

# Description of larvae of two species of *Coeliccia* Selys, 1865 from Sarawak, identified using DNA barcoding (Odonata: Platycnemididae)

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**Abstract.** The final stadium (F) larva of *Coeliccia flavostriata* Laidlaw, 1918, is described and illustrated based on a mature male specimen, collected at Gunung Serapi, Sarawak, East Malaysia. The larva of *Coeliccia campioni* Laidlaw, 1918, is described from an immature (F-2?) female specimen from Gunung Mulu, Sarawak, East Malaysia. Larvae were identified by matching the mitochondrial marker COI with that of known adult specimens from several localities throughout Sarawak. The specimens presented close matches with all adults in this gene. Despite the disparity in maturity of the specimens several morphological differences, likely to be reflected in the mature larva of *C. campioni*, are identified. Comparisons with known larval descriptions of other *Coeliccia* species are provided. It is concluded that molecular analysis will eventually provide the most reliable practical method of determining the species of larvae of many species from this diverse genus.

**Further key words.** Dragonfly, damselfly, Zygoptera, *Coeliccia campioni*, *C. flavostriata*, larval taxonomy, COI sequencing, Borneo.

## Introduction

The genus *Coeliccia* Kirby, 1890 includes 62 species occurring in tropical and subtropical Asia east of Wallace's Line (SCHORR & PAULSON 2015). Currently ten described species are known from Borneo (LIEFTINCK 1954; ORR 2003; DOW 2010; DOW & REELS 2011) although others await description and more probably remain undiscovered. Known species fall into two clear groups, with *C. arcuata* Lieftinck, 1940, *C. borneensis* (Selys 1886), *C. campioni* Laidlaw, 1918, *C. flavostriata* Laidlaw, 1918, *C. kenyah* Dow, 2010, and *C. southwelli* Dow & Reels, 2011 forming a well-defined assemblage,

the *borneensis*-group, well separated from the other members of the genus found in Borneo (Dow 2010).

Among other members of the genus larval descriptions with illustrations have previously been provided for *C. cyanomelas* Ris, 1912 and *C. flavicauda flavicauda* Ris, 1912 from Taiwan (MATSUKI & LIEN 1984); *C. flavicauda masakii* Asahina, 1951, *C. ryukyuensis ryukyuensis* Asahina, 1951 and *C. ryukyuensis amamii* Asahina, 1962 (ISHIDA 1996); and *C. mingxiensis* Xu, 2006 from China (XU 2013).

The present paper describes in detail the larva of *C. flavostriata* based on a fully developed F stadium male specimen from Gunung Serapi, Sarawak, and provides comparative information on *C. campioni* based on an immature, probably F-2 larvae, from Gunung Mulu, Sarawak. Both specimens were identified by matching the mitochondrial marker COI with that of known adult specimens from several localities throughout Sarawak, a technique previously shown to be effective with larvae of *Drepanosticta ?attala* Lieftinck, 1934 (ORR & DOW 2015a) and *Onychargia atrociana* (Selys, 1865) (ORR & DOW 2015b). These species are compared with previously described *Coeliccia* larvae and those of some other Platycnemididae. The potential usefulness of DNA barcoding in identification of larvae is discussed.

## Methods

### Site and collection of material

Larval specimens were collected by sieving of leaf litter and gravel from small forest streams on Gunung Serapi just outside of Kubah National Park near Kuching, Sarawak, East Malaysia, and another small forest stream on the lower slopes of Gunung Mulu in the national park of the same name, Sarawak, by the second author (RAD). Adult specimens from the *borneensis*-group for comparison were collected from various locations in Sarawak (see Table 2) by RAD and G.T. Reels. Larval specimens were killed and preserved in 96 % ethanol; a leg was removed from adult specimens and preserved in 96 % ethanol while the voucher specimen was treated with acetone and then dried.

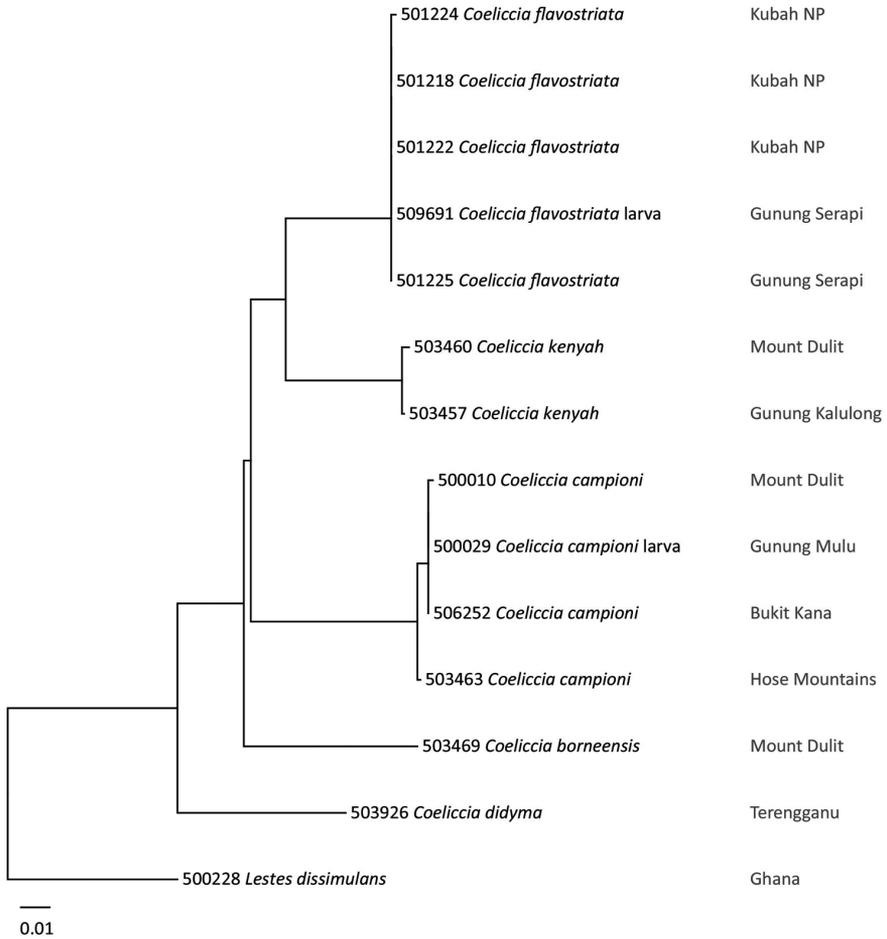
Voucher material is deposited in the collection of the Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH).

### Molecular analyses

Genomic DNA was extracted from legs using a NucleoMag 96 Tissue kit (Macherey-Nagel) on a KingFisher Flex magnetic particle processor (Thermo Scientific). A volume of 150  $\mu$ l was used for elution. Fragments of the mitochondrial COI gene (658 bp) were amplified using primer combinations provided in Table 1. Twenty-five microlitres of PCR reaction mix for COI contained 5  $\mu$ l of 5 $\times$  Phire II Reaction Buffer (Thermo Scientific), 1  $\mu$ l of each primer (10 pM), 0.5  $\mu$ l of Phire Hot Start II DNA Polymerase (Thermo Scientific), 0.5  $\mu$ l of dNTPs and 1  $\mu$ l of DNA template. The amplification protocol consisted of 30 sec at 98°C followed by 40 cycles of 5 s at 98°C, 5 s at 50°C and 15 s at 72°C, and a final 5 min at 72°C. Bi-directional Sanger sequencing was performed at Macrogen Europe and at BaseClear, Leiden, The Netherlands.

Sequences were edited with Sequencher 4.10.1 (Gene Codes Corporation). COI sequences were checked for stop-codons using the invertebrate mitochondrial genetic code in Geneious pro 6.1.8 (KEARSE et al. 2012). Association of larva with adult was based on similarity of COI sequences. Analysis for this publication was made using Geneious pro 6.1.8. Sequence data and additional geographic and ecological data for the specimens were uploaded to the Barcode of Life Data System (BOLD; RATNASINGHAM & HEBERT 2007). BOLD process IDs are given in Table 2.

For *C. campioni* the larva differs in just 0–3 base pairs from the adults, all of which are from separate populations from each other and from the larva. In the case of *C. flavostriata* the pairwise identity of the larva differs in 0–1 base pairs from the four adults, although in this case all are from what can be considered to be a single population in and just outside Kubah National Park. A neighbour joining COI gene tree (uncorrected p-distance) illustrating the relationship of larvae to adults of both species is shown in Figure 1; two other *Coelliccia borneensis*-group species are included and the non-*borneensis*-group species *C. didyama* (Selys, 1863) and *Lestes dissimulans* Fraser, 1955 are used as outgroups. All *C. borneensis*-group species for which COI data is available are clearly distinguishable from each other using this marker.



**Figure 1.** Neighbour joining COI gene tree using uncorrected p-distance for species of the *Coeliccia borneensis*-group, including both adults and larvae of *C. campioni* and *C. flavostriata*, and their sampling sites (except for *Lestes dissimulans* all from Sarawak, East Malaysia). *Coeliccia didyma* and *Lestes dissimulans* are used as out-groups.

All voucher specimens have a six digit collection number with an RMNH.INS. prefix; this prefix is omitted in the figure for clarity.

**Table 1.** Primer combinations used for amplification of COI.

Primer name	Target	Direction	Sequence (5' to 3')	Reference
ODO_LCO1490d	COI	F	TTTCTACWAACCAAYAAAGATATTGG	DIJKSTRA et al. (2014)
ODO_HCO2198d	COI	R	TAAACTTCWGGRTGTCCAAARAATCA	DIJKSTRA et al. (2014)
LepF1	COI	F	ATTCAACCAATCATAAAGATATTGG	HEBERT et al. (2004)
LepR1	COI	R	TAAACTTCTGGATGTCCAAAAAATCA	HEBERT et al. (2004)
LCO1490	COI	F	GGTCAACAAATCATAAAGATATTGG	FOLMER et al. (1994)
HCO2198	COI	R	TAAACTTCAGGGTGACCAAAAAATCA	FOLMER et al. (1994)

## Description of larvae

### *Coeliccia flavostriata* Laidlaw, 1918 (Figs 2; 3a, b; 4a)

**Material studied.** One F larva, ♂ (RMNH.INS.509691), stream on Gunung Serapi, just outside of Kubah National Park, Kuching Division, Sarawak, Borneo, 1.58943N, 110.19487E. 01-vii-2013, ca 650 m a.s.l., leg. R.A. Dow, in RMNH.

**Description.** In general appearance a small, moderately long-legged zygopteran of light build and typical coenagrionoid appearance (Fig. 2). Post-ocular lobes of head smoothly rounded. Caudal lamellae paddle shaped, with distinct petiole, the central lamella being considerably longer than the laterals (Fig. 4). Coloration overall neutral brown, with darker brown banding on legs and deeper purplish mottling on the caudal lamellae. Body surface relatively smooth, bearing only sparse, very fine setae.

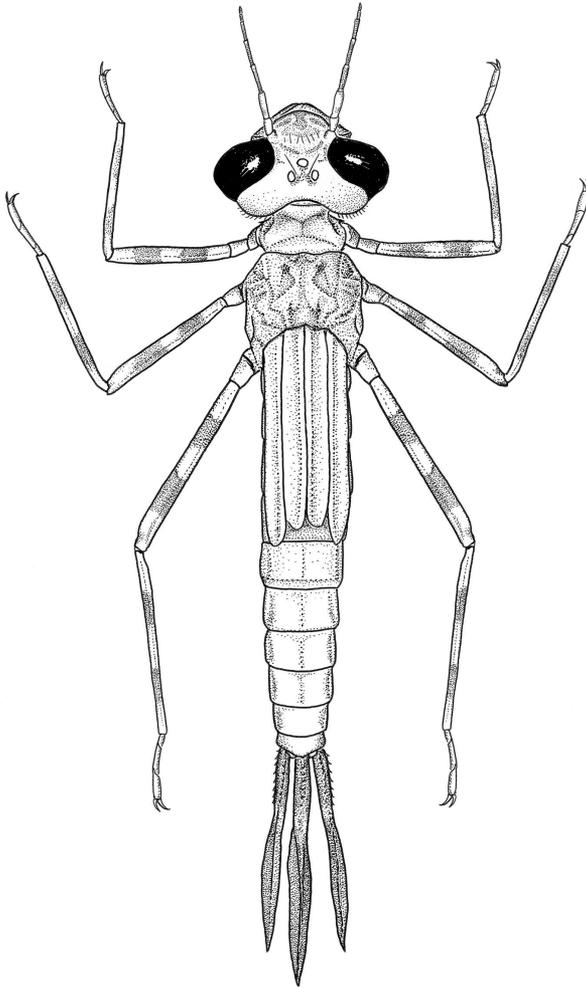
Head in dorsal view (Fig. 2), forming roughly a squashed pentagon in outline, with well rounded occipital lobes and a deep rounded excavation posteriorly. Labrum produced and rounded. In dorsal view labial palps and anterior corners of prementum clearly visible projecting beyond labrum and face. Occipital triangle only slightly raised. Antennae long, seven-segmented, final segment very short; segments 2–6 slightly darkened except at base and apex, but not so strongly as to produce a clear pattern. Compound eyes moderately large. Mandibles not evident in dorsal view. Articulation

**Table 2.** Locations where voucher specimens were collected and BOLD Process IDs for COI sequences used in the analysis. Sequences for *Coelliccia flavostriata* (RMNH.INS.501225), *C. borneensis* (RMNH.INS.503469), *C. didyama* (RMNH.INS.503926), and *Lestes dissimulans* (RMNH.INS.500228) were previously published in DIJKSTRA et al. (2014).

Species	RMNH.INS.	Country	Location	BOLD Process ID
<i>Coelliccia campioni</i>	503463	Malaysia	Hose Mountains, Kapit division, Sarawak	COBOR001-15
<i>Coelliccia campioni</i>	506252	Malaysia	Bukit Kana, Bintulu division, Sarawak	COBOR002-15
<i>Coelliccia campioni</i>	500010	Malaysia	Mount Dulit, Miri division, Sarawak	COBOR003-15
<i>Coelliccia campioni</i>	500029	Malaysia	Gunung Mulu National Park, Miri division	COBOR004-15
<i>Coelliccia flavostriata</i>	501225	Malaysia	Gunung Serapi, Kuching division, Sarawak	ODOPH251-13
<i>Coelliccia flavostriata</i>	509691	Malaysia	Gunung Serapi, Kuching division, Sarawak	COBOR005-15
<i>Coelliccia flavostriata</i>	501222	Malaysia	Kubah National Park, Kuching division, Sarawak	COBOR006-15
<i>Coelliccia flavostriata</i>	501218	Malaysia	Kubah National Park, Kuching division, Sarawak	COBOR007-15
<i>Coelliccia flavostriata</i>	501224	Malaysia	Kubah National Park, Kuching division, Sarawak	COBOR008-15
<i>Coelliccia kenyah</i>	503460	Malaysia	Mount Dulit, Miri division, Sarawak	COBOR009-15
<i>Coelliccia kenyah</i>	503457	Malaysia	Gunung Kalulong, Miri division, Sarawak	COBOR010-15
<i>Coelliccia borneensis</i>	503469	Malaysia	Mount Dulit, Miri division, Sarawak	ODOPH246-13
<i>Coelliccia didyama</i>	503926	Malaysia	Sekayu Recreational Forest, Terengganu	ODOPH249-13
<i>Lestes dissimulans</i>	500228	Ghana		ODOPH201-13

lation of mask almost reaching posterior margin of mesocoxae when retracted. Prementum (Fig. 3a) flat ventrally, only slightly concave dorsally, with three pairs of long, strong setae seated post medially; in outline broad distally and tapered to a narrow base about one quarter its maximum distal

breadth; median lobe (ligula) strongly produced with the two slightly convex sides forming an angle of about  $100^\circ$ ; median cleft absent; lateral margins bearing short strong setae in distal half, bare in basal half. Labial palp long, terminating in a strong ventral curved hook and a dorsal, straight, slightly serrate securiform edge (Fig. 3c), the two separated by a deep cleft;



**Figure 2.** *Coeliccia flavostriata*, larva habitus, also showing form of head, in dorsal view. Drawing by AGO

four long, strong, inwardly directed palpal setae and a rather short movable hook, seated well back from its apex. Mandibles: right mandible incisor with four long, well defined teeth and shorter basal one ventrally; molar field unarmed except for small basal prominence; left incisor similar but left molar field with short, prominent, blunt tooth. Legs, moderately long; femora and tibiae with distinct dark marking and bearing only very fine sparse setae. Wing pads reaching just beyond hind-margin of S4; unmarked. Abdomen unmarked, smooth, gradually tapered posteriorly. Caudal lamellae clearly dorso-ventrally flattened (arranged vertically), foliate and rounded apically with extensive dark basal marking, mottled around the periphery; median lamella slightly more than half length of abdomen; lateral lamellae (Fig. 4b) ca  $\frac{1}{10}$  length of median lamella; petiole with outer lateral, as well as dorsal and ventral spines. Cerci small and bud-like. Male gonapophyses well developed; extending a little beyond posterior margin of S9.

Measurements [mm]: Total length (without caudal lamellae) 12.45; maximum width of head 3.68; hind femur 3.70; cercus 0.15; right lamella 4.13; median lamella 3.68.

Remark. The larva was found in a small pack of leaf litter between rocks in the stream bed; adult *C. flavostriata* were moderately abundant at the same site.

### ***Coellicia campioni* Laidlaw, 1918**

(Figs 3c, d; 4b)

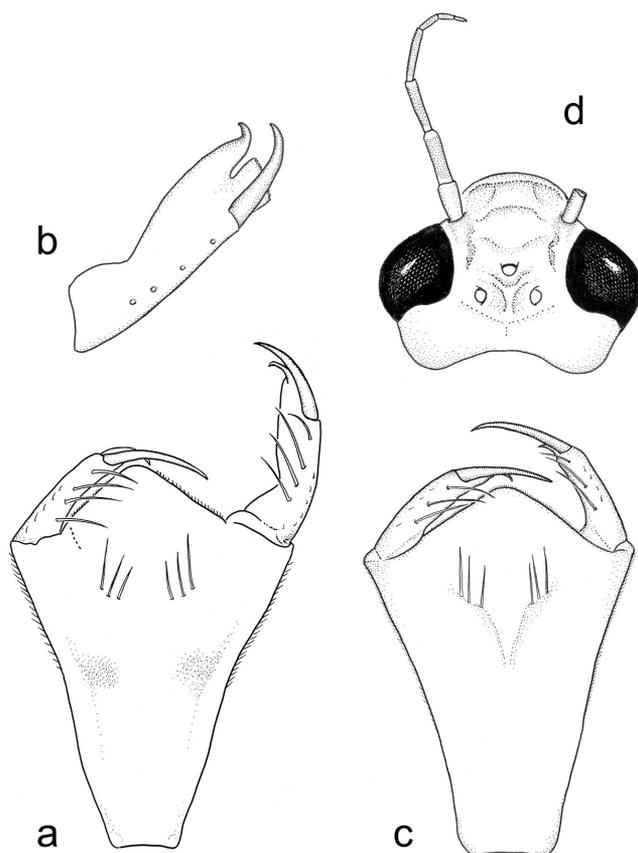
**Material studied.** One F-2? larva, ♀ (RMNH.INS.500029), “Camp 2” stream, lower slopes of Gunung Mulu, Gunung Mulu National Park, Miri Division, Sarawak, Borneo, 4.04751N 114.86622E. 12-ix-2008, ca 500 m a.s.l., leg R.A. Dow, in RMNH.

**Description.** In general appearance a small, moderately long-legged zygopteran of light build and typical coenagrionoid appearance, a little more squat with proportionally longer legs than the larva of *C. flavostriata* (Fig. 2). Marked as *C. flavostriata*. Otherwise very similar to that species except in points noted below.

Head (Fig. 3d) rather robustly built, in dorsal profile relatively long, with rounded occipital lobes widely separated. Labrum rounded. Mandibles not visible in dorsal view but labial palps and anterior corners of prementum protruding well beyond labrum and face so as to be visible in dorsal view. Ocellar triangle prominently raised, but not excessively so. Antennae long, seven-segmented, final segment very short; segments 2–6 very slightly darkened except at base and apex, without any clear pattern. Articulation of mask reaching almost to hind margin of mesocoxae when retracted. Prementum with three pairs of long, strong setae seated post medially. In outline (Fig. 3c) broad distally and tapered to a narrow base a little less than one third its maximum distal breadth; median lobe (ligula) strongly produced to form an obtuse angle, the two slightly convex sides forming an angle of about 125°; median cleft absent; lateral margins of prementum bearing short stout setae in distal half, bare in basal half; Labial palp long, bearing just three long, strong, inwardly directed setae. Mandibles: right mandible incisor with four long, well defined teeth and shorter basal one ventrally; molar field unarmed except for small basal prominence; left incisor similar but left molar field with short, prominent, blunt tooth. Legs moderately long; femora and tibiae with distinct dark marking and bearing only very fine sparse setae. Abdomen tapered smoothly and gradually; outer wing pads reaching a little beyond hind margin of S2, inner wing pads terminating well before hind margin S2. Caudal lamellae (Fig. 4b) dorso-ventrally flattened (arranged vertically); strongly petiolated with posterior lamina foliate with acuminate tip; basal half of lamina strongly pigmented dark brown; median lamella ca  $\frac{7}{10}$  length of abdomen; lateral lamellae, ca  $\frac{4}{5}$  length of median lamella and with much more developed terminal filament; petiole fringed with fine setae, lacking spines. Cerci small and bud-like. Female gonapophyses well developed; outer pair extending about halfway to posterior margin of S10; inner pair distinctly shorter.

Measurements (mm): Total length (without caudal lamellae) 7.20; maximum width of head 2.10; hind femur 2.85; cercus 0.10; right lamella 3.00; median lamella 3.50.

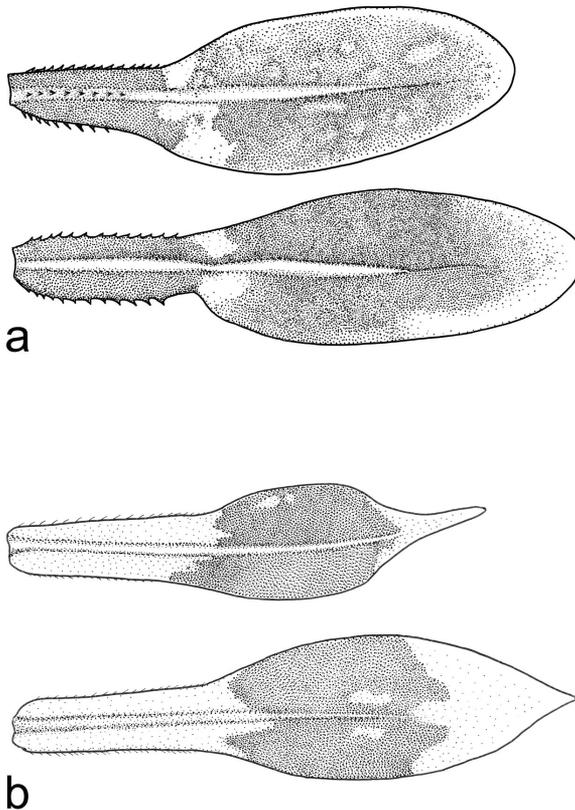
Remarks. Because the specimen is not a final instar some characters may not correspond to the condition found in the F larvae. These include in particular the development of the gonapophyses as well as the overall stature of the larva, which is slightly squatter than the F stadium of *C. flavostriata*. It seems probable the heavier build of the head, and the different shape of the mask, would also occur in the mature larva of *C. campioni* as would the strikingly differently shaped caudal lamellae. It is unclear if the smaller number of palpal setae would also be present in the F stadium larva.



**Figure 3.** a, b – *Coeliccia flavostriata*: a – overview of mask; b – lateral detail of labial palp (setae not removed); c, d – *C. campioni*: c – overview of mask; d – dorsal view of head. Drawing by AGO

The larva was found in a small pack of leaf litter in the bed of a small, high gradient stream. Adult *C. campioni* have been found at the same location as the larva.

**Differential diagnosis.** In *C. campioni* the head (Fig. 3d) is more robustly built than in *C. flavostriata*, in dorsal profile distinctly longer, with rounded occipital lobes more widely separated, making the whole head wider posteriorly and overall more robust. Ocellar triangle more prominently raised in *C. campioni* than *C. flavostriata*. Prementum broader basally and ligula less



**Figure 4.** Lateral view of caudal lamellae (right above, median below): a – *Coeliccia flavostriata*; b – *C. campioni*. Drawing by AGO

produced in *C. campioni* than in *C. flavostriata*. Caudal lamellae strongly acuminate in *C. campioni*, spatulate in *C. flavostriata*.

### Discussion

The two species described here, *Coelliccia campioni* and *C. flavostriata*, exhibit clear morphological differences despite their relatively close relationship. The exact range of these differences is unclear due to the differing stages of development of the two specimens, as well as the small sample size. Nevertheless it is almost certain that in *C. campioni* the head is much more robust with commensurate differences in the form of the prementum (less narrow basally with anterior margin less acute). The reduction in the number of long setae on the labial palps of this species may also be diagnostic. The form of the caudal appendages differs significantly, with those of *C. campioni* acute posteriorly, especially the lateral appendages which bear a terminal filament, whereas those of *C. flavostriata* are rounded and spatulate. This character seems to show some intra-specific variation in other members of the genus; for example, ISHIDA (1996) illustrates the lamellae of two specimens of *C. flavicauda*, one in which the tips are rounded, the other in which the tips are acute, but not with a terminal filament. However, as the differences between *C. campioni* and *C. flavostriata* are so pronounced we believe this character would enable separation of the two species. Moreover the petiole of the lamellae is better defined and bears strong spines dorso-ventrally and laterally in *C. flavostriata*, but not in *C. campioni*.

Compared with larvae of other species of *Coelliccia*, the two Bornean species described here differ as follows: All other species described bear 4–5 pairs of setae anteriorly on the upper surface of the prementum even if one of these is quite short, whereas *C. campioni* and *C. flavostriata* bear only three pairs; other species bear 5–6 long setae on the labial palp (3–4 in *C. campioni* and *C. flavostriata*); in other species the caudal lamellae tend to be long and thin, either acuminate or rounded terminally, but lacking a distinct petiole, although there is a weakly defined petiole evident in *C. flavicauda* (ISHIDA 1996) and other species show some terminal expansion in the lamellae; by contrast the petiole is particularly well defined in *C. flavostriata* and also clearly present in *C. campioni*. A strongly developed petiole occurs sporadi-

cally throughout the Platycnemididae, being present for example in *Onychargia atrocyana* (ORR & DOW 2015b). In terms of general habitus *C. flavostriata* conforms well to other species, particularly in the gracile form of the head with post-ocular lobes tapering posteriorly. Other species such as *C. cyanomelas* have a slightly more robust head but not nearly so much as in *C. campioni* (MATUSKI & LIEN 1984). It might be suggested that the different form of the head reflects the immaturity of the larva but our experience suggests that in other Platycnemididae, e.g., *Copera* sp., the characteristic gracile form found in several genera is established well before the F-2 stage (AGO unpubl. obs.). The general form of the mask is rather similar in all species thus far studied, with *C. flavostriata* presenting an extreme in terms of extension of the anterior margin and posterior tapering of the prementum.

This work demonstrates once more the value of molecular analysis in associating larvae, which are difficult to locate and difficult to breed, with their adults. It is of interest to find such clear morphological differences between two closely related species, and for workers in the field we hope this information will prove useful. Nevertheless it seems likely that in such a large and speciose genus as *Coellicia* identification of larvae by molecular analysis may be the only reliable method of determining many species; in the case of the *borneensis*-group of species it appears that the standard barcoding marker COI can be used for this purpose. However a note of caution must be sounded on the use of COI-based barcoding for larval identification outside of the *borneensis*-group; for instance some other species of *Coellicia* from Borneo are not well-differentiated from each other in COI (unpublished Naturalis data). Other markers will need to be found if DNA barcoding is to be used to deal with such cases.

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used for molecular analysis. We are grateful to Günther Fleck and Gunther Theischinger for many helpful comments on the manuscript. Field work on Gunung Kalulong was partly supported by a grant from the Worldwide Dragonfly Association, field work on Mount Dulit and in the Hose Mountains by grants from the International Dragonfly Fund, fieldwork in the Hose Mountains was also partly supported by grant SUB.2010.12.03 from Uyttenboogaart-Eliassen Stichting.

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