In addition, DNA variation especially third nucleotide substitution in codon formation does not always produce different putative protein (Avise 1994). The examples based on Ak2 KB sample (haplotype 2) at nucleotides no. 117-119 and no. 123-125, Ak1 BL sample at nucleotides no. 252-254 (haplotype 4), and Ak5 BL sample at nucleotides no. 270-272 (haplotype 5).

The alignment between current samples (refer to Table 3) and published A. koschevnikovi COI databases in Genebank has resulting 685 bp of nucleotides long (Table 5), which fulfilled 648 nucleotides as a minimum number of nucleotide requirement to be submitted to DNA Barcode (www.ncbi.nlm.nih.gov). None of the current COI A. koschevnikovi study samples showed the same sequence with the published nucleotide sequence from Genebank databases (Table 5). Hence, these five haplotypes from this research were the new haplotypes for A. koschevnikovi COI database.

The results of this research can be used to accomplish information about molecular ecology studies of A. koschevnikovi as well. Based on current studies alignment, Ak1 HST and Ak1 TB has a same sequence, although the distance of HST and TB regencies approximately 150 km (Figure 1). However, sequences variations were found in two samples both from Balangan Regency (Ak1 BL and Ak5 BL). These results indicate that mixed population occurred in A. koschevnikovi distribution.

Genetic distances and phylogenetic analysis were performed to observe genetic relationship between current studies and published A. koschevnikovi COI databases in Genebank as well. Genetic distance analysis grouped all samples in this study in one cluster, with the highest genetic distance in Ak2 KB sample i.e 0.003-0.004 (Table 7). Ak2 KB sample was collected not from Kalimantan mainland but from Sebuku island (see sampling location number 24 in Figure 1). Hence, these highest number might be due to geographical barrier which separate by Laut strait and Laut island (see Figure 1).

Phylogenetic analysis grouped Ak samples from this previous study in three clusters (Figure 6). All samples in this study and several haplotypes from Sabah and Brunei were clustered in cluster A (0.000-0.009 value of genetic distances), while haplotype 2 from Sarawak, haplotype 10 from West Kalimantan, and haplotype 11 from Sabah were clustered in cluster B (0.000-0.022 value of genetic distances) (Table 7). However, both were classified in one group due to their bootstrap value which is 100 (Figure 6).

However, Ak haplotypes 3 and 4 from Sabah were grouped in different cluster with 100 of bootstrap value, while these samples were collected from same region with several haplotypes in cluster A and B. Genetic distances of haplotypes 3 and 4 compared with the other haplotypes showed the highest value (0.001-0.077) (Table 7). Therefore, these 2 haplotypes need to be reconfirmed for the species validity.

In the case of these two anomalous haplotypes from Genebank, therefore, one need to be aware with the published DNA database. In addition, further studies from the other areas in Kalimantan is necessary to have a complete picture of A. koschevnikovi COI gene database.

CONCLUSION

The exploration of COI gene of A. koschevnikovi from six samples taken from five regencies in South Kalimantan showed the genetic differentiation. There were five haplotypes of A. koschevnikovi COI gene were found in this study i.e. haplotype 1, 2, 3, 4, and 5. Haplotype 1 was a common haplotype was found in sample Ak1 HST and Ak1 TB, while others specific in other places. None of the current samples showed the same sequence with the nucleotide sequences of Genebank database, hence these five haplotypes were the new haplotypes of A. koschevnikovi COI gene.

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