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Original Article

Ovipositor morphology and host relations of the *Bactrocera tau* complex (Diptera: Tephritidae) in Thailand

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Abstract

The taxon, *Bactrocera tau*, is a complex of fruit flies that infest fruits of many species in the family Cucurbitaceae as well as fruits from very different plant families in southeast Asia. Past mitotic karyotype studies of *B. tau* flies from different geographic location- and/or host-associated populations indicate there are nine forms present within the taxon in Thailand, which have been designated as *B. tau* forms A to I. In this study, ovipositor morphology was compared among seven members of the *B. tau* complex using scanning electron microscopy. The flies could be placed into two main groups based on the shape of the aculeus apex. The first group comprised *B. tau* forms C and I which have trilobed aculeus apices. The second group included *B. tau* forms A, D, E, F and G, all of which have single-pointed apices. The latter five forms were further divided on the basis of the sharpness of the aculeus apex into "medium" (A and E), "sharp" (D and G) and "blunt" (F) apices. Host fruit associations, fly aculeus apex shape and geographical region were overlain onto a molecular phylogeny previously published for the *B. tau* group in Thailand. Cucurbitaceae fruits appear to be ancestral hosts for the *B. tau* complex whereas the use of fruits of other plant families appeared late in the evolutionary history of this group. Forms with trilobed and single-pointed aculeus apices separated early in *B. tau* evolutionary history, but the split does not seem host related. Flies with medium, sharp and blunt, simple-pointed aculeus apices showed no evident associations, being randomly distributed across the phylogenetic tree. *Bactrocera tau* form A which infested fruits of nine Cucurbitaceae species was found in all five surveyed regions, whereas each of the other forms, which were restricted to 1-3 fruit species, were found in 1-2 regions.

Keywords: sibling species, fruit flies, aculeus, host fruit relations, evolution

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1. Introduction

The taxon *Bactrocera* (*Zeugodacus*) *tau* (Walker) (formerly *Dacus tau*) includes flies that are major pests of cucurbit crops in Southeast Asia (Drew and Romig, 1997) and is widespread throughout the Oriental region (White and Elson-Harris, 1992). Although these flies commonly attack fruits of plant species within the family Cucurbitaceae, e.g., *Cucumis* spp., *Luffa* spp., *Momordica* spp., *Trichosanthes* spp., etc., they have also been found infesting fruits from very different families, e.g., Leguminoseae (*Phaseolus vulgaris*), Moraceae (*Ficus racemosa*), Myrtaceae (*Psidium guajava*), Oleaceae (*Myxopyrum smilacifolium*), and Sapotaceae (*Manilkara zapota*) (Allwood *et al.*, 1999).

Considerable taxonomic confusion exists regarding the species status of *B. tau*-like flies (Mahmood, 1999). The wide host range and variable morphology among *B. tau* flies indicate that the taxon comprises a complex of species, as has been suggested by Drew and Romig (1997). In Thailand, Baimai *et al.* (2000) provided evidence for a species complex

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with *B. tau*-like flies from different fruit species and/or locations being clearly separated based on their metaphase karyotypes. The karyotype forms were designated as *B. tau* forms A-G by Baimai *et al.* (2000). Two other karyotype forms have since been added to the list and have been designated as *B. tau* form H and *B. tau* form I (V.B., unpublished). Later molecular genetic and allozyme electrophoretic studies (Jamnongluk *et al.*, 2003; Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006) provided strong support for these fruit- and/or location-associated *B. tau* flies being separate species. In addition, these later studies placed *B. tau* flies into groups that are in general, although not perfect conformity, with the karyotype groups recognized by Baimai *et al.* (2000).

Within the genus *Bactrocera*, the shape of the ovipositor apical segment, termed the aculeus, and the size, shape and arrangement of spicules on the distal end of the eversible membrane of the ovipositor are frequently found to differ among species and so are often used in species comparisons (e.g., White and Elson-Harris, 1992; Drew and Hancock, 1994). In this paper, we describe and compare these characters and other aspects of the ovipositor morphology to determine their utility for separating forms within the *B. tau* complex in Thailand.

The *B. tau* forms of this study were reared from fruits in the Family Cucurbitaceae as well as fruits from two other plant families (Table 1). In addition, several phylogenies for the *B. tau* complex in Thailand have recently been published (Jamnongluk *et al.*, 2003; Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006). These phylogenetic studies were mainly concerned with the efficacy of using molecular and electrophoretic markers for distinguishing forms within the complex. However, the phylogenetic patterns determined in these studies can allow us to find the genetic relationships among *B. tau* forms that use fruit species of different plant families. This, in turn, may allow us to hypothesize the way in which the host plant relations of flies in the *B. tau* complex evolved, especially when ecological and ovipositor morphological data are included in the analysis. We, therefore, review these phylogenetic studies and in combination with data on host plant species, geographic location and ovipositor characters hypothesize the evolutionary history of Thai *B. tau* flies.

2. Materials and Methods

2.1 Sources of *Bactrocera tau* complex flies

Fruits infested with tephritid larvae were collected from many localities throughout Thailand from January 1996 to November 1997 (Table 1) as part of a large tephritid fruit fly survey. Infested fruits of each plant species from each locality were placed on sawdust at the bottom of a plastic box with a gauze-covered hole in the lid for air ventilation. Sawdust served as a pupation medium. Each collection box was labeled with collection code, locality and host plant species and then brought back to an insectary at Mahidol University for rearing flies to the adult stage. Adult flies were fed with artificial food (3:1 sugar:yeast hydrolysate) and water for about one week to allow color patterns used for identification to develop. The flies were killed and then identified to species based on morphological characters using the keys of Hardy (1973) and White and Elson-Harris (1992). Adult flies of the same species from each fruit and locality were stored together in 70% ethanol at room temperature. *Bactrocera tau*-like flies were identified from several fruit

Table 1. Collection details for fruits from which females of the *B. tau* complex in Thailand used in this study were reared. Regions: n – Northern, w – Western, s – Southern.

Form	Province (Region)	Locality code	Host plant species (Family)*	Collection date
A	Chiangmai (n)	CM(X2)	*Cucurbita moschata* (Cucurbitaceae)	Oct. 97
C	Kanchanaburi (w)	KB(S)50	*Momordica cochinchinensis* (Cucurbitaceae)	Dec. 97
D	Ranong (s)	RN(H)22	*Trichosanthes tricuspidata* (Cucurbitaceae)	Feb. 97
E	Ranong (s)	RN(M/2)492	*Strychnos thorelli* (Strychnaceae)	Jan. 97
F	Ranong (s)	RN223	*Hydnocarpus anthelminthicus* (Flacourtiaceae)	Jun. 96
G	Kanchanaburi (w)	KB(S)3	*Hydnocarpus anthelminthicus* (Flacourtiaceae)	Jan. 96
I	Yala (s)	YL(C)5	*Trichosanthes tricuspidata* (Cucurbitaceae)	Nov. 97

* Plant nomenclature and classification based on Smitinand (2001).

species (Table 1). These *B. tau*-like flies were classified into several host-fruit associated forms on morphological grounds (S. Tigvattananont, unpublished data). Based on clear differences in metaphase kayotypes, these forms have been temporarily designated as *B. tau* forms A-I (Baimai *et al.* 2000, V.B. unpublished). The forms of the *B. tau* complex used for ovipositor study in this paper were forms A, C, D, E, F, G and I. *Bactrocera tau* forms B and H female adults were not available at the time of this ovipositor study and so were not included. Adult specimens of all forms are held by Assoc. Prof. S. Tigvattananont, King Mongkut University of Technology, Lat Krabang, Bangkok, Thailand.

2.2 Specimen preparation for SEM

Bactrocera tau female adults were removed from 70% ethanol and their ovipositors were dissected out from abdomens using forceps and dissecting needles under a stereomicroscope. Preparation of ovipositors for SEM was based on the methods of Bozzola and Russell (1999). The ovipositors were prefixed with 4% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 2 hours at 4°C and washed three times in 0.1M cacodylate buffer (pH 7.4) at 4°C. They were then postfixed with 1% osmium tetroxide in 0.1M cacodylate buffer (pH 7.4) for 1 hour at 4°C and washed three times in cold filtered water at 4°C. Specimens were dehydrated in ascending ethanol concentrations through to absolute alcohol as follows: 70% ethanol for 5-15 minutes at 4°C, 80% ethanol for 5-15 minutes at 4°C, 90% ethanol for 5-15 minutes at 4°C, twice in 95% ethanol for 5-15 minutes at room temperature and twice in 100% ethanol for 5-15 minutes at room temperature. Specimens were critical point dried with liquid carbon dioxide. They were then mounted on aluminium stubs and sputter coated with platinum and palladium. Ovipositors of one to two specimens of each *B. tau* form were prepared for SEM.

2.3 Scanning electron microscopy

Ovipositors were observed under a Hitachi S-2500 scanning electron microscope. Aculei were photomicrographed at a magnification of 600x and spicules at the distal end of the eversible membrane were photomicrographed at a magnification of 3000x.

3. Results

3.1 Ovipositor morphology

Several characters of the external morphology of the ovipositor have been previously used for taxonomic description of *Bactrocera* fruit flies including the shape of the aculeus apex (e.g., Kapoor *et al.*, 1989, Drew and Hancock, 1994), and the dentation of spicules at the distal end of the eversible membrane and the numbers of preapical setae (Drew and Hancock, 1994). White (2000) considered the shape of the aculeus of species in the tribe Dacini, to which *Bactrocera* belongs, to be under such strong genetic control that it is virtually invariable among specimens of the same species. We, therefore, classified aculeus apices into four groups based on shape as medium simple-pointed, blunt simple-pointed, sharp simple-pointed and trilobed.

In this study, the relative lengths of preapical setae were also found useful for differentiating among *B. tau* forms. Descriptions of ovipositor morphology for all forms are given below.

1) *B. tau* form A (Figure 1.1, Figure 2.1)

Apex of aculeus medium simple-pointed with 4 pairs of preapical setae. The 2 pairs nearest the apex are much longer than the 2 pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.1). Spicules at the distal end of the eversible membrane are short and with small teeth approximately subequal in size (Figure 2.1).

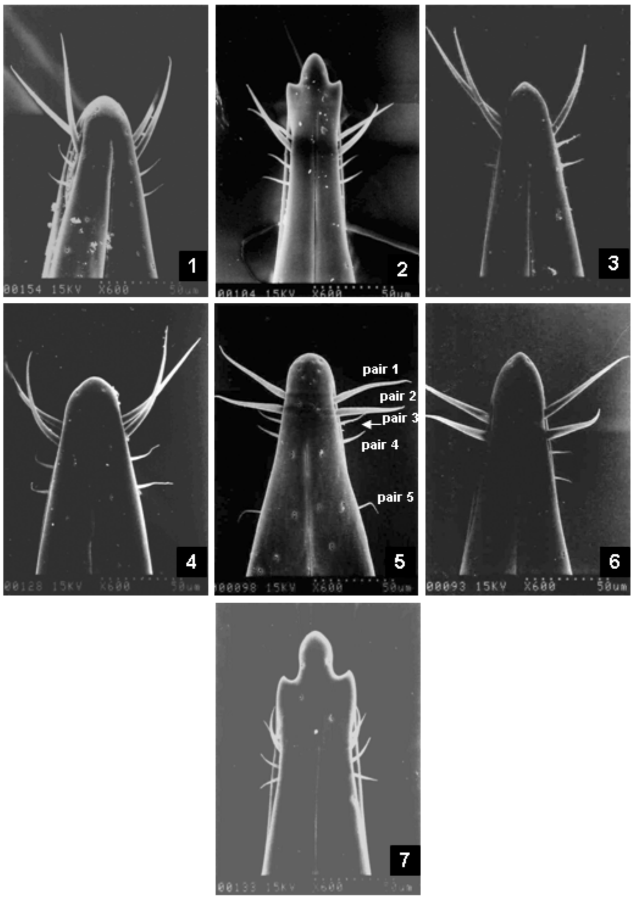

Figure 1. Scanning electron micrographs of the aculei of flies in the *Bactrocera tau* complex in Thailand: 1) form A, 2) form C, 3) form D, 4) form E, 5) form F, 6) form G, 7) form I. Form F illustrates setal pair terminology.

2) *B. tau* form C (Figure 1.2, Figure 2.2)

Apex of aculeus trilobed with 4 pairs of preapical setae. The 2 pairs nearest the apex are much longer than the 2 pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.2). Spicules at the distal end of the eversible membrane are long with small teeth approximately subequal in size (Figure 2.2).

3) *B. tau* form D (Figure 1.3, Figure 2.3)

Apex of aculeus sharp simple-pointed with 4 pairs of preapical setae. The 2 pairs nearest the apex are much longer than the 2 pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.3). Spicules at the distal end of the eversible membrane are long and wide with teeth approximately subequal in size (Figure 2.3).

4) *B. tau* form E (Figure 1.4)

Apex of aculeus medium simple-pointed with 4 pairs of preapical setae. The 2 pairs nearest the apex are much longer than the 2 pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.4). Spicules at the distal end of the eversible membrane are long with teeth approximately subequal in size. [Note that a micrograph of spicules is not presented here because the specimen was distorted, possibly during the dehydration process, and no other good quality specimens were available.]

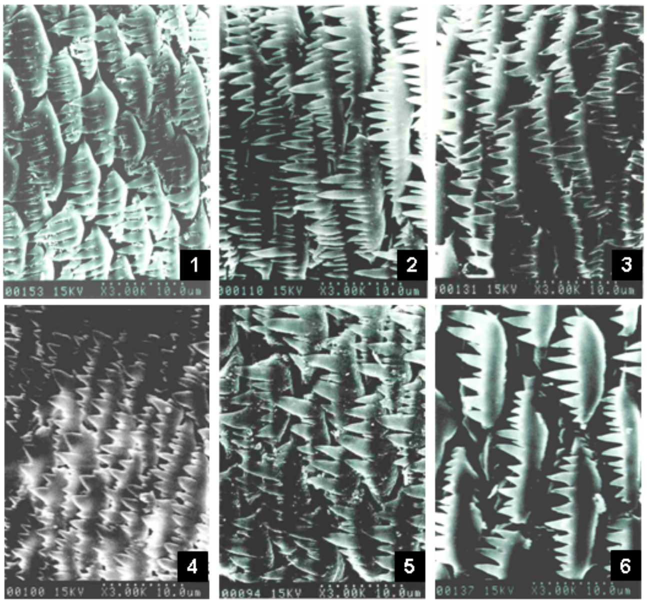

Figure 2. Scanning electron micrographs of spicules on the eversible membranes of ovipositors of flies in the *Bactrocera tau* complex in Thailand: 1) form A, 2) form C, 3) form D, 4) form F, 5) form G, 6) form I.

5) *B. tau* form F (Figure 1.5, Figure 2.4)

Apex of aculeus blunt simple-pointed with 4 pairs of preapical setae plus another pair of setae (Pair 5, Figure 1.5) proximally and well away from the preapical setae. The 2 preapical pairs nearest the apex (pairs no. 1-2) are much larger than the 2 preapical pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and the lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.5). Spicules at the distal end of the eversible membrane with short teeth approximately subequal in size (Figure 2.4).

6) *B. tau* form G (Figure 1.6, Figure 2.5)

Apex of aculeus sharp simple-pointed with 4 pairs of preapical setae. The 2 pairs nearest the apex are much longer than the 2 pairs further away. Lengths of setal pair 1 are approximately equal to those of setal pair 2 and the lengths of setal pair 3 are approximately equal to those of setal pair 4 (Figure 1.6). Spicules at the distal end of the eversible membrane are long with large, thick teeth of different size (Figure 2.5).

7) *B. tau* form I (Figure 1.7, Figure 2.6)

Apex of aculeus trilobed with 4 pairs of preapical setae. The 4 pairs of preapical setae are approximately the same size with pairs 1 and 2 of this form being the shortest and smallest among all the forms studied here (Figure 1.7). Spicules at the distal end of the eversible membrane are long with teeth approximately subequal in size (Figure 2.6).

4. Discussion

The tephritid ovipositor makes intimate contact with the fruit during oviposition. It seems likely therefore that within the *B. tau* complex each form may have an ovipositor adapted to penetrating and ovipositing within its particular host fruit. As such, ovipositor morphology may be expected to vary among forms in the complex. Indeed, differences in ovipositor morphology were found among *B. tau* complex flies in this study, especially in relation to the shape of the aculeus apex, the part of the ovipositor that pierces the fruit surface before oviposition. Here, we discuss the usefulness of ovipositor characteristics for distinguishing species within the *B. tau* complex in Thailand. We then assimilate phylogenetic information from previously published studies (Jamnongluk *et al.*, 2003; Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006) with known host fruit associations and geographical locations from this study and Baimai *et al.*

(2000) and aculeus apex morphology (this study) to elucidate evolution of the *B. tau* complex.

4.1 Ovipositor morphology for distinguishing forms in the *Bactrocera tau* complex

The shape of the aculeus apex is an important character frequently used in descriptions of and in distinguishing tephritid species (e.g., White and Elson-Harris, 1992; Drew, 1989; Drew and Hancock, 1994; Norrbom, *et al.*, 2000). Within the *B. tau* complex, we could classify forms into two main groups based upon aculeus apex shape (Table 2), that is, forms C and I with trilobed apices and forms A, D, E, F and G with simple-pointed apices.

Contrasting reports of *B. tau*-like flies with either simple-pointed or trilobed aculeus apices have resulted in considerable confusion in the taxonomic literature. *Bactrocera tau sensu stricto* is most frequently characterized as having a simple-pointed aculeus apex, e.g., Hardy (1973), Kapoor *et al.* (1989), White and Elson-Harris (1992) and Mahmood (1999). However, Liang *et al.* (1993) reported a species from Gansu Province in mainland China, which they called *Bactrocera* sp. near *tau*, with a trilobed aculeus apex. Liang *et al.* (1993) considered this species to be the same as specimens with trilobed aculeus apices from Taiwan that Hardy (1973) had concluded were *B. nubila* (Hendel). However, White and Wang (1992) examined a female *B. nubila* paralectotype held at the Natural History Museum, London, and determined that it had a simple-pointed and not a trilobed aculeus apex. They therefore placed *B. nubila* in synonymy with *B. tau*, and then suggested that the specimens examined by Hardy (1973) were an undescribed species with a trilobed aculeus that occurred in the same locality as the *B. nubila* type locality.

Mahmood (1999) also considered aculeus tip shape to be an important taxonomic character for distinguishing species within the *B. tau* complex. He determined a *B. tau*-like fly with a trilobed aculeus tip originating from Sichuan Province in China to be a separate species from *B. tau sensu stricto* and re-erected a species name, *B. bezziana* (formerly *Dacus bezziana*), for this fly. Mahmood (1999) further suggested that the Taiwanese specimens determined as *B. nubila* by Hardy (1973) were misidentifications of *B. bezziana*. Clearly, the current taxonomy of the *B. tau* complex is confused and the shape of the aculeus apex is an important character that can help resolve this confusion.

The forms of spicules on the distal ends of the eversible membranes of ovipositors have been investigated for their utility in separating species in another *Bactrocera* species complex, i.e., the *B. dorsalis* complex, by Drew and Hancock (1994). These authors found a diversity of shapes and dentations among *B. dorsalis* complex flies; in some cases the forms of spicules seemed species-specific. Although there was some variability in spicules of *B. tau* flies (Figure 2), the diversity of spicule shapes evident within the *B. dorsalis* complex (Drew and Hancock, 1994) was not evident in *B. tau* flies of this study. Spicule shape and dentation, therefore, do not appear to be useful for separating species within the *B. tau* complex in Thailand.

Like other tephritids (Kozánek and Belcari, 2002), *B. tau* complex flies possessed 4 pairs of preapical setae located in lateral grooves along the apex of the aculeus. These lateral grooves containing the preapical setae can be seen on the aculeus apex in *B. tau* forms A, C, D, F, G and I (Figures 1.1-1.3, 1.5-1.7) but are hidden from view for *B. tau* form E (Figure 1.4). An extra pair of setae is evident on the aculeus of *B. tau* form F (Pair 5, Figure 1.5) situated basally well away from the other 4 pairs. It is not clear whether this pair is situated in or separate from the lateral grooves. A fifth pair is also displayed in the *B. tau* aculeus diagram of White and Elson-Harris (1992), and, like the pair seen in *B. tau* form F, appears separate from those setae situated in the lateral grooves. Kapoor *et al.* (1989) and Hardy (1973) also illustrate 5 pairs of preapical setae in their diagrams of the aculeus apex of *B. tau*, but, in contrast to *B. tau* form F (this study) and White and Elson-Harris (1992), the 5th setal pair appears to be grouped with the other four pairs. White (2000) states that small aculeus tip setae seen using optical microscopy may not be apparent using scanning electron microscopy under some conditions. The existence of fifth setal pairs in other members of the complex and their importance to *B. tau* taxonomy need further investigation.

The relative lengths of the preapical setae may vary considerably in tephritids (Kozánek and Belcari 2002). We investigated the relative lengths of these setae for their suitability in separating *B. tau* forms. We found that six of the seven *B. tau* complex forms were similar with the two distal pairs of preapical setae appearing much longer than the two basal pairs. However, in *B. tau* sp. I of the "trilobed" group, all four pairs of preapical setae were approximately the same length. This could be a useful feature for separating *B. tau* form I from form C of the trilobed group in Thailand, but needs further investigation.

4.2 Host-plant relationships within the *Bactrocera tau* complex

Several phylogenies have been proposed for the *B. tau* complex in Thailand based on molecular and electrophoretic markers (Jamnongluk *et al.*, 2003; Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006). These phylogenetic studies have laid the basis for grouping and distinguishing *B. tau* forms. All phylogenies are very similar, although there are some minor differences in branching patterns. The use of these phylogenies for elucidating the evolutionary history of the *B. tau* complex has only been done in very general terms (see the respective "Discussion" sections of Jamnongluk *et al.* (2003), Thanaphum and Thaenkham (2003), and Saelee *et al.* (2006)). Inferences of *B. tau* complex evolution that include ecological and morphological factors are presented here. In particular, we overlay host fruit relations and geographic location (based on the data of Table 1 and those of

Baimai *et al.* (2000) and Saelee *et al.* (2006)) as well as aculeus apex shape (Table 2) on to a representative phylogeny (Figure 3A of Jamnongluk *et al.* 2003) to develop an evolutionary perspective of the *B. tau* complex in Thailand.

Figure 3 shows the phylogenetic tree simplified from Figure 3A of Jamnongluk *et al.* (2003). The most basal branches of this phylogenetic tree form lineages for *B. tau* forms D, C and I; this is also evident in the other phylogenies (Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006). The karyotype study of Baimai *et al.* (2000) also supports the close relations of *B. tau* forms C with D (*B. tau* form I was not included in their study). *Bactrocera tau* forms C, D and I have only ever been reared from Cucurbitaceae fruit. It therefore seems likely that fruits of species of the family Cucurbitaceae form the ancestral hosts for the *B. tau* complex in Thailand. In the phylogenetic tree of Figure 3, lineages that use Cucurbitaceae host fruits are depicted by black-colored branches. The only other fly in the complex reared from Cucurbitaceae fruit was *B. tau* form A, which appears distantly related to *B. tau* forms D, C and I in all phylogenies. If Cucurbitaceae host fruit use is ancestral, then the most parsimonious explanation of the phylogenetic pattern in Figure 3 is that the *B. tau* form A lineage represents the main lineage from which all other branches derive.

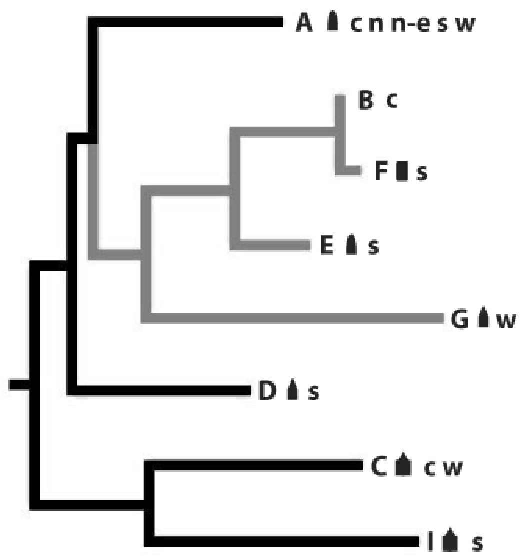

Figure 3. Phylogenetic tree for flies in the *Bactrocera tau* complex in Thailand (redrawn from Jamnongluk *et al.*, 2003) overlain with host fruit, aculeus apex shape and geographical region. Upper case letters represent forms A, C, D, E, F, G and I of the complex used in this study. Form B was not included in the present study of ovipositor morphology but is in the phylogenetic study of Jamnongluk *et al.* (2003), and so is included in the tree here. Lower case letters represent geographical regions from which flies of that form have been collected: c – central; n – northern; n-e – north-eastern; s – southern; w - western. Black branches represent fly lineages that use fruit species in the Family Cucurbitaceae. Gray branches represent fly lineages that use fruit species in non Cucurbitaceae plant families. Symbols represent shapes of aculeus apices: trilobed and simple-pointed – blunt, medium, sharp.

After the lineages of *B. tau* forms C, D and I branched off from that of *B. tau* form A in what appears to be a first radiation, a second radiation onto fruits of non Cucurbitaceae host species occurred (Figure 3). Such a second radiation is indicated in all other published phylogenies as well (Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006). In the phylogeny of Figure 3, a single branch splits off from the main *B. tau* form A lineage comprising *B. tau* forms that use non Cucurbitaceae fruits (gray branch of Figure 3); this branch includes *B. tau* forms F and G, both of which use fruit of *Hydnocarpus anthelminthicus* (Family Flacourtiaceae), *B. tau* form B, fruit of *Siphonodon celastrineus* (Family Celastraceae), and *B. tau* form E, fruit of *Strychnos thorelli* (Family Strychnaceae). This pattern is slightly different to that of other phylogenies (Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006), in which a branch comprising *B. tau* forms B, F and G split from the main *B. tau* form A line first, followed later by another branch splitting from the main line, which included *B. tau* form species E. Although there is disagreement among phylogenies about the order in which branching occurred, all phylogenies consistently indicate that radiation onto non Cucurbitaceae hosts occurred late in the evolutionary history of the *B. tau* complex (Jamnongluk *et al.*, 2003; Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006).

In this study, aculeus tip shape was shown to vary among *B. tau* complex flies. When tip shape is superimposed onto the phylogenetic tree of Jamnongluk *et al.* (2003) as shown in Figure 3, it can be seen that the two trilobed forms, *B. tau* forms C and I, are very closely related and form a branch that is positioned basally within the phylogenetic tree. This same pattern is evident in all other phylogenetic trees published for the *B. tau* complex in Thailand (Thanaphum and Thaenkham, 2003; Saelee *et al.*, 2006). It seems, therefore, that forms with trilobed apices branched off from the main *B. tau* form A lineage relatively early in the evolution of the complex. However, this split does not appear to be host related. *Bactrocera tau* form C has been collected from *Momordica cochinchinensis* fruit and *B. tau* form I from *Trichosanthes cordata* and *T. tricuspidata* (Table 1; Baimai *et al.*, 2000). However, *B. tau* form A with a simple-pointed aculeus apex has also been collected from fruit of these same three plant species (Baimai *et al.*, 2000). The difference in aculeus apex shapes could be related, for example, to oviposition by the trilobed *B. tau* forms C and I into parts of fruits different to those used by the simple-pointed *B. tau* form A. Detailed studies of oviposition behaviour in different *B. tau* flies, particularly in relation to the use of trilobed and simple-pointed aculeii, may reveal the significance of different aculeus apex shapes to the host relations of flies in the *B. tau* complex. For those *B. tau* forms with simple-pointed aculeus apices, the degree of sharpness of the apices does not relate clearly to any branching patterns in Figure 3 and this is also evident in the phylogenetic trees of Thanaphum and Thaenkham (2003) and Saelee *et al.* (2006).

It is not certain what initiated the two radiations of *B. tau* forms. Examination of geographic locations (Figure 3) indicates that *B. tau* forms B, C, D, E, F, G and I that split from the main *B. tau* form A lineage all occur in southern, central or western Thailand. None of these forms occur in north or northeast Thailand, even though numerous collections of fruit species were made in these latter two regions (Baimai *et al.*, 2000). It seems possible, therefore, that past, unstable, environmental conditions in southern, central and western Thailand may have resulted in the narrowing of distributions and isolation of *B. tau* fly populations with novel fruit species causing the formation of new fly-fruit associations followed by the evolutionary development of new *B. tau* forms. In the case of *B. tau* forms C, D and I, *B. tau* form A utilizes the same fruit species as these forms even in the same provinces (see data in Baimai *et al.*, 2000). It is postulated, therefore, that this first *B. tau* radiation involved environmental changes that isolated *B. tau* form A populations with their Cucurbitaceae hosts. Environmental pressures in these isolated populations may have resulted in the need for penetrating host fruit in a different way when ovipositing, causing the evolution of trilobed aculeus apices in *B. tau* forms C and I. Later environmental changes may then have brought *B. tau* forms C and I back into sympatry with form A resulting in the current patterns of *B. tau* form distribution, host-plant relations and ovipositor morphology.

5. Conclusions

Table 2 summarizes the results of our ovipositor morphological study of the *B. tau* complex in Thailand. Ovipositors of flies in the *B. tau* complex were classified into two main types based on the shape of the aculeus tip as either trilobed or simple-pointed. Aculeus tip shape has frequently been invoked to settle disputes regarding the species status of *B. tau* taxa (see Section 4.1). Other characters of ovipositors, such as the positions and relative lengths of preapical setae may also prove useful in characterizing *B. tau* species with further investigation. Based on aculeus apex shape, there are at least two species within the *B. tau* complex in Thailand, that is, a trilobed species (forms C and I) and a simple-pointed species (forms A, D, E, F and G). However, Baimai *et al.* (2000), Jamnongluk *et al.* (2003), Thanaphum and Thaenkham (2003) and Saelee *et al.* (2006) all considered there were sufficient karyotype, molecular genetic and electrophoretic differences among *B. tau* forms to warrant separating them into more than two species. Morphological differences have also been suggested to distinguish these *B. tau* forms (Saen Tivatananont unpublished). It is now imperative to publish taxonomic descriptions and names for species that occur within the *B. tau* complex in Thailand.

Superimposition of aculeus apex shape, host fruit species and geographic location onto a previously published phylogenetic tree (Figure 3) allowed the postulation of the evolutionary history of the *B. tau* complex. It was found that fruits in the Family Cucurbitaceae appeared to be ancestral hosts for the *B. tau* complex and that the use of fruits in other plant families stemmed late in the complex's evolutionary history. Phylogenies also indicated that the trilobed condition of the aculeus formed a unique lineage that branched from the main simple-pointed main lineage early in the evolutionary history of the complex. Other than the polyphagous *B. tau* form A, other *B. tau* forms seem confined to the southern, central and western regions of Thailand and it is postulated that radiations that resulted in branching from the main *B. tau* lineage occurred in these regions. Despite extensive sampling of fruits for *B. tau* complex flies throughout Thailand (Baimai *et al.*, 2000), other members of the complex undoubtedly await discovery. The inclusion of additional flies within the *B. tau* complex into phylogenies would allow more robust tests of evolutionary hypotheses as well as provide further insights into the evolutionary history of this interesting complex.

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Table 2. Summary of ovipositor characters used to delineate forms within the *B. tau* complex in Thailand.

Form	Aculeus apex		Lengths of setal pairs 1 & 2 *cf* lengths of setal pairs 3 & 4
	Shape	Sharpness	
A	simple	medium	greater than
C	trilobed	-	greater than
D	simple	sharp	greater than
E	simple	medium	greater than
F	simple	blunt	greater than
G	simple	sharp	greater than
I	trilobed	-	approx. equal

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