AUSTRALIAN JOURNAL of ENTOMOLOGY

Australian Journal of Entomology (2011) **50**, 86–92

Review of the planthopper genus *Zophiuma* Fennah (Hemiptera: Fulgoromorpha: Lophopidae) with first description of the male of *Zophiuma pupillata* Stål

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Abstract An examination of the five described species of the New Guinean genus *Zophiuma* Fennah (Hemiptera: Fulgoromorpha: Lophopidae), has confirmed that *Zophiuma guineae* (Lallemand) is a new synonym of *Zophiuma pupillata* (Stål) and that *Zophiuma lobulata* Ghauri is a new synonym of *Zophiuma butawengi* (Heller). The third species of the genus, *Zophiuma doreyensis* Distant, is known only from the male holotype. The morphology of the male genitalia and mitochondrial cytochrome oxidase subunit I (COI) gene sequences were used to compare *Z. pupillata* and *Z. butawengi*. Both the male genitalia and the COI sequences showed clear cut differences between the two species with little intraspecific variation in comparison to interspecific variation. Sequence data demonstrated that males collected with the distinctively coloured *Z. pupillata* females are the males of that species. Male genitalia of *Z. pupillata* are described and illustrated for the first time and a key for the discrimination of the three species of the genus is provided.

Key words Fulgoroidea, morphology, nomenclature, taxonomy.

INTRODUCTION

The planthopper genus *Zophiuma* Fennah, 1955, was one of three genera recognised by Fennah (1955) when he split the genus *Kasserota* Distant, 1906, (Hemiptera: Fulgoromorpha: Lophopidae), the others being *Kasserota* Distant *sensu stricto* and *Onycta* Fennah, 1955. Fennah (1955) placed *Acarna pupillata* Stål, 1863, and *Kasserota doreyensis* Distant, 1906, in *Zophiuma* and designated the former as the type species of the genus. Ghauri (1967) added a third species, *Zophiuma lobulata* Ghauri, and provided a key for the separation of all three species as well as illustrating the male genitalia of *Z. lobulata* and *Zophiuma doreyensis*. He differentiated *Zophiuma pupillata* from the other two species on the basis that the known specimens (all females) were dark red in colour while the other species are smaller and brown in colour.

These species are all native to West Papua (Indonesia) and Papua New Guinea (PNG) where *Z. lobulata* is associated with Finschhafen Disorder (FD) in coconut *Cocos nucifera* L. and oil palm *Elaeis guineensis* Jacq. (Smith 1980a,b; Prior *et al*. 2001) in PNG. Betel nut, *Areca catechu* (L.), is also recorded as a host plant for *Z. lobulata* (Prior *et al*. 2001).

Lallemand (1962) described a new genus *Hellerides* Lallemand, 1962, in the family Fulgoridae to contain the single New Guinean species *Hellerides guineae* Lallemand, 1962, and, 4 years later, Heller (1966) added a second species, *Hellerides butawengi* Heller, 1966. Both species of *Hellerides* were based on single female holotypes from PNG. Liang (1995) transferred *Hellerides* to the Lophopidae and synonymised it with *Zophiuma*. Liang (1995) suggested that possible synonymies of the two transferred species with the other described species of *Zophiuma* might be revealed with further study.

In the course of a study on FD of oil palms, we have collected specimens of *Zophiuma* extensively on palms in West New Britain and neighbouring areas of mainland PNG (see Fig. 1) and this has given us the opportunity to review the species in the genus.

MATERIALS AND METHODS

Morphological techniques

Field collected specimens were collected into propylene glycol for transport to Australia. They were then washed in 70% ethanol to remove propylene glycol. The abdomens of all male specimens were removed and boiled for 5 min in 10% potassium hydroxide (KOH). Once cleared, each abdomen was washed in water to remove KOH residues. Muscles and soft connective tissue surrounding the genitalia were removed and the genitalia transferred to glycerine for examination. After examination, the abdomen was placed in glycerine in a

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doi:10.1111/j.1440-6055.2010.00791.x

Fig. 1. Map of Papua New Guinea showing sites where *Zophiuma butawengi* and *Zophiuma pupillata* were collected.

small plastic vial attached to the pin of the specimen from which the abdomen had been removed.

Abbreviations for institutions used in this paper are: ASCU, Agricultural Scientific Collections Unit, Industry & Investment NSW, Orange, NSW; BMNH, The Natural History Museum, London, UK; SMNS, Staatlichen Museums für Naturkunde, Stuttgart, Germany.

Molecular techniques – COI sequence analysis

The left hind leg of each planthopper was removed for DNA extraction. DNA extraction was conducted using a Corbett Robotics CAS-1820 robotic DNA platform (Corbett Robotics, Mortlake, NSW) and the manufacturer's recommended DNA extraction kit (Sigma-Aldrich, Castle Hill, NSW). PCR was carried out in a total volume of 15 μL. The reaction mixture contained 1 μL of genomic DNA, 1X PCR buffer (20 mmol/L Tris-HCL, pH 8.4; 50 mmol/L KCL), 3 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs (0.3 μL of 10 mmol/L) (0.3 μL of 5 μmol/L), 0.375 units of Platinum Taq DNA polymerase (all reagents supplied by Invitrogen, Mount Waverly, Australia) and 1.5 pmol of each primer. The primers used were BC1Fm and BC3Rm (Cho *et al*. 2008). PCR was carried out on an Eppendorf eps thermocycler under the following conditions: 94°C for 2 min, 40 cycles of (94°C for 30 s, 52°C for 30 s, 72°C for 60 s) and 72°C for 7 min. PCR products were directly sequenced in both directions using the ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit and an ABI 3730xl Genetic Analyzer (Applied Biosystems).

Sequence trace files were assembled using Staden v.1.7.0 (Bonfield *et al*. 1995). Consensus sequences were exported to BioEdit v.7.0.9 (Hall 1999) and aligned by eye, and then imported into MEGA 4 (Tamura *et al*. 2007). MEGA was used to calculate uncorrected 'p' distances within and between species and a neighbour-joining (NJ) analysis, with 500 bootstrap replicates (Felsenstein 1985), was performed using the Kimura two-parameter distance. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. In order to conduct a parsimony analysis we constructed a second data set that consisted of unique haplotypes only. This data set was made by trimming the ragged ends of the alignment so that all sequences were of equal length with no missing data and using the online software FABOX (Villesen 2007) to reduce the alignment to unique haplotypes only. This haplotype data set was analysed in MEGA 4 under the maximum parsimony criterion by implementing the Max-mini branch and bound search option (Mitchell & Maddox 2010).

RESULTS

Morphology examination

In the material collected from all sites, there were two different males based on male genitalia. One matched the illustrations of *Z. lobulata* provided by Ghauri (1967). The other matched neither *Z. lobulata* nor *Z. doreyensis* based on Ghauri's (1967) illustrations. As the males of this latter species were collected in association with the distinctively coloured females of *Z. pupillata*, they were presumed to be males of that species, even though they lacked the red coloration used by Ghauri (1967) to distinguish *Z. pupillata* from other species of the genus. Supporting this proposal is the fact that these males were much larger than those of other described species and they also bear short transverse black banding on the basal half of the tegmen, a feature also found in females of *Z. pupillata* but not in other species.

An examination of the female holotypes of the two species originally described in the genus *Hellerides* Lallemand has shown that *Zophiuma guineae* is synonymous with *Z. pupillata* based on the red colour of the body and tegmina and the presence of short transverse black bands on the basal half of the tegmen and on the distinctive shape of the anal segment. Similarly, *Z. butawengi* and *Z. lobulata* show no differences in size, coloration, head shape and morphology of the anal segment which was also illustrated for *Z. lobulata* by Ghauri (1967).

Below, we provide notes on the three species and a key that allows them to be distinguished from each other.

COI sequence analysis

Zophiuma COI sequences group into two major clusters, corresponding with the two species recognised by male genitalia morphology. The maximum p-distance within *Z. butawengi* is 0.7% and 4.3% within *Z. pupillata*. The minimum p-distance between the two species is 14.8%. There were nine unique

haplotypes of 507 nt each in the haplotype data set and 93 parsimony-informative sites. Parsimony analysis yielded two most parsimonious (MP) trees with a length of 105 steps, consistency index (for parsimony-informative characters) of 0.92 and a retention index of 0.95. One of the MP trees recovered identical relationships to the NJ tree and the other differed only in the relationship among the three haplotypes of *Z. butawengi*.

More importantly, the larger brown coloured males collected with the large red females of *Z. pupillata* are shown to group with those females and this confirms the hypothesis that these males are *Z. pupillata.* The COI sequence data showed some variation within *Z. pupillata* which corresponded with the origins of the specimens (Fig. 2).

Attempts at PCR from the female holotypes of *Z. guineae* and *Z. butawengi* were not successful, probably because of the age of the specimens (>40 years and >100 years). It is likely that DNA extracted was degraded, explaining failure of PCR with external primers. It was therefore not possible to test the proposed synonymies using COI sequence data.

Taxonomy

Genus *Zophiuma* Fennah

Zophiuma Fennah 1955: 170; type species: *Acarna pupillata* Stål by original designation.
Hellerides Lallemand 1962: 1; type species: *Hellerides guineae* Lallemand, by original designation, synonymised by Liang 1995: 163.
Notes. The genus was described in detail by Fennah (1955) who also provided three views of the head of *Z. pupillata*. It was characterised in a key by Soulier-Perkins (1998) with the following features: apical spines of first hind tarsal segment forming a triangular zone; ocellar carinae present; tegmina with at least 80% of their surface coloured; costal vein and costal margin distinct from each other; labium long, extending beyond hind trochanters; tegmina with apical white eyespot confined by dark rings. Soulier-Perkins (1998) also provided a dorsal *habitus* illustration of *Z. pupillata*.

Zophiuma pupillata (Stål) (Figs 3–9)

Acarna pupillata Stål 1863: 586
Kasserota pupillata (Stål), Distant 1906: 350
Zophiuma pupillata (Stål), Fennah 1955: 171
Hellerides guineae Lallemand 1962: 3, **New Synonym**
Types. Holotype, female, Dory (*sic.*) New Guinea (BMNH).
Material examined. 1 male, 1 female, 1 nymph, PNG, Madang province, Keki, 4°41′40″S 145°25′05″E, 5.vii.2006, C.F. Dewhurst; 3 males, 8 females, 1 nymph, PNG, Madang province, Omoru coconut nursery, 5°18′38″S 145°42′04″E, 5.xi.2008, C.F. Dewhurst (ASCU); 1 female, holotype of *H. guineae*, New Guinea, leg. Ludeking 1867 (SMNS).

Description

Length. Males ($n = 4$) 17.5–20.0 mm (mean = 18.9), females ($n = 10$) 21.5–24.0 mm (mean = 22.7).

Coloration. Male (Fig. 3), brown, paler on the head. Tegmen with clavus and apical half dark brown, basal half translucent pale brown with short, irregular, transverse black bands, longitudinal veins reddish. Corium beyond claval apex dark brown with two pale brown patches on costal margin and apex smoky brown and bearing circular 'eyespot', round black spot with white dot near dorsal margin of spot. Female (Figs 4,5), head, thorax, legs and abdomen dark red, eyes black. Tegmen red with very dark clavus and apical third; basal corium with short black transverse bands; apex tending to pale brown with black eyespot with white dot near dorsal margin of spot.
Male genitalia (Figs 6,7). Pygofer short, hind margin sinuate and lined with short hairs. Pygofer processes absent. Anal segment long with apical half deeper than basal half, ventral margin of apical half, in lateral view, roundly excavate, apex with a series of short hairs. Subgenital plates ovate, dorsal margin obliquely divided near apex, inner lobe transverse with short hook at midlength, outer lobe roundly triangular. Aedeagus (Fig. 8) complex, with apical bifurcate process on each side, dorsal margin near apex with two short spinose processes and ventral surface with single median spine extending towards base of aedeagus and bearing line of short spicules.
Female genitalia. Anal segment large, apically extending ventrally on either side of ovipositor, terminated with two flat plates flanking ovipositor, each kidney-shaped in outline when viewed ventrally (Fig. 9).
Notes. This is the first description of the male of this species. Stål (1863) stated that the type specimen in BMNH was a male but neither Fennah (1955) nor Ghauri (1967), both of whom worked at BMNH, made mention of male genitalia. A check of the original specimen has revealed that Stål (1863) was in error and the specimen is a female (M.D. Webb pers. comm. 2009). Ghauri's (1967) differentiation of this species from other described species of the genus was on the basis of colour but this only applies to the female. The male is basically brown as in the males and females of other described species although both males and females have dark transverse speckling on the basal half of the tegmen, a feature not found in the other two known species. This species is also considerably larger than either *Z. butawengi* or *Z. doreyensis.* The synonymy of *H. guineae* with *Z. pupillata* is made on the basis that the holotype female (Fig. 5) of the former matches females of *Z. pupillata* in their distinctive size and coloration and in the shape of the anal segment, particularly the kidney-shaped apical plates (Fig. 9).

Zophiuma butawengi (Heller) (Figs 10–14)

Hellerides butawengi Heller 1966
Zophiuma butawengi (Heller), Liang 1995: 163
Zophiuma lobulata Ghauri 1967: 557, **New Synonym**
Types. 1 female, holotype of *H. butawengi* Heller (examined), Butaweng, 8.x.1965, H. Pyka leg. (SMNS), 1 male, holotype of *Z. lobulata* Ghauri (not examined), on *Cocos nucifera*, Saki's plantation, Nisingtalatu Village, near Finschhafen, Morobe District, New Guinea, 20.i.1966, T.L. Fenner (BMNH).
Other material examined. 180 specimens, male and female, from the following localities in New Guinea. **West New**

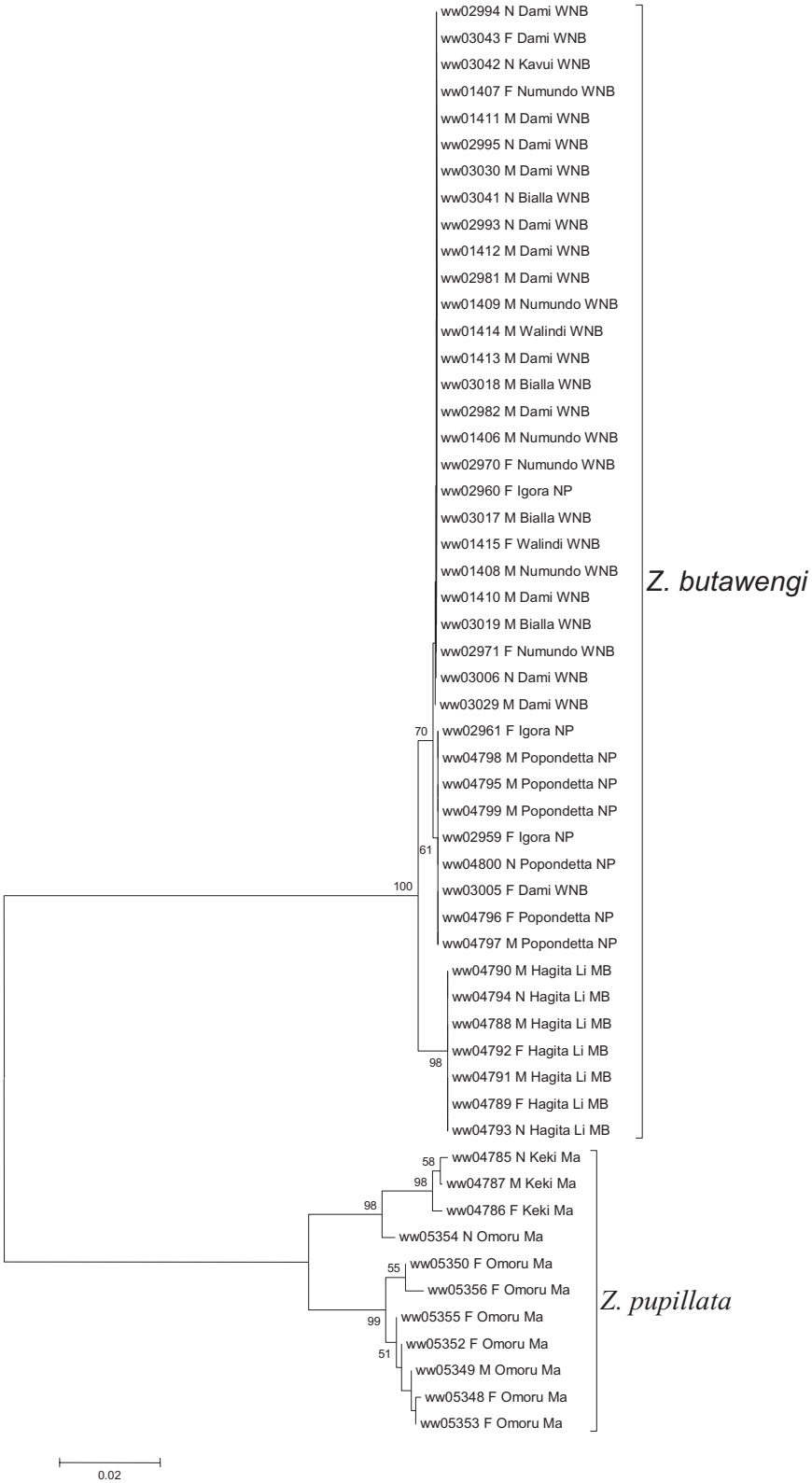


Fig. 2. Neighbour-joining tree showing distinct clustering of *Zophiuma butawengi* and *Zophiuma pupillata.* Numbers to the left of nodes are bootstrap percentages. *Z. butawengi* were collected from both mainland and West New Britain province of PNG. *Z. pupillata* were collected only from Madang Province in mainland PNG. Names of localities from which *Zophiuma* were collected are followed by an abbreviation of the province. F, female; M, male; Ma, Madang; MB, Milne Bay; N, nymph; NP, Northern Province; WNB, West New Britain.

Britain: Hoskins 5°27′S 150°24′E; Dami 5°32′S 150°20′E; Kimbe 5°33′S 150°09′E; Numundo 5°31′S 150°05′E; Walindi 5°26′S 150°05′E; Kavui 5°35′S 150°18′E; Biala 5°18′28″S 150°59′58″E; **Papua New Guinea mainland**: Popondetta (Igora Plantation, Joroba Plantation) 8°46′S 148°14′E; Hagita Li, Milne Bay, 10°19′S 150°19′E.

Known hosts. Oil palm, *Elaeis guineensis* Jacq.; coconut palm, *Cocos nucifera* and betel nut, *Areca catechu* Linnaeus (all Arecaceae) (Ghauri 1967).

Notes. Both Heller (1966) and Ghauri (1967) gave comprehensive descriptions of this species. Heller (1966) illustrated the dorsum, face, tegmen and hindwing and three views of

the female terminalia. Ghauri (1967) illustrated the dorsum, lateral and facial views of the head, tegmen, hindwing, male genitalia, ventral and lateral view of the female terminalia, bursa copulatrix and spermatheca. Ghauri (1967) also gave a number of size measurements for the species including length of body as 15.75–16.50 mm for males and 17.50–18.00 mm for females. Our length measurements of the species (from apex of head to tip of wing) are males ($n = 10$) 14.0–15.5 mm (mean, 14.9), females ($n = 10$) 15.5–17.0 mm (mean, 16.5).

Figs 3–5. *Zophiuma pupillata.* (**3**) Male habitus; (**4**) female habitus; (**5**) holotype of *Hellerides guineae*.

Zophiuma butawengi and *Z. lobulata* are synonymised on the basis that the female type of the former (Fig. 12) matches precisely with females of the latter, particularly the markings on the tegmen, shape of the head and the shape of the female anal segment (Fig. 10).

To determine priority, the publication date of both Heller (1966) and Ghauri (1967) needed to be determined. Reprints of both papers are dated 1966: Heller's is dated '15 December 1966' and Ghauri's is dated 'September to December 1966'. An enquiry to The Natural History Museum in London to determine if the original volume in which Ghauri published his paper bore a date of publication led to the discovery that this volume was not published until March 1967 (M.D. Webb pers. comm. 2009). This means that *Z. lobulata*, believed to have been published in 1966 was not, in fact, published until 1967 and *Z. butawengi* therefore has priority.

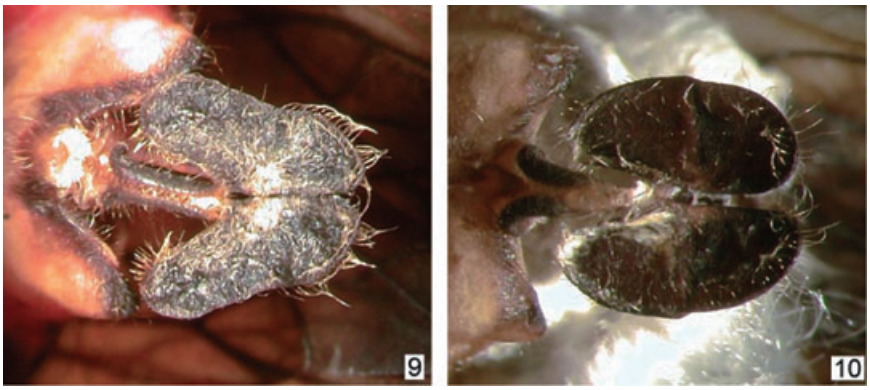
Zophiuma doreyensis (Distant)

Kasserota doreyensis Distant 1906: 350
Zophiuma doreyensis (Distant), Fennah 1955: 171

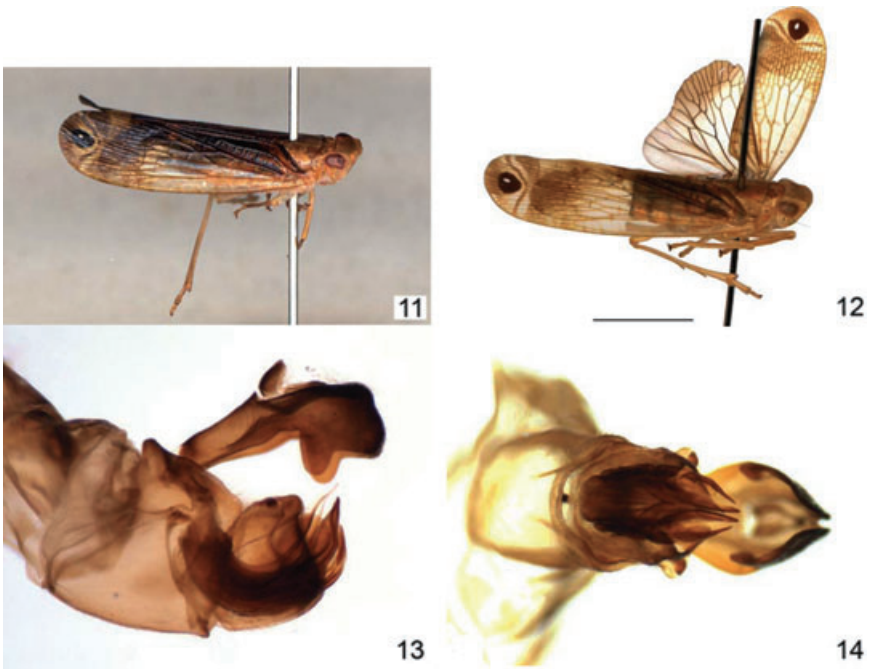
Description (from Distant 1906)

> Body and legs brownish ochraceous; abdomen above with the posterior segmental margins fuscous; vertex of head, face, clypeus, femora, and apex of mesonotum paler or more ochraceous; tegmina pale ochraceous, with the venation brown, posterior basal half and apical third umber-brown, the latter with two greyish-white spots at costal margin and a similar spot near apex of hind margin, and before apex a black spot with a white eye and an ochraceous margin; wings very pale fuliginous, the venation and apical area fuscous; face with the lateral carinae very convex, broadly rounded and united anteriorly, angles behind eyes strongly acutely pro-

Figs 6–8. *Zophiuma pupillata*, male. (**6**) Terminalia, lateral view; (**7**) terminalia, ventral view; (**8**) aedeagus, lateral view.



Figs 9,10. Female, anal segment, ventral view. (**9**) *Zophiuma pupillata*. (**10**) *Zophiuma butawengi.*



Figs 11–14. *Zophiuma butawengi.* (**11**) Male habitus; (**12**) holotype habitus; (**13**) male terminalia, lateral view; (**14**) male terminalia, ventral view.

duced; pronotum distinctly tricarinate; mesonotum with a central double carination, not extending beyond anterior half, the lateral carinations united anteriorly. Long., excl. tegm., 13½ mm.; exp. tegm. 36 mm.

Types. Holotype, male (not examined), Dorey, Wallace (BMNH).

Notes. Distant (1906) did not indicate how many specimens he had available but Ghauri (1967) notes that 'the only specimen of *Z. doreyensis* (Distant) is the holotype male'. Ghauri (1967) provided a comprehensive redescription of this species which he differentiated from *Z. butawengi* (as *Z. lobulata*) on the structure of the male anal segment, this species lacking the anterolateral lobe characterising *Z. butawengi*. From *Z. pupillata* males, it can be separated on the basis of size, as well as the structure of the anal lobe. Males of all species are clearly differentiated by the complex structure of the aedeagus.

The precise location of Dorey was problematic until reference to Wallace's original paper presented to the Royal Geographical Society of London in 1859 (Wallace 1860) gave the location on the northwest coast of West Papua, the New Guinean province of Indonesia. The village of Dorey, where Wallace spent 3 months, is now called Manokwari and is located at 0°51′35″S 134°04′38″E. It is interesting to note that the holotype of *A. pupillata* was presumably also collected at Dorey, although Stål (1863) spelt it 'Dory', and it may well therefore also be a Wallace specimen.

The following key to males of the known species of *Zophiuma* is modified from Ghauri (1967).

1. Tegmen red (females) or brown (males) with short, transverse black bands on basal half (Figs 3,4)……
……*Z. pupillata*
Tegmen brown, without black bands on basal half (Figs 11,12)……2
2. Postero-ventral margin of male anal segment produced as a lobe (Fig. 13)……*Z. butawengi*
Postero-ventral margin of male anal segment entire, broadly rounded (Fig. 15)……*Z. doreyensis*

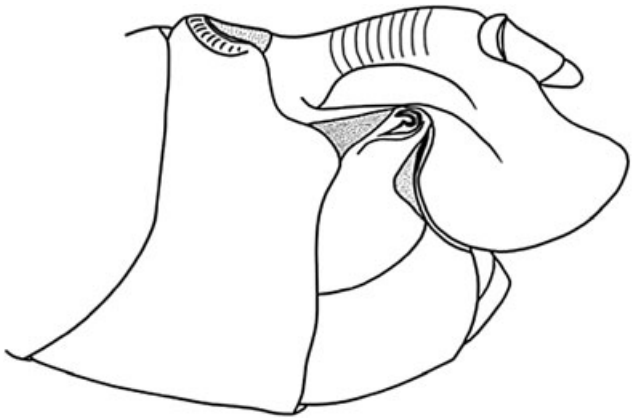


Fig. 15. *Zophiuma doreyensis.* Male terminalia, lateral view (after Ghauri 1967).

DISCUSSION

This paper returns the genus *Zophiuma* back to three species after the addition of the two species from the genus *Hellerides* by Liang (1995). The best known species is associated with FD on coconut and oil palms (Smith 1980a; Gitau *et al*. 2009) and it is unfortunate that the rules of priority require that this species has a change of name from *Z. lobulata* to *Z. butawengi*. All references to *Z. lobulata* previously published actually apply to *Z. butawengi*. All three species are restricted to New Guinea with *Z. doreyensis* still only known from a single male specimen from West Papua. Further collecting in the western areas of New Guinea and in the islands of Indonesia east of the Wallace Line may reveal other species in this interesting genus.

Results from COI gene sequences demonstrate that examination of the male genitalia provides a sufficient method for differentiating the species. There is no basis for suspecting cryptic species within the morphologically defined species of *Z. butawengi* although some intraspecific variation in COI that contrasts with morphological homogeneity appears to exist in *Z. pupillata*. COI also allows otherwise unidentifiable females and nymphs to be linked to males in groups like planthoppers in which the morphological taxonomy is based on male genitalia. As the technology improves, obtaining sequences from older specimens will become more successful and allow definitive determinations of species that are based solely on female specimens.

ACKNOWLEDGEMENTS

We wish to thank Dr Wolfgang Schawaller (SMNS) for the loan of the type specimens of *H. guineae* and *H. butawengi* and for permission to remove a leg from each for possible DNA extraction. We also thank Mick Webb (BMNH) for determining the date of publication of Ghauri's description of *Z. lobulata* and for checking the sex of the holotype of *A. pupillata*. Special thanks go to Cecilia Lawler (ASCU) for tracing the New Guinean locality Dorey through Wallace's publications and to Vicki Glover (OAI Library) for finding Wallace's original publication. We also thank Holger Löcker (ASCU) for producing the Automontage® images and the entomology staff at PNGOPRA for help with field collections. This paper has been an outcome of a project on Finschhafen Disorder in Oil Palms funded by the Australian Centre for International Agricultural Research (ACIAR) grant CP/2006/063.

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Accepted for publication 5 October 2010.