Molecular Phylogeny of Bactrocera Species (Diptera: Tephritidae: Dacini) Inferred from Mitochondrial Sequences of 16S rDNA and COI Sequences

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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* (*Tetradacus*) *minax* and *Bactrocera* (*Zeugodacus*) *diaphora* sequences for the first time, and the subgenus *Bactrocera* (*Tetradacus*), 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*) *tau* 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 1lineage 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 de 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 tefrítidos, *Anastrepha ludens* y *Ceratitis capitata,* que fueron usadas como grupos externos para el análisis filogenético. Reportamos las secuencias de *Bactrocera* (*Tetradacus*) *minax* y *Bactrocera* (*Zeugodacus*) *diaphora* por la primera vez, y el subgénero *Bactrocera* (*Tetradacus*), aqui 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-*

phora y *Bactrocera* (*Zeugodacus*) *scutellata* estan estrechamente relacionados con *Bactrocera* (*Zeugodacus*) *tau* y *Bactrocera* (*Zeugodacus*) *cucurbitae*. Estos resultado indican que los subgéneros *Austrodacus* y *Zeugodacus*, que atacan plantas cucurbitas, estan estrechamente relacionados a las especies en los subgéneros *Afrodacus*, *Bactrocera*, y *Gymnodacus*, que atacan plantas en un gran número de familias. Además, el subgénero *Paratridacus* es un grupo hermano de subgénero *Tetradacus,* y 7 de las especies de complejo de *Bactrocera* (*Bactrocera*) *dorsalis* (definido por Drew & Hancock 1994) incluidas en este estudio formaron un grupo monofilético. El subgénero *Daculus* tiene su propio linaje, que no cae dentro de los grupos de *Bactrocera* o de *Zeugodacus*.

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 preceeding 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 cladisticly *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* (Coquillett), *B. tau* (Walker), *B. dia-*

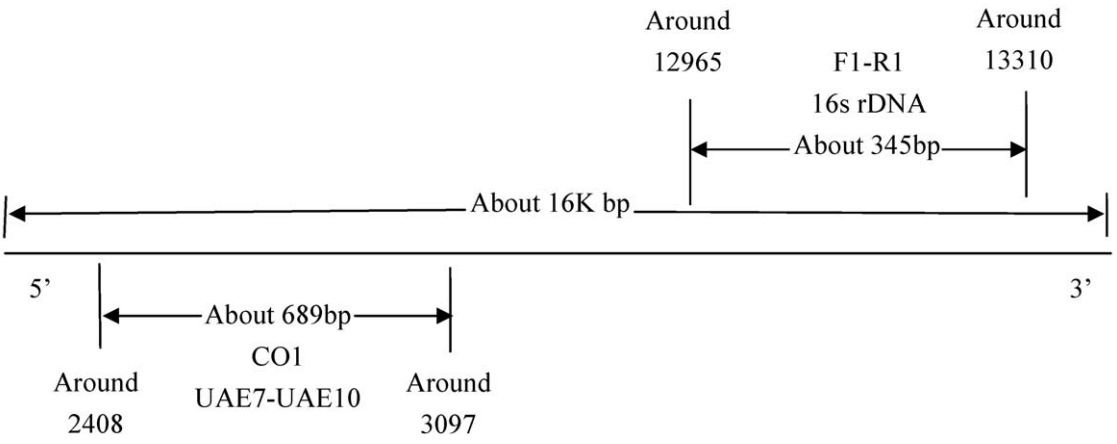

Fig 1. The position plot of *16s rDNA* and *CO1*gene fragment used in present study.

phora (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 H_2O, 3.75 µL 10*PCR buffer, 1.5 µL 25 mM $MgCl_2$, 0.5 µL dNTPs (10 mM each),1.5 µL of 20 µM forward and reverse primers and 1 unit of Taq DNA polymerase (Promega). The temperature profile for the amplification of the gene fragments included an initial denaturation step of 94°C for 3 min followed by 35 cycles of 94°C for 45 s, 56°C for 60 s, 72°C for 90 s, and a final extension step at 72°C for 10 min. A 690-bp long *COI* fragment was polymerized with the sense primer UEA 7 and the antisense primer UEA 10, both of which were developed by Lunt et al. (1996). PCR amplification was done in 20 µL reaction volume, as follows: 12.5 µL ddH2O, 2 µL 10×PCR buffer (Promega, Madison, Wis.), 2 µL of 25 mM MgCl2, 0.5 µL dNTP (10 mM each), 0.5 µL of 20 µM forward and reverse primers, and 1 U Taq DNA polymerase (Promega). PCR amplification was done with initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and final extension step at 72°C for 30 min. Ten microliters of each PCR product were run on a 1% agarose gel to determine the presence and size of amplified DNA. Both strands of the PCR product were sequenced for all samples. The sequencing of inserts in both directions was carried out on an ABI 377 automated sequencer. Sequences of all 7 tephritid fruit fly species have been deposited in GenBank under accession Nos. FJ866820-FJ866826 (*16s rDNA*) and GQ458042-GQ458048 (*COI*) (Table 1).

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

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*	AY530904*	AU
Bactrocera (*Austrodacus*) *cucumis*	AB074019*	AB192448*	AU
Bactrocera (*Bactrocera*) *curvipennis*	AB074020*	AY530895*	AU
Bactrocera (*Bactrocera*) *frauenfeldi*	AB074021*	AB192428*	AU
Bactrocera (*Bactrocera*) *kandiensis*	AB048738*	AB192431*	OR
Bactrocera (*Bactrocera*) *occipitalis*	AB048742*	AB192435*	OR
Bactrocera (*Bactrocera*) *psidii*	AB074027*	AB192440*	AU
Bactrocera (*Bactrocera*) *tryoni*	AB074029*	AY530892*	AU
Bactrocera (*Bactrocera*) *umbrosa*	AB048749*	AY530897*	OR, AU
Bactrocera (*Daculus*) *oleae*	AY210702*	AY210702*	PA, AF, OR
Bactrocera (*Gymnodacus*) *calophylli*	AB035109*	AB192419*	OR, AU
Bactrocera (*Paratridacus*) *expandens*	AB035110*	AB192427*	UK
Bactrocera (*Tetradacus*) *minax*	FJ866821	GQ458044	PA, OR
Bactrocera (*Tetradacus*) *tsuneonis*	DQ419809*	AB192447*	PA, OR
Bactrocera (*Zeugodacus*) *caudate*	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 = Afrotropical; AU = Australasian; HO = Holarctic; NE = Nearctic; NT = Neotropical; OR = Oriental; PA = Palearctic; and UK = Unknown.

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 2. OLIGONUCLEOTIDE PRIMERS USED FOR POLYMERASE CHAIN REACTION (PCR) AMPLIFICATIONS.

Name	Sequence
(16s rDNA-F) F1	5'-ATCCAACATCGAGGTCGCAAAC-3'
(16s rDNA-R) R1	5'-GGCTGGTATGAACGGTTGGACGAG-3'
(CO1-F) UEA-7	5'-TACAGTTGGAATAGACGTTGATAC-3'
(CO1-R) UEA-10	5'-TCCAATGCACTAATCTGCCATATTA-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 rDNA* and *COI* among the 27 *Bactrocera* species. Within the subgenus *Bactrocera*, 46 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 phylogennetic 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

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	

TABLE 4. MAXIMUM COMPOSITE LIKELIHOOD ESTIMATE OF THE PATTERN OF NUCLEOTIDE SUBSTITUTION FROM 27 *BACTROCERA* SPECIES.

	A	T	C	G
A		*5.5*	*3.09*	4.96
T	*5.35*		17.93	*1.98*
C	*5.35*	31.87		*1.98*
G	13.4	*5.5*	*3.09*	

Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

in NJ and MP phylogennetic 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 cladisticly 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 that 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* (*Bactro-*

TABLE 5. SUMMARY OF CHARACTER STATISTICS AND RESULTS OF PARSIMONY ANALYSIS OF DATA FROM 27 *BACTROCERA* SPECIES.

Data partition	Characters (including gaps)	Characters constant	Variable sites	PIC	TL	CI	HI	RI	RCI
16S	352	265	87	53	206	0.54	0.46	0.61	0.33
CO1	698	429	271	228	1095	0.37	0.63	0.50	0.19
16S+CO1	1049	699	350	283	1311	0.39	0.61	0.50	0.20

PIC, number of parsimony informative characters; TL, most parsimonious tree length; EPT, number of equally parsimonious trees; CI, consistency index; HI, homoplasy index; RI, retention index; RCI, rescaled consistency index.

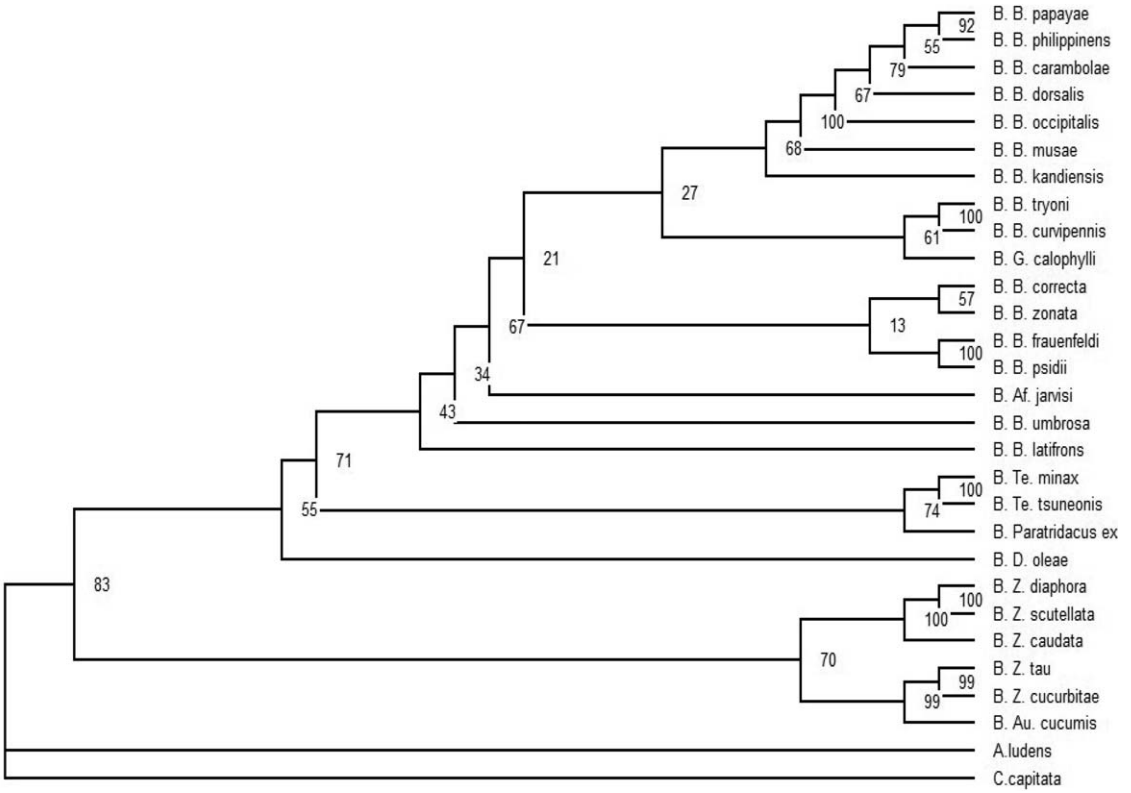

Fig. 2. The strict consensus of the most parsimonious trees (Tree length: 1311; CI: 0.39) for 27 species of *Bactrocera* and two out groups *Anastrepha ludens* and *Ceratitis capitata* based on the combined DNA sequence (1049-bp including gaps) of mitochondrial *16S rRNA* and C*ytochrome Oxidase I*. Numbers above branches are bootstrap values (%).

cera) *papaya*, *Bactrocera* (*Bactrocera*) *carambolae,* 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 phylogentic studies before, it is necessary that further molecular phylogentic studies should be done to examine taxonomic status of subgenus *Tetradacus*.

Although there are some molecular evolutional 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 1species. 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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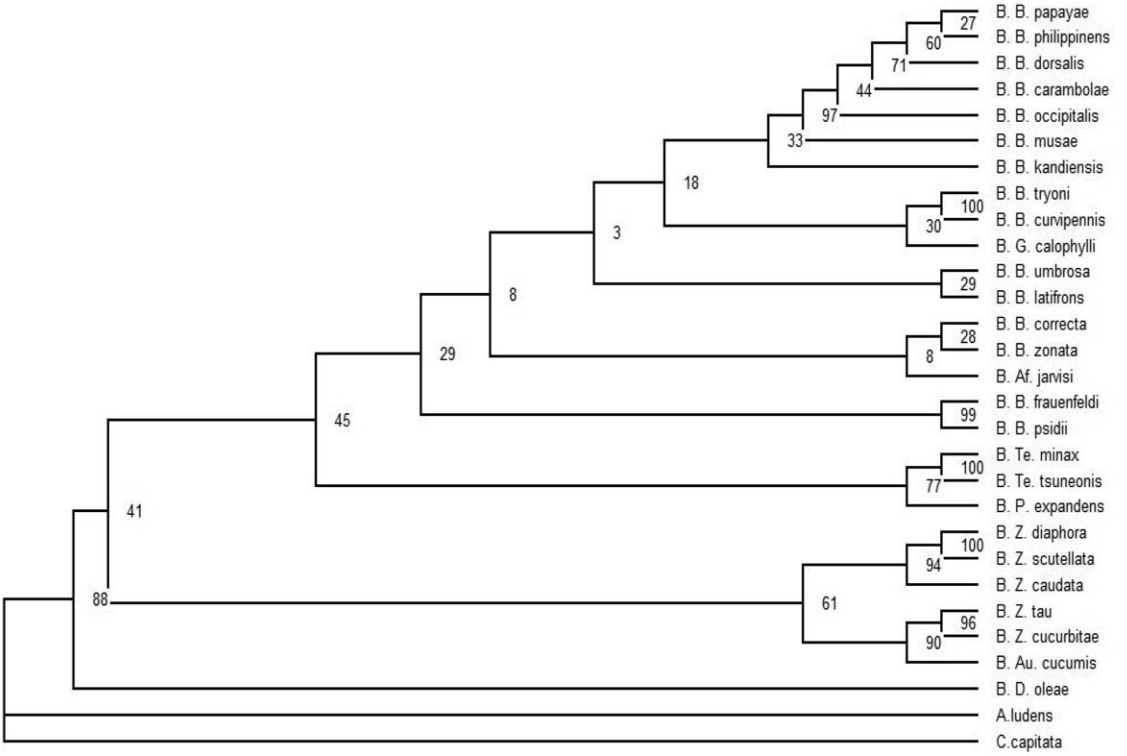

Fig. 3. Neighbour-joining dendrogram The strict consensus of the most parsimonious trees for 27 species of *Bactrocera* and 2 out groups *Anastrepha ludens* and *Ceratitis capitata* based on the Jukes-Cantor distances with combined DNA sequence (1049-bp including gaps) of the mitochondrial *16S rRNA* and C*ytochrome Oxidase1*. Numbers above branches are bootstrap values (%).

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