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A Preliminary Introduction to the Phylogeny of Opiine Parasitoids (Braconidae: Opiinae) with Larvae of Fruit flies, *Bactrocera* spp. (Tephritidae: Dacinae) Based on Four Molecular Data

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Abstract. *Bactrocera* Macquart is a fruit fly genus, infesting many fruits and vegetables species. It is parasitized by the braconid wasps, Opiinae, the potential parasitoid species for controlling the infestation of the fruit flies in the agricultural fields. In this paper, the relationships of the opiine species and the *Bactrocera* were illustrated in the tree phylogeny using of combined data four molecular markers (*COI, 16S, 12S* and Cytb) by implementing Maximum parsimony (MP) and Bayesian Inference (BI) analyses. The *Bactrocera* species also extracted and amplified using *COI* marker for confirming the identity molecularly. As preliminary data, resolved phylogenies were obtained from the analyses. Three genera viz. *Diachasmimorpha, Fopius* and *Psyttalia* formed 3 monophyletic clades and supported with high value of bootstraps and posterior probabilities. Besides that, each species belongs to every genus also clustered together and supported with moderately to highly support values. The interaction between species was proven molecularly and seems there is no strict coevolution among them. It is because the *Bactrocera* and opiines species are polyphagus and switch many times their hosts.

Keywords: opiine parasitoids, *Bactrocera*, mitochondrial DNA, co-speciation, interaction

1. Introduction

Fruit fly is known as a main insect pest infesting many of fruit species in Malaysia and worldwide. The infestations of the fruit flies greatly reduced the yield and affect the yearly production of the crops. So, the using of insecticides help so much, but the using of the natural enemy as biocontrol agent to control the infestation is more significant due to the environmental friendly [1]. Therefore, to know the potential parasitoid species is the first step before continuation with the behavioral study, ecological study etc. for mass production of the parasitoids. Braconid wasps under the subfamily Opiinae are recognized as highly promising to be potential biological control agents against fruit flies (Diptera: Tephritidae) [2]. Hence, the data on the opiine parasitoids and also the pest species, *Bactrocera* are very important, which basically involving the interaction and the coevolutionary process.

In this paper, the coevolution between the opine wasps and the *Bactrocera* species was understood and helped based on the molecular data of four mitochondrial DNA namely *COI, 16S, 12S* and *Cytb*. The intra- and interspecies of the parasitoids were estimated based on the phylogeny resulted from the phylogenetic analyses of Maximum Parsimony (MP) and Bayesian Inference (BI). Furthermore, the information regarding the pests, larval stages of *Bactrocera* spp. was determined using molecular data of *COI*. So, the

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coevolutionary process on both taxa was understood to clarify the tritrophic or multitrophic interactions in the agricultural fields.

2. Material and Methods

2.1. Samples Collection

Sample of pests; larval stages of fruit flies were obtained and randomly collected from infested crops species viz. carambola or star fruit (*Averrhoa carambola*), wax apple (*Syzygium samarangense*), guava (*Psidium guajava)* and ridge gourd or luffa *(Lufa acutangula*). A larva of fruit fly was collected for molecular work from each group of fruit sample (several species from different localities). The rests of infested fruits were kept in the laboratory (specific rearing condition) until emergence of the parasitoids. The opiines and fruit flies species extracted and used in the analyses (Table 1).

Table 1. Lists of braconids species emerged from the reared fruit flies species and the information of the locality of collection and the infested crops species used for the phylogenetic analyses (only an individual of the tephritid randomly extracted).

TEPHRITIDS	CODE OF OPIINES	SPECIES OF OPIINES	LOCALITY	CROP SPECIES
Bactrocera papayae	2.1.1	*Psyttalia* sp.	Malaysia: Dengkil, Selangor	*Syzygium samarangense*
Bactrocera papayae	2.1.2	*Psyttalia* sp.	Malaysia: Dengkil, Selangor	*Syzygium samarangense*
Bactrocera papayae	2.2.2	*Fopius arisanus*	Malaysia: Dengkil, Selangor	*Averrhoa carambola*
Bactrocera papayae	2.2.3	*Fopius arisanus*	Malaysia: Dengkil, Selangor	*Averrhoa carambola*
Bactrocera papayae	2.3.2	*Fopius vandenboschi*	Malaysia: Dengkil, Selangor	*Syzygium samarangense*
Bactrocera papayae	2.4.1	*Fopius arisanus*	Malaysia: Dengkil, Selangor	*Averrhoa carambola*
Bactrocera papayae	2.4.2	*Fopius arisanus*	Malaysia: Dengkil, Selangor	*Averrhoa carambola*
Bactrocera papayae	2.5.1	*Fopius arisanus*	Malaysia: Dengkil, Selangor	*Averrhoa carambola*
Bactrocera carambolae	34.1	*Diachasmimorpha longicaudata*	Malaysia: Kluang, Johor	*Averrhoa carambola*
Bactrocera carambolae	34.5	*Diachasmimorpha longicaudata*	Malaysia: Kluang, Johor	*Averrhoa carambola*
Bactrocera carambolae	34.7	*Diachasmimorpha longicaudata*	Malaysia: Kluang, Johor	*Averrhoa carambola*
Bactrocera papayae	47.1.1	*Fopius vandenboschi*	Malaysia: Lanchang, Pahang	*Psidium guajava*
Bactrocera papayae	47.2	*Fopius vandenboschi*	Malaysia: Lanchang, Pahang	*Psidium guajava*
Bactrocera papayae	54.2	*Diachasmimorpha longicaudata*	Malaysia: Sg. Baging, Pahang	*Averrhoa carambola*
Bactrocera cucurbitae	57.1	*Psyttalia fletcheri*	Malaysia: Setiu, Terengganu	*Luffa acutangula*
Bactrocera cucurbitae	57.2	*Psyttalia fletcheri*	Malaysia: Setiu, Terengganu	*Luffa acutangula*
Bactrocera cucurbitae	57.3.2	*Psyttalia fletcheri*	Malaysia: Setiu, Terengganu	*Luffa acutangula*
Bactrocera cucurbitae	57.3.3	*Psyttalia fletcheri*	Malaysia: Setiu, Terengganu	*Luffa acutangula*
Bactrocera cucurbitae	57.3.4	*Psyttalia fletcheri*	Malaysia: Setiu, Terengganu	*Luffa acutangula*

2.2. DNA Extraction, PCR and DNA Sequencing

DNA of the braconids was extracted using Qiagen Kit (DNeasy® Blood and DNA Tissue) with small modification named as freezing method [3], [4], while DNA of tephritids was obtained using the similar Kit as braconids with general procedures from the manufacturer. Polymerase Chain Reaction (PCR) analysis was conducted on 4 molecular markers (*COI, 16S, 12S* and *Cytb*) using several pairs of primers; 16S Wb [5] and 16S outer [6]; SR-J-14199 [7] and SR-N-14594 [7]; LCO2198[8] and HCO1490 [8] and CB-J-10933 [7] and CB-N-11367 [7] for *16S, 12S, COI* and *Cytb*. The PCR conditions and the reactions differed between markers. PCR optimization for each marker was successfully done. The PCR products were purified using GeneAid purification kit and the PCR products were sent for sequencing analysis to First Base Sdn. Bhd., Shah Alam, Selangor, Malaysia. The BioEdit version 7.0.2 software was used for sequences alignment [9]. Dataset for each marker consists of 19 Opiinae samples using Clustal W and were aligned individually and then combined for the phylogenetic analyses.

2.3. Phylogenetic Analyses

Two analyses were run on the combined data of four markers namely the Maximum Parsimony (parsimony) and Bayesian Inference analyses (likelihood) using PAUP 4.0b10 and MR BAYES 3.1.2. For

maximum parsimony, 1000 stepwise addition replicates in a heuristic search with tree-bisection-reconnection (TBR) option for branch-swapping algorithm. The MP tree was subjected to bootstrap analysis with 1000 replicates. For Bayesian approach, phylogenetic reconstruction [10] was analyzed with two Markov Chain Monte Carlo (mcmc) and runs simultaneously for 510000 generations with a sample frequency of 100 generations. The first 1275 trees have been discarded as burnin.

3. Results and Discussion

Combined data of four mitochondrial genes; *COI, 16S, 12S* and *Cytb* resulted in 2100 bp of fragment length. It consists of 544 (26%) of parsimony informative, 151 (7.2%) of parsimony non-informative and 1405 (67%) of constant. From the MP analysis, the consistency index (CI) is 0.7755, homoplasy index (HI) is 0.2255, retention index (RI) is 0.9179 and tree length is 1038. For the Bayesian inference, number of frequency is 0.006283 with 510000 generations by implemented the best-fit model (GTR+I+G) by AIC using MrModelTest version 2.2 [11].

Combined data of four markers resulting more resolved trees compared to the individual trees (not shown here). Each genus (*Diachasmimorpha longicaudata*, *Fopius arisanus* + *F. vandenboschi*, *Psyttalia* sp. + *P. fletcheri*) and every species formed monophyletic and supported with 100% bootstrap and posterior probability values (Fig. 1). However, the nodes supported with low posterior probability and bootstrap values in every individual trees.

Both trees have been developed using Maximum Parsimony (MP) and Bayesian Inference (BI) analyses. For the MP, using the parsimony criterion, the minimum evolutionary changes have been determined by obtaining the most parsimonious trees [12]. According to Faubet et al. (2007), likelihood criterion by implementing Bayesian Inference analysis took into account the mutation rate, migration rate (in island population) and population size.

Basically, the combined data increased the length of fragment that automatically increasing and maximizing the number and percentage of parsimony informative characters [13]. It is because phylogeny can be improved by using data with high amount of parsimony characteristics [14], [15]. Besides that, the combined data showed low amount of homoplasy in the phylogenetic tree [16] that can be traced from the high value of consistency index and the low value of homoplasy index, which is presented in MP analysis. Furthermore, Remsen and DeSalle [17] also have proven that the accuracy of the phylogeny will be improved by combining data from several genes.

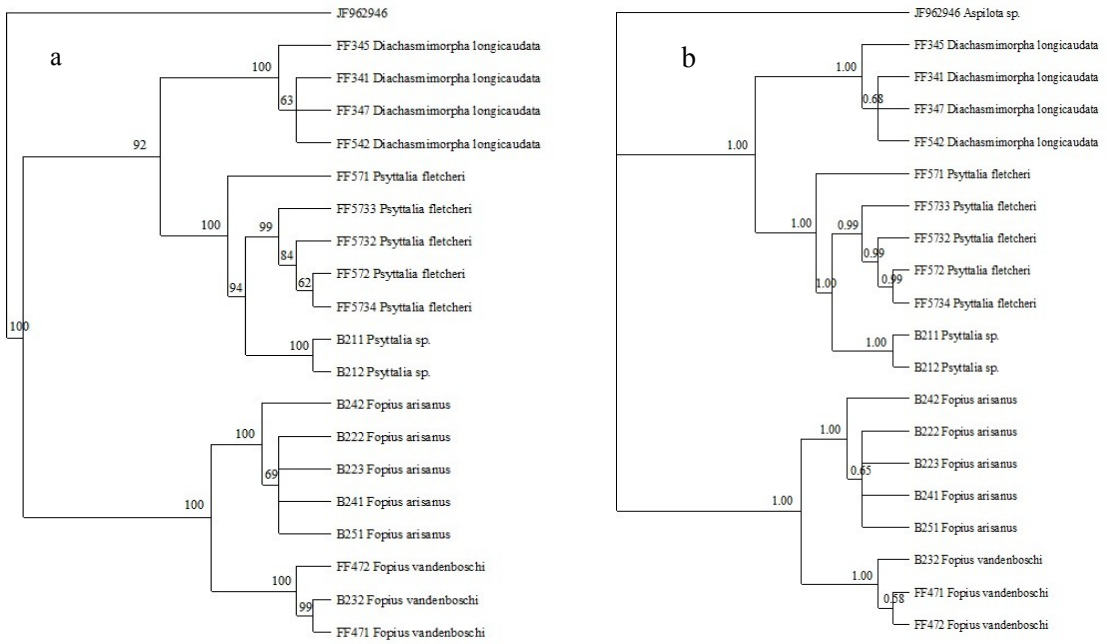

Fig. 1: 50% majority rule consensus tree of combined four markers a) maximum parsimony analysis (15 parsimonious trees) - bootstrap values above branches (1000 replications); b) bayesian inference analysis - posterior probability values at the interior nodes.

In this preliminary study, the phylogenies obtained (Fig. 1) illustrated pattern of the coevolutionary process between *Bactrocera* and several species of opiines. However, it has shown no strict coevolution because several *Bactrocera* species have been parasitized by several opiines species and this pattern also similar in the *Bactrocera* that infesting many of host plants (if we based on the survey done by Chinajariyawong et al. [18]. In summary, the opines are determined as polyphagus parasitoids that searching different species of hosts and it also switches many times of their hosts. Besides that, the host, the *Bactrocera* species also are polyphagus species e.g. in the *Bactrocera carambolae and B. papayae* that infesting many species of fruits and vegetables. Eventhough, there were very limited data obtained because of limited samples collected from several localities and host fruits. However, information based on the survey data also was referred for result interpretation.

This paper has proven by combining of four mitochondrial data (*COI, Cytb, 12S* and *16S*) has successfully resolved the phylogeny on both associated species, as model samples to study the coevolutionary process. All these markers have high ability to show a high degree of variations in nucleotide to distinguish among species [19], [20]. Preliminary data on this paper very much helps in species identification. Precise identification of species is very essential for the successful management of pests and parasitoids in the agricultural fields under the biological control programme.

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