Research project of counterparts funded at IPB

Name	Counterpart	Title
Purnama Hidayat, Rawati Panjaitan, Damayanti Buchori	**Z02**	Identification of the Arhopala spp. (Lepidoptera: Lycaenidae) from Bukit Duabelas and Harapan Forest Landscape, Jambi, Sumatra Based on Morphological Characteristics and mtCOI DNA Sequences

Background and Objectives

Arhopala is a group with cryptic members and is somewhat difficult to identify morphologically (Bickford *et al*. 2007). The cryptic species in *Arhopala* are influenced by diverse and isolated geographical regions, making it is possible to form variations in the ecotypic expression of *Arhopala* species. Geographic isolation that occurs in Indonesia also causes high species endemicity. The high variation in morphological expression on butterfly wings makes it difficult to identify using morphological characteristics only. Thus, molecular analysis is needed to support morphological identification. There are some *Arhopala* species from Jambi, Sumatra which are cryptic and therefore careful identification must be applied. The aim of this research is to identify *Arhopala* spp. collected from the Bukit Duabelas and Harapan Forest landscapes in Jambi, Sumatra using morphological characteristics and mitochondrial CO-1 gene (mtCOI) DNA sequences.

Methodology

Analysed specimens of *Arhopala* spp. were collected from the Bukit Duabelas and the Harapan Forest landscapes in 2017. Observed morphological characteristics were wing size, color pattern and point pattern, and male genitalia. Specimens were temporarily given names based on morphological characteristics identification based on Seki *et al*. (1991), Bethune-Baker (1903) and D'Abrera (1990). Molecular identification was done by analysing the mtCOI DNA sequences of collected samples using Identity Matrix and the Phylogeny Tree. The DNA extraction process and PCR referred to the modified methods by Megens *et al*. (2004) and Takats and Mølgaard (2015). The primers used referred to Takáts and Mølgaard (2015) using specific LepF1 primers (5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3 ') and LepR1 (5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3') amplifying DNA samples at 679-715 bp. The PCR products were sent to Genetika Science Malaysia for DNA sequencing. The 'contig' process was performed with the CLC Sequence software, then analyzed using BLAST with other deposited insect nucleotide sequences in GenBank. The identity matrix was carried out using Sequence Demarcation Tool Ver. 1.2, while the phylogeny analysis was done using MEGA X software with Maximum Likehood algorithm, Bootstrap 1000x.

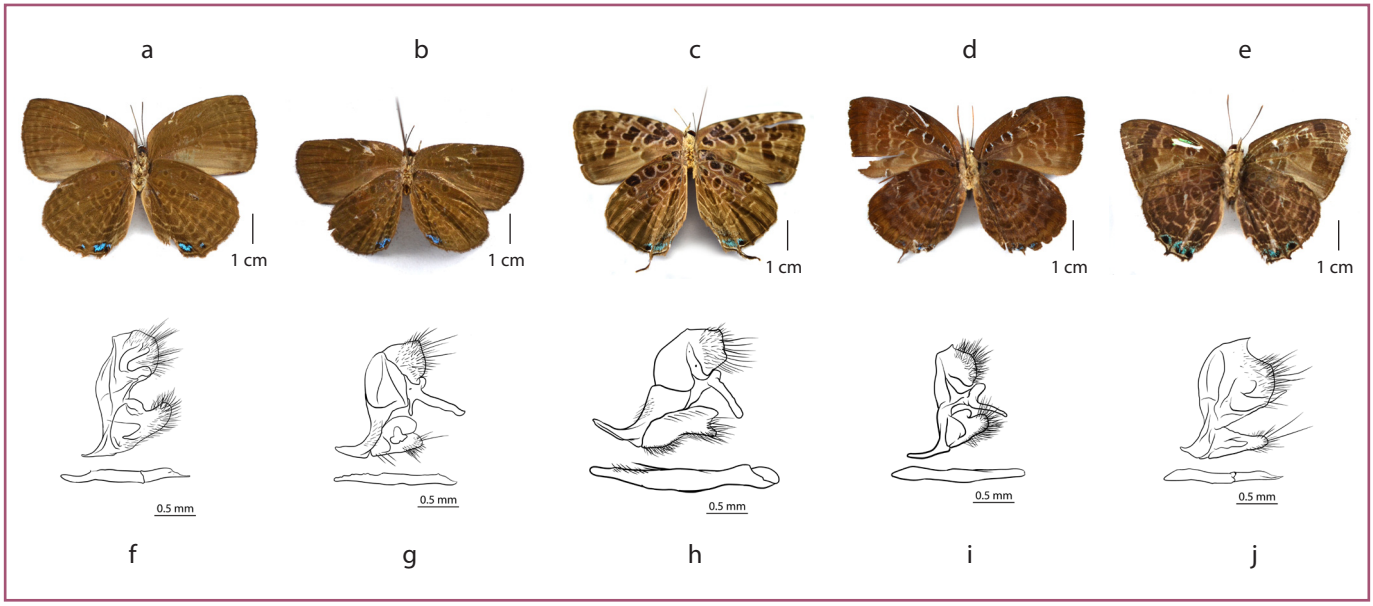

Figure 1. Ventral wings and male genitalia: (a,f) *A. agesias*; (b,g) *A. agesilaus*; (c,h) *A. paraganesa*; (d,i) *A. pseudocentaurus*; (e,j) *A. trogon*

Results and Conclusion

Identification based on morphological characteristics of *Arhopala* spp. collected from the Bukit Duabelas and Harapan Forest landscapes in Jambi, Sumatra resulted in five species namely *Arhopala agesias, A. agesilaus, A. paraganesa, A. pseudocentaurus*, and *A. Trogon* (Figure 1 a-j). The male genitalia are the most consistent characteristics compare to the size and spot pattern of wings. There were 32 specimens which were extracted for genomic DNA: *A. agesias* (7 samples), *A. agesilaus* (8 samples), *A. paraganesa* (10 samples), *A. pseudocentaurus* (5 samples), and *A. trogon* (2 samples). However only 11 samples which consisted of 5 species made it into the sequencing stage. The identity matrix analysis (Figure 2A) shows that all three samples of *A. agesias* from Jambi have high percentages of identity (95–97%) and formed one clade as a sister group with the DNA sequence from Malaysia, GenBank KF 226296 (Figure 2B). All two samples of *A. agesilaus* shows a very high percentage identity (99%) and formed a single clade as a sister group with the DNA sequence from Thailand, GenBank HQ962188 (Figure 2B). One of two samples of *A. paraganesa* shows 96% identity and formed a single clade, while the other sample shows only 93% identity with the sample from China, GenBank KT236371 (Figure 2B). Two species of *Arhopala*, *A. pseudocentaurus* and *A. trogon,* show lower DNA sequence identity (<90%) with other DNA sequences in GenBank.

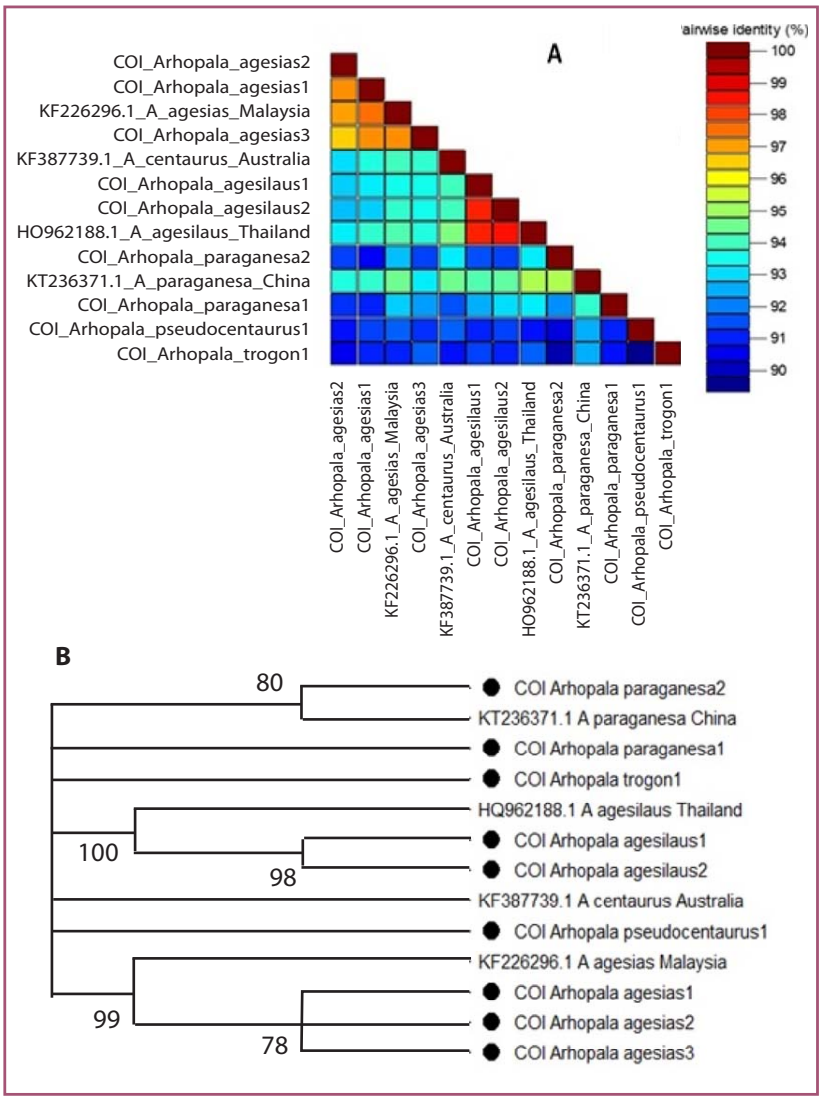

Figure 2. Analysis results of the mtCOI DNA sequences: (A) Identitiy matrix of *A. a*gesias, *A. agesilaus, A. paraganesa, A. paraganesa*, *A. pseudocentaurus*, and *A. trogon* with some deposited mtCOI DNA sequences in GenBank using Sequence Demarcation Tool Ver. 1.2.; (B) Phylogenetic tree of collected *Arhopala* spp. constructed using MEGA X with Maximum Likehood algorithm and Bootstrap 1000x.

As a conclusion of this study, species identification based on the male genitalia and mtCOI DNA sequences shows that three species of *Arhopala* from Jambi, *A. agesias*, *A. agesilaus, and A. paraganesa,* can be identified conclusively; while identification of two other species, *A. pseudocentaurus* and *A. trogon* remains to be inconclusive.

References

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