PENSOFT

SENCKENBERG
world of biodiversity

Leibniz Association

Complete mitochondrial genomes of *Bactrocera* (*Bulladacus*) *cinnabaria* and *B.* (*Bactrocera*) *propinqua* (Diptera: Tephritidae) and their phylogenetic relationships with other congeners

Hoi-Sen Yong[1], Sze-Looi Song[2,3], Kah-Ooi Chua[1,4], Yvonne Jing Mei Liew[4,5,6], Kok-Gan Chan[1], Phaik-Eem Lim[3], Praphathip Eamsobhana[7]

1 *Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
2 *Institute for Advanced Studies, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
3 *Institute of Ocean and Earth Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
4 *Centre for Research in Biotechnology for Agriculture (CEBAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
5 *University of Malaya Centre for Proteomics Research, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
6 *Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
7 *Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

https://zoobank.org/818E345F-E3FD-4346-B463-BADBFE97B465

Corresponding authors: Sze-Looi Song (szelooi@um.edu.my), Kah-Ooi Chua (kahooi@um.edu.my)

Received 29 November 2023
Accepted 28 May 2024
Published 2 July 2024

Academic Editors Marianna Simões, André Nel

Citation: Yong H-S, Song S-L, Chua K-O, Liew YJM, Chan K-G, Lim P-E, Eamsobhana P (2024) Complete mitochondrial genomes of *Bactrocera* (*Bulladacus*) *cinnabaria* and *B.* (*Bactrocera*) *propinqua* (Diptera: Tephritidae) and their phylogenetic relationships with other congeners. Arthropod Systematics & Phylogeny 82: 515–526. https://doi.org/10.3897/asp.82.e115954

Abstract

Bactrocera (*Bulladacus*) *cinnabaria* and *B.* (*Bactrocera*) *propinqua* are tephritid fruit flies of the subfamily Dacinae, tribe Dacini. The whole mitogenomes of these two species (first report for the subgenus *Bulladacus*) possess 37 genes (13 protein-coding genes – PCGs, 2 rRNA and 22 tRNA genes). The mitogenome of *B. cinnabaria* (15,225 bp) is shorter than that of *B. propinqua* (15,927 bp), mainly due to the smaller size of the control region and intergenic spacers in *B. cinnabaria*. Molecular phylogeny based on mitochondrial genes (mt-genes) reveals two clades of the genus *Bactrocera*: one comprising the subgenus *Bactrocera* and the other comprising the subgenera *Bulladacus*, *Daculus*, *Tetradacus* and unassigned *Bactrocera* sp. '*yunnanensis*'. The subgenera represented by two or more taxa are monophyletic. *B.* (*Bulladacus*) *cinnabaria* forms a sister group with the subgenus *Tetradacus* (*B. minax* and *B. tsuneonis*) and *B.* sp. '*yunnanensis*', in a clade containing also the basal sister lineage of the subgenus *Daculus* (*B. oleae* and *B. biguttula*). *B. propinqua* forms a sister group with *B. ritsemai* and *B. limbifera* in a subclade containing also *B. umbrosa*, *B. curvifera* and *B. moluccensis* of the monophyletic subgenus *Bactrocera*. The present study supports the synonymy of *B. ruiliensis* with *B. thailandica*. It also shows a high genetic similarity between (a) *B. melastomatos* and *B. rubigina*, (b) *B. papayae* and *B. philippinensis*, (c) *B. dorsalis* and *B. invadens*, (d) *B. tryoni* and *B. neohumeralis*, and (e) *B. cheni* and *B. tuberculata*; and *B. cheni* is distinct from and not a synonym of *B. tsuneonis* or *B. lombokensis*.

Keywords

Bactrocera subgenera; Dacinae; Fruit flies; Mitogenomes; Molecular phylogeny

1. Introduction

True fruit flies of the genus *Bactrocera* Macquart are members of the family Tephritidae, subfamily Dacinae, tribe Dacini. Based on the classification with *Zeugodacus* Hendel as a genus, the genus *Bactrocera* consists of 461 species worldwide, with 451 species in the Asia-Pacific and 13 species in Africa (Doorenweerd et al. 2018). The actual number of species is much higher as many new species are being described, and there are unnamed species awaiting to be described (Leblanc et al. 2021a; Drew and Romig 2022; Singh et al. 2022; Starkie et al. 2022).

Fifteen subgenera are recognized under the genus *Bactrocera*: (1) *Bactrocera* group of subgenera – *Apodacus* Perkins, *Bactrocera* Macquart, *Bulladacus* Drew & Hancock, *Calodacus* Hancock, *Queenslandacus* Drew, *Semicallantra* Drew, and *Trypetidacus* Drew; (2) *Melanodacus* group of subgenera – *Daculus* Speiser, *Gymnodacus* Munro, *Hemizeugodacus* Hardy, *Neozeugodacus* May, *Notodacus* Perkins, *Paratridacus* Shiraki, and *Parazeugodacus* Shiraki; and (3) *Tetradacus* Miyake (Hancock and Drew 2018). Members of this genus are among the world's most economically important and invasive insect pests of agriculture (Vargas et al. 2015); some 55 species have been listed as fruit pests (Doorenweerd et al. 2018).

Several aspects of the *Bactrocera* fruit flies have been widely studied, such as taxonomy and systematics (Drew and Romig 2013, 2022; Leblanc et al. 2019), molecular phylogeny (San Jose et al. 2018; Starkie et al. 2022; Zhang et al. 2010), male lures (Leblanc et al. 2021a; Chen et al. 2022; Fan et al. 2022; Starkie et al. 2022), microbiota (Yong et al. 2017a,b,c; Khan et al. 2019; He et al. 2022; Majumder et al. 2022; Ravigné et al. 2022;), invasion biology (Duyck et al. 2022), and biological control (Dias et al. 2022).

Most of the studies on the molecular phylogeny of the genus *Bactrocera* (and other tephritid fruit flies) are based on partial sequences of single or multiple mitochondrial and nuclear genes (Zhang et al. 2010; San Jose et al. 2018; Leblanc et al. 2021a; Starkie et al. 2022). In contrast, there are relatively few studies based on the complete mitochondrial genomes (Yong et al. 2021; Zhang et al. 2022). As of February 2023, 32 species of the genus *Bactrocera* (excluding the three taxa of *Bactrocera dorsalis* complex considered by some as conspecific and others as valid species) are available in the NCBI GenBank. Of these, 27 species belong to the subgenus *Bactrocera*.

In view of the potential application of mitochondrial genomes (mitogenomes) in studies regarding phylogeny and evolution (Cameron 2014), the present study reports the mitogenomes of *Bactrocera* (*Bulladacus*) *cinnabaria* Drew & Romig and *Bactrocera* (*Bactrocera*) *propinqua* (Hardy & Adachi) and their phylogenetic relationships with other congeners. This is the first report on the mitogenome for the subgenus *Bulladacus*.

Bactrocera cinnabaria is found in Andaman and Nicobar Islands (David and Ramani 2011), Peninsular Malaysia (Yong 1994) and Singapore (Hardy and Adachi 1954). When first discovered in these countries, it was named as *Bactrocera mcgregori* (Bezzi), a taxon found in the Philippines, but was subsequently described as a new species (Drew and Romig 2013). Its larva host plant is *Gnetum gnemon* (Hardy and Adachi 1954; Yong 1994). The male flies are not attracted to methyl eugenol and cue-lure/raspberry ketone.

Bactrocera propinqua has been documented in Bangladesh, China, Thailand, Cambodia, Laos, Vietnam, Malaysia, Singapore, and Indonesia (Leblanc et al. 2021b). The larva host plants include six species of the genus *Garcinia* (family Clusiaceae) (Leblanc et al. 2021b); the host plant *Garcinia lauena* (Hardy 1973) was emended to *Garcinia bancana* (Yong 1992). The male flies are attracted to cue-lure (Yong 1992).

The larvae of *B. cinnabaria* and *B. propinqua* feed on a variety of fruits, including both cultivated and wild species. They are economically important pests, posing significant challenges to agriculture and fruit production.

2. Materials and Methods

2.1. Specimen collection and mitochondrial DNA extraction

Both *B. cinnabaria* and *B. propinqua* were collected by H-S Yong from the garden of the Institute of Biological Sciences, Universiti Malaya, Malaysia (3°07′9.00″N, 101°39′13.79″E). Fruit flies of *B. cinnabaria* hatched from the infested fruits of *Gnetum gnemon*. Male fruit flies of *B. propinqua* were collected by application of cue-lure on the surface of a green leaf. Cue-lure is a synthetic pheromone that attracts male fruit flies, and when applied to the leaf, it effectively lures them in for collection. The specimens were preserved in absolute ethanol and stored in a −20°C deep freezer until use for DNA extraction. They were identified according to existing literature (Drew and Romig 2013). Photographs of the fruit flies are available to interested party as whole insects were used for DNA extraction. The extraction of mitochondrial DNA followed the method of Yong et al. (2016a).

2.2. Mitogenomes from GenBank, library preparation and genome sequencing

The complete mitogenomes of the genus *Bactrocera* (n = 32 species) available from the GenBank (Table S1) were used for phylogenetic comparison. Three other tephritid mitogenomes (*Ceratitis capitata* NC_000857, *Ceratitis fasciventris* NC_035497, and *Ceratitis rosa* NC_053847) available from the GenBank were used as the outgroup taxa.

Sample and library preparation (using Nextera DNA Sample Preparation Kit) and genome sequencing using the Illumina MiSeq Desktop Sequencer (150 bp paired-end reads) (Illumina, USA) were as described in Song et al.

(2018). The mitogenome sequences have been deposited in the GenBank, under the accession numbers OR085849 (*B. cinnabaria*) and OR085850 (*B. propinqua*).

2.3. Analysis of mitogenome

A contig identified and established as mitogenome was annotated with MITOS (Donath et al. 2019) on the Galaxy platform (https://usegalaxy.eu). The circular map of the mitogenome was created using Blast Ring Image Generator (BRIG) (Alikhan et al. 2011). Transfer RNA (tRNA) genes were identified by MITOS (Donath et al. 2019).

MEGA X was used to determine the nucleotide composition, amino acid frequency and relative synonymous codon usage (RSCU) (Kumar et al. 2018). The ratios of non-synonymous substitutions (Ka) and synonymous (Ks) substitutions for the PCGs were estimated by DnaSP 6 (Rozas et al. 2017). The AT and GC skewness were determined according to Perna and Kocher (1995). Palindromes (inverted repeats) in the control region were checked with Tandem Repeats Finder (Benson 1999).

2.4. Phylogenetic analysis

Alignment of nucleotide sequences and reconstruction of phylograms based on 13 concatenated PCGs and 15 mt-genes (13 PCGs and 2 rRNA genes) followed that described in Song et al. (2018) and Yong et al. (2015, 2016a,b). Briefly, the gene sequences were aligned by MAFFT version 7 (Katoh and Standley 2013), using the Q-INS-I algorithm and subsequently edited and trimmed using BioEdit v.7.0.5.3 (Hall 1999). Kakusan v.3 (Tanabe 2007) was used to determine the best-fit nucleotide substitution models for maximum likelihood (ML) analysis selected using the corrected Akaike Information Criterion (Akaike 1973). Bayesian analysis was conducted using the Markov chain Monte Carlo (MCMC) method via Mr. Bayes v.3.1.2 (Huelsenbeck and Ronquist 2001), with two independent runs of 2×10^6 generations with four chains, and with trees sampled every 200th generation. Convergence and burn-in of likelihood values for all post-analysis trees and parameters were evaluated using the "sump" command in MrBayes and the computer program Tracer v.1.5 (http://tree.bio.ed.ac.uk/software/tracer). The first 200 trees from each run were discarded as burn-in (where the likelihood values were stabilized prior to the burn-in), and the remaining trees were used for the construction of a 50% majority-rule consensus tree. Phylograms of the mt-genes were constructed using TreeFinder. Phylogenetic trees were viewed and edited by FigTree v.1.4 (Rambaut 2012). Uncorrected pairwise ('*p*') genetic distances were estimated using PAUPb10 software (Swofford 2002).

3. Results

3.1. Mitogenome features

The mitogenomes of *B. cinnabaria* and *B. propinqua* had similar gene order and contained 37 genes (13 protein-coding genes – PCGs, 2 rRNA genes, and 22 tRNA genes) and a non-coding region (A + T-rich control region) (Table 1; Fig. 1). There were 28 intergenic regions with spacing sequence totalling 150 bp in *B. cinnabaria*, and 222 bp in *B. propinqua* (Table 1). The region between *trnQ* and *trnM* genes had the longest sequence of 69 bp in *B. propinqua*; it was 11 bp in *B. cinnabaria*. The longest intergenic sequence in *B. cinnabaria* was 29 bp between

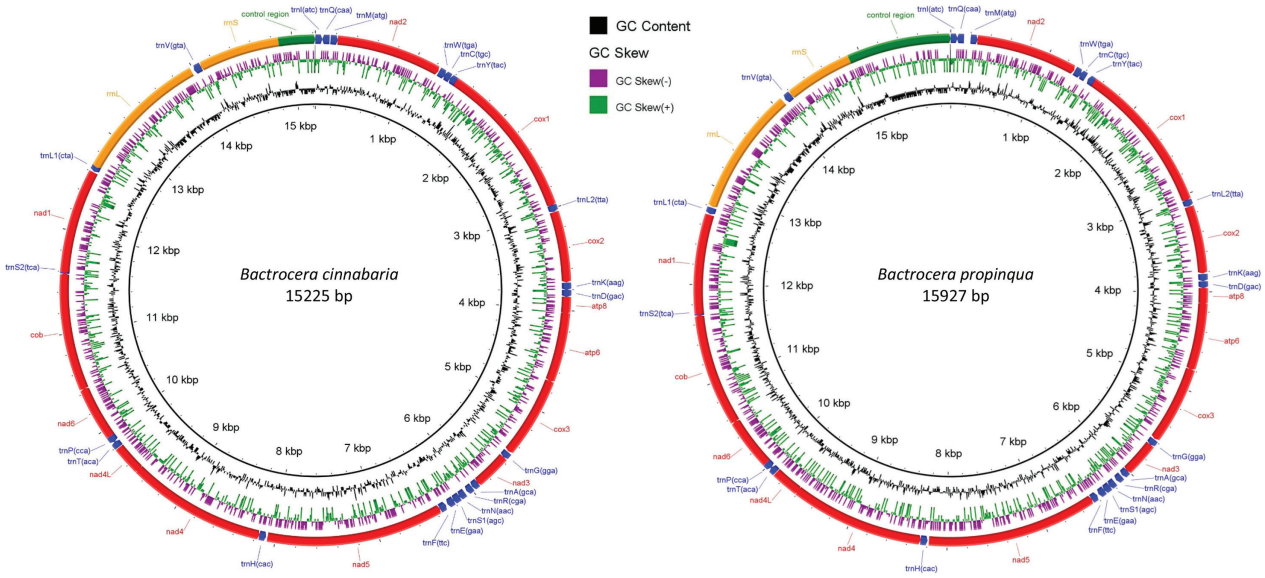

Figure 1. Complete mitogenomes of *Bactrocera cinnabaria* and *B. propinqua* with BRIG visualization showing the protein coding genes, rRNAs, tRNAs and non-coding region. GC skew is shown on the outer surface of the ring whereas GC content is shown on the inner surface. The anticodon of each tRNA gene is shown in parentheses.

Table 1. Gene order and organization of the mitochondrial genomes of *Bactrocera cinnabaria* (Bc) and *B. propinqua* (Bp). *Minus sign indicates overlap. J (+) or N (–) indicates gene directions.

Gene	Strand	Size (bp)	Intergenic sequence*	Start codon	Stop codon
		Bc/Bp	Bc/Bp	Bc/Bp	Bc/Bp
trnI(gat)	J	66/66	1/–3		
*trn*Q(ttg)	N	69/69	11/69		
*trn*M(cat)	J	69/69			
nad2	J	1023/1023	8/10	ATT/ATT	TAG/TAA
trnW(tca)	J	68/69	–8/–8		
trnC(gca)	N	62/63	1/31		
trnY(gta)	N	67/67	–2/–2		
cox1	J	1539/1539	–5/–5	TCG/TCG	TAA/TAA
trnL2(taa)	J	66/66	4/4		
cox2	J	690/687	4/7	ATG/ATG	TAA/TAA
trnK(ctt)	J	70/71	0/3		
trnD(gtc)	J	67/67			
atp8	J	162/162	–7/–7	ATC/GTG	TAA/TAA
atp6	J	678/678	–1/–1	ATG/ATG	TAA/TAA
cox3	J	789/789	9/9	ATG/ATG	TAA/TAA
trnG(tcc)	J	65/65			
nad3	J	354/354	–2/–2	ATT/ATT	TAG/TAG
trnA(tgc)	J	64/65	5/14		
trnR(tcg)	J	63/64	24/26		
trnN(gtt)	J	65/65			
trnS1(gct)	J	68/68			
trnE(ttc)	J	66/67	18/19		
trnF(gaa)	N	65/65	1/0		
nad5	N	1720/1720	15/15	ATT/ATT	T/T
trnH(gtg)	N	65/69			
nad4	N	1341/1341	–7/–7	ATG/ATG	TAA/TAG
nad4L	N	291/291	8/8	ATG/ATG	TAA/TAA
trnT(tgt)	J	65/65			
trnP(tgg)	N	66/66	2/2		
nad6	J	525/525	–1/–1	ATT/ATT	TAA/TAA
cob	J	1137/1137	–2/–2	ATG/ATG	TAG/TAG
trnS2(tga)	J	67/67	–65/–65		
nad1	N	1020/1020	10/10	ATG/ATG	TAA/TAA
trnL1(tag)	N	65/65	–23/10		
rrnL	N	1322/1291	29/30		
trnV(tac)	N	72/72	–1/–1		
rrnS	N	790/790			
Control region	J	358/947			

rrnL and *trnV* genes; it was 30 bp in *B. propinqua*. Sequences with 18, 24 and 29 bases in *B. cinnabaria*, and 26, 30, 31, and 69 bases in *B. propinqua* had clear stem-loop structures (Fig. S1). *B. cinnabaria* had overlaps in 13 regions totalling 125 bp, and *B. propinqua* had overlaps in 11 regions totalling 124 bp. Both species had the longest overlap of 65 bp between *trnS2* and *nad1* genes.

3.2. Protein-coding genes and codon usage

The A + T content for the whole mitogenome was 71.2% for *B. cinnabaria* and 74.1% for *B. propinqua*, with positive AT and negative GC skewness values (Table 2). Likewise, the A + T content was higher in *B. propinqua* than *B. cinnabaria* for 13 PCGs (including the three codon positions), tRNA genes, rRNA genes, control region, and both the J and N strands. Most of the regions had negative AT skewness value, except the control region with positive AT skewness values for both *B. cinnabaria* and *B. propinqua*, and the N strand had positive value for *B. propinqua*. Unlike the whole mitogenome, the 1st codon position of PCGs, tRNA genes and rRNA genes had positive GC skewness values for both *B. cinnabaria* and *B. propinqua*, and the N strand had positive GC skewness value for *B. cinnabaria* (Table 2); the other regions had negative GC skewness values.

For the individual PCGs, the A + T content ranged from 62.9% for *cox1* to 76.3% for *nad4L* in *B. cinna-*

Table 2. A + T content (%), AT and GC skewness of *Bactrocera cinnabaria* (Bc) and *B. propinqua* (Bp) mitogenomes.

	A+T%		AT skew		GC skew	
Region	**Bc**	**Bp**	**Bc**	**Bp**	**Bc**	**Bp**
Whole mitogenome	71.2	74.1	0.084	0.069	–0.250	–0.228
Protein coding genes	69.1	71.6	–0.152	–0.153	–0.049	–0.018
1st codon position	63.8	65.1	–0.069	–0.076	0.166	0.198
2nd codon position	65.1	65.5	–0.382	–0.380	–0.167	–0.165
3rd codon position	78.2	84.1	–0.028	–0.039	–0.211	–0.182
tRNA genes	73.9	75.3	–0.009	–0.012	0.088	0.109
rRNA genes	77.4	77.7	–0.083	–0.094	0.304	0.309
Control region	84.9	89.5	0.178	0.068	–0.373	–0.135
J strand	67.4	69.8	–0.045	–0.060	–0.206	–0.166
N strand	74.3	76.2	0.147	0.134	–0.094	–0.084

baria, and 65.1% for *cox3* to 80.1% for *nad4L* in *B. propinqua* (Table S2). Most of the PCGs had negative AT and GC skewness values (Table S2); *cox2* and *nad6* had positive AT skewness values in *B. cinnabaria*, *nad4L* had positive GC skewness value in *B. cinnabaria*, and *nad1*, *nad4* and *nad5* had positive GC skewness values in both *B. cinnabaria* and *B. propinqua*.

B. cinnabaria and *B. propinqua* shared an identical start codon for their respective PCGs, except *atp8* with ATC for *B. cinnabaria* and GTG for *B. propinqua* (Table 1). The commonest start codon was ATG (in 7 PCGs – *cox2*, *atp6*, *cox3*, *nad4*, *nad4L*, *cob*, *nad1*), followed by four ATT (*nad2*, *nad3*, *nad5*, *nad6*), and one TCG (*cox1*). The two species had an identical TAA stop codon for eight PCGs (*cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad4L*, *nad6*, *nad1*) and TAG for two PCGs (*nad3*, *cob*), and incomplete T-- stop codon for one PCG (*nad5*); one PCG (*nad4*) had TAA in *B. cinnabaria* and TAG in *B. propinqua*, and one PCG (*nad2*) had TAG in *B. cinnabaria* and TAA in *B. propinqua* (Table 1).

The frequency of individual amino acid was quite similar between *B. cinnabaria* and *B. propinqua* (Fig. 2). The predominant amino acids (with frequency above 200) in the two mitogenomes were glycine, isoleucine, leucine2, phenylalanine, serine2, threonine, and valine (Table S3); in addition, leucine1 had a frequency of 106 in *B. cinnabaria*, and methionine had a frequency of 203 in *B. propinqua*. Cysteine had the lowest frequency of 45 in *B. cinnabaria* and 43 in *B. propinqua*.

Analysis of the relative synonymous codon usage (RSCU) revealed that there was no biased usage of A/T than G/C at the third codon position (Table S4; Fig. 2). The frequency of each codon varied between the two *Bactrocera* mitogenomes. The most commonly used codon was UUA encoding for leucine2, and the least commonly used codon was CUG encoding for leucine1 (Table S4; Fig. 2).

The Ka/Ks ratio (an indicator of selective pressure on a PCG) was less than 1 for all the 13 PCGs in the two *Bactrocera* mitogenomes, indicating purifying selection (Table S5; Fig. 3). The *cox3* gene had the lowest ratio (Ka/Ks = 0.010) followed by *cox1* gene (Ka/Ks = 0.012); the *atp8* gene had the highest ratio of 0.221.

3.3. Ribosomal RNA genes and transfer RNA genes

The cloverleaf structure for some tRNAs was dissimilar in *B. cinnabaria* and *B. propinqua* (Fig. 4). Asparagine (*trnA*) lacked the TΨC-loop in both species. Arginine (*trnA*) lacked the TΨC-loop in *B. cinnabaria*, and had a reduced loop in *B. propinqua*. Isoleucine (*trnI*) had reduced TΨC-loop in *B. cinnabaria*, while phenylalanine (*trnF*) lacked the TΨC-loop in *B. propinqua*. Serine S1 (*trnS1*) lacked the DHU stem in *B. cinnabaria*, and lacked the DHU loop in *B. propinqua*.

3.4. Control region

The control region was flanked by *rrnS* and *trnI* genes respectively, with 358 bp in *B. cinnabaria* and 947 bp in *B. propinqua*. In the control region of *B. propinqua*, a long poly-A stretch of 12 bp was present in the anterior region, and a 24 bp poly-A stretch was present in the posterior region; a long poly-T stretch of 24 bp was present in the middle region. Long poly-A and poly-T stretches were not present in *B. cinnabaria* control region.

Simple tandem repeats and palindromes were present in the control region of *B. cinnabaria* and *B. propinqua* (Table 3). Some were common in the two mitogenomes, while some were present only in *B. cinnabaria* or *B. propinqua*. There were more tandem repeats and palindromes in the control region of *B. propinqua*. Some palindromes (ATAATA, TATTAT, ATTAATTA, and TAAAATTAAAAT) are also tandem repeats.

3.5. Phylogenetic analysis/relationship

Phylogenetic analysis, based on 15 mt-genes (13 PCGs and 2 rRNA genes) and 13 PCGs of available complete mitogenomes, revealed two major clades of the *Bactrocera* taxa: (A) subgenus *Bactrocera*, and (B) subgenera *Bulladacus*, *Daculus*, *Tetradacus* and unassigned *Bactrocera* sp. '*yunnanensis*' (Fig. 5). The subgenus *Bactroc-*

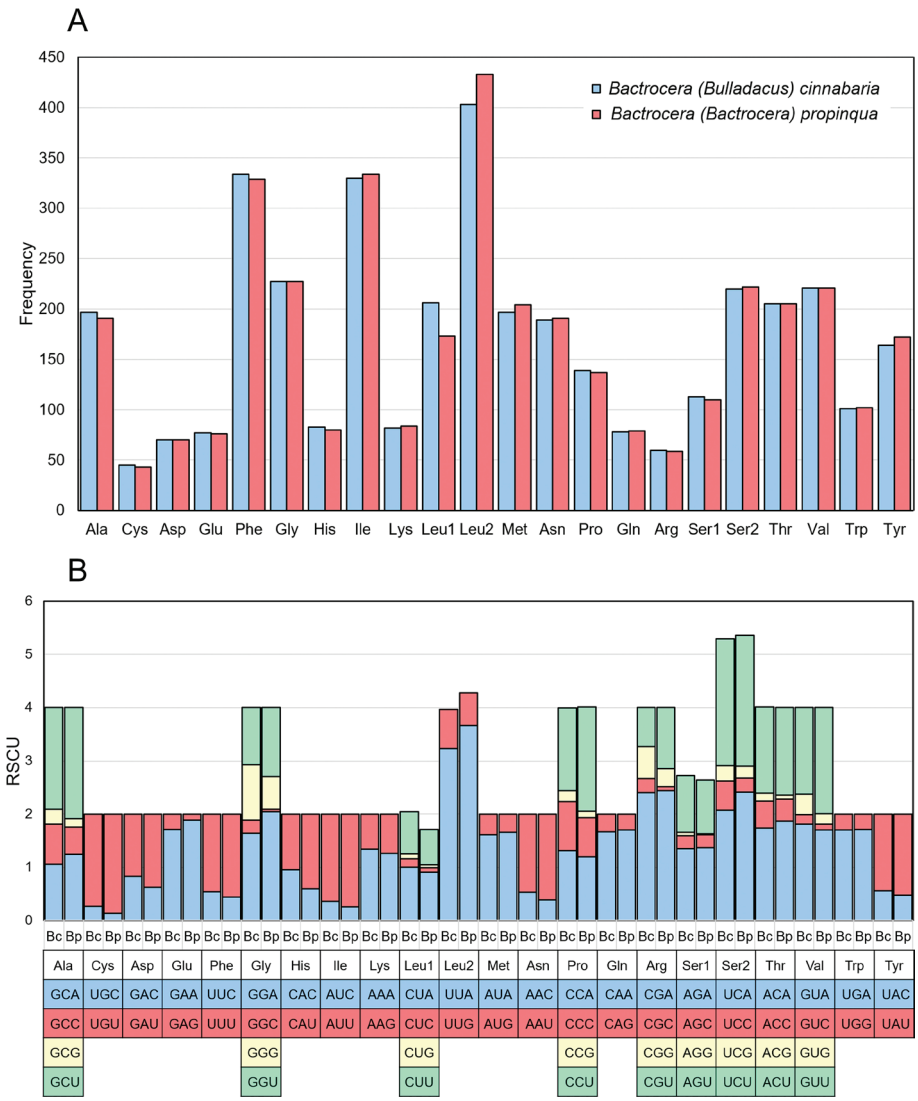

Figure 2. Amino acid frequency (**A**) and relative synonymous codon usage (**B**) of protein-coding genes in *Bactrocera cinnabaria* and *B. propinqua* mitogenomes.

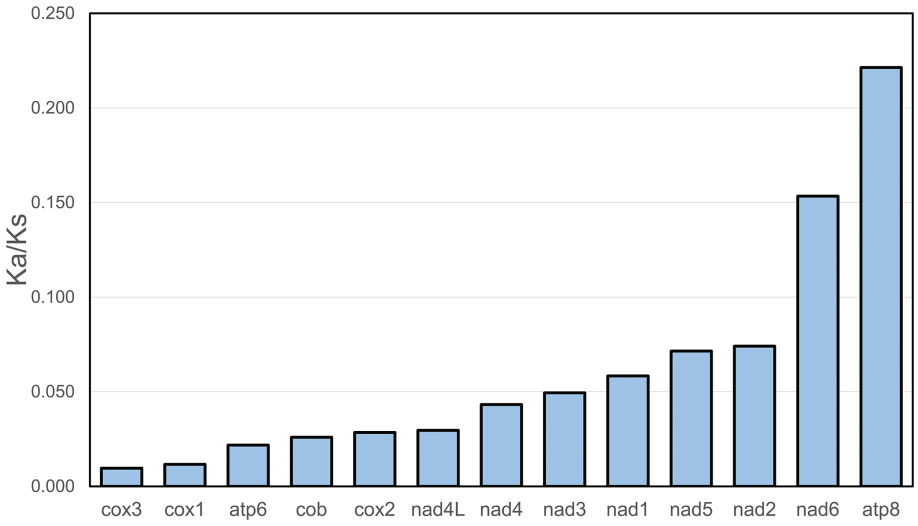

Figure 3. Ka/Ks ratio for 13 protein-coding genes of *Bactrocera cinnabaria* and *B. propinqua* mitogenomes.

era clade (Clade A) consisted of many more taxa than Clade B.

B. cinnabaria was basal to the sister lineage of *B. minax* and *B. tsuneonis*, indicating that the subgenus *Bulladacus* was closer related to subgenus *Tetradacus* than to subgenus *Daculus*. *B. propinqua* was basal to the sister lineage of *B. ritsemai* and *B. limbifera*, forming a subclade with *B. umbrosa*, *B. curvifera* and *B. moluccensis* (Fig. 5). The sister lineage of *B. latifrons* and *B. bryoniae* was basal to the other subgenus *Bactrocera* lineages.

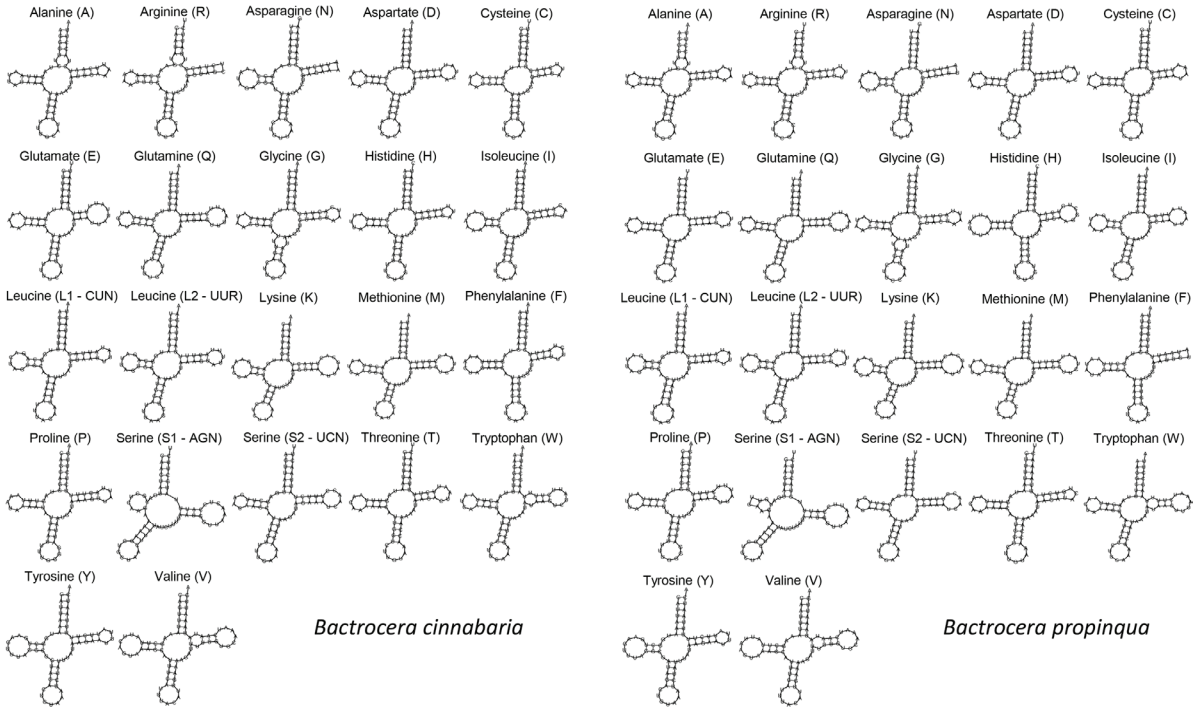

Figure 4. Cloverleaf structure of the 22 inferred tRNAs in the mitogenomes of *Bactrocera cinnabaria* and *B. propinqua*.

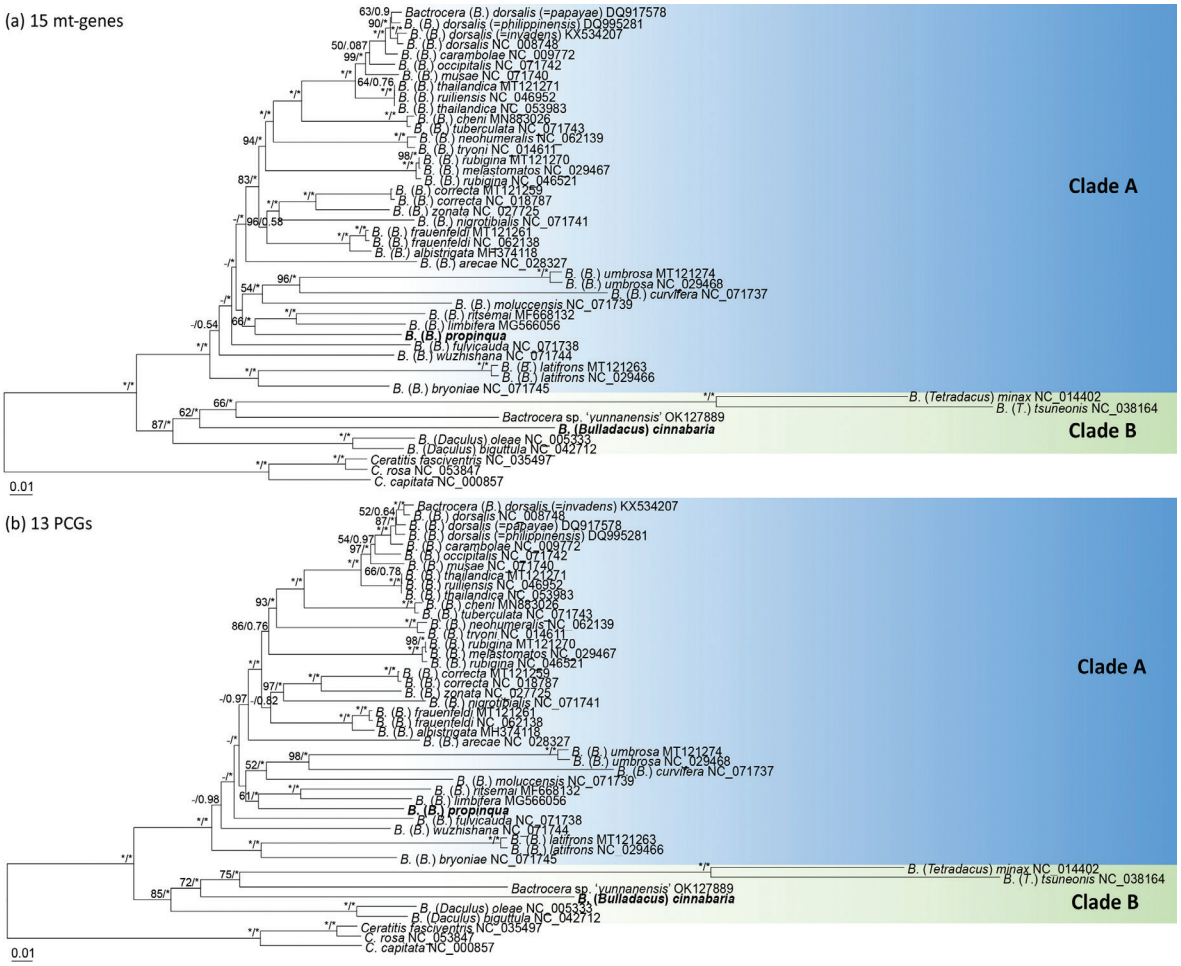

Figure 5. Phylogenetic trees (ML/BI) of (**a**) 15 mt-genes (13 PCGs + 2 rRNA genes), and (**b**) 13 PCGs of the whole mitogenomes of *Bactrocera* fruit flies with *Ceratitis capitata*, *C. fasciventris*, and *C. rosa* as the outgroup taxa. Numeric values at the nodes are ML bootstrap and Bayesian posterior probabilities. Support values labelled with a "*" have 100% bootstrap support or 1.0 posterior probability.

Table 3. Number of different repetitive sequences in the control regions of *Bactrocera cinnabaria* and *Bactrocera propinqua* mitogenomes.

		No. of repeat	
Type of repeat	**Repetitive sequence**	***B. cinnabaria***	***B. propinqua***
Simple sequence repeat	$(A)_{12}$	0	1
	$(A)_{24}$	0	1
	$(T)_{24}$	0	1
	$(TA)_3$	0	4
	$(TA)_6$	0	1
	$(ATA)_2$	0	1
	$(AAT)_2$	2	3
	$(TAA)_2$	5	4
	$(TAG)_2$	1	1
	$(TTA)_2$	2	3
	$(TAAA)_2$	2	1
	$(AAAT)_2$	1	1
	$(ATAA)_2$	0	1
	$(TTAA)_2$	0	2
	$(TTTA)_2$	0	2
	$(AAATA)_2$	0	1
	$(AATTT)_2$	0	1
	$(TTTAA)_2$	0	1
	$(TTTAAA)_2$	1	0
Palindromes	AATTAA	2	4
	ATAATA	0	1
	ATTTTA	0	1
	TAAAAT	3	2
	TATTAT	0	3
	TTAATT	1	5
	AATTTTAA	0	1
	ATTAATTA	0	1
	TTAAAATT	0	1
	AAATTTTAAA	0	1
	TAAAATTAAAAT	0	1

4. Discussion

In the present study, the mitogenomes of *B. cinnabaria* and *B. propinqua* have three main clusters of characteristic tRNAs (Fig. 1), as in other insect taxa: (1) I-Q-M (isoleucine, glutamate and methionine); (2) W-C-Y (tryptophan, cysteine and tyrosine); and (3) A-R-N-S1-E-F (alanine, arginine, asparagine, serine S1, glutamate and phenylalanine). The atypical cloverleaf structure of serine S1 (*trnS1*) in these *Bactrocera* mitogenomes is common in all Metazoa (Jühling et al. 2012).

The 358-bp control region of *B. cinnabaria* mitogenome is exceptionally short for tephritid fruit flies. It aligns with the anterior portion of the long control region of other *Bactrocera* species. It is, however, not the shortest control region for *Bactrocera* species. *Bactrocera rubigina* (NC_046521) has a 235-bp control region (Wang et al. 2020b); it has, however, also been reported to have a long control region (MT121270 with 954 bp; Zhang et al. 2023). Another *Bactrocera* species with short control region is *B. neohumeralis* (NC_062139 with 595 bp; Towett-Kirui et al. 2022). Both long and short control regions have been reported: *B. tryoni* – NC_014611 with 951 bp (Nardi et al. 2010) and NZ520737 with 595 bp (Towett-Kirui et al. 2022); and *B. frauenfeldi* – MZ520731 with 596 bp (Towett et al. 2022) and MT121261 with 952 bp (Zhang et al. 2023). More studies are needed to clarify the occurrence of both long and short control regions in the same species.

In the present study, the subgenera of genus *Bactrocera*, particularly the subgenus *Bactrocera* represented by a large number of taxa, are monophyletic. Apart from the subgenus *Bactrocera*, a broader taxon sampling is needed to confirm the monophyletic status of the subgenera *Bulladacus*, *Daculus* and *Tetradacus*. A recent study, however, indicates that the subgenus *Bactrocera* based on current taxonomic classification is not monophyletic (Starkie et al. 2022). Some studies also indicate that the *Bactrocera* group and *Melanodacus* group of subgenera within the genus *Bactrocera* are not monophyletic (San Jose et al. 2018; Satrkie et al. 2022).

An earlier study based on partial COXI and 16S sequences shows that the subgenus *Tetradacus* is a sister group to the subgenus *Paratridacus* of the *Melanodacus* group (Zhang et al. 2010). In the present study, the subgenus *Bulladacus* of the *Bactrocera* group of subgenera (Hancock and Drew 2018) forms a clade with the subgenera *Daculus* and *Tetradacus* of the *Melanodacus* group, concurring with the clustering of the subgenera [(*Bulladacus – Parazeugodacus*) – (*Notodacus – Bactrocera – Tetradacus*)] based on partial sequences of six genes (COXI, COXII, 16S, DDOSTs2, RPA2 and EIF3L) (Starkie et al. 2022). The basal position of the subgenus *Daculus* to the subgenus *Tetradacus* is congruent with that of Zhang et al. (2010). However, the findings of San Jose et al. (2018) show *B.* (*Tetradacus*) *tsuneonis* to be basal to other *Bactrocera* taxa, including *B.* (*Daculus*) *oleae* which is closer related to *Parazeugodacus*, and the study of Starkie et al. (2022) shows the subgenus *Hemizeugodacus* (*Melanodacus* group) to be basal to the other subgenera. In an earlier study with limited taxon sampling, *Daculus* forms a lineage with *Gymnodacus* of *Melanodacus* group, which is distinct from the subgenus *Bactrocera* (Virgilio et al. 2015).

The sister lineage of *B. oleae* and *B. biguttula* (Fig. 5; da Costa et al. 2019; Zhang et al. 2023) is congruent with the proposal to name the subgenus *Afrodacus* Bezzi of the African taxa as a synonym of the subgenus *Daculus* Speiser; the Asian taxa of *Afrodacus* are members of the subgenus *Bactrocera* (Copeland et al. 2004). Based on the DNA sequences of three mitochondrial genes (NADH dehydrogenase – *nad1*, cytochrome *c* oxidase subunit I – *cox1*, and 16S rRNA), *B. biguttula* is basal to the sister lineage of *B. oleae* and *B. munroi* (Bon et al. 2016). In the present study, the *Daculus* lineage is basal to the lineage consisting of the subgenera *Bulladacus* and *Tetradacus* (and unassigned *B.* sp. '*yunnanensis*') (Fig. 5).

The very small genetic difference (lack of genetic differentiation, '*p*' = 0.03% based on 15 mt-genes) between

B. ruiliensis and *B. thailandica* supports the synonymy of *B. ruiliensis* with *B. thailandica* (Drew and Romig 2013). This synonymy was also indicated by the small COXI genetic distance of 0.00% to 1.18% between *B. ruiliensis* and *B. thailandica* (Jiang et al. 2014). The genetic distance of the near complete COXI gene in the present study is '*p*' = 0.00%.

In the present study, a small genetic difference is observed between *B. melastomatos* and *B. rubigina* with '*p*' = 0.08% and 0.40%, and intra *B. rubigina* genetic distance of 0.37% (Table S6; Fig. 5). A recent study based on a single specimen of *B. rubigina* also shows its sister lineage with *B. melastomatos* (Zhang et al. 2023). Further studies are needed to determine the taxonomic relationship of these closely related taxa.

Likewise, *B. tryoni* and *B. neohumeralis* (each with one specimen) are genetically very similar with '*p*' = 0.69% (Table S6; Fig. 5). The phylogenetic analysis of Starkie et al. (2022) shows two subclades of the *B. tryoni* complex. The two *B. tryoni* specimens do not form a sister lineage but are members of the lineage containing a *B. neohumeralis* specimen; two other *B. neohumeralis* specimens are members of another lineage.

The present phylogenetic analysis based on 15 mt-genes concurs with the finding of Wang et al. (2020a) that *B. cheni* and *B. tsuneonis* are clearly two different species; *B. cheni* is a member of the subgenus *Bactrocera* while *B. tsuneonis* is a member of the subgenus *Tetradacus* (Fig. 5), with '*p*' = 19.33% (Table S6). The synonymy of *B. cheni* with *B. lombokensis* (Drew and Romig 2013) is also not supported by a genetic distance of 9.79% based on the partial COXI sequence of *B. lombokensis* (KT594922) and that of *B. cheni* (MN883026). In the present study, *B. cheni* forms a lineage with *B. tuberculata* (Fig. 5), with a small genetic distance of '*p*' = 0.45% based on 15 mt-genes (Table S6). The taxonomic status of *B. cheni* therefore remains to be resolved.

Likewise, the present finding of '*p*' = 1.70-1.74% (Table S6) based on 15 mt-genes does not support the synonymy of *B. albistrigata* with *B. frauenfeldi* (Doorenweerd et al. 2023), as opined by Drew and Hancock (2022) and evident in the phylogenetic trees of Starkie et al. (2022) and Zhang et al. (2023).

A recent mitogenomic study on specimens of the *B. dorsalis* complex from various geographic regions indicates that they do not group together, and is therefore paraphyletic (Zhang et al. 2023). In an earlier study, one *B. invadens* mitogenome sequence forms a lineage with *B. dorsalis* while another sequence forms a lineage with *B. papayae* containing also *B. philippinensis*; both the *B. invadens* specimens are from Kenya (Drosopoulou et al. 2019). Based on the structure of the male genitalia (aedeagus) and the relationship of the structure to mating, Drew and Hancock (2022) conclude that *B. papayae* and *B. philippinensis* are conspecific, and *B. papayae* and *B. invadens* are good species separate from *B. dorsalis*. On the other hand, an integrative molecular and morphological study of *B. invadens* and *B. dorsalis* from across a wide geographic distribution supports the hypothesis that they represent a single biological species (Schultze et al. 2015).

The present phylogenetic analysis supports the high genetic similarity of *B. papayae* and *B. philippinensis* which form a sister lineage with '*p*' = 0.85%; *B. papayae* has a genetic distance of 1.00% with *B. dorsalis* and 1.18% with *B. invadens* (Table S6; Fig. 5). *B. dorsalis* and *B. invadens* form a sister lineage with '*p*' = 0.65%, indicating high genetic identity and therefore these two taxa/specimens may be conspecific. It is evident that an integrative study on broad representative samples from various geographic regions is needed to resolve the taxonomy of this and other species complexes.

In summary, we have successfully sequenced the whole mitochondrial genomes of *B.* (*Bulladacus*) *cinnabaria* (the first report for the subgenus *Bulladacus*) and *B.* (*Bactrocera*) *propinqua* from Peninsular Malaysia by next generation sequencing. The genome features are similar to other *Bactrocera* fruit flies, excepting the short control region (358 bp) in *B. cinnabaria*. Phylogenetic analysis based on the mt-genes reveals two major clades of the *Bactrocera* taxa: (A) subgenus *Bactrocera*, and (B) subgenera *Bulladacus*, *Daculus*, *Tetradacus* and unassigned *Bactrocera* sp. '*yunnanensis*'. The subgenera represented by two or more species are monophyletic. A broad taxon sampling, including taxa of all the subgenera, will help to clarify their phylogeny. The present study supports the synonymy of *B. ruiliensis* with *B. thailandica*. It also shows a high genetic similarity between (a) *B. melastomatos* and *B. rubigina*, (b) *B. papayae* and *B. philippinensis*, (c) *B. dorsalis* and *B. invadens*, (d) *B. tryoni* and *B. neohumeralis*, and (e) *B. cheni* and *B. tuberculata*; and *B. cheni* is distinct from and not a synonym of *B. tsuneonis* or *B. lombokensis*. The phylogenomics will serve as a useful dataset for studying the genetics, systematics (including species differentiation) and phylogenetic relationships of the many species/species complexes and subgenera of the genus *Bactrocera* in particular, and tephritid fruit flies in general.

5. Acknowledgments

We thank our respective institutions for their support of our research on tephritid fruit flies. H-S Yong is supported by MoHE-HIR Grant (H-50001-00-A000025) and Universiti Malaya (H-5620009).

6. References

Akaike H (1973) Information Theory and an Extension of the Maximum Likelihood Principle. In: Petrov BN, Csaki F (editors). Proceedings of the 2nd International Symposium on Information Theory pp. 267–281. Akademia Kiado, Budapest. https://doi.org/10.1007/978-1-4612-0919-5_37

Alikhan N-F, Petty NK, Zakour NLB, Beatson SA (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genome 12: 402. https://doi.org/10.1186/1471-2164-12-402

Benson G, (1999) Tandem repeats finder: a program to analyse DNA sequences. Nucleic Acids Research 27: 573–580. https://doi.org/10.1093/nar/27.2.573

Bon M-C, Hoelmer KA, Pickett CH, Kirk AA, He Y, Mahmood R, Daane KM, (2016) Populations of *Bactrocera oleae* (Diptera: Tephritidae) and its parasitoids in Himalayan Asia. Annals of the Entomological Society of America 109: 81–91. https://doi.org/10.1093/aesa/sav114

Cameron SL (2014) Insect mitochondrial genomics: implications for evolution and phylogeny. Annual Review of Entomology 59: 95–117. https://doi.org/10.1146/annurev-ento-011613-162007

Chen X, Yang H, Wu S, Zhao W, Hao G, Wang J, Jiang H (2022) BdorOBP69a is involved in the perception of the phenylpropanoid compound methyl eugenol in Oriental fruit fly (*Bactrocera dorsalis*) males. Insect Biochemistry and Molecular Biology 147: 103801. https://doi.org/10.1016/j.ibmb.2022.103801

Copeland RS, White IM, Okumu M, Machera P, Wharton RA (2004) Insects associated with fruits of the Oleaceae (Asteridae, Lamiales) in Kenya, with special reference to the Tephritidae (Diptera). Bishop Museum Bulletin in Entomology 12: 135–164.

da Costa LT, Powell C, van Noort S, Costa C, Sinno M, Caleca V, Rhode C, Kennedy RJ, van Staden M, van Asch B (2019) The complete mitochondrial genome of *Bactrocera biguttula* (Bezzi) (Diptera: Tephritidae) and phylogenetic relationships with other Dacini. International Journal of Biological Macromolecules 126: 130–140. https://doi.org/10.1016/j.ijbiomac.2018.12.186

David KJ, Ramani S (2011) An illustrated key to the fruit flies (Diptera: Tephritidae) from peninsular India and the Andaman and Nicobar Islands. Zootaxa 302: 1–31. https://doi.org/10.11646/zootaxa.3231.1.4

Dias NP, Montoya P, Nava DE (2022) A 30-year systematic review reveals success in tephritid fruit fly biological control research. Entomologia Experimentalis et Applicata 170: 370–384. https://doi.org/10.1111/eea.13157

Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M (2019) Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Research 47: 10543–10552. https://doi.org/10.1093/nar/gkz833

Doorenweerd C, Leblanc L, Norrbom AL, San Jose M, Rubinof D (2018) A global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). ZooKeys 730: 19–56. https://doi.org/10.3897/zookeys.730.21786

Doorenweerd C, San Jose M, Geib S, Dupuis J, Leblanc L, Barr N, Fiegalan E, Morris KY, Rubinoff D (2023) A phylogenomic approach to species delimitation in the mango fruit fly (*Bactrocera frauenfeldi*) complex: A new synonym of an important pest species with variable morphotypes (Diptera: Tephritidae. Systematic Entomology 48: 10–22. https://doi.org/10.1111/syen.12559

Drew RAI, Hancock DL (2022) Biogeography, Speciation and Taxonomy within the genus *Bactrocera* Macquart with application to the *Bactrocera dorsalis* (Hendel) complex of fruit flies (Diptera: Tephritidae: Dacinae). Zootaxa 5190(3): 333–360. https://doi.org/10.11646/zootaxa.5190.3.2

Drew RAI, Romig MC (2013) Tropical fruit flies (Tephritidae: Dacinae) of South-East Asia. CAB International, Wallingford. 653 pp. https://doi.org/10.1079/9781780640358.0000

Drew RAI, Romig MC (2022) The fruit fly fauna (Diptera: Tephritidae: Dacinae) of Papua New Guinea, Indonesian Papua, associated islands and Bougainville. CAB International, Wallingford. https://doi.org/10.1079/9781789249514.0000

Drosopoulou E, Syllas A, Goutakoli P, Zisiadis G-A, Konstantinou T, Pangea D, Sentis G, van Sauers-Muller A, Wee S-K, Augustinos AA, Zacharopoulou A, Bourtzis K (2019) The complete mitochondrial genome of *Bactrocera carambolae* (Diptera: Tephritidae): genome description and phylogenetic implications. Insects 10: 429. https://doi.org/10.3390/insects10120429

Duyck P-F, Jourdan H, Mille C (2022) Sequential invasions by fruit flies (Diptera: Tephritidae) in Pacific and Indian Ocean islands: A systematic review. Ecology and Evolution 12: e8880. https://doi.org/10.1002/ece3.8880

Fan Y, Zhang C, Qin Y, Yin X, Dong X, Desneux N, Zhou H (2022) Monitoring the methyl eugenol response and non-responsiveness mechanisms in Oriental fruit fly *Bactrocera dorsalis* in China. Insects 13: 1004. https://doi.org/10.3390/insects13111004

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hancock DL, Drew RAI (2018) A review of the subgenera *Apodacus* Perkins, *Hemizeugodacus* Hardy, *Neozeugodacus* May, stat. rev., *Semicallantra* Drew and *Tetradacus* Miyake of *Bactrocera* Macquart (Diptera: Tephritifae: Dacinae). Australian Entomologist 45(1): 105–132.

Hardy DE (1973) The fruit flies (Tephritidae–Diptera) of Thailand and bordering countries. Pacific Insects Monograph 31: 1–353.

Hardy DE, Adachi M (1954) Studies in the fruit flies of the Philippine Islands, and Malaya. Part 1. Dacini (Tephritidae–Diptera). Pacific Science 8: 147–204.

He M, Chen H, Yang X, Gao Y, Lu Y, Cheng D (2022) Gut bacteria induce oviposition preference through ovipositor recognition in fruit fly. Communications Biology 5: 973. https://doi.org/10.1038/s42003-022-03947-z

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Jiang F, Jin Q, Liang L, Zhang AB, Li ZH (2014) Existence of species complex largely reduced barcoding success for invasive species of Tephritidae: a case study in *Bactrocera* spp. Molecular Ecology Resources 14: 1114–1128. https://doi.org/10.1111/1755-0998.12259

Jühling F, Pütz J, Bernt M, Donath A, Middendorf M, Florentz C, Stadler PF (2012) Improved systematic tRNA gene annotation allows new insights into the evolution of mitochondrial tRNA structures and into the mechanisms of mitochondrial genome rearrangemnts. Nucleic Acids Research 40: 2833–2845. https://doi.org/10.1093/nar/gkr1131

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Khan M, Seheli K, Bari MA, Sultana N, Khan SA, Sultana KF, Hossain MA (2019) Potential of a fly gut microbiota incorporated gel-based larval diet for rearing *Bactrocera dorsalis* (Hendel). BMC Biotechnology 19 (Suppl 2): 94. https://doi.org/10.1186/s12896-019-0580-0

Kumar K, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Leblanc L, Hossain MA, Doorenweerd C, Khan SA, Momen M, San Jose M, Rubinoff D (2019) Six years of fruit fly surveys in Bangladesh: a new species, 33 new country records and discovery of the highly invasive *Bactrocera carambolae* (Diptera, Tephritidae). ZooKeys 876: 87–109. https://doi.org/10.3897/zookeys.876.38096

Leblanc L, Tsatsia F, Doorenweerd C (2021a) Novel lures and COI sequences reveal cryptic new species of *Bactrocera* fruit flies in the

Solomon Islands (Diptera, Tephritidae, Dacini). ZooKeys 1057: 49–103. https://doi.org/10.3897/zookeys.1057.68375

Leblanc L, Hossain MA, Momen M, Seheli K (2021b) New country records, annotated checklist and key to the dacine fruit flies (Diptera: Tephritidae: Dacinae: Dacini) of Bangladesh. Insecta Mundi 0880: 1–56.

Majumder R, Taylor PW, Chapman TA (2022) Dynamics of the Queensland Fruit Fly microbiome through the transition from nature to an established laboratory colony. Microorganisms 10: 291. https://doi.org/10.3390/microorganisms10020291

Nardi F, Carapelli A, Boore JL, Roderick GK, Dallai R, Frati F (2010) Domestication of olive fly through a multi-regional host shift to cultivated olives: comparative dating using complete mitochondrial genomes. Molecular Phylogenetics and Evolution 57(2): 678–686. https://doi.org/10.1016/j.ympev.2010.08.008

Perna NT, Kocher TD (1995) Patterns of nucleotide composition in fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41: 353–358. https://doi.org/10.1007/BF00186547

Rambaut A (2012) FigTree (version 1.4.0). Available at. http://tree.bio.ed.ac.uk/software/figtree.

Ravigné V, Becker N, Massol F, Guichoux E, Boury C, Mahé F, Facon B (2022) Fruit fly phylogeny imprints bacterial gut microbiota. Evolutionay Applications 15: 1621–1638. https://doi.org/10.1111/eva.13352

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large datasets. Molecular Biology and Evolution 34: 3299–3302. https://doi.org/10.1093/molbev/msx248

San Jose M, Doorenweerd C, Leblanc L, Barr N, Geib S, Rubinoff D (2018) Incongruence between molecules and morphology: a seven-gene phylogeny of Dacini fruit flies paves the way for reclassification (Diptera: Tephritidae). Molecular Phylogenetics and Evolution 121: 139–149. https://doi.org/10.1016/j.ympev.2017.12.001

Schutze MK, Mahmood K, Pavasovic A, Bo W, Newman J, Clarke AR, Krosch MN, Cameron SL (2015) One and the same: integrative taxonomic evidence that the African Invasive Fruit Fly *Bactrocera invadens* (Diptera: Tephritidae), is the same species as the Oriental Fruit Fly *Bactrocera dorsalis*. Systematic Entomology 40: 472–486. https://doi.org/10.1111/syen.12114

Singh MP, Sharma I, Hancock DL, Prabhakar CS (2022) A new species of *Bactrocera* Macquart and a new distribution record of *Dacus* Fabricius (Diptera: Tephritidae: Dacinae) from India. Zootaxa 5168(2): 237–250. https://doi.org/10.11646/zootaxa.5168.2.9

Song S-L, Yong H-S, Suana IW, Lim P-E (2018) Complete mitochondrial genome of *Bactrocera ritsemai* (Insecta: Tephritidae) and phylogenetic relationship with its congeners and related tephritid taxa. Journal of Asia-Pacific Entomology 21: 251–257. https://doi.org/10.1016/j.aspen.2017.12.009

Starkie ML, Strutt F, Royer JE (2022) New records and a description of a new species of fruit fly (Diptera: Tephritidae) from eastern Australia. Australian Entomologist 49(3): 169–180.

Swofford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA. https://doi.org/10.1002/0471650129.dob0522

Tanabe AS (2007) Kakusan: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. Molecular Ecology Notes 7: 962–964. https://doi.org/10.1111/j.1471-8286.2007.01807.x

Towett-Kirui S, Morrow JL, Riegler M (2022) Substantial reaarangements, single nucleotide frameshift deletion and low diversity in mitogenome of Wolbachia-infected strepsipteran endoparasitoid in comparison to its tephritid hosts. Scientific Reports 12: 477. https://doi.org/10.1038/s41598-021-04398-y

Vargas RI, Piñero JC, Leblanc L (2015) An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific Region. Insects 6: 297–318. https://doi.org/10.3390/insects6020297

Virgilio M, Jordaens K, Verwimp C, White IM, De Meye M (2015) Higher phylogeny of frugivorous flies (Diptera, Tephritidae, Dacini): localised partition conflicts and a novel generic classification. Molecular Phylogenetics and Evolution 85: 171–179. https://doi.org/10.1016/j.ympev.2015.01.007

Wang T, Ren Y-L, Zhong Y, Yang M-F (2020a) Sequencing and analysis of the complete mitochondrial genome of *Bactrocera cheni* from China and its phylogenetic analysis. Mitochondrial DNA Part B 5(1): 780–781. https://doi.org/10.1080/23802359.2020.1715882

Wang T, Ren Y-L, Shao W-Z, Yang M-F, Wu G-H (2020b) Characterization of the complete mitochondrial genome of *Bactrocera rubigina* from China and its phylogenetic analysis. Mitochondrial DNA Part B 5(2): 1372–1373. https://doi.org/10.1080/23802359.2020.1735956

Yong HS (1992) *Garcinia cowa* (Guttiferae): host plant for fruit fly *Bactrocera propinqua* (Tephritidae). Nature Malaysiana 17: 66–67.

Yong HS (1994) The Gnemon fruit fly. Nature Malaysiana 19: 37–40.

Yong H-S, Song S-L, Lim P-E, Chan, K-G, Chow W-L, Eamsobhana P (2015) Complete mitochondrial genome of *Bactrocera arecae* (Insecta: Tephritidae) by next-generation sequencing and molecular phylogeny of Dacini tribe. Scientific Reports 5: 15155. https://doi.org/10.1038/srep15155

Yong H-S, Song S-L, Lim P-E, Eamsobhana P, Suana IW (2016a) Complete mitochondrial genome of three *Bactrocera* fruit fies of subgenus *Bactrocera* (Diptera: Tephritidae) and their phylogenetic implications. PLoS ONE 11: e014820. https://doi.org/10.1371/journal.pone.0148201

Yong H-S, Song S-L, Lim P-E, Eamsobhana P, Suana IW (2016b) Differentiating sibling species of *Zeugodacus caudatus* (Insecta: Tephritidae) by complete mitochondrial genome. Genetica 144: 513–521. https://doi.org/10.1007/s10709-016-9919-9

Yong H-S, Song S-L, Chua K-O, Lim P-E (2017a) Microbiota associated with Bactrocera carambolae and *B. dorsalis* (Insecta: Tephritidae) revealed by next-generation sequencing of 16S rRNA gene. Meta Gene 11: 189–196. https://doi.org/10.1016/j.mgene.2016.10.009

Yong H-S, Song S-L, Chua K-O, Lim P-E (2017b) High diversity of bacterial communities in developmental stages of *Bactrocera carambolae* (Insecta: Tephritidae) revealed by Illumina MiSeq sequencing of 16S rRNA gene. Current Microbiology 74: 1076–1082. https://doi.org/10.1007/s00284-017-1287-x

Yong H-S, Song S-L, Chua K-O, Lim P.-E (2017c) Predominance of *Wolbachia* endosymbiont in the microbiota across life stages of *Bactrocera latifrons* (Insecta:Tephritidae). Meta Gene 14: 6–11. https://doi.org/10.1016/j.mgene.2017.07.007

Yong H-S, Chua K-O, Song S-L, Liew YJ-M, Eamsobhana P, Chan K-G (2021) Complete mitochondrial genome of *Dacus vijaysegarani* and phylogenetic relationships with congeners and other tephritid fruit flies (Insecta: Diptera). Molecular Biology Reports 48: 6047–6056. https://doi.org/10.1007/s11033-021-06608-2

Zhang B, Liu YH, Wu WX, Wang ZL (2010) Molecular phylogeny of *Bactrocera* species (Diptera: Tephritidae: Dacini) inferred from mi-

tochondrial sequences of 16S rDNA and COI sequences. Florida Entomologist 93(3): 369–377. https://doi.org/10.1653/024.093.0308

Zhang Y, Li H, Feng S, Qin Y, De Meyer M, Virgilio M, Singh S, Jiang F, Kawi AP, Susanto A, Sañudo IM, Wu J, Badji K, Davaasambuu U, Li Z (2023) Mitochondrial phylogenomics reveals the evolutionary and biogeographical history of fruit flies (Diptera: Tephritidae). Entomologia Generalis 43: 359–368. https://doi.org/10.1127/entomologia/2022/1594

Supplementary Material 1

Figure S1

Authors: Yong H-S, Song S-L, Chua K-O, Liew YJM, Chan K-G, Lim P-E, Eamsobhana P (2024)
Data type: .pdf
Explanation notes: Stem-loop structures of intergenic sequences in the mitogenomes of *Bactrocera cinnabaria* and *B. propinqua*.

Link: https://doi.org/10.3897/asp.82.e115954.suppl1

Supplementary Material 2

Tables S1–S6

Authors: Yong H-S, Song S-L, Chua K-O, Liew YJM, Chan K-G, Lim P-E, Eamsobhana P (2024)
Data type: .pdf
Explanation notes: **Table S1.** List of *Bactrocera* mitogenomes from GenBank. — **Table S2.** Base composition, A + T content (%), AT and GC skewness of the 13 protein coding genes in *Bactrocera cinnabaria* (Bc) and *B. propinqua* (Bp) mitogenomes. — **Table S3.** Amino acid frequency for the protein-coding genes of *Bactrocera cinnabaria* (Bc) and *B. propinqua* (Bp) mitogenomes. — **Table S4.** Relative synonymous codon usage for the 13 protein coding genes of *Bactrocera cinnabaria* (Bc) and *B. propinqua* (Bp) mitogenomes. — **Table S5.** Ka, Ks, Ka/Ks values for the 13 protein coding genes of *Bactrocera cinnabaria* and *B. propinqua* mitogenomes. — **Table S6.** Pair-wise genetic distance (%) of *Bactrocera* taxa based on 15 mt-genes (13 protein-coding genes and 2 rRNA genes).

Link: https://doi.org/10.3897/asp.82.e115954.suppl2