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The geometric approach to explore the *Bactrocera tau* complex (Diptera: Tephritidae) in Thailand

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ABSTRACT

Specimens of the genus *Bactrocera* were collected from several host plants in northern and western Thailand. They were morphologically recognized as *Bactrocera tau* and were subdivided into eleven samples according to host plant, geographic origin and time of collection. Twelve landmarks of the right wing were described in a total of 264 males and 276 females. An exploratory analysis using kernel density estimates was performed on the multivariate morphometric space. Non-parametric classification highlighted the existence of two non-overlapping clusters within both males and females. The clusters were not congruent with geography. One cluster (cluster I) contained only one plant, *Momordica cochinchinensis*, the other one (cluster II) contained five different plants including *M. cochinchinensis*. Further morphometric analyses on selected samples indicated that the influence of the plants on the shape of the wing could not explain satisfactorily the presence of two clusters. Genetic techniques identified the presence of *B. tau* cryptic species C in *M. cochinchinensis* from cluster I, and of *B. tau* cryptic species A in *Coccinia grandis* from cluster II. Our working hypothesis is that the two clusters identified by geometric morphometrics were species A and C, respectively.

1. Introduction

Fruit flies are the world's worst pests of fruits causing enormous economic loss every year (White and Elson-Harris, 1992; Aluja et al., 1996; Armstrong and Jang, 1997). Tephritid flies of the genus *Bactrocera* (Family Tephritidae) are of particular concern throughout much of Asia and Australia, where they constitute a significant threat to agricultural resources (Nagappan et al., 1971; Fletcher, 1987; Han et al., 1994; White, 1996; Kinnear et al., 1998; Kim et al., 1999).

In Thailand and other South-East Asian (SEA) countries, the genus *Bactrocera* is known for being one of the major pests of tropical fruits and vegetables (Hardy, 1973; Drew and Romig, 1997). Most host plants belong to the family Cucurbitaceae, e.g., species of *Coccinia*, *Cucurbita*, *Cucumis*, *Luffa*, *Momordica*, *Trichosanthes*, etc. (Hardy, 1973; White and Elson-Harris, 1992). Various species are of great concern, such as *Bactrocera dorsalis* (Hendel), the oriental fruit fly, infesting a very wide range of fruits (Drew, 1989; Baimai et al., 2000); *Bactrocera* (*Zeugodacus*) *tau* (Walker), infesting a more restricted range of host plants, and the melon fly *Bactrocera cucurbitae* (Areekul, 1986; Yang et al., 1994). Compared with *B. cucurbitae*, *B. tau* is a more destructive species, especially in Taiwan and China (Yang et al., 1994; Chen, 2001).

Morphological variation within *B. tau* led some authors to suspect the presence of various species within the taxon (Hardy, 1973; White and Elson-Harris, 1992). Particularly, Drew and Romig (1997) suggested that *B. tau* is a large complex of sibling species in the SEA region. Cytogenetic studies (Baimai et al., 2000), multilocus enzyme electrophoresis (MLEE) (Saelee et al., 2006) and DNA studies (Jamnongluk et al., 2003; Thanaphum and Thaenkham, 2003) confirmed this theory and recognized at least 7 species in the *B. tau* complex in Thailand. The members of the complex are cryptic species, i.e. morphologically very close, and have been labeled as species A, C, D, E, F, G and I (Baimai et al., 2000), with species A being *B. tau sensu stricto*.

Although the *B. tau* members were well classified by cytogenetics, MLEE and DNA techniques, their systematics still requires intensive investigation. Using the non-parametric kernel density estimates and the principal component analyses of shape, the present study explores the venation geometry of the wings as a character employable for pattern recognition. Examining the clusters as defined in our dataset by the kernel density technique, we suggest that the geometric approach can help in the identification of cryptic taxa. It also raises interesting questions about the possible effects of host plants and species competition on morphology.

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Fig. 1. Geographic origin of the fruit flies: 186 flies from Nan (NA) and 149 flies from Chiangmai (CM) in northern Thailand; 205 flies from Kanchanaburi (KN) in western Thailand.

2. Materials and methods

2.1. Insect samples

Oviposited eggs and larvae of the *B. tau* complex were collected from infested fruits of five host plant species in the family Cucurbitaceae: *Coccinia grandis* (CG), *Cucurbita moschata* (CMo), *Cucumis sativus* (CS), *Momordica cochinchinensis* (MC) and *Trichosanthes tricuspidata* (TT). They were obtained from three localities: Nan (NA) and Chiangmai (CM) in northern Thailand, and Kanchanaburi (KN) in western Thailand (Fig. 1). Fruits with ovipositional scars or marks of larval infestation were collected and kept in the laboratory with a code indicating location, host plant and time of collection (Table 1). The temperature of the laboratory was maintained at 27 ± 2 °C, with 70 ± 10% relative humidity and a photoperiod of 12L:12D. Newly emerged adults were reared in transparent plastic cages (12 cm × 33 cm × 18 cm). They were provided with 10% honey/distilled water solution and sugar mixed with yeast hydrolyzate for at least 2 weeks to ensure all morphological characters developed well, especially the color and shape of abdominal bands typical of the *B. tau* complex. The population density by fruit was not scored.

Table 1
Material of the *Bactrocera tau* complex used in this study. M, males; F, females; KN, Kanchanaburi; CM, Chiangmai; NA, Nan; CG, *Coccinia grandis*; CMo, *Cucurbita moschata*; MC, *Momordica cochinchinensis*; TT, *Trichosanthes tricuspidata*; CS, *Cucumis sativus*. Numbers after plant abbreviations (-1, -16, -19, -19/3, -20, etc.) are codes referring to the time of collection.

Host plant species	Locality	Code of the fruit	M	F
Coccinia grandis	Kanchanaburi	KN(CG)-26	16	16
Cucurbita moschata	Kanchanaburi	KN(CMo)-20	21	21
Cucurbita moschata	Kanchanaburi	KN(CMo)-30	21	20
Momordica cochinchinensis	Kanchanaburi	KN(MC)-27	21	35
Momordica cochinchinensis	Kanchanaburi	KN(MC)-31	20	14
Momordica cochinchinensis	Chiangmai	CM(MC)-1	67	82
Momordica cochinchinensis	Nan	NA(MC)-19	20	20
Momordica cochinchinensis	Nan	NA(MC)-16	21	21
Momordica cochinchinensis	Nan	NA(MC)-19/3	18	9
Trichosanthes tricuspidata	Nan	NA(TT)-38	19	17
Cucumis sativus	Nan	NA(CS)-32	20	21
			264	276

2.2. Specimen preparation and data collection

The left and right wings of the specimens were removed with forceps and mounted in Hoyer medium on glass microscopic slides. All slides were photographed by using a dissection stereo-microscope connected to a digital camera system with a 4× lens (40×). Twelve landmarks were digitized on the wings (Fig. 2) according to "type I" classification (venation intersections) (Bookstein, 1991). Only the right wing was used unless damaged, in which case the left wing was used. To avoid possible optical distortion at the periphery of the optical lens, each wing was located at the center of the visual field (Caro-Riaño et al., 2008).

To reduce error at digitizing the landmarks, the same person collected the landmarks for all the wings. The precision was estimated by comparing two sets of measurements on a subset of 42 individuals (21 males and 21 females). It was computed as the "repeatability" index (R) (Arnqvist and Mårtensson, 1998) of the first two principal components of shape ("relative warps", or RW, see Section 2.3.2), where R is provided by the ratio of the between-individual variance and the total variance.

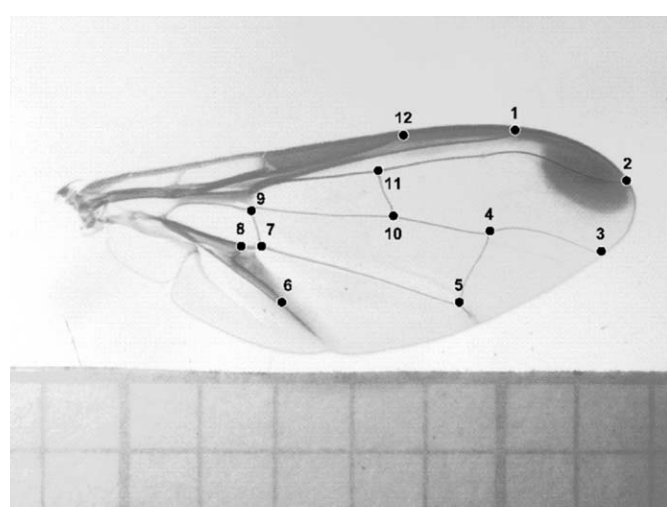

Fig. 2. Fore wing of *Bactrocera tau* showing the 12 landmarks whose coordinates were used in morphometric analyses. Each landmark is located at the junction of two different veins, as required by type I landmarks (Bookstein, 1991). Each picture contains a millimeter paper (see bottom) for true size scaling.