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They are different: molecular approach on *Tirathaba* pest infesting oil palm and coconut tree

Abstract

There are some confusion among agriculturists on the species of *Tirathba* beetles that are infesting on oil palm and coconut trees. Many thought they are the same species. In this study, the mitochondrial DNA Cytochrome oxidase subunit I (COI) of *Tirathaba* pest infested oil palm and coconut tree were compared. The mitochondrial DNA Cytochrome oxidase subunit I (COI) gene of the targeted *Tirathaba* sp. infesting on oil palm and coconut tree were sequenced. The sequences were trimmed to remove gaps and produce a final aligned fragment of 603bp for oil palm *Tirathaba* sample and 602bp for coconut pest sample. The DNA sequences were analyzed with other *Tirathaba* sp. sequences available in Gene bank using phylogenetic tree constructed with Neighbor-Joining (NJ) and genetic distance analysis algorithms. The result of this study indicates they were two different species. This knowledge will provide important data elements in the development of pest management strategy.

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Introduction

High infestation rate of *Tirathaba mundella* was reported from many oil palm plantations established on peat Masijan et al.[1] The infestation is identified by the presence of long tubes of silk and frass which is built by the pest. The larvae stage of pest does most of the damage by feeding or scraping leaving holes on immature fruitlets. Larvae of early instar stage often attack on male inflorescences while larvae of older instar stage attack on female inflorescences and bunch Yaakop et al.[2] A new damage caused by *Tirathaba mundella* is visible as moist and reddish brown faeces on brunches while an old damage is characterized as dry and brownish black faeces. Another *Tirathaba* that also regarded as agricultural pest is *Tirathaba fructivora*. It was first observed in Fiji and Java, Indonesia Paine[3] and later in Sumatra, Indonesia and Malaysia (Kalshoven, I981). The pest causes destructive effect to the coconut plantation in Philippines. *Tirathaba fructivora* together with another coconut spike moth, *Tirathaba rufivena* Walker are making damage to coconut flower. Both pests attack the inflorescence of coconut, causing the young and soft flower to drop off from the plant. Falling of flower prevents the formation of nuts Alouw et al.[4] *Tirathaba fructivora* has a total development period of around 25days from the stage of egg deposition to adult emergence. The newly laid egg of *Tirathaba fructivora* is white in colour and it turns dark yellow or dark orange when it is about to hatch. Hatching took about 4days from egg deposition. The larvae have five instars level with a larval stadium of around I7days and pupal period of around 8days.

The first instar larvae are light brown and it turns into darker colour slowly in the following instars. Thoracic shield was developed on the first thoracic segment from first instars and becomes more apparent in the later instars. Prolegs are well grown from the second to fifth instars. The longevity of female and male *Tirathaba fructivora* is around 8-9days Alouw et al.[4] Many authors believe that the *Tirathaba* pest found in both plantation were the same species. This notion needs to be clarified. Therefore this study aims to compare the genetic markers CO1 of both *Tirathaba* sp. infesting on the oil palm and coconut tree. The finding of this study provides essential data to determine if the *Tirathaba* species from both type of plantation belongs to same species.

Methods

Tirathaba mundella were collected in the Sarawak Oil Palms Berhad (SOP) plantation site, located in Miri, Sarawak. The specimens were collected from the male flower. For the coconut pest, the insect were collected from immature nuts of coconut tree. *Tirathaba* sp. from both plantations were DNA extracted, amplified with primers coding for the cytochrome oxidase I (COI) gene, which were "LepFI" 5'-ATTCAACCAATCATAAAGATATTGG-3' and "LepRI" 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hajibabaei et al. 2006). The amplified DNA was sequenced and blasted against public available Gene bank. The sequences were further analyzed their genetic distance with other *Tirathaba* spp. using the Kimura two parameter Hosoishi et al.[5] Kimura.[6] A phylogeny tree was constructed by using Neighbor-Joining (NJ) method Saitou et al.,[7] in the program MEGA version 7 Hosoishi et al.[5]

Result

The sequenced DNA fragments for both samples collected from oil palm and coconuts were approximately 602 base pairs (bp). The BLAST result showed only *Tirathaba* sp from oil palm plantation had only 92% of similarities with *Tirathaba parasiticus* sequence in the Gene bank while coconut pest sample showed less than 95% similarities with other *Tirathaba* spp available in the database (similarities>95%). The genetic distances for both *Tirathaba* spp were analyzed with other six *Tirathaba* sp. The six species included *Tirathaba pseudocomplana, Tirathaba rufivena, Tirathaba parasiticus, Tirathaba ruptilinea, Tirathaba cissinobaphes* and *Tirathaba psolopasta.* All *Tirathaba* sp. COI sequences that contained more than 600bp were aligned by Multiple Sequence Alignment Tool (MUSCLE) and result as shown in Table 1. A phylogeny tree was generated (Figure 1) and shows that the oil palm *Tirathaba* sample was under the same clade with *Tirathaba pseudocomplana*, *Tirathaba rufivena*, *Tirathaba parasiticus* and *Tirathaba ruptilinea.*

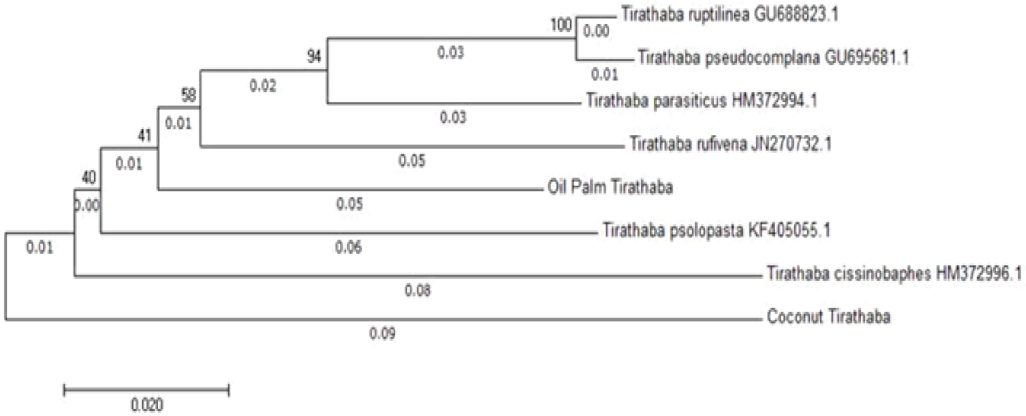

Figure 1 Evolutionary relationships of taxa.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch.

Length = 0.44666809 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was shown next to the branches. The tree was drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were Ist +2nd +3rd +Non coding. All positions containing gaps and missing data were eliminated. There were a total of 602 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Table 1 Estimates of evolutionary divergence between sequences

S. No	Genetic diversity of Cytochrome c Oxidase subunit i sequence	1	2	3	4	5	6	7
1	Tirathaba *ruptilinea*							
GU688823.1								
2	Tirathaba *rufivena*	0.1015						
JN270732.1								
3	Tirathaba *psolopasta*	0.1129	0.1133					
KF405055.1								
4	Tirathaba *pseudocomplana*	0.0118	0.0976	0.1149				
GU695681.1								
5	Tirathaba *parasiticus*	0.0644	0.1015	0.1229	0.07			
HM372994.1								
6	Tirathaba *cissinobaphes*	0.1449	0.1470	0.1486	0.1512	0.1367		
HM372996.1								
7	Oil Palm *Tirathaba mundella*	0.1131	0.1036	0.1213	0.1111	0.0899	0.1424	
8	Coconut *Tirathaba*	0.1732	0.1820	0.1628	0.1775	0.1712	0.1840	0.1485

Discussion and conclusion

Tirathaba mundella showed a genetic distance with the coconut *Tirathaba* at 0.1485. This indicates that the two species were not from the same genus. The Blast result also indicates that the pest infested the coconut plantation could be misidentified as *Tirathaba rufivena* as their COI sequence were distantly related. The coconut pest may be a newly discovered *Tirathaba* species which needed to further study.[8]

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None.

Conflict of interest

The author declares no conflict of interest.

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