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Reported ovo-viviparity in *Heliocypha perforata* (Odonata: Chlorocyphidae) – reassessment of the evidence, based partly on examination of the female reproductive system and mature eggs

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Heliocypha perforata (*sensu lato*) is a common stream-dwelling damselfly widespread in mainland tropical Asia. Recently a report has been published suggesting possible ovo-viviparity in this species, based on the interpretation of evidence from a short video sequence. This video is re-evaluated. The internal and external anatomy of the *H. perforata* female reproductive system, including mature eggs, is examined and illustrated, to the extent that this information casts light on the observations. Three competing hypotheses are considered: (1) a prolarva or larva was expressed from the female's oviduct, due to abnormal retention of the fertilized egg in the oviduct for several days; (2) an egg, deep in the oviposition substrate, previously laid and near hatching, was disturbed by the female's probing ovipositor and the prolarva hatched, becoming briefly caught in the in valves of the ovipositor; and (3) a small unidentified aquatic insect, probably of a different species and different order, was disturbed and similarly briefly caught in the valves. Based on the size and colour of the object relative to that of a mature egg and the likelihood of the event, hypothesis 3 is favoured.

Keywords: *Heliocypha perforata;* viviparity; Zygoptera; Chlorocyphidae; female reproductive tract

Introduction

The chlorocyphid damselfly *Heliocypha perforata* (Percheron, 1835) is a common inhabitant of small clear streams throughout much of tropical and subtropical Asia east of Burma. Several subspecies are recognised, which may in some cases represent distinct species, but as far as is known all share a similar general biology. The ecology and reproductive behaviour of the species were discussed at some length by Furtado (1966), and also by Günther (2008), who included an analysis of male agonistic behaviour and courtship based on behavioural studies in southern Thailand, Malaysia and Hong Kong. In these studies key behavioural events were documented with video recordings. In 2009 the second author studied the reproductive behaviour in southern Thailand by using high-speed cinematography (Günther, unpublished). In 2003 the first author observed at some length, territorial behaviour, courtship, mate guarding and oviposition in localities in Thailand (Orr, unpublished).

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As with many Oriental region chlorocyphids (Orr, 1996), males of *H. perforata* maintain territories around oviposition substrates, typically larger semi-submerged pieces of fallen timber, which they guard against rivals (Günther, 2008). Females arriving at the substrate are typically courted at length by the male while perched (Günther, 2008 and unpublished, Orr, unpublished). Receptive females accept matings, which are of about 1–2 minutes in duration, then commence laying. It is usual for the male to guard the female by perching nearby and driving off other males, at least for 15–30 minutes. It is also common for newly arrived females to repulse a courting male by a clear rejection display (similar to that figured for *Libellago semiopaca* Selys by Orr, 2009), and ovipositing females often congregate in groups, which may include other chlorocyphid species, where they are seldom subjected to unwanted attention from males. Once established at an oviposition site females often remain for more than one hour (Orr, unpublished).

Recently, a claim was made of possible ovo-viviparity in *H. perforata* based on video evidence of an ovipositing female in south-western China (Dayananda & Kitching, 2014a). This claim, even as a report of an aberrant or pathological event, is so extraordinary it deserves close examination. The authors have provided for public scrutiny the video footage on which these claims are based. Here we analyse this footage in detail, and also provide information on the genital anatomy and egg morphology of female *H. perforata*, which adds considerably to the forensic analysis of the images. We examine, in turn three conflicting hypotheses: (1) a prolarva or larva was expressed from the female's oviduct, due to abnormal retention of the fertilized egg in the oviduct for several days allowing time for maturation; (2) an egg, deep in the oviposition substrate, previously laid and near hatching, was disturbed by the probing ovipositor and the prolarva hatched, becoming briefly caught in the valves of the female's ovipositor; and (3) a small unidentified aquatic insect probably of a different species and different order was disturbed and similarly briefly caught in the valves.

Material and methods

Video footage provided by Dayananda and Kitching (2014b) was viewed frame by frame by AGO using Quicktime 10.4 (Apple Inc., Cupertino, CA, USA) on a MacBook Pro using a 15-inch Retina display (5 million pixels) and by AG using QuickTime Player version 7.7.1 (Apple Inc.) and a 21.5″. BenQ display E2200HD DVI (1920 × 1080 pixels). Dayananda and Kitching (2014a) do not state the equipment used, nor other technical details, but analysis showed the film speed was 25 fps (frames per second).

Female anatomy and egg morphology was studied by AGO using a Wild dissecting microscope (max magnification ×50) and a specimen from northern Thailand, collected while ovipositing, preserved in kerosene-acetic acid solution (KAA; see Gibb & Oseto, 2006, p. 44) and dissected in 30% alcohol; collection data are as follows: THAILAND, Chiang Mai, Hangdong-Samoeng Rd., Ban Pong, Huay Mae Ta Chang at Lanna Resort, 20 March 2003–18° 45′ 16″ N, 98° 52′ 40″ E, alt. 380 m, leg A.G. Orr.

Female anatomy and egg morphology

The anatomy of the zygopteran female genitalia has been described by Tillyard (1917), Westfall and May (1996) and more recently by Matushkina and Lambret (2011) and Matushkina and Gorb (2002). These publications use slightly varying terminology but that of Westfall and May (1996) is used here.

In *H. perforata* the main paired elements of the ovipositor are the outer sheathing valve bearing a sensory stylus subterminally, and the long curved, sickle-like posterior gonapophysis which in

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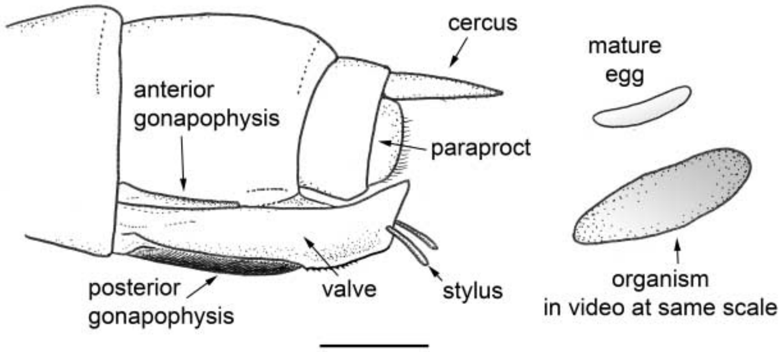

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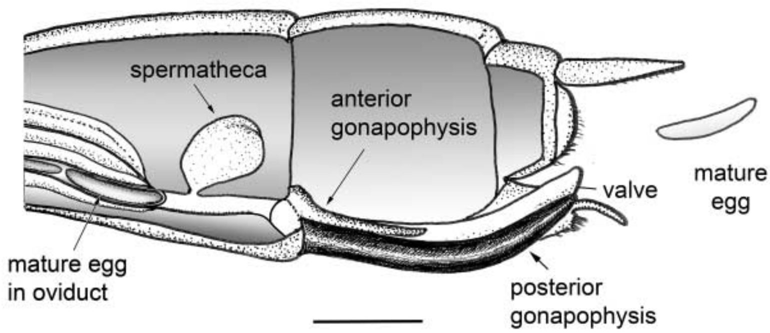
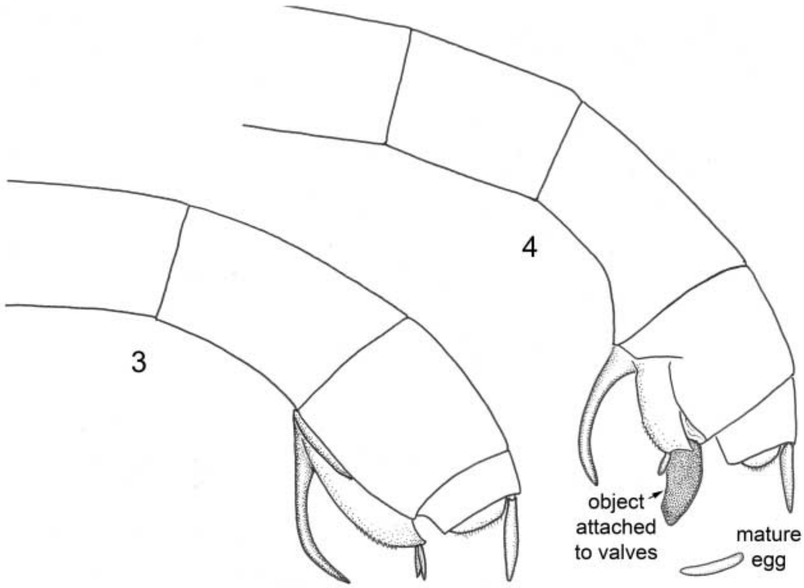


Figures 1–2. (1) Detail of posterior abdominal segments of *H. perforata* female, showing ovipositor in resting position; egg and organism from Dayananda and Kitching (2014a) shown to right (scale bar 1 mm). (2) Parasagittal section of posterior abdominal segments of *H. perforata* female, showing the relation of the oviduct and spermatheca to the ovipositor (scale bar 1 mm).

conjunction with the valves forms the egg tube and the shorter anterior gonapophysis (Figure 1). The function of these structures has recently been discussed in *Lestes macrostigma* (Eversmann, 1836) by Matushkina and Lambret (2011) and more generally in Zygoptera by Matsukina and Gorb (2002). In particular the stylus of the valve was shown to have an important sensory function, as earlier recognised by Tillyard (1917). *Heliocypha perforata* differs somewhat from other Zygoptera that have been studied in detail in that the anterior gonapophysis is considerably reduced. Figure 1 also shows a mature egg, dissected from the female, and an outline of the organism shown in the video of Dayananda and Kitching (2014a), both at the same scale as the main drawing.

The relationship between the external structures and distal part of the internal genitalia is shown in parasagittal section in Figure 2, with some simplification of the skeletal anatomy at the base of the ovipositor. The spermatheca was extremely simple and packed with sperm. In general in Chlorocyphidae two small spermathecal appendices are developed as shallow buds (Orr, unpublished data) and it is possible in the present case that the dense packing with sperm has obliterated the junction between these and the bursa copulatrix. To clarify this point a series of specimens should be dissected. A mature egg is shown entering the common oviduct as it nears the position in which insemination will occur, and outside, a mature egg on the same scale.

When the ovipositor is deployed its separate parts become more obvious as seen in the video (Figure 3). Figure 4 shows a detail of frame 649 (25.96 s), in which the organism identified by



Figures 3–4. (3) Ovipositor in action, just before being inserted into wood (traced from Dayananda & Kitching, 2014b). (4) Detail of frame 649, traced from Dayananda and Kitching (2014b), showing organism purporting to represent newly eclosed larva or prolarva.

Dayananda and Kitching (2014a) is shown trapped in the valves next to a mature egg drawn at the same scale for comparison.

Analysis of video

The events shown in the video are interpreted in detail in Table 1. By this analysis the organism previously suggested to be a prolarva or larva is considered to be a larger non-odonate invertebrate, possibly a coleopteran larva.

Discussion

The only plausible circumstance in which regular ovo-viviparity could function would be if the females were parthenogenetic. We reject immediately this possibility, and indeed Dayananda and Kitching (2014a) are equivocal about it. Not only are the ecological conditions unlikely to select for this rare phenomenon, the female is guarded by a male, who very likely had mated with her.

Of the more likely explanations, this leaves three hypotheses that might reasonably be considered:

1. Aberrant ovo-viviparity; that is to say, ovo-viviparity by a rare accidental event, such as a fertilized egg having been lodged in the oviduct for several days prior to the incident, or by some other pathology. Firstly it should be noted that the oviduct is quite short between the spermatheca and the lumen opening into the valves (Figure 2). It is difficult to see how such an elongated egg could become lodged sideways. It is equally unlikely that the female would voluntarily retain the egg within her reproductive tract for the 4–5 days until hatching, and likewise difficult to see how a female may have been prevented from ovipositing by males. Female odonates regularly mate to gain access to oviposition sites. If need be they will accept mating even when their spermatheca is full (Waage, 1984). Chlorocyphid females with mating systems of the sort found

Table 1. Analysis of actions throughout video sequence for time periods and frame numbers (#) measured from first frame (film speed 25 fps), video footage from Dayananda and Kitching (2014b).

Time (s) (frame #)	Action
0–7.96 (#1–199)	Opening title
8.00–11.16 (#200–279)	Oviposition obscured behind stick
11.20–14.16 (#280–354)	Oviposition probing in plain view Small insect on surface disturbed
14.20–20.24 (#355–506)	Probing moved back by extension of abdomen and again obscured behind stick
20.28–25.84 (#507–646)	Concerted deep probing in plain view
25.88–27.40 (#647–685)	Retracts ovipositor with large elongate dark object attached to end of valves
27.44–29.32 (#686–733)	Drags ovipositor with object to side and washes off, raises abdomen free
29.36–41.00 (#734–1025)	No adult odonates, but small organism visible moving on surface of wood
41.04–44.60 (#1026–1115)	Perched male depicted, probably guarding mate
44.64–73.32 (#1116–1833)	Close-up sequence of oviposition.
73.36–102.40 (#1834–2560)	No insects in film, slow motion replay and closing credits

in *H. perforata* are normally able to gain access to oviposition sites without mating even when harassed by males (Günther, 2008; Orr, 2009, AGO, AG pers. obs.).

However without engaging in further speculation, it must be pointed out that the organism photographed, is at least eight times the volume of a mature egg, and also sclerotized (Figure 4). The prolarva would be about the same size as the egg, and probably pale creamish in colour, that is to say, unsclerotized. It must be acknowledged that the egg may increase in size slightly after oviposition as noted by Corbet (1999, p. 50): “Eggs commonly increase in linear dimension between oviposition and hatching. Those of *Calopteryx virgo* increased by about 10% (Degrange 1974) . . . .” Therefore we might expect a slight increase in the size of the egg from that shown in Figures 1, 2 and 4, but this would not be nearly enough to match in size the organism depicted by Dayananda and Kitching (2014a). Even if the organism were a first instar larva (i.e. second instar, *sensu* Corbet, 1999), it would be only slightly larger. Tillyard (1917, p. 68) illustrates the hatching of the larva of *Anax papuensis* Burmeister from which it can be calculated that the larva is about 25% longer than the original egg, an increase presumably mainly resulting from absorption of water. If it hatched within the female’s body it would have had no chance to find nourishment in order to grow beyond this size. The female tract is, like other chlorocyphids examined by the first author, extremely simple and unspecialized. On this basis we can definitely reject the ovo-viviparity hypothesis.

2. An egg laid several days previously (presumably) by another female induced to hatch on contact with ovipositor: for the reasons given above, especially the size of the organism which is trapped in the tips of the valves, this cannot be a pro-larva and hence the hypothesis is rejected.

3. Some other organism, possibly a coleopteran larva, has become wedged in the valves of the female and she has disengaged with it. It is certainly animate, and may well be the same as the indistinct creature seen in frames 734–1025 at 29.36–41.00 s. It appears to have appendages, but the resolution is too poor to be certain of this.

If this interpretation is accepted, the follow questions arise: What was the organism that was involved in the encounter? How did it become entangled with the valves of the female damselfly?

Was the female deliberately removing it from the site, because for example it was a coleopteran larva which preys on eggs, or did she accidentally collect it on her valves and went to some effort to wash it off? These questions could have been better answered had the specimen been collected, but this is easily said with hindsight. The video footage is both remarkable and spectacular, and open to various interpretations when considered on its own. It remains a very interesting observation and we are grateful to the authors (Dayananda & Kitching, 2014a) for putting it on record and providing open access to their video source.

Acknowledgements

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