THE TAXONOMY AND PHYLOGENETIC RELATIONSHIPS OF SPECIES IN THE
BACTROCERA MUSAE COMPLEX OF FRUIT FLIES
(DIPTERA: TEPHRITIDAE: DACINAE) IN PAPUA NEW GUINEA

R. A. I. Drew
International Centre for the Management of Pest Fruit Flies, Griffith School of Environment, Griffith University, Nathan Qld 4111, Australia.
Email: d.drew@griffith.edu.au

Jing Ma
Griffith School of Environment, Griffith University, Nathan Qld 4111, Australia.

S. Smith
Griffith School of Environment, Griffith University, Nathan Qld 4111, Australia.

J. M. Hughes
Griffith School of Environment, Griffith University, Nathan Qld 4111, Australia.

ABSTRACT. – Bactrocera (Bactrocera) musae (Tryon) is a major pest species of some banana varieties in northern Queensland, the Torres Strait Islands, Papua New Guinea and associated islands. It is the only pest species in a complex of 17 species described herein. Five of these are new species and are described and illustrated: Bactrocera (Bactrocera) balagawii, B. (B.) parabancroftii, B. (B.) ramuensis, B. (B.) rufivitta, B. (B.) uvariae. The remaining 12 species have been previously described and are revised with up-to-date information on host plants, attractant records and geographic distributions. Further, a molecular study was able to explore the distinction between six of the described species. A phylogeny based on 562 base pairs of the COI gene showed strong agreement between morphological and molecular data. Furthermore, for three of the species that occurred in sympatry and were represented by sufficient sample numbers (namely, B. contermina, B. musae, B. rufivitta), analysis of eight new microsatellite loci using STRUCTURE also supported them as distinct species.

KEY WORDS. – Bactrocera, fruit fly, cryptic species, mtDNA, microsatellites.

INTRODUCTION

The tephritid subfamily Dacinae contains a number of large species complexes, based primarily on morphological characters. There are 12 known species in Papua New Guinea that Drew (1989) placed in the assita and musae complexes. These have been separated only on male lure responses, the assita group responding to cue lure and the musae group to methyl eugenol. However, on the basis of morphology, all 12 species could be placed in the musae complex, similar to the combination of the aemula and dorsalis groups into the dorsalis complex by Drew and Hancock (1994).

A knowledge of the taxonomy and biology of the musae complex of Papua New Guinea is of great importance because Bactrocera musae (Tryon) is a major pest of some banana varieties. All other morphologically similar species in the complex have non-pest status. In 2000, B. musae was introduced into East New Britain where it is causing serious food crop losses. Therefore, it is necessary to be able to identify B. musae accurately and to define its geographic distribution within Papua New Guinea. Locations, especially islands, where B. musae does not occur could become important banana growing and export centres.

Traditionally, descriptions of new species are based on morphological characters and decisions as to whether or not they represent true biological species (sensu Mayr, 1963; Paterson, 1985) are not, in most cases, included. However, recent evidence combining molecular with morphological data has demonstrated that morphological characters alone may not always be the best means of defining species status. For example, recent studies of the dorsalis complex of fruit flies have shown that some morphologically defined species
occur within complexes of closely related species where the status of some of these species had been confirmed on biological and molecular criteria (Drew & Hancock, 1994). By combining morphological and biological evidence with that derived from molecular data, a much clearer picture of true species relationships can be obtained (Drew & Hancock, 1994). Not only can phylogenetic relationships between different taxa be assessed, but molecular data can also provide evidence as to whether or not taxa are interbreeding in nature, a condition of the biological species concept (Paterson, 1985).

Firstly, this paper presents morphological descriptions that support the notion that there are 17 species in the musae complex, along with information on male lure responses and host plants where they are known. Secondly, by using a subset of the species from which DNA could be extracted, sequence data from a fragment of the mitochondrial cytochrome c oxidase 1 (CO1) gene have been studied in order to examine the congruence between molecular and morphological data. This gene has been used in many other studies of insect phylogenetic relationships and is particularly useful for investigating closely related species (Eastwood and Hughes, 2003). It has also been used in other studies of tephritids, (e.g. Virgilio et al., 2009), although it has not always identified relationships without additional information from nuclear genes. In this study, we used both sequence data from the CO1 gene and nuclear data from microsatellites. Specifically the hypothesis that individuals of each morphologically defined species form a monophyletic clade within the gene tree has been tested. Thirdly, for the three species of sufficient sample size and which occur in sympatry, eight new microsatellite loci have been isolated and used to investigate the hypothesis that gene flow is restricted between them.

MATERIAL AND METHODS

Specimen collection, preservation and morphology.

Specimens were collected by male lure trapping and host fruit rearing, at localities along the northern coastline of Papua New Guinea from Madang to the West Sepik, at Lae and East New Britain. For baseline data on B. musae, confirmed specimens were reared at Tully, North Queensland.

For molecular studies, specimens were preserved in 70% ethyl alcohol. For morphological examination and permanent record, specimens were dried, pinned and labelled. Morphological examinations, descriptions and illustrations were carried out using stereomicroscopy, at the International Centre for the Management of Pest Fruit Flies, Griffith University, Brisbane.

The nomenclature for the morphological characters is consistent with McAlpine (1981) except for the term “mesopleural stripe” which has been commonly used for many years by a range of authors.

Abbreviations of institutions where specimens have been deposited: ANIC = Australian National Insect Collection, CSIRO, Canberra; BMNH = The National History Museum, London; QDPI = Queensland Department of Employment, Economic Development and Industry, Brisbane; QM = Queensland Museum Insect Collection, Queensland Museum, Brisbane.

Molecular methods. – For molecular analyses, specimens were aggregated into morphologically similar groups. However, correctly preserved specimens of all species were not available for these studies. The following species were included: B. bancroftii (Tryon), B. contermina Drew, B. musae (Tryon), B. prolixa Drew, B. rajivitta new species, B. tinomisci Drew. In addition, B. endiandrae (Perkins & May) and B. tryoni (Froggatt) were included as representatives outside the musae complex with B. tryoni used as the outgroup.

mtDNA analysis. – For each fly sample, one leg was removed and used for DNA extraction using the QIAGEN DNeasy mini-kit (QIAGEN, Germany). DNA concentrations ranged from 20–50 ng/µl. A 562 bp fragment of the CO1 gene was amplified using the published primers CO1-L and CO1-H (Folmer et al., 1994). Polymerase chain reaction (PCR) was performed in 50 µl volumes and contained 100 ng of DNA, 1 × PCR Buffer, 200 µM of each dNTP (Bioline, UK), 2 mM MgCl2 (Bioline, UK), 1 unit of Taq polymerase (Invitrogen, USA), and 400 nM of each primer. PCR cycle conditions were: 1 cycle of 3 min at 95°C, then 15 cycles of 30 s at 94°C, 30 s at 40°C, 1 min at 72°C, then 25 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C, and a final extension of 5 min at 72°C. The PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN) before sequences were determined using the ABI Big-Dye Terminator Cycle Sequencing Kit 2.0 and electrophoresis on an ABI 377 automated sequencer (Applied Biosystems, USA).

The molecular sequence data was trimmed to remove primer sequences and end sections of low quality bases before being aligned using the program BioEdit (Hall, 1999). Phylogenetic analysis of the aligned sequences was performed using the software package PAUP* (V. 4.0b4a, Swofford, 1999). A DNA distance matrix was calculated based on the Kimura two-parameter model as suggested by Modeltest. This matrix was used to construct a maximum likelihood tree using B. tryoni (Froggatt) and B. endiandrae (Perkins & May) as outgroups. Bootstrap support for this tree was generated by performing 1000 iterations of the original data set, prior to the distance matrix and tree calculations and then creating a majority rule consensus tree. Bayesian posterior probabilities were also calculated for this data set using the software program Mr Bayes Version 2.01 (Huelsenbeck and Ronquist, 2001) with a burn-in of 35,000 and 450,000 repetitions.
Offprint requests to: S. Drew

The Raffles Bulletin of Zoology • Volume 69, Number 1, 2011

147

Microsatellite development and analysis. – Total genomic DNA was extracted from two samples using the standard CTAB method (Doyle & Doyle, 1987). Extracted DNA was then digested using the restriction enzyme Apal. DNA fragments of 200–700 bp were isolated in a 1.3% agarose gel and then ligated into the HindIII site of the pUC18 plasmid vector (Amersham-Pharmacia). The recombinant plasmids were electroporated into Escherichia coli cells (strain JM109, Promega) and the transformed E. coli cells were then grown on LB-Ampicillin plates at 37°C overnight. Colonies were then transferred to new LB-Ampicillin plates in a new grid array and incubated at 37°C overnight before being blotted on to nylon membrane (Amersham-Pharmacia). The recombinant plasmid DNA was then denatured and cross-linked to the membrane. Radiolabelled [γ32P]-ATP(Perkin-Elmer) probes [(AAC)₈, (ACC)₈, (AAG)₈, (AGC)₈, (ACG)₈, (ACT)₈, (CA)₁₅, (AG)₁₂] were used to identify positive clones containing the repeat sequences. Plasmid DNA of the positive clones was extracted using the miniprep method and sequenced using the ABI Big-Dye Terminator Cycle Sequencing Kit 2.0 and electrophoresis on an ABI 377 automated sequencer (Applied Biosystems, USA). These sequences were used to design PCR primers flanking the repeat regions for subsequent analysis.

One hundred and thirty six flies were used to screen for polymorphic loci. One of each primer set was fluorescently labelled with HEX (Geneworks, Australia). The PCR amplifications were performed in 12.5 µl volumes containing 50 ng of DNA, 1 × PCR Buffer, 200 µM of each dNTP (Bioline, UK), 2 mM MgCl₂, 0.25 units of Taq polymerase (Invitrogen, USA), and 400 nM of each primer. Before loading, both the PCR products and a TAMRA-labelled 350 bp size marker (PE Applied Biosystems), were diluted with formamide loading dye (1:1) and denatured at 95°C for 5 min. A 5% acrylamide denaturing gel was cast and loaded into a Gelscan 2000 fragment analyser (Corbett Research, Australia). Each lane of the gel was loaded with 1 µl of PCR product with every third lane containing 1 µl of the TAMRA 350 size-marker. Fragment size data was scored using the One-Dscan software supplied with the Gelscan 2000.

Microsatellite genotype data was analysed using the programme STRUCTURE (ver. 2.1, Pritchard et al., 2000) to identify (K) distinct clusters of individuals following the Delta-K method prescribed in Evanno et al. (2005). Multilocus genotypes for the 136 individuals were analysed using the admixture model with correlated allele frequencies and no prior population information supplied. The burn-in period was set to 30,000 and the number of repetitions at 100,000. The analysis was repeated 20 times for each proposed value of K from 1 to 10. We then plotted the second order rate of change of the log probability of the data (ΔK) with respect to the number of clusters using the programme Structure Harvester (Earl, 2011). The number of clusters with the highest ΔK value is a good predictor of the actual number of clusters in the data set (Evanno et al., 2005). This method is based on the notion that populations that are freely interbreeding should be in Hardy-Weinberg equilibrium and in linkage equilibrium. It therefore assigns individuals to populations, according to the level of admixture suggested by their multilocus genotypes, such that the resulting groupings match the optimal balance of equilibrium (Pritchard, 2000).

RESULTS

Seventeen species were differentiated, placed and described within the musae complex on the basis of morphological analyses. Another species, Bactrocera endiandrae (Perkins & May), belongs to the dorsalis complex and was selected for the molecular study because it is morphologically close to B. musae. Twelve species have been described previously and five are new. Diagnoses are given below for the 12 known species and full descriptions of the five previously undescribed. A remaining undescribed species, coded m-3, cannot be described at present due to insufficient intact specimens.

TAXONOMY

Definition of Bactrocera musae complex. – Bactrocera (Bactrocera) species with a black scutum, two lateral postnotal yellow vittae present, medial postnotal yellow vitta absent, wing colourless except for a narrow fuscous costal band (confluent with or overlapping R₈ but not to R₉), and cubital streak, abdominal terga predominantly orange-brown. See Drew & Hancock (1994) for definitions of terminologies used and interpretation of size of characters.

Species included in Bactrocera musae complex

Bactrocera (Bactrocera) assita Drew
Bactrocera (Bactrocera) balagawii Drew, new species
Bactrocera (Bactrocera) bancroftii (Tryon)
Bactrocera (Bactrocera) brevistriata (Drew)
Bactrocera (Bactrocera) circamusae Drew
Bactrocera (Bactrocera) commina Drew
Bactrocera (Bactrocera) contermina Drew
Bactrocera (Bactrocera) contigua Drew
Bactrocera (Bactrocera) finitima Drew
Bactrocera (Bactrocera) musae (Tryon)
Bactrocera (Bactrocera) parabancroftii Drew, new species
Bactrocera (Bactrocera) prolisa Drew
Bactrocera (Bactrocera) ramuensis Drew, new species
Bactrocera (Bactrocera) robertsi Drew
Bactrocera (Bactrocera) rufivitta Drew, new species
**Bactrocera (Bactrocera) tinomiscii** Drew

**Bactrocera (Bactrocera) uvariae** Drew, new species

### Key to species in the *Bactrocera musae* complex in Papua New Guinea

1. Notopleuron with anterior one half black and posterior one half yellow; scutellum yellow with broad black basal band ........................................... **Bactrocera robertsi** Drew

   Notopleuron entirely yellow; scutellum yellow with narrow black basal band ................................................................. 2

2(1) Lateral postnotural yellow vitta ending before *ia.* seta .... ......................................................................................... 3

   Lateral postnotural yellow vitta ending at or behind *ia.* seta ......................................................................................... 6

3(2) Wing with cells *bc* and *c* pale fulvous ......................... **Bactrocera comminata** Drew

   Wing with cells *bc* and *c* colourless or with an extremely pale tint .................................................................................. 4

4(3) Abdomen shape elongate-oval . **Bactrocera prolisla** Drew

   Abdomen shape oval ........................................................................... 5

5(4) Scutum entirely black; wing with cubital streak broad .... **Bactrocera brevistriata** Drew

   Scutum black with a broad medial longitudinal dark red-brown band over anterior one half; wing with cubital streak narrow ........................................... **Bactrocera rufivitta**, new species

6(2) Scutum with a lanceolate black pattern interspersed with red-brown ... **Bactrocera musae** (Tryon)(some specimens)

   Scutum entirely black or if pale then tending towards dark fuscous centrally ........................................................................ 7

7(6) Wing with cells *bc* and *c* colourless ................................. 8

   Wing with cells *bc* and *c* coloured ......................................................................................................................... 12

8(7) Abdomen shape elongate-oval ........................................... **Bactrocera balagawii**, new species

   Abdomen shape oval ........................................................................... 9

9(8) Wing with costal band just overlapping *R*₂₃, .................. 10

   Wing with costal band distinctly overlapping *R*₂₃, almost confluent with *R*₄₅, ................................................................. 11

10(9) Lateral postnotural yellow vitta ending at *ia.* seta; lateral margins of abdominal terga III–V or fuscous to dark fuscous patterns ................................................................................................................................. 12

   Lateral postnotural yellow vitta ending behind *ia.* seta; lateral margins of abdominal terga III–V with fuscous to dark fuscous patterns ................................................................................................................................. 11

11(9) Wing with costal band overlapping midway between *R*₂₃ and *R*₄₅, ........................................... **Bactrocera musae** (Tryon)(some specimens)

   Wing with costal band almost confluent with *R*₄₅, ................................................................................................................................. 10

12(7) Wing with cells *bc* and *c* fuscous and covered with dense microtrichia ........................................... **Bactrocera assita** Drew

   Wing with cells *bc* and *c* pale fulvous to fulvous; microtrichia in outer corner to outer one half of cell *c* only .................. 13

13(12) Wing with cells *bc* and *c* fulvous; mesopleural stripe reaching to anterior *npl.* seta dorsally; face with spots extremely small and pale ................ **Bactrocera circumanusae** Drew

   Wing with cells *bc* and *c* pale fulvous; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta dorsally; face with spots large and distinct ................................................................................................................................. 14

14(13) Scutellum with fuscous to dark fuscous spot on apex .... **Bactrocera bancroftii** (Tryon)(some specimens)

   Scutellum entirely yellow ................................................................. 15

15(14) Wing with costal band narrow, confluent with or just overlapping *R*₂₃ ................................................................. 16

   Wing with costal band almost confluent with *R*₄₅, ............ 18

16(15) Abdominal terga III–V with shining spots (ceromae) fuscous to dark fuscous ........ **Bactrocera uvariae**, new species

   Abdominal terga III–V with shining spots (ceromae) orange-brown ...................................................................................... 17

17(16) Abdominal terga III–V entirely orange-brown .......... ........ **Bactrocera bancroftii** (Tryon)(some specimens)

   Abdominal terga III–V orange-brown with a narrow fuscous to black medial longitudinal band and narrow fuscous lateral margins ........ **Bactrocera parabancroftii**, new species

18(15) Abdominal terga entirely orange-brown ............................ ........ **Bactrocera contermina** Drew

   Abdominal terga III–V orange-brown with a narrow medial longitudinal pale fuscous line and pale fuscous anterolateral corners on tergum III ........ **Bactrocera contigua** Drew

---

### DIAGNOSES AND DESCRIPTIONS OF SPECIES

**Bactrocera (Bactrocera) assita** Drew


**Diagnosis.** – A small species (wing length 5.3 mm); face with a pair of medium sized oval black spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postnotural vitta of medium width and narrowing slightly posteriorly to end at *ia.* seta; mesopleural stripe slightly wider than notopleuron dorsally; wings with cell *c* pale fulvous, cell *bc* paler fuscous, dense microtrichia covering cells *bc* and *c*, costal band almost confluent with *R*₄₅, and of uniform dark fulvous colouration, cubital streak broad and dark fuscous; legs with all segments entirely fuscous; abdominal terga III–V orange-brown except for fuscous margins on tergum III and sometimes on terga IV and V, shining spots orange-brown to pale fuscous.

**Attractant.** – Cue lure.

**Hosts.** – No known record.

**Distribution.** – Type locality: Papua New Guinea (Gurney, Milne Bay).

 Previous recorded: Papua New Guinea (Milne Bay Province, Morobe Province).

**Bactrocera (Bactrocera) balagawii**, new species

(Fig. 1)


**Location of Types.** – Holotype (T. 152247) in QM.
**Diagnosis.** – A medium sized species; face fulvous with a pair of small circular black spots; postpronotal lobe and notopleuron yellow; scutum black; two lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching anterior npl. seta dorsally; scutellum yellow; wings with a narrow dark fuscous costal band overlapping R_{2+3}, broad fuscous cubital streak present, cells bc and c colourless, microtrichia in outer \( \frac{1}{3} \) of cell c only; abdominal terga III–V entirely orange-brown.

**Description.** – Male.

**Head.** – Vertical length 1.6 mm. Frons length 1.5 times breadth; fulvous with dark fuscous around orbital setae and on anteromedial hump; latter with a small number of short dark hairs; orbital setae black: 1 s.or.; 2 i.or.; lunule red-brown. Ocellar triangle black. Vertex dark fuscous. Face fulvous with a pair of small circular black spots; length 0.48 mm. Genae fulvous, large dark fuscous subocular spot; dark seta present. Occiput red-brown, fulvous along eye margins; occipital row with five strong black setae. Antennae with segments 1 and 2 red-brown, segment 3 red-brown with fuscous on apex and outer surface; length of segments: 0.25 mm, 0.29 mm, 0.88 mm.

**Thorax.** – Scutum black with red-brown markings as follows: below and behind lateral postsutural vitta, around mesonotal suture, between notopleuron and postpronotal lobe and inside postpronotal lobe. Pleural area dark fuscous to black except red-brown below postpronotal lobe. Yellow markings as follows: postpronotal lobe; notopleuron; mesopleural stripe reaching anterior npl. seta dorsally, not continuing onto katepisternum, anterior margin slightly convex; anatergite (posterior apex black); anterior \( \frac{3}{4} \) katatergite (remainder black); two parallel sided lateral postsutural vittae of medium width and ending at ia. setae. Postnotum black. Scutellum yellow except for a narrow black basal band. Setae: sc. 2; prsc. 2; ia. 1; p.sa. 1; a.sa. 1; mpl. 1; npl. 2; scp. 4.

**Wings.** – Length 6.2 mm; cells bc and c colourless; microtrichia in outer third of cell c only; remainder of wings colourless except dark fuscous cell sc, narrow costal band which is dark fuscous to R_{2+3}, and around costal margin to apex of wing and pale fuscous over R_{2+3} almost to R_{4+5}, broad fuscous cubital streak; dense aggregation of microtrichia around A_{1+CuA_{2}}; supernumerary lobe weak.

**Abdomen.** – Elongate oval; terga free; pecten present on tergum III. Tergum I and sterna I and II wider than long. Tergum I dark fuscous except for a narrow orange-brown transverse band across posterior margin but not reaching lateral margins; tergum II orange-brown except for a narrow transverse fuscous band sub-anteriorly but not reaching lateral margins; terga III–V entirely orange-brown. A pair of oval orange-brown shining spots on tergum V. Posterior lobe of surstylus short, sternum V with a deep concavity on posterior margin.

**Attractant.** – Methyl eugenol.

**Distribution.** – East Sepik Province, Papua New Guinea.

**Hosts.** – No known record.

**Etymology.** – This species is named after Dr. Solomon Balagawi in recognition of his extensive contributions to fruit fly research in Papua New Guinea.

**Remarks.** – Bactrocera balagawii new species is similar to B. prolixa in possessing an elongate-oval abdomen, costal band overlapping R_{2+3}, and cells bc and c colourless. It differs from B. prolixa in having each lateral postsutural vitta ending at ia. seta, a wider mesopleural stripe reaching to anterior npl. seta dorsally and abdominal terga III–V entirely orange-brown.

---

**Bactrocera (Bactrocera) bancroftii (Tryon)**


**Diagnosis.** – A medium sized species (wing length 6.0 mm); face with a pair of medium sized circular black spots; scutum dull black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta broad, parallel sided and ending at ia. seta; mesopleural stripe of medium width ending midway between anterior margin of notopleuron and anterior npl.
Drew et al.: Phylogeny of Bactrocera fruit flies in Papua New Guinea

seta dorsally; scutellum yellow except for a pale fuscous apical spot in some specimens; wings with cells bc and c very pale fulvous, microtrichia in outer corner of cell c only, costal band only just overlapping R_{2+3} and very narrow beyond extremity of R_{2+3}, cubital streak broad and fuscous; legs with all segments entirely fulvous; all abdominal terga generally orange-brown, shining spots orange-brown.

Attractant. – Methyl eugenol (a weak response).

Hosts. – Maclura cochinchenesis (family Moraceae) and Morus nigra (mulberry – family Moraceae).

Distribution. – Type locality: Australia (Gympie District). Previously recorded: Australia (Coastal areas of Queensland), Torres Strait Islands (Baxter, Yam, Yorke) (Drew, 1989). New Record: Papua New Guinea (Lae, Morobe Province).

Bactrocera (Bactrocera) brevistriata (Drew)


Diagnosis. – A medium sized species (wing length 6.3 mm); face with spots either absent or very pale and small; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta of medium width and narrowing to end before ia. seta; mesopleural stripe of medium width ending midway between anterior margin of notopleuron and postpronotal lobe dorsally; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, costal band fuscous and confluent with R_{2+3}, cubital streak broad and fuscous; legs with all segments fulvous; abdominal terga mostly orange-brown except for a narrow medial longitudinal fuscous band on tergum V, shining spots orange-brown.

Attractant. – Cue lure.

Hosts. – No known record.

Distribution. – Type locality: Papua New Guinea (Oomsis, Morobe Province).

Bactrocera (Bactrocera) contermina Drew


Diagnosis. – A medium sized species (wing length 5.5 mm); face with a pair of small circular black spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta of medium width and narrowing slightly posteriorly to end at ia. seta; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally; wings with cells bc and c pale fulvous, microtrichia covering most of cell c and along anterior margin of cell bc, broad fuscous costal band almost confluent with R_{4+5}, broad fuscous cubital streak; legs fulvous except fore and mid tibiae fuscous and hind tibiae dark fuscous; abdominal terga orange-brown with a narrow black ‘T’ pattern and moderately broad lateral longitudinal fuscous bands over all three terga, shining spots orange-brown.

Attractant. – No known record.

Hosts. – No known record.

Distribution. – Type locality: Papua New Guinea (Bulolo, Morobe Province).

Bactrocera (Bactrocera) circamusae Drew


Diagnosis. – A medium sized species (wing length 6.6 mm); face with a pair of small pale fuscous spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta broad and narrowing slightly posteriorly to end at ia. seta; mesopleural stripe almost reaching anterior npl.
Attractant. – Methyl eugenol (new record).

Hosts. – Pangium edule (family Flacourtiaceae) (new record).

Distribution. – Type locality: Papua New Guinea (Bulolo, Morobe Province).

New record: Papua New Guinea (Madang Province).

Bactrocera (Bactrocera) contigua Drew


Diagnosis. – A medium sized species (wing length 7.0 mm); face with a pair of small to medium sized spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta of medium width and narrowing slightly to end at ia. seta; mesopleural stripe reaching midway between anterior margin of notopleuron and postpronotal lobe dorsally; wings with cells bc and c very pale fulvous, microtrichia in outer corner of cell c only, fuscous costal band almost confluent with R_{4+5} and of uniform colouration, broad fuscous cubital streak; legs fulvous except fore and mid tibiae pale fuscous and hind tibiae fuscous; abdominal terga III–V orange-brown except anterolateral corners of tergum III fuscous and a narrow broken medial longitudinal line over all three terga, shining spots orange-brown.

Attractant. – No known record.

Hosts. – No known record.

Distribution. – Type locality: Papua New Guinea (Bulolo, Morobe Province).

Bactrocera (Bactrocera) finitima Drew


Diagnosis. – A medium sized species (wing length 6.5 mm); face with a pair of medium sized pear shaped spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta of medium width and narrowing slightly posteriorly to end at ia. seta; mesopleural stripe ending midway between anterior margin of notopleuron and postpronotal lobe dorsally; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, broad fuscous costal band almost confluent with R_{4+5} and of uniform colouration, broad fuscous cubital streak; legs with all segments fulvous except hind tibiae pale fuscous; abdominal terga mostly orange-brown except for a very narrow medial longitudinal dark line on tergum V and posterior part of tergum IV, shining spots orange-brown.

Attractant. – No known record.

Hosts. – No known record.

Distribution. – Type locality: Papua New Guinea (Bulolo, Morobe Province).

Bactrocera (Bactrocera) musae (Tryon)

Chaetodacus musae: Tryon, 1927: 197–199.  
See Drew (1989) for complete list of synonyms.

Diagnosis. – A medium sized species (wing length 6.0 mm); face with a pair of medium sized spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta medium width and narrowing slightly to end at ia. seta; mesopleural stripe reaching midway between anterior margin of notopleuron and postpronotal lobe dorsally; wings with cells bc and c of uniform colouration, broad fuscous cubital streak; legs with segments fulvous except hind tibiae dark fuscous; abdominal terga III–V generally orange-brown with narrow anterolateral dark markings on tergum III but may have a range of colours between a narrow black ‘T’ and a narrow medial longitudinal vitta over terga IV and V, shining spots orange-brown.

Attractant. – Methyl eugenol.

Hosts. – Musa species (major hosts) (family Musaceae). Also recorded occasionally from fruits of eight other plant families (Hancock et al., 2000).

Distribution. – Type locality: Australia (Meringa, North Queensland).

Previously recorded: Australia (Northeast Queensland, Torres Strait Islands), Papua New Guinea (East New Britain, mainland Papua New Guinea).

Bactrocera (Bactrocera) parabancroftii, new species (Fig. 2)

Material examined. – Holotype male, PAPUA NEW GUINEA: Central Province, Kainau Settlement (Hiri Tano Highway), 3 Dec.1998, D. Tenakanai, attracted to cue lure, (trap P022).

Diagnosis. – A medium sized species; face fulvous with a pair of medium sized oval black spots; postpronotal lobe and notopleuron yellow; scutum black; two lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching anterior npl seta dorsally; scutellum yellow; wing with a narrow fuscous costal band just overlapping R_{2+3}, broad fuscous cubital streak present, cells bc and c pale fulvous, microtrichia in outer corner of cell c only; abdominal terga III–V orange-brown with a narrow medial longitudinal fuscous to black band from centre of tergum III to posterior margin of tergum V.

Description. – Male.

Head. – Vertical length 1.7 mm. Frons length 1.53 times breadth; red-brown with fulvous along lateral and ventral margins and a dark fuscous around orbital setae and on anteromedial hump; latter with a small number of short dark hairs; orbital setae black: 1 s.or.; 2 i.or.; lunule red-brown. Ocellar triangle black. Vertex fuscous. Face fulvous with a pair of medium sized oval black spots; length 0.5 mm. Genae fulvous, small fuscous subocular spot; red-brown seta present. Occiput red-brown, fulvous along eye margins; occipital row with 4–8 dark setae. Antennae with segments 1 and 2 red-brown, segment 3 red-brown with dark fuscous on apex and outer surface; length of segments: 0.16 mm, 0.32 mm, 1.0 mm.

Thorax. – Scutum black with dark red-brown below lateral postsutural vitta, around mesonotal suture, between postpronotal lobe and notopleuron, inside postpronotal lobe. Pleural area fuscous to black except red-brown below postpronotal lobe. Yellow markings as follows: postpronotal lobe; notopleuron; mesopleural stripe reaching anterior npl seta dorsally, continuing to katepisternum as a transverse spot, anterior margin straight; anatergite (posterior apex black); ocular fuscous. A pair of oval orange-brown shining spots interrupted at intersegmental lines; in some specimens the fuscous to black band over terga III–V which is sometimes interrupted at intersegmental lines; in some specimens the lateral margins of terga III, IV and V have very narrow fuscous patterns. A pair of oval orange-brown shining spots.

Wings. – Length 5.8 mm; cells bc and c pale fulvous (cell c slightly paler); microtrichia in outer corner of cell c only; reminder of wings colourless except fuscous cell sc, narrow fuscous costal band just overlapping R_{2+3} with a pale infuscation and remaining narrow before ending between extremities of R_{4+5} and m, broad fuscous cubital streak; dense aggregation of microtrichia around A_{1+CuA}; supernumerary lobe weak.

Abdomen. – Oval; tergum free; pecten present on tergum III. Tergum I and sternum I and II wider than long. Terga I–V orange-brown with a very narrow medial longitudinal fuscous to black band over terga III–V which is sometimes interrupted at intersegmental lines; in some specimens the lateral margins of terga III, IV and V have very narrow fuscous patterns. A pair of oval orange-brown shining spots.

Fig. 2. Bactrocera (Bactrocera) parabancroftii, new species.
on tergum V. Posterior lobe of surstylus short, sternum V with a moderate concavity on posterior margin.

**Attractant.** – Cue lure.

**Distribution.** – Central Province, Papua New Guinea.

**Hosts.** – No known record.

**Etymology.** – This species is named *parabancroftii* because of its close morphological resemblance to *Bactrocera bancroftii* (Tryon).

**Remarks.** – *Bactrocera parabancroftii* new species is similar to *B. contermina* and *B. contigua* in possessing the typical musae complex characters, each lateral postsutural vitta reaching to or behind the ia. seta, costal cells with a pale fulvous tint and microtrichia in outer corner of cell c only. It differs from *B. contermina* in having some dark colouration on the abdominal terga, from *B. contigua* in having the broad cubital streak and from both species in having each broad parallel sided lateral postsutural vitta ending behind the ia. seta and a narrow costal band just overlapping R$_{2+3}$.

**Bactrocera (Bactrocera) prolixa** Drew


**Diagnosis.** – A medium sized species; face fulvous with a pair of small oval fuscous spots; postpronotal lobe and notopleuron yellow; scutum black, tending fuscous to dark fuscous centrally; two lateral postspiracular vittae present; medial postspiracular vitta absent; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally; scutellar yellow; wings with a narrow fuscous costal band slightly overlapping R$_{2+3}$, moderately broad fuscous cubital streak present, cells bc and c colourless, microtrichia in outer corner of cell c only; abdominal terga III–V orange-brown with large fuscous to dark fuscous areas along anterior margin of tergum III which expand around lateral margins of this tergum, pale fuscous to fuscous on anterolateral corners of terga IV and V.

**Description.** – Male.

**Head.** – Vertical length 1.47 mm. Frons length 1.42 times breadth; fulvous with fuscous around orbital setae and pale fuscous on anteromedial hump; latter with a small number of short pale hairs; orbital setae black: 1 s.or.; 2, i.or.; lunule fulvous. Ocellar triangle black. Vertex fuscous. Face fulvous

**Attractant.** – Methyl eugenol.

**Hosts.** – No known record.

**Distribution.** – Type locality: Papua New Guinea (Nomad, Western District).

**Bactrocera (Bactrocera) ramuensis**, new species

(Fig. 3)

**Material examined.** – Holotype male, Papua New Guinea: Madang Province, Ramu Sugar Residential Area, 1 Sep.1999, attracted to cue lure, (trap P411).


**Location of Types.** – Holotype (T. 152255) in QM; 2 paratypes in ANIC; 2 paratypes in BMNH; 3 paratypes in QDPI.

**Diagnosis.** – A medium sized species; face fulvous with a pair of small oval fuscous spots; postpronotal lobe and notopleuron yellow; scutum black, tending fuscous to dark fuscous centrally; two lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally; scutellar yellow; wings with a narrow fuscous costal band slightly overlapping R$_{2+3}$, moderately broad fuscous cubital streak present, cells bc and c colourless, microtrichia in outer corner of cell c only; abdominal terga III–V orange-brown with large fuscous to dark fuscous areas along anterior margin of tergum III which expand around lateral margins of this tergum, pale fuscous to fuscous on anterolateral corners of terga IV and V.

**Description.** – Male.

**Head.** – Vertical length 1.47 mm. Frons length 1.42 times breadth; fulvous with fuscous around orbital setae and pale fuscous on anteromedial hump; latter with a small number of short pale hairs; orbital setae black: 1 s.or.; 2, i.or.; lunule fulvous. Ocellar triangle black. Vertex fuscous. Face fulvous
with a pair of small oval fuscous spots; length 0.49 mm. Genae fulvous, fuscous subocular spot; red-brown seta present. Occiput red-brown, fulvous along eye margins; occipital row with 3–7 dark setae. Antennae with segments 1 and 2 fulvous, segment 3 fulvous with fuscous on apex and outer surface; length of segments: 0.15 mm, 0.3 mm, 0.75 mm.

**Thorax.** – Scutum black, tending dark fuscous centrally and with red-brown below lateral postspiracular vitta and dark red-brown around mesonotal suture, between notopleura and postpronotal lobe and inside postpronotal lobe. Pleural area fuscous to dark fuscous except red-brown below postspiracular lobe. Yellow markings as follows: postspiracular lobe; notopleuron; mesopleural stripe of medium width, reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally, continuing to katepisternum as a transverse spot, anterior margin slightly convex; anatergite (posterior apex black); anterior ¼ katatergite (remainder dark fuscous); two broad parallel sided lateral postsutural vittae ending behind ia. setae. Postnotum dark fuscous tending dark red-brown centrally. Scutellum yellow except for a narrow black basal band. Setae: sc. 2; prsc. 2; ia. 1; p.sa. 1; a.sa. 1; mpl. 1; npl. 2; scp. 4.

**Legs.** – All segments fulvous except hind tibiae pale fuscous; mid tibiae each with an apical black spur.

**Wings.** – Length 5.4 mm; cells bc and c colourless; microtrichia in outer corner of cell c only; remainder of wings colourless except fuscous cell sc, narrow fuscous costal band slightly overlapping R2+3 where it is slightly paler and diffuse and ending between extremities of R4+5 and m, broad fuscous cubital streak; dense aggregation of microtrichia around A1+CuA2; supernumerary lobe of medium development.

**Abdomen.** – Oval; terga free; pecten present on tergum III. Tergum I and sternum I and II wider than long. Tergum I fuscous except for a narrow fulvous band along posterior margin which does not reach lateral margins; tergum II red-brown except for large fulvous areas posterolaterally and a narrow transverse fuscous band anteriorly but not reaching dark fuscous anterolateral margins; terga III–V orange-brown except for large fuscous to dark fuscous areas laterally on tergum III which usually join along anterior margin of this tergum and pale fuscous to fuscous anterolateral corners on terga IV and V. In some specimens there is a narrow diffuse pale fuscous to fuscous median longitudinal line on tergum III and anteriorly on tergum IV. A pair of oval orange-brown shining spots on tergum V. Posterior lobe of surstylistus short, sternum V with a deep concavity on posterior margin.

**Attractant.** – Cue lure.

**Distribution.** – Morobe and Madang Provinces, Papua New Guinea.

**Hosts.** – No known record.

**Etymology.** – The name *ramuensis* is with reference to the type locality, ‘Ramu’ in the Madang province, Papua New Guinea.

**Remarks.** – *Bactrocera ramuensis* new species is similar to *B. finitima, B. musae* and *B. tinomiscii* in possessing lateral postspiracular vitta that end at or behind ia. seta, colourless cells bc and c and an oval shaped abdomen. It differs from *B. finitima* and *B. musae* in having the costal band barely overlapping R2+3 (only as a pale, diffuse tint when it does), from all these species in having each parallel sided lateral postspiracular vitta ending behind ia. seta and from *B. tinomiscii* in having dark colour patterns on terga III, IV and V, particularly laterally.

**Bactrocera (Bactrocera) robertsi** Drew

*Bactrocera (Bactrocera) robertsi* Drew, 1989: 42–43.

**Diagnosis.** – A medium sized species (wing length 7.0 mm); face with dark facial spots either absent or very small and pale; scutum black; postspiracular lobe yellow; notopleuron with anterior ½ to ¼ black and remainder yellow; each lateral postspiracular vitta broad and narrowing slightly posteriorly to end before ia. seta; mesopleural stripe reaching midway between anterior margin of notopleuron and postspiracular lobe dorsally; wings with cell bc pale fulvous and cell c fuscous, microtrichia in outer corner of cell c only, fuscous costal band confluent with R2+3 and of uniform colouration, broad fuscous cubital streak; legs fulvous except hind tibiae fuscous; terga III–V red-brown with a narrow transverse fuscous to dark fuscous band across anterior margin of tergum III and fuscous on lateral margins of all three terga and a narrow medial longitudinal fuscous to black band over all three terga, shining spots red-brown.

**Attractant.** – Cue lure.

**Hosts.** – No known record.

**Distribution.** – Type locality: Papua New Guinea (Wau, Morobe Province). Previously recorded: Papua New Guinea (Central, Morobe and Western Highlands Provinces).

**Bactrocera (Bactrocera) rufivittia**, new species

(Fig. 4)


**Paratypes.** – 1 male, same data as holotype; 1 male, PNG, Morobe Province, Forest Res Inst. Lae Botanical Gardens, 27 Apr.1999, A. Mararuai, (trap P402); 3 males, Morobe Province, 21–24 Aug.1999, S. Sar, used for DNA analyses coded Alc 19, Alc 93, Alc 99.

**Location of Types.** – Holotype (T. 152256) in QM; 5 paratypes in QDPI.
Diagnosis. – A medium sized species; face fulvous with a pair of medium sized oval black spots; postpronotal lobe and notopleuron yellow; scutum dull black; two lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally; scutellum yellow; wings with a narrow dark fuscous costal band slightly overlapping R2+3, narrow fuscous to dark fuscous cubital streak, cells bc and c colourless, microtrichia in outer corner of cell c only; abdominal terga III–V orange-brown with a narrow to medium width medial longitudinal black band and pale fuscous to fuscous anterolateral corners on each of the three terga.

Description. – Male.

Head. – Vertical length 1.7 mm. Frons length 1.5 times breadth; red-brown without dark markings; anteromedial hump with a small number of short dark hairs; orbital setae black; 1 s.or; 2 i.or.; lunule pale fuscous. Ocellar triangle black. Vertex red-brown. Face fulvous with a pair of medium sized oval black spots; length 0.5 mm. Genae red-brown, pale fuscous subocular spot; seta absent. Occiput red-brown, with occasional pale fuscous blotches, fulvous along eye margins; occipital row with a few short red-brown setae. Antennae with segments 1 and 2 red-brown, segment 3 absent; length of segments: 0.14 mm, 0.34 mm.

Thorax. – Scutum dull black with paler markings as follows: red-brown below lateral postsutural vitta, around mesonotal suture, between notopleuron and postpronotal lobe, inside postpronotal lobe; dark red-brown along posterior margin and a broad red-brown medial longitudinal band beginning at anterior margin of scutum and ending at level of mesonotal suture (within this band is a narrow medial longitudinal black line). Pleural area dull black except red-brown below postpronotal lobe. Yellow markings as follows: postpronotal lobe; notopleuron; mesopleural stripe of medium width, reaching midway between anterior margin of notopleuron and anterior npl. seta dorsally, continuing to katepisternum as a transverse spot, anterior margin slightly convex; anatergite (posterior apex black); anterior 1/3 katatergite (remainder black); two moderately broad lateral postsutural vittae, narrowing slightly posteriorly to end just before ia. setae. Postnotum black laterally, dark red-brown centrally. Scutellum yellow except for a narrow black basal band. Setae: sc. 2; prsc. 2; ia. 1; p.sa. 1; a.sa. 1; mpl. 1; npl. 2; sep. 4.

Legs. – All segments fulvous except fore tibiae pale fuscous and hind tibiae dark fuscous; mid tibiae each with an apical black spur.

Wings. – Length 6.2 mm; cells bc and c colourless; microtrichia in outer corner of cell c only; remainder of wings colourless except dark fuscous cell sc, narrow dark fuscous costal band slightly overlapping R2+3 and ending between extremities of R4,5 and m (the costal band remains dark fuscous over R2+3, and of uniform width beyond apex of R2+3); a narrow fuscous to dark fuscous cubital streak; a dense aggregation of microtrichia around A1+CuA2; supernumerary lobe of medium development.

Abdomen. – Oval; terga free; pecten present on tergum III. Tergum I and sterna I and II wider than long. Tergum I dark fuscous except for a narrow transverse orange-brown margin posterocentrally; tergum II orange-brown except for a narrow transverse sub-anterior band that is mostly black except fuscous centrally, and anterolateral corners fuscous; terga III–V orange-brown except for a narrow to medium width medial longitudinal black band, anterolateral corners of tergum III dark fuscous and anterolateral corners of terga IV and V narrowly pale fuscous to fuscous. A pair of oval orange-brown shining spots on tergum V. Posterior lobe of surstylus short, sternum V with a deep concavity on posterior margin.

Attractant. – Cue lure.

Female. – No known record.

Distribution. – Morobe Province, Papua New Guinea.

Hosts. – No known record.

Etymology. – The name rufi vitta refers to the red-brown medial longitudinal band on the anterior portion of the scutum.

Remarks. – Bactrocera rufi vitta new species has been distinguished through the molecular studies. It is similar
to *B. brevistriata*, *B. commina* and *B. prolixa* in possessing the general colour patterns of the *musae* complex, yellow notopleuron and each lateral postsutural vitta ending before the *ia* seta. It is distinct from these species in having a broad dark red-brown medial longitudinal band on the anterior half of the scutum and a narrow cubital streak in the wing.

**Bactrocera (Bactrocera) tinomiscii** Drew


**Diagnosis.** – A medium sized species (wing length 6.3 mm); face with a pair of small oval spots; scutum black; postpronotal lobe and notopleuron yellow; each lateral postsutural vitta of medium width and narrowing slightly posteriorly to end at *ia* seta; mesopleural stripe reaching midway between anterior margin of notopleuron and postpronotal lobe dorsally; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, fuscous costal band just overlapping *R*₂₃₄, broad fuscous cubital streak; legs fulvous except basal ½ of hind tibiae fuscous; abdominal terga mostly orange-brown with fuscous anterolateral corners on tergum III and a narrow dark fuscous to black medial longitudinal band over terga III–V, shining spots orange-brown.

**Attractant.** – Cue lure.

**Hosts.** – *Tinomiscium phytocrenoides* (family Menispermaceae) and *Chlaenandra ovata* (family Menispermaceae).

**Distribution.** – Type locality: Papua New Guinea (Rouna Power Station, Central District). Previously recorded: Papua New Guinea (Central Province, Morobe Province, Eastern Highlands District, Sepik District).

**Bactrocera (Bactrocera) uvariae**, new species

(Fig. 5)


**Location of Types.** – Holotype (T. 152257) and 5 paratypes (T. 152258 – T. 152262) in QM; 10 paratypes in BMNH; 10 paratypes in ANIC; 41 paratypes in QDPI.

**Diagnosis.** – A medium sized species; face fulvous with a pair of medium sized oval dark fuscous to black spots; postpronotal lobe and notopleuron yellow; scutum black; two lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching almost to anterior *npl* seta dorsally; scutellum yellow; wing with a narrow fuscous costal band confluent with *R*₂₃₄, broad fuscous cubital streak, cells bc and c with a pale fuscous tint, microtrichia in outer ¼ of cell c; all abdominal terga bright orange-brown with fuscous to dark fuscous oval shining spots on tergum V.

**Description.** – Female.

**Head.** – Vertical length 1.7 mm. Frons length 1.6 times breadth; fulvous except red-brown anterocentrally, dark fuscous around orbital setae and dark fuscous to black on anteromedial hump; latter with a large number of small dark hairs; orbital setae black: 1 *s.or*.; 2 *i.or*.; lunule red-brown. Face fulvous with a pair of medium sized oval dark fuscous to black spots; length 0.49 mm. Genae red-brown, large dark fuscous subocular spot; dark seta present. Occipital row red-brown, fulvous along eye margins, 4–5 dark setae present. Antennae with segments 1 and 2 red-brown, segment 3 red-brown with dark fuscous on apex and outer surface; length of segments: 0.17 mm, 0.29 mm, 0.84 mm.

**Thorax.** – Scutum black with red-brown below and behind lateral postsutural vitta, around mesonotal suture, between...
notopleuron and postpronotal lobe, inside postpronotal lobe and along anterior margin. Pleural area red-brown except pale fuscous along anterior margin of mesopleural stripe. Yellow markings as follows: postpronotal lobe; notopleuron; mesopleural stripe of medium width reaching almost to anterior npl. seta dorsally, continuing to keatepistemum as a transverse spot, anterior margin straight; anatergite (posterior apex red-brown); anterior ¼ katatergite (remainder red-brown); two broad parallel sided lateral postsutural vittae reaching to ia. setae. Postnotum red-brown. Scutellum yellow except for a narrow black basal band. Setae: sc. 2; prsc. 2; ia. 1; p.s. 1; a.sa. 1; mpl. 1; npl. 2; scp. 4.

**Legs.** – All segments fulvous except apical fore segments of all tarsi fuscous and hind tibiae pale fuscous; mid tibiae each with an apical black spur.

**Wings.** – Length 6.0 mm; cells bc and c with a pale fulvous tint; microtrichia in outer one half of cell c only; remainder of wings colourless except fuscous cell sc, narrow fuscous costal band confluent with R₂,³ and remaining of uniform width but very narrow till apex of wing, broad fuscous cubital streak; no dense aggregation of microtrichia around A₁+CuA₂; supernumerary lobe weak.

**Abdomen.** – Oval; terga free; no pecten on tergum III. Tergum I and sterna I and II wider than long. All terga bright orange-brown except fuscous dark fuscous, dorsoventrally flattened and tapering posteriorly in dorsal view; ratio of length of oviscape to length of tergum V, 0.8:1; apex of aculeus needle-shaped.

**Male.** – As for female except dense aggregation of microtrichia around A₁+CuA₂; supernumerary lobe of medium development; pecten present on abdominal tergum III.

**Attractant.** – Cue lure.

**Distribution.** – Morobe Province, Papua New Guinea.

**Hosts.** – This species has been reared from the genus *Uvaria* (family Annonaceae).

**Etymology.** – The name *uvariae* refers to the plant genus *Uvaria*, the endemic rainforest host plant.

**Remarks.** – *Bactrocera uvariae* new species possesses the typical *musae* complex characters of black scutellum, orange-brown abdominal terga and wings with a narrow costal band. It is similar to some specimens of *B. bancroftii* and *B. parabancroftii* new species in having a pale tint in cells bc and c, and entirely yellow scutellum and the costal band just overlapping R₂,³ but is unique in having fuscous to dark fuscous shining spots on abdominal tergum V and a dark fuscous basal segment of the ovipositor.

**MITOCHONDRIAL DNA**

Phylogenetic analyses were performed on the 562 bp region of the CO1 gene. This region contained mean base frequencies of: A=0.291; C=0.196; G=0.174; and T=0.339. There were 189 variable sites of which 110 sites were informative for parsimony analysis. Table 1 shows the percentage sequence divergences for the morphological groups identified in this study. Sequence divergences were high between all clades (mean=12.5%) and were less than 3% within any individual group. Each one of these clades also corresponded to a species as defined morphologically.

The trees produced from the maximum parsimony (not shown), neighbour joining (not shown) and maximum likelihood methodologies (Fig. 6) had concordant topologies indicating the presence of a number of strongly supported lineages. There was a remarkable congruence between the species identified on the basis of morphological characters and the occurrence of distinct clades in the gene tree. All six species identified morphologically either formed distinct monophyletic clades supported by high bootstrap values (i.e. *B. bancroftii*, *B. contermina*, *B. musae* and new species, *B. rufivitta*) or were isolated as single ungrouped sequences (*B. prolixa*, and *B. tinomiscii*). In the gene tree, *B. musae* and *B. rufivitta* which could be separated morphologically, appeared to be more closely related to each other than to other species in the complex.

**MICROSATELLITE DNA**

Eight polymorphic loci were developed and screened in this study (Table 2). The data from the eight microsatellite loci also provides support for the notion that the major clades, *B. musae* (Clade A), *B. rufivitta* (Clade B) and *B. contermina* (Clade C), are distinct species. By following the methodology of Evanno et al. (2005), it was evident that a hierarchical population structure was present in the microsatellite data. The original data set was divided into two major groupings based on the highest ΔK value: cluster A corresponding to the *B. musae* group, and cluster B containing the *B. rufivitta* group and the *B. contermina* group combined (Fig. 7A). Subsequent analysis of the two clusters separately further revealed that cluster B should be split into two sub-clusters representing the *B. rufivitta* and *B. contermina* groups respectively (Fig. 7B). Interestingly and in contrast to the mitochondrial data, this hierarchical structure suggests the *B. rufivitta* group is more closely related to the *B. contermina* group than the *B. musae* group. Also, individual 104 was included in the *B. contermina* cluster, albeit with 20% mixed ancestry with the *B. rufivitta* cluster which it groups closer to in the *CO1* gene tree (Fig 7B).
Table 1. Average percentage sequence divergence for lineages of fruit flies in the genus *Bactrocera* for the 562 base-pairs of the mtDNA CO1 gene. SD in brackets.

<table>
<thead>
<tr>
<th>Clade A</th>
<th>Clade B</th>
<th>Clade C</th>
<th>Bm105</th>
<th>Bbanc</th>
<th>Bprol</th>
<th>Bendri</th>
<th>Btin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td></td>
<td></td>
<td>4.0</td>
<td>5.4</td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.1)</td>
<td></td>
<td></td>
<td>(1.1)</td>
<td>(2.2)</td>
<td>(2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.0</td>
<td>12.6</td>
<td>2.4</td>
<td>11.0</td>
<td>11.0</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.6)</td>
<td>(0.4)</td>
<td></td>
<td>(1.8)</td>
<td>(3.5)</td>
<td>(2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>11.3</td>
<td>12.2</td>
<td>11.0</td>
<td>11.0</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.9)</td>
<td>(2.2)</td>
<td>(2.9)</td>
<td>(5.2)</td>
<td>(5.2)</td>
<td>(0.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clade A = specimens of *Bactrocera musae* (Tryon)
Clade B = specimens of *Bactrocera rufivitta* n. sp.
Clade C = specimens of *Bactrocera contermina* Drew
Bm105 = unknown species similar morphologically to *Bactrocera endiandrae* (Perkins & May)
Bbanc = *Bactrocera bancroftii* (Tryon)
Bprol = *Bactrocera prolixa* Drew
Bendri = *Bactrocera endiandrae* (Perkins & May)
Btin = *Bactrocera tinomiscii* Drew
Btri = *Bactrocera tryoni* (Froggatt)

**DISCUSSION**

The mitochondrial DNA data clearly supports species status for each of the morphologically described taxa that was available for molecular analyses. Not only are each of the taxa monophyletic within the tree but sequence divergence, even between the two most closely related species, is approximately 4.0%. Based on molecular clock calibrations for CO1 in insects, this equates to separation of approximately two million years (Brower, 1994). This result is also supported by the nuclear microsatellite data where the specimens of each species were clearly assigned, on the basis of their multilocus genotypes, to their respective species. *Bactrocera contermina, B. musae* and *B. rufivitta* occur sympatrically and, for this study, were collected from within the same geographic area. An additional advantage of the nuclear data is that, if the three groups were interbreeding, sharing of alleles would be expected and assignment of individuals to one or other group would be difficult. This was clearly not the case. All individuals were assigned to clusters corresponding to their respective mitochondrial clades (Fig. 7). The slight discordance in the inter-relationships of the three clusters inferred from the mitochondrial and microsatellite data is likely a result of differences in the rate of molecular evolution between the DNA sources (Dirienzo et al., 1994).

Morphologically, *B. contermina* is distinct in having coloured cells bc and c whereas in *B. musae* and *B. rufivitta* these cells are colourless. Further, *B. rufivitta* can be separated from *B. musae* in having a broad red-brown medial longitudinal band on the scutum between the level of the mesonotal suture and the anterior margin and in the lateral postsutural vittae ending before the ia. setae. The other three species studied in the molecular analyses, *B. bancroftii, B. prolixa* and *B. tinomiscii* are more distinct from one another and from *B. contermina, B. musae* and *B. rufivitta* morphologically and were distinctly separate as Clade D on the molecular data (Fig. 6).

Within the tephritid subfamily Dacinae, there are a number of important sibling species complexes which contain species of major economic importance. The most significant are the *dorsalis* complex, the *frauenfeldi* complex, the *musae* complex, the *scutellaris* complex and *tau* complex (Drew, 1989, and also unpublished data). The data reported herein provides some evidence that species within these complexes can be diagnosed using a combination of biological, molecular and morphological data.

**ACKNOWLEDGEMENTS**

Specimens for this study were collected in Papua New Guinea by staff from the National Agricultural Research Institute (NARI) and the National Agricultural Quarantine and Inspection Authority (NAQIA). Mr Richard Piper, Scientific Advisory Services, North Queensland, supplied host reared specimens of *Bactrocera musae* for DNA studies. Michelle Baker, Griffith University, prepared the illustrations. All this assistance is gratefully acknowledged.
Table 2. Microsatellite loci developed and used to screen populations of *Bactrocera musae* (Tryon).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences</th>
<th>Repeat motif</th>
<th>Ta (°C)</th>
<th>Detected alleles</th>
<th>Allele size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F20</td>
<td>Fwd – GT TGT ATG AAG GTA</td>
<td>(CAC)₇</td>
<td>52</td>
<td>8</td>
<td>144 – 168</td>
</tr>
<tr>
<td></td>
<td>Rev – TGT CTC GGC GTG TCG AAA TTTGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F24</td>
<td>Fwd – AG TTG CAG GCC GCC</td>
<td>(CA)₉, (GCA)₃ATT(GCA)₃(CAA)₇</td>
<td>55</td>
<td>4</td>
<td>114 – 151</td>
</tr>
<tr>
<td></td>
<td>Rev – TTG TAG AGC TGA CGC AAC AGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F73</td>
<td>Fwd – AC ATT GTC AGC ACT</td>
<td>(CA)₁₃</td>
<td>52</td>
<td>13</td>
<td>95 – 123</td>
</tr>
<tr>
<td></td>
<td>Rev – TG CTC TCC TAC GTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F77</td>
<td>Fwd – CAT TAC AAC ACC CTC GTC GTC</td>
<td>(CA)₉</td>
<td>55</td>
<td>5</td>
<td>152 – 167</td>
</tr>
<tr>
<td></td>
<td>Rev – GT GCT AAT AAC TAG AC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F83D</td>
<td>Fwd – AGC GCA GAT GTG AGA CA</td>
<td>(TTG)₈</td>
<td>52</td>
<td>8</td>
<td>117 – 144</td>
</tr>
<tr>
<td></td>
<td>Rev – CAT TAC AAC ACC CTC GTC GTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F84B</td>
<td>Fwd – CAT ACG CAT ATG TGG</td>
<td>(CA)₉CCC(CAA)₇</td>
<td>52</td>
<td>6</td>
<td>103 – 128</td>
</tr>
<tr>
<td></td>
<td>Rev – TG TGG GGG TGG GGG TGG CTG A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F42B</td>
<td>Fwd – AG GTG CTC CAA ACG AAC ATG G</td>
<td>(CAG)₅</td>
<td>52</td>
<td>5</td>
<td>86 – 102</td>
</tr>
<tr>
<td></td>
<td>Rev – ATG TGG AGA CTT TAT GCT GC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F97</td>
<td>Fwd – AG TGC AAG CTT GCA TGG</td>
<td>C(AAC)₇</td>
<td>52</td>
<td>7</td>
<td>90 – 110</td>
</tr>
</tbody>
</table>


Fig. 6. Neighbour joining tree showing relationships between CO1 haplotypes from species in the *Bactrocera musae* complex. Values at nodes are for 1000 bootstrap replicates of the maximum likelihood calculations using the Kimura two-parameter model of sequence evolution (left) and Bayesian posterior probability (right).


Note: the numbers at the branch tips represent the field collection codes given to individual specimens.
Fig. 7. Diagram showing clustering of individuals at (A) the highest hierarchical level of structuring in the *Bactrocera musae* complex using STRUCTURE, and (B) the sub-group structuring into two further clusters of the individuals from the red cluster in A. Vertical bars represent individuals and colours denote the proportion of ancestry from each cluster based on eight microsatellite loci. Note at the highest level (A), individuals are clearly assigned to either the *B. musae* or the ‘others’ cluster. At the next level (B), individuals from the ‘others’ cluster are assigned to either the *B. rufiventris* cluster (red) or the *B. contermina* cluster (green).

Note: The numbers below the vertical bars represent the field collection codes given to individual specimens.

**LITERATURE CITED**


