รายงานการวิจัย

การศึกษาอนุกรมวิธานและการวิเคราะห์สัณฐานของแมลงวันทอง
(*Bactrocera*: Tephritidae)

(Taxonomic and morphometric studies of fruit flies (*Bactrocera*: Tephritidae))

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ผลงานวิจัยเป็นความรับผิดชอบของหัวหน้าโครงการวิจัยแต่เพียงผู้เดียว

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คณะผู้วิจัย

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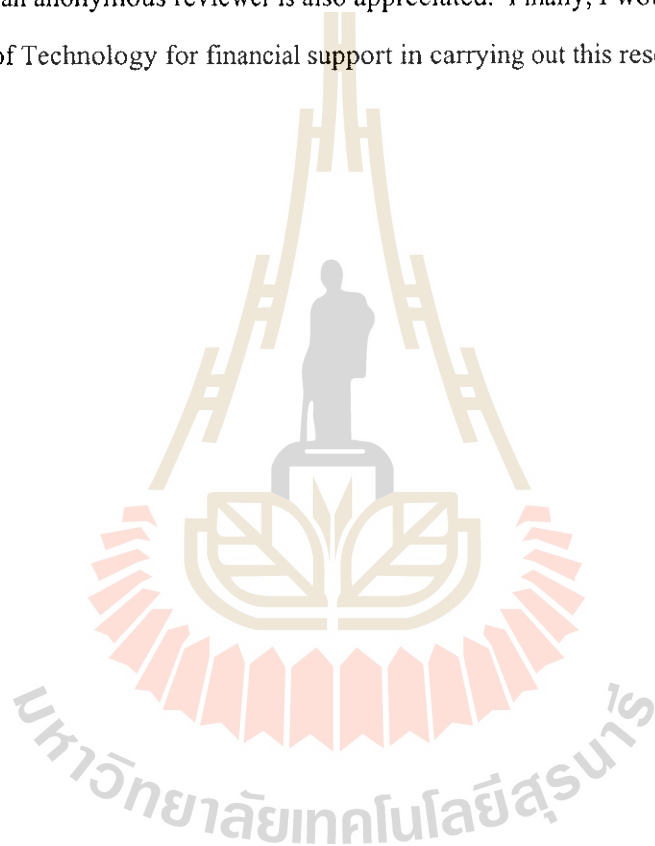
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มหาวิทยาลัยเทคโนโลยีสุรนารี

Acknowledgements

Abstract

Fruit flies of the genus *Bactrocera* (Tephitidae) are major agricultural pests in Thailand and other countries in Asia, Australia, and the Pacific region because of the destruction of fruits and flowers by the larvae of these flies. Many of the species of these flies are morphologically similar and difficult to separate. In this research project, attempts were made to use morphometric analysis of wings and scanning electron microscopic studies of male and female genitalia to separate selected species occurring in Thailand. Morphometric analysis was performed using 13 wing measurements (lengths of veins or distances between veins) on *Bactrocera dorsalis* and *B. carambolae* by means of discriminant function analysis. For females, 28 of 29 individuals (96.6%) could be separated, as determined by cross-validation using 3 selected wing measurements. For males, 20 of 26 individuals (76.9%) could be separated, as determined by cross-validation using 2 wing measurements. SEM of male genitalia from 19 species allowed separation of individuals between 2 subgenera, but not clear separation of flies within a subgenus. SEM of females from 14 species allowed separation of most species by characters of the ovipositor. Morphometric analysis using additional characters of flies are necessary for more accurate identification, especially for male flies. Additionally, studies are necessary of immature stages of the life cycle, including larvae.

บทคัดย่อ

แมลงวันทองสกุล *Bactrocera* (Tephritidae) เป็นแมลงที่เป็นปันหาใหญ่ปันหาหนึ่งด้าน
การเกษตรในประเทศไทย และประเทศอื่น ๆ ใน เอเชีย ออสเตรเลีย และภูมิภาคแถบแปซิฟิค โดย
แมลงวันทองจะวางไข่บนผลไม้ของพืช ซึ่งไข่ของแมลงวันทองเจริญเติบโต เป็นตัวหนอนทำความ
เสียหายให้กับพืชผลนั้น ๆ มีแมลงวันทองหลายชนิดซึ่งมีรูปร่างลักษณะที่คล้ายกันมากยากต่อการ
จำแนกชนิด งานวิจัยนี้เป็นการวิจัยเพื่อวินิจฉัยและจำแนกชนิดของแมลงวันทอง ซึ่งเลือกจากใน
ประเทศไทยโดยวิเคราะห์ทางสัณฐานวิทยาของปีกรวมทั้งการศึกษาอวัยวะเพศของเพศผู้และเพศเมีย
โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

จากเทคนิควิธี discriminant function analysis โดยวิเคราะห์จากเส้นปีกและระยะห่างระหว่าง
เส้นปีกจำนวน 13 เส้นของแมลงวันทอง 2 ชนิด คือ *Bactrocera dorsalis* และ *B. carambolae* สำหรับ
เทคนิควิธี cross-validation จากการจำแนกชนิดแมลงวันทองเพศเมียสามารถจำแนกชนิดได้ 28 ตัว
จาก 29 ตัว คิดเป็นร้อยละ 96.6 โดยการวัดเส้นปีก 3 เส้น ส่วนเพศผู้สามารถจำแนกชนิดได้ 20 ตัว
จาก 26 ตัว คิดเป็นร้อยละ 76.9 โดยการวัดเส้นปีก 2 เส้น สำหรับการศึกษาโดยใช้กล้องจุลทรรศน์
อิเล็กตรอนแบบส่องกราดศึกษาอวัยวะเพศผู้จาก 19 ชนิด สามารถจำแนกได้เพียง 2 สกุลย่อย
(subgenera) แต่ไม่สามารถจำแนกได้ชัดเจนว่าเป็นชนิดใดในสกุลย่อยนั้นๆ ส่วนเพศเมียโดยดูจาก
ลักษณะตอนปลายของอวัยวะวางไข่ (aculeus) จาก 14 ชนิด สามารถจำแนกได้เกือบทั้งหมด การ
วิเคราะห์ทางสัณฐานวิทยาโดยใช้ลักษณะของอวัยวะส่วนอื่นและ จำแนกตัวอย่างที่มากขึ้นในอนาคต
อาจช่วยให้สามารถจำแนกชนิดได้อย่างแน่นอนเพิ่มขึ้น การใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่อง
กราด เพื่อศึกษาลักษณะเพิ่มเติมของแมลงวันทองเป็นสิ่งที่จำเป็นในการช่วยวินิจฉัยชนิดได้ถูกต้อง
โดยเฉพาะเพศผู้ การศึกษาเพิ่มเติมที่ควรทำคือศึกษาวงจรชีวิต และระยะตัวอ่อน เป็นต้น

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Chapter I

Introduction

Fruit flies of the genus *Bactrocera* Macquart (Tephritidae) are major pests of fruits and vegetables in Thailand and other countries in Asia, Australia, and Pacific regions because of the destruction of these plant parts by the larvae (Drew, 1989; Drew and Hancock, 1994; Baimai et al., 1995).

An early study by Hardy (1973) described 43 species of *Bactrocera* in Thailand and surrounding countries, giving host fruit information where known. In a more recent study based on more extensive collecting, Drew and Hancock (1994) described 52 species in the *Bactrocera* (*Bactrocera*) *dorsalis* complex alone from Asia, forty of these being newly described species. Of these 52 species, 14 were collected from Thailand. Additionally, in a three-year study by Baimai et al. (1996), fruit flies were collected extensively throughout Thailand, resulting in collection of at least 50 species of *Bactrocera*, several of which are probably undescribed species. Many of the species could be placed in the *B. dorsalis* group of sibling and morphologically similar species, and studies of mitotic karyotypes (Baimai et al., 1995; Phinchongsakuldit, 1998) and isozyme electrophoresis (Satayalai, 1995; P.J. Grote, unpublished data) confirmed that the flies comprised many reproductively isolated species. Additional species collected as part of the above-mentioned three-year project and later by S. Tigvattananont were similar morphologically to *B.* (*Zeugodacus*) *tau*. Isozyme electrophoresis and studies of the mitotic karyotype of these flies show that they make up a complex of reproductively isolated sibling species (Saelee, 1999).

Some species, such as *B.* (*B.*) *dorsalis* and *B.* (*B.*) *carambolae*, have very broad host ranges, with larvae being found in fruits of many species belonging to many families. Other species, such as *B.* (*B.*) *kanchanaburi* and *B.* (*B.*) *verbascifoliae*, have very restricted host ranges, with larvae of the former species only found in fruits of *Artabotrys* (Annonaceae) and larvae of the latter occurring in fruits of *Solanum erianthum* and possibly other species of *Solanum* (Solanaceae) (Baimai et al., 1995).

Because of the economic importance of flies of the genus *Bactrocera*, it is essential that knowledge of the taxonomy and the biology of the flies be obtained. Control techniques, such as the sterile insect technique, in which males are reared in the laboratory, sterilized by exposure to gamma radiation, and released to mate with wild females, require that the exact species being

reared is known and that the geographic range of the wild females is likewise correctly known. Also, species of flies brought into the country as exotic species need to be identified, an essential duty of the quarantine office.

As early as 1954, Hardy and Adachi mentioned the difficulty in distinguishing *Bactrocera dorsalis* from related species. Likewise, much difficulty has been encountered in identifying individual flies to the species determined by mitotic chromosomes or isozyme electrophoresis (P.J. Grote, unpublished data). Often, female adults could be distinguished by detailed structures of the ovipositor, whereas a high percentage of the males could not be separated into species when looking at external morphology. Drew and Hancock (1994) provide a key to separate the 52 species of the *B. (B.) dorsalis* complex in Asia, much of it based on external color markings. However, I found that not all of the flies I observed could be identified using the key because of intra-specific variation not accommodated by the key. For example, *B. (B.) dorsalis* keys out as having entirely fulvous femora. Most individuals show this character, but some specimens have apical dark markings on the fore femora. Likewise, the narrowness or broadness of the costal streak, a band on the leading edge of the wing, is used to separate species in the key. However, I have found this character to be somewhat variable within species, such as *B. (B.) dorsalis* and *B. (B.) carambolae*.

Hardy (1979) described the known species of fruit flies in Thailand, and Drew and Hancock provided an updated description of flies of the *B. (B.) dorsalis* complex in Asia, including Thailand. Scanning electron microscopic studies of ovipositors of *Bactrocera* female adults are presented in Drew and Hancock (1994) for some of their 52 species of fruits flies. SEM is also used in the studies of *Bactrocera* species in Drew and Hardy (1981), Drew and Lambert (1986), and Drew (1981) for flies in Australasia and the Pacific. The results of these researchers show that many morphologically similar species can be separated by detailed morphology of the ovipositor in females. Useful characters are the length of the aculeus, or piercer, the length and number of subapical setae on the aculeus, and the shape of denticles, or spicules, which occur on the eversible membrane of the ovipositor, especially the denticles on the distal part of this membrane. Drew and Hancock (1994) provide descriptions of male genitalia (the surstylus) for some species of *Bactrocera*, and Hardy (1973) also describes the male genitalia of some species of *Bactrocera* (which he includes in the genus *Dacus*), accompanied by line drawings. Drew and Hancock (2000) describe how four groups of subgenera, the *Queenslandacus*, *Bactrocera*, *Zeugodacus*, and

Melanodacus groups, each has a distinctive combination of shape of abdominal sternum V, the number of scutellar bristles, and the length of the posterior lobe of the lateral surstylus.

Morphometric analysis using discriminant function analysis has often been used in the study of morphologically similar species. The analysis searches for the most suitable characters that can be used to separate two or more species. For example, ten linear measurements were made of female adult tree hoppers (Homoptera: Membracidae) from the Neotropics in an attempt to discriminate among sibling species (Dietrich et al., 1991). In a first group of two genera, 98.11% of the individuals could be correctly assigned to species using three measurements. In a second group of 4 taxa, 94.94% of individuals could be assigned to the correct taxon using ten measurements. In another study (McNamee and Dytham, 1993), nine linear measurements were made of adult fruit flies of the sibling species *Drosophila melanogaster* and *D. simulans* (Diptera: Drosophilidae). By canonical variates analysis (basically the same as discriminant function analysis), all individuals could be correctly assigned to sex and to species. In a study of parasitic wasps (Hymenoptera: Ichneumonidae), 100% of 50 adults comprising five species of *Itoplectis* from Canada could be assigned to the correct species using 49 wing measurements (Yu et al., 1992).

In this research project, morphological features of male and female adult flies of selected species of the genus *Bactrocera* will be studied, including probably undescribed species. Scanning electron microscopy will allow a detailed investigation of the ovipositor of females and genitalia of males, to determine the usefulness of these features in distinguishing species. The results will be compared with studies of the same species using cytotaxonomy (Baimai et al., 1995; Saelee, 1999) and isozyme electrophoresis (Satayalai, 1995; Phinchongsakuldit, 1998; P.J. Grote, unpublished data).

In addition, morphometric analysis (discriminant function analysis) will be carried out using wing characters of two sibling species of *Bactrocera*, *B. (B.) dorsalis* and *B. (B.) carambolae*.

The expected benefits of this study are that morphologically similar species including economic pest species, can be more accurately and easily identified to species. The results may also prove useful in analyzing the evolutionary relationships and biology of these flies.

Chapter 2

Materials and methods

1. Morphometric analysis

Discriminant function analysis is a type of multivariate analysis used to increase the discrimination between groups. Group identity and membership of the individuals must be known *a priori*. Discrimination is obtained by transforming the variables to maximize the between-group variation while minimizing the within-group variation. When two groups are analyzed, the result is a discriminant function in the form of a linear equation derived from the original variables:

$$F_1 = a_1x_1 + a_2x_2 + a_3x_3 + ... + a_nx_n$$

$x_1, x_2, x_3, ... , x_n$ are the original variables, and $a_1, a_2, a_3, ..., a_x$ are "weighing" coefficients. An F_1 value is obtained for each individual; the individuals can then be separated into groups by the F_1 value (Lestrel, 2000).

    1.1   Preparation of specimens: The flies used for morphometric analysis were collected as larvae from Thai guava (*Psidium guajava* L.) fruits from one locale in Sadao District, Songkhla Province, on 18 June 1994 by S. Tigvattananont and assistants as part of the research project, "Population genetics and sexual behavior in the management of *Dacus* species of fruit flies in Thailand", headed by Visut Baimai and supported by the Thailand Research fund (Baimai et al., 1996). The adults, after rearing, were identified as two sympatric species, *Bactrocera dorsalis* and *B. carambolae*, based on external morphology. Many but not all of the flies could be identified to species. Adults of *B. dorsalis* generally show a more narrow streak near the leading margin of the wing and lack a marking on the fore femur. Adults of *B. carambolae* usually show a broader streak on the wing and a spot on the fore femur. However, some of the flies showed intermediate characteristics and could thus not be identified to either one or the other species. The morphometric discriminant analysis was thus an attempt to separate the two species by analytical means.

Flies to be used for morphometric analysis were killed by placing them into liquid nitrogen. They were then removed from liquid nitrogen, and both wings, and the posterior half of

the abdomen if the fly was female, were removed with a razor blade. The wings, and part of the abdomen for females, were stored at room temperature while the rest of the body was returned to liquid nitrogen for later use in electrophoresis. The abdomens were allowed to dry and were later used to prepare slides of the ovipositor.

1.2 Electrophoresis: Because group membership is necessary *a priori* in discriminant analysis, isozyme electrophoresis was carried out in an attempt to separate individuals of the two species, *B. dorsalis* and *B. carambolae*. Appoximately 100 flies, both male and female, of the two species were used in polyacrylamide isozyme electrophoresis after removal of the wings, and abdomen of females.

Flies were removed from liquid nitrogen, and an extract from each fly was used for isozyme electrophoresis, using polyacrylamide gels and separating the following isozymes; aspartate aminotransferase (AAT), alcohol dehydrogenase 1 (ADH1), alcohol dehydrogenase 2 (ADH2), alcohol dehydrogenase 3 (ADH3), glucose phosphate isomerase 1 (GPI1), glucose phosphate isomerase 2 (GPI2), malic enzyme 1 (ME1), malic enzyme 2 (ME2), isocitrate dehydrogenase (IDH), and lactate dehydrogenase (LDH) (Satayalai, 1995; P.J. Grote, unpublished data).

1.3 Measurement of wing vein lengths: For each fly, both wings were placed on a microscope slide and covered with a cover slip held in place with a drop of Canada balsam at each corner. In the case of females, the extended ovipositor was placed on the same slide with the wings, by using Canada balsam and a cover slip (see below for preparation of the ovipositor). Wings mounted on microscope slides were measured using a stereomicroscope and a micrometer placed directly over the wings. Fourteen vein lengths were measured as shown in Fig. 1.
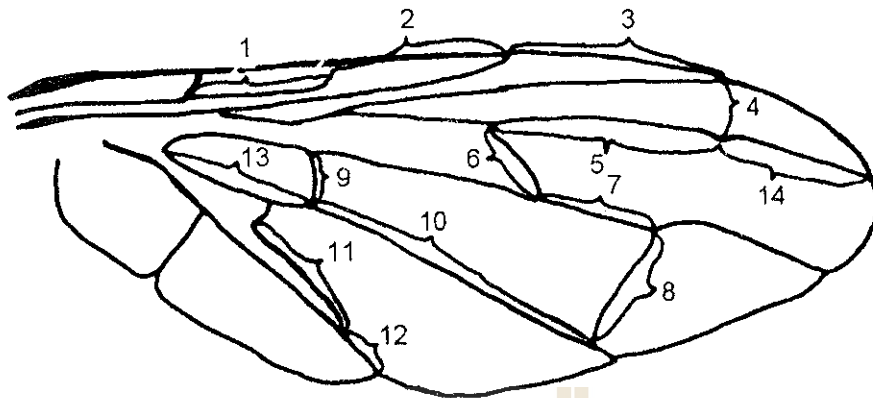
Figure 1. Right wing of fruit fly, showing position of 14 measurements

1.4 Discriminant function analysis: The wing characteristics (vein lengths or distances between veins) were used for discriminant analysis to separate two species of flies, *Bactrocera dorsalis* and *B. carambolae*, using SPSS version 9.

2. Use of male and female genitalia in taxonomy

2.1 Specimens: Adult flies were obtained from the laboratory of Dr. Visut Baimai, Department of Biology, Faculty of Science, Mahidol University, Bangkok. The flies had been collected as larvae infesting various species of fruits and flowers and reared to adulthood by S. Tigvattananont, King Mongkut's Institute of Technology Lad Krabang. The adults were then reared at Mahidol University. The flies had been collected from throughout Thailand as part of the research project, "Population genetics and sexual behavior in the management of *Dacus* species of fruit flies in Thailand", headed by Visut Baimai and supported by the Thailand Research Fund (Baimai et al., 1996). Additional flies were later collected by S. Tigvattananont and were kept at the Department of Biology, Mahidol University, and were the subjects of research of graduate students (Phinchongsakuldit, 1998). Some of the remaining flies were used for this study.

2.2 Male and female genitalia preparation for slides and SEM: Preparation of female and male genitalia of fruit flies was modified from the techniques in Ibrahim and Ibrahim (1990), as follows:

2.2.1 Male genitalia: The abdomen was removed by razor blade from a dried fly, first placed in distilled H_2O, then placed in boiling 10% NaOH for approximately 7-10 minutes, and left to soak in 10% NaOH at room temperature. Next the abdomen was placed into distilled H_2O and cleaned out in a Petri dish; the genitalia, tergum, and sternites were saved, and other internal matter discarded. The saved material was soaked in dilute acetic acid and washed three time in distilled H_2O. The tergum was allowed to air dry, the sternites were placed into 95% ethanol, and the genitalia placed into 1% merbromin solution for more than 10 minutes. The genitalia were next placed into absolute alcohol for 2 or more minutes, then in clove oil for more than 15 minutes. The genitalia were brought through 3 or 4 washes of absolute alcohol, then stored in absolute alcohol.

2.2.2 Ovipositors: The abdomen and ovipositor were placed in boiling 10% NaOH for 5-10 minutes. They were then washed at least twice in distilled H_2O. The ovipositor was then placed in a saturated phenol solution for 20 minutes, then washed three times in H_2O. Next, the ovipositor was transferred to two washes of absolute alcohol.

2.2.3 Preparation of slides: The specimen in absolute alcohol was transferred to 50% absolute alcohol: 50% xylene, then to 100% xylene, before placing into a drop of Canada balsam on a microscope slide, positioning with a metal wire, and covering with a cover slip.

2.2.4 Preparation of specimens for SEM: The specimen in absolute alcohol was dried by critical point drying, then coated with gold using a JEOL JFC-1100E Fine Coat Ion Sputtering Device for 4 minutes. SEM was done using a JEOL JSM-6400 Scanning Microscope.

2.3 Scanning electron microscopy: Male genitalia and the aculei of female flies were observed at 5, 15, or 20 kV. Photographs were taken with 120 Verichrome black and white film.

Chapter 3

Results

1. Morphometric analysis

1.1 Preparation of specimens: Approximately 100 flies collected from Thai guava from Sadao District, Songkhla Province, were used in isozyme electrophoresis. Of these flies, wings of 37 *B. dorsalis* adults (19 females, 18 males) and 18 *B. carambolae* adults (10 females, 8 males) were measured for morphometric analysis.

1.2 Electrophoresis: Separation was done for the following isozymes: AAT, ADH1, ADH2, ADH3, GPI1, GPI2, ME1, ME2, IDH, and LDH (O. Satayalai, unpublished data; P.J. Grote, unpublished data). The two species were best separated by using the isocitrate dehydrogenase (IDH) results (P.J. Grote, unpublished results). Most *Bactrocera dorsalis* individuals were homozygous for the slow allele, and most *B. carambolae* were homozygous for the fast allele. Out of 100 individuals, only one was possibly heterozygous for the slow and fast allele of IDH, though the bands were unclear with this result being inconclusive. Thus, there appeared to be very little, if any, gene flow between the two sympatric species. The electrophoretic bands were scored and the individual flies were assigned to either *B. dorsalis* or *B. carambolae*. Bands that were not clear were disregarded, and the flies from which these unclear bands were obtained were not used in the discriminant analysis.

1.3 Measurement of wing vein lengths: Wings of the flies were used for morphometric analysis because they were easily preserved and easier to measure than other parts of the fly. Measurements were made on the right wing of each fly by placing a micrometer directly on top of the coverslip holding the wing and observing with a stereomicroscope. The micrometer had lines at intervals of 0.1 mm, and the distances on the wings were measured directly, estimating to the nearest 0.01 mm. Measurements were made on the right wing of the following number of flies: *B. dorsalis*, 19 females, 18 males; *B. carambolae*, 10 females, 8 males. Measurement no. 14 (fig. 1) was not used in the analysis because some of the wings were broken at the tip and this measurement could not be made.

1.4 Discriminant function analysis: Because male and female adults of *Bactrocera* can be easily separated from each other (the female has a large ovipositor near the tip of the abdomen), and because even individual wings can be identified as to male or female (vein A_1 + CuA_2, measurement 12 in fig. 1, is much shorter in females, with a notch in the wing margin near the terminus of this vein), discriminant analysis was performed separately on the females of the two species and on the males of the two species.

1.4.1 Female flies: Discriminant analysis and other analyses were performed using SPSS version 9. Thirteen wing measurements of 29 female flies (19 *B. dorsalis*, 10 *B. carambolae*) were used in discriminant analysis, using equal prior probability of both groups and within-groups covariance matrix. The following unstandardized discriminant function coefficients were obtained (table 1).

Canonical Discriminant Function Coefficients for Female *Bactrocera* Flies			
	Function 1		Function 1
WING1	32.057	WING8	-3.515
WING2	1.221	WING9	3.552
WING3	0.757	WING10	8.754
WING4	-10.598	WING11	7.499
WING5	-7.286	WING12	11.74
WING6	-39.411	WING13	-4.942
WING7	10.685	(Constant)	-34.610
Unstandardized coefficients			

Table 1. Canonical discriminant function coefficients for female *Bactrocera* flies based on 13 wing measurements

All 29 individuals were correctly classified to species (100% classification accuracy). Cross-validation, or jackknife validation, was also performed. In this technique, one sample is removed, the rest of the samples are used to derive the classification function, then the sample is classified. This procedure is repeated for each of the samples. The results of the cross-validation were that three individuals were misclassified giving an accuracy of 89.7%; in each of these three cases, *B. dorsalis* was predicted to be *B. carambolae*.

The results of classification are summarized below (table 2):

Classification Results (13 Variables)					
		Predicted Group Membership		Total	Accuracy (%)
	Species	*B. dorsalis*	*B. carambolae*		
Original Count:	*B. dorsalis*	19	0	19	100
	B. carambolae	0	10	10	100
%	*B. dorsalis*	100.0	0.0	100.0	100
	B. carambolae	0.0	100.0	100.0	
Cross-validated[a] Count:	*B. dorsalis*	16	3	19	84.2
	B. carambolae	0	10	10	100
%	*B. dorsalis*	84.2	15.8	100.0	89.7
	B. carambolae	0.0	100.0	100.0	

[a]Cross-validation is done only for those cases used in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

Table 2. Classification results of 2 *Bactrocera* species based on 13 wing measurements of females

Next, stepwise selection of variables was performed to choose an optimal subset of variables. Some variable may be weak discriminators or may be highly correlated with equally powerful discriminators and can be removed. Removal of variables in cases like this can actually increase the percent of correct classifications. The variables were selected one-by-one using the Wilks' Lambda statistics. The F value for entry and for removal were 3.84 and 2.71, respectively. By this technique, only three variables were selected, wing measurements 6, 1, and 10 (table 3).

Variables Entered/Removed[a,b,c,d]									
Wilks' Lambda									
							Exact F		
Step	Entered	Statistic	df1	df2	df3	Statistic	df1	df2	Sig.
1	WING6	0.586	1	1	27.000	19.085	1	27.000	0.000
2	WING1	0.345	2	1	27.000	24.724	2	26.000	0.000
3	WING10	0.208	3	1	27.000	31.738	3	25.000	0.000

At each step, the variable minimizing the overall Wilks' Lambda is entered.

[a]Maximum number of steps is 26.

[b]Minimum partial F to enter is 3.84

[c]Maximum partial F to remove is 2.71

[d]Insufficient F level, tolerance, or VIN for further computation

Table 3. Entering and removal of variables from the original 13 wing measurements of *Bactrocera* females

The following unstandardized discriminant function coefficients were obtained (table 4):

Canonical Discriminant Function Coefficients for Female *Bactocera* Flies	
	Function 1
WING1	26.547
WING6	-40.032
WING10	13.852
(Constant)	-33.078
Unstandardized coefficients	

Table 4. Canonical discriminant function coefficients for female *Bactrocera* flies based on 3 wing measurements

Using three selected variables, the following classification results were obtained (table 5):

Classification Results (Three Variables)					
		Predicted Group Membership		Total	Accuracy (%)
	Species	B. dorsalis	B. carambolae		
Original Count:	B. dorsalis	19	0	19	100
	B. carambolae	0	10	10	100
%	B. dorsalis	100.0	0.0	100.0	100
	B. carambolae	0.0	100.0	100.0	
Cross-validated[a] Count:	B. dorsalis	18	1	19	94.7
	B. carambolae	0	10	10	100
%	B. dorsalis	94.7	5.3	100.0	96.6
	B. carambolae	0.0	100.0	100.0	

[a]Cross-validation is done only for those cases used in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

Table 5. Classification results of 2 *Bactrocera* species based on 3 wing measurements of females

In cross-validation, only one fly was misclassified, *B. dorsalis* classified as *B. carambolae*, for an accuracy of 96.6%.

1.4.2 Male flies: Next, 13 wing measurements of 26 male flies (18 *B. dorsalis* and 8 *B. carambolae*) were used in discriminant analysis, using equal prior probability of both groups and within-groups covariance matrix. The following unstandardized discriminant function coefficients were obtained (table 6):

Canonical Discriminant Function Coefficients for Male *Bactrocera* Flies			
	Function 1		Function 1
WING1	-60.502	WING8	-53.639
WING2	-12.280	WING9	27.400
WING3	-21.579	WING10	14.674
WING4	35.398	WING11	8.497
WING5	18.326	WING12	31.527
WING6	31.494	WING13	7.329
WING7	-5.944	(Constant)	20.535
Unstandardized coefficients			

Table 6. Canonical discriminant function coefficients for male *Bactrocera* flies based on 13 wing measurements

The results of classification are as follows, using all 13 wing measurements (table 7):

Classification Results (13 Variables)					
		Predicted Group Membership		Total	Accuracy (%)
	Species	*B. dorsalis*	*B. carambolae*		
Original Count:	*B. dorsalis*	18	0	18	100
	B. carambolae	0	8	8	100
%	*B. dorsalis*	100.0	0.0	100.0	100
	B. carambolae	0.0	100.0	100.0	
Cross-validated[a] Count:	*B. dorsalis*	15	3	18	83.3
	B. carambolae	2	6	8	75.0
%	*B. dorsalis*	83.3	16.7	100.0	80.8
	B. carambolae	25.0	75.0	100.0	

[a]Cross-validation is done only for those cases used in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

Table 7. Classification results of 2 *Bactrocera* species based on 13 wing measurements of males

As can be seen, in cross-validation three *B. dorsalis* flies were incorrectly classified as *B. carambolae*, and two *B. carambolae* flies were misclassified as *B. dorsalis.*

Next, stepwise selection of variables was performed using the Wilks' Lambda statistics. The F values for entry and for removal were 3.84 and 2.71, respectively. Of the 13 original variables, only two were selected, wing7 and wing12 (table 8).

Variables Entered/Removed[a,b,c,d]									
Wilks' Lambda									
							Exact F		
Step	Entered	Statistic	df1	df2	df3	Statistic	df1	df2	Sig.
1	WING7	0.658	1	1	24.000	12.498	1	24.000	0.002
2	WING12	0.536	2	1	24.000	9.936	2	23.000	0.001

At each step, the variable minimizing the overall Wilks' Lambda is entered.

[a]Maximum number of steps is 26.

[b]Minimum partial F to enter is 3.84

[c]Maximum partial F to remove is 2.71

[d]Insufficient F level, tolerance, or VIN for further computation

Table 8. Entering and removal of variables from the original 13 wing measurements of *Bactrocera* males

Discriminant analysis was performed on the male flies using the two variables, wing7 and wing 12, giving the following unstandardized discriminant function coefficients (table 9):

Canonical Discriminant Function Coefficients for Male *Bactrocera* Flies	
	Function 1
WING7	19.398
WING12	-20.333
(Constant)	-8.233
Unstandardized coefficients	

Table 9. Canonical discriminant function coefficients for male *Bactrocera* flies based on 2 wing measurements

The classification results are listed below (table 10):

Classification Results (Two Variables)					
		Predicted Group Membership		Total	Accuracy (%)
	Species	B. dorsalis	B. carambolae		
Original Count:	B. dorsalis	14	4	18	77.8
	B. carambolae	2	6	8	7.5
%	B. dorsalis	77.8	22.2	100.0	76.9
	B. carambolae	25.0	75.0	100.0	
Cross-validated[a] Count:	B. dorsalis	14	4	18	77.8
	B. carambolae	2	6	8	75.0
%	B. dorsalis	77.8	22.2	100.0	76.9
	B. carambolae	25.0	75.0	100.0	

[a]Cross-validation is done only for those cases used in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

Table 10. Classification results of 2 *Bactrocera* species based on 2 wing measurements of males

Classification using two variables was less successful than when using all 13 variables.

2. Use of male and male genitalia in taxonomy

2.1 Specimens: Male genitalia and female ovipositors were prepared for SEM observation from the following species (table 11):

Adult Flies of *Bactrocera* Used for Study of Genitalia and Ovipositors		
Species	Male genitalia	Ovipositor
Bactrocera (B.) carambolae Drew & Hancock	X	
B. (B.) correcta (Bezzi)	X	X
B. (B.) dorsalis (Hendel)	X	
B. sp. nr. *B. (B.) dorsalis* sp. C	X	
B. sp. nr. *B. (B.) dorsalis* sp. E	X	
B. sp. nr. *B. (B.) dorsalis* sp. I	X	X
B. sp. nr. *B. (B.) dorsalis* sp. J	X	X
B. sp. nr. *B. (B.) dorsalis* sp. K	X	X
B. sp. nr. *B. (B.) dorsalis* sp. L	X	X
B. sp. nr. *B. (B.) dorsalis* sp. M	X	
B. sp. nr. *B. (B.) dorsalis* sp. O	X	X
B. sp. nr. *B. (B.) dorsalis* sp. P		X
B. sp. nr. *B. (B.) dorsalis* sp. S	X	X
B. sp. nr. *B. (B.) dorsalis* sp. U		X
B. sp. nr. *B. (B.) dorsalis* sp. V		X
B. (B.) kanchanaburi Drew & Hancock	X	
B. (B.) irvingiae Drew & Hancock		
B. (B.) melastomatos Drew & Hancock	X	
B. (B.) propinqua (Hardy & Adaschi)	X	
B. (B.) pyrifoliae Drew & Hancock	X	X
B. (B.) verbascifoliae Drew & Hancock	X	
B. (Zeugodacus) diaphoropsis (Hering)		X
B. (Z.) tau Walker	X	
B. sp. nr. *B. (Z.) tau* sp. G		X
B. sp. nr. *B. (Z.) tau* sp. J		X
B. (Z.) sp. K	X	

Table 11. Adult male and female flies of *Bactrocera* used for study of genitalia and ovipositors

2 2 Male and female genitalia preparation for slides and SEM: Selected specimens were prepared as mounts on a microscope slide and observed with a compound microscope.

Male genitalia of 19 species and ovipositors of 14 species of *Bactrocera* were observed and photographed using a scanning electron microscope.

2.3 Scanning electron microscopy

2.3.1 Male genitalia: Genitalia of male flies of 19 species of *Bactrocera* were observed and photographed with a scanning electron microscope. The lateral surstylus and at least part of the epandrium were photographed for each species (figure 2). The distiphallus and glans were photographed for several specimens but not all because of the difficulty in preparing for SEM.
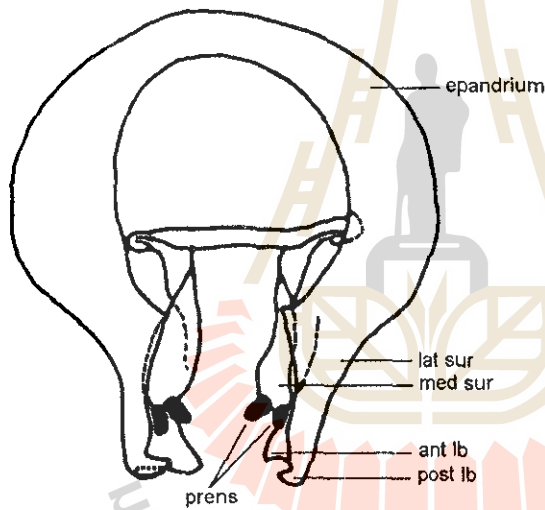
Figure 2. Male genitalia, in part; ant lb = anterior lobe of lateral surstylus; post lb = posterior lobe of lateral surstylus; lat sur = lateral surstylus; med sur = medial surstylus; redrawn from White *et al.* (2000)

2.3.2 Ovipositors: Ovipositors of female flies of 14 species of *Bactrocera* were observed and photographed with a scanning electron microscope (figure 3).

oviscape

eversible membrane

aculeus

1 mm
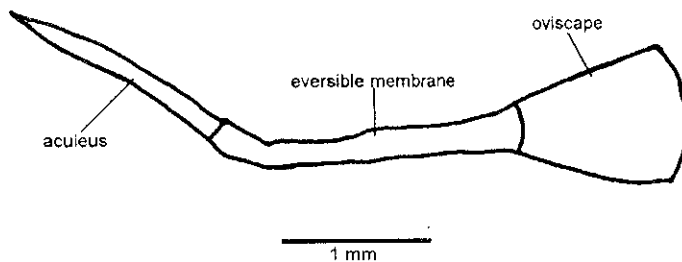
Figure 3. Ovipositor, fully extended, from female *Bactrocera* sp. near *B. (B.)* sp. L

2.3.3 Description of species:

2.3.3.1 *Bactrocera (B.) carambolae*

Male genitalia: the posterior lobe of the lateral surstylus is triangular, narrowly acute, with a rounded apex.

Aculeus: the apex is pointed with 2 long and 2 short subapical setae (Drew and Hancock, 1994). The distal denticles on the emersible membrane are rounded with many small dentitions (Baimai et al., 1996; P.J. Grote, personal observation)

This species is similar to *B. (B.) dorsalis* but can often, but not always, be distinguished by markings on the wing and fore femur. The mitotic karyotype is distinct from that of other species studied (Baimai et al., 1995). The host range is very broad; larvae have been collected from fruits of many families (Drew and Hancock, 1994; Baimai et al., 1996).

2.3.3.2 *B. (B.) correcta*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 5) is triangular, acute, with a rather sharp apex.

Aculeus: the apex is somewhat rounded, with 2 long and 3 short subapical setae (fig. 23) (perhaps this specimen is an abnormality, as 2 short and 2 long setae is much more common in *Bactrocera*). The distal denticles on the eversible membrane have a rounded or straight toothed edge, with small and medium-sized dentitions (fig. 24).

This species is in the subgenus *Bactrocera*, but is morphologically distinct from members of the *B. (B.) dorsali* group. The hosts include a wide range of families.

2.3.3.3 *B. (B.) dorsalis*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 6) is acute with a rounded apex.

Aculeus: the apex is pointed with 2 long and 2 short subapical setae. The distal denticles on the eversible membrane are broad, shallow, often slightly rounded on the toothed margin, with many small dentitions, slightly unequal in size (Baimai et al., 1996; P.J. Grote, pers. obs.).

This species has a mitotic karyotype distinct from other species studied (Baimai et al., 1995). The host range is very wide, comprising fruits of many families (Drew and Hancock, 1994; Baimai et al., 1995).

2.3.3.4 *B.* sp. nr. *B. (B.) dorsalis* sp. C

Male genitalia: the posterior lobe of the lateral surstylus (fig. 7) is triangular, with a sharp acute apex and slightly convex outer margin.

This species is similar morphologically to *B. dorsalis*, but is distinctive in its mitotic karyotype from *B. dorsalis* and other studied species in the size of the X and Y chromosomes and the amount and distribution of constitutive heterochromatin (Baimai et al., 1995). This species is similar to *B. raiensis* Drew & Hancock morphologically and in the shape of the denticles of the eversible membrane of the aculeus (Drew and Hancock, 1994), but the type specimens of *B. raiensis* have not been studied. The host fruits are mostly species of *Artocarpus* (Moraceae), but the larvae have also been recovered from fruits of Annonaceae (Baimai et al., 1996).

2.3.3.5 *B.* sp. nr. *B. (B.) dorsalis* sp. E

Male genitalia: the posterior lobe of the lateral surstylus (fig. 8) is triangular, subobtuse, with a rounded apex.

This species is similar morphologically to *B. dorsalis*, but is distinctive in its mitotic karyotype (Baimai et al., 1995). This species does not appear to agree with any species of *Bactrocera* described in Hardy (1973) or Drew and Hancock (1994). These flies have a very narrow host range, the larvae having been found only in *Anthocephalus chinensis* and a species of *Nauclea*, both in Rubiaceae.

2.3.3.6 *B. sp. nr. B. (B.) dorsalis* sp. I

Male genitalia: the posterior lobe of the lateral surstylus (fig. 9) is triangular, acute, with a rounded apex and small convexity of the outer margin.

Aculeus: the apex is pointed with 2 medium-length and 2 short subapical setae (fig. 25). The distal denticles on the eversible membrane are rounded with rather small, slightly unequal-sized dentitions (fig. 26).

This species is similar morphologically to *B. (B.) pyrifoliae*, but appears to be a genetically isolated species based on the results of isozyme electrophoresis (P.J. Grote, unpublished data). Mitotic karyotypes have not been investigated. This species appears to be distinct from all species of *Bactrocera* in Hardy (1973) and Drew and Hancock (1994).

This species is only known from fruits of *Heliciopsis terminalis* (Proteaceae).

2.3.3.7 *B. sp. nr. B. (B.) dorsalis* sp. J

Male genitalia: the posterior lobe of the lateral surstylus (fig. 10) is triangular, narrowly acute, with a sharp apex.

Aculeus: the apex is pointed with 4 short subapical setae.

This species is similar morphologically to *B. (B.) cognata* (Hardy & Adachi), a species in the *B. (B.) dorsalis* group, but differs in some characters (Phinchongsakuldit, 1998). *B. (B.) cognata* is only known from the Philippines (Drew and Hancock, 1994); sp. J may be a geographical variant race of this species or may be a new undescribed species (Phinchongsakuldit, 1998). The mitotic karyotype is dissimilar from that of *B. (B.) dorsalis* and other species studied (Phinchongsakuldit, 1998). The chromosomes of *B. (B.) cognata* from the Philippines were not studied. The host fruits are *Cleistocalyx operculatus* and perhaps *Syzygium* sp., both in Myrtaceae.

2.3.3.8 *B. sp. nr. B. (B.) dorsalis* sp. K

Male genitalia: the posterior lobe of the lateral surstylus (fig. 11) is triangular, acute, with a large rounded apex.

Aculeus: the apex is trilobed with a small notch at the tip; 4 short subapical setae are present (fig. 27). The distal denticles of the eversible membrane have one very large dentition with small dentitions of varying size on either side (fig. 28).

This species is morphologically distinct, based on markings on the femora and other characteristics, from all species of *Bactrocera* in Hardy (1973) and Drew and Hancock (1994). This species shows a mitotic karyotype distinct from all species studied (Baimai et al., 1995; Phinchongsakuldit, 1998). This species is only known from fruits of *Payena* sp. (Sapotaceae).

2.3.3.9 *B. sp. nr. B. (B.) dorsalis* sp. L

Male genitalia: the posterior lobe of the lateral surstylus (fig. 12) is triangular, acute, with a rounded apex and convex inner margin.

Aculeus: the apex is pointed (fig. 29), with 2 long and 2 short subapical setae. The distal denticles of the eversible membrane have a slightly rounded or straight toothed edge, with small, somewhat unequal-sized dentitions (fig. 30).

This species is morphologically similar to *B. (B.) thailandica* Drew & Hancock, but differs in some characters (Phinchongsakuldit, 1998). The mitotic karyotype is similar but not identical to that of *B. (B.) carambolae* (J. Phinchongsakuldit, pers. comm.). This species has been collected from various fruit types, including species of *Syzygium* (Myrtaceae) and *Platea latifolia* (Icacinaceae) (Phinchongsakuldit, 1998) and perhaps from other species.

2.3.3.10 *B. sp. nr. B. (B.) dorsalis* sp. M

Male genitalia: the posterior lobe of the lateral surstylus (fig. 13) is triangular with a sharp apex.

This species is morphologically distinct from other species in the *B. (B.) dorsalis* group. It also differs in its mitotic karyotype from the other species investigated (Phinchongsakuldit, 1998). This species has been collected from fruits of *Fibraurea tinctoria* (Menispermaceae) (Phinchongsakuldit, 1998).

2.3.3.11 *B. sp. nr. B. (B.) dorsalis* sp. O

Male genitalia: the posterior lobe of the lateral surstylus (fig. 15) is triangular with a large rounded apex.

Aculeus: the apex is pointed with 2 short subapical setae plus 1 or 2 additional setae missing (fig. 39). The distal denticles of the eversible membrane have a slightly rounded toothed edge with large dentitions (fig. 40).

This species is similar morphologically to *B. (B.) papayae* Drew and Hancock, but shows slight differences (Phinchongsakuldit, 1998). This species differs in its mitotic karyotypes from the other species studied (Phinchongsakuldit, 1998). This species has been found in fruits of many species, including a species of *Willughbeia* (Apocynaceae).

2.3.3.12 *B. sp. nr. B. (B.) dorsalis* sp. P

Aculeus: the apex is rounded with 2 long and 2 short subapical setae close to the tip of the apex (fig. 31). The distal denticles of the eversible membrane have large dentitions (fig. 32).

This species can be placed in the *B. (B.) dorsalis* group and is characterized by the large size of the ovipositor of females. It has been collected from a species of *Nauclea* (Rubiaceae).

2.3.3.13 *B. sp. nr. B. (B.) dorsalis* sp. S

Male genitalia: the posterior lobe of the lateral surstylus (fig. 14) is triangular, narrowly acute, with a rounded apex.

Aculeus: the aculeus is bilobed (notched at the tip) with 2 long and 2 short subapical setae (fig. 33). The distal denticles of the eversible membrane is rounded with many small dentitions (fig. 34).

This species can be placed based on morphology into the *B. (B.) dorsalis* group. The bilobed aculeus is also found in *B. holtmanni* (Hardy) known from peninsular Malaysia and the Philippines (Drew and Hancock, 1994), but specimens of that species have not been available for study. This species has been collected from one fruit type, possibly indentified as *Azadirachta excelsa* (Anacardiaceae) (Baimai et al., 1996).

2.3.3.14 *B. sp. nr. B. (B.) dorsalis* sp. U

Aculeus: the apex is pointed with 2 long and 2 short subapical setae (fig. 35). The distal denticles of the eversible membrane are rounded with many small teeth (fig. 36).

This species is similar morphologically to *B. (B.) dorsalis*, but the females have a black marking on the fore femur. This species has been collected from a species of *Diospyros* (Ebenaceae) (Baimai et al., 1996).

2.3.3.15 *B. sp. nr. B. (B.) dorsalis* sp. V

Aculeus: the apex is pointed with 2 long and 2 short subapical setae (fig. 37). The distal denticles of the eversible membrane are rounded with small, slightly unequal-sized dentitions (fig. 38).

This species is similar morphologically to *B. (B.) carambolae* but has a distinctive mitotic karyotype (J. Phinchongsakuldit, pers. com.). This species has been collected from fruits of a species of *Ziziphus* (Rhamnaceae).

2.3.3.16 *B. (B.) kanchanaburi*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 16) is triangular, acute, with a rounded apex.

This species is a member of the *B. (B.) dorsalis* group but can usually be indentified by a black marking on the face. This species shows a distinctive mitotic karyotype (Baimai et al., 1995).   This species shows a narrow host range, having been found in species of *Artabotrys* (Annonaceae).

2.3.3.17 *B. (B.) melastomatos*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 17) is triangular, narrowly acute, with a rounded apex.

This species has a mitotic karyotype distinct from other species studied (Phinchongsakuldit, 1998). It is known from fruits and flowers of *Melastoma* (Melastomataceae) (Drew and Hancock, 1994; Phinchongsakuldit, 1998).

2.3.3.18 *B. (B.) propinqua*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 18) is triangular, narrowly acute, with a rounded apex.

This species can be placed morphologically in the *B. (B.) dorsalis* group, but can be separated from most species by the trilobed apex of the aculeus. This species is known from several species of *Garcinia* (Clusiaceae) (Drew and Hancock, 1994; Baimai et al., 1996).

2.3.3.19 *B. (B.) pyrifoliae*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 19) is triangular, narrowly acute, with a rounded apex. The outer margin of the posterior lobe is convex basally.

Aculeus: the apex is pointed with 2 long and 2 short subapical setae (fig. 41). The distal denticles of the eversible membrane have a straight toothed edge, with mostly large, uneven-sized dentitions (fig. 42).

This species has been placed in the *B. (B.) dorsalis* group. Its mitotic karyotype is distinctive, with 2 X chromosome types (Phinchongsakuldit, 1998). This species has a wide host range, with larvae being found in fruits in several families, including in economically important fruits such as guava (*Psidium guajava*) and peaches (*Prunus persica*).

2.3.3.20 *B. (B.) verbascifoliae*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 20) is triangular, acute, with a rounded apex.

This species can be placed into the *B. (B.) dorsalis* group. The denticles of the eversible membrane are distinctive, being transversely broadly ovate with many small teeth (Baimai et al., 1996). The mitotic karyotype is distinctive (Baimai et al., 1995). The host range is very narrow; larvae are known from *Solanum erianthum* (Solanaceae) and possibly another species of the same genus (Drew and Hancock, 1994; Baimai et al., 1996).

2.3.3.21 *B. (Z.) diaphoropsis*

Aculeus: the apex is rounded with 2 long and 2 short subapical setae close to the tip of the apex (fig. 43). The distal denticles of the eversible membrane have a straight or slightly rounded toothed edge with large dentitions (fig. 44).

This species can be distinguished from other members of the subgenus *Zeugodacus* by its wing markings and number of bristles on the fronto-orbital bristles (Hardy, 1973). Hardy (1973) reported this species from Laos, but not from Thailand, but S. Tigvattananont

has collected larvae from central and northeastern Thailand (Baimai et al., 1996) from a species of *Strychnos* (Loganiaceae).

2.3.3.22 *B. (Z.) tau*

Male genitalia: the posterior lobe of the lateral surstylus (fig. 21) is long and tapering, much longer than the anterior lobe.

This species is in the subgenus *Zeugodacus* and has markings quite distinctive from those found in the subgenus *Bactrocera*. Larvae are most commonly found in fruits or flowers of species of Cucurbitaceae, but have been found in other families (Baimai et al., 1996).

2.3.3.23 *B.* sp. near *B. (Z.) tau* sp. G

Aculeus: the apex is rounded with 1 (or maybe 2) long and 2 short subapical setae near the tip of the apex; it is uncertain whether a second long subapical seta is missing (fig. 45). The distal spicules of the eversible membrane have a straight or slightly rounded toothed edge with medium and large denticles (fig. 46).

This species is morphologically similar to *B.* sp. near *B. (Z.) tau* sp. J, but is distinct in its mitotic karyotype (J. Phinchongsakuldit, pers. com.).

2.3.3.24 *B.* sp. near *B. (Z.) tau* sp. J

Aculeus: the apex is rounded with 2 long and 2 short subapical setae near the tip of the apex (fig. 47). The distal denticles of the eversible membrane have a rounded toothed edge with large broad teeth (fig. 48).

This species is morphologically similar to *B. (Z.) tau*, but differs both in its mitotic karyotype and in the banding patterns of isozymes in electrophoresis (A. Saelee, pers. com.)

2.3.3.25 *B. (Z.)* sp. K

Male genitalia: the posterior lobe of the lateral surstylus (fig. 22) is long and curved and slightly expanded at the apex. The posterior lobe is much larger than the anterior lobe.

This species is known from a species of *Strychnos* (Loganiaceae) (Baimai et al., 1996).

Figure 5. *B. (B.) correcta* male genitalia, showing surstyli and part of epandrium and proctiger

Figure 7. *B. B.* sp. nr. *B. (B.) dorsalis* sp. C male genitalia, showing surstylis and part of proctiger

Figure 4. *Bactrocera (B.) carambolae* male genitalia, showing surstyli and part of epandrium and protiger

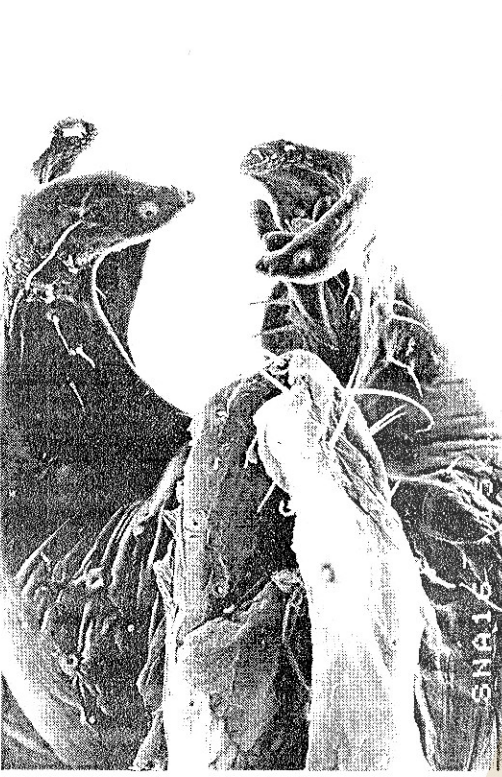Figure 6. *Bactrocera dorsalis* male genitalia, showing surstyli

Figure 8. *B. sp. nr. B. (B.) dorsalis* sp. E male genitalia, showing surstyli
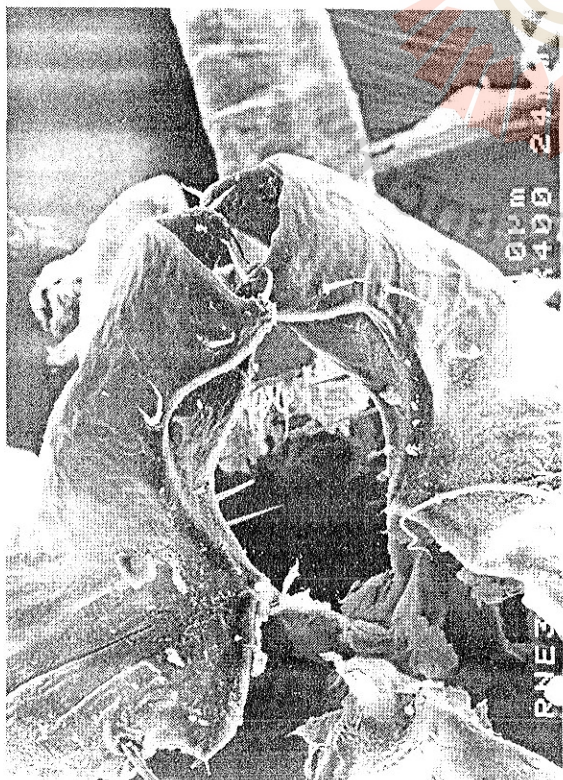

Figure 9. *B. sp. nr. B. (B.) dorsalis* sp. I male genitalia, showing surstyli

Figure 10. *B. sp. nr. B. (B.) dorsalis* sp. J male genitalia, showing surstyli and part of proctiger

Figure 11. *B. sp. nr. B. (B.) dorsalis* sp. K male genitalia, showing surstyli and part of proctiger

Figure 12. *B. sp. nr. B. (B.) dorsalis* sp. L male genitalia, showing surstyli and part of proctiger

Figure 13. *B. sp. nr. B. (B.) dorsalis* sp. M male genitalia, showing surstyli

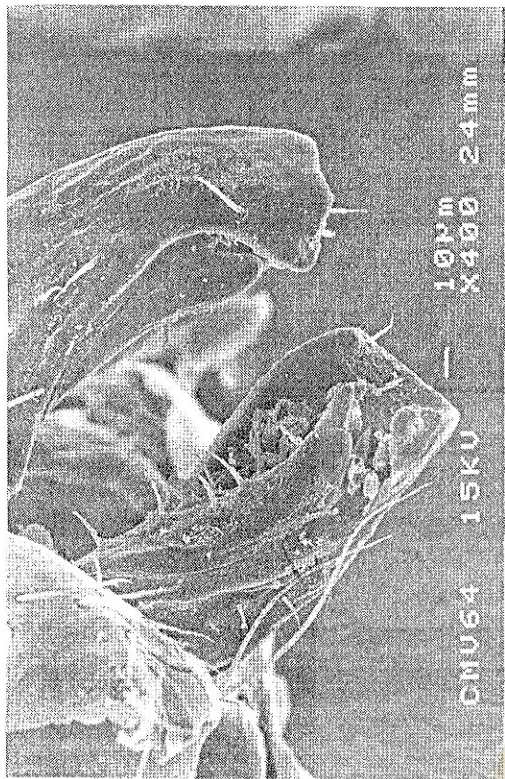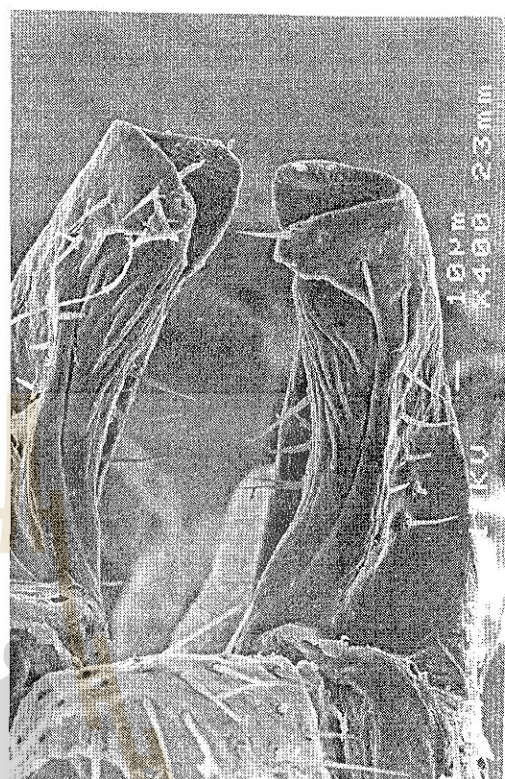Figure 14. *B. sp. nr. B. (B.) dorsalis* sp S. male genitalia, showing surstyli and part of proctiger

Figure 15. *B. sp. nr. B. (B.) dorsalis* sp. O male genitalia, showing surstyli
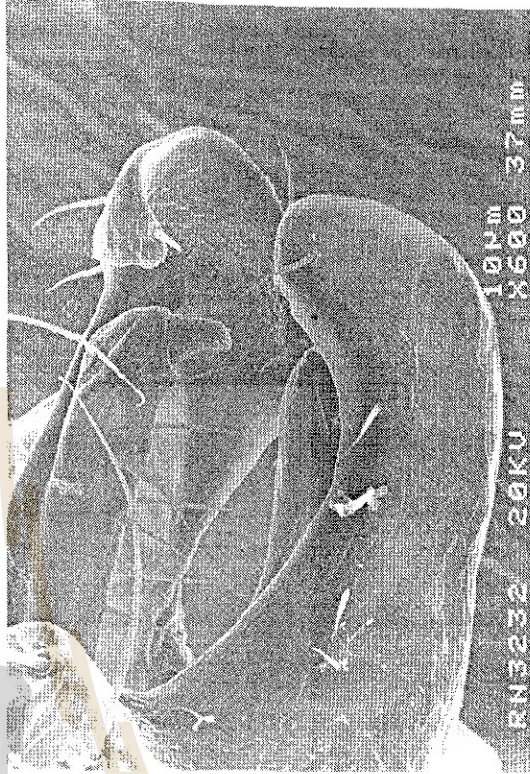
Figure 16. *B.* (*B.*) *kanchanaburi* male genitalia, showing surstyli and part of epandrium and proctiger

Figure 17. *B.* (*B.*) *melastomatos* male genitalia, showing surstyli and part of proctiger
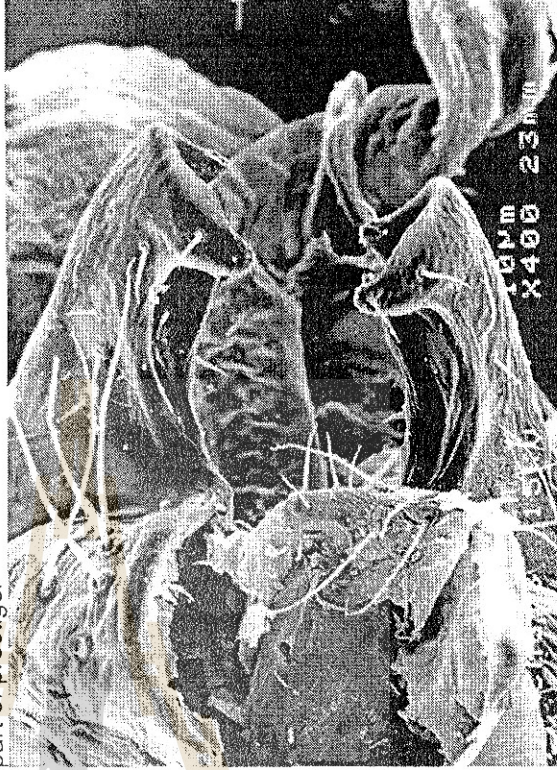
Figure 18. *B.* (*B.*) *propinqua* male genitalia, showing surstyli and part of proctiger

Figure 19. *B.* (*B.*) *pyrifoliae* male genitalia, showing surstyli and part of proctiger

Figure 20. *B.* (*B.*) *verbascifoliae* male genitalia, showing surstyli
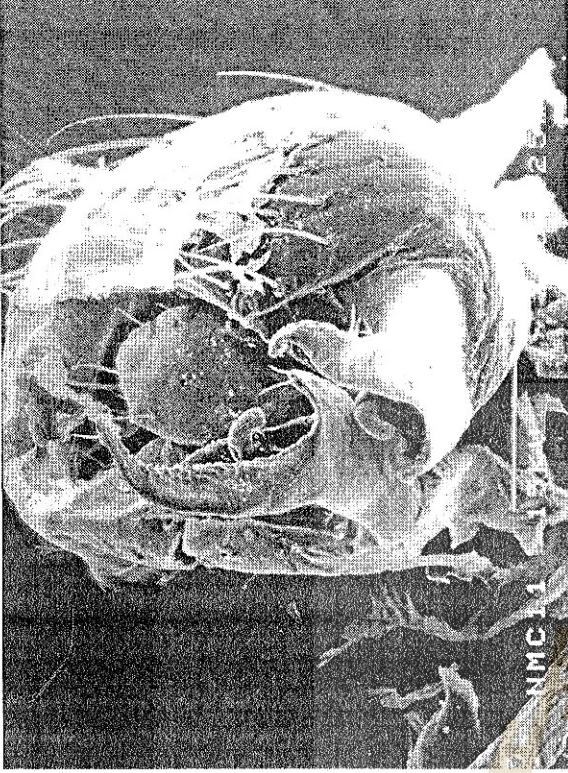
Figure 21. *B.* (*Zeugodacus*) *tau* male genitalia, showing surstyli, epandrium, and proctiger

Figure 22. *B.* (*Z.*) sp. K male genitalia, showing surstyli and part of proctiger

Figure 24. *B. correcta*, denticles at distal end of eversible membrane of ovipositor

Figure 26. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. I, denticles at distal end of eversible membrane of ovipositor

Figure 23. *Bactrocera correcta*, apex of aculeus

Figure 25. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. I, apex of aculeus

Figure 28. *B.* sp. nr. *B.* *(B.)* *dorsalis* sp. K, denticles at distal end of eversible membrane of ovipositor

Figure 30. *B.* sp. nr. *B.* *(B.)* *dorsalis* sp. L, denticles at distal end of eversible membrane of ovipositor
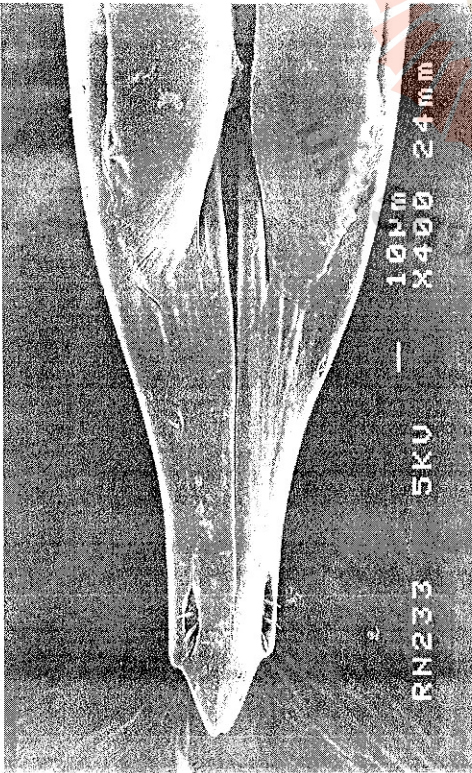
Figures 27. *B.* sp. nr. *B.* *(B.)* *dorsalis* sp. K, apex of aculeus
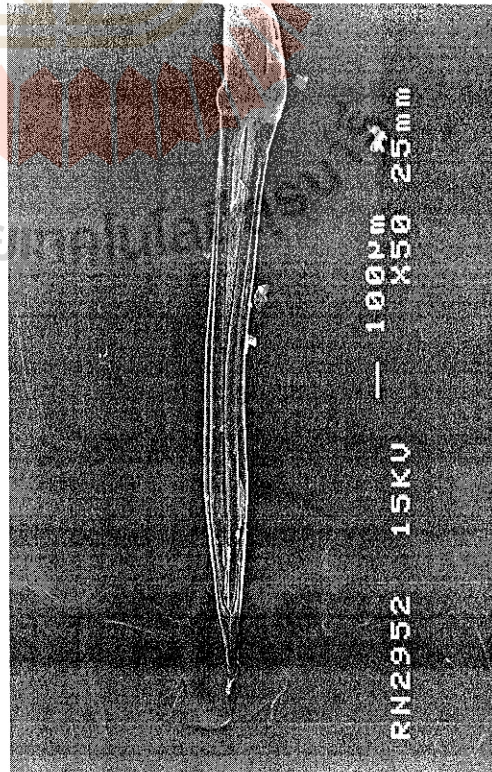
Figure 29. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. L aculeus and part of eversible membrane

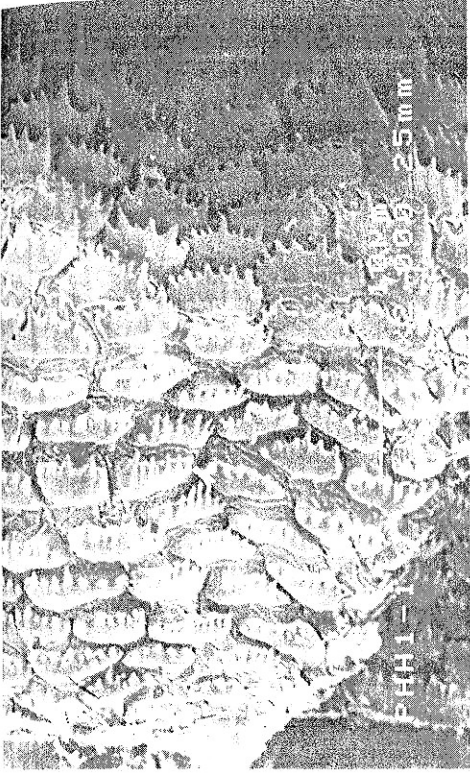Figure 32. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. P, denticles at distal end of eversible membrane of ovipositor
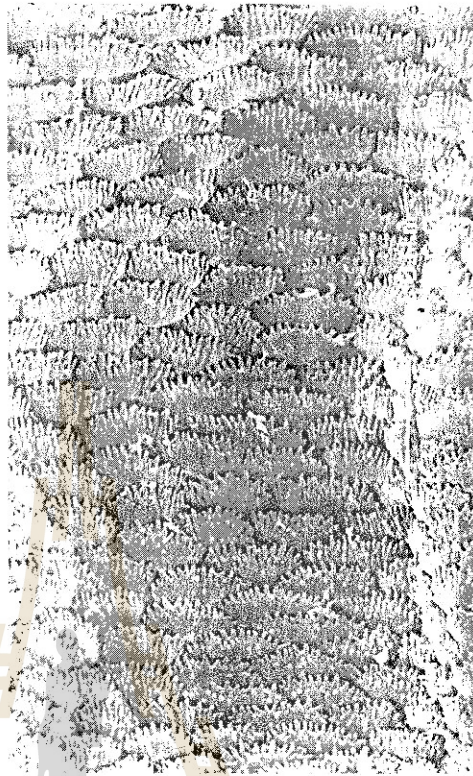
Figure 34. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. S, denticles at distal end of eversible membrane of ovipositor

Figure 31. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. P, apex of aculeus

Figure 33. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. S, apex of aculeus

Figure 36. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. U, denticles at distal end of eversible membrane of ovipositor
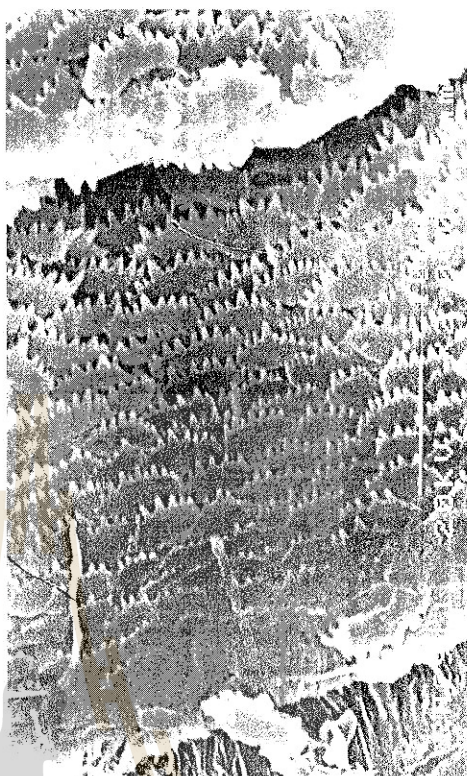
Figure 38. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. V, denticles at distal end of eversible membrane of ovipositor
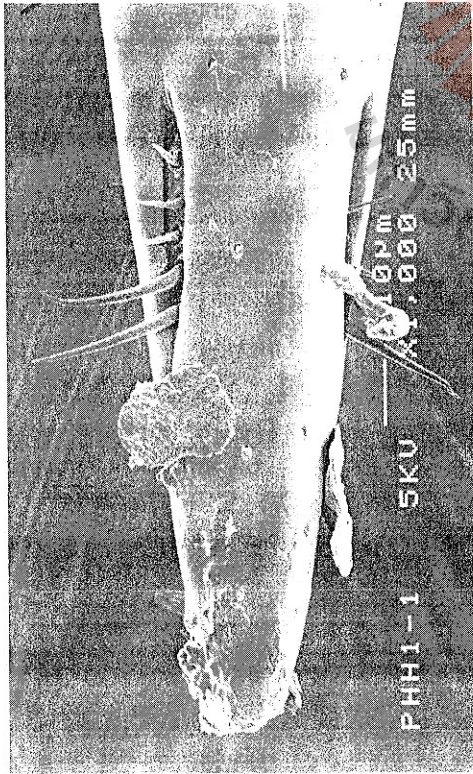

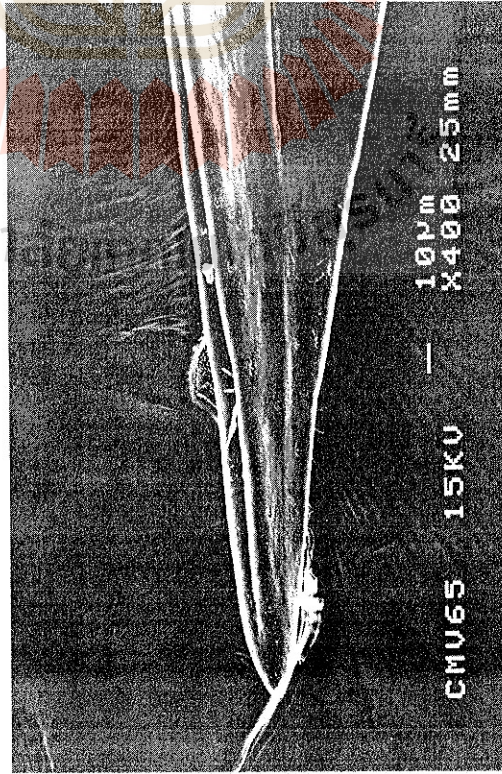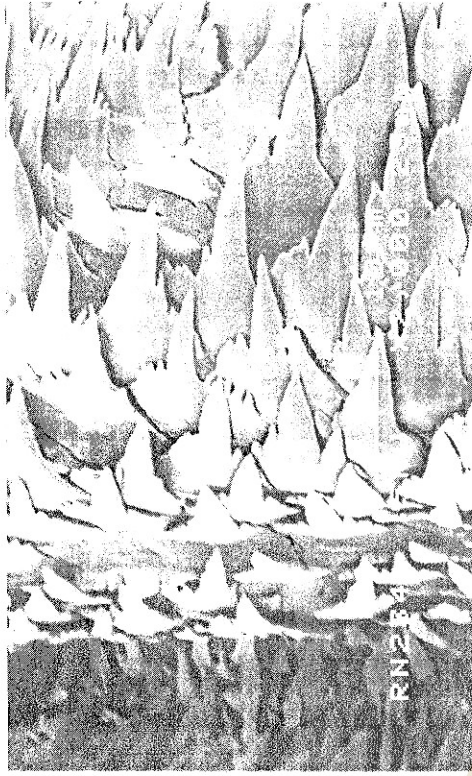Figure 35. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. U, apex of aculeus

Figure 37. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. V, apex of aculeus
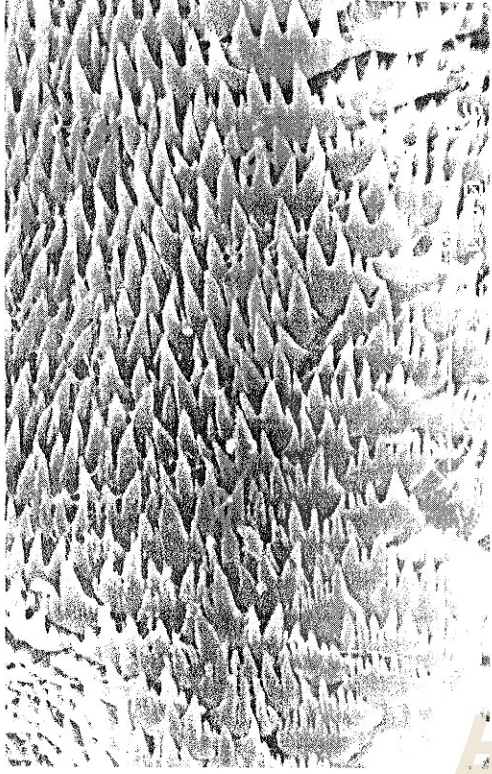
Figure 40. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. O, denticles at distal end of eversible membrane of ovipositor

Figure 42. *B.* (*B.*) *pyrifoliae*, denticles at distal end of eversible membrane of ovipositor
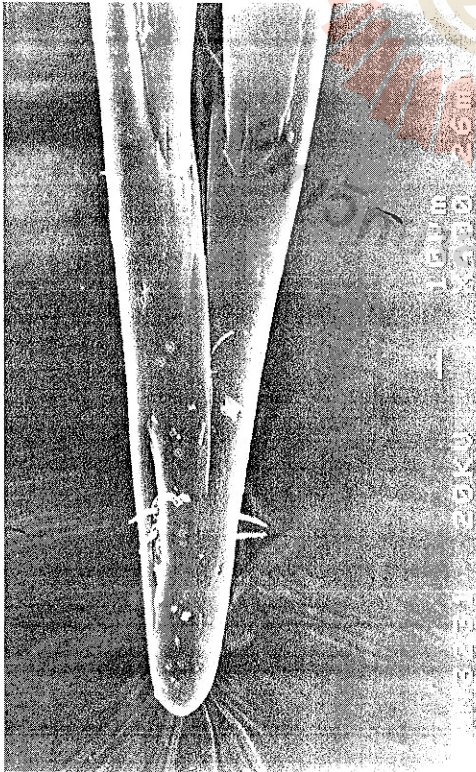
Figure 39. *B.* sp. nr. *B.*(*B.*) *dorsalis* sp. O, apex of aculeus
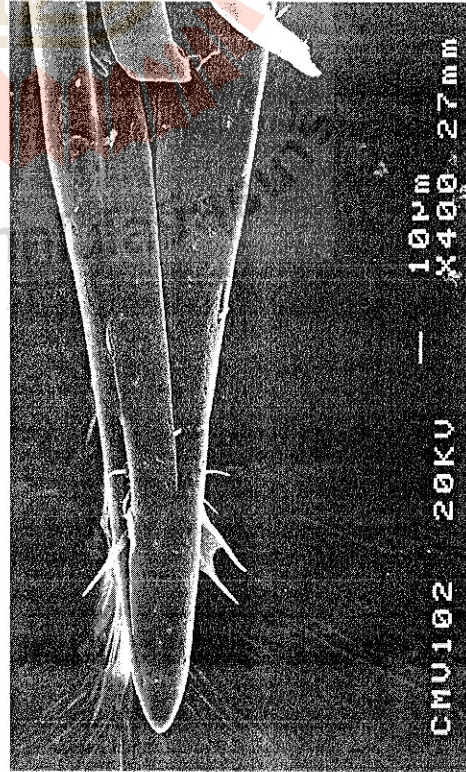
Figure 41. *B.* (*B.*) *pyrifoliae*, apex of aculeus

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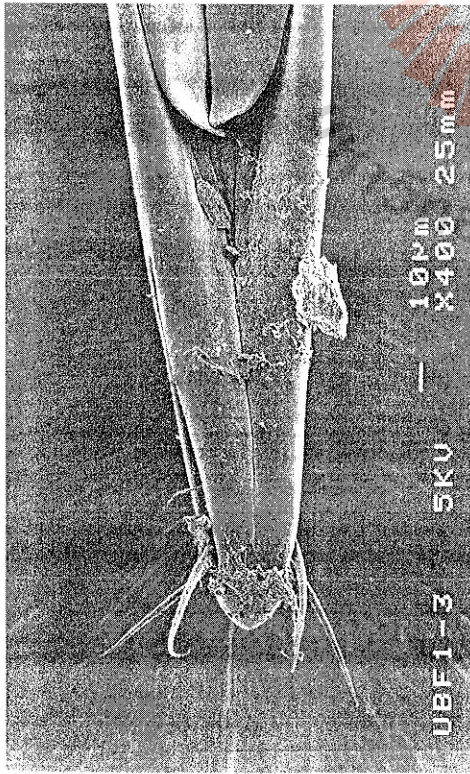
Figure 43. *B.* (Zeugodacus.) *diaphoropsis*, apex of aculeus

Figure 44. *B.* (Z.) *diaphoropsis*, denticles at distal end of eversible membrane of ovipositor

Figure 45. *B.* sp. near *B.* (Z.) *tau* sp. G, apex of aculeus
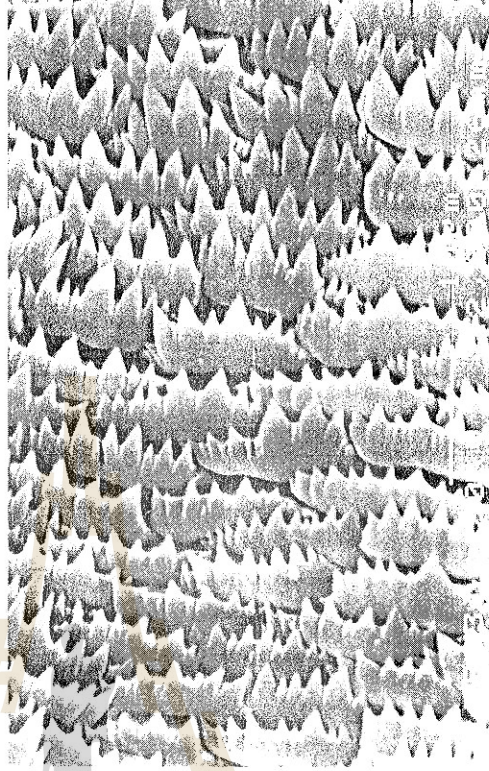
Figure 46. *B.* sp. near *B.* (Z.) *tau* sp. G, denticles at distal end of eversible membrane of ovipositor

Figure 48. *B.* sp. near *B. (Z.) tau* sp. J, denticles at distal end of eversible membrane of ovipositor
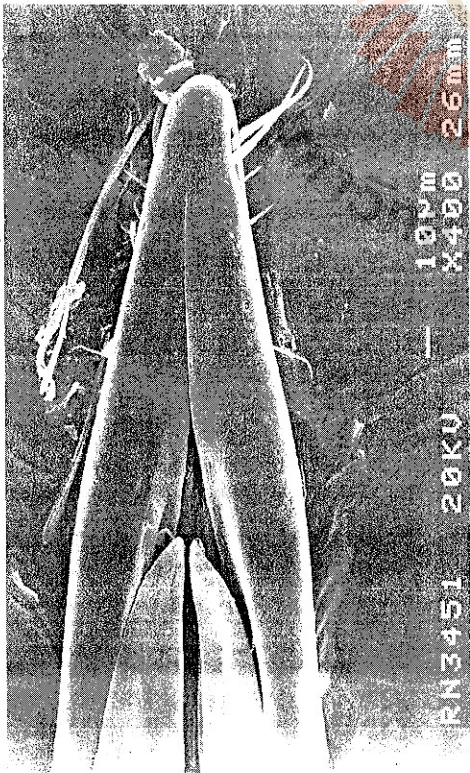
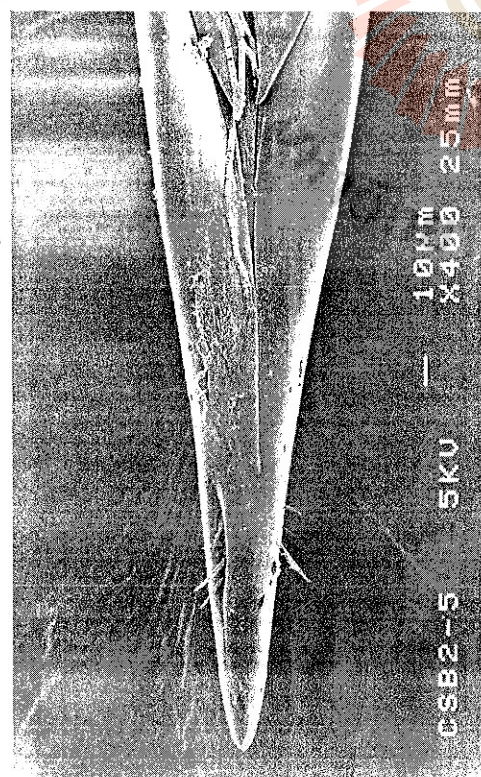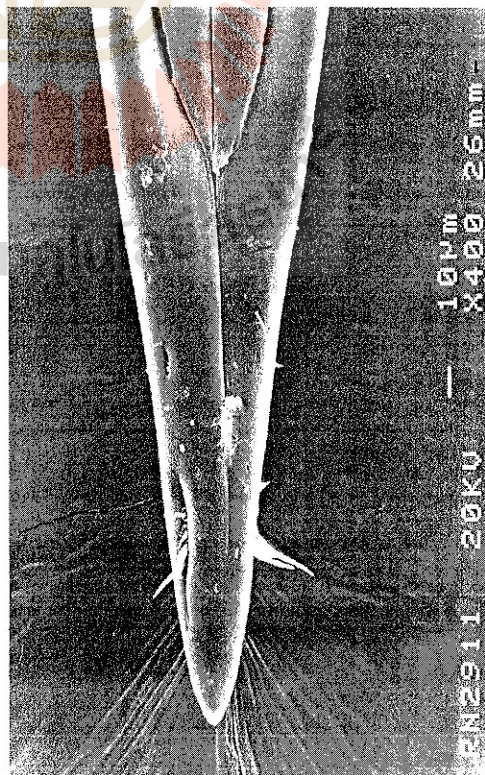Figure 47. *B.* sp. near *B. (Z.) tau* sp. J, apex of aculeus

Chapter 4

Discussion and Conclusions

Discriminant function analysis using 13 wing measurements of *B.* (*B.*) *dorsalis* and *B.* (*B.*) *carambolae* females allowed separation of 26 out of 29 species (89.7%) upon cross-validation (jackknifing). The difference in percent correct identification between using the original full data set (29 correct out of 29) and the data sets used in jackknifing, where N-1 sets are used, indicates that the sample size is probably too small, and a larger sample size is needed (McGarigal et al., 2000). In this study, the sample size was limited by the number of flies that could be analyzed by isozyme electrophoresis. In addition, some of the electrophoretic bands were blurred and could not be used, and some of the wings broke and could not be measured.

When three wing measurements were used instead of 13 in analysis of females of the two species of fly, correct identification upon cross-validation (jackknifing) was 28 out of 29 (96.6%). The higher degree of accuracy with three characters rather than 13 may indicate that some of the 13 measurements are highly correlated and that "noise", such as slight errors in measurement, may reduce the accuracy.

When 13 measurements were used in discriminant function analysis of adult males of *B.* (*B.*) *dorsalis* and *B.* (*B.*) *carambolae*, 26 out of 26 flies (100%) were correctly identified using the original full data set, whereas only 21 out of 26 (80.8%) were identified upon cross-validation (jackknifing) with N-1 data sets. This again indicates that the sample size is probably too small and should be enlarged. When two wing measurements are used rather than 13, accuracy is reduced to 20 out of 26 (76.9%) for both the original full data set and when performing cross-validation (jackknifing). There does not appear to be a problem with sample size when using two measurements as the degree of accuracy did not change between using the full data set and jackknifing.

Use of three and two measurements, respectively, for females and males, is recommended. Accuracy is higher for females, 96.6% compared to 89.7% when using 13 measurements. Accuracy is only slightly lower for males, 76.9% compared to 80.8% when using 13 measurements.

Adults of the two species can be correctly separated based on external morphology approximately 90% of the time for females and 80% of the time for males by the author. Females can be separated to a level of nearly 100% if the aculei are prepared and studied with a compound

microscope or by SEM. Thus, for an experienced worker, the accuracy of separation is not different between visual inspection and use of discriminant function analysis using wing measurements. However, use of discriminant function analysis would be useful for those inexperienced in identifying these flies. It is not known whether performing the study described in this project with a larger sample size would allow derivation of a more accurate discriminant function or allow selection of a better subset of variables when performing stepwise selection of variables. Discriminant function analysis using additional characters such as measurements of the legs or head may also provide greater accuracy.

A benefit of using discriminant function analysis of external characters is that the flies do not need to be specially prepared, nor do they need to be kept alive or stored at a low temperature. Wings are easy to measure, since being flat they can be held in place on a microscope slide with a cover slip.

The second part of this research project was to use a scanning electron microscope to study male genitalia and the aculei of selected species of *Bactocera* occurring in Thailand. The shape of the lateral surstylus was used in study of the males. Other parts of the male genitalia, such as the distiphallus, were more difficult to prepare for SEM studies. However, the lengths of the aedeagus (the intromittent organ that includes the distiphallus) of *Bactrocera* males have been successfully measured and compared by others (Drew et al., 2008). Male flies could easily be separated to the subgenus *Bactrocera* or the subgenus *Zeugodacus* because in the latter subgenus the posterior lobe of the lateral surstylus is much larger than the anterior lobe. However, no major differences were observed in the genitalia among the 17 species of the subgenus *Bactrocera*. The species seemed to vary in having a more acute or more obtuse angle of the posterior lobe of the lateral surstylus or a more rounded or pointed apex of the lobe. Little is known of the variation within a species since only one or two specimens were observed per species in this study. However, aculei and spicules of the eversible membrane of females of four of the species studied in this project, *B.* sp. nr. *B.* (*B.*) *dorsalis* I, K, L, and O, were also studied and photographed by Phinchonsakuldit (1998). The shapes were more or less similar to those of the flies in this study. Observations of a greater number of specimens per species may show the constancy or lack of constancy of features such as having a very narrow posterior lobe in *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. J (fig. 10). Furthermore, observation of more specimens may indicate whether the number and position of setae on the lateral surstylus can vary among the species.

Characters of the ovipositors of females investigated were the shape of the apex of the aculeus, the number and size of the subapical setae, and the shape of the denticles on the eversible membrane.

At the level of subgenus, the three species of subgenus *Zeugodacus* studied had large aculei with a rounded apex and subapical setae closer to the apex than found in the species of subgenus *Bactrocera*. The distal denticles of the eversible membrane of the ovipositor have larger dentitions than in most species of the subgenus *Bactrocera*. The only species of the subgenus *Bactrocera* with a similar aculeus and denticles is *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. P.

Within a subgenus, some species have clearly distinct ovipositors but others do not. For example, *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. K has an aculeus in which the apex is trilobed and has a small notch at the tip (fig. 27). In addition, the distal spicules have a large central tooth (fig. 28). I am not familiar with any other species in the genus with similar characteristics. The external morphology is also different from other species in the subgenus *Bactrocera*. All six femora have distal black markings and the wings have a broad dark streak near the leading edge, a combination of characters not seen in any other species known from Thailand. Distinctive characters, however, were not observed in the male genitalia (fig. 11). *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. S is distinctive among the species studied in this project in having a notched aculeus. *B.* (*B.*) *holtmanni* from Peninsular Malaysia and the Philippines has a notched aculeus, but details concerning the shape of the distal denticles were not reported (Drew and Hancock, 1994).

B. sp. nr. *B.* (*B.*) *dorsalis* sp. I and *B.* (*B.*) *pyrifoliae* are very similar in external morphology. However, differences can be seen in the aculei and the distal denticles. *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. I has two medium-length and two short subapical setae (fig. 25), whereas *B.* (*B.*) *pyrifolia* has two long and two short setae (fig. 41). In addition, the former species has distal denticles with a rounded toothed margin, while the latter species has denticles with straight teeth margins. These two species also differ electrophoretically. In an isozyme electrophoretic study of five loci, the species differed in allelic frequency at four loci (P.J. Grote, unpublished results).

B. sp. nr. *B.* (*B.*) *dorsalis* sp. O has an aculeus with a pointed apex (fig. 39) and distal spicules with large dentitions (fig. 40). This combination is not seen in the ovipositors of other species presented in this study, but is similar to that of female flies of *B.* sp. nr. *B.* (*B.*) *dorsalis* sp. C as illustrated in Baimai et al. (1996).

B. sp. nr. *B*. (*B*.) *dorsalis* sp. P differs from the other species of the subgenus *Bactrocera* in this study in having an aculeus with a blunt apex and subapical setae very close to the apex (fig. 31) and having dentitions on the distal denticles larger than in most other species. These characters are also found in the subgenus *Zeugodacus*, but the two subgenera can be separated by external characters, such as markings on the abdomen.

B. sp. nr. *B*. (*Z*.) *tau* sp. G and *B*. sp. nr. *B*. (*Z*.) *tau* sp. J are morphologically similar to each other. However, differences can be seen in their ovipositors. The former species has distal denticles with a straight tooth margin and medium and large dentitions (fig. 46). The latter species has distal denticles with a more rounded tooth margin and large dentitions in the center and small dentitions on either side (fig. 48). The number of subapical setae in the former species is uncertain (one or two long setae and two short setae), precluding comparison with the latter species. These two species also differ from each other in their mitotic karyotype (J. Phinchongsakuldit, pers. com.).

Research on the taxonomy of fruit flies is best carried out with a multifaceted approach. Many fruit flies of the genus *Bactrocera* can be separated by external morphology. However, some species are very similar morphologically and are considered to be sibling species. Use of SEM to look at detailed anatomy can help separate sibling species. For example, the sibling species *B*. sp. nr. *B*. (*Z*.) *tau* sp. G and sp. J can be separated, for adult females, by the shape of the denticles of the eversible membrane (see above). SEM of male genitalia may allow separation of *B*. sp. nr. *B*. (*B*.) *dorsalis* sp. J (fig. 10) from other members of the *B*. (*B*.) *dorsalis* group because of the narrow posterior lobe of the lateral surstylus.

Use of discriminant function analysis in conjunction with various measurements may also be useful in distinguishing similar species. In this study, measurements of the length of segments of wing veins or the distance between two veins were used in a search for characters to allow separation of two sympatric species, *B*. (*B*.) *dorsalis* and *B*. (*B*.) *carambolae*. The separation rates achieved were 96.6% for females and 76.9% for males. Adsavakulchai et al. (1998) carried out discriminant and cluster analyses of nine species of Bactrocera using digital images of wings. The species included *B*. (*B*.) *dorsalis* but not *B*. (*B*.) *carambolae*.

Modern molecular and cytotaxonomic techniques can be used in conjunction with morphological analyses. Study of the mitotic karyotypes of larvae of *Bactocera*, especially those thought to be sibling species, is very useful in deciding whether populations of flies are conspecific

or separate biological species (Baimai et al., 1996; Phincholsakuldit, 1998). Isozyme electrophoresis can also provide evidence as to whether flies collected from one area form an interbreeding population or whether they comprise two or more genetically isolated populations (Saelee, 1999). Mitochondrial DNA was used to study eight species in the *Bactrocera tau* complex (Jamnongluk et al., 2003) and 27 species of *Bactrocera* from six subgenera (Nakahara and Muraji, 2008). Once populations of flies are identified as biologically distinct species by molecular methods, detailed anatomical studies, such as studies of the genitalia or morphometric analyses can be made to find suitable ways of separating individuals to species. Cytotaxonomic studies of mitotic karyotypes is especially beneficial in that larvae collected directly from infested fruits can be used.

1. *B. (B.) dorsalis* and *B. (B.)* papayae Drew & Hancock

In the collection of fruit flies under the research project of Baimai et al. (1995), flies of similar morphology were collected throughout Thailand and were considered to be *B. (B.) dorsalis*. This included the flies from Songkhla used in the present research project involving discriminant function analysis. Drew and Hancock (1994) proposed a new species, *B. (B.) papayae*, of flies with a distribution that included peninsular Malaysia, Singapore, Borneo, Sulawesi, Christmas Island, as well as peninsular Thailand. I had considered the flies from Songkhla used in the present study as either *B. dorsalis*, and not *B. papayae*, or as matching *B. papayae*, but with *B. papayae* being conspecific with *B. dorsalis*. More recent studies have shown that *B. papayae* is likely a biologically distinctive species from *B. dorsalis* (Drew et al., 2008). Drew et al. (2008) report that these two species, along with three other species of *Bactrocera*, differ at the specific level based on molecular evidence. Studies of mitochondrial DNA by Nakahara and Muraji (2008) also indicate differences between the two species, with one of the *B. papayae* specimens having been obtained from Pattani, southern Thailand. The aedeagus of the males differ greatly in length, and male pheromones show minor though consistent differences between the two species (Drew et al., 2008). Hybrids of the two species have been observed, but these occurred with caged flies (Drew et al., 2008).

Because it is likely that *B. papayae* is a distinct species from *B. dorsalis* and that *B. papayae* occurs in southern Thailand, further study is needed to determine if the flies used for discriminant function analysis in this study are *B. dorsalis* or *B. papayae*. However, this

uncertainty does not change the results of the ability to distinguish the two species, *B. dorsalis* or *B. papayae* and *B. carambolae*, by morphological characters of the wing.

2. Conclusions

1) Wing measurements can be used in discriminant function analysis to separate individual adult flies between the two species *Bactrocera (B.) dorsalis* and *B. (B.) carambolae* at a level of accuracy of 96.6% for females and 76.9% for males.

2) Male genitalia were studied by means of scanning electron microscopy of 19 species of *Bactocera* occurring in Thailand. By observing characters of the lateral surstylus, flies could be distinguished as belonging to the subgenus *Bactocera* or the subgenus *Zeugodacus*. However, only some flies could be identified to species by the characters observed.

3) Female ovipositors of 14 species of *Bactrocera* were studied with a scanning electron microscope. Some flies could be identified to species by the shape of the apex of the aculeus, including the number and length of subapical setae, by the shape of distal spicules on the eversible membrane, or by a combination of the two. Other species, however, were not clearly separable by this means.

4) Measurement of organs or body parts in addition to wings, such as legs or the head, when used in discriminant function analysis may provide a better means of classifying unknown adult flies to species. Additionally, egg, larvae, or pupal stages may provide characters that permit identification to species.

5) Proper taxonomic study of fruit flies should be multifaceted, incorporating morphological, cytotaxonomic, and molecular studies, as well as study of the basic biology of the flies.

6) Accurate identification of pest species or potential pest species is necessary for control techniques, such as the sterile male technique.

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Born: 26 November 1954, Ohio, USA

Educational background:

Ph.D., 1989, Indiana University, Bloomington, Indiana, USA, major: Biology; minor: Geology.

M.S., 1979, University of Cincinnati, Cincinnati, Ohio, USA, major: Biological Sciences.

B.S. (Cum Laude), 1977, Xavier University, Cincinnati, Ohio, USA, major: Biology.

Experience:

Lecturer, School of Biology, Suranaree University of Technology, 1995-present.

Lecturer, Department of Biology, Mahidol University, Bangkok, 1991-1995.

Lecturer, Department of English, Rangsit University, PathumThani, 1991.

Lecturer, Department of English, Dhurakijpundit University, Bangkok, 1990-1991.

Associate Instructor, Department of Biology, Indiana University, Indiana, USA, 1982-1986, 1987, 1989.

Research Assistant, University of Cincinnati Medical Center, Ohio, USA 1979-1981

Teaching Assistant, Department of Biological Sciences, University of Cincinnati, Ohio, USA, 1977-1978.

Selected Publications:

Grote, Paul J. 2007. Studies of fruits and seeds from the Pleistocene of northeastern Thailand. *Courier Forschungsinstitut Senckenberg 258*: 171-181.

Grote, P.J. 1998. Separation of fruit fly species of the Bactrocera dorsalis group (Tephritidae) using SEM. *Journal of electron microscopy society of Thailand 12 (1)*: 73-74 (Abstract).

Baimai, Visut, Wachareeporn Trinachartvanit, Saen Tigvattananont, and Paul J. Grote. 1996. Metaphase karyotypes of fruit flies of Thailand II. five species in four subgenera of Bactrocera. *Journal of the Science Society of Thailand 22*: 97-104.

Baimai, V., W. Trinachartvanit, S. Tigvattananont, P.J. Grote, R. Poramarcom, and U. Kijchalao. 1995. Metaphase karyotypes of fruit flies of Thailand. I. five sibling species of the Bactrocera dorsalis complex. *Genome 38*:1015-1022.