HAYATI
Journal of Biosciences

Vol. 31 No. 4, July 2024 768-780
DOI:10.4308/hjb.31.4.768-780
ISSN: 1978-3019
EISSN: 2086-4094

Apis cerana Fabricius, 1793 in Sumatra: Haplotype Variations of Mitochondrial DNA and the Molecular Relationship with the Asian Honey Bees (Hymenoptera: Apidae)

Juniarto Gautama Simanjuntak[1], Windra Priawandiputra[1], Rika Raffiudin[1*], Nurul Insani Shullia[2], Jauharlina Jauharlina[3], Mahardika Gama Pradana[4], Araz Meilin[5], Jasmi Jasmi[6], Yulia Pujiastuti[7], Puji Lestari[8], Rustem Ilyasov[9], Rahmadi Sitompul[10], Tri Atmowidi[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia*
[2]*Biology Education Study Program, Faculty of Teacher Training and Education, University of Jember, Jember, Indonesia*
[3]*Department of Plant Protection, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia*
[4]*Indonesian Oil Palm Research Institute, Medan, Indonesia*
[5]*Research Center for Horticulture, National Research and Innovation Agency, Republic of Indonesia*
[6]*Study Program Industrial Hygiene and Occupational Safety and Health, College of Health Sciences, Padang, Indonesia*
[7]*Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia*
[8]*Department of Plant Protection, Faculty of Agriculture, Lampung University, Bandar Lampung, Indonesia*
[9]*Department of Biological Sciences, Koltzov Institute of Developmental Biology, Moscow, Russia*
[10]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan, Indonesia*

ARTICLE INFO

Article history:
Received October 1, 2022
Received in revised form March 1, 2024
Accepted March 15, 2024

KEYWORDS:
cytochrome c oxidase subunits 1,
genetic diversity,
mtDNA markers,
Pleistocene,
North Sumatra,
Sundaland

ABSTRACT

Honey bee *Apis cerana* is widely distributed in Asia and the Indonesian archipelago, including Sumatra. We studied the molecular variations of *A. cerana* using *cytochrome c oxidase subunits 1* and *2* genes (*cox1* and *cox2*) and the *cox1*/*cox2* intergenic spacers (igs) in several altitudes in the six provinces of Sumatra. We explored the haplotype distributions of those three mtDNA markers for *A. cerana* in the low-, mid-, and highlands of Sumatra. We also analyzed their relationship with *A. cerana* in Sundaland and Asia using those markers. Our study revealed 12 new haplotypes of *A. cerana cox1* in Sumatra, while nine and eight new haplotypes for *cox2* and igs, respectively. *Apis cerana* in North Sumatra, Lampung, and South Sumatra had the three highest haplotype variations. Most of the specific haplotypes of inter-colony *A. cerana* from Sumatra were found in the lowlands, while most were in the highlands for intra-colony variations. We found low gene flow among populations of *A. cerana* in Sumatra. One haplotype, Sumatra4 *cox2* from North Sumatra, was the same as Java3 haplotype, presumably due to anthropogenic impact. The molecular phylogenetic tree of *A. cerana* in the Sundaland revealed that *A. cerana* from Sumatra has a close relationship to those of Borneo compared to Java.

1. Introduction

Sumatra Island formed a geological part of Sundaland that united with Java, Borneo, Thai-Malay Peninsula and other small islands in Asia continental (de Bruyn *et al.* 2014). The formation of Sumatra Island was due to the increase in sea level, thus emerging in the shelf area of Sunda and Australia during the Pleistocene (Hall 2013). The unity of the plains with mainland Asia before the Pleistocene can affect the distribution of mammals to the Sunda shelf (Heaney 1986).

Asian honey bee *Apis cerana* is distributed widely in Asia, from Afghanistan, Pakistan, China, Korea (Ruttner 1988), and the southernmost areas of the former Soviet Union and south to northern Vietnam (Engel 1999). Further, this species is also discovered in Sundaland of Indonesia: Borneo, Sumatra, and Java, lesser Sunda of Indonesia, Sulawesi, Malaysia, Thailand, Myanmar, India, and Sri Lanka (Damus and Otis 1997). The bees are extensively adapted to massive areas, from Russia (Ilyasov *et al.* 2019) to the archipelago of Indonesia (Raffiudin *et al.* 2022) and the Philippines (Smith *et al.* 2000). In Indonesia, *A. cerana* were commonly used in beekeeping (Buchori *et al.* 2022).

* Corresponding Author
E-mail Address: rika.raffiudin@apps.ipb.ac.id

Four subspecies of *A. cerana* were first recognized, i.e., *A. c. cerana, A. c. indica, A. c. japonica*, and *A. c. himalaya* (Ruttner 1988). Furthermore, based on the whole mitochondrial genome, nuclear genes, and morphological measurements, several new subspecies were discovered, i.e. *A. c. koreana* in Korea (Ilyasov *et al.* 2018) and *A. c. ussuriensis* in Russia Far-East (Ilyasov *et al.* 2019).

Based on morpho-cluster, *A. cerana* in Sundaland as parts of Indonesia was classified in morphocluster VI cluster (Hepburn and Radloff 2011). The cluster consisted of the Indo-Malayan cerana and those from southern Thailand, Malaysia and Indonesia (Radloff *et al.* 2010; Hepburn and Radloff 2011). Moreover, Engel (1999) classified eight subspecies of *A. cerana*, two of which exist in Indonesia, i.e., *A. c. javana* distributes from Java to Timor and *A. c. johni* in Sumatra.

The forewing of *A. cerana* with complete distal venation is the morphological key to differentiating the Apini Tribe from other Corbiculate bees. However, the presence of the distal abscissa vein M in the hyaline hind wing in *A. cerana* is the specific character that differs from other *Apis* (Engel 2012). Based on the geometric morphometric of *A. cerana* wing venations, the bees from Sumatra have a high bending energy as the result of the forewing landmark variety (Nisa *et al.* 2022). *Apis cerana* in the highland showed longer in all morphometrics characters (except the proboscis length) than in the lowland (Raffiudin *et al.* 1999). However, there is no extensive study using molecular markers of this cavity-nesting honey bee in the low- and highlands of Sumatra.

Mitochondrial DNA (mtDNA) has been used to study the population structure in honey bees (Tanaka *et al.* 2003; Songram *et al.* 2006; Tan *et al.* 2016). Based on mtDNA of *cox1/cox2* intergenic spacer (igs) region, *A. cerana* was separated into Sundaland, Asian mainland, Palawan, and Luzon-Mindanao clades (Smith *et al.* 2000). Furthermore, the Sundaland *A. cerana* phylogenetic tree based on *cox1* showed that the Indonesian (Java, Bali, Lombok, South Kalimantan, and Central Sulawesi) was separated from the Indo-Malayan lineage (Sabah, Sarawak, and Kalimantan) (Raffiudin and Shullia 2020). However, a molecular study using the mtDNA markers of *A. cerana* has not been conducted in Sumatra. Thus, the current study investigates the molecular variations of *A. cerana* in the six provinces in Sumatra using *cox1, cox2*, and igs of mtDNA and explores the relationship with those of *A. cerana* from Sundaland and East Asia.

2. Materials and Methods

2.1. Sample Collection

Workers of *Apis cerana* honey bee were collected from 17 locations (eight colonies in the lowlands <300 m asl, three colonies in the midland 301–1,000 m asl, and six colonies in the highland >1,000 m asl (altitude classification follow Bourke 2010) in the six provinces of Sumatra (Table 1). Honey bees were preserved in absolute ethanol and placed at 4°C before DNA extraction.

Table 1. Colony ID, locations, and collectors of *Apis cerana* samples from Sumatra

Colony ID	Location	Altitude (m asl)	Habitat	Coordinates	Collectors*
Aceh					
Ac_PnB_AcB Ach	Peukan Bada, Aceh Besar	30	Human settlement near forest	5°32'28.436"N 95°13'39.857"E	JHL, TAF, KFH
Ac_Bkt_BnM Ach	Bukit, Bener Meriah	1,343	Coffee plantation	4°42'29.764"N 96°51'46.098"E	JHL, AFY, MDS
North Sumatra					
Ac_StS_Pmt NS	Siantar Sitalasari, Pematangsiantar	425	Human settlement with gardens and river	2°56'31.131"N 99°2'25.792"E	MGP, GNS, AHS
Ac_GSB_Sml NS	Girsang Sipangan Bolon, Simalungun	1,175	*Calliandra* sp. and *Styrax* sp. plantation	2°43'13.847"N 98°56'21.257"E	MGP, GNS, SMS
Ac_Prb_Sml NS	Purba, Simalungun	1,301	Human settlement with gardens	2°52'34.408"N 98°42'44.263"E	MGP, GNS, RHM
Ac_Krj_PkB NS	Kerajaan, Pakpak Bharat	1,047	Human settlement near forest and *Shorea* sp. plantation	2°40'17.269"N 98°19'37.254"E	MGP, GNS, JMS
Ac_SlL_Ash NS	Silo Lama, Asahan	15	Coconut plantation	3°6'24.327"N 99°43'20.003"E	MGP, GNS, MRN

Table 1. Continued

Colony ID	Location	Altitude (m asl)	Habitat	Coordinates	Collectors*
Jambi					
Ac_KlJ_TJT Jmb	Kuala Jambi, East Tanjung Jabung	200	Human settlement near forest	-1°2'39,40425"S 103°47'33,96229"E	AMN, CLA, MRD
Ac_GnK_Krc Jmb	Mount Kerinci, Kerinci	1,157	Human settlement near forest	-1°54'51,009"S 101°14'15,181"E	AMN, CLA, JKR
West Sumatra					
Ac_SnG_PdP WS	Sungai Garingging, Padang Pariaman	15	Human settlement near plantation	-0°25'37,46892"S 100°4'34,17258"E	JSI, BYC, YAS
Ac_Btp_TnD WS	Batipuh, Tanah Datar	1,116	Feral colonies with coffee and woody plant plantation	-0°26'16,02816"S 100°26'47,42538"E	JSI, ZZI, FHI
South Sumatra					
Ac_BTS_MsR SS	Bulang Tengah Suku Ulu, Musi Rawas	79	Human settlement near gardens	-3°23'13,338"S 103°21'40,788"E	YPI, ASI, HSO
Ac_GnM_MrE SS	Mount Megang, Muara Enim	75	Human settlement near gardens	-3°32'6,008"S 103°57'4,267"E	YPI, ASI, SYN
Ac_SDL_MrE SS	Semedo Darat Laut, Muara Enim	676	Human settlement near gardens	-4°4'19,853"S 103°38'56,021"E	YPI, ASI, TKO
Lampung					
Ac_PsS_PsB Lmp	South Pesisir, West Pesisir	80	Littoral with coconut plantation	-5°25'17,454"S 105°42'24,632"E	PLI, DAA, SHM
Ac_Jbg_LmT Lmp	Jabung, East Lampung	14	Human settlement near gardens	-5°19'17,551"S 103°59'56,61"E	PLI, DAA, SYO
Ac_WyR_Psw Lmp	Way Rate, Pesawaran	510	Human settlement near gardens	-5°33'9,156"S 105°6'12,531"E	PLI, DAA, YSA

*Colony ID Ac: *Apis cerana*, AcB: Aceh Besar, Ach: Aceh, Ash: Asahan, Bkt: Bukit, BnM: Bener Meriah, Btp: Batipuh, BTS: Bulang Tengah Suku Ulu, GnK: Gunung Kerinci, GnM: Gunung Megang, GSB: Girsang Sipangan Bolon, Jbg: Jabung, Jmb: Jambi, KlJ: Kuala Jambi, Krc: Kerinci, Krj: Kerajaan, Lmp: Lampung, LmT: East Lampung, MrE: Muara Enim, MsR: Musi Rawas, NS: North Sumatra, PdP: Padang Pariaman, PkB: Pakpak Bharat, Pmt: Pematangsiantar, PnB: Peukan Bada, Prb: Purba, PsB: West Pesisir, PsS: South Pesisir, Psw: Pesawaran, SDL: Semendo Darat Laut, SlL: Silo Laut, Sml: Simalungun, SnG: Sungai Geringging, SS: South Sumatra, StS: Siantar Sitalasari, TJT: East Tanjung Jabung, TnD: Tanah Datar, WS: West Sumatra,WyR: Way Ratai

*Collectors AFY: Afriyani, AHS: Aam Hasanudin, AMN: Araz Meilin, ASI: Arsi, BYC: Buti Yohenda, Christy, CLA: Chandra Lela, DAA: Denny Achmad Akbar Roswandi, FHI: Fahmi, GNS: Ganesa, HSO: Heru Supriyanto, JHL: Jauharlina, JSI: Jasmi, JKR: Joko Rasmono, JMS: Julkermin Simanjuntak, KFH: Khairil Fatah, MGP: Mahardika Gama Pradana, MRN: Marmin, MDS: Muhammad Darussalam, MRD: Mursyid, PLI: Puji Lestari, RHM: Rohman, SHM: Suherman, SMS: Samalam Saragih, SYO: Suyarto, SYN: Suryadin, TAF: Teuku Andrio Febrian, TKO: Tukino, YAS: Yance Andrianus, YPI: Yulia Pujiastuti, YSA: Yayat Supriatna, ZZI: Zamzami. Lowland: <300 m asl, Midland: 501– 1,000 m asl, Highland: >1,000 m asl

2.2. DNA Extraction, Amplification, and Sequencing

The whole genomic DNA of *A. cerana* was extracted from thorax tissue using DNA Mini Kit GenAid. The *cox1* gene was amplified using forward and reverse primers, i.e., Am_*cox1*b_F and Am_*cox1*b_R, respectively (Raffiudin *et al.* 2022). The igs and *cox2* gene amplifications were carried out using forward primer E2 (Cornuet *et al.* 1991) and a reverse primer H1 (Estoup *et al.* 1996). The 25 µl final volumes of PCR mix were composed of 7 µl of distilled water (dH_2O), 1.5 µl of 25 mM $MgCl_2$, 12.5 µl of MyTaq™ HS Red Mix 2x (Bioline Reagents Ltd, United Kingdom), 1.0 µl of 10 µM each forward and reverse primer, and 1-2 µl of up to 250 ng/µl DNA template. The PCR conditions were conducted as follows: pre-denaturation at 95°C for 2.5 min, 35 cycles of denaturation at 95°C for 15 s, annealing at 51°C (*cox1*) and 55°C (igs-*cox2*) for 15 s, and elongation at 72°C for 1 min. Then post elongation at 72°C for 2 min, and final at 15°C for 5 min. PCR products were visualized in 1% agarose gel, stained using Diamond Nucleic Acid Dye (Promega, Madison, USA), and sequenced in 1st BASE, Selangor, Malaysia. The current study of *A. cerana cox1* and igs-*cox2* in Sumatra have been listed in GenBank accession numbers: LC728498 to LC728558 and LC739435 to LC739501, respectively (Supplementary 1 doi.org/10.5281/zenodo.10639197).

2.3. Bioinformatics, Haplotype Analysis, and Phylogenetics

Homology analysis of *A. cerana* sequences was carried out using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences of *A. cerana cox1, cox2,* and igs were aligned using ClustalX 2.0 (Larkin *et al.* 2007). Gene haplotype variations, haplotype diversity, nucleotide diversity, and Fixation index value (F_{ST}) were analyzed using DnaSP 5.10 (Rozas *et al.* 2003). Further, the common and specific haplotypes were determined in the low-, mid- and highlands of Sumatra. Nucleotide and putative amino acid variations, the number of transitions and transversions, and pairwise distances of each gene were analyzed using MEGA X (Kumar *et al.* 2018). The nucleotide-based phylogenetic trees of *A. cerana* from current Sumatra samples and sequences from GenBank data, such as *A. cerana* from other islands in Indonesia, Sabah, Sarawak, East Asia, and Russia (Supplementary 1), were inferred using the Maximum Likelihood (ML) method implemented in MEGA X using suggested the best model with 1,000 bootstrap replicates.

3. Results

3.1. The New and Specific Haplotypes of *Apis cerana cox1, cox2,* and igs in Sumatra

The amplicons of the *cox1* and *cox2* genes of *A. cerana* were 593 and 464 bp, respectively, and the igs non-coding region was from 84-88 bp. BLASTN results showed that the *cox1* and igs-*cox2* of *A. cerana* in Sumatra were 96.35–99.27% and 95.45–99.51%, respectively. Those sequences showed homology to *A. cerana* from Sabah, Borneo (GenBank Acc. Num. AP018149.1) (Supplementary 2a–b doi.org/10.5281/zenodo.10464107).

Our exploration found 12 new haplotypes of *A. cerana cox1*, while nine and eight new haplotypes were found in *cox2* and igs, respectively, of *A. cerana* in Sumatra. Three haplotypes of *cox2*, i.e., Sumatra4 was the same as Java3 (Raffiudin *et al.* 2022), Sumatra11 was the same as *A. cerana* from Sabah, Borneo AY587544.1, and Sumatra12 was the same as *A. cerana* from Sabah, Borneo AP018149.1. One haplotype of igs, i.e., Sumatra2, was the same as Java1 (Smith and Hagen 1996). Therefore, 12 and nine haplotypes of *cox2* and igs, respectively, have been recorded.

Among the three markers, the highest common haplotype (Sumatra1) of *A. cerana* in Sumatra was igs (66%), while 59% of *cox1* and the least 37% of *cox2*. The specific haplotype of *A. cerana cox2* was also found to be the lowest (22%), whereas 26% and 27% for *cox1* and igs, respectively. The specific haplotypes were only found in one location of *A. cerana* in Sumatra, mostly in the lowlands (Supplementary 3b, e, g doi.org/10.5281/zenodo.10642694).

Our first exciting finding was that North Sumatra has the highest haplotype variations of *A. cerana*, which were six, seven, and four haplotypes for *cox1, cox2* and igs, respectively (Figure 1). These were followed by *A. cerana cox1, cox2*, and igs in Lampung with six, two, and four, and South Sumatra with four, four, and three haplotypes for *cox1, cox2* and igs, respectively.

In terms of the specific haplotypes, *A. cerana* from each North Sumatra and Lampung had the highest of four specific haplotypes of *cox1* (Supplementary 3b). The specific haplotypes of *A. cerana* in the North Sumatra were Sumatra3 (colony 2 Siantar Sitalasari), Sumatra4 (colony 1 Purba), Sumatra5 (colony 1 Kerajaan), and Sumatra6 (colony 1 Silo Lama) (Supplementary 3b). The specific haplotypes of *A. cerana* in Lampung consisted of Sumatra9 (colony 1 South Pesisir) and Sumatra10 (colony 2 South Pesisir), Sumatra11 (colony 1 Way Ratai), and Sumatra12 (colony 2 Way Ratai) (Supplementary 3b). We found only one specific *cox1* haplotype, Sumatra7, in South Sumatra in colony 1 Bulang Tengah Suku Ulu (Supplementary 3b). However, Aceh, Jambi, and West Sumatra did not show specific haplotypes for *cox1* (Figure 1, Supplementary 3b). The specific haplotypes are essential and can be used as the marker of *A. cerana* from a particular region. On the other hand, Aceh had a specific haplotype Sumatra2 of the *cox2* gene in colony 2 Bukit (Supplementary 3e). North Sumatra also had the highest three specific haplotypes of *cox2* those are Sumatra4, Sumatra5, and Sumatra6 in colony 2 Siantar Sitalasari, colony 1 Siantar Sitalasari, and colony 1 Silo Lama, respectively (Supplementary 3e). One specific haplotype *cox2* gene was found in Jambi, West Sumatra, and South Sumatra (Supplementary 3e). The specific haplotype Sumatra8 was from *A. cerana* colony 2 Kuala Jambi, Sumatra9 from colony 2 Sungai Geringging, Sumatra10 from colony 1 Bulang Tengah Suku Ulu, and Sumatra11 from colony 1 Semendo Darat Laut (Supplementary 3e).

In line with the *cox1* gene, *A. cerana* igs from North Sumatra had the highest (two) specific haplotypes (Supplementary 3g). We found Sumatra2 from *A. cerana* colony 1 Silo Lama and Sumatra3 from colony 2 Siantar Sitalasari were the specific haplotype for igs in North Sumatra, *Apis cerana* from Jambi, West Sumatra, South Sumatra, and Lampung had one specific haplotype of igs (Figure 1, Supplementary 3g). Haplotype Sumatra8 of *A. cerana* in Sumatra was

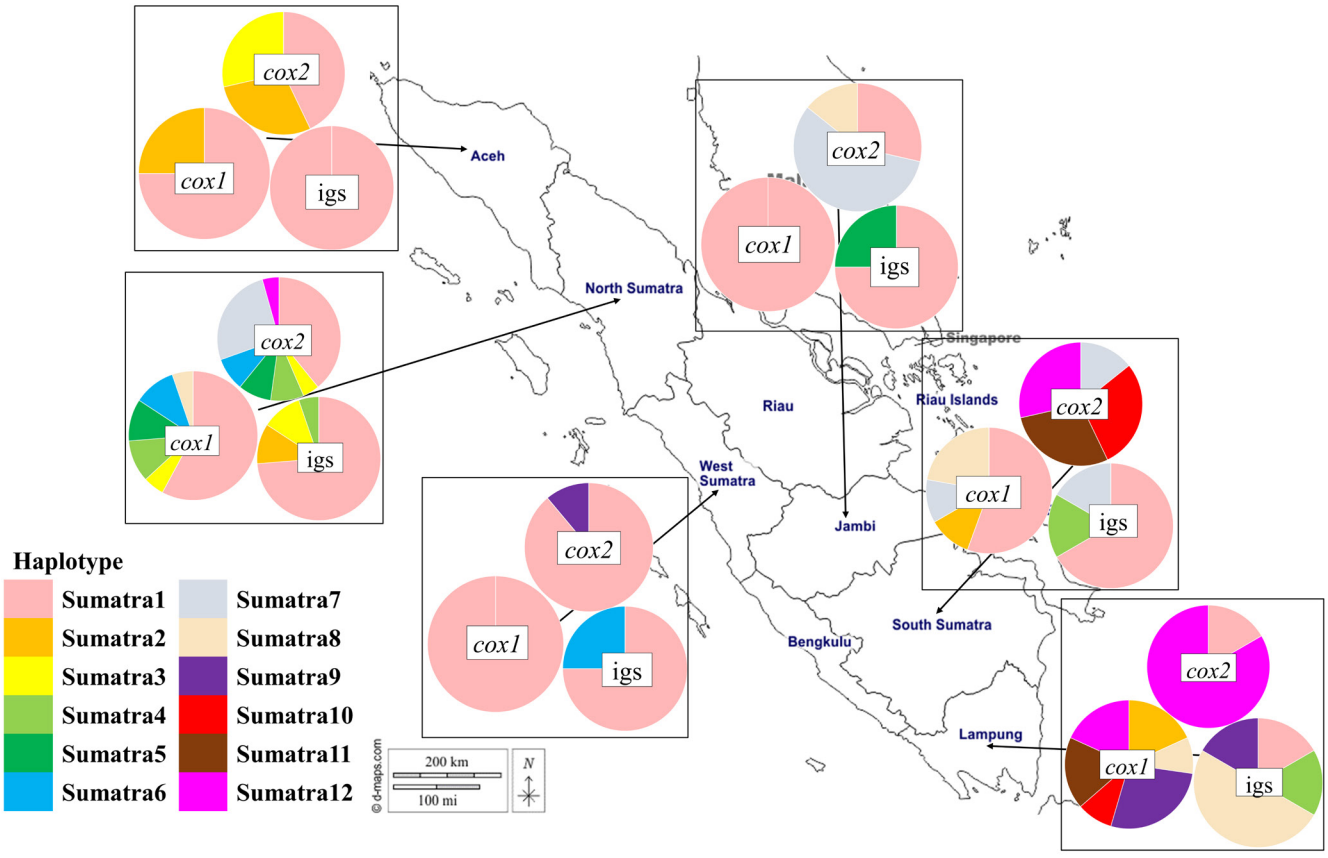

Figure 1. The distribution of haplotypes of *Apis cerana* in Sumatra based on the *cox1*, *cox2* genes, and igs from 17 locations in Sumatra. The haplotypes of *A. cerana* refer to Supplementary 3a, 3c, 3f doi.org/10.5281/zenodo.10642694

found only in Lampung with two locations: lowland (colony 1 and 2 Pesisir Selatan) and midland (colony 1 Way Ratai) (Supplementary 3g). In accordance with the *cox1* gene, no specific haplotypes of igs were found in Aceh (Figure 1, Supplementary 3g).

3.2. Genetic Diversity, Differentiation, and Nucleotide Variations of *Apis cerana cox1, cox2,* and igs in Sumatra

Genetic diversity is shown by the diversity of haplotype (Hd) and nucleotide (Л) (Freeland *et al.* 2011). The average Hd of *cox2 A. cerana* in Sumatra showed high values (0.799±0.034), whereas both *cox1* and igs were moderate, i.e. 0.643±0.069 and 0.545±0.066, respectively (Table 2). Furthermore, the highest Hd values were found in Lampung (*cox1*), South Sumatra (*cox2*), and Lampung (igs), respectively. We found zero (0.000) Hd values for *A. cerana* in Jambi and West Sumatra (*cox1*) and Aceh (igs) (Table 2).

The average Л values of *A. cerana* based on *cox1*, *cox2*, and igs markers all showed relatively low, were $390 \times 10^{-5} \pm 96 \times 10^{-5}$, $570 \times 10^{-5} \pm 174 \times 10^{-5}$, and $112 \times 10^{-5} \pm 180 \times 10^{-5}$, respectively (Table 2), with the highest Л values, were found in North Sumatra. The lowest Л values were found in Jambi-West Sumatra and Aceh based on *cox1*, *cox2*, and igs (Table 2).

The F_{ST} value showed genetic differentiation for the *A. cerana cox2* gene, i.e. 0.01282-0.76110 ($P<0.001$) and 0-0.61143 for the *cox1* gene ($0.01<P<0.05$) (Table 3). The igs was -0.00312 to 0.69697 ($P<0.001$) (Table 4). Generally, the F_{ST} values of those three mtDNA markers gradually increased from Aceh to Lampung, indicating differentiation among *A. cerana* populations in Sumatra (Tables 3 and 4).

Apis cerana cox2 was also the highest (28 nucleotide variations) among the other two markers (Supplementary 3c, 3e doi.org/10.5281/zenodo.10642694). The 1st, 2nd, and 3rd codon percentage variations of *cox2* were 11%, 0%, and 89%, respectively. *Apis cerana* Sumatra4 *cox2* gene from colony 2 Siantar Sitalasari, North Sumatra, showed a putative amino acid substitution at amino acid position 97 (Valine-Isoleucine) (Supplementary 3d). The second mutation at amino acid position 151 of *cox2* haplotype Sumatra9 occurred in individual 1 of *A. cerana* colony 2 in Sungai Geringging (West Sumatra) (Supplementary 3d), which changed

Table 2. Genetic diversity of *Apis cerana* Sumatra based on the *cox1*, igs, and *cox2* sequences

Population	Hd ± SD			Л ± SD		
	cox1	*cox2*	igs	*cox1*	*cox2*	igs
Ac_Ach	0.429±0.169	0.714±0.123	0.000±0.000	0.429±0.169	0.714±0.123	0.000±0.000
Ac_NS	0.661±0.114	0.779±0.082	0.477±0.134	0.661±0.114	0.779±0.082	0.477±0.134
Ac_Jmb	0.000±0.000	0.667±0.160	0.356±0.159	0.000±0.000	0.667±0.160	0.356±0.159
Ac_WS	0.000±0.000	0.250±0.180	0.429±0.169	0.000±0.000	0.250±0.180	0.429±0.169
Ac_SS	0.694±0.147	0.800±0.089	0.582±0.142	0.694±0.147	0.800±0.089	0.582±0.142
Ac_Lmp	0.891±0.063	0.303±0.147	0.727±0.109	0.891±0.063	0.303±0.147	0.727±0.109
Average	0.643±0.069	0.799±0.034	0.545±0.066	390×10^{-5} $\pm 96 \times 10^{-5}$	570×10^{-5} $\pm 174 \times 10^{-5}$	$1{,}112 \times 10^{-5}$ $\pm 180 \times 10^{-5}$

Hd = haplotype diversity, л = nucleotide diversity, SD = standard deviation. The details of population abbreviations are given in the note of Table 1

Table 3. Fixation index value (F_{ST}) of *Apis cerana* Sumatra populations based on the *cox1* (above diagonal) and *cox2* (below diagonal) and *cox2* (below diagonal) genes

Population	Ac_Ach	Ac_NS	Ac_Jmb	Ac_WS	Ac_SS	Ac_Lmp
Ac_Ach		0.06986*[a)]	0.14286*[a)]	0.14286*[a)]	0.14536*[a)]	0.55980*[b)]
Ac_NS	0.07463***[a)]		0.05903*[a)]	0.05903*[a)]	0.02766*[a)]	0.30877*[b)]
Ac_Jmb	0.33333***[b)]	0.06284***[a)]		0.00000[a)]	0.21154*[b)]	0.61143*[b)]
Ac_WS	0.28571***[b)]	0.09917***[a)]	0.37559***[b)]		0.21154*[b)]	0.61143*[b)]
Ac_SS	0.33333***[b)]	0.10053***[a)]	0.01282***[a)]	0.46377***[b)]		0.17780*[b)]
Ac_Lmp	0.49282***[b)]	0.18607***[b)]	0.29781***[b)]	0.76110***[b)]	0.12919***[a)]	

Zero values should be interpreted as no genetic differentiation between two populations. Details on the population code are given in Table 1. [a)]indicate weak and moderate differentiation (F_{ST}: 0-0.15, Wright 1978). [b)]indicate great and extremely great differentiation (F_{ST}: >0.15, Wright 1978). *cox1*: *0.01 < P < 0.05; *cox2*: ***P < 0.001. The F_{ST} values, 0-0.05: weak differentiation, 0.05-0.15: moderate differentiation. 0.15-0.25: great differentiation, and >0.25: extremely great differentiation among the populations (Wright 1978)

Table 4. Fixation index value (F_{ST}) of *Apis cerana* Sumatra populations based on the IGS

Population	Ac_Ach	Ac_NS	Ac_Jmb	Ac_WS	Ac_SS	Ac_Lmp
Ac_Ach						
Ac_NS	0.11146***[a)]					
Ac_Jmb	0.11111***[a)]	-0.00312[a)]				
Ac_WS	0.14286***[a)]	0.11747***[a)]	0.12045***[a)]			
Ac_SS	0.20000***[b)]	0.06493***[a)]	0.07591***[a)]	0.19162***[b)]		
Ac_Lmp	0.69697***[b)]	0.37780***[b)]	0.45743***[b)]	0.63540***[b)]	0.17746***[b)]	

Negative values should be interpreted as no genetic differentiation between two populations. Details on the population code are given in Table 1. [a)]indicate weak and moderate differentiation F_{ST}: 0-0.15, Wright 1978). [b)]indicate great and extremely great differentiation (F_{ST}: >0.15, Wright 1978). IGS: ***P < 0.001. The F_{ST} values indicator refers to Table 3

Isoleucine to Valine (Supplementary 3d). We found a second interesting phenomenon, *cox2* haplotype Sumatra4 in colony 2 Siantar Sitalasari, North Sumatra, which was the same haplotype as Java3 from Raffiudin *et al.* (2022).

Moreover, the *cox2* gene variation of *A. cerana* in North Sumatra revealed an intra-colony variation in nucleotide 402 of colony 1 Girsang Sipangan Bolon, which was in Sumatra1 and Sumatra3 (Supplementary 3c). However, this nucleotide mutation did not alter the putative Leucine amino acid (in position 134). *Apis cerana* colony 2 Sungai Geringging West Sumatra (Sumatra1 and Sumatra9) showed intra-colony variation in nucleotide 451 (Supplementary 3c). This mutation changed the putative amino acid Isoleucine to Valine in position 151 (Supplementary 3d). As predicted, putative amino acid variation was observed only in colony 2 Sungai Geringging intra-colony nucleotide variations.

The number of transitions of the Sumatra *cox1*, *cox2* genes, and igs of *A. cerana* from Sumatra was consistently higher than those of transversion (Figure 2A–G). The number of substitutions is higher in the third codon, followed by the first codon, and no mutation in the second codon (Figure 2A–G). The substitutions are higher in the igs followed by *cox2* and slowest in the *cox1* genes (Figure 2A–G).

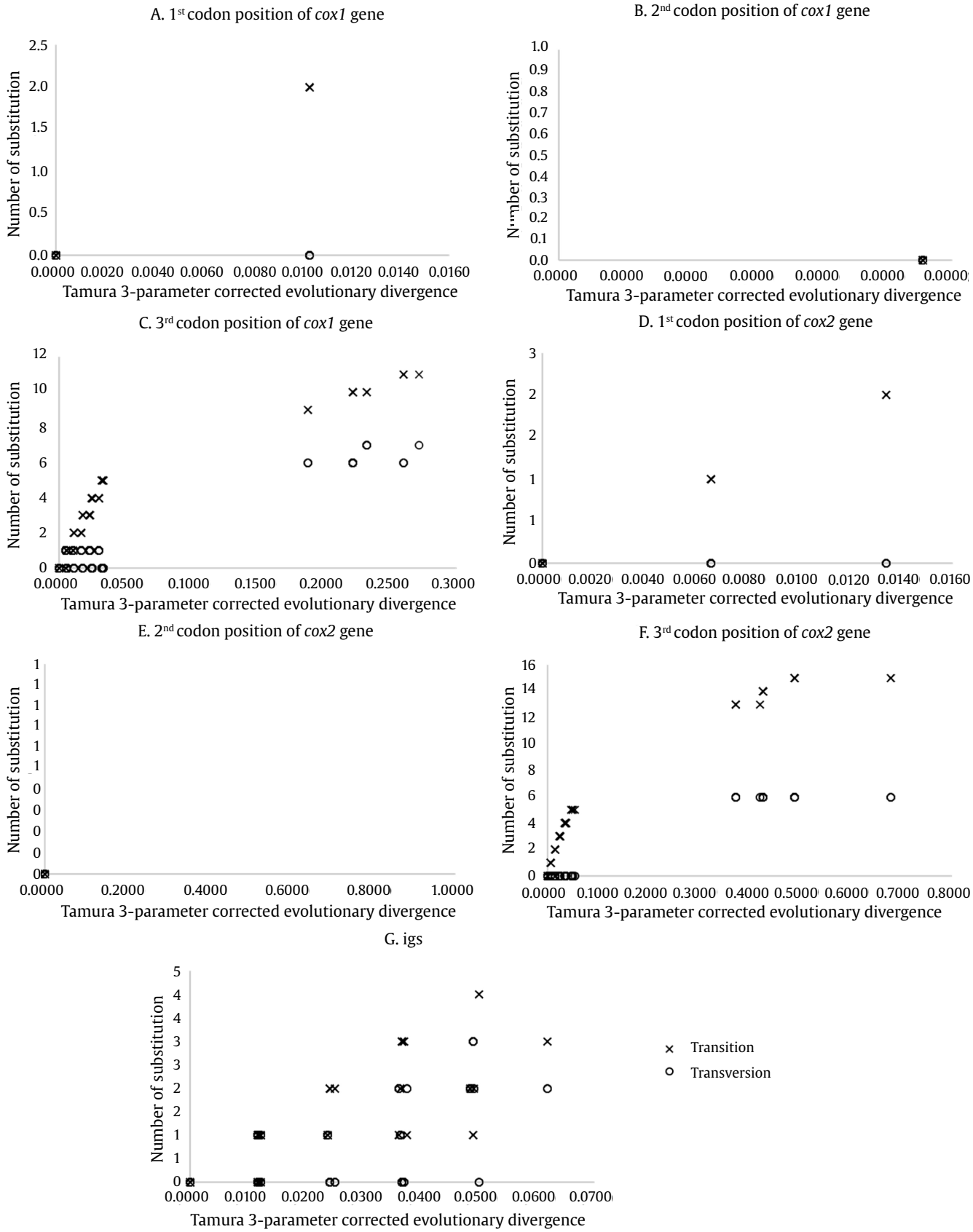

Figure 2. The number of transitions and transversions in the three codon positions of *Apis cerana cox1, cox2* genes in Sumatra, and transition and transversion in igs. The average divergence, (A) 0.000338, (B) 0, (C) 0.01753, (D) 0.001, (E) 0, (F) 0.03673, and (G) 0.01301

This study found 22 nucleotide variations of the *cox1* gene, with the highest variations (91%) shown in the third codon, followed by the 1st (9%), and no variations in the second codon. Similar to the *cox2* gene, we found intra-colony nucleotide variations in the *cox1* gene, leading to haplotype variations (Supplementary 3a). Colony 2 Purba (North Sumatra) had two *cox1* haplotypes of Sumatra1 and Sumatra8. Furthermore, haplotype variations were shown in colony 2 Semendo Darat Laut (South Sumatra) for Sumatra1 and Sumatra2, and colony 2 South Pesisir (Lampung) had Sumatra9 and Sumatra10 of *cox1* gene (Supplementary 3a–b).

Apis cerana igs showed the least 11 nucleotide variations (Supplementary 3f). In North Sumatra, we observed intra-colony nucleotide variations of *A. cerana* igs in nucleotides 14, 41, and 56 for colony 2 Purba, which was in Sumatra1 and Sumatra4 (Supplementary 3f–g).

3.3. Genetic Distance of *Apis cerana cox1, cox2,* and igs in Sumatra and Phylogenetic Relationship with Asian Honey Bees

The genetic distances of intra *A. cerana* from Sumatra, except colony 2 Siantar Sitalasari, were ranged from 0–0.97%, 0–1.12%, 0–5.00%, and 0.08–5.58% based on *cox1, cox2*, igs, and *cox1*-igs-*cox2*, respectively (Supplementary 4a–d doi.org/10.5281/zenodo.10642762). We also compared the genetic distance of *A. cerana cox1* Sumatra to Borneo and found that it was closer (0.38–1.37%) than to Java (2.99–4.07%) (Supplementary 4a). Furthermore, the latter is similar to the genetic distance between *A. cerana* from Sumatra and *A. cerana* from East Asia (4.28–5.82%) (Supplementary 4a).

Following the genetic distance data, the ML phylogenetic tree based on the *cox1, cox2*, igs, and *cox1*-igs-*cox2* showed that *A. cerana* from Sumatra was closely related to *A. cerana* from Borneo and separated from *A. cerana* from Java (Figure 3A–D). Colony 2 from Siantar Sitalasari was confirmed by the ML tree based on the *cox1, cox2*, and igs that grouped with the clade of *A. cerana* from Java (Figure 3A–D).

4. Discussion

This study reveals the genetic structure based on *cox1, cox2*, and igs of the *A. cerana* in Sumatra and their relationship with *A. cerana* from Sundaland and East Asia. The sequences of the targeted mtDNA markers showed a total of 33 haplotypes haplotype variations. All *cox1* haplotypes, nine of 12 *cox2* haplotypes, and eight of nine igs haplotypes were new and specifically found only in *A. cerana* in Sumatra.

4.1. Highly Number of Haplotype Variations and Specific Haplotype of *Apis cerana* in Different Altitudes of North Sumatra, Lampung, and South Sumatra

This study found that North Sumatra, Lampung, and South Sumatra had the highest haplotype variation of *A. cerana* from Sumatra based on *cox1, cox2*, and igs in all altitudes (Figure 1). Moreover, the genetic variation that was determined by haplotype diversity (Hd) and nucleotide diversity (JI) (Freeland *et al.* 2011) of *A. cerana* from Sumatra supported these results (Table 2). The wide range of altitude (23–1,307 m asl) of *A. cerana* traditional apiaries in North Sumatra with habitat variations (Table 1) presumably shaped the haplotype variations in these regions. The high variation of *A. cerana* from North Sumatra occurred in four out of five habitat variations in the low-, mid-, and highlands (Table 1). Similar high variations and specific haplotypes of tRNA-Leu-*cox2* of mtDNA were derived from various altitudes in *A. cerana* from Qinghai-Tibet plateau-valley landforms, China (Yu *et al.* 2019). The molecular results were supported by the morphometrics of *A. cerana* in different altitudes, that *A. cerana* in highlands have longer body parts than those in lowlands in all characters except the proboscis (Raffiudin *et al.* 1999). This phenomenon increased the length of body parts with the altitude, also seen in the morphology data of the mean centroid size values of *A. cerana* forewings and hindwings in five regions in China (Nannan *et al.* 2022). Those reports indicate that altitude influences genetic variations and morphology.

We also found that North and South Sumatra in the low- and highlands had specific haplotypes based on the three mtDNA markers, while the highland of Aceh had only a specific haplotype of *cox2* (Figure 1, Table 1, Supplementary 3b, e, g). The lowland of Jambi and West Sumatra had specific haplotypes in two mtDNA markers, i.e., igs and *cox2*, while Lampung in the low- and midlands had specific haplotypes of *cox1* and igs (Figure 1, Table 1, Supplementary 3b, e, g). In the Philippines Islands, de la Rúa *et al.* (2000) also found the specific

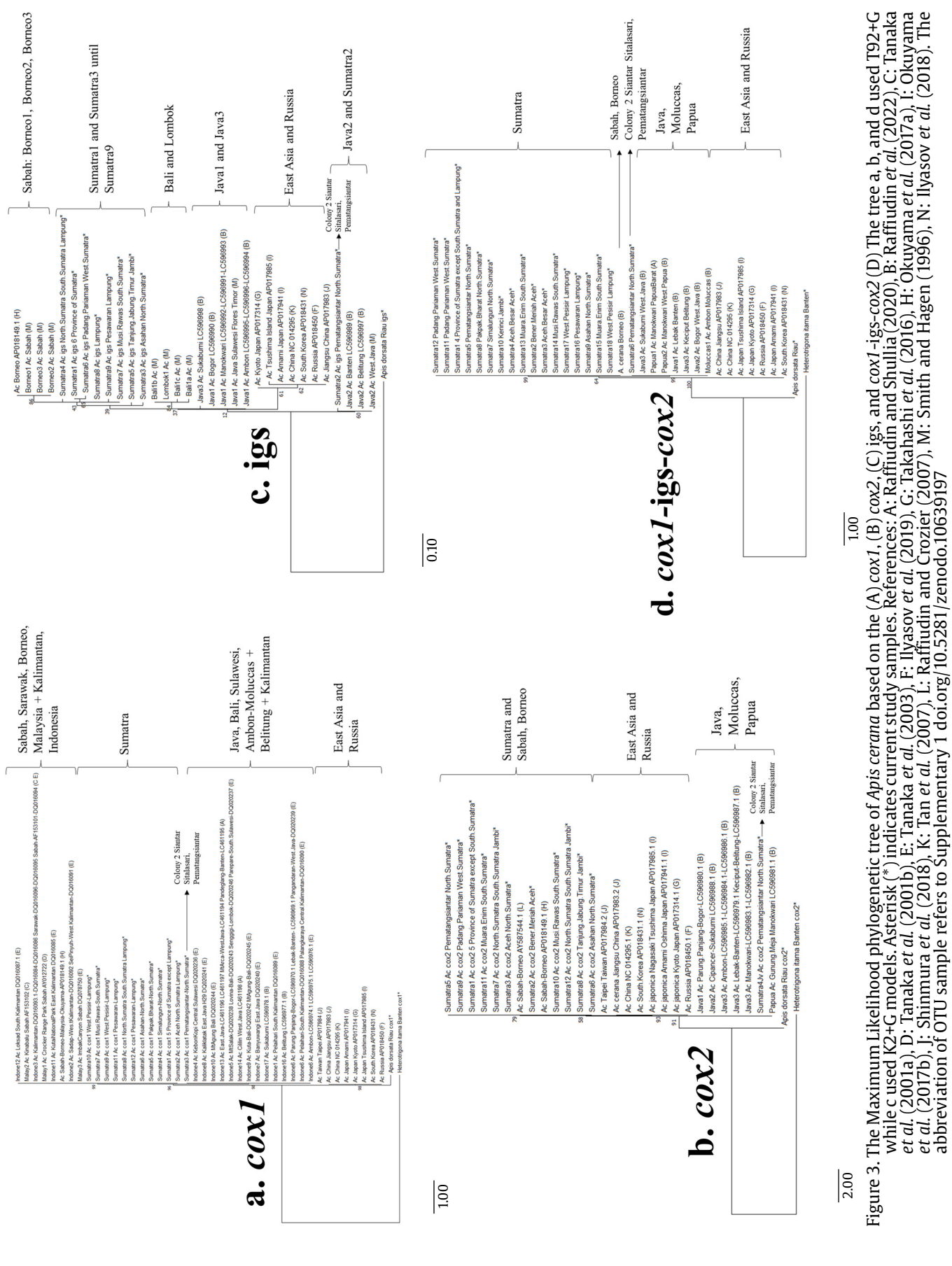

Figure 3. The Maximum Likelihood phylogenetic tree of *Apis cerana* based on the (A) *cox1*, (B) *cox2*, (C) igs, and *cox1*-igs-*cox2* (D) The tree a, b, and d used T92+G while c used K2+G models. Asterisk (*) indicates current study samples. References: A: Raffiudin and Shullia (2020), B: Raffiudin *et al.* (2022), C: Tanaka *et al.* (2001a), D: Tanaka *et al.* (2001b), E: Tanaka *et al.* (2003), F: Ilyasov *et al.* (2019), G: Takahashi *et al.* (2016), H: Okuyama *et al.* (2017a), I: Okuyama *et al.* (2017b), J: Shinmura *et al.* (2018), K: Tan *et al.* (2007), L: Raffiudin and Crozier (2007), M: Smith and Hagen (1996), N: Ilyasov *et al.* (2018). The abbreviation of OTU sample refers to Supplementary 1 doi.org/10.5281/zenodo.10639197

haplotypes of *A. cerana* with low- and highlands, i.e., Ce2 in Luzon Island, Ce3 and Ce4 in Palawan Island. Moreover, specific haplotypes Sichuan1, Sichuan2, Sichuan3, Yunnan1, and Yunnan2 were found to be the markers of *A. cerana* from Sichuan and Yunnan, China (Tan *et al.* 2016).

The specific haplotypes of *A. cerana* in Sumatra can be used as the origin marker, and we found that a total of 12 specific haplotypes were mostly in lowland areas of <300 m asl (Figure 1, Table 1, Supplementary 3b, e, g). Future studies of nuclear gene markers for adaptation in high temperatures in the lowlands of Sumatra are needed to answer our finding of the high specific haplotypes in these regions.

We found another exciting result: the intra-colony variations occurred mostly in the highland >1,000 m asl (Supplementary 3a, c, f). Mitochondrial DNA in honey bees is inherited from the queen bees. However, these variations occurred because of mitochondrial recombination by paternal leakage in fertilization (Ballard dan Whitlock 2004). Intra-colony variations were also found in *A. cerana* from Thailand peninsular and Samui Island in southern Thailand (Songram *et al.* 2006) and found ±27% in *A. mellifera capensis* and *A. m. carnica* reciprocal crosses through artificial insemination (Meusel and Moritz 1993).

4.2. Genetic Differentiation and Genetic Distance of *Apis cerana* in Sumatra in High Distance Range

Apis cerana from Sumatra had high haplotype variations of *cox1, cox2*, and igs, i.e., 12, 12, and nine haplotypes, respectively (Figure 1). This high haplotype variation might be due to the high distance range of the traditional apiaries in Sumatra (Table 1). In supporting this case, the haplotype variations of *A. cerana* from China have been the impact of the high distance range, such as an isolated island outside of the mainland, i.e., Hainan Island (Zhao *et al.* 2014). Moreover, the high distance range also made up the high genetic differentiation of *A. cerana* in Sumatra supported by the low gene flow among the populations (F_{ST} value Wright 1978, see Table 3, 4; The high F_{ST} value indicates low gene flow, and vice versa, see Freeland *et al.* 2011).

The F_{ST} values of *A. cerana* from Sumatra *cox1*, *cox2*, and igs gradually increased in parallel with the location distances among *A. cerana* from Sumatra population from Aceh to Lampung with moderate to high population difference of the F_{ST} values or with a low gene flow (Wright 1978, see Table 3, 4). We found that the highest gene flow of *A. cerana* populations was between Aceh-North Sumatra and North Sumatra-Jambi, while moderate gene flow was between North Sumatra-West Sumatra-South Sumatra (Table 3, 4). All provinces to Lampung had the lowest gene flow based on those three mtDNA markers (Table 3, 4). *Apis cerana* from Qinghai-Tibet, China, showed the same result: a high distance range emerged from a small discrete population and presented a high genetic differentiation based on the F_{ST} value of the tRNA-Leu-*cox2* mtDNA gene (Yu *et al.* 2019). In parallel, the F_{ST} values of SNP nucleotide were positively correlated with the geographical distance in *A. cerana* in Hubei Province, China (Fang *et al.* 2022).

Based on the genetic distance (Supplementary 4) and Maximum likelihood (ML) phylogenetic tree of *cox1, cox2*, and igs, *A. cerana* from Sumatra was relatively closer to those in Borneo compared to Java, in Sundaland (Figure 3). The same phenomena as the relationship of *A. cerana* from Sundaland also occurred in the relationship of flora Malesiana (Welzen *et al.* 2011), *Erionota thrax thrax* (Lepidoptera: Hesperiidae) (Wiyati *et al.* 2022), and mammals in Sundaland and the Philippines (Heaney *et al.* 1986), although the Merrill-Dickerson/Huxley Line encompassed Sumatra, Borneo, and Java into one group. Our mitochondrial studies were also supported by the geometric morphometric of wing venation, that *A. cerana* from Sumatra mostly separated from those from Java, Bali, Sulawesi, and Moluccas (Nisa *et al.* 2022).

The genetic distance of *A. cerana* from Sumatra to East Asia based on *cox1* was 4.28–5.82% (Supplementary 4), which is close to the genetic distance of *A. cerana* from Java to East Asia based on *cox1*, i.e., 4.03–5.14% (Supplementary 4). The genetic distance of Indonesian *A. cerana* was 4–5.1% from East Asia *A. cerana* based on the *cox1* gene (Raffiudin *et al.* 2022). Using a complete mitochondria genome combined with nuclear genes and morphological parameters can propose that *A. cerana* from the Korean Peninsula was differentiated into a new subspecies as *A. c. koreana* (Ilyasov *et al.* 2018).

4.3. High Haplotype Variations in *Apis cerana* in North Sumatra: Geological and Anthropogenic Impact?

The super-eruption of the Lake Toba caldera in North Sumatra covering 100 × 30 km^2 and distributed the Toba fragmental materials (tephra) from the north-eastern Indian Ocean to India 75,000 years ago (Ninkovich *et al.* 1978a, 1978b). Thus, a massive extinction of 26% of mammal species occurred due to this super-eruption (Louys 2007). Apart from the

extinction of the eruption, the ancestor of *A. cerana* in North Sumatra could survive up to now and currently shows the highest haplotype variation compared to other regions in Sumatra with a total of 17 haplotypes of *cox1*, *cox2*, and igs (Figure 1), Furthermore, *A. cerana* from Changbai Mountain, China, showed high observed heterozygosity (Ho) of SNP nucleotide (Nannan *et al.* 2022) that might be resulted from a powerful eruption in that region during 1,100 years ago. The eruption formed Tianchi Lake, 373 m deep and 4 km diameter at 2,192 m asl (Wei *et al.* 2013).

A. cerana colony 2 from Siantar Sitalasari, North Sumatra, showed a low genetic distance to those of Java based on the three mtDNA markers (Supplementary 4). Moreover, the haplotype Sumatra4 *cox2* gene from *A. cerana* colony 2 Siantar Sitalasari was the same as Java3 from Lebak, Banten as well (Raffiudin *et al.* 2022), which could be due to the anthropogenic impact. This result was supported by the ML tree that colony 2 from Siantar Sitalasari was clustered with those from Java (Figure 3). The phenomenon was presumably due to the transfer of the bees from Java to Siantar Sitalasari, the urbanized area. Smith *et al.* (2004) also found one haplotype of *A. cerana* in Sundaland in Myanmar, which might be due to the relic of a formerly widespread Sundaland population, honey bee migration and human transfer.

Acknowledgements

We expressed our acknowledgement to The Ministry of Research and Technology /National Research and Innovation Agency of Indonesia for the research grant to the corresponding author entitled "Evolution of honey bee *Apis cerana* in Indonesia: Morphology and Molecular Approach" (No. 3614/IT3. L1/PT.01.03/P/B/2022). Our appreciation also goes to the beekeepers who gave support during *A. cerana* collections. We also acknowledged the Government basic research program of the Ministry of Education and Science of Russia in 2024 No. 0088-2024-0009 for supporting Rustem Ilyasov in providing scientific research on honey bees in collaboration with the corresponding author.

References

Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. *Mol. Ecol.* 13, 729-744. https://doi.org/10.1046/j.1365-294X.2003.02063.x

Bourke, R.M., 2010. Altitudinal limits of 230 economic crop species in Papua New Guinea, in Haberle, S.G, Stevenson, J., Prebble, M. (Eds.), *Altered Ecologies: Fire, Climate and Human Influence on Terrestrial Landscapes (Terra Australis 32). 1st ed.* Canberra, ANU ePress, pp. 473-512. https://doi.org/10.22459/TA32.11.2010.27

Buchori, D., Rizali, A., Priawandiputra, W., Raffiudin, R., Sartiami, D., Pujiastuti, Y., Jauharlina, Pradana, M.G., Meilin, A., Leatemia, J.A., Sudiarta, I.P., Rustam, R., Nelly, N., Lestari, P., Syahputra, E., Hasriyanti, Watung, J.F., Daud, I.D.A., Hariani, N., Jihadi, A., Johannis, M., 2022. Beekeeping and managed bee diversity in Indonesia: perspective and preference of beekeepers. *Diversity.* 14, 52. https://doi.org/10.3390/d14010052

Cornuet, J.M., Garnery, L., Solignac, M., 1991. Putative origin and function of the intergenic region between *cox1* and *cox2* of *Apis mellifera* L. mitochondrial DNA. *Genetics.* 128, 393-403. https://doi.org/10.1093/genetics/128.2.393

Damus, M.S., Otis, G.W., 1997. A morphometric analysis of *Apis cerana* F and *Apis nigrocincta* smith populations from Southeast Asia. *Apidologie.* 28, 309-323. https://doi.org/10.1051/apido:19970507

de Bruyn, M.D., Stelbrink, B., Morley, R.J., Hall, R., Carvalho, G.R., Cannon, C.H., van den Bergh, G., Meijaard, E., Metcalfe, I., Boitani, L., Maiorano, L., Shoup, R., von Rintelen, T., 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. *Syst Biol.* 63, 879-901. https://doi.org/10.1093/sysbio/syu047

de la Rúa, P., Simon, U.E., Tilde, A.C., Moritz, R.F.A., Fuchs, S., 2000. MtDNA variation in *Apis cerana* populations from the Philippines. *Heredity.* 84, 124-130. https://doi.org/10.1046/j.1365-2540.2000.00646.x

Engel, M.S., 2012. The honey bees of Indonesia (Hymenoptera: Apidae). *Treubia.* 39, 1-85.

Engel, M.S., 1999. The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae; Apis). *J. Hymen Res.* 8, 165-196.

Estoup, A., Solignac, M., Cornuet, J.M., Goudet, J., Scholl, A., 1996. Genetic differentiation of continental and island population of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Mol. Ecol.* 5, 19-31. https://doi.org/10.1111/j.1365-294X.1996.tb00288.x

Fang, F., Chen, X., Lv, J., Shi, X., Feng, X., Wang, Z., Li, X., 2022. Population structure and genetic diversity of Chinese honeybee (*Apis cerana cerana*) in Central China. *Genes.* 13, 1007. https://doi.org/10.3390/genes13061007

Freeland, J.R., Kirk, H., Petersen, S., 2011. *Molecular Ecology*. John Wiley & Sons, Ltd, Oxford. https://doi.org/10.1002/9780470979365

Hall, R., 2013. The palaeogeography of Sundaland and Wallacea since the Late Jurassic. *J. Limnol.* 72, 1-17. https://doi.org/10.4081/jlimnol.2013.s2.e1

Heaney, L.R., 1986. Biogeography of mammals in SE Asia: estimates of colonization, extinction, and speciation rates. *Biol. J. Linn. Soc.* 28, 127-165. https://doi.org/10.1111/j.1095-8312.1986.tb01752.x

Hepburn, H.R., Radloff, S.E., 2011. *Honeybees of Asia*. Springer-Verlag, Berlin. https://doi.org/10.1007/978-3-642-16422-4

Ilyasov, R.A., Park, J., Takahashi, J., Kwon, H.W., 2018. Phylogenetic uniqueness of honeybee *Apis cerana* from the Korean Peninsula inferred from the mitochondrial, nuclear, and morphological data. *J Apic Sci.* 62, 189-214. https://doi.org/10.2478/jas-2018-0018

Ilyasov, R.A., Youn, H.G., Lee, M., Kim, K.W., Proshchalykin, M.Y., Lelej, A.S., Takahashi, J., Kwon, H.W., 2019. Phylogenetic relationships of Russian far-east *Apis cerana* with other north Asian populations. *J. Apic. Sci.* 63, 289-314. https://doi.org/10.2478/jas-2019-0024

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evo.* 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Larkin, M., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P., McWilliam, H., Valentin, F., Wallace, I.M., Wilm A, Lopez, R., Thompson. J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and clustal X version 2.0. *Bioinformatics.* 23, 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Louys, J., 2007. Limited effect of the quaternary's largest super-eruption (Toba) on land mammals from Southeast Asia. *Quaternary Sci Rev.* 26, 3108-3117. https://doi.org/10.1016/j.quascirev.2007.09.008

Meusel, M.S., Moritz, R.F.A., 1993. Transfer of paternal mitochondrial DNA during fertilization of honeybee (*Apis mellifera* L.) eggs. *Curr. Genet.* 24, 539-543. https://doi.org/10.1007/BF00351719

Nannan, L., Huamiao, L., Yan, J., Xingan, L., Yang, L., Tianjiao, W., Jinming, H., Qingsheng, N., Xiumei, X., 2022. Geometric morphology and population genomics provide insights into the adaptive evolution of *Apis cerana* in Changbai Mountain. *BMC Genomics.* 23, 64.https://doi.org/10.1186/s12864-022-08298-x

Ninkovich, D., Abdel-Monem, A.A., Obradovich, J.D., Izett, G., 1978a. K-Ar Age of the late Pleistocene eruption of Toba, North Sumatra. *Nature.* 276, 574-577. https://doi.org/10.1038/276574a0

Ninkovich, D., Sparks, R.S.J., Ledbetter, M.T., 1978b. The exceptional magnitude and intensity ofthe Toba eruption, Sumatra: an example of the use of deep-sea tephra layers as a geological tool. *Bull. VoIcanol.* 41, 286-298. https://doi.org/10.1007/BF02597228

Nisa, N.R., Juliandi, B., Raffiudin, R., Jauharlina, J., Pradana, M.G., Meilin, A., Jasmi, J., Pujiastuti, Y., Fahri, F., Priawandiputra, W.M., Atmowidi. T., 2022. Intra- and interspecies wing venation variations of *Apis cerana* and *Apis nigrocincta* species in Indonesia. *HAYATI J. Biosc.* 29, 222-233. https://doi.org/10.4308/hjb.29.2.222-233

Okuyama, H., Tingek, S., Takahashi, J., 2017a. The complete mitochondrial genome of the cavity-nesting honeybee, *Apis cerana* (Insecta: Hymenoptera: Apidae) from Borneo. *Mitochondr. DNA Part B Resour.* 2, 475-476. https://doi.org/10.1080/23802359.2017.1361344

Okuyama, H., Wakamiya, T., Fujiwara, A., Washitani, I., Takahashi, J., 2017b. Complete mitochondrial genome of the honeybee *Apis cerana* native to two remote islands in Japan. *Conserv Genet Resour.* 9, 557-560. https://doi.org/10.1007/s12686-017-0721-5

Radloff, S.E., Hepburn, C., Hepburn, H.R., Fuchs, S., Hadisoesilo, S., Tan, K., Engel, M.S., Kuznetsov, V., 2010. Population structure and classification of *Apis cerana*. *Apidologie*. 41, 589-601. https://doi.org/10.1051/apido/2010008

Raffiudin, R., Crozier, R.H., 2007. Phylogenetic analysis of honey bee behavioral evolution. *Mol. Phylogenet. Evol.* 43, 543-552. https://doi.org/10.1016/j.ympev.2006.10.013

Raffiudin, R., Shullia, N.I., 2020. Phylogenies of Asian honey bees, in: Ilyasov, R.A., Kwon., H.W. (Eds.), *Phylogenetics of Bees.* CRC Press Taylor & Francis Group, Boca Raton, pp. 28-57. https://doi.org/10.1201/b22405-2

Raffiudin, R., Shullia, N.I., Damayanti, A.U., Wahyudi, D.T., Febiriani, T.V., Atmowidi, T., Lamerkabel, J.S.A., Widjaja, M.C., 2022. New haplotypes of *Apis cerana* in Indonesia: identification from mitochondrial and major royal jelly protein 2 genes. *Int. J. Trop. Insect Sc.* 42, 389-401. https://doi.org/10.1007/s42690-021-00556-x

Raffiudin, R., Sosromarsono, S., Ratna, E.S., Solihin, D.D., 1999. Morphological variation of the Asian Honeybee *Apis cerana* (F.) (Hymenoptera: Apidae) in West Java. *Bull. Hama dan Penyakit Tumbuhan.* 11, 20-25.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analysis by the coalescent and other methods. *Bioinformatics.* 19, 2496–2497. https://doi.org/10.1093/bioinformatics/btg359

Ruttner, F., 1988. *Biogeography and Taxonomy of Honeybees*. Springer-Verlag, Berlin. https://doi.org/10.1007/978-3-642-72649-1

Shinmura, Y., Okuyama, H., Kiyoshi, T., Lin, C., Kadowaki, T., Takahashi, J., 2018. The complete mitochondrial genome and genetic distinction of the Taiwanese honey bee, *Apis cerana* (Hymenoptera: Apidae). *Conserv. Genet. Resour.* 10, 621-626. https://doi.org/10.1007/s12686-017-0879-x

Smith, D.R., Hagen, R.H., 1996. The biogeography of *Apis cerana* as revealed by mitochondrial DNA sequence data. *J. Kansas. Entomol. Soc.* 69, 294-310.

Smith, D.R., Villafuerte, L., Otis, G., Palmer, M.R., 2000. Biogeography of *Apis cerana* F. and *A. nigrocincta* Smith: insights from mtDNA studies. *Apidologie.* 31, 265-279. https://doi.org/10.1051/apido:2000121

Smith, D.R., Warrit, N., Hepburn, H.R., 2004. *Apis cerana* from Myanmar (Burma): unusual distribution of mitochondrial lineages. *Apidologie*. 35, 637-644. https://doi.org/10.1051/apido:2004058

Songram, O., Sittipraneed, S., Klinbunga, S., 2006. Mitochondrial DNA diversity and genetic differentiation of the honeybee (*Apis cerana*) in Thailand. *Biochem. Genet.* 44, 256-269. https://doi.org/10.1007/s10528-006-9030-5

Takahashi, J., Wakamiya, T., Kiyoshi, T., Uchiyama, H., Yajima, S., Kimura, K., Nomura, T., 2016. The complete mitochondrial genome of the Japanese honeybee, *Apis cerana japonica* (Insecta: Hymenoptera: Apidae). *J. Mitochondr. DNA Part B Resour.* 1, 156-157. https://doi.org/10.1080/23802359.2016.1144108

Tan, K., Warrit, N., Smith, D.R., 2007. Mitochondrial DNA diversity of Chinese *Apis cerana. Apidologie.* 38, 238-246. https://doi.org/10.1051/apido:2007008

Tan, K., Qu, Y., Wang, Z., Liu, Z., Engel, M.S., 2016. Haplotype diversity and genetic similarity among populations of the Eastern honey bee from Himalaya-Southwest China and Nepal (Hymenoptera: Apidae). *Apidologie.* 47, 197-205. https://doi.org/10.1007/s13592-015-0390-x

Tanaka, H., Roubik, D.W., Kato, M., Liew, F., Gunsalam, G., 2001a. Phylogenetic position of *Apis nuluensis* of northern Borneo and phylogeography of *Apis cerana* as inferred from mitochondrial DNA sequences. *Insect Soc.* 48, 44-51. https://doi.org/10.1007/PL00001744

Tanaka, H., Suka, T., Kahono, S., Samejima, H., Mohamed, M., Roubik, D.W., 2003. Mitochondrial variation and genetic differentiation in the honey bees (*Apis cerana, A. koschevnikovi*, and *A. dorsata*) of Borneo. *Tropics.* 13, 107-117. https://doi.org/10.3759/tropics.13.107

Tanaka, H., Suka, T., Roubik, D.W., Mohamed, M., 2001b. Genetic differentiation among geographic groups of three honeybee species, *Apis cerana, A. koschevnikovi*, and *A. dorsata*, in Borneo. *Nat Hum Act.* 6, 5-12.

Wei, H., Liu, G., Gill, J., 2013. Review of eruptive activity at Tianchi volcano, Changbaishan, northeast China: implications for possible future eruption. *Bull. Volcanol.* 75, 706. https://doi.org/10.1007/s00445-013-0706-5

Welzen, P.C.V., Parnell, J.A.N., Slik, J.W.F., 2011. Wallace's Line and plant distributions: two or three phytogeographical areas and where to group Java?. *Biol. J. Linn. Soc.* 3, 531-545. https://doi.org/10.1111/j.1095-8312.2011.01647.x

Wiyati, S.Y., Raffiudin, R., Sutrisno, H., 2022. The genetic diversity of banana leaf roller Erionota thrax thrax (Lepidoptera: Hesperiidae) in Indonesia. *J Hun Uni Nat Sci*. 49, 251-256. https://doi.org/10.55463/issn.1674-2974.49.3.28

Wright, S., 1978. *Evolution and the Genetics of Population, Variability within and Among Natural Populations.* University of Chicago Press, Chicago.

Yu, Y., Zhou, S., Zhu, X., Xu, X., Wang, W., Zha, L., Wang, P., Wang, J., Lai, K., Wang, S., Hao, L., Zhou, B., 2019. Genetic differentiation of eastern honey bee (*Apis cerana*) populations across Qinghai-Tibet plateau-valley landforms. *Front. Genet.* 10, 1-11. https://doi.org/10.3389/fgene.2019.00483

Zhao, W., Tan, K., Zhou, D., Wang, M., Cheng, C., Yu, Z., Miao, Y., He, S., 2014. Phylogeographic analysis of *Apis cerana* populations on Hainan Island and southern mainland China, based on mitochondrial DNA sequences. *Apidologie.* 45, 21-33. https://doi.org/10.1007/s13592-013-0223-8