



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

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## 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. molluccensis* of the monophyletic subgenus *Bactrocera*. The present study supports the synonymy of *B. ruliensis* 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.



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

wise, 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 1<sup>st</sup> 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.

Region	A+T%		AT skew		GC skew	
	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
1 <sup>st</sup> codon position	63.8	65.1	-0.069	-0.076	0.166	0.198
2 <sup>nd</sup> codon position	65.1	65.5	-0.382	-0.380	-0.167	-0.165
3 <sup>rd</sup> 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*.

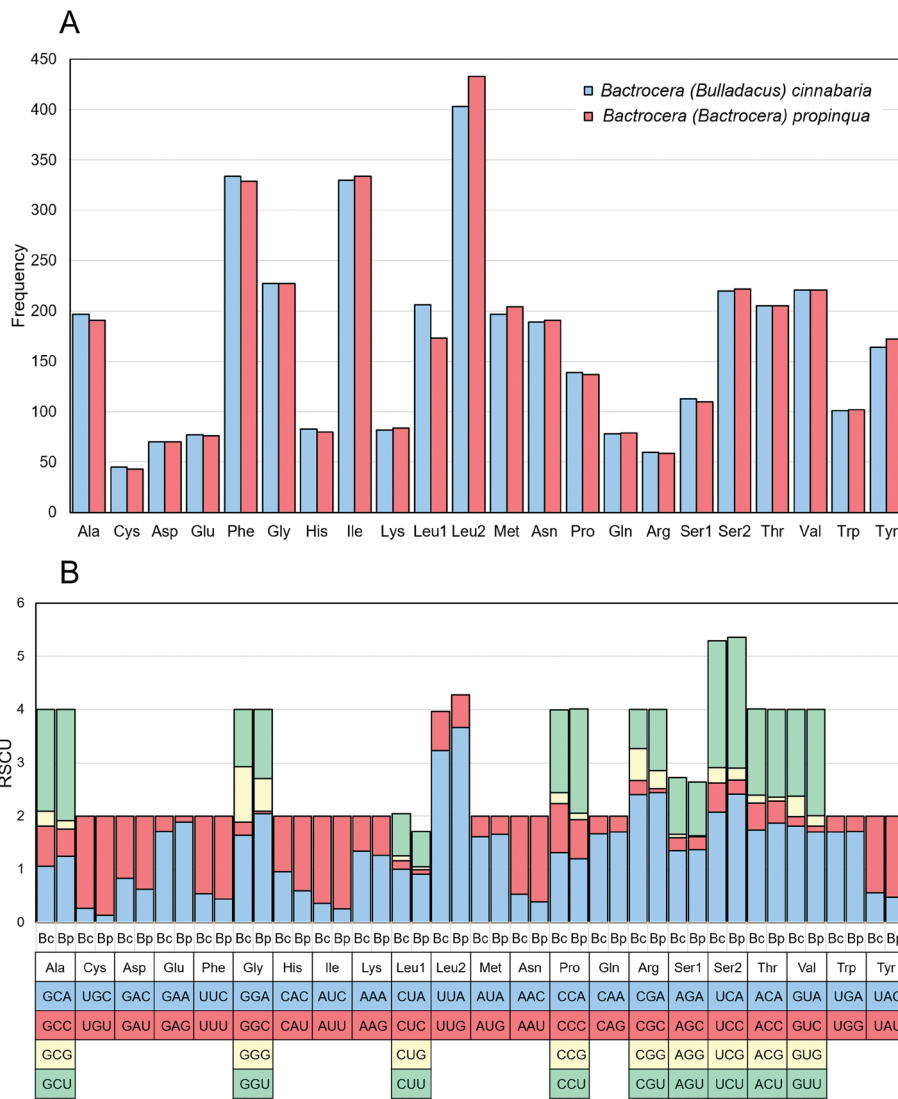
### 3.4. Control region

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.

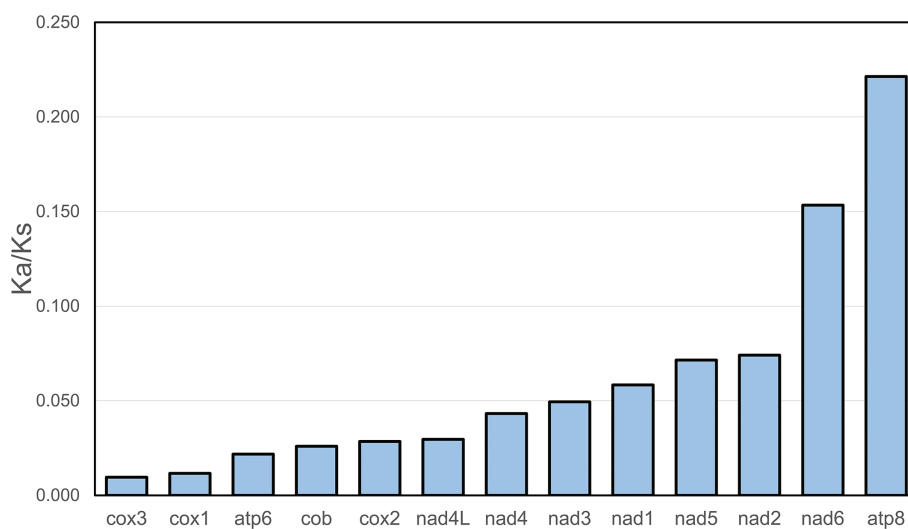
Simple tandem repeats and palindromes were present in the control region of *B. cinnabaria* and *B. propinqua* (Table 3). Some were common in the two mitogenomes, while some were present only in *B. cinnabaria* or *B. propinqua*. There were more tandem repeats and palindromes in the control region of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



**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.

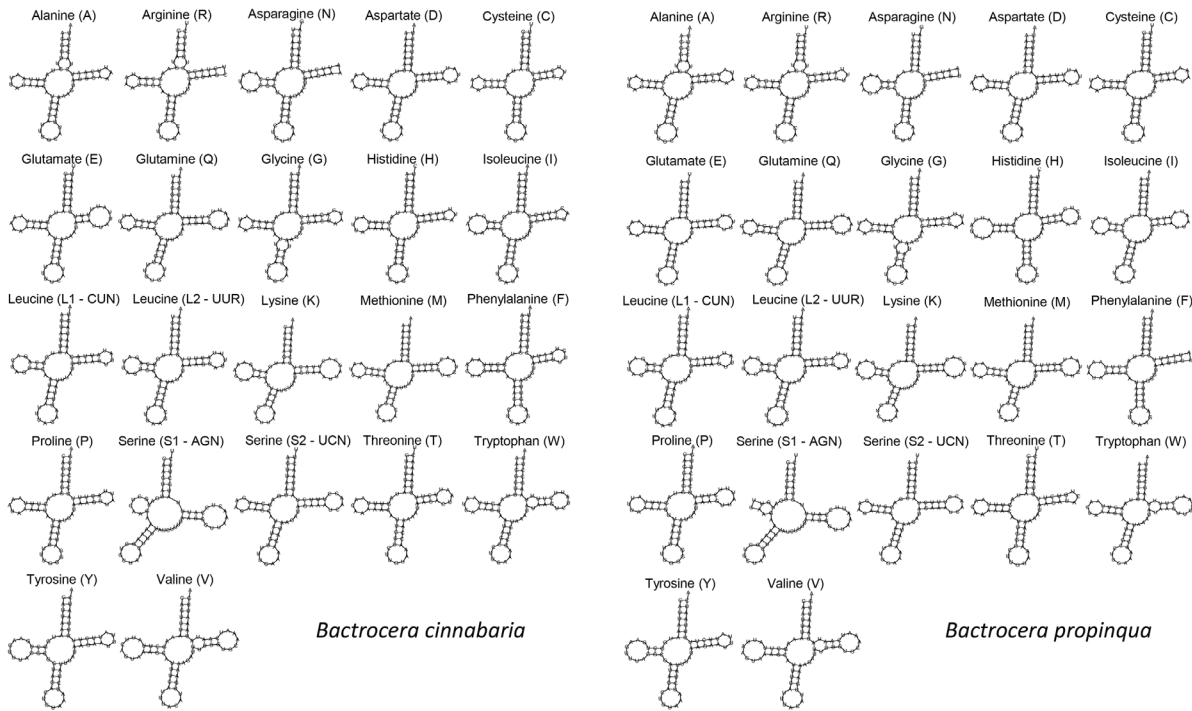


Figure 4. Cloverleaf structure of the 22 inferred tRNAs in the mitogenomes of *Bactrocera cinnabaria* and *B. propinqua*.

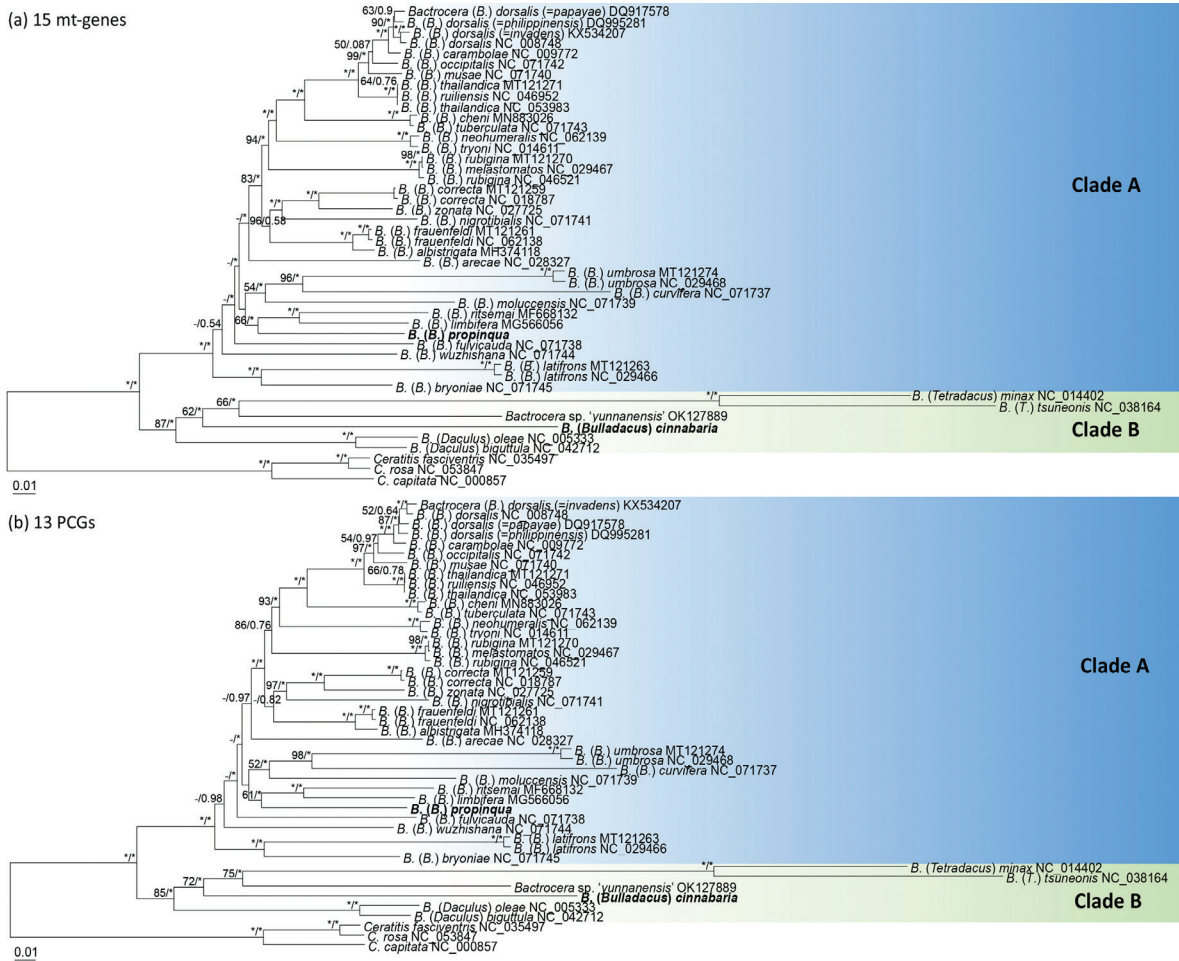


Figure 5. Phylogenetic trees (ML/BI) of (a) 15 mt-genes (13 PCGs + 2 rRNA genes), and (b) 13 PCGs of the whole mitogenomes of *Bactrocera* fruit flies with *Ceratit*s *capitata*, *C. fasciventris*, and *C. rosa* as the outgroup taxa. Numeric values at the nodes are ML bootstrap and Bayesian posterior 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.

Type of repeat	Repetitive sequence	No. of repeat	
		<i>B. cinnabaria</i>	<i>B. propinqua</i>
Simple sequence repeat	(A) <sub>12</sub>	0	1
	(A) <sub>24</sub>	0	1
	(T) <sub>24</sub>	0	1
	(TA) <sub>3</sub>	0	4
	(TA) <sub>6</sub>	0	1
	(ATA) <sub>2</sub>	0	1
	(AAT) <sub>2</sub>	2	3
	(TAA) <sub>2</sub>	5	4
	(TAG) <sub>2</sub>	1	1
	(TTA) <sub>2</sub>	2	3
	(TAAA) <sub>2</sub>	2	1
	(AAAT) <sub>2</sub>	1	1
	(ATAA) <sub>2</sub>	0	1
	(TTAA) <sub>2</sub>	0	2
	(TTTA) <sub>2</sub>	0	2
	(AAATA) <sub>2</sub>	0	1
	(AATTT) <sub>2</sub>	0	1
	(TTTAA) <sub>2</sub>	0	1
(TTTAAA) <sub>2</sub>	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
TAAAATTTAAAAT	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.

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## 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*.

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**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).

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