DIVERSITY AND ABUNDANCE OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) AT TUNKU ABDUL RAHMAN NATIONAL PARK, SABAH, MALAYSIA

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A thesis submitted in partial fulfillment of the requirements for the degree of

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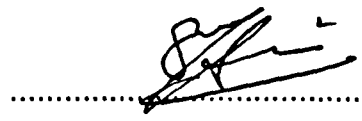

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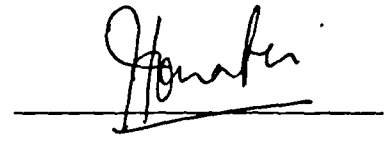

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ABSTRACT

A preliminary study of fruit flies was conducted at five islands within Tunku Abdul Rahman National Park (TARP) from February 2014 to April 2014. The objectives were to investigate the diversity of fruit flies (Diptera: Tephritidae) and to construct a set of pictorial identification key for species found at TARP. Three methods were applied: Bait trapping with parapheromones Methel Eugenol (ME) and Cue lure (CUE), manual collection and the Malaise trap. The bait traps and Malaise trap serviced for three days in each island. A total of 37 fruit fly species was obtained, 33 of them from the genus *Bactrocera* and another four belong to other genera, namely *Trupanae*, *Euphranta*, *Adrama*, and *Dacus*. The most abundance species is the *Bactrocera albistrigata* (50.36%), following by the *B.papayae* (13.89%), and *B.carambolae* (10.71%). The fruit fly diversity of TARP was found high with the highest Shannon Diversity Index (H'=2.19) shown from Gaya Island. From the collection, 20 *Bactrocera* species were assigned to morphospecies due to the lack of identification material. A pictorial dichotomous key of generic level and species level were constructed. This first TARP fruit fly key is important to provide general identification guide of fruit flies at TARP. The key could be further upgraded to the key of fruit fly identification in Borneo as more studies going on.

ABSTRAK

Kajian awal ini telah dilaksanakan di kelima-lima pulau Taman Tunku Abdul Rahman dari bulan Februari 2014 hingga bulan Mac 2014. Tujuan kajian ini adalah untuk mengkaji kepelbagaian lalat buah (Diptera: Tephritidae) dan membina kekunci dikotomi bergambar untuk lalat buah yang berada di Taman Tunku Abdul Rahman. Tiga kaedah pengumpul digunakan iaitu perangkap umpan yang mengandungi parapheromone Methyl Eugenol (ME) dan Cue lure (CUE), pengumpulan secara manual, dan perangkap Malaise. Perangkap umpan dan perangkap Malaise telah dipasang selama tiga hari di setiap pulau. Sebanyak 37 spesis lalat buah telah ditemui, antaranya 33 dari genus *Bactrocera*, empat lain dari genera lain, iaitu *Trupanae*, *Euphranta*, *Adrama*, dan *Dacus*. Spesis yang paling banyak ialah *Bactrocera albistrigata* (50.36%), diikuti oleh *B. papayae* (13.89%), dan *B. carambolae* (10.71%). Diversiti lalat buah Taman Tunku Abdul Rahman didapati tinggi, Pulau Gaya mempunyai indeks diversiti Shannon yang paling tinggi (H'=2.19). Daripada koleksi, 20 spesis *Bactrocera* telah diberikan nama morfospesis kerana kekurangan bahan identifikasi. Kekunci dikotomi bergambar untuk tahap genus dan tahap spesis telah dibinakan. Kekunci yang pertama ini dibina khas untuk lalat buah Taman Tunku Abdul Rahman adalah penting sebagai runjukan untuk proses identifikasi lalat buah yang umum di Taman Tunku Abdul Rahman. Kekunci identifikasi ini boleh dinaik taraf untuk lalat buah yang berada di kepulauan Borneo pada masa hadapan selaras dengan banyak kajian yang akan dijalankan.

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LIST OF ABBREVIATION

ACIAR	Australia Centre for International Agriculture Research
AWPM	Area-wide Pest Management
CUE	Cue-lure
GDP	Gross Domestic Product
i.a.	Intra-alar
ITBC	Institute for Tropical Biology and Conservation
IPM	Integrated Pest Management
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
ME	Methyl Eugenol
MVSP	MultiVariate Statistical Package
TARP	Tunku Abdul Rahman National Park
SIT	Sterile Insect Technique
R	Radian
UMS	University Sabah Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background

Dietary patterns of human have been changed over years, majority nowadays prefer healthy, high natural fibre content meal such as fresh fruits (Bateman, 1989). The demand of fresh fruits rises with the increase of human population. This in turn encourages more production of fresh fruits, especially in third world countries upon the demand from first and second world countries. As fruits are the main nutrient source of many insects, these fruit associated pests hitchhike and distribute themselves worldwide with the growth and globalisation of world trade.

One of the major fruit pests is fruit fly, belonging to the family Tephritidae, which causes fruit producers to lose significant direct economic losses. For instance, the European cherry fruit fly, *Rhagoletis cerasi* (L.) can infest more than 90% of sour cherries in Mediterranean areas (Fimiani, 1989). The oriental fruit fly, *Bactrocera dorsalis* (Hendel) in South-East Asia and Japan, attacks citrus, mango, guava and more than 40 families of tropical and subtropical fruits (Allwood *et al.*, 1999).

In order to prevent entry of exotic fruit fly some countries impose stringent quarantine barriers to the imported fruits (White & Elson, 1992). Producers must invest in expensive post-harvest facilities and treatments such as fumigation to disinfect fruit cargoes before exportation of fresh fruits (Hendrichs, 1996). Their

market range becomes narrower due to closed export markets in fly-free countries. These direct and indirect losses bring financial burden to fruit producers, as well as to the government.

The economic importance of Tephritid is significant and can be generalized with following reasons (Koyama, 1989). Firstly it attacks commercial fruits. Secondly, some species spread far away from native area and establish as major pests in countries which depend economically on fruit production. Thirdly, quarantine restriction and quarantine regulation of fruit fly are expensive and require much professional effort.

Agriculture sector is important to Malaysia as it contributes around 7.7 percentages in Gross Domestic Product (GDP) (Malaysia Economic Report 2012-2013). Many fruits are grown to meet the demand of consumers and thus, it is necessary to know the species diversity and abundance of fruit fly. The information of existence species and diversity is extremely useful in implementing integrated pest management (IPM) on fruit flies.

1.2 Fruit flies of Family Tephritidae

Tephritidae is one of the largest families in the order Diptera. Species of this family usually possess colourful marking and spotted or banded wings often forming attractive and unique wing pattern (Chua, 2010). The size of fruit fly varies and relatively small, with body length from 2mm to over 20mm (Christenson & Foote, 1960). There are about 4,550 described species worldwide from 500 genera (Jackson *et al.*, 2011), about 200 tephritid species are considered as pests. Larvae of tephritids feed on phyto-materials and can be classified into two categories, frugivorous and non-frugivorous. Frugivorous larvae feed on fleshy fruit whereas non-fruginorous feed on plant parts other than fruit. Some non-frugivorous are even seed predators, gall makers, and leaf miners (Christenson & Foote, 1960).

Mature female inserts its ovipositor to deposit eggs on ripening fruit of host plant. Larva hatches and feeds on the fleshy fruit which eventually causes direct damage on fruit. This larval period lasts three to twenty-one days. The fruit is further damaged by penetration of microorganisms and decomposers through the opening holes which may causes early fall of fruit in field (Uchôa, 2012). The third instar or full-grown larva emerges and falls on ground into soil for pupation. Pupal period lasts for six to nine days and the survival depends on soil texture and moisture. The immature fruit fly is vulnerable to different predators such as ants, carabid beetles and spiders (Ansari *et al.*, 2012). This pressure becomes almost none when comes to adult fruit fly but it begins to create "pressure" to sector of agriculture.

1.2 Justifications

Fruit flies (Diptera:Tephritidae) include some of the world's most serious agricultural pests. It has been causing billion losses of dollars for a country such as Japan in the efforts to control it (Koyama,1989). Malaysia is another important agricultural country, especially in Sabah as it contributes to state's economy (Kuncinas, 2013). Thus there is a need to obtain base data for ecological study of fruit flies and the fruit fly diversity in Sabah. It builds up the foundation for further effort in conservation of biodiversity, agriculture management and integrated pest management.

Identification of tephritid is extremely difficult to non-expert because species of different areas are different. There is a need to provide identification materials of fruits flies to non-taxonomists, agriculturists, and non-specialists. As fruit flies in Sabah are not well studied, this study will contribute to appropriate fruit fly information and management.

The large population and expanding distribution of fruit flies plays significant ecological role. There is a need to raise the concern of public in fruit flies as part of conservation biodiversity. Besides, tephritid may possess undiscovered value and scientific research value.

The outcome of this study could provide us a better understanding of the diversity of fruit flies in Borneo, with a complete pictorial identification guide. All of these are not known unless study is carried out.

1.3 Objectives

The objectives of the study were:

a. To examine the diversity and abundance of fruit flies at Tunku Abdul Rahman National Park (TARP), Sabah.

b. To provide general identification guide of fruit flies of TARP by constructing pictorial dichotomous key.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and classification

Order Diptera is classified into two suborders, Nematocera and Bachycera (Triplehorn & Johnson, 2005). Bachycera composed of 10 superfamilies, namely Nerioidea, Diopsoidea, Conopoidea, Tephritoidea, Lauxanioidea, Sciomyzoidea, Opomyzoidea, Carnoidea, Sphaeroidea and Ephydroidea (Yeates *et al.*, 2007). Tephritoidae includes the familes of Lonchaeidae, Piophilidae, Pallopteridae, Richardiidae, Ulidiidae, Platystomatidae, Pyrgotidae, and Tephritidae (Korneyev, 2000a).

Tephritidae are a monophyletic group with hypothesis supported by some characters that appear to be autapomorphies (Korneyev, 2000a). Firstly, the development of frontal plates appears to be prolongation of the parafacialon the frontal surface. Secondly, frontal setae developed on the frontal surface. It is always much longer than surrounding setulae. Thirdly, the costal vein has a deep constriction or a break before the apex of the subcostal vein. Fourth and lastly, two or three costal spines with enlarged and thickened setae guarding such a break that appear to be a synapomorphy of Tephritidae.

The phylogenetic relationships of family Tephritidae was revised by Korneyev (2000a, 2000b) and he concluded 6 subfamilies as shown in Table 2.1 based mainly on morphological evidence.

Table 2.1: Subfamilies and tribes of Tephritidae.

Order/ Sub-order	**Superfamily**	**Family**	**Subfamily**	**Tribe**
Diptera/ Bachycera	Tephritoidea	Tephritidae	Tachiniscinae	Tachiniscini Ortalotrypetini
			Blepharoneurinae	(No tribe)
			Phytalmiinae	Acanthonevrini, Phytalmini Phascini Expacrocerini
			Trypetinae	Xarnutini Hexachaetini Toxotrypanini Rivelliomimini Adramin Carpomyni Nitrariomyiini Trypetini
			Tephritinae	Terelliini Xyphosiini Myopitini Cecidocharini Dithrycini Tephritini Tephrellini Eutretini (including Acrotaeniini) Schistopterini
			Dacinae	Gastrozononini Dacini Ceratitidini

2.2 Morphology of fruit fly

General morphology of fruit fly is shown (Figure 2.1). Three major body regions of fruit fly are head, thorax, and abdomen. Many parts of these three major regions are important identification keys, associating with components of compound eye, antenna, mouthparts, wings, legs, and terminalia (Cumming & Wood, 2009). Approximately 90% of the Dacine pest species can be identified accurately based on morphospecies (Plant Health Australia, 2011).

Head has been divided into few regions. The frons above is the antennae, locating at the between of compound eyes. Ocellar triangle is present on the vertex at top of the head. A spherical of oval head capsule bearing proboscis mouthpart makes up the other region of the head (Cumming & Wood, 2009).

Thorax is fused by the prothorax, mesothorax, and metathorax into a single rectangular capsule. Mosothorax associates with the wings and leg muscles. Prothorax and metathorax support the leg musculature of first and third pairs of legs (Cumming & Wood, 2009). The notum of mesothorax is mesonotum. It occupies most of the dorsal surface of thorax. Four areas dividing mesonotum are the prescutum, scutum, scutellum and postnotum (Cumming & Wood, 2009). The front wings are developed for flight. The venation of wings has high taxonomic value as it varies throughout the family (Cumming & Wood, 2009). All tephritids wings has costal vein broken at two positions bordering second costal cell (Drew, 1989). The hind wings are reduced into small structures called halters which act as organs of equilibrium (Triplehorn & Johnson, 2005).

The abdomen is composed of five terga. Basal segments are referred to preabdomen whereas the remaining complex of modified genital and anal segments is referred as terminalia or postabdomen (Cumming & Wood, 2009). Male abdomen is not elongated and possesses developed surstyli and claspers (Drew, 1989). Female has an elongate abdomen with a developed ovipositor.

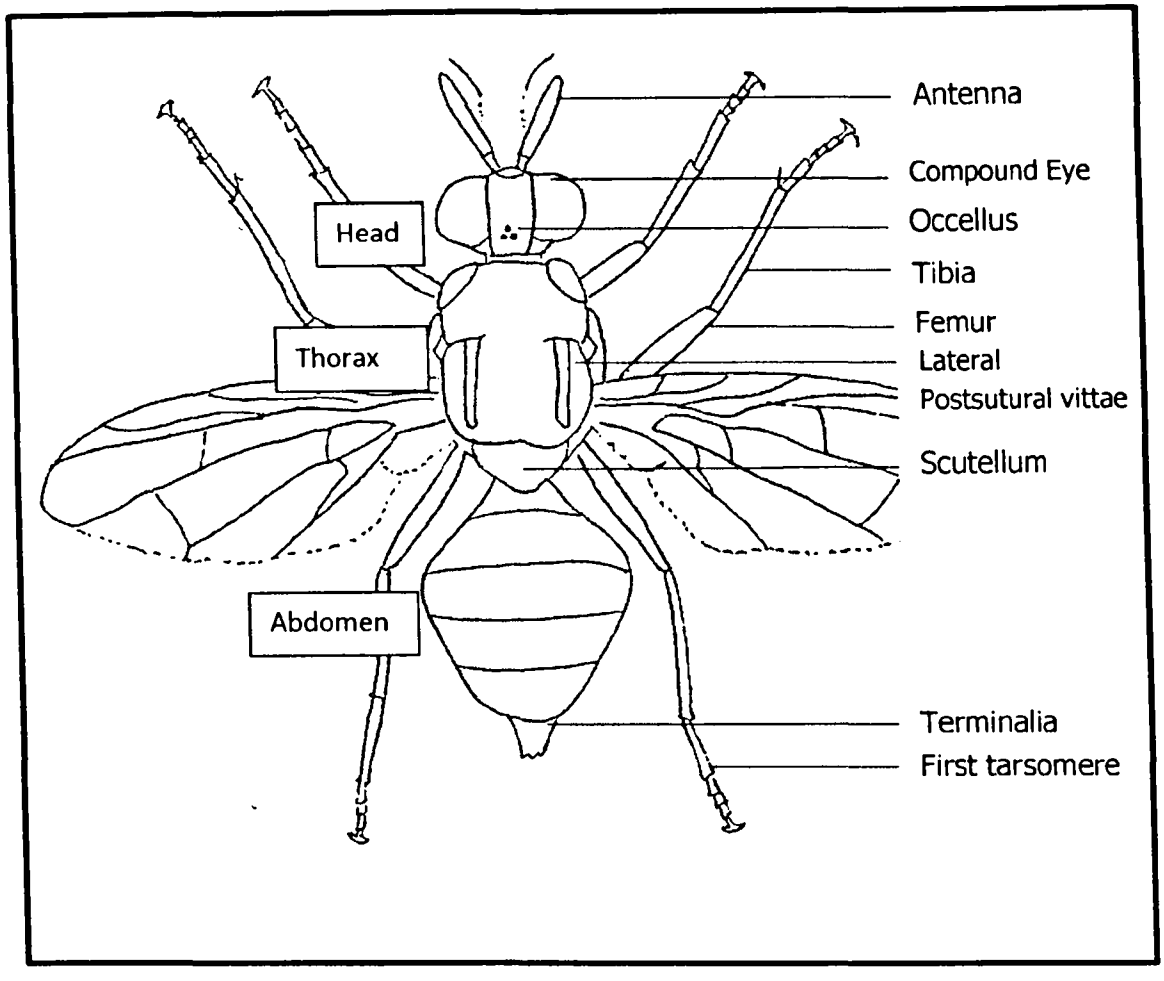

Figure 2.1: Dorsal view of *Bactrocera* fruit fly

2.3 Biology, ecology and behaviour

2.3.1 Life cycle

Relationship between the host plant and fruit fly may determine the duration and timing of various life stage of tephritid. Subfamily Dacinae has short life cycle, approximately 6-8 generations per year from subtropics to topic area (Drew & Romig, 2000). The melon fruit fly, *Bactrocera cucurbitae* has 8-10 generations per year, with life cycle last from 21 to 179 days (Dhillon *et al.*, 2005). Subfamily Trypetinae has longer life cycles, such as the *Rhagoletis* and *Anastrepha* species, take approximately 80 days from egg to adult and 160 days of adult longevity, with average one generation per year (Bauer, 1986; Ansari *et al.*, 2012).

The life cycle of most fruit flies in field is still unknown (Ansari *et al.*, 2012). Most studies are under laboratory conditions. It involves four stages: Egg, larva, pupa, and adult. All life stages take place throughout the year. Egg of fruit fly is typically white in colour and elongate-cylindrical shape, sometimes with long, tail-like extension. Egg stage lasts a few days, depending on species. The larva of *B. cucurbitae* hatches initially as delicate first instar and feeds actively inside the host plant tissue. It moults into more robust second instar and then in turn moults into third instar (Headrick & Goeden, 1998). Some exceptions are *Urophora jaceana* and *U. cardui* which the first instar remains in the egg and moults into second instar (Headrick & Goeden, 1998). The body shape of instar varies. It ranges from vermiform (found in stem-mining species), elongate ellipsoidal shape, to globose (found in gall-makers) (Headrick & Goeden, 1998). When comes to pupa stage, non-frugivore pupates inside the host plant whereas frugivore emerges and leaves the fruit, then it pupates in the soil. In pupation, the skin of larva becomes barrel-shapes, hard and tanned brown puparium. True pupa is living inside the puparium. When it becomes mature, it splitting opens the anterior end of puparium and squeezes out. Female fly does not develop egg and it needs protein feed. Most fruit flies are facultative breeders that only lay eggs when host plants are available.

2.3.2 Feeding behaviour

Vast majority of tephritids are phytophagous (frugivourous or nonfrugivourous), except Tachniscinae are parasitoids (zoophagous) and Phytalmiinae are

saprophagous (Korveyev, 2000a; Díaz-Fleischer *et al.*, 2000). Feeding behaviour of adult and larva are different. Tephritid larva spends most of the time feeding on fleshy tissue of host plant. Only few studies were done on feeding behaviour of larva. It is known that hydrolyzed protein in form of brewer's yeast was added to larva culture media (Drew & Yuval, 2000). Not all larvae are frugivores. Frugivorous larva (Tephritinae) has a pair of mouth hooks and a well-developed median oral lobe. Function of median oral lobe is to uptake fluid exuding from plant tissues that have been scraped by mouth hooks.

Feeding strategy is important to physiological processes of adult such as sexual maturation, courtship and mating. Adult forages for reproductive resource that cannot be acquired during the larval stage. *Bactrocera cucurbitae*, *B. dorsalis*, and *Ceratitis capitata* require carbohydrate, protein, minerals, B-complex vitamins and water for both reproduction and longevity (Díaz-Fleischer *et al.*, 2000).

2.3.3 Reproductive and mating behaviour

Various mating strategies are found in different species. Courtship interaction are complicated, often involve combinations of chemical, visual, auditory signaling system, and competitive, territorial and agonistic interactions with conspecific flies (Landolt & Quilici, 1996). Many Dacinae exhibit characteristics rendezvous behaviour, utilising the host-plants as specific waiting places and territories (Drew & Romig, 2000).

Host plant plays vital role as mating site for fruit flies. Male establishes position on host plants, scanning for arriving of conspecific. If a female is spotted male walked along the leaves and stems to intercept her. Meanwhile only virgin female would wave wings to attract male while mated female may resist male advances (Sivinski & Burk, 1989). Only 65% of copulation was found successful (Sivinski & Burk, 1989), where the male gained a 'leglock' on female using enlarged fore femora. Another interesting behaviour is lekking behaviour. Leks usually occur in host plants but away from host fruits (Sivinski & Burk, 1989). This was shown by laboratory study of tropical Dacines (Koyama, 1989). All males aggregate into loose

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