NOTE

A New Tritrophic Association in Malaysia between *Fopius arisanus*, *Bactrocera carambolae*, and *Syzygium samarangense*, and Species Confirmation using Molecular Data[1]

Salmah Yaakop[2,3] and A. Z. Aman[2]

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Fruit flies belonging to the family Tephritidae (Diptera) are pests of many crops in Malaysia and other parts of the tropics. *Bactrocera papayae* Drew & Hancock, *B. carambolae* Drew & Hancock, *B. cucurbitae* (Coquillett), *B. umbrosa* F., *B. latifrons* Hendel, *B. caudata* (F.), and *B. tau* Walker infest papaya (*Carica papaya* L.), carambola (*Averrhoa carambola* L.), melon (Cucurbitaceae), jackfruit (*Artocarpus heterophyllus* Lam.), tomato (*Solanum lycopersicum* L.), and chilli (*Capsicum annum* L.), respectively (Chua et al. 2010). These important fruit fly pests reduce yields and hinder the expansion of fruit production areas. There have been some studies on the ecology and taxonomy of fruit fly parasitoids used in biological control programmes and integrated pest management (IPM), while controlled breeding processes have been developed for fruit flies and their parasitoid species (Clausen 1978, Harris & Bautista 2001, Joyce et al. 2010).

A total of 13 Opiinae species (Hymenoptera: Braconidae) are recognized as parasitoids of tephritid species in Thailand and Malaysia (Chinajariyawong et al. 1999). Moreover, Allwood et al. (1999) provided a complete list of tephritids and their host crops in south-eastern Asia. Many species of Braconidae have been used successfully as biological control agents of fruit flies (Harris et al. 2010). One of these species, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), which is native to the Indo-Pacific Region, has been used successfully against *Bactrocera dorsalis* (Hendel) in Hawaii (Vargas et al. 2012). Because of its economic importance, the biology of *F. arisanus* has been extensively studied (Bautista et al. 2001). *Fopius arisanus* is known to parasitize *B. carambolae* on *Magnifera indica* L., *Annona montana* L., *Terminalia catappa* L., *Fagraea ceilanica* Thunberg, *Artocarpus heterophyllus* Lamarck, *Eugenua* sp., *Psidium guajava* L., *Syzygium aqueum* (Burm. f.) Alston, *S. malaccense* (L.) Merr. & L. M. Perry, *Averrhoa carambola* L., and *Manilkara zapota* (L.). To date, there are as yet no records of *F. arisanus* associated with *B. carambolae* infesting the wax apple fruit, *Syzygium samarangense* (Blume) Merrill & Perry (Myrtales: Myrtaceae) from Malaysia, but this association has been recorded from Thailand (Yu et al. 2005).

[1]Accepted for publication 27 March 2013.
[2]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangaan Malaysia, 43600 Bangi, Selangor, Malaysia.
[3]Corresponding author, salmah78@ukm.my; phone: 0060389215698.

In this study, we report *F. arisanus* as a new record on *B. carambolae* infesting *S. samarangense* in Malaysia. We also confirmed the species identification of these insects using both morphological and molecular characters. Species identifications of *Bactrocera carambolae* and *B. papayae* are problematic when based solely on morphological features (Chua et al. 2010). In addition, species identifications are complicated further by ongoing changes in the general classification of Opiinae proposed by various taxonomists (Fischer 1972, Shi et al. 2005). Traditionally, taxonomic classifications of insects based on morphological attributes have been widely used and accepted. However, molecular techniques have been used lately to confirm the taxonomic status of specific taxons, particularly those with morphological ambiguities (Yaakop et al. 2009, 2010). Some difficult or arbitrary morphological characters have been resolved recently by these molecular techniques (Krug et al. 2008).

In this study, a total of 60 fleshy wax apple fruits were collected from a commercial farm (D-Paradise Tropical Fruits World, Malacca, Malaysia) and brought to the laboratory. The *S. samaragence* were put into 12 transparent containers with netting (7 cm tall, 6 cm diameter) and kept at room temperature for adult emergence. A total of six braconid and two tephritids adults were recovered from *S. samaragense* from one of the containers. These specimens were identified following Achterberg's (1993) and Drew & Hancocok's (1994) keys. The DNA of each of the adult tephritids and braconids was isolated by DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA) using the 'freezing method' for PCR amplification, sequencing, and BLAST analysis.

The braconid and tephritid species were initially identified as *Diachasmimorpha* sp. and *B. papayae*, respectively, based on their external morphology. However, identification using only braconid males is problematic because there is lack of species characters based on male specimens for the key species (C. van Achterberg, Key to species of the genus *Fopius* Wharton, unpublished). Furthermore, there could be some confusion in separating *B. carambolae* and *B. papayae* because of the presence of intermediate morphological characters in both (Chua et al. 2010). Results of BLAST analysis showed that the 28S data (JN833636) was identical to *F. arisanus* with values for maximum score, total score, query covery, E-value, and maximum identical being 829, 829, 91%, 0, and 100%, respectively. Surprisingly, there are no data in the Genbank for ND1 sequences for this species, therefore, the ND1 sequence data for *F. arisanus* will be a new submission to the database (JN833637). For the tephritid species (JN833638), the results of BLAST showed that the COI referred to *Bactrocera carambolae* with maximum score, total score, query covery, E-value, and maximum identical being 765, 765, 89%, 0, and 98%, respectively. The ND1 sequence data (JN833639) was also identical to *B. carambolae*, with maximum score, total score, query covery, E-value, and maximum identical values of 810, 810, 95%, 0, and 98%, respectively.

Our data extends the information reported by Chinajariyawong et al. (1999). To date, there has been no record of *F. arisanus* associated with *B. carambolae* infesting *S. samarangense* fruit from Malaysia. However, *F. arisanus* was reported to be infesting *S. aqueum* from Malaysia and *S. malaccense* from Thailand. Furthermore, many braconid parasitoids were reared from several *Bactrocera* spp., but, interestingly, their results indicated that *S. samarangense* was not a preferred host fruit for *F. arisanus* in Malaysia. However, other

tephritid species associated with *F. arisanus* have been found to infest other families of fruits in both countries (Chinajariyawong et al. 1999).

The result of Chinajariyawong et al. (1999) may be biased because it only focussed on the sampling of soft fleshy fruits that are normally *Bactrocera* hosts. However, in our study, only one fruit species, the wax apple (*Syzygium samarangense*), was sampled and our investigation was made only on the parasitoid (*Fopius arisanus*as) of the main pest (*B. carambolae*) of this fruit.

The molecular technique was also pertinent in clarifying and confirming the taxonomic status of the parasitoid species. In this study, we present *F. arisanus* as a new record of a braconid wasp in Malaysia, parasitizing *B. carambolae*, the common fruit fly pest of the wax apple fruit, *S. samarangense*. We have also clarified the taxonomical status of species, *F. arisanus* and *B. carambolae* by using the three molecular markers, 28S, COI and ND1. The molecular verification has helped to overcome the problems of identification based solely on morphological characters. This study has also added the ND1 sequence for *F. arisanus* to the genomic library, which is of significance because braconid species are often used for the biological control of fruit flies, and updated information on their taxonomy, ecology, and behavior are crucial in the effective integrated management of fruit flies in Malaysia and other countries in the Pacific rim.

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