Apis koschevnikovi: Distribution in South Kalimantan and Cytochrome b Mitochondrial DNA Variations

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ABSTRAK

***Apis koschevnikovi*: Distibusi di Kalimantan Selatan dan Variasinya pada Mitokondria Cytochrome b.** Variasi mitokondria berdasarkan *Cytochrome* b (*Cyt* b) pada *Apis koschevnikovi* di lakukan di tujuh lokasi di Kalimantan Selatan Indonesia. Di koleksi 29 koloni *A. koschevnikovi* yang diperoleh di kawasan hutan primer dan sebagian koloni tidak dijumpai dihutan bekas penebangan. Hasil analisis *Cyt* b juga mengindikasikan bahwa terjadi variasi genetik dari jenis ini walaupun secara morfologi menunjukkan bentuk yang sama

Keywords : *Apis koschevnikovi*, penebangan hutan, variasi haplotipe

INTRODUCTION

A. koschevnikovi that is sympatric with *A. cerana*, distributed in several regions Sumatra, the Malay Peninsula, Borneo and Java. *A. koschevnikovi* is now rarely seen, except in Borneo, due to the destruction of forest habitat (Otis 1991). This species is usually found mostly at sea level, but has also been discovered at elevations up to 1000 m (Otis 1996). Further data mentioned that the altitudinal distributions show that *A. koschevnikovi* mainly occurs between sea level and about 1200 m: for 102 recorded localities, 98 were < 1200 m, and 4 between 1200 to 2700 m (Hadisoesilo *et al.* 2008).

A. koschevnikovi workers have their distinctive yellow-orange color in most body parts while the drone is mostly brown (Woyke 1997). However different color was found for *A. koschevnikovi* in the Malay Peninsula and Sumatera, Indonesia, which is a dark, coppery color (Otis 1997). Workers are medium sized (body length approximately 10-11 mm), forewing length of 8.46 $\pm$ 0.11 mm, with cubital index 7.64 $\pm$ 1.40 (Tingek *et al.* 1996). Overall, it is larger than *A. cerana* (Ruttner *et al*. 1989). *A. koschevnikovi* drone mating flight is at 16.15 h - 18.15 h, which clearly separate it from sympatric *A. cerana* at 13.45 h - 15.30 h (Koeniger *et al.* 1988).

Multivariate morphometric analyses were performed on workers of *Apis koschevnikovi* from throughout their distribution in Malaysia, Borneo and Indonesia. Principal component analysis showed one morphocluster comprising bees from Kalimantan Indonesia,

Sarawak, Sabah and the Malay Peninsula. The population is more homogeneous than *A. cerana* over the same geographical area, as seen from the average coefficient of variation in 12 characters in *A. koschevnikovi* (1.8%) compared to those same characters in *A.cerana* (4.3%) .

Mitochondrial DNA analysis started that *A. koschevnikovi* differs in both nuclear and mitochondrial DNA regions (Arias *et al*. 1996; Takahashi *et al*. 2002; Raffiudin and Crozier 2007) from other species of *Apis* with which it has a sympatric distribution.

Here we report the variations from the intergenic region of *cytochrome oxidase*1 and 2 and from cytochrome b mitochondrial DNA (mtDNA) in South Kalimantan both in mainland and in Laut Island based on PCR-RFLP analysis.

METHODS AND MATERIALS

Apis koschevnikovi were surveyed in seven districts in South Kalimantan, i.e. Tanah Laut, Banjar, Tapin, Hulu Sungai Selatan (HSS), Hulu Sungai Tengah (HST), Balangan (BL), Kota Baru (KB) and Tanah Bumbu (TB) (Figure 1). Furthermore, we also compare the *Apis koscehvnikovi* from South Kalimantan with that Hepburn collection from Sarawak and our collection from East Kalimantan. Bees were anesthetized and preserved in absolute ethanol**.**

Out of 29 colonies obtained, 22 colonies were used for mtDNA analysis. Genomic DNA was extracted from the thoraxes of single bees using phenol–chloroform extraction and ethanol precipitation (Raffiudin & Crozier 2007).

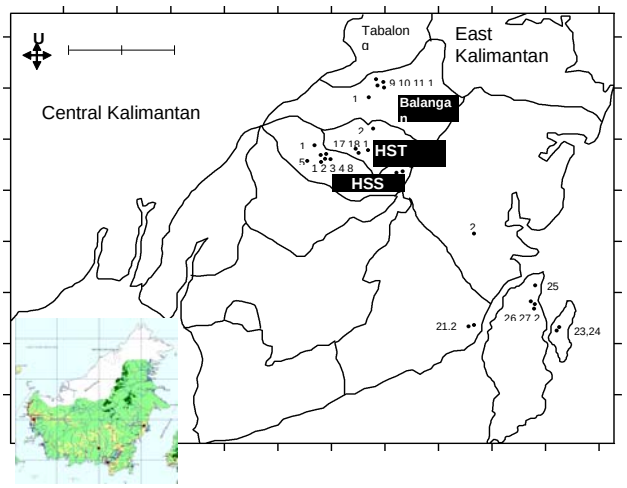

Figure 1. Location of *A. koschevnikovi* colonies in South Kalimantan: black dot and number represents the bee colony number; Locations / district in South Kalimantan were written in black box.

Part of the *cyt* b region was amplified using primers. Numbers in brackets indicate the position of *A. mellifera cyt* b in the mtDNA. *Cytochrome* b Forward: 5'-TATGTACTACCTTTGAGGACAAATATC-3' (11400); *Cytochrome* b Reverse 5'-ATTACACCTCCTAATTTATTAAGGAAT-3' (11859) (Crozier *et al*. 1991).

DNA was sequenced by using the same primer as the amplification. All sequence were gathered in database in Genetyx program. Subsequently the sequences were analysed their homology comparing to the Genbank database by sending to BLAST (www.ncbi.nlm.nih.gov/BLAST). DNA alignment was carried out by using Clustal X (Thompson *et al.* 1997) and analysed the GC content of the sequences.

RESULTS

Distribution of *A. koschevnikovi* in South Kalimantan

Twenty nine colonies of *Apis koschevnikovi* were found in our exploration in five districts in South Kalimantan, Indonesia, i.e. HSS, HST BL, TB, KB respectively for 11, 4, 5, 2 and 7 colonies (Figure 1). Hulu Sungai Selatan (HSS) district was located in the range of primary forest of Meratus Mountain; hence provided a natural ever green habitat for this species. We found high population *A. koschevnikovi* in Laut Island and Sungai Bali Island which had primary forest as well.

However, *A. koschevnikovi* were not found in three districts, i.e., Tanah Laut, Banjar, Tapin, due to deforestation in those locations. Those areas was covered with oil palm plantation and acacia besides the existed of coal mining existed (R. Raffiudin, unpubl. data).

Variations of *A. koschevnikovi* cytochrome b

We obtained a 206 bp of cytochrome subunit b gene *A. koschevnikovi* (AK). South and East Kalimantan and Sarawak (Figure 2). The DNA sequence was highly AT rich (73%), which was the same of insect DNA mitochondria sequences (Crozier 1989). Based on the alignment of the cytochrome subunit b gene *A. koschevnikovi* sequence, we observed variations of AK from South and East Kalimantan and Sarawak (Figure 2). The first haplotype was the most common sequences found in samples of AK1 TB, AK5HSS, AK5BL, AK1HST, AK7HSS. The second haplotype occurred in AK1BR and AK2TB both has had a transition of C'T at the nucleotide number 61 and 160, compared to the haplotype 1. Another variation occurred in the third haplotype ie. AK2BR only C'T at nucleotide 61. The fourth and the fifth haplotype occurred at AK1KB and AK3KB repectively both have the same variations except nuc 37 T'C at haplotype 4. Haplotype 6 from Sarawak has mostly different points mutations among the South and East Kalimantan is *Apis koschevnikovi*. Hence this study resulted six haplotypes of cytochrome subunit b gene *A. koschevnikovi* in South and East Kalimantan and Sarawak Borneo (Table 2).

Table 1. Collection of *A. koschevnikovi* (AK) in South Kalimantan used in this study; AK1 = *Apis koschevnikovi* colony number 1; HSS = Hulu Sungai Selatan, HST = Hulu Sungai Tengah, BL = Balangan; TB = Tanah Baru, KB= KotaBaru; "-"= could not obtain data due to tree canopy thickness.

No.	*A. koscevnikovi* database (referred to Figure 1)	*A. koschevnikovi* for mtDNA analysis	LS	BT
1	1	AK1HSS	02°.49’	115 °.17’
2.	4	AK4HSS	02°.49’	115 °.17’
3.	5	AK5HSS	02°.50’	115 °.18’
4	7	AK7HSS	02°.49’	115 °.25’
5.	9	AK1BL	02°.20’	115 °.25’
6.	10	AK2BL	02°.20’	115 °.25’
7.	12	AK4BL	02°.20’	115 °.25’
8.	13	AK5BL	02°.20’	115 °.25’
9.	17	AK1HST	02°.38’	115 °.26’
10.	18	AK 2HST	02°.38’	115 °.25’
11.	19	AK3HST	02°.38’	115 °.25’
12	20	AK4HST	02°.31’	115 °.27’
13	21	AK1TB	03º29’13.3”	116º00’03.2
14	22	AK 2TB	03º29’13.3”	115º59’41.3”
15	23	AK1KB	03º30’0”	116º21’03.0”
16	24	AK2KB	03º29’17.7”	116º21’16.8”
17	25	AK3KB	03º14’03.0”	116º15’06.8”
18	26	AK4KB	03º14’23.5”	116º13’31.9”
19	27	AK5KB	-	-
20	28	AK6KB	-	-
21	29	AK7KB	03º02’34.9”	115º58’59.6”

DISCUSSION

As shown above, none of *A. koschevnikovi* colony was found in deforested area such as in district of Tapin, Banjar and Tanah Laut. This result was in agreement with numerous excursions of S. Hadisoesilo, H.R. Hepburn, G.W. Otis, M. Pianchaeron, P.H. Thai, D.W. Roubik, S Wongsiri, (all unpubl. data) in tropical forests over the last decade in Thailand, Myanmar, Cambodia and Vietnam (Hadisoesilo *et al*. 2008). Those excursions failed to find this species and concluded that *A. koschevnikovi* is probably restricted to

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AK1BL    CTCATTACAT TTTATTTTAC CATTAGTAAT TTTATTTATA GTTATTCTTC ATTTATTTGC  [ 60]

AK5HSS   .......... .......... .......... .......... .......... ..........  [ 60]
AK5B     .......... .......... .......... .......... .......... ..........  [ 60]
AK1HST   .......... .......... .......... .......... .......... ..........  [ 60]
AK7HSS   .......... .......... .......... .......... .......... ..........  [ 60]
AK2.2TB  .......... .......... .......... .......... .......... ..........  [ 60]
AK1BR    .......... .......... .......... .......... .......... ..........  [ 60]
AK1.2KB  .......... .......... .......... ......C... .......... .......C..  [ 60]
AK2BR    .......... .......... .......... .......... .......... ..........  [ 60]
AK3.2KB  .......... .......... .......... .......... .......... .......C..  [ 60]
AK2SRW   T...C..... .......... .......... ......C... .......... ..........  [ 60]
A.cerana T...C.T..C .......... .T........ .......... ..A....... ..........  [ 60]

AK1BL    CCTACATTTA ACTGGTTCAT CAAATCCTTT AGGATCAAAT TATAATAATT ATAAAATTTC  [120]
AK5HSS   .......... .......... .......... .......... .......... ..........  [120]
AK5B     .......... .......... .......... .......... .......... ..........  [120]
AK1HST   .......... .......... .......... .......... .......... ..........  [120]
AK7HSS   .......... .......... .......... .......... .......... ..........  [120]
AK2.2TB  T......... .......... .......... .......... .......... ..........  [120]
AK1BR    T......... .......... .......... .......... .......... ..........  [120]
AK1.2KB  T......... .......... .......... .......... .......... ..........  [120]
AK2BR    T......... .......... .......... .......... .......... ..........  [120]
AK3.2KB  T......... .......... .......... .......... .......... ..........  [120]
AK2SRW   T......... .......... .......... .......... .......... .C........  [120]
A.cerana AT........ .....A.... .......... .........C .......... ..........  [120]

AK1BL    ATTTCATCCA TATTTTTCAA TTAAAGATTT ACTTGGTTTT TATATTATTT TATTTATTTT  [180]
AK5HSS   .......... .......... .......... .......... .......... ..........  [180]
AK5B     .......... .......... .......... .......... .......... ..........  [180]
AK1HST   .......... .......... .......... .......... .......... ..........  [180]
AK7HSS   .......... .......... .......... .......... .......... ..........  [180]
AK2.2TB  .......... .......... .......... .........C .......... ..........  [180]
AK1BR    .......... .......... .......... .........C .......... ..........  [180]
AK1.2KB  ......C... .......... .......... ......A... .......... ..........  [180]
AK2BR    .......... .......... .......... .......... .......... ..........  [180]
AK3.2KB  ......C... .......... .......... ......A... .......... ..........  [180]
AK2SRW   .......... .....C.... .......... .......... .......... ..........  [180]
A.cerana .........T ..C.....T. .......... .T.A..A... .......... ..........  [180]

AK1BL    TATATTTATT AATTTTCAAT ATCCTT [206]
AK5HSS   .......... .......... ...... [206]
AK5B     .......... .......... ...... [206]
AK1HST   .......... .......... ...... [206]
AK7HSS   .......... .......... ...... [206]
AK2.2TB  .......... .......... ...... [206]
AK1BR    .......... .......... ...... [206]
AK1.2KB  .......... .......... ...... [206]
AK2BR    .......... .....C.... ...... [206]
AK3.2KB  .......... .......... ...... [206]
AK2SRW   .......... .......... ...... [206]
A.cerana ....A.A... .......... ...... [206]
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Figure 2. DNA alignment of cytochrome b gene of *Apis koschevnikovi* (AK). Numbers following "AK" were samples number and the sample location source (Refer to Table 1)

the evergreen forests of the Malay Peninsula, Borneo and Sumatera. Hence, great effort should be taken for the conservation of *A. koschevnikovi* habitat. Otis (1997) based on collections in various museums, mentioned that A. koschevnikovi were widely collected in the Sundaland region of Southeast Asia. He also stated that the decline of *A. koschevnikovi* population due to habitat changes resulting from deforestation and the establishment of tea, oil palm, rubber

Table 2. Haplotypes of Apis koschevnikovi in Kalimantan based on cytochrome b and nucleotides variations

No	Haplotype	Ak samples	Nucleotida position	Variations
1	Haplotype 1	AK1BL		
		AK5HSS		
		AK5BL		
		AK1HST		
		AK7HSS		
2	Haplotype 2	AK1BR	**61**	C-T
		AK2TB	160	T-C
3	Haplotype 3	AK2BR	**61**	C-T
4	Haplotype 4	AK1KB	37	T-C
			59	T-C
			61	C-T
			127	T-C
			157	G-A
5	Haplotype 5	AK3KB	**59**	T-C
			61	C-T
			127	T-C
			157	G-A
6	Haplotype 6	AK2SRW	1	C-T
			5	T-C
			37	T-C
			61	C-T
			112	T-C
			136	T-C

and coconut plantations. Based on our observations, *A. koschevnikovi* lives in primary forest with densely covered canopy.

During *A. koschevnikovi* exploration, we can still found *A. cerana* the sympatric species of *A. koschevnikovi* in the open and destructive area where *A. koschevnikovi* could not survive. This observation was congruence with that of Hadisoesilo *et al*. (2008) that *A. koschevnikovi* habitat is in evergreen rain forests, of Sundaland while *A. cerana* can be abundant in agricultural and even urban settings.

Mitochondrial DNA is a suitable for tracing the evolution of an organisms due to the high rate mutation in several genes (Crozier & Crozier 1993). We observed variation of *A. koschevnikovi* MboI restriction sites of cox1/cox2 in colonies which located in the most north area of South Kalimantan, i.e. District Balangan.

A genetic variations study for *A. meliifera* in Spain (Garnery *et al*. 1995)

showed a gradient changes of dominant haplotype based on cox1/cox2. In Spain, *A. mellifera* was found to be M and A haplotype. The A haplotype was dominant in South Spain *A. mellifera* (13.6% of M haplotype). However, in centre of Spain (Segovia), both haplotypes showed equal percentage. Interestingly, in the north of Spain, the M haplotype turned to be the dominant (97.3%) haplotype and become 100 % in the far north Spain (San Sebastian).

In the future, exploration further out of South Kalimantan to the Central, East and West Kalimantan will be important to explain more of the genetic variations of this restricted distribution honey bee. Besides that, tracing the variations in both genes can be carried out by examining their DNA sequences; hence one can found detail variation of all haplotypes and might be possible can find other DNA variations besides the restriction sites used in this study.

ACKNOWLEDGMENTS

We expressed our gratitude to Directorate General of Higher Education (DGHE), Minister of Education, Republic of Indonesia, for the Competitive Grant in 2004-2005 and Competitive grant for International Publication 2009. To Ahmad Jundar the beekeeping in Hulu Sungai Selatan, Kalimantan and Rusdi Hidayat BSc., Ruth Martha Winnie and Apriani we also thank for accompanied us in *A. koschevnikovi* collection. Special thanks addressed to Zulfarida Syamsi, BSc. for technical assistance in the mitochondrial analysis in the Zoology Laboratory, Dept. of Biology, Bogor Agricultural University

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Received: Juni 2010
Accepted: Oktober 2011