Draft Annex to ISPM 27 – *Bactrocera dorsalis* complex (2006-026)

**Status box**

*This is not an official part of the standard, and it will be modified by the IPPC Secretariat after adoption.*

**Date of this document**

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**Document category**

Draft new annex to ISPM 27 (*Diagnostic protocols for regulated pests*)

**Current document stage**

To consultation

**Major stages**

- 2006-05: Standard Committee (SC) added original subject: *Bactrocera dorsalis* complex (2006-026)
- 2016-10: Expert consultation
- 2017-02: Technical Panel on Diagnostic Protocols (TPDP) review
- 2017-05: TPDP e-decision for consultation
- 2017-06: SC e-decision for consultation

**Consultation on technical level**

The first draft of this protocol was written by (lead author and DP drafting group):
- Mr Kenji TSURUTA (JA) (lead author)
- Mr Sujinda THANAPHUM (TH)
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In addition, the draft has also been subject to expert review and the following international experts submitted comments: Jane Royer (AU), Mark Schutze (AU), Josephine Moraa Songa (KE), George Momanyi (KE), Sharon Reid (GB), Yuji Kitabara (JA), Ken Hong Tan (MY), Alvin Hee (MY), Eddy Dijkstra (NL) and Elizabeth Minchinton (AU).

**Main discussion points on during the development of the diagnostic protocol**

Comment:

This diagnostic protocol has been placed in pending status for several years because of concurrent studies to revise the taxonomy of *B. dorsalis* and close relatives. A 2015 publication created synonyms for several pest species. This revision is still not accepted by all experts. Recent publication in 2016 argues against revision.

There has been confusion in scope because of use of *Bactrocera dorsalis* in the name of the *B. dorsalis* complex and the *B. dorsalis* sibling species complex. The *B. dorsalis* complex is not a true evolutionary unit and to make it useful in screening major pests a redefinition according to Schutze et al. (2015a) has been included.

Given that a new classification has been proposed but not adopted by all experts, synonyms are currently treated as subjective (ICZN rules). As IPPC is supposed to develop DPs for recognized species, the DP is not intended to instruct on revision debates.

Larval morphology information has been removed as it is not reliable for diagnosis of flies to the complex or species levels.

Footnotes and brand names (based on SC decision and according to TPDP instruction to authors): If in the DP there is more than one mention to a brand name, the second mention (and the sub sequential mentions) to a brand name shall be associated with the footnote number with the full text (e.g. if the first mention to a brand name is “footnote 1”, the subsequent mentions to brand names should be accompanied by the same footnote number).

**Notes**

This is a draft document. The final formatting will be adjusted at a later stage.

2017-04 Edited
Adoption

This diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in 20-- [to be completed after adoption].

The annex is a prescriptive part of ISPM 27 (Diagnostic protocols for regulated pests).

I. Pest Information

Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus Bactrocera Macquart consists of over 650 described species that are distributed mostly in regions of Asia and Australasia and subtropical islands of the southern Pacific Ocean (Drew and Romig, 2013). Within the genus is a group of flies named the Bactrocera dorsalis complex (Drew and Hancock, 1994; Drew, 2004; Clark et al., 2005). This complex comprises 85 described species (Vargas et al., 2015) that share a very similar appearance, but the complex as a whole does not represent a monophyletic lineage and is merely a group of convenience (Leblanc et al., 2015). The complex is named after one of its member species, Bactrocera dorsalis (Figure 1) which is a polyphagous pest of commercial fruits. Several other species in the complex are also recognized as pests, based on plant host use and pest records (White and Elson-Harris, 1992; Clarke et al., 2005; Vargas et al., 2015; Plant Health Australia, 2016).

The scope of the current protocol is to diagnose adult fruit flies for six species of the Bactrocera dorsalis complex that are found in commercial fruits and vegetables associated with international trade. These species are: B. dorsalis, B. carambolae, B. caryae, B. kandiensis, B. occipitalis and B. pyrifoliae. Distributions of these species are mapped with their pest status and invasion history by Vargas et al. (2015).

A lack of characters that can be used reliably to distinguish B. dorsalis from two other species (i.e. B. papayae Drew and Hancock, 1994, and B. invadens Drew et al., 2005) has resulted in debate regarding the valid taxonomy of the species (Clarke et al., 2005; Chen and Hui, 2007; Schutze et al., 2015a, 2015b; Drew & Romig, 2016). These three species have been treated as members of a sibling species complex, not to be confused with the Bactrocera dorsalis complex (Clarke and Schutze, 2014). It is not possible to reliably distinguish among these three species because an accurate identification requires both evaluation of species distribution information and analysis of morphological characters that are not discrete for the species. Species distribution information may not be reliable when examining specimens collected outside its known range. Published molecular data cannot distinguish these species (Schutze et al., 2015a). In a review of available evidence, Schutze et al. (2015a) concluded that these three species are in fact a single biological species called Bactrocera dorsalis. Drew and Romig (2016) disagree with that revision. In this protocol, the three species are collectively treated as B. dorsalis sensu lato.

Evidence has been reported of hybridization among some of these six Bactrocera species under laboratory conditions (McInnis et al., 1999; Ebina and Ohto, 2006; Schutze et al., 2013) and of morphological intermediates in the wild (Delomen et al., 2013; Jalani et al. 2014). The frequency of hybrids between these species in nature has not been estimated. Although methods for detecting hybrids between B. dorsalis and B. carambolae have been reported (Ebina and Ohto, 2006) it is currently not possible to measure impacts of hybridization events over time, such as genome introgression or detection of progeny of backcrossed populations.
B. carambolae attacks a wide range of fruits from 20 plant families, particularly Averrhoa carambola (carambola) (CABI, 2016). It is found in the southern peninsular area of southeast Asia through Indonesia and the nearby Andaman Islands. It is also present in some South American countries (CABI, 2016).

B. caryae attacks Mangifera spp. (mango), Malpighia emarginata (acerola), Psidium spp. (guava), Citrus spp. and Pouteria spp. (mamey sapote), and is endemic to southern India (CABI, 2016).

Bactrocera dorsalis s.l. attacks over 270 plant species (Vargas et al. 2015) in over 50 families of commercial fruits and wild fruits (CABI, 2016). It has the largest species range of the six pests included in the protocol, and is found on some islands in the Pacific Ocean, and most of continental Africa (sub-Saharan countries) in addition to its original Asian range (Drew and Hancock, 1994; Drew et al., 2005; White, 2006; Drew and Romig, 2013; Schutze et al., 2015a, b).

B. kandiensis attacks a wide range of fruits including Mangifera indica (mango), Garcinia spp., Carica papaya (papaya), Persea americana (avocado) and Psidium spp. (guava) (CABI, 2016). It has a limited distributional range, being endemic to Sri Lanka.

B. occipitalis attacks Mangifera spp. (mango), Psidium spp. (guava), Spondias purpurea (red mombin), Averrhoa carambola (carambola), Citrus spp. and Manilkara zapota (sapodilla) (CABI, 2016). It has a relatively narrow range in southeast Asia (Drew and Romig, 2013).

B. pyrifoliae attacks Psidium spp. (guava) and Prunus persica (peach) (Allwood et al., 1999). It is known from parts of southeast Asia (Drew and Romig, 2013).

2. Taxonomic Information

Name: Bactrocera dorsalis complex

Synonyms:

Taxonomic position: Insecta, Diptera, Tephritidae, Bactrocera

The species included in the Bactrocera dorsalis complex are in the subgenus Bactrocera (Bactrocera). According to ICZN (1999), three species are treated as subjective synonyms under Bactrocera dorsalis s.l.: Bactrocera papayae, Bactrocera invadens and Bactrocera philippinensis. Drew and Romig (2013) placed B. philippinensis as a synonym of B. papayae. Revision by Schutze et al. (2015a) places B. invadens and B. papayae as junior synonyms of B. dorsalis. Drew and Romig (2016) provide an argument for treating these as separate species. Note that Bactrocera invadens was not formally placed into the Bactrocera dorsalis complex by Drew et al. (2013), but based on Schutze et al. (2015a) is considered a sibling species of, or synonym of, Bactrocera dorsalis. The current protocol treats these names (B. papayae, B. invadens and B. philippinensis) as part of Bactrocera dorsalis s.l.

Table 1. Common names and synonyms of six species in the Bactrocera dorsalis complex included in the protocol

<table>
<thead>
<tr>
<th>Bactrocera species</th>
<th>Common name</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bactrocera (Bactrocera) carambola (Drew and Hancock, 1994)</td>
<td>Carambola fruit fly</td>
<td>None</td>
</tr>
<tr>
<td>Bactrocera (Bactrocera) caryae (Kapoor, 1971)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dacus (Strumeta) caryae Kapoor, 1971</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dacus (Bactrocera) caryae Kapoor, 1971; Hardy, 1977</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dacus dorsalis Hendel, 1912</td>
<td></td>
</tr>
</tbody>
</table>
3. Detection

Fruit flies of the genus *Bactrocera* are detected mainly by male lure trap or in fruits. Only male adult fruit flies are captured by male lure trapping, while all immature stages such as eggs (Figure 2(a)), early to final instar larvae (Figures 2(b) to (d)), and pupae and puparia (Figures 2(e) to (f)) can be found during inspection of fruits.

3.1 Trapping

Guidance on trapping *Bactrocera* fruit flies is given in Appendix 1 of ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae)*). Additional information on trapping methods is provided by Drew (1982), Drew and Romig (2010), and FAO and IAEA (2003). The *Bactrocera dorsalis* complex includes species that respond to different male lures. When the lure responsiveness information is available, it can be used as supporting information for species identification. Five of the target species in this diagnostic protocol are methyl eugenol responding species. The only exception is *B. pyrifoliae*, which has been reported to respond to an alternative lure: cue lure (Drew and Romig, 2013).

Additional information on attractants for trapping, such as synthetic food attractants and hydrolysed protein substances, are available in ISPM 26 Appendix 1: Fruit fly trapping, 2011.

3.2 Inspection of fruits

Fruits with soft areas, dark stains, rot, orifices or injuries that might have originated from female oviposition or larval feeding activities are targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Second and third instar larvae and pupae are not likely to occur when unripe fruits are collected and packed; however, these fruits
might host eggs and first instar larvae, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositioning female flies should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits.

Once detected, immature larvae can be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species or as part of the *Bactrocera dorsalis* complex. The incubation of infested fruits is a common practice to obtain adult flies which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize.

### 3.3 Rearing larvae to obtain adults

Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g. damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. It is recommended that each fruit be incubated separately. Each sample should be observed and pupae gathered daily. The pupae are placed in containers with the pupation medium, and the containers are covered with a tight lid that enables proper ventilation. Once the adults emerge, they must be kept alive for several days to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. Prior to mounting (before they harden), it is useful to gently squeeze the apical part of the preabdomen with forceps, then squeeze the base and apex of the oviscape to expose the aculeus tip for females, and to pull out the aedeagus for males. Alternatively, this will need to be dissected later in flies.

### 4. Identification

Identification at the level of the species or the *Bactrocera dorsalis* complex requires morphological examination of adult flies. It is generally difficult and not reliable to morphologically identify eggs, larvae or pupae to the species level. It is not possible to identify a fly to the *Bactrocera dorsalis* complex using immature life stages.

Molecular methods of *Bactrocera* species identification have been reported and provide additional information to support morphological identifications of specimens. DNA sequencing of the cytochrome oxidase I DNA barcode does not provide adequate resolution to identify many species in the *B. dorsalis* complex (details in section 4.3). Other molecular methods lack the specificity data needed to demonstrate that a test is accurate for species identification. For example, the molecular profiles of all six pest species targeted in the protocol are not known using rDNA analysis (section 4.3). DNA can be used to distinguish *B. carambolae* from *B. dorsalis s.l.* and this test is provided in the protocol (section 4.3.2).

### 4.1 Preparation of adults for identification

Proper preparation of specimens is essential for accurate morphological identification. General instructions on preparation of adult fruit fly specimens are given by Drew (1991) and White and Elson-Harris (1992).

Every attempt should be made to preserve all characters on at least one side of the centre line, regardless of the mounting method (Foote *et al.*, 1993).

Characters on the head, wing, leg, thorax and abdomen of a fly can be examined from pinned specimens under magnification using a stereomicroscope. This magnification level is appropriate for observation of spot and colour patterns and wing morphology (Figure 1). Microscopic examination is required to measure characters on the genitalia that are described in section 4.1.1.

Structures of the ovipositor such as oviscope, eversible membrane and aculeus have been used as important taxonomic characters at species level (Hardy, 1949, 1969; Hardy and Adachi, 1954; Drew and Hancock, 1994). Since the review by Drew and Hancock (1994), aculeus length has been used in
particular for distinguishing some of the fruit fly species within the _Bactrocera dorsalis_ complex, and male aedeagus length, which is highly correlated with aculeus length, has also been used because only males are trapped in lure trapping surveys. Preparation methods for male genitalia are included in section 4.1.1.

To assist in identification of characters under a stereomicroscope, the following can be applied:
- Examination of the costal band below the _R_{2+3} vein will be made easier by putting white paper underneath the wing or by using transmitted light.
- When black markings on abdominal tergites 3–5 are difficult to observe due to damage such as colour change, observation may be made easier by wetting with a paintbrush dipped in 70% ethanol or clearing with 10% potassium hydroxide (KOH).
- When the inner yellow membrane in lateral vittae (Figure 3) is partially removed, which makes the boundary of the lateral vittae difficult to see, an alternative is to measure the width of the translucent window in the scutum (Figure 4(b)).
- In measuring the width of lateral vittae (example of measuring indicated in Figure 4(b)), adjustment of the angle to give the widest value of the vittae is important.

Preparation of adults for microscopic examination of genitalia

The procedures for dissection of the genitalia are mainly based on White and Elson-Harris (1992), White and Hancock (1997) and Foote _et al._ (1993). When measuring the length of genitalia, it is recommended that the relative length to body size also be calculated. The length of the _CuA_1 vein along the discal medial cell of the wing has been used as an index of body size in prior studies (Ebina and Ohto, 2006).

Preparation of the abdomen for dissection and examination of genitalia can be accomplished by first removing the abdomen from the specimen and soaking it in a 10% solution of KOH at 95 °C for 10 to 20 minutes depending on the condition of the specimen. Once the KOH soak is complete, the digested abdomen can be transferred to a spot of glycerol.

For aculeus examination, the dissection should be done in a drop of glycerol with two fine forceps (or dissection needles). The oviscape should be broken from the rest of the abdomen and then it is possible to telescope the aculeus out of the oviscape by gently squeezing the oviscape with one pin (Figure 5(b)). It is necessary to finish removal of the aculeus by holding the oviscape with one pin and pulling the aculeus out with the other (for more details, see Foote _et al._, 1993). If the telescoping method fails, the oviscape will need to be torn open to remove the aculeus.

For aedeagus examination, it is recommended that the epandrium–surstylus assemblage (Figure 6(c)) be pulled from the rest of the abdomen. Using two pins, it is possible to straighten the aedeagus (Figure 7). It is then recommended that a small coverslip be placed over the aedeagus, leaving the epandrium, hypandrium and aedeagus base outside of the coverslip. The coverslip is carefully moved away from the epandrium so as to stretch the aedeagus out into a straight line. It is then measured from the base of the basiphallus (enclosed by the hypandrium) to just before the aedeagal glans (Figure 7(d)). In general, the aedeagus should be preserved in glycerol. However, if the specimens are to be used only for measurement, it is sufficient to glue onto a paper stage.

Morphological identification of adults

Members of the _Bactrocera dorsalis_ complex are identified using a combination of morphological characters. The diagnostic characters required to complete an identification to the six species covered by this protocol and to the _Bactrocera dorsalis_ complex as a whole are provided below. Additional resources on general characters for tephritid fruit fly identification are provided in White and Elson-Harris (1992).
4.2.1  **Characters to identify the subgenus Bactrocera (Bactrocera)**

Methods to identify fly specimens to the genus *Bactrocera* are not within the scope of the current protocol. However, proper screening of specimens is important to ensure that flies being diagnosed are within the subgenus *Bactrocera* (*Bactrocera*). The work of White and Elson-Harris (1992) provides a useful resource for those general identifications. Characters used to identify fruit flies to the tribe Dacini, including the genus *Bactrocera*, are useful in the identification of flies to the subgenus *Bactrocera* (*Bactrocera*). These flies have reduced chaetotaxies on the head, with ocellar (Figure 8(b)) and postocellar (Figure 8(b)) bristles absent (atrophied); the first flagellomere (Figure 8(a)) is at least three times as long as broad; and wing cell cup extension is very long (Figure 9(a)). In addition to these characteristics, fruit flies of the genus *Bactrocera* have separate abdominal tergites (Figures 6(a) and (d)) (except for first and second tergites). In addition to the above characteristics of the genus *Bactrocera*, the subgenus *Bactrocera* also has the characteristics listed below.

The presence of diagnostic characters of other *Bactrocera* subgenera is useful in diagnosing flies as not being members of the *Bactrocera dorsalis* complex via exclusion. For example, flies in the subgenus *Bactrocera* (*Afrodacus*) lack anterior supra-alar bristles (Figure 10) and flies in the subgenus *Bactrocera* (*Gymnodacus*) lack pectens on tergite 3 (Figure 6). The characters listed below are used for defining the subgenus *Bactrocera*. In starting identification, it is important to confirm that the fruit flies in question meet the definition. At this stage of identification, superficially similar species in other subgenera such as *Afrodacus* or *Gymnodacus* that could be intercepted during plant inspection can be excluded.

**List of diagnostic characters of subgenus Bactrocera (Bactrocera):**

- [140] posterior lobe of male surstylus short (Figure 6(c))
- [141] abdominal sternite 5 of male deeply concave on posterior margin (Figure 6(b))
- [142] abdominal sternite 5 of male with pecten (Figure 6(a))
- [143] postpronotal bristles absent (Figure 10)
- [144] anterior supra-alar (a. sa.) bristles present (Figure 10)
- [145] prescutellar acrostichal (prsc.) bristles usually present (Figure 10)
- [146] one pair of scutellar (sc.) bristles present (Figure 10).

4.2.2  **Characters to identify the Bactrocera dorsalis complex**

Characters useful for the identification of adult flies following the terminology of Drew and Romig (2013) are listed in Table 2. The definition of the *Bactrocera dorsalis* complex in this protocol follows Drew and Romig (2013) except for scutum colour. Scutum colour in Drew and Romig (2013) is black, but herein black and red-brown are included in the description of the complex. A specimen must have characters that match the descriptions provided in Table 2 to confidently identify the fly as a *B. dorsalis* complex species.

**Table 2.** A combination of characters to diagnose the *Bactrocera dorsalis* complex

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>Distinct facial spots present (Figures 8(a), 8(b), 11)</td>
</tr>
<tr>
<td>Scutum</td>
<td>Colour mostly black to mostly red-brown (inter-regionally variable) (Figure 12)</td>
</tr>
<tr>
<td></td>
<td>Lateral vittae present and yellow (Figures 10 and 13)</td>
</tr>
<tr>
<td></td>
<td>Medial vittae absent (Figure 10)</td>
</tr>
<tr>
<td>Scutellum</td>
<td>Yellow colour (Figures 1 and 12)</td>
</tr>
<tr>
<td></td>
<td>With a dark basal band (Figures 10 and 12)</td>
</tr>
<tr>
<td></td>
<td>Never with other dark patterns (Figure 11)</td>
</tr>
<tr>
<td>Femora</td>
<td>Entirely or mostly fulvous (reddish-yellow or tawny) colour but may possess dark patterns particularly on and around apices (Figure 14)</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Wing</td>
<td>Cells bc and c hyaline (colourless) or, at most, with an extremely pale tint (Figures 9 and 15)</td>
</tr>
<tr>
<td></td>
<td>Without dense microtrichia covering cells bc and c (Figure 9)</td>
</tr>
<tr>
<td></td>
<td>Costal band narrow (never confluent with R&lt;sub&gt;4+5&lt;/sub&gt;) (Figure 9)</td>
</tr>
<tr>
<td></td>
<td>Narrow anal streak present (diagonal marking that is above anal lobe) (Figures 9 and 15)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>With a “T” pattern on tergites 3–5 (Figures 6 and 16)</td>
</tr>
</tbody>
</table>

**Morphological identification of six economically important species of *Bactrocera dorsalis* complex**

Morphological identification of species in the *Bactrocera dorsalis* complex is difficult in part because of a high level of character variability within species and overlap in characters between species. Ranges of variations in each diagnostic character shown in Table 3 are compiled from various sources including Drew and Hancock (1994), Drew and Romig (2013, 2016), and Schutze *et al.* (2015a, b). In Table 3, some character descriptions are recorded with indications of being “inter-regionally” or “intra-regionally” variable because some of the regional populations seem to have clearly unique variations in qualitative or quantitative characters.

Identification at species level is generally difficult when specimens lack a combination of characteristics typical for one of the species. This is particularly evident in diagnosis of *B. dorsalis* s.l. and *B. carambolae* when genitalia lengths can match either species. As mentioned, hybrids are possible between these species but cannot be diagnosed with confidence using morphology.

An identification to one of the six species in the protocol requires the adult specimen to be examined for the characters provided in Table 3. This can be accomplished using the key in section 4.2.4 to screen specimens and then identification can be confirmed by comparing fly morphology to information in Table 3. If one or more characters are inconsistent between the specimen and the descriptions provided in Table 3, then the specimen cannot be diagnosed as one of these species. Morphometric examination of genitalia does not always provide a clear diagnosis because of overlap in the range of aedeagus and aculeus sizes between *B. dorsalis* s.l. and *B. carambolae* (Table 3). These characters are included because they can be informative in distinguishing some specimens of *B. dorsalis* s.l. from *B. carambolae*. When specimens match both *B. dorsalis* s.l. and *B. carambolae* based on morphology, then a molecular test (section 4.3) should be run to distinguish between these species.
Table 3. Diagnostic morphological characters of adult fruit flies of six economically important species of the *Bactrocera dorsalis* complex

<table>
<thead>
<tr>
<th>Structure</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td><em>Bactrocera carambolae</em></td>
<td><em>Bactrocera caryae</em></td>
<td><em>Bactrocera dorsalis</em> s.l.</td>
<td><em>Bactrocera kandiaiensis</em></td>
<td><em>Bactrocera occipitalis</em></td>
<td><em>Bactrocera pyrifoliæ</em></td>
</tr>
<tr>
<td>Facial</td>
<td>Medium-sized, oval</td>
<td>Large, elongate oval</td>
<td>Medium to large, circular to oval (inter-regionally variable)</td>
<td>Large, oval (Figure 11(c))</td>
<td>Large, oval (Figure 11(e))</td>
<td>Medium-sized, circular (Figure 11(f))</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Tergites 3–5 (Figures 6(a), 16, 17)</td>
<td>Tergite 3</td>
<td>Tergite 4</td>
<td>Tergite 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tergite 3</td>
<td>With medium-width medial longitudinal black stripe (Figures 16(a) and 17(a))</td>
<td>With narrow to medium-width medial longitudinal black stripe (Figures 16(b) and 17(b))</td>
<td>With rectangular anterolateral (occasionally triangular) black markings</td>
<td>With anterolateral black markings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tergite 4</td>
<td>With a narrow transverse black band across anterior margin (constituting a &quot;T&quot; pattern) widening to cover lateral margins</td>
<td>With a broad transverse black band across anterior third to half</td>
<td>With broad lateral black bands</td>
<td>With broad lateral black bands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tergite 5</td>
<td>With anterolateral black markings</td>
<td>With any marking or with anterolateral black markings (occasionally rectangular in shape)</td>
<td>With any marking or with anterolateral black marking</td>
<td>With very narrow anterolateral black markings to broad lateral bands</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Facial spots**: (Figures 8(a), 8(b), 11) Medium-sized, oval (Figure 11a)  
Large, elongate oval (Figure 11(b))  
Medium to large, circular to oval (inter-regionally variable) (Figure 11(c))  
Large, oval (Figure 11(d))  
Large, oval (Figure 11(e))  
Medium-sized, circular (Figure 11(f))

**Abdomen Tergites**:  
3: With a narrow transverse black band across anterior margin (constituting a "T" pattern) widening to cover lateral margins  
With a broad transverse black band across anterior third to half  
With rectangular anterolateral (occasionally triangular) black markings  
With broad lateral black bands  
With anterolateral black markings |

4: With any marking or with anterolateral black markings (occasionally rectangular in shape)  
With very narrow anterolateral black marking  
With very narrow anterolateral black markings to broad lateral bands  
With broad lateral black bands that cover lateral margins  
With broad lateral black bands that cover lateral margins
<table>
<thead>
<tr>
<th>Structure</th>
<th>Species</th>
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<td></td>
<td><strong>Bactrocera carambolae</strong></td>
<td><strong>Bactrocera caryeae</strong></td>
<td><strong>Bactrocera dorsalis s.l.</strong></td>
<td><strong>Bactrocera kandiensis</strong></td>
<td><strong>Bactrocera occipitalis</strong></td>
<td><strong>Bactrocera pyrifoliae</strong></td>
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<tr>
<td><strong>Thorax</strong></td>
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<tr>
<td><strong>Scutum colour</strong> (Figure 12)</td>
<td><strong>Dull black</strong> (Figure 12(a))</td>
<td><strong>Pure black</strong> (Figure 12(b))</td>
<td><strong>Black to red-brown (inter or intra-regionally variable)</strong> (Figure 12(c))</td>
<td><strong>Black</strong> (Figure 12(d))</td>
<td><strong>Black with clear central stripe</strong> (Figure 12(e))</td>
<td><strong>Pure black</strong> (Figure 12(f))</td>
</tr>
<tr>
<td><strong>Postpronotal lobe</strong> (Figures 4, 10, 12, 18)</td>
<td><strong>Entirely yellow</strong> (Figure 18(a))</td>
<td><strong>Yellow with dark anteromedial corner</strong> (Figure 18(b))</td>
<td><strong>Entirely yellow</strong> (Figure 18(c))</td>
<td><strong>Yellow with dark anteromedial corner</strong> (Figure 18(d))</td>
<td><strong>Entirely yellow</strong> (Figure 18(e))</td>
<td><strong>Entirely yellow</strong> (Figure 18(f))</td>
</tr>
<tr>
<td><strong>Anterior margin of mesopleural stripe</strong> (Figures 4(a) and 13)</td>
<td><strong>Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin)</strong> (Figure 13(a))</td>
<td><strong>Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight (anterior margin)</strong> (Figure 13(b))</td>
<td><strong>Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight to convex (anterior margin)</strong> (Figure 13(c))</td>
<td><strong>Slightly wider than notopleuron, equal in width to notopleuron; straight (anterior margin)</strong> (Figure 13(d))</td>
<td><strong>Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin)</strong> (Figure 13(e))</td>
<td><strong>Equal in width to notopleuron; convex (anterior margin)</strong> (Figure 13(f))</td>
</tr>
<tr>
<td><strong>Basal band of scutellum</strong> (Figures 10 and 12)</td>
<td><strong>Narrow</strong> (Figure 12(a))</td>
<td><strong>Moderately broad</strong> (Figure 12(b))</td>
<td><strong>Narrow</strong> (Figure 12(c))</td>
<td><strong>Narrow</strong> (Figure 12(d))</td>
<td><strong>Narrow</strong> (Figure 12(e))</td>
<td><strong>Narrow</strong> (Figure 12(f))</td>
</tr>
<tr>
<td><strong>Lateral vittae</strong> (Figures 3, 4, 10)</td>
<td><strong>Broad, parallel-sided, ending at or behind ia. bristles</strong> (Figure 3(a))</td>
<td><strong>Very narrow; either entirely parallel-sided or narrowing posteriorly; ending at or just before ia. bristles</strong> (Figure 3(b))</td>
<td><strong>Narrow to broad (inter-regionally variable), parallel-sided, ending at or just behind ia. bristles</strong> (Figure 3(c))</td>
<td><strong>Narrow, parallel-sided, ending at ia. bristles or (in some specimens) ending behind ia. bristles</strong> (Figure 3(d))</td>
<td><strong>Broad, parallel- or subparallel-sided; either ending at ia. bristles or (in some specimens) parallel-sided and ending at ia. bristles</strong> (Figure 3(e))</td>
<td><strong>Narrow; either subparallel-sided and ending before ia. bristles or (in some specimens) parallel-sided and ending at ia. bristles</strong> (Figure 3(f))</td>
</tr>
<tr>
<td>Structure</td>
<td>Species</td>
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<tr>
<td><strong>Wing</strong></td>
<td>[306]<strong>Bactrocera carambolae</strong></td>
<td>[307]<strong>Bactrocera caryeae</strong></td>
<td>[308]<strong>Bactrocera dorsalis</strong> s.l.</td>
<td>[309]<strong>Bactrocera kandiensis</strong></td>
<td>[310]<strong>Bactrocera occipitalis</strong></td>
<td>[311]<strong>Bactrocera pyrifoliae</strong></td>
</tr>
<tr>
<td><strong>Costal band</strong> (Figures 9 and 15)</td>
<td>[313]Narrow, slightly overlapping R&lt;sub&gt;2+3&lt;/sub&gt;, moderately broad around apex of wing (Figure 15(a))</td>
<td>[314]Very narrow, confluent with R&lt;sub&gt;2+3&lt;/sub&gt;, very narrow around apex of wing (Figure 15(b))</td>
<td>[315]Narrow, generally confluent with R&lt;sub&gt;2+3&lt;/sub&gt; (inter- or intra-regionally variable), narrow to moderately broad around apex of wing (Figure 15(c))</td>
<td>[316]Narrow, confluent with R&lt;sub&gt;2+3&lt;/sub&gt;, narrow around margin of wing (Figure 15(d))</td>
<td>[317]Narrow, distinctly overlapping R&lt;sub&gt;2+3&lt;/sub&gt;, broad around apex of wing extending to mid-point between R&lt;sub&gt;2+3&lt;/sub&gt; and R&lt;sub&gt;4+5&lt;/sub&gt; (Figure 15(e))</td>
<td>[318]Narrow, confluent with R&lt;sub&gt;2+3&lt;/sub&gt;, narrow but slightly expanding around apex of wing (Figure 15(f))</td>
</tr>
<tr>
<td><strong>Femora</strong> (Figure 14)</td>
<td>[327]Fulvous, generally with a large elongate oval black marking on outer surface of fore femora (Figure 14(a))</td>
<td>[328]Fulvous with large dark fuscous markings on all femora (Figure 14(b))</td>
<td>[329]Generally fulvous, occasionally with a small dark marking on outer surface of fore femora (inter-regionally variable) (Figure 14(c))</td>
<td>[330]Fulvous with large dark markings on all femora (Figure 14(d))</td>
<td>[331]Generally fulvous, occasionally with a small preapical dark spot on outer surface of fore femora (Figure 14(e))</td>
<td>[332]Fulvous with a small apical marking on fore femora and dark fuscous around apices of mid and hind femora (Figure 14(f))</td>
</tr>
<tr>
<td><strong>Genitalia</strong></td>
<td>[334]Aculeus length (mm) (Figure 5)</td>
<td>[335]Ratio (Cu&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;1&lt;/sub&gt;/Acul.)</td>
<td>[336]Aedeagus length (mm) (Figure 7)</td>
<td>[337]Ratio (Aed./Cu&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>[338]Aculeus length (mm) (Figure 5)</td>
<td>[339]Ratio (Cu&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;1&lt;/sub&gt;/Acul.)</td>
</tr>
<tr>
<td>[341]1.3–1.6</td>
<td>[342]n/a</td>
<td>[343]1.4–2.2 (inter or intra-regionally variable)</td>
<td>[344]n/a</td>
<td>[345]n/a</td>
<td>[346]n/a</td>
<td>[347]n/a</td>
</tr>
<tr>
<td>[348]1.4–1.6</td>
<td>[349]n/a</td>
<td>[350]1.0–1.8</td>
<td>[351]n/a</td>
<td>[352]n/a</td>
<td>[353]n/a</td>
<td>[354]n/a</td>
</tr>
<tr>
<td>[355]2.0–2.7</td>
<td>[356]n/a</td>
<td>[357]2.3–3.5 (inter or intra-regionally variable)</td>
<td>[358]n/a</td>
<td>[359]n/a</td>
<td>[360]n/a</td>
<td>[361]n/a</td>
</tr>
<tr>
<td>[362]1.2–1.3</td>
<td>[363]n/a</td>
<td>[364]1.2–1.4</td>
<td>[365]n/a</td>
<td>[366]n/a</td>
<td>[367]n/a</td>
<td>[368]n/a</td>
</tr>
</tbody>
</table>

[11] [369]Acul., aculeus length; Aed., aedeagus length; Cu<sub>A</sub><sub>1</sub>, first anterior branch of cubitus vein (see Figure 9); ia., intra-alar; n/a, not available; npl., notopleural; R<sub>2+3</sub>, R<sub>4+5</sub>, posterior branches of radial vein (see Figure 9).
4.2.4 Diagnostic key to six species of economic importance belonging to the Bactrocera dorsalis complex (adult)

1. Postpronotal lobe yellow with dark anteromedial corner (Figures 18(b) and (d))..........................2

2. Postpronotal lobe entirely yellow (Figures 18(a), (c), (e), (f))..................................................3

3. Scutum pure black (Figure 12(b)), abdominal tergites 3–5 with broad black dorsolateral markings (Figures 16(b) and 17(b)); lateral vittae very narrow (Figure 3(b))..............................................B. caryeae

4. Costal band distinctly overlapping R$_{2+3}$ and expanding broadly around apex of wing reaching mid-point between R$_{2+3}$ and R$_{4+5}$ (Figure 15(e)).................................................................B. occipitalis

5. Abdominal tergites 3–5 with broad black dorsolateral markings (Figures 16(f) and 17(f))......B. pyrifoliae

6. Abdominal tergites 3–5 without broad black dorsolateral markings..........................................5

7. Costal band confluent with R$_{2+3}$, narrow to moderately broad around apex of wing (Figure 15(c)); abdominal tergite 3 exhibits variations from black band across anterior margin (constituting a “T” pattern) to broad lateral bands, tergite 4 without markings or with anterolateral or narrow lateral black margins (occasionally rectangular), tergite 5 without markings or with anterolateral black markings (Figures 16(c) and 17(c))......................................................B. dorsalis s.l.

4.3 Molecular identification of Bactrocera carambolae

Molecular identification of the six target species has been confounded by their very close genetic relationships and uncertain taxonomy (Boykin et al., 2014; Hendrichs et al., 2015). Molecular tests alone are not recommended for identification of the six species. However, molecular methods can provide useful information to support morphological identifications when new records are reported from the morphological diagnosis. When identifying B. carambolae and B. dorsalis s.l. specimens using this protocol, a molecular test is necessary for accurate identification whenever adult morphology alone cannot distinguish between the two species.

DNA sequencing of either the internal transcribed spacer 1 (ITS1) or 2 (ITS2) nuclear DNA regions has been proposed as a reliable test to distinguish between the species B. carambolae and B. dorsalis s.l. (Boykin et al., 2014; Schutze et al., 2015a). The internal transcribed spacer 1 (ITS1) test as described by
Boykin et al. (2014) for distinguishing between the two species is included in the current protocol. This test is designed to diagnose a fly as *B. carambolae* based on the presence of a unique DNA insertion. Specificity of the test for *B. carambolae* has been examined using four additional species in the *Bactrocera dorsalis* complex: *B. dorsalis* s.l., *B. occipitalis*, *B. opiliae* and *B. cacuminata*.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

### 4.3.1 DNA extraction for molecular tests

Boykin et al. (2014) and Ball and Armstrong (2008) provide protocols for DNA extraction using commercial kits that are useful because small starting material such as one fruit fly leg can give enough DNA yield and quality for PCR reactions. The methods used to preserve fruit flies for morphological and molecular examination are not the same. Ethanol is a common preservative for fruit fly DNA. Although fruit fly specimens can be preserved in ≥95% ethanol at −20 °C or colder for long-term storage, ethanol can alter the colouring of adult specimens, which can hinder morphological identification. All identifications performed using this protocol require morphological examination. In cases where molecular methods are to be used, it is therefore recommended that a leg be removed and stored in ethanol for DNA extraction and that the remaining specimen be prepared for morphology work. Further examples of methods are provided by Plant Health Australia (2016).

### 4.3.2 ITS1 PCR and DNA sequencing test for distinguishing *B. carambolae* from *B. dorsalis* s.l.

The Boykin et al. (2014) study compared a large collection of ITS1 sequences from *B. dorsalis* s.l. and *B. carambolae* specimens. Although many primer sets for analysis of ITS1 have been reported in the scientific literature (e.g. Plant Health Australia, 2016), the ITS7/ITS6 primer set reported by Boykin et al. (2014) is reported here to simplify comparison with reference sequences from that study and stored in GenBank. Other primer sets that target the same region of ITS1 could also function adequately. None of the published primer sets for this target gene have been tested for reproducibility or sensitivity.

The ITS7 (forward) and ITS6 (reverse) primers are:

\[\text{ITS7 (5'}\text{- GAA TTT CGC ATA CAT TGT AT-3')}\text{ (Boykin et al., 2014)}\]
\[\text{ITS6 (5'}\text{- AGC CGA GTG ATC CAC CGC T-3')}\text{ (Armstrong and Cameron, 2000)}\]

PCR can be carried out in 30 µl reactions according to Boykin et al. (2014), using the master mix and cycling parameters given in Table 4.
Table 4. Master mix composition for PCR and cycling conditions for a final reaction of 30 µl

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR grade water</td>
<td>–†</td>
</tr>
<tr>
<td>PCR buffer</td>
<td>1×</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2.0 mM</td>
</tr>
<tr>
<td>dNTPs</td>
<td>200 µM of each</td>
</tr>
<tr>
<td>Primer (forward)</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>Primer (reverse)</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>0.6 U</td>
</tr>
<tr>
<td>DNA sample</td>
<td>2 µl</td>
</tr>
</tbody>
</table>

Cycling parameters

- Initial denaturation: 94 °C for 2 min
- Number of cycles: 35
- Denaturation: 94 °C for 15 s
- Annealing: 60 °C for 20 s
- Elongation: 69 °C for 60 s
- Final elongation: 68 °C for 5 min
- Expected Amplicon: 500-550 bp

Description: the amplicon size varies for species and individuals

† For a final reaction volume of 30 µl.

Sanger sequencing of PCR products should be carried out using each primer to generate two independent DNA sequences read in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T, or G). The final edited sequence should be at least 400 base pairs (bp) in length for data interpretation.

### Controls for molecular tests

For the test result obtained to be considered reliable, the following controls should be considered for each series of nucleic acid extractions and PCR amplifications of the target pest. As a minimum, a positive nucleic acid control and a negative amplification control (no template control) should be used for the ITS1 PCR test.

**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA may be used.

**Negative amplification control (no template control).** This control is necessary to rule out false positives due to contamination with other genetic material during the preparation of the reaction mixture. PCR grade water that was used to prepare the reaction mixture is added in place of template DNA.

**Negative extraction control.** This control is used to monitor contamination during nucleic acid extraction. This requires processing extraction blanks alongside the samples to be tested.
4.3.4 Interpretation of molecular test results

The size of ITS1 is different for *B. carambolae* and *B. dorsalis* because of a 44-bp insertion in *B. carambolae* located near one end of the gene located near the ITS7 primer. The inserted DNA is identical in all *B. carambolae* studied. The sequence of the insertion is: 5´-GAAAAATTAATAAAAGTTAAATGATCTTTTTTATAAAAAT-3´.

The ITS1 sequence is variable between conspecific specimens of these two species (Boykin et al., 2014). Consequently, an identical match for sites outside of the insertion region is not expected. However, the test sequence should be at least 99% similar to one of the reference sequences for the interpretation to proceed. It is possible to distinguish between *B. carambolae* and *B. dorsalis* s.l. after comparing the DNA sequence of the tested specimen with a representative sequence of each species: GenBank KC446737 for *B. carambolae* and KC446776 for *B. dorsalis*. If the tested sequence is most similar to *B. carambolae* and has the 44-bp insertion region, then it can be diagnosed as *B. carambolae*. If the tested sequence is most similar to *B. dorsalis* and lacks the insertion region, then it is diagnosed as not *B. carambolae*. Several other species in the *B. dorsalis* complex lack the insertion and a match with *B. dorsalis* s.l. cannot exclude those as a possible identification.

4.4 Other molecular methods of identification

Plant Health Australia (2016) has compiled a resource for identification of *Bactrocera* species using DNA methods. That resource summarizes three molecular options for identification: conventional PCR and restriction fragment length polymorphism (RFLP) of the ITS1 region (Plant Health Australia, 2016), PCR-RFLP analysis of a segment of rRNA array including the ITS1 and 18S gene regions (Armstrong et al., 1997; Armstrong and Cameron, 2000), and DNA barcoding of the cytochrome oxidase subunit I (COI) gene (Armstrong and Ball, 2005) based on the Barcode of Life Data Systems (BOLD) resource (Ratnasingham and Hebert, 2007). The species *B. caryae*, *B. kandtensis*, *B. occipitalis* and *B. pyrifoliae* do not have molecular profiles available for either of the PCR-RFLP tests described in the Plant Health Australia resource, precluding their use as a diagnostic test for the pests. For the species *B. dorsalis*, the resource provides expected PCR product sizes of ITS1 and the expected fragment sizes of digested PCR products of the rDNA fragment including ITS1+18S. These rDNA tests lack specificity data to support diagnosis of a fly as *B. dorsalis* s.l. using genetic profiles alone. However, rDNA profiles that do not match recorded results of *B. dorsalis* s.l. can be used to reject diagnosis of a fly as *B. dorsalis* s.l.

DNA barcode records are not available for *B. pyrifoliae*. The *cytochrome oxidase I (COI)* DNA barcode records for the other five species cannot distinguish at the species level (Armstrong and Ball, 2005). To date, no study has provided information on how to use COI sequence data to accept or reject a diagnosis of a specimen as part of the *Bactrocera dorsalis* complex or as one of the 85 species within the complex. The work by Leblanc et al. (2015) demonstrates that this complex is not a monophyletic group and a molecular diagnosis of the complex is not possible. The standard DNA Barcode COI region cannot be used reliably to differentiate *B. dorsalis* s.l. from other species in the *Bactrocera dorsalis* complex including *B. carambolae* (Armstrong and Ball, 2005).

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27.

In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, as appropriate) should be kept for at least one year.

6. Contact points for further information

Further information on this protocol can be obtained from:
A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

Acknowledgements

The original draft of this protocol was written by Kenji Tsuruta (Ministry of Agriculture, Forestry and Fisheries, Japan (see preceding section)), Sujinda Thanaphum (Mahidol University, Thailand (see preceding section)), Luc Leblanc (University of Idaho, United States of America (see preceding section)) and Norman Barr (United States Department of Agriculture, United States of America). The following experts provided comments on earlier versions that improved the quality of the protocol: Jane Royer (Queensland Department of Agriculture and Fisheries, Australia), Mark Schutze (Queensland University of Technology, Australia), Josephine Moraa Songa (Kenya Agricultural & Livestock Research Organization, Kenya), George Momanyi (Kenya Plant Health Inspectorate Service, Kenya), Sharon Reid (Fera Science Ltd., Sand Hutton, York, United Kingdom), Yuji Kitabara (Ministry of Agriculture, Forestry and Fisheries, Japan), Eddy Dijkstra (Plant Protection Service, Netherlands), and Ken Hong Tan (Tan Hak Heng, Penang, Malaysia).

References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispm.


[495] Vargas, R.I., Piñero, J.C. & Leblanc, L. 2015. An overview of pest species of Bactrocera fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific region. Insects, 6: 297–318.


![Figure 1. Bactrocera dorsalis, female (habitus)](source)

[501] Source: Photo courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan
Figure 2. Immature stages of Bactrocera dorsalis: (a) egg; (b) first instar larva; (c) second instar larva; (d) third instar larva; (e) puparium; (f) pupa.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 3. Lateral vittae: (a) *Bactrocera carambolae*; (b) *Bactrocera caryae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 4. (a) Lateral view of Dacinae thorax. (b) Damaged lateral vitta, showing translucent window.

mpl., mesopleural bristle.

Source: Photo and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 5. Dacinae abdomen: (a) female in dorsal view; (b) genitalia (fully extended). syntg 1 + 2, syntergites 1 + 2; tg 3, tergite 3; tg 4, tergite 4; tg 5, tergite 5; ovscp, oviscape; ev memb, eversible membrane; acul, aculeus; cm, ceromata.

Source: Line drawing (a) adapted from Ito (1988) and photo (b) courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 6. Dacinae abdomen: (a) male in dorsal view; (b) male in ventral view; (c) epandrium and lateral surstylus, showing short posterior lobe; (d) epandrium and lateral surstylus, showing long posterior lobe. syntg 1 + 2, syntergites 1 + 2; tg3, tergite 3; tg4, tergite 4; tg5, tergite 5; pect, pecten; cm, ceromata.

Source: Photos and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 7. Male abdomen and aedeagus (*B. dorsalis*): (a) abdomen in ventral view (KOH treated); (b) part of aedeagus appearing rightside (when base of abdomen set upside-down and viewed from ventral side) of epandrium; (c) pulling out aedeagus using hooked micropin; (d) extended aedeagus, showing the part to be measured.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 8. (a) Lateral view of Dacinae head. (b) Frontal view of Dacinae head. (c) Dorsal view of Dacinae head (vertex). i. or. b., inferior fronto-orbital bristles; s. or. b., superior fronto-orbital bristles.

Source: Photo and line drawings courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 9. Wing of Dacinae. Veins: A1, branch of anal vein; C, costa; CuA1, CuA2, anterior branches of cubitus; M, media; R1, anterior branch of radius; R2+3, R4+5, combined posterior branches of radius; Sc, subcosta; bm-cu = basal medial-cubital crossvein; dm-cu, discal medial-cubital crossvein; r-m, radial-medial crossvein. Cells: bc, basal costal; c, costal; sc, subcostal; bm, basal medial; br, basal radial; cup, posterior cubital; dm, discal medial. Anal streak, areas around cup and cup extension indicated red outline.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 10. Dorsal view of Dacinae thorax. a. sa., anterior supraalar bristle; ia., intraalar bristle; mpl., mesopleural bristle; a. npl., anterior notopleural bristle; p. npl., posterior notopleural bristle; ppn., postpronotal bristle; prsc., prescutellar bristle; p. sa., posterior supraalar bristle; sc., scutellar bristle; scp., scapular bristle.

Source: Line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 11. Head in anterolateral view: (a) *Bactrocera carambola*; (b) *Bactrocera caryae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 12. Thorax in dorsal view: (a) Bactrocera carambolae; (b) Bactrocera caryae; (c) Bactrocera dorsalis s.l.; (d) Bactrocera kandiensis; (e) Bactrocera occipitalis; (f) Bactrocera pyrifoliae. Basal band indicated by red circle in image (a).

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 13. Thorax in lateral view: (a) *Bactrocera cambolae*; (b) *Bactrocera caryae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 14. Legs: (a) *Bactrocera carambola*; (b) *Bactrocera caryae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifolia*. 1, fore leg (outer surface); 2, mid leg; 3, hind leg (inner surface, when folded back alongside abdomen).

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 15. Wings: (a) *Bactrocera carambolae*; (b) *Bactrocera caryaeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 16. Abdomen in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifolia*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 17. Abdomen in dorsolateral view: (a) Bactrocera carambola; (b) Bactrocera caryae; (c) Bactrocera dorsalis s.l.; (d) Bactrocera kandiensis; (e) Bactrocera occipitalis; (f) Bactrocera pyrifolia.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.
Figure 18. Postpronotal lobes in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. 

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.