

Mitochondrial Barcodes of Dragonflies and Damselflies Originated from Taman Negara Endau Rompin, Johor, Malaysia

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Abstract. Dragonflies and damselflies (Odonates) are important biological indicators in freshwater ecosystems. However, identification among Odonates is often challenging due to their similar morphological features. Therefore, the incorporation of morphological identification by taxonomists and validation using mitochondrial barcodes such as cytochrome *c* oxidase subunit I (COI) can be a more reliable approach to enhance the accuracy in species identification. In this study, four COI barcodes for Malaysian dragonflies (*Neurothemis fluctuans*) and damselflies (*Neurobasis chinensis*, *Aristocypha fenestrella* and *Sundacypha petiolata*) were generated. Three of the generated barcodes (D2 COI, D4 COI and D5 COI) supported the species identified by taxonomists meanwhile D3 COI deduced that the damselfly species was misidentified due to the very similar morphology between the same genus of damselfly. All of the COI barcodes are now available in the GenBank with the accession numbers of MT266926.1 (D2 COI), MT266925.1 (D3 COI), MT269676.1 (D4 COI) and MT266924.1 (D5 COI).

1. Data Description

Dragonflies and damselflies (Odonates) are important as biological indicators of freshwater and terrestrial ecosystems [1]. Besides that, dragonflies and damselflies also play a vital role as prey and predator to maintain the balance of the trophic level of the food chain and have a potential application in providing pest-control services to agricultural systems [2]. According to Malaysia Biodiversity Information System (MyBIS), there are approximately 428 species of dragonflies and damselflies in Malaysia. However, out of these 428 dragonfly and damselfly species, there are only 87 dragonflies and 95 damselflies cytochrome *c* oxidase subunit I (COI) reference barcodes available in the GenBank. Therefore, more effort is needed to develop and expand the reference barcodes for Malaysian dragonflies and damselflies in the GenBank for future species identification. The COI gene encodes for the cytochrome *c* oxidase I enzyme which is a mitochondrial protein located in the inner mitochondrial membrane. This enzyme plays a vital role in electron transport chain where it responsible in the metabolism of eukaryotic aerobic organisms. In this study, four COI barcodes for dragonflies and damselflies from Taman Negara Endau Rompin, Johor, Malaysia were developed with the information shown in Table 1.



Table 1. Information of four COI barcodes with reference species retrieved from BLASTn

Sample ID	Species identified by taxonomists	Size (bp)	Top Hit Species retrieved from BLAST				
			Species name	Identity (%)	Query cover (%)	GenBank Accession No.	Origin
D2	<i>Neurothemis fluctuans</i>	631	<i>Neurothemis fluctuans</i>	100	71	AB709004.1	Peninsular Malaysia
D3	<i>Neurobasis longipes</i>	638	<i>Neurobasis sp.</i>	100	97	MF804737.1	Myanmar
			<i>Neurobasis chinensis</i>	100	91	MG518624.1	Not-stated
D4	<i>Aristocypha fenestrella</i>	639	<i>Aristocypha fenestrella</i>	99.11	70	AB708423.1	Laos
D5	<i>Sundacypha petiolata</i>	644	<i>Sundacypha petiolata</i>	97.78	98	KF369551.1	Borneo (Sarawak)

A phylogenetic tree included all the COI barcodes generated in this study and COI reference barcodes available in the GenBank was constructed as shown in Figure 1. All the COI barcodes were aligned by using ClustalW (Jalview) prior to the construction of Neighbour-Joining (NJ) phylogenetic tree using MEGA-X [3]. A butterfly species named *Parantica aspasia* (accession no.: MF804749.1) was used as an outgroup for the NJ-tree. Based on the phylogenetic tree constructed (Figure 1), all the COI barcodes generated in this study formed a monophyletic group with their respective species with 100% bootstrap support.

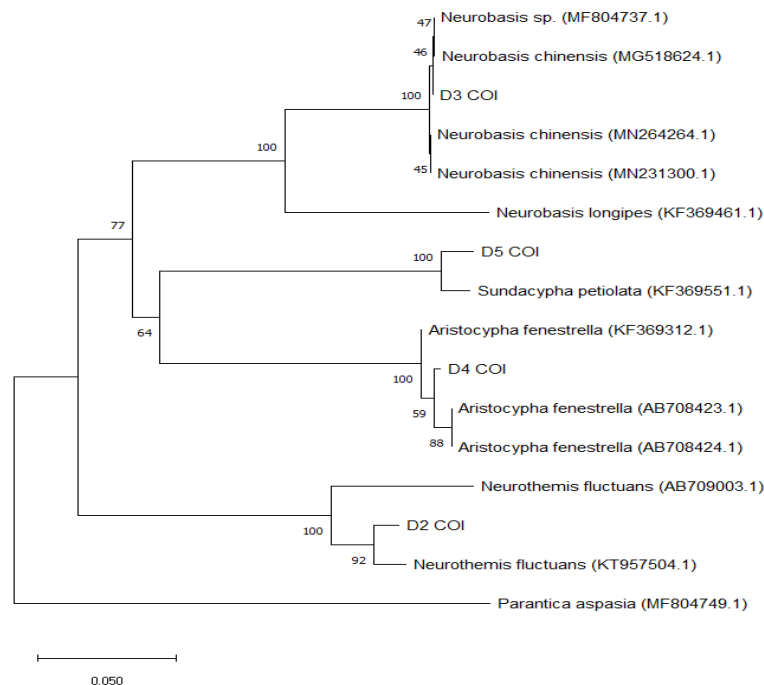


Figure 1. Phylogenetic tree of sample D2 COI (accession no.: MT266926.1), D3 COI (accession no.: MT266925.1), D4 COI (accession no.: MT269676.1) and D5 COI (accession no.: MT266924.1) with reference barcodes available in GenBank. Numbers on nodes indicate bootstrap supports.

From the BLASTn result in Table 1, D2 COI barcode (MT266926.1) from Peninsular Malaysia showed 100% identity to *Neurothemis fluctuans* (AB709004.1) originated from Malaysia. However, the query coverage between these barcodes was low (71%) as the size of the barcode generated in this study was bigger than the reference barcode deposited in the GenBank (451 bp). As both species had 100% identity, the BLASTn result further supported the species identification for D2 by taxonomists from Universiti Tun Hussein Onn Malaysia (UTHM).

Meanwhile, D3 was identified as *Neurobasis longipes* based on its morphology. However, the COI barcode for D3 (MT266925.1) had the closest match to *Neurobasis* sp. (MF804737.1) originated from Myanmar and *Neurobasis chinensis* (MG518624.1) originated from unknown provenance with 100% identity thus suggesting that the *Neurobasis* sp. (MF804737.1) may be *Neurobasis chinensis*. The damselfly under the genus of *Neurobasis* are commonly known as metalwing. As both *Neurobasis longipes* and *Neurobasis chinensis* share a common distribution area (Peninsular Malaysia) and have similar morphology, misidentification might occur as it is a very difficult and challenging to build identification keys to separate all *Neurobasis* species [4]. A morphological comparison of *Neurobasis chinensis* and *Neurobasis longipes* is shown in Figure 2. To date, there are no COI barcodes for Malaysian *Neurobasis chinensis* available in the GenBank.



Figure 2. Morphology of *Neurobasis chinensis* (common green metalwing) (left) and *Neurobasis longipes* (long-legged metalwing) (right)

Source: BOLD Record Number (RMNH.INS.506833 and RMNH.INS.501097)

The D4 COI barcode (MT269676.1) showed 99.11% identity to the *Aristocypha fenestrella* (AB708423.1) originated from Laos with 70% query coverage [5]. This finding agrees with the morphology-based identification by the UTHM taxonomists and suggests that there may be some genetic variation between the Peninsular Malaysia and Laos populations. The low query coverage is due to the low coverage of overlapping regions as the size of the D4 COI barcode was bigger than the reference barcode (AB708423.1) in the GenBank (451 bp).

Finally, the D5 COI barcode (MT266924.1), which was identified as *Sundacypha petiolata* based on its morphology showed 97.78% identity with 98% query coverage to the *Sundacypha petiolata* (KF369551.1) sample originated from Borneo (Sarawak). The slight difference between our Peninsular Malaysia sample and the Sarawak sample are likely due to intra-specific genetic variation between the geographically isolated populations [6][7]. However, this data must be interpreted with caution as there is only one *Sundacypha petiolata* reference barcode is available in GenBank.

2. Experimental Design

A total of four Odonates samples consist of one dragonfly and three damselflies were collected from Taman Negara Endau Rompin, Johor, Malaysia. All of these dragonfly and damselfly samples were identified by taxonomists from UTHM based on their morphological features. The legs of the samples were used for genomic DNA extraction using QIAGEN DNeasy® Blood and Tissue Kit. Polymerase chain reaction (PCR) amplification of COI gene of the genomic DNA was done by using universal COI primers and the PCR products spotted using 2% agarose gel electrophoresis prior to sequencing [8]. The sequences obtained were trimmed by using BioEdit and used for species identification through BLASTn in the GenBank. Then, the sequences were aligned using Clustal W (Jalview) and a phylogenetic tree was constructed using the Neighbour-Joining method in MEGA-X. A bootstrap analysis of 1000

replicates was conducted to estimate the statistical supports of the topology of the consensus tree and the bootstrap values displayed as node labels.

3. Conclusion

In this work, a total of four COI barcodes for Malaysian *Neurothemis fluctuans*, *Neurobasis chinensis*, *Aristocypha fenestrella* and *Sundacypha petiolata* were generated. The verification using mitochondrial barcodes in species identification was shown to help overcome the limitation of morphological-based approach. To further enhance the species identification, complete mitogenome sequencing of the Malaysian Odonates and increase the sampling of Odonates from different geographical locations can be done to obtain more accurate information about the genome, phylogeny and evolutionary relationship between the Odonates.

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