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# Phylogenetic and Haplotype Analyses Offer Insights Towards Conservation of *Pteroptyx tener* Olivier, 1907 (Coleoptera: Lampyridae) Populations from Malaysia

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## ABSTRACT

The synchronous-flashing firefly, *Pteroptyx tener* Olivier, 1907 is a congregating firefly with high economic value for the ecotourism industry. However, the populations of *P. tener* are increasingly being threatened due to habitat destruction. The assessment of genetic variation within and among populations of the species from Malaysia might play a major role in conservation and management programs. Objectives of this study were to investigate the relationships among *P. tener* from five populations in Malaysia (Peninsular Malaysia and Borneo (Sabah) using cytochrome oxidase subunit I (*COI*), and to obtain its haplotype information. A total of 50 sequences of *P. tener* were used in the Neighbour-Joining (NJ), Maximum Parsimony (MP), and Bayesian Inference (BI) phylogenetic analyses and successfully formed several distinct clades of West and East Malaysia (East Coast and West Coast of Peninsular Malaysia) with moderately to highly supported bootstrap values and posterior probabilities. Several haplotype analysis parameters are presented; low nucleotide diversity (PiT, 0.01893), haplotype number (Hn, 19), and high haplotype diversity (Hd, 0.89469). Our findings assumed that the geographical isolation due to the inability for flying long distances without help by the wind and flooding in tidal rivers. The data

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presented here are important genetic information on the *P. tener* populations and are important for the conservation programme in Malaysia by conducting the breeding program among populations that have similar genetic information.

*Keywords:* COI, beetle, conservation, mangrove, mtDNA, Luciolinae, Malaysia

## INTRODUCTION

*Pteroptyx tener* is a species of congregating fireflies that flash synchronously with the purpose of finding mating partners. This species is commonly found in Malaysia (Jusoh, Ballantyne, & Chan, 2013) as well as the southern part of Thailand (Sriboonlert, Swatdipong, Wonnapinij, Teerasak, & Thancharoen, 2015). *Pteroptyx tener* shows high affinity towards *Sonneratia caseolaris*, a tree commonly found in mangrove area of tropical regions such as Peninsular Malaysia (Shahara, Nura Adila, Maimon, & Norela, 2017). The flashing of *P. tener* is generated through a chemical reaction catalyzed by luciferase (Nur Khairunnisa, Nurul Wahida, & Norela, 2016). The flashing of fireflies as they congregate on trees at the riverbank creates a spectacular view at night. This unique beetle is an important ecotourism asset for Malaysia as it captivates many foreign and local tourists alike. The Kampung Kuantan Firefly Park in Kuala Selangor has received a total of 57,368 visitors in 2018 (Kuala Selangor District Council 2019). *Pteroptyx tener* has become a huge commodity for Malaysia, generating income for the local communities through their ecotourism programs.

Anthropogenic activity near the firefly habitat has a huge impact on these populations. Through study and population monitoring, *P. tener* has been observed to have declined during the previous few decades (Seri, Abd Rahman, & Kassim, 2021). This happened as the mangrove areas were extensively converted for other land uses such as agriculture, aquaculture and urbanization (Jusoh & Hashim, 2012; Prasertkul, 2018). The effect is especially inevitable when it concerns land clearing through the logging of mangrove trees, pollution of river by petrol and diesel by-product as well as agricultural chemicals and pesticides. There is also an obvious effect of artificial light on firefly populations as the communication between individuals is being disturbed. Treatment of artificial light on *Photinus* firefly by Owens & Lewis (2021) for example, has led to change in its flashing pattern leading to suppressed courtship activity. Artificial lighting near *P. tener* habitat may have threatened the population to local extinction. This shows the urgent need for conservation of these firefly population.

Study of fireflies in Malaysia involves a lot of firefly monitoring in various localities. Molecular studies have also been done on Malaysian mangrove fireflies to delineate different firefly species in an effort to assist in firefly species identification (Jusoh, Hashim, Saaksjarvi, Adam, & Wahlberg, 2014; Jusoh, Ballantyne, & Chan, 2020). It is also important to note that molecular genetics may help in conservation efforts (Frankham, 2010). With the rate of urbanisation occurring around the natural habitat of *P. tener*, it is very important to have a better understanding of the genetic information of each population through *COI* phylogeny and the haplotype analyses. It is because the conservation measure by implementing the genetic of the *P. tener* may be proposed which is important for conservation and species exploitation by presenting the information on phylogeography and evolution of the species (Wang, 2020). Intraspecific genetic variation depicted by genetic diversity value is a fundamental parameter in conservation practice. A unit within populations with higher genetic diversity will have greater tolerance against human perturbations (Dincă et al, 2018). The molecular genetic approach allows for the determination of significant units within species that are genetically strong to adapt in a changing environment. This may facilitate the conservation effort of *P. tener* where a specific unit within the populations can be selected for the reintroduction of this species to areas from which it has been lost. Moreover, the study on the population genomics of fireflies able to estimate the capacity of populations to evolve and adapt in response to environmental change and manage adaptive variation which was important in a long run conservation efforts (Hohenlohe, Funk, & Rajora, 2021). Hence, the aim of this study is to investigate the phylogeny of the *P. tener* populations from different regions in Malaysia, and to obtain haplotype data and a haplotype network to visualize and support the population relationships. Therefore, the breeding program among populations that having similar genetic information can be conducted effectively.

## MATERIAL AND METHODS

### Sampling sites

Fresh samples of *P. tener* individuals were collected from East Malaysia (Sungai Klias, Beufort, Sabah; 5°27'07.02"N 115°38'25.10"E, West Coast of Peninsular Malaysia (Sungai Sepetang, Taiping, Perak; 4°54'6.56"N 100°39'35.38"E and Sungai Rembau, Rembau, Negeri Sembilan; 2°26'31.31"N 102° 3'43.60"E) and East Coast of Peninsular Malaysia (Sungai Chukai, Kemaman, Terengganu; 4°18'3.93"N 103°22'58.51"E and Sungai Johor, Kota Tinggi, Johor; 1°39'18.35"N 103°55'37.90"E (Fig. 1). Samples were collected from November 2018 through May 2019. Each set of ten consisted of five males and females were sampled from each location which totalled 50 *P. tener* individuals altogether. The list of samples and localities is in Table 1. The samples were preserved in 70% alcohol to maintain its DNA and to avoid contamination.

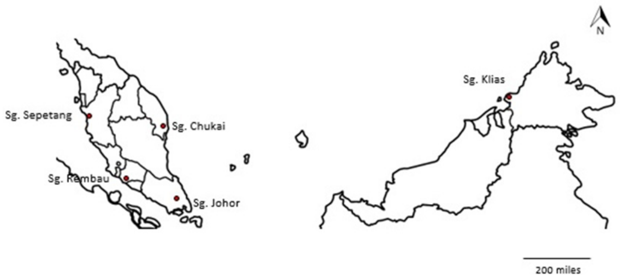

Figure 1. Localities of *Pteroptyx tener* collected in Peninsular Malaysia and Borneo (Sabah).

Table 1. Details of *Pteroptyx tener* used in the study, voucher code, GenBank accession numbers and

sampling locality.

| No | Code | GenBank Accession No. | Locality |
|---|---|---|---|
| 1 | TF1 | MN199184 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 2 | TF2 | MN199185 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 3 | TF3 | MN199186 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 4 | TF4 | MN199187 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 5 | TF5 | MN199188 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 6 | TM1 | MN199189 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 7 | TM2 | MN199190 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 8 | TM3 | MN199191 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 9 | TM4 | MN199192 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 10 | TM5 | MN199193 | East PM: Terengganu: Kemaman, Sungai Chukai |
| 11 | JF1 | MN199194 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 12 | JF2 | MN199195 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 13 | JF3 | MN199196 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 14 | JF4 | MN199197 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 15 | JF5 | MN199198 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 16 | JM1 | MN199199 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 17 | JM2 | MN199200 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 18 | JM3 | MN199201 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 19 | JM4 | MN199202 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 20 | JM5 | MN199203 | East PM: Johor: Kota Tinggi, Sungai Johor |
| 21 | SF1 | MN199204 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 22 | SF2 | MN199205 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 23 | SF3 | MN199206 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 24 | SF4 | MN199207 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 25 | SF5 | MN199208 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 26 | SM1 | MN199209 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 27 | SM2 | MN199210 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 28 | SM3 | MN199211 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 29 | SM4 | MN199212 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 30 | SM5 | MN199213 | East Malaysia: Sabah, Beufort, Sungai Klias |
| 31 | NF1 | MN199214 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 32 | NF2 | MN199215 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 33 | NF3 | MN199216 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 34 | NF4 | MN199217 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 35 | NF5 | MN199218 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 36 | NM1 | MN199219 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 37 | NM2 | MN199220 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 38 | NM3 | MN199221 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 39 | NM4 | MN199222 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 40 | NM5 | MN199223 | West PM: Negeri Sembilan, Rembau, Sungai Rembau |
| 41 | PF1 | MN199224 | West PM: Perak, Taiping, Sungai Sepetang |
| 42 | PF2 | MN199225 | West PM: Perak, Taiping, Sungai Sepetang |
| 43 | PF3 | MN199226 | West PM: Perak, Taiping, Sungai Sepetang |
| 44 | PF4 | MN199227 | West PM: Perak, Taiping, Sungai Sepetang |
| 45 | PF5 | MN199228 | West PM: Perak, Taiping, Sungai Sepetang |
| 46 | PM1 | MN199229 | West PM: Perak, Taiping, Sungai Sepetang |
| 47 | PM2 | MN199230 | West PM: Perak, Taiping, Sungai Sepetang |
| 48 | PM3 | MN199231 | West PM: Perak, Taiping, Sungai Sepetang |
| 49 | PM4 | MN199232 | West PM: Perak, Taiping, Sungai Sepetang |
| 50 | PM5 | MN199232 | West PM: Perak, Taiping, Sungai Sepetang |

## Species identification

The samples were identified to the species level by examining morphological characters under a microscope (Stereomicroscope Stemi 4). Identification was done by referring to Ballantyne (1987) and Jusoh, Ballantyne, Lambkin, Hashim, & Wahlberg (2018).

## DNA extraction and PCR amplification

DNA was extracted from adult firefly samples collected from the field, using Nucleospin DNA Insect Kit (Düren, Germany) according to the manufacturer's protocol. Firefly specimens had a small incision done in their abdomen in order to expose the tissue for DNA extraction. Standard DNA extraction protocols and the methods of Syarifah-Zulaikha, Halim, Aman, & Yaakop (2021) were followed. A 750 bp sequence of *COI* was amplified via a polymerase chain reaction (PCR) using Mastercycler Nexus (Eppendord North America, Inc). Primer regions used for *COI* were HCO2198 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3 and LCO1490 5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3 (Folmer et al. 1994). The PCR was carried out by using a profile with 12.5 µl of Green Mastermix, 7.5 µl of $DDH_2O$, 3µl of template DNA, and 1.5µl of primer region each from forward and reverse site with the total quantity of 25 µl. The parameters for the PCR process were as follows: 3 minutes of 95 $^{o}$C pre-denaturation, 30 cycle of denaturation for 30 seconds at 95 $^{o}$C, 1 minute of annealing at 47 $^{o}$C, 30 seconds of elongation at 72 $^{o}$C and 10 min of final elongation at 72 $^{o}$C (Halim, Aman-Zuki, & Yaakop, 2017). PCR products were visualized via electrophoresis on a 1.5% agarose gel.

## Sequencing, BLAST and BOLD analyses.

All PCR products of the fireflies were then sent to Apical Sdn. Bhd., Petaling Jaya, Selangor, Malaysia for sequencing. The sequences were manually edited using BioEdit version 7.0.4 (Hall, 1999). The Barcode of Life Data System (BOLD) and Basic Local Alignment Search Tool (BLAST) were applied for comparisons to database sequences (Altschul, Gish, Miller, Myers, & Lipman, 1990) based on several criteria namely expected value, maximum identical, total score, query coverage and maximum score.

## Phylogenetic analysis (NJ, MP and BI)

Fifty sequences were analyzed using three phylogenetic analyses which are the Neighbour-Joining (distance criterion), the Maximum Parsimony (parsimony criterion) and the Bayesian Inference (likelihood criterion). For the NJ tree, the Kimura-2- Parameter model was selected for phylogenetic reconstructions. The analysis was constructed by undergoing 1000 bootstrap replications to obtain the bootstrap confidence level. Bayesian tree was generated using MrBayes 3.1.2 software (Ronquist et al. 2012). Prior to tree generating with Bayesian inference, the best nucleotide substitution model was chosen using jModeltest 2.1.4 (Posada, 2008). The robustness of the tree was estimated using bootstrap analysis of 1000 replications; all codons containing gaps or missing data were eliminated. The NJ, MP and BI trees were constructed with the out group of *Luciola cruciata* as the closest related genus to *Pteroptyx* species based on Cheng et al (2019).

## Genetic distance and haplotype analyses

Genetic distance between firefly populations from different locations was analyzed using MEGA 7.0 through Kimura-2-Parameter (K2P). Haplotype analysis was conducted on each *P. tener* sequence using DNA Sequence Polymorphism (DnaSP) version 5.10.01 (Librado & Rozas, 2009). The distribution of haplotypes was mapped through Maximum Spanning Network (MNS) in Network 5.0

# RESULTS

## Sequence variation and genetic distance

A total of 50 samples of *P. tener* were successfully extracted in this study and a 750 bp sequence of *COI* was successfully amplified from each individual. Haplotype diversity differed amongst locality where the lowest was in the east coast region of Penisular Malaysia which is in Sungai Chukai, Terengganu while the highest was in west coast region of Peninsular Malaysia which is Sungai Sepetang, Perak. Correspondingly, the average pairwise distance within population and variation in nucleotide diversity reflect the same trend as haplotype diversity (Table 2). Meanwhile, average pairwise distance among population showed the lowest genetic distance (0.003) between Sungai Chukai-Sungai Johor and Sungai Rembau-Sungai Sepetang while the highest genetic distance was shown between Sungai Sepetang-Sungai Chukai (0.038) (Table 3).

Table 2. Estimates of genetic diversity amongst locality based on different genetic parameter.

| Locality | N | h | Hd | π | i |
|---|---|---|---|---|---|
| Sungai Chukai, Tererengganu | 10 | 2 | 0.20000 | 0.00031 | 0.0003 |
| Sungai Johor, Johor | 10 | 3 | 0.37778 | 0.00061 | 0.0006 |
| Sungai Klias, Sabah | 10 | 3 | 0.37778 | 0.00061 | 0.0006 |
| Sungai Rembau, Negeri Sembilan | 10 | 3 | 0.37778 | 0.00085 | 0.0009 |
| Sungai Sepetang, Perak | 10 | 9 | 0.97778 | 0.00385 | 0.0039 |

Note: N= number of individuals, h= number of haplotypes, Hd- haplotype diversity, π = nucleotide diversity, i = average pairwise distance within population

Table 3. Average pairwise distance among populations of *Pteroptyx tener* in Peninsular Malaysia and Borneo (Sabah).

| Locality | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sungai Chukai, Tererengganu | | | | | |
| Sungai Johor, Johor | 0.003 | | | | |
| Sungai Klias, Sabah | 0.011 | 0.011 | | | |
| Sungai Rembau, Negeri Sembilan | 0.037 | 0.034 | 0.032 | | |
| Sungai Sepetang, Perak | 0.038 | 0.034 | 0.033 | 0.003 | |

## Phylogenetic analysis

For the NJ tree (Fig. 2), the bootstrap values ranged from 63 to 100. The NJ tree was separated into three clades (A, B, C) which consisted of *P. tener* individuals from different population. The three clades were supported by moderate to high bootstrap values of 79, 95 and 98% respectively. Clade A includes the populations of *P. tener* from Sungai Chukai (Terengganu) and Sungai Johor (Johor). *P. tener* populations were separated in Clade B while *P*. *tener* from Sungai Rembau (Negeri Sembilan) and Sungai Sepetang (Perak) were grouped in Clade C (Fig. 2). The topologies of the MP (Fig. 3) and BI (Fig. 4) trees were similar where the trees were separated into three large clades. Clade A includes *P. tener* population from Sungai Rembau (Negeri Sembilan) and Sungai Sepetang (Perak) while Clade B includes population of *P. tener* from Sungai Klias (Sabah) while Clade C grouped population of *P. tener* from Sungai Johor (Johor) and Sungai Chukai (Terengganu).

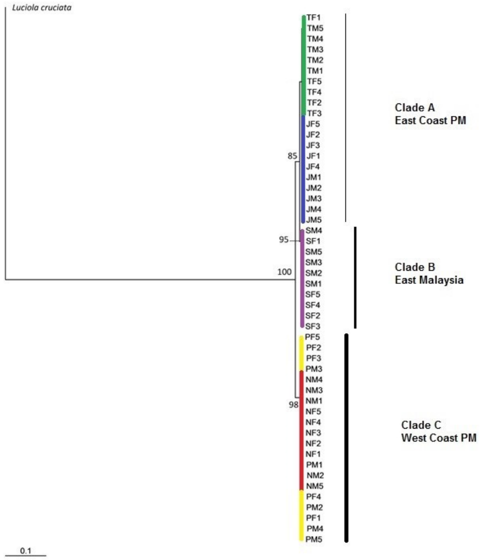

Figure 2. Phylogenetic tree of Neighbour Joining (NJ) analysis of *Pteroptyx tener* using *COI* marker. Bootstrap value was shown on branch of the tree (1000 replications).

## Haplotype analyses

A total of 19 haplotypes consisted of 34 nucleotides have been discovered from this study. Populations of *P. tener* in Sungai Johor (Johor), Sungai Rembau (Negeri Sembilan) and Sungai Klias (Sabah) recorded three haplotypes. There were two haplotypes in Sungai Chukai (Terengganu) while Sungai Sepetang (Perak) recorded nine haplotypes (Table 4). The haplotype analysis presented; low nucleotide diversity (PiT, 0.01893), and haplotype number (Hn, 19) with high haplotype diversity (Hd, 0.89469).

Table 4. Haplotype distribution of *Pteroptyx tener* populations using *COI* marker in Peninsular and Malaysia and Borneo (Sabah).

| **Locality** | **Haplotype** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| Sungai Sepetang | | | | | | | | | 1 | | | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| Sungai Johor | | | 1 | 8 | 1 | | | | | | | | | | | | | | |
| Sungai Chukai | 1 | 9 | | | | | | | | | | | | | | | | | |
| Sungai Rembau | | | | | | | | | 8 | 1 | 1 | | | | | | | | |
| Sungai Klias | | | | | | 1 | 8 | 1 | | | | | | | | | | | |

Based on the minimum spanning network (MNS) analysis, there were 18 unique haplotypes comprised of Hap1 and Hap2 (Chukai), Hap3, Hap4 and Hap5 (Johor), Hap6, Hap7 and Hap8 (Klias), Hap10 and Hap11 (Rembau), Hap12, Hap13, Hap14, Hap15, Hap16, Hap17, Hap18 and Hap19 (Sepetang). Only Hap9 was being shared between Sepetang and Rembau showing mixture of *P. tener* population from Sungai Sepetang (Perak) and Sungai Rembau (Negeri Sembilan) (Fig. 5).

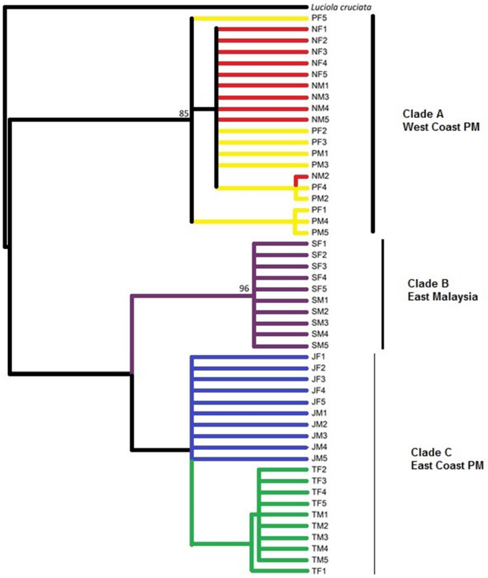

Figure 3. Phylogenetic tree of Maximum Parsimony (MP) analysis of *Pteroptyx tener* using *COI* marker. Bootstrap value was shown on branch of the tree (1000 replications).

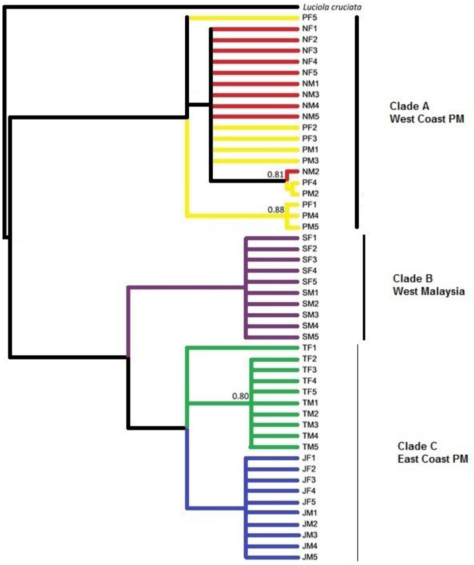

Figure 4. Phylogenetic tree of Bayesian Inference (BI) analysis of *Pteroptyx tener* using *COI* marker. Posterior probabilities value was shown on branch of the tree.

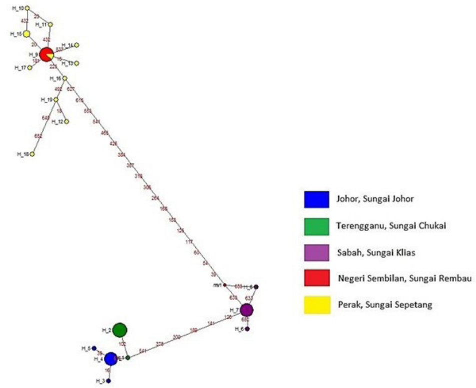

Figure 5. Minimum Spanning Network (MSN) based on sequence analysis of *Pteroptyx tener* using *COI* marker. The size of each circle corresponds to its relative frequency in the total sample.

## DISCUSSION

Phylogenetic and haplotype analyses have been conducted on *P. tener* populations from Malaysia using *COI* marker. Lee et al, (2003) also used *COI* to successfully separate populations of *Pyrocoelia rufa* on Jeju do Island, Korea and Tsushima Island, Japan. The use of *COI* data also helped in the identification of various firefly species of the genus *Pteroptyx,* which revealed the existence of cryptic species (Jusoh et al, 2020). This study presents the NJ, MP and BI trees of *COI* sequences of *P. tener* from Malaysia, while *Luciola cruciata* (Luciolinae) was chosen as an outgroup in order to construct comprehensive phylogenetic trees by referring to the previous phylogenetic studies of fireflies (Cheng, Munian, Tan, Mohd Azahari, & Shah-Fadir, 2019).

All trees showed a distinct separation of *P. tener* populations from the West Coast of Peninsular Malaysia in Sungai Sepetang (Perak) and Sungai Rembau (Negeri Sembilan, and the East Coast populations from Sungai Chukai (Terengganu) and Sungai Johor (Johor). The high average pairwise distance between Sungai Sepetang (Perak) and Sungai Chukai (Terengganu) was also manifested in coherent with geographical distance between east coast and west coast. The population of *P. tener* from Sungai Klias (Sabah) was also clearly separated from the Peninsular area. This study is in coherent with Cheng et al (2019) which detected three distinct populations of *P. tener* in Malaysia, one each from east and west coast of Peninsular Malaysia as well as Borneo. There were also numerous other studies showing similar geographic separations in other taxa (Yusof et al, 2016; Ith et al, 2016; Chan, Abraham, Grismer, & Grismer, 2018).

According to Thancharoen (2021), the genetic differentiation of fireflies can be caused by the limitation of dispersal activity, which has a higher probability of reproductive isolation. For example, the discontinuous habitats of desert firefly, *Microphotus octarthrus* that has winged males and apterous larviform females have resulted in genetic isolation (Usener & Cognato, 2005). The isolation of populations in Peninsular was often hypothesized due to the Titiwangsa mountain range acting as a physical barrier that limits the movement of organisms. This isolation was observed in insect species such as *Anopheles* mosquitoes (Sum et al, 2014), and *Coptotermes* termites (Cheng et al, 2014) Nevertheless, since *P. tener* is highly associated with mangrove areas, it was also suggested that the distribution of this species was due to the ancient river network during Pleistocene era (Cheng et al, 2019). However, isolation over distance was not applied to the populations of *P. tener* in Sungai Rembau and Sungai Sepetang. The distance shown to be high at 0.034 suggesting other factors affecting its population structure. As such, it is important to note that the dispersal of *P. tener* may occur through the semi-aquatic larval movement. The female *P. tener* lay their eggs on the wet soil of the riparian area. The hatching larvae of this firefly will easily be dispersed through irrigations of river channels especially during high tide. It has been reported that the eggs of aquatic firefly *Sclerotia aquatilis* in Thailand were found on aquatic vegetation which can be transported with the flow of water (Lewis et al, 2020). Thus, there is a higher possibility for the *P. tener* to establish in a new area if a huge number of larvae survives in the flow of the river.

Apart from the geographical factor, the mtDNA gene flow of *P. tener* may be limited by its flight ability. Considering the small body size of *P. tener*, the dispersal through flight may only occur over short distances explaining the lower average pairwise distance between population in Sungai Sepetang and Sungai Rembau. This mtDNA gene flow is hampered due to *P. tener*'s flight ability as most small insects are easily affected by wind speed (Gatehouse, 1997). As Peninsular Malaysia is subjected to monsoons during long periods of the year (Southwest Monsoon from April to September and Northeast monsoon from October to March), *P. tener* will not be able to overcome the strong moving air to move a larger distance thus limiting its movement.

The haplotypes distribution also corresponds to the others results of this study. Haplotypes from different geographic regions occupied different and non-overlapping parts of the minimum-spanning network suggesting strong phylogeographic structuring. Every sampling site revealed locality-specific haplotype reflecting high diversity even at a small scale. There were 18 unique haplotypes while only one haplotype was being shared between population in Sg. Sepetang and Sg. Rembau. Spatially close populations have a higher tendency sharing haplotypes. For instance, all individuals of *Exocelina manokwariensis* (Dytiscidae) from two different populations shared the same haplotype as the populations located near each other (Lam et al, 2018).

On the other hand, unique haplotypes reflect variation in populations where they do not overlap with other individuals from different populations. *Pteroptyx tener* can be considered as a specialist by referring to its strong association to mangrove habitat, a resource that is distributed rather patchily across Peninsular Malaysia and Sabah. Thus, as weak dispersers, the isolation of extant populations was further emphasized. A similar situation occurred in a deciduous tree specialized beetle, *Osmoderma* (Coleoptera: Scarabaeidae) for which its distribution is highly affected by distribution of its resource (Landvik et al, 2017). All of the information is important in managing populations of congregating fireflies in this region.

From the results, it shows that in conservation and management efforts for *P. tener*, it is important to consider each population in Malaysia as a single genetical unit since there is only scarce overlap between populations of different localities. The populations of *P. tener* are considered unique as they become regionally adapted. As such, the unique populations tend to be very sensitive towards environmental changes (Allendorf, Luikart, & Aitken, 2013). Nevertheless, variations shown in the results were based on a single mitochondrial marker. It is suggested for additional studies to be done using different markers with the possibility of discovering other variations.

The population of *P. tener* in Sungai Chukai requires more attention in respect to the low haplotype diversity in the area. As the population becomes isolated with low gene flow, the rate of inbreeding will increase. Thus, at any influence of genetic drift with detrimental effect, population may experience regional extinction (Allendorf, Luikart, & Aitken, 2013; Allendorf et al, 2013). However, looking at the current status of land use around the area, Sungai Chukai is still largely surrounded by natural mangrove forest habitat which indirectly safeguard the firefly population for the time being. It is suggested for mangrove area around Sungai Chukai to be protected by law in prevention of land use changes which can be detrimental in effect.

In relation to the future prospect of introducing this species to a new area, the population of *P. tener* in Sungai Sepetang will have a higher chance to sustain in reference to its high genetic diversity. It is hypothesized that *P. tener* in Sungai Sepetang will be more resilient towards change in the environment, making it possible to introduced it to a new mangrove habitat. Yet, it is more crucial to focus on protecting the local habitat of this firefly species while considering it an individual genetic pool. Given the specialization of *P. tener* towards mangrove habitats, the species is facing multiple challenges. The most serious threats to mangroves are directly and indirectly a result of increasing human population density (Romañach et al, 2018).

## CONCLUSION

Based on our results, we conclude that *P. tener* may only be protected by conservation of the mangrove habitat at the local level. Thus, management and conservation practices should focus on breeding of the species. Genetic diversity and population structure studies are important to be conducted in the near future to measure precisely the genetic movement between populations and the supporting reasons.

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