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MOLECULAR PHYLOGENY OF BACTROCERA SPECIES (DIPTERA: TEPHRITIDAE: DACINI) INFERRED FROM MITOCHONDRIAL SEQUENCES OF 16S rDNA AND COI SEQUENCES

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ABSTRACT

Many fruit flies in the genus Bactrocera (Diptera: Tephritidae: Dacini) are economically important insects. However, little attention has been given to the molecular phylogenetic relationship among Bactrocera subgenera. We explored the phylogenetic relationship among the 8 subgenera Afrodacus, Austrodacus, Bactrocera, Daculus, Gymnodacus, Paratridacus, Tetradacus, and Zeugodacus based on the sequences of 2 mitochondrial DNA fragments with a combined length of 1034 base pairs. The 2 mtDNA fragments are a 689-bp segment of the COI gene and a 345-bp segment of the 16S rDNA gene. Thirty-five individuals representing 7 Bactrocera species found in the Chongqing region in China were sequenced for both fragments, and sequences of the same gene regions were acquired from GenBank for another 20 Bactrocera species and 2 other tephritid species, Anastrepha ludens and Ceratitis capitata, which were used as outgroups for the phylogenetic analyses. We reported Bactrocera (Tetracus) minax and Bactrocera (Zeugodacus) diaphora sequences for the first time, and the subgenus Bactrocera (Tetracus), here represented by B. (T.) minax and B. (T.) tsuneonis, was included for the first time in an analysis of the genus Bactrocera phylogeny. Results of our analyses showed within-subgenus nucleotide diversity ranged from 9.1 to 19.0% among the subgenera, and the net divergence among subgenera ranged from 4.6 to 12.7%. Results of phylogenetic analyses based on maximum parsimony method supported that subgenus Bactrocera (Bactrocera) and Bactrocera (Zeugodacus) are paraphyletic. The subgenus Zeugodacus, Bactrocera (Zeugodacus) caudate, Bactrocera (Zeugodacus) diaphora, and Bactrocera (Zeugodacus) scutellata are closely related to Bactrocera (Zeugodacus) minax and Bactrocera (Zeugodacus) cucurbitae. This results indicated that subgenus Austrodacus and Zeugodacus, which attack cucurbit plants, are closely related to species of the subgenus Afrodacus, Bactrocera, and Gymnodacus, which attack plants of numerous families. In addition, subgenus Paratridacus is a sister group to subgenus Tetradacus, and 7 species of the Bactrocera (Bactrocera) dorsalis complex (as defined by Drew & Hancock 1994) included in this study formed a monophyletic clade. Subgenus Daculus is lineage by itself, which does not fall into the Bactrocera group or Zeugodacus group.

Key Words: Bactrocera spp., 16s rDNA, COI, mitochondrial DNA, molecular phylogeny

RESUMEN

Muchas moscas de la fruta en el género Bactrocera (Diptera: Tephritidae: Dacini) son insectos economicamente importante. Sin embargo, se han puesto poca atención en cuanto a la relación filogenética molecular entre los subgéneros de Bactrocera. Exploramos la relación filogenética entre los 8 subgéneros Afrodacus, Austrodacus, Bactrocera, Daculus, Gymnodacus, Paratridacus, Tetradacus, y Zeugodacus basado en las secuencias de 2 fragmentos de ADN mitocondrial con un longitud combinado total de 1034 pares de bases. Los 2 fragmentos de mtADN son un segmento de 689-pb del gene COI y un segmento de 345-pb de gene 16S rADN. Se secuenciaron treinta y cinco individuos representando 7 especies de Bactrocera encontrados en la región de Chongqing en China para ambos fragmentos, y secuencias de las mismas regiones de los genes fueron adquiridas del GenBank para otras 20 especies de Bactrocera y otras 2 especies de tefritidos, Anastrepha ludens y Ceratitis capitata, que fueron usados como grupos externos para el análisis filogenético. Reportamos las secuencias de Bactrocera (Tetracus) minax y Bactrocera (Zeugodacus) diaphora por la primera vez, y el subgénero Bactrocera (Tetracus), aquí representado por B. (T.) minax y B. (T.) tsuneonis, fueron incluidos por la primera vez en el análisis de la filogenia del género Bactrocera. Los resultados de nuestro análisis mostraron una diversidad de 9.1 a 19.0% entre los nucleótidos dentro de los subgéneros, y una divergencia total entre los subgéneros de 4.6 a 12.7%. Los resultados del análisis filogenético basado en el método de parsimonia maxima apoyaron que ambos subgéneros Bactrocera (Bactrocera) y Bactrocera (Zeugodacus) son parafiléticos. Los subgéneros Zeugodacus, Bactrocera (Zeugodacus) caudate, Bactrocera (Zeugodacus) dia-
The genus *Bactrocera* (Diptera: Tephritidae: Dacini) is widespread in Asia and Australia and is one of the largest genera within Tephritidae with about 500 described species arranged in 28 subgenera (Drew 1989; Drew & Hancock 2000). Several *Bactrocera* species are serious pests of fruits and vegetables (Allwood et al. 1999; White et al. 1992).

*Bactrocera* and *Dacus* are sister taxa which share the following apomorphies: radial veins crowded anteriorly and medial cells very broad; female abdominal tergite 6 separate from preceding tergites; and tergite 5 of both sexes with glandular areas (“ceromae”) (Munro 1984). Similar to many other tephritid genera, classification and taxonomy of the group is controversial. Taxonomic status of this group has been repeatedly revised since it was first recognized in 1835, (Drew 1972; Hardy 1955, 1976), and its current status as a genus was established by Drew (1989). Taxonomic positions of related groups have also been subject to changes. This situation results from differences in the morphological features used in the various taxonomic studies, some of which are quite questionable (Drew 1989; Drew & Hancock 1994; White & Hancock 1997; Drew & Hancock 2000; White 2000). After White (2000) chose 37 morphological characters from 51 economically important species and quantitatively analyzed cladistically *Bactrocera* species, representing 9 *Bactrocera* subgenera, he pointed out that independent characters, such as DNA sequences, should play a more important role in rigorous phylogenetic analyses. Many closely related sibling species are not morphologically distinct. For example, the *B. dorsalis* complex presumably includes more than 60 geographically diverse species (Drew & Hancock 1994), the majority of which were treated as a single species before a revisionary report by Drew & Hancock (1994). Therefore, it is highly desirable to search for more stable and reliable methods to study the evolutionary relationships among *Bactrocera* taxa and use this information to solve the taxonomic placement of the problematic species. Mitochondrial DNA sequences have been used as common molecular markers in phylogenetic analyses and population genetic studies in animals (Boyce et al. 1994; Langor & Sperling 1997). The advantage of using mitochondrial genes in evolutionary study is that mutations that create new haplotypes are rare. Therefore, 2 individuals that share the same haplotype are likely to have a common ancestor (Li 1997). An A+T bias has been found in most insect mtDNA genes (Lunt et al. 1996; Han & McPheron 1997; Langor & Sperling 1997) and it has been suggested that regions with high A+T content might be useful for studying phylogenetic relationships among closely related insect species (Lunt et al. 1996). The phylogenetic relationships of some tephritid taxa have been resolved with strong support based on mtDNA sequence data (Han & McPheron 1997; Han 2000), especially at the generic level (Smith & Bush 1997).

In the present study, we conducted combined analyses of 16s rDNA and COI mtDNA sequence data in order to resolve the phylogenetic relationships of *Bactrocera* fruit flies. In most cases, combined analyses are more likely to recover a phylogenetic tree close or identical to the “true” tree, because the amount of information available to infer a phylogenetic tree is maximized (Smith 2002, 2003; Muraji & Nakahara 2001). All the genes used were mitochondrial, and thus, presumably share the same evolutionary history. The approximately 1040-bp long fragment of the mtDNA contains the 16S rDNA (about 345-bp) and COI (about 690-bp) (Fig. 1). Both 16s rDNA and COI sequences of *B. (T.) minax* and *B. (Z.) diaphora* are reported for the first time and subgenus *Tetradacus* including *B. (T.) minax* and *B. (T.) tsuneonis* also is introduced into *Bactrocera* phylogenetic analysis for the first time. The results are discussed in relation to the phylogenetic and diagnostic utility of the mtDNA fragment, and to the taxonomic positions of each species included in this study.

**MATERIALS AND METHODS**

Collection and Handling of Fruit Flies

A list of analyzed taxa including origin and associated GenBank accession number is presented in Table 1. The specimens were collected from different host plants growing in various areas of Chongqing region from May to Nov 2007. All of them are *Bactrocera* species, including *Bactrocera cucurbitae* (Coquillet), *B. tau* (Walker), *B. dio-
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B. phor a (Hendel), B. caudate (Fabricius), B. scutellata (Hendel), B. dorsalis (Hendel), and B. minax (Enderlein). Specimens were stored in absolute ethyl alcohol at -4°C until required for molecular analysis.

Template Preparation and DNA Manipulation

Total DNA was extracted from individual fruit fly adults by the crude boiling methods (O’Neill et al. 1992). Thoracic tissue was homogenized with a sterilized pestle in a 1.5-mL microcentrifuge tube filled with 100 μL of STE buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8.0)), and 1 mM EDTA (pH 8.0). The homogenate was heated at 95°C for 10 min before being centrifuged at 4000 rpm for 1 min at room temperature. Two microliters of supernatant were used as the DNA template for the polymerase chain reaction (PCR).

Two different DNA fragments comprising portions of the 16S rRNA and Cytochrome Oxidase I mitochondrial genes were amplified and sequenced with the oligonucleotide primers listed in Table 2. The 16s rDNA primers used in this study were designed by Simon (1994) and Muraji (2002). The primers were used to amplify a 350-bp fragment from 35 individuals of the 7 species noted above. Polymerase chain reaction (PCR) amplifications were performed in 20 μL-volumes. The specific volumes were 5μL DNA template, 6.05 μL dd H2O, 3.75 μL 10×PCR buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8.0)), and 1 mM EDTA (pH 8.0). The homogenate was heated at 95°C for 10 min before being centrifuged at 4000 rpm for 1 min at room temperature. Two microliters of supernatant were used as the DNA template for the polymerase chain reaction (PCR).

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Fig 1. The position plot of 16s rDNA and CO1 gene fragment used in present study.

Data Analysis

In addition to mitochondrial 16s rDNA and COI gene sequences of the 27 Bactrocera species, 16s rDNA and COI sequences of the same region for Ceratitis capitata and Anastrepha ludens were used in the analysis as outgroups.

A consensus sequence of 16s rDNA and COI fragments combined from 1 specimen of each fruit fly species was constructed by using the SeqMan program (DNastar, Lasergene). The sequences were initially aligned in the Clustal X 1.81 program (Thompson et al. 1997) and manually adjusted as needed. Nucleotide sequence differences and the overall transition-transversion ratio...
among the Bactrocera species were calculated with MEGA software 4.1. The Jukes-Cantor distance method was used to calculate nucleotide sequence differences. We conducted Maximum Parsimony (MP) and Neighbour-joining (NJ) analysis with PAUP* 4.1 and heuristic search procedure with TBR (tree bisection reconnection) swapping and 100 maxtree options. The gaps were treated as missing data. Bootstrap analyses were done with 1000 replicates.

Jukes-Cantor distance is recommended when the value is lower than 0.3 (Kumar et al. 1993), Kimura 2-parameter distance was used when the transition/transversion ratio was high, and Tamura and Tamura-Nei distances were used when A + T content bias was obviously high. In the present study, the number of nucleotide substitutions per site ranged from 0.09 to 0.18, overall transition (ti)/transversion (tv) ratio was 1.451 and A + T content occupied 68.1%. We chose all 3 methods and we found similar result. In the present study, we use the result by Jukes-Cantor to generate Neighbour-joining tree.

TABLE 1. LIST OF TAXA EXAMINED WITH GEOGRAPHIC ORIGIN AND GENBANK ACCESSION NUMBERS.

| Species | 16s rDNA | COI | Original region |
|---------|----------|-----|-----------------|
| Bactrocera (Bactrocera) carambolae | EF014414* | EF014414* | NT, OR |
| Bactrocera (Bactrocera) correcta | AB048752* | AY530905* | OR |
| Bactrocera (Bactrocera) dorsalis | FJ866822 | GQ458045 | OR, AU |
| Bactrocera (Bactrocera) latifrons | FJ009200* | FJ903498* | OR, AU |
| Bactrocera (Bactrocera) musae | AB074023* | AB192432* | AU |
| Bactrocera (Bactrocera) papayae | DQ917578* | DQ917578* | OR |
| Bactrocera (Bactrocera) philippinensis | DQ995281* | DQ995281* | OR |
| Bactrocera (Bactrocera) zonata | AB048757* | AB192445* | AF, OR, AU |
| Bactrocera (Afrodacus) jarvisi | AB074022* | AB192448* | AU |
| Bactrocera (Austrodacus) cucumis | AB074019* | AB192448* | AU |
| Bactrocera (Bactrocera) curcipennis | AB074020* | AY530895* | AU |
| Bactrocera (Bactrocera) frauenfeldi | AB074021* | AB192428* | AU |
| Bactrocera (Bactrocera) kandiensis | AB048738* | AB192431* | OR |
| Bactrocera (Bactrocera) occipitalis | AB048742* | AB192435* | OR |
| Bactrocera (Bactrocera) psilii | AB074027* | AB192440* | AU |
| Bactrocera (Bactrocera) tryoni | AB074029* | AT530892* | AU |
| Bactrocera (Bactrocera) umbrosa | AB048749* | AY530897* | OR, AU |
| Bactrocera (Daculus) oleae | AB1210702* | AY210702* | PA, AF, OR |
| Bactrocera (Gymnodacus) calophylli | AB035109* | AB192419* | OR, AU |
| Bactrocera (Paratridacus) expansens | AB035110* | AB192427* | UK |
| Bactrocera (Tetradacus) minax | FJ866821 | GQ458044 | OR |
| Bactrocera (Tetradacus) tsuneonis | DQ419809* | AB192447* | PA, OR |
| Bactrocera (Zeugodacus) cadiate | FJ866826 | GQ458048 | PA, OR |
| Bactrocera (Zeugodacus) diaphora | FJ866824 | GQ458043 | OR |
| Bactrocera (Zeugodacus) scutellata | FJ866825 | GQ458046 | PA, OR |
| Bactrocera (Zeugodacus) tau | FJ866823 | GQ458047 | OR |
| Bactrocera (Zeugodacus) cucurbitae | FJ866820 | GQ458042 | PA, AF, OR, AU |
| Anastrepha ludens | AB035102* | AB192462* | NE, NT |
| Ceratitis capitata | AJ242872* | AB192447* | NT, PA, AF, AU |

*Represent the data is previously published sequence obtained from GenBank.
AF = Afrotopical; AU = Australasian; HO = Holarctic; NE = Nearctic; NT = Neotropical; OR = Oriental; PA = Palearctic; and UK = Unknown.

TABLE 2. Oligonucleotide primers used for polymerase chain reaction (PCR) amplifications.

| Name | Sequence |
|------|----------|
| (16s rDNA-F) F1 | 5'-ATCCACATCGAGGTGCAAAC-3' |
| (16s rDNA-R) R1 | 5'-GGCTGTTGATGAGGTTGACGAG-3' |
| (CO1-F) UEA-7 | 5'-TACAGTTGGAATAGACGTTGATAC-3' |
| (CO1-R) UEA-10 | 5'-TCAATGCATAAATCTGCAATTATA-3' |
RESULTS

Characterization of the Nucleotide Data

Through MEGA 4.1, a total of about 1,049 bp nucleotide sequences of the 16s rDNA and COI combined genes among the genus Bactrocera were employed in the analyses. The overall mean sequence divergence among the Bactrocera species was 11.0%. Between different subgenera, the highest nucleotide sequence divergence was found between Tetradacus and Gymnodacus (18.1%) and the lowest between Bactrocera and Afrodacus, Zeugodacus and Austrodacus (9.0%). (Table 3)

The nucleotide frequencies are 0.336 (A), 0.345 (T), 0.194 (C), and 0.124 (G). The base composition of the 2 mitochondrial gene fragments was biased toward adenine (A) and thymine (T), which together constituted an estimated 68.1% of the total. The overall transition (ti)/transversion (tv) ratio was 1.451. Among transitions, 18.36% were A-G transitions and 49.8% were C-T transitions. The estimated relative proportions of the 8 types of transversions were: A-T; 10.85%; A-C; 8.44%; G-T; 7.48%; and G-C; 5.07%. Summary statistics for the different substitutional changes are shown in Table 4.

Amino acids varied at 83 locations across the 347 amino acid sequences of the segment of 16s rRNA. Amino acid variation sites were found, and fewer amino acid variation sites (17 sites) were found within the subgenus Zeugodacus.

Phylogenetic Analyses

In the 1,049 characters including two out groups, 350 (33.4%) were variable, and 283 (27.0%) were parsimony informative. The character statistics and results of parsimony analysis are shown in Table 5. The consensus tree generated by Maximum parsimony indicated the following relationships: (1) subgenus Bactrocera is paraphyletic, (2) subgenus Zeugodacus is paraphyletic, (3) subgenus Paratridacus is a sister group to subgenus Tetradacus, (4) subgenus Daculus, represented here by Bactrocera (Daculus oleae) show different classification positions in NJ and MP phylogenetic trees (Fig. 2 and Fig. 3), but subgenus Daculus is 1 lineage by itself, (5) subgenus Austrodacus and Zeugodacus were closely related to the subgenus Afrodacus, Bactrocera, and Gymnodacus, and (6) seven species of the B. (B.) dorsalis complex (as defined by Drew & Hancock 1994) included in this study form a monophyletic clade (Fig. 2 and Fig. 3).

DISCUSSION

Some researchers have proposed a phylogenetic analysis of the Bactrocera subgenera groupings based on morphological characters (Drew 1989; Drew & Hancock 2000; White 2000). According to Drew (1989), the subgenera of Bactrocera were divided into 4 groups, the Bactrocera group, Queenslandacus group, Zeugodacus group, and Melanodacus group. In the present study, the subgenera Afrodacus, Tetradacus, and Gymnodacus are placed in the Bactrocera group and the subgenus Austrodacus in the Zeugodacus group. This agrees with the classification by Drew (1989). However, subgenus Paratridacus is located within the Bactrocera group in our study, and this differs from Drew (1989), who classified Paratridacus in the Zeugodacus group. Muraji & Nakahara (2001) used mitochondrial DNA sequences from 18 Bactrocera species in 4 subgenera to investigate the evaluation of Bactrocera, and their study supported our result that subgenus Paratridacus should not be in the Zeugodacus group but in the Bactrocera group. Drew's (1989) classification was not based on cladistic principles but only on the shape of male sternite 5 and length of male surstylus lobe. We suggest that Paratridacus should be put in the Bactrocera group of subgenera.

The subgenus Daculus, represented here by B. (D.) oleae, shows different classification positions

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Table 3: Nucleotide sequence differences of the combined data sets by 16s rDNA and Cytochrome oxidase I gene based on the Jukes-Cantor distance method.

| Subgena       | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|---------------|----|----|----|----|----|----|----|----|
| Bactrocera    |    |    |    |    |    |    |    |    |
| Afrodacus     | 0.091 |    |    |    |    |    |    |    |
| Gymnodacus    | 0.101 | 0.121 |    |    |    |    |    |    |
| Zeugodacus    | 0.122 | 0.127 | 0.154 |    |    |    |    |    |
| Austrodacus   | 0.126 | 0.122 | 0.155 | 0.092 |    |    |    |    |
| Daculus       | 0.120 | 0.127 | 0.138 | 0.125 | 0.131 |    |    |    |
| Paratridacus  | 0.135 | 0.141 | 0.142 | 0.153 | 0.157 | 0.143 |    |    |
| Tetradacus    | 0.168 | 0.177 | 0.190 | 0.176 | 0.183 | 0.178 | 0.178 |    |

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in NJ and MP phylogenetic trees. According to Drew (1989), B. (D.) oleae belongs to the Melanodacus group, and never falls into the Bactrocera group or Zeugodacus group. Smith et al. (2003), however, indicated that B. (D.) oleae fell within the Bactrocera group.

The question whether subgenus Bactrocera is monophyletic or paraphyletic has been debated. White (2000) chose 37 morphological characters from 51 economically important species to quantitatively analyze cladistically genus Bactrocera species, which represented 9 Bactrocera subgenera. White’s (2000) study indicated that B. (Bactrocera) is paraphyletic in both unweighted and weighted analyses, and based on DNA sequences data, Muraji & Nakahara (2001) also proposed that subgenus Bactrocera was paraphyletic. However, Smith et al. (2003) reported that subgenus Bactrocera was monophyletic. Drew (1989) reported that there were various characters in the presence of a medial postsutural vitta and the absence of a prescutellar bristle among species belonging to subgenus Bactrocera. Absence of a prescutellar bristle was an important diagnostic character to discriminate among genera of Bactrocera and may be a reason for the debate whether subgenus Bactrocera is monophyletic or paraphyletic. Both NJ trees and MP phylogenetic trees in this study indicated subgenus B. (Bactrocera) is paraphyletic because both subgenus Gymnodacus and subgenus Afrodacus locate within the clade of subgenus Bactrocera. Further analyses must be conducted to examine phylogenetic classification of subgenus Bactrocera.

Our study demonstrated subgenus Zeugodacus is paraphyletic, and is based on the result that subgenus Austrodacus is located within the clade of subgenus Zeugodacus. This result is supported by Smith et al. (2003), who proposed that subgenus Zeugodacus is paraphyletic. However, Muraji & Nakahara (2001) believed that the subgenus Zeugodacus is a monophyletic clade, but they were uncertain of the conclusion because of limitation of samples. The subgenus Zeugodacus, the main tephritid species in Chongqing region, was divided into 2 groups, with one as B. (Z.) caudate, B. (Z.) diaphora, and B. (Z.) scutellata, and the other as B. (Z.) tau and B. (Z.) cucurbitae. Muraji & Nakahara (2001) reported that B. (Z.) tau and B. (Z.) cucurbitae were closely related to B. (Z.) scutellata.

Phylogenetic tree analysis also showed that the subgenera Austrodacus and Zeugodacus were closely related to the subgenera Afrodacus, Bactrocera, and Gymnodacus. An interesting phenomenon is that the former clade includes subgenera Austrodacus and Zeugodacus usually attack cucurbit plants, but the latter clade that includes subgenera Afrodacus, Bactrocera, and Gymnodacus prefers to attack plants of numerous families.

The 7 members of the B. (B.) dorsalis complex species (as defined by Drew & Hancock 1994) included in this study are monophyletic. Smith et al. (2003) once analyzed 4 members of the B. (B.) dorsalis complex species (as defined by Drew & Hancock 1994) and also found that the B. (B.) dorsalis complex species are monophyletic. Muraji & Nakahara (2001) supported that B. (B.) dorsalis complex species are monophyletic. All these results show that although some B. (B.) dorsalis complex species have quiet different biological features from each other, the B. (B.) dorsalis complex species seem to have a common ancestor.

The complete sequence of mitochondrial genome of B. (B.) dorsalis complex species (Bactrocera (Bactrocera) dorsalis, Bactrocera (Bactrocera (Bactrocera...
cera) papaya, Bactrocera (Bactrocera) carambola, and Bactrocera (Bactrocera) philippinensis) has been deposited in GenBank. More and more complete sequence of mitochondrial genome of B. (B.) dorsalis complex species sequenced will contribute to rigorous phylogenetic analyses about B. (B.) dorsalis complex species.

In present study, 2 subgenus Tetradacus species were included in a phylogenetic analysis for the first time. We found that subgenus Tetradacus is a sister group to subgenus Paratridacus, and the subgenus Tetradacus has greater genetic distance to other subgenera in Bactrocera group. Because the subgenus Tetradacus has not been introduced into phylogenetic studies before, it is necessary that further molecular phylogenetic studies should be done to examine taxonomic status of subgenus Tetradacus.

Although there are some molecular evolutionary studies of genus Bactrocera phylogeny, how to choose appropriate gene sections to infer taxonomic clades is still a problem. Some researchers tended to use longer gene sequences to study genus Bactrocera phylogeny (Smith 2002, 2003; Muraji & Nakahara 2001), but too long sequences require much work and time. Additionally, the main reason for debate on the phylogeny of Bactrocera species is limited taxon samples, e.g., many taxon sites only were represented 1 species. Morphological classification is still the basic of phylogenetic analysis for the Bactrocera. More cladistic characters should be investigated in the future; e.g., Smith et al. (2003) suggested that the male surstylus lobe is the more important in phylogenetic analysis of Bactrocera. They also pointed out that a short posterior lobe of the male surstylus and a shallow V-shaped emargination of male sternite 5 are plesiomorphic and a deep V-shaped emargination of male sternite 5 is apomorphic. Some researchers have proposed that different attraction reactions to methyleugenol and cue-lure can be a reliable way to discriminate species within Bactrocera (White & Hancock 1997; Drew & Hancock 2000; White 2000; Smith et al. 2003).

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REFERENCE CITED

ANNA, Z., ANTIGONE, Z., AND ZACHARIAS, G. S. 1999. The genome of the olive fruit fly Bactrocera oleae: localization of molecular markers by in situ hybridization to the salivary gland polytene chromosomes. Genome 42: 744-751.

BAIMAI, V., PHINCHONGSAKULDIT, J., AND SUMRANDEE, C. 2000. Cytological evidence for a complex of species within the taxon Bactrocera tau (Diptera: Tephritoideae) in Thailand. Biol. J. Linnean Soc. 69: 399-409.

BEARD, C. B., HAMM, D. M., AND COLLINS, F. H. 1993. The mitochondrial genome of the mosquito Anopheles gambiae: DNA sequence, genome organization and comparisons with mitochondrial sequences of other insects. Insect Mol. Biol. 2: 103-124.

BERLOCHER, S. H. 1980. An electrophoretic key of distinguishing species of the genus Rhagoletis (Diptera: Tephritoideae) as larvae, pupae or adults. Ann. Entomol. Soc. America 73: 131-137.

BOYCE, T. M., ZWICK, M. E., AND AQUADRO, C. F. 1994. Mitochondrial DNA in the bark weevils: phylogeny and evolution in the P. strobi group. Mol. Biol. Evol. 11: 183-194.

BUSH, G. L. 1975. Modes of animal speciation. Annu. Rev. Ecol. Syst. 6: 339-364.

DAVID, H., TINA, T., AND CHARI, T. 1994. DNA probes can be used to discriminate between tephritid species at all stages of life cycle (Diptera: Tephritoideae). J. Econ. Entomol. 87: 741-746.

DREW, R. A. I., AND HARDY, D. E. 1981. Docus (Bactrocera opiliae), a new sibling species of the dorsalis complex of fruit flies from Northern Australia (Diptera: Tephritoideae). J. Australian Entomol. Soc. 20: 131-137.

DREW, R. A. I. 1989. The Tropical Fruit Flies (Diptera: Tephritoideae: Dacinae) of the Australian and Oceanian Regions. Memoirs Queensland Museum 26: 1-521.

DREW, R. A. I., AND HANCOCK, D. L. 1994. The Bactrocera dorsalis complex of fruit flies (Diptera: Tephritoideae: Dacinae) in Asia. Bull. ent. Res. Supplement, 2: 68.

DREW, R. A. I., AND HANCOCK, D. L. 2000. Phylogeny of the tribe Dacini (Dacinae) based on morphological, distributional, and biological data. In M. Aluja and A. L. Norrbom (eds.), Fruit Flies (Tephritoideae).
Zhang et al.: Molecular Phylogeny of Bactrocera species

Phylogeny and Evolution of Behavior, CRC Press, New York.
Flook, P. K., Rowell, C. H., and Gellissen, G. 1995. The sequence, organization and evolution of the Locusta migratoria genome. J. Mol. Evol. 41: 928-941.
Han, H. Y., and McPherson, B. A. 1997. Molecular phylogenetic study of Tephritidae (Insecta: Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. Mol. Phylogenetic Evol. 7: 17-32.
Han, H. Y. 2000. Molecular phylogenetic study of the tribe Trypetini (Diptera: Tephritidae) using mitochondrial 16S ribosomal DNA sequences. Biochem. Syst. Ecol. 28: 501-513.

HARDY, D. E. 1973. The Fruit Flies (Tephritidae: Diptera) of Thailand and Bordering Countries. Pacific Insects Monogr. 31: 1-353.

He, M., and Haymer, D. S. 1997. Polymorphic intron sequence detected within and between populations of the oriental fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. America 90: 825-831.
He, M., and Haymer, D. S. 1999. Genetic relationships of populations and the origins of new infestations of the Mediterranean fruit fly. Mol. Ecol. Syst. 8: 1247-1257.
He, M., and Haymer, D. S. 2003. The use of intron sequences for molecular taxonomic and systematic studies of species in the Bactrocera dorsalis complex. Plant Prot. Bull. Spec. Publ. New 5: 35-47.

Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01. The Pennsylvania State University, University Park, PA 16802.

LANGOR, D. W., AND SPERLING, F. A. H. 1997. Mitochondrial DNA sequence divergence in weevils of the Pisododes strobi species complex (Coleoptera: Curculionidae). Insect Mol. Biol. 6: 255-265.
Lewis, D. L., Farr, C. L., and Kacuni, L. S. 1995. Drosophila melanogaster mitochondrial DNA: completion of the nucleotide sequence and evolutionary comparisons. Insect Mol. Biol. 4: 263-278.

Li, W. H. 1997. Molecular Evolution. Sinauer Associates, Sunderland, MA.

Lunt, D. H., Zhang, D. X., Szymura, J. M., and Hewitt, G. M. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol. Biol. 5: 153-165.
Munro, H. K. 1984. A taxonomic treatise on the Dacidae (Tephritoidae: Diptera) of Africa. Entomol. Mem. South Africa Dept. Agric. 61: 313.

Muraji, M., and Nakahara, S. 2001. Phylogenetic relationships among fruit flies, Bactrocera (Diptera: Tephritidae), based on the mitochondrial rDNA sequences. Insect Mol. Biol. 10: 549-559.

Naelo, C. K. M., and Haymer, D. S. 2003. Use of oligonucleotide arrays for molecular taxonomic studies of closely related species in the oriental fruit fly (Bactrocera dorsalis) complex. Mol. Ecol. Notes 3: 662-665.

Nei, M., and Gojobori, T. 1986. Simple methods for estimating the number of synonymous and nonsynonymous substitutions. Mol. Biol. Evol. 3: 418-426.
O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L., and Robertson, H. M. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. USA 89: 2699-2702.
Posada, D., and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Saeloe, A. 1999. Electrophoretic Study of Bactrocera tau Complex (Diptera: Tephritidae) in Certain Populations in Thailand. M.S. Thesis, Mahidol University, Bangkok, Thailand.

Smith, P. T., McPherson, B. A., and Kambhampati, S. 2002. Phylogenetic analysis of mitochondrial DNA supports the monophyly of dacini fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 95(6): 658-664.
Smith, P. T., Kambhampati, S., and Armstrong, K. A. 2003. Phylogenetic relationships among Bactrocera species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. Mol. Phylogenetic Evol. 26: 8-17.

Smith, J. J., and Bush, G. L. 1997. Phylogeny of the genus Rhagoletis (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxi-
dase II. Mol. Phylogenetic Evol. 7: 33-43.
Spanos, L. G., Kouroumbas, M., and Louis, K. C. 2000. The mitochondrial genome of the Mediterrane-
nan fruit fly, Ceratitis capitata. Insect. Mol. Biol. 9: 139-144.

Thompson, J. D., Gibson, T. J., Plewniak, F.,Jeanmougin, F., and Higgins, D. G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24: 4876-4882.
Warwisa, J., and Visut, B. 2003. Molecular phylogeny of Tephritid fruit flies in the Bactrocera tau complex using the mitochondrial CO1 sequences. Genome, 46: 112-118.
White, I. M. 2000. Morphological features of the Dacini (Dacinae): their significance to behavior and classification, pp. 505-533 In M. Aluja and A. L. Norrbom (eds.), Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Ratons, FL.
White, I. M., and Hancock, D. L. 1997. CABIKEY to the Indo-Australasian Dacini Fruit Flies. CAB International, Wallingford, CDROM.
White, I. M., and Elson-Harris, M. M. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. Centre for Agriculture and Biosciences International.
Yang, A. 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. 39: 105-111.
Zhao, J. T., Formmer, M., and Sved, J. A. 2003. Genetic and molecular markers of the Queensland fruit fly, Bactrocera tryoni. J. Heredity, 94: 416.