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Dorsolateral cuticular outgrowths in second stadium larvae of *Gomphus flavipes* (Odonata: Gomphidae)

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Dedicated to Georg Rüppell, who always inspires us with his enthusiasm for dragonflies.

Second and third stadium larvae of *Gomphus flavipes* have dorsolateral cuticular outgrowths in the form of small basal tubercles bearing fan-shaped setae. These sensilla are aligned in two rows on each side of the thorax and abdomen. European species of *Gomphus*, *Onychogomphus* and *Ophiogomphus* that we examined lack these structures, having instead, at most, short hairlike setae in double rows. The fan-shaped setae of *G. flavipes* are present only in second and third stadia, apparently being lost later in larval development. We speculate that this loss might be due to changes in microhabitat or might be some kind of phylogenetic constraint.

Keywords: dragonfly; larvae; abdominal protuberance; *Gomphus flavipes; Gomphus graslini; Gomphus pulchellus; Gomphus simillimus; Ophiogomphus cecilia; Onychogomphus uncatus*

Introduction

To distinguish larvae of different species of the family of Gomphidae morphological characters like lateral abdominal spines or dorsal hooks were used (e.g. Suhling & Müller, 1996). Within European members of the genus *Gomphus*, only the larva of *Gomphus flavipes* (Charpentier) has developed a small single-scaled structure at the posterior margin of abdominal segment 9 (Suhling & Müller, 1996, p. 169), so it can be distinguished from other European *Gomphus* species. In this work we describe dorsolateral outgrowths on the thorax and abdomen of second stadium larvae of *G. flavipes*. None of the European species of *Gomphus* are known to have such outgrowths, neither from second stadium nor from later larvae.

Similar structures on the abdominal tergites in exuviae of anisopteran larvae are known in Petaluridae, e.g. in *Phenes raptor* Rambur (Fleck, 2011) and *Tanypteryx pryeri* Selys (Taketo 1971). According to Schmidt (1941, as cited by Fleck, 2011) these “Zottenhöcker” are at least present in *Tachopteryx*, *Phenes* and *Petalura*. In second stadium larvae they are shown by Watanabe (1995) for *Stylurus oculatus* (Asahina) and *Stylurus nagoyanus* (Asahina) and by Dunkle (1980) in three North American species of *Stylurus*.

Compared to other *Gomphus* species, the larvae of *G. flavipes* also differ in later stadia, as Schmidt (1987) described. They only have shorter tibial hooks on the fore- and midlegs, shortened hind legs and an elongated abdominal segment 9 like the larvae of the genus *Stylurus*

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(Schmidt 1987). On the basis of several characters of the imagines and these specifics in the larval morphology, Schmidt (1987) reclassified *G. flavipes* in *Stylurus flavipes*. However, this classification has been questioned (Heidemann, 1988, 1989) and has not become established (Dijkstra & Kalkman, 2012). If dorsolateral outgrowths are common only in early stadia larvae of *Stylurus* species this could be a new argument for a reclassification of *G. flavipes*.

Methods

In order to obtain data on diapause regulation in gomphids (e.g. Schütte 1998) larvae of *G. flavipes*, *Gomphus pulchellus* Sélys, *Gomphus simillimus* Sélys, *Gomphus graslini* Rambur, *Onychogomphus uncatus* (Charpentier) and *Ophiogomphus cecilia* (Fourcroy) were reared from eggs in the laboratory. Larvae were examined for morphological details at 50 × magnification with a binocular microscope. Any second stadium larvae that died were preserved in 70% alcohol in order to find potential identification characters. External morphology of the larvae was examined microscopically at 100 × magnification (Carl-Zeiss Laboval 4, Jena) and all larvae were drawn using a WACOM Intuos graphics tablet (Krefeld, Germany).

Results

G. flavipes larvae possess a double row of dorsolateral fan-shaped outgrowths (Figure 1A, B). Except in *G. simillimus*, which lacked any prominent dorsolateral setae, the larvae of the other species had only a double row of single hairs in the same place (Figure 1C, E–G). The outgrowths in *G. flavipes* larvae are located on prominent cuticular tubercles whereas the hairs in the other species are not. The outgrowths were found on each segment of the thorax and the abdomen, except for abdominal segment 10. We did not find any other distinct morphological features in the second stadium, e.g. abdominal dorsal hooks or lateral spines, which could be used to distinguish the different species examined.

Discussion

There are no apparent differences in the shapes of the second stadium larvae of the six species examined. One should expect that the larvae of the four *Gomphus* species look somewhat different from the larvae of *O. uncatus* or *O. cecilia*. Typical characters used for identifying the genera, such as presence and shape of the dorsal hooks and the specific shapes of the third antennal segments seem to occur only in later stadia larvae (e.g. Suhling & Müller, 1996). By contrast, *G. flavipes* could be distinguished from the other genera as well as from its congenerics by the shape of the last abdominal segment. Second stadium larvae of *G. flavipes* already show the almost square shape of later stadia. This character is used to distinguish this species from other *Gomphus* species.

There is a minor difference between *G. flavipes*, *O. cecilia* and *O. uncatus* on the one hand and *G. simillimus*, *G. graslini* and *G. pulchellus* on the other hand. In the first group the eyes are located in the foremost third of the head (viewed from above as in Figure 1). In the second group the eyes are located at about midlength of the head. This does not correspond with the characteristics used for identification of later stadia larvae, however (Suhling & Müller, 1996).

The most obvious difference between the second stadium larvae of the six species is the presence of outgrowths on the thorax and abdomen of *G. flavipes*. Watanabe (1995) showed in second

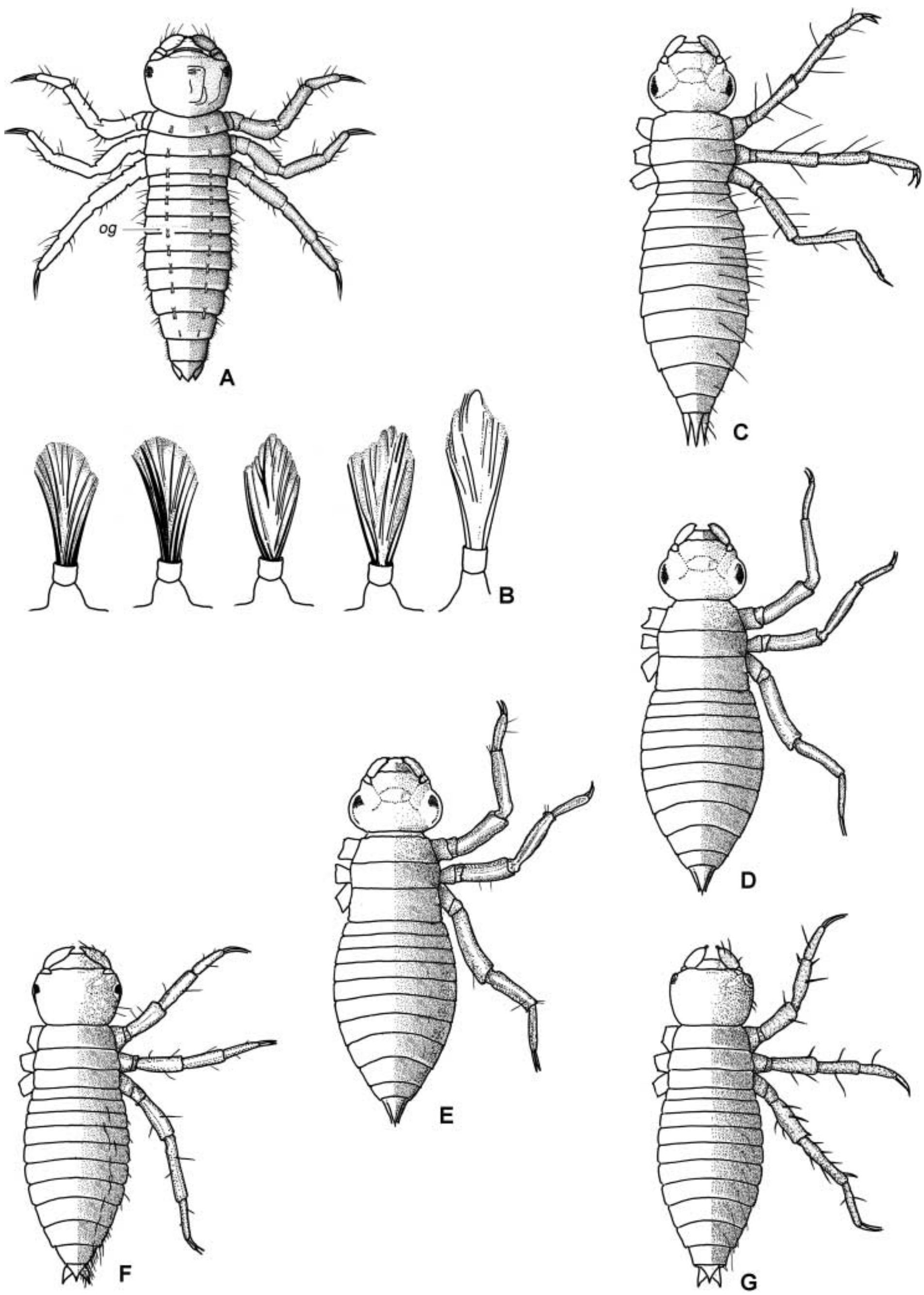

Figure 1. Drawings of body shape and some details of second stadia larvae of six European species of gomphids. (A) *Gomphus flavipes* habitus; (B) *Gomphus flavipes*, og – outgrowth; (C) *Gomphus pulchellus*; (D) *Gomphus simillimus*; (E) *Gomphus graslini*; (F) *Onychogomphus uncatus*; (G) *Ophiogomphus cecilia*.

stadium larvae of *Stylurus oculatus* or *Stylurus nagoyanus* outgrowths that are rather similar to those described here for *G. flavipes*. Such outgrowths have been also described from a number of other Odonate families. Brooks & Jackson (2001) described fan-shaped dorsal setae on early instar libellulid larvae which have exactly the same shape as the dorsal cuticular outgrowths in

G. flavipes. *Epophthalmia vittata sundana* (Burmeister) (Corduliidae) also has composite spine-like setae on the head and thorax (Corbet 1962). The structures we found in first and second instar larvae of *G. flavipes* might function as sensory organs. For example, they might be mechanoreceptors, as Corbet (1999, p. 94) assumed for similar but smaller fan-shaped setae on the head of last instar *Cordulegaster insignis* Schneider larvae. He also described similar "structural peculiarities" (Corbet, 1962) that vanish during larval development. These mechanoreceptors could help the larvae to detect prey and avoid predators.

Field studies showed that the larvae of *G. flavipes* avoid river sections with strong currents and prefer a fine substratum with a high share of detritus (Müller, 1995). The preferred microhabitats of all instars are soft sediments that consist of mineral matter, especially sandy particles with grain sizes of 0.125–0.250 mm. The larvae are typical "burrowers" (Corbet, 1962), mainly buried in the sediment. Microhabitats with these characteristics of substratum are probably not suitable as microhabitats for the early instars because the average grain size of the fine sandy sediments might be too coarse in relation to the size of early and thus small larvae. This could be a reason for the larvae not to bury themselves but to stay on the surface of the substratum. In the field the fine substratum is often covered with a layer of detritus in which early instar larvae may hide. If the first instars live on the surface the described protuberances could function to keep fine detritus particles at the surface of the dorsal cuticula. This might contribute to their camouflage as Miller (1964) also assumed for larvae of *Ictinogomphus ferox* Rambur that live at the surface of substratum as well.

Unfortunately we do not know the preferred microhabitat of the second instar larvae of this species. Research on the habitat selection of this stadium needs to be done to understand the function of this outgrowth.

Another explanation derives from the general observation that early developmental stages of organisms may reveal similarities that are masked by features acquired later during ontogeny. Thus the second larval stadium of *G. flavipes* might show morphological characters present in more distant ancestors than does the last stadium. Therefore Dunkle (1980) recommended that characters of the second larval stadium should be considered along with all other characters of a species when establishing taxonomic relationships. We show new strong data to support reclassification of *G. flavipes* to *Stylurus flavipes* here, in agreement with recent unpublished results of genetic sequence analysis (M. May, personal communication, December 2014).

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