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Distinguishing Features of *Bactrocera occipitalis* (Bezzi) and *Bactocera philippinensis* Drew & Hancock Occurring in the Philippines*

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Abstract.- Identification of pest species in Oriental fruit fly complex commonly encountered in Asia is difficult. The pest species in the Oriental fruit fly complex, occurring in the Philippines (*Bactrocera occipitalis* and *Bactrocera philippinensis*) are separated in this paper. Different identification techniques, colour pattern on wing and abdominal tergites, aculeus length to wing discal cell length ratio, aedeagus length to discal cell length ratio and scanning electron microscopy of tomentum pattern on prescutum are used.

Keywords: *Bactrocera occipitalis, Bactrocera philippinensis*, Oriental fruit fly complex, Aedeagus and aculeus length, Tomentum pattern, the Philippines.

INTRODUCTION

Bezzi (1919) described *Bactrocera occipitalis* as *Chaetodacus ferrugineus* var. *occipitalis* and *Bactrocera philippinensis* was recently described by Drew and Hancock (1994). These species are sympatric and serious pests of fruits in the Philippines. There is a chance of introduction of these notorious pests to the other countries engaged in trade with the Philippines unless quarantine regulations are vigorously enforced. Unfortunately, the identification in some cases has been notoriously difficult, even to the specialists or even to the authors. The main reason for this is the degree of inter- and intra-specific variability exhibited within this complex. Drew and Hancock (1994) described *B. philippinensis* and re-described *B. occipitalis* in the revision of the Oriental fruit fly complex in Asia with fifty other species. Although this was a good quality scientific work, it is extremely difficult to use it without a lot of experience in studying the complex and access to a good reference collection. Moreover, they dealt with all the species without regard to economic importance, which makes the work confusing and difficult for inexperienced users. Usually the requirement of the local workers is to identify a couple of locally occurring economically important species. It is difficult to use the key with more than fifty couplets and closely related species, and the chances of making a wrong identification are high.

White and Mahmood (1995) presented an identification system based on the aculeus length and discal cell length for female specimens and applied an additive scoring method for the identification of both sexes of *B. philippinensis* and *B. occipitalis*, which was developed for the use of local worker. Recently, DNA analysis for these two species was being carried out in USA, however, the specimens sent by local workers for DNA analyses were misidentified, indicating the difficulty in identification of these two species. Different identification methods presented in this article are additive scoring, aculeus length to wing discal cell length ratio, aedeagus length to wing discal cell length ratio and scanning electron microscopy of tomentum pattern on prescutum. The methods presented here have been developed keeping in view the local needs of extension and other workers with little experience in taxonomy.

MATERIALS AND METHODS

Specimens of *B. occipitalis* from the Philippines and Brunei, and *B. philippinensis* from the Philippines were examined. The following identification techniques were applied.

Additive scoring method

In each case, each character was given a numeric code, "0" being the typical state of one

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species and "1" for the state typical of other species. The intermediate states of any character were coded "0.5". The overall score of each specimen was calculated by addition of its numeric states for each character. For example, the most extreme *B. philippinensis*- like specimens scored "0" and the most extreme *B. occipitalis*- like specimens scored "4". No prior assumption was made about identification of each specimen. Wald-Wolfowitz Runs test was carried out on the total scores. In this test, the null hypothesis was that two independent samples have been drawn from the same population against the alternative hypothesis that they differ in respect of their medians, variability or skewness. The scores for each individual were plotted in the form of frequency histograms. The specimens were examined using a "Wild Herrbrugg" microscope.

Aculeus length and the discal cell length

Female specimens were dissected for aculeus length and wing discal cell length (the lower vein enclosing the discal cell) was measured. The dissection procedure described by White and Elson-Harris (1992) was followed.

Aedeagus length and the discal cell length

Male specimens were dissected from several localities and the aedeagus length and wing discal cell length was measured. The dissection procedure of White (1988) was followed.

The measurements were carried out with the help of video camera and video plan software belonging to the Medical and Veterinary Division. Department of Entomology, the Natural History Museum, London. The numerical data were saved in the spread sheet module of the Smart-II, software. The graphs were generated using the same software. The Student's t-test was carried out on the means of measurements of the aculeus length and aedeagus length, discal cell length, aculeus length to discal cell length ratio and aedeagus length to discal cell length ratio using Unistate statistical package. The null hypothesis that the two population means are equal was tested.

Scanning electron microscopy

Critical point drying (where necessary), mounting and coating was carried out according to a standard procedure, for observing specimens in a Scanning Electron Microscope. Scanning electron microscopy was carried out in the SEM unit of the Department of Mineralogy, the Natural History Museum, London, using a Hitachi S 2500 Scanning Electron Microscope.

The results and discussion are presented in a manner that likely to be of practical interest to field entomologists.

RESULTS

Additive scoring method

Specimens of both these sympatric species were separated using an additive scoring method for four characters. These characters (Table I) are diagnostic of *B. occipitalis* and *B. philippinesis* when used in combination.

The frequency histogram of the specimen (Fig. 1) clearly shows two peaks, one around the score 0.0 – 0.5 and the other around the score 3.5 – 4.0. The specimens with the score from 0.0 – 2.0 were identified as *B. philippinensis* and the specimens with scores 3.0 – 4.0 were identified as *B. occipitalis* (Figs. 2, 3). The dissected male and female specimens followed a similar pattern as the undissected specimens.

Thus 85% specimens of *B. occipitalis* were separated by costal band depth over lapping vein R_{2+3} and 87% specimens of *B. philippinensis* were separated by the costal band not over-lapping vein R_{2+3} and similarly other characters (Table II).

Aculeus length and the discal cell length

Female specimens of *B. occipitalis* and *B. philippinensis* could also be separated by the relationship of aculeus length and discal cell length. When the aculeus length was plotted against the discal cell length, there was clear separation between the two species (Fig. 2). The graph clearly indicates two groups, *B. philippinensis* corresponding with a long aculeus (1.65 – 2.14 mm, mean 1.89 mm) and *B. occipitalis* with a short aculeus (1.34 – 1.60 mm, mean 1.50 mm). The aculeus length to discal cell length ratio provided an even clearer separation between the two species (*B. occipitalis* 0.61 – 0.70, *B. philippinensis* 0.76 – 0.98). The females of both the species were first separated by the four characters (described above),

Fig. 1. Frequency histogram of male and female specimens of *B. philippinensis* and *B. occipitalis* with respect to score.

Fig. 2. Relationship of aculeus length and discal cell length between *B. philippinensis* and *B. occipitalis*.

Fig. 3. Relationship of aedeagus length and discal cell length between *B. philippinensis* and *B. occipitalis*.

Table I.- The typical conditions of the characters for each species.

Characters	State typical of *B. philippinensis* coded as "0"	State typical of *B. occipitalis* coded as "1"
Overlap of costal band on wing	Not below Vein R_{2+3} except at the apex (Fig. 4A)	Below vein R_{2+3} for entire length (Fig. 4B)
Transverse black band on abdominal tergite III	Narrow, not coming down the lateral margins except at the extreme sides (Fig. 4C)	Broad and coming down the lateral margins (Fig. 4D)
Lateral black markings on abdominal tergite IV	Triangular, or narrow longitudinal line, or absent (Fig. 4C)	Rectangular, broad or sometimes narrow (Fig. 4D)
Lateral black markings on abdominal tergite V	Triangular, or a narrow longitudinal line, or without any mark (Fig. 4C)	Rectangular, or triangular and extending towards the medial band (Fig. 4D)

the female specimens with score 1-2 corresponding to the long aculeus, *i.e. B. philippinensis*, and the specimens with score 3 – 4 corresponded to the short aculeus, *i.e. B. occipitalis* (Fig. 2).

Aedeagus length and discal cell length

Male specimens of these two species were separated by aedeagus length expressed as a ratio to discal cell length. When a graph was plotted of aedeagus length with respect to discal cell length, there were no clear groups (Fig. 3). Specimens with a long aedeagus (3.24 – 3.95 mm, mean 3.51 mm) corresponding to score 0-1.5 were *B. philippinensis* and specimens with a short aedeagus (2.69 – 3.25 mm, mean 3.08 mm) corresponding to the score 2.5–4 were *B. occipitalis*. The aedeagus length to discal cell length ratio was generally higher (more than 1.5 in 90% specimens) for *B. philippinensis* and was generally lower (less than 1.5 in 90% specimens) for *B. occipitalis*.

Tomentum pattern on the prescutum

Both the species were studied under the scanning electron microscope. The tomentum

Table II.- Average score for each character of each species.

Species	Costal band overlap	Tergite III basal transverse band	Tergite IV anterolateral markings	Tergite V anterolateral markings
B. philippinensis	0.13	0.06	0.01	0.03
B. occipitalis	0.85	0.92	0.85	0.89

Statistical Analysis

Species	Cases	Mean	Std. Dev.	Std. Err.	t-value
a) Wald-Wolfowitz test for total scores					
B. philippinensis	75	3.6533	0.4861	5.6132	15.70***
B. occipitalis	175	0.3343	0.5783	4.3717	
b) t-test for aculeus length					
B. philippinensis	76	1.8933	0.09916	0.01137	17.28***
B. occipitalis	22	1.5037	0.06741	0.01437	
c) t-test aculeus length to discal cell length ratio					
B. philippinensis	76	0.8403	0.03988	0.004574	19.31***
B. occipitalis	22	0.6633	0.02952	0.006293	
d) t-test for the aedeagus length					
B. philippinensis	65	3.4938	0.1599	0.01984	16.20***
B. occipitalis	61	3.0828	0.1207	0.01545	
e) t-test for the aedeagus length to discal cell length ratio					
B. philippinensis	65	1.5882	0.08272	0.01026	11.81***
B. occipitalis	61	1.4078	0.08885	0.01138	

Degrees of freedom: Runs = 2, b, c = 96, d,e = 124, $P<0.001$
The differences between the means for total scores (a), for aculeus length (b), for aculeus length to discal cell length ratio (c), for aedeagus length (d), and for aedeagus length to discal cell length ratio (e) were highly significant.

pattern on the prescutum was observed. The tomentum pattern on the prescutum of *B. occipitalis* (Fig. 5A) has a wide longitudinal gap in the centre, while that of the *B. philippinensis* (Fig. 5B) lacks a gap. This character separated all the specimens of these two species.

DISCUSSION

Drew and Hancock (1994) separated *B. occipitalis* from *B. carambolae, B. papayae* and *B. philippinensis* by "the combination of the broad parallel sided postsutural vittae, broad lateral dark markings on abdominal tergites III-V, lack of any dark spots around frontal setae and moderately short aculeus". In the present study, it was not possible to separate *B. occipitalis* from *B. carambolae, B. papayae* and *B. philippinensis* by the postsutural vittae, the absence of dark spots around frontal setae and moderately short aculeus. However, the broad lateral markings on tergites III-V were a good combination of characters to differentiate *B. occipitalis* from *B. philippinensis* and *B. papayae*. In addition to the lateral abdominal markings, the depth of the costal band was another characters that separated *B. occipitalis* from *B. philippinensis* and *B. papayae*. The lateral markings on abdominal tergites IV and V and the depth of the basal transverse

Fig. 4. Wings of *B. philippinensis* (A), and *B. occipitalis* (B); abdomens of *B. philippinensis* (C) and *B. occipitalis* (D).

band on abdominal tergite III were defined in the present study. The clear definition of these markings was vital for correct identification. The depth of the costal band in combination with the abdominal markings on tergites III-V separated *B. occipitalis* from *B. philippinensis* in the present study. The present study showed that there was no single external character to separate these two species. Therefore, it was necessary to use the combination of characters. The highly significant value of t (15.70, P<0.001) indicated hat the populations were different.

According to Drew and Hancock (1994) the aculeus length of *B. occipitalis* and *B. philippinensis* were 1.4 – 1.6 mm and 1.77 – 2.12 mm, respectively. In the present study the known minimum aculeus length in *B. philippinensis* and *B. occipitalis* was found to be 1.65 mm and 1.34 mm, respectively. In the present study the difference in mean values was highly significant for aculeus length (t = 17.28, P<0.001) and aculeus length when expressed as a ratio to discal cell length (t = 19.31, P<0.001).

It was very important to separate male specimens as the males of many species of dacines are attracted to either methyl eugenol or cue lure and male lure traps are designed to collect and monitor populations. Therefore, aedeagus length clearly separated the two species. Although there was no clear grouping and slight (less than 2%) overlap in the aedeagus lengths of each species, difference in means for the two species was highly significant (t = 16.20, P<0.001). The difference in means for aedeagus length when expressed as a ratio to discal length was also highly significant (t = 11.81, P<0.001). Aedeagus length has not previously been used for dacine identification.

Although commonly found useful in Diptera identification, tomentum patterns have not previously been studied in the Dacini. Using the SEM, differences between the tomentum pattern on prescutum of the two species were observed and it found to be the best character to separate. In *B. occipitalis* the white scale- like structures (tomentum) were absent from the sides and from the centre of the prescutum, forming three longitudinal black bands (Fig. 5A). In *B. philippinensis* the white scale- like structures were absent only from the sides of the prescutum, leaving only longitudinal black bands (Fig. 5B). About five hundred specimens of both the species were examined for tomentum pattern, under light microscope and this character separated 100% specimens. Aculeus

Fig. 5. Tomentum pattern on prescutum of *B. occipitalis* (A), and *B. philippinensis* (B).

length, aedeagus length and additive scoring method supported the identification by the tomentum pattern.

This character could easily be used with the light microscope or even sometimes with a naked eye, when the specimen was viewed antero-dorsally with oblique light (lighting also from in front). In *B. occipitalis* the central clear area shines, while in *B. philippinensis* there was no central shiny area. It is important to view this character from the correct direction and with correct direction of light source (as mentioned above) for accurate separation of these two species. When the specimens were preserved in alcohol or when the tomentum pattern was damaged, it was impossible to see this character.

In the present study it was also observed that there was slight variability in *B. philippinensis* between backyard and secondary forest populations. Ninety percent of the backyard population had a black triangular marking on the abdominal tergite IV, while only 25% of secondary forest individuals had that form of marking (75% of specimens had a narrow lateral longitudinal black hand). Similarly, 15% of the backyard population had the wing costal band over-lapping vien R_{2+3}, while in the secondary forest samples none of the specimens had the costal hand overlapping vein R_{2+3}.

These observations suggest that colour the pattern of *B. philippinensis* depends on the host. It is quite reasonable to assume that the specimens from the backyard population emerged from cultivated commercial host fruits (these were dark in colour). Similarly the secondary forest population probably emerged from more wild hosts (these were lighter in colour). So it can be suggested that the body colouration depend upon the host.

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