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The feeding action of *Forcipomyia paludis* (Diptera: Ceratopogonidae), a parasite of Odonata imagines

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Abstract

Females of *Forcipomyia paludis* were studied microscopically during their feeding action on Odonata wings where they were mostly attached to main veins in the basal half of the wings. In some individuals rhythmic nodding of the head was noted. Conspicuously many midges lifted the abdominal tip every one or two minutes and from the anus fast growing gas bubbles appeared that burst after about half a second. We suppose that the insects, having punctured the host's veins with their stout proboscis, sucked much air (as well as haemolymph) from the tracheae which they had to get rid of afterwards. From these observations, combined with further indications, it is inferred that *F. paludis* acts as a true parasite of Odonata and that the association is not only phoretic as previously assumed.

Introduction

Females of the ceratopogonid genus *Forcipomyia* Meigen (subgenus *Pterobosca* Macfie) have repeatedly been reported to be attached to various body parts of many Odonata species originating from different continents and islands of tropical and temperate regions (e.g. Macfie 1932; Cowley 1936, 1940; Clastrier & Legrand 1991). As various ceratopogonids (biting midges) are blood-sucking parasites on mammals, birds and other vertebrates, thus constituting pests and vectors of infections, many authors took it for granted that the *Forcipomyia* species found on Odonata would feed on the haemolymph of their host (e.g. Mayer 1937, 1955; Wirth 1956; Clastrier & Legrand 1984; Naraoka 1999). This assumption is obviously based on morphological studies of the midges' mouthparts (e.g. Mayer 1936) and on the knowledge of ceratopogonid parasitising mammals (e.g. Downes 1958; Kettle 1977; Bishop et al. 1994). However, in recent works true ectoparasitic habits have been doubted as no signs of puncture were found on wing veins or other parts of the exoskeleton after the midges had become separated from the site of attachment (Dell 'Anna et al. 1995; Orr & Cranston 1997). Due to the lack of any confirmation of host feeding these authors hypothezised that the association of *Forcipomyia* females with Odonata may be restricted to phoresis. However, all findings so far available have been based only on photographic documents, microscopic studies of dry museum specimens, or macroscopic inspection in the field. Strangely enough, no living ceratopogonids attached to Odonata have been examined in the laboratory with respect to their behaviour. Here we report on stereomicroscopic observations of *F. paludis* (Macfie, 1936) females that yield firm evidence for haemolymph-sucking from wing veins of two European Anisoptera species.

Material and methods

Adult males of *Cordulia aenea* (Linnaeus) from a pond near Hombrechtikon, Switzerland (47°15'30"N, 08°48'00"E) and of *Cordulegaster boltonii* (Donovan) from fen-ditches near Hinwil, Switzerland (47°15'27"N, 08°49'42"E) were netted and instantly examined for ceratopogonids on the wings. Biting midges were usually found on only a small part of the captured Odonata. In the years 2000, 2001 and 2004 the infestation rate varied greatly, depending on the species, locality, year and season of the year. Many midges detached from their host's wings in the net and escaped, and few remained firmly attached in captivity for hours up to nearly two days. Their behaviour was studied under a stereomicroscope with magnifications up to 70x and successive movements were measured with a stop watch. In order to keep the odonates immobile they were fixed by the wings with narrow strips of paper pinned on pieces of styropor, leaving the basal wing parts with the midges free. During observation periods of up to 80 mins no negative reactions of the midges towards artificial illumination were noted. As the odonates did not suffer injury they could be released after examination. A number of ceratopogonid individuals was retained for later identification.

Results

All ceratopogonid specimens identified conformed to *Forcipomyia paludis*. On their host's wings they were firmly attached to the basal parts of the main veins, almost exclusively in the clefts of the longitudinally folded wing, in a few cases also directly on one of the joint membranes (Fig. 1). Generally, the midges remained motionless and kept their wings closed in the typical ceratopogonid manner. The tips of the tarsi adhered firmly to the wing membranes with the aid of the empodium that was bent almost perpendicularly to the tarsal segments. Only occasionally was the position of the legs changed. Mutual cleaning of both fore and hind legs respectively occurred rarely. The head was either kept straight or somewhat tilted, probably in order to obtain better access to the substrate with the mouthparts. No typical position of the antennae was recognized although one or both of them were often held backward, sometimes being moved rhythmically. The relatively short but sturdy proboscis was inserted deeply up to the head capsule into the host's integument.

Haemolymph sucking was not observed directly, but rhythmic movements of the distal body parts indicated that feeding was going on. In some individuals weak, nodding head movements were noted with a frequency of 4-5 Hz, giving the impression that imbibing was taking place. In the normal position the tapered abdominal tip was bent downward, the cerci being invisible from above. Occasionally the two terminal segments were repeatedly stretched telescopically, bent slightly upwards and withdrawn again. The first of these 'pumping movements' was weak and slow, then they became stronger and faster, the last two proceeding in about one second.

After five to six events, featuring maximal extension of the abdominal tip and while the cerci were often slightly spread laterally, a rapidly growing bubble was formed from the anus and burst within about half a second (Fig. 1). Subsequently the tip of the abdomen was bent downward, attaining the normal position again. Few tiny droplets of colourless liquid were spread over the host's wing membrane up to 8 mm away from the midge, drying up quickly afterwards.

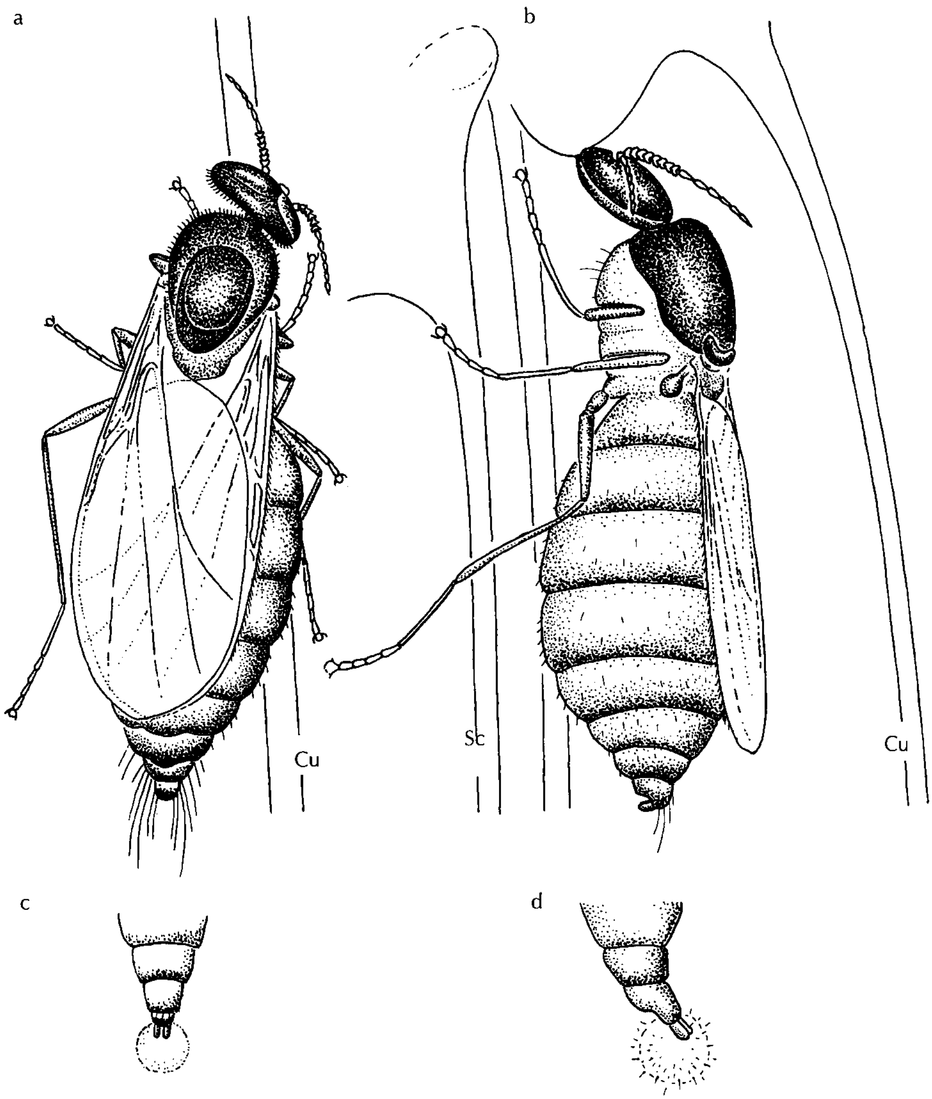

Figure 1: Biting midge *Forcipomyia paludis* females — piercing with their mouthparts (a) the cubital vein of *Cordulegaster boltonii* and (b) a wing joint membrane of *Cordulia aenea*; (c, d): posture of the abdominal tip during formation and burst of a gas bubble viewed from above (c) and from the side (d). Cu: cubital vein, Sc: subcostal vein. Length of *F. paludis* female: 1.8 mm.

The frequency of bubbling varied enormously between individuals. In three of four individuals investigated simultaneously and that were attached to the same host close together only two to six bubbles appeared in the course of ca 80 mins while in a fourth individual 53 bubbles were counted at the same time. The intervals between the appearance of two subsequent bubbles were measured 48-58 times within 47-81 min in three individuals that produced them about every one to two minutes. The interval between two bubbles averaged 52, 69, and 91 s (Fig. 2), the shortest period being 25 and the longest 210 s. The volume of the discharged gas figured out for a realistic example with a frequency of 74 bursts per h and a bubble diameter of 70 µm amounted 0.104 mm^3 h^{-1}, i.e. 1.6-1.9 times the volume of a moderately distended abdomen calculated as a sphere or double cone respectively.

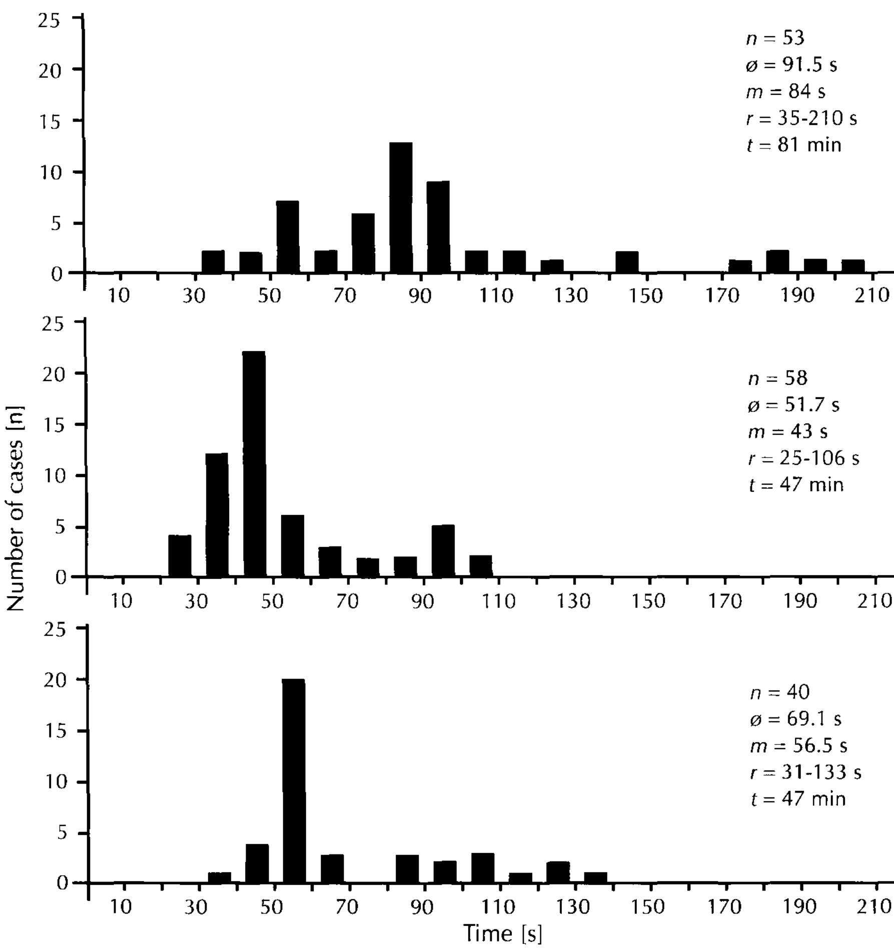

Figure 2: Frequency of bubble formation in three individuals of *Forcipomyia paludis* — abscissa: time elapsing between two bubbling events [s], ordinate: number of cases [n], *n* = total number of bubbling events, Ø: mean time between bubbling events, *m*: median, *r*: range, *t*: observation time.

Detachment of the biting midge from the attachment site was followed in two cases. The abdomen of the females were strongly swollen. After having withdrawn the proboscis from the host an individual remained on the site for 15-20 s, cleaning its mouthparts and antennae with the fore legs. The antennae were directed ventrally and drawn through both fore legs one after the other, the tarsi being put together like hands. Subsequently the midge walked away a short distance and immediately flew off spontaneously, without having been disturbed by the observer. At the site of attachment no injuries caused by the midges' mouthparts could be detected on the wing veins.

The distention of the abdomen varied in freshly captured females. Some appeared rather thin, and others moderately distended or even fully engorged. In a few individuals it was obvious that their abdomen swelled overnight. In captivity most midges left their host after 10-20 h, but one individual stayed for 43 h.

Discussion

Direct observations of living *Forcipomyia paludis* females attached to the wings of Anisoptera revealed clear evidence of haemolymph sucking. The most striking evidence is the regular excretion of bubbles of which the total volume per hour may reach 1.5-2 times the volume of a moderately distended midge abdomen. We assume that haemolymph sucking is accompanied by gas intake and subsequent excretion of gas. The parasite inserts the short and stout proboscis into the wing veins that contain nerves, haemolymph and tracheae (cf. Pflugfelder 1970). During the feeding action the midges not only obtain fluid from the vein but also much air from the trachea that may simultaneously be pierced by the mandibles. Thus, the midge takes into its gut not only liquid but also much air which has to be got rid of. By injecting $^{32}P\text{-}Na_2HPO_4$ into the abdomen of some Anisoptera spp. and subsequent autoradiography, Münchberg (1963) showed that haemolymph circulates in wing veins, especially in their proximate sections, but also in the fine network of the wing tips. Strikingly, the midges cling mostly near the wing bases, thus minimizing the centrifugal forces to which they are exposed during wing beats. But this need not be the only reason for their site choice. The main veins near the bases are thicker and contain more haemolymph than those in the distal sections, thus yielding a better supply for the parasite. No biting midges are found on the costa, probably because this strongly sclerotised vein cannot be penetrated by the midges' mouthparts (cf. Münchberg 1963).

Curiously and in contrast to water mites (e.g. Münchberg 1935: 88), the feeding action of biting midges do not seem to leave visible lesions on the host's integument. This finding is advanced as an argument against parasitic behaviour of *F. paludis* that were found on the wings and rarely on the thorax of five Odonata spp. in Italy (Dell'Anna et al. 1995) and also of *F. debenhamae* Cranston that was observed exclusively on the thorax of *Libellago hyalina* Selys in Brunei (Orr & Cranston 1997). However, we assume that the cuticle of the wing veins is elastic and does not leave behind visible scars. This is especially true for the flexible joints between the longitudinal and cross veins that contain resilin, a rubberlike protein (Gorb 1999). Macfie (1932) also states that it is not known to what extent the midges cause injury to their hosts. Obviously he did not notice any conspicuous marks either. Ceratopogonid parasitism of insect imagines is well known in other cases, the midges sucking haemolymph mainly at thin parts of the cuticle such as the pleural and intersegmental membranes (e.g. Havelka 1979, 1980).

The conclusion that *Forcipomyia* spp. are true parasites of Odonata is supported by several indications: (a) So far, only females have been recorded as being attached to odonate adults; (b) the mouthparts are stinging and sucking devices (Mayer 1936); and (c) as demonstrated in this paper, the abdomen may swell largely over the time of attachment (Orr & Cranston 1997; this paper). Yet all of these findings do not exclude the option that the association between ceratopogonids and odonates is also phoretic.

F. paludis is the only ceratopogonid species that has hitherto been reported to be associated with European Odonata (e.g. Macfie 1936; Edwards 1937; Remm 1967; Clastrier et al. 1994). In contrast, six *Forcipomyia* spp. have been found attached to odonates in Japan (Naraoka 1999). With respect to their choice of host species biting midges are opportunistic. For *F. tokunagai* Oka & Asahina 18 Odonata host species are recorded in Japan (Naraoka 1999) and so far, for *F. paludis* the number of species attacked in Europe exceeds 50 (A. Martens, H. Wildermuth unpubl.). We assume that the behaviour of the various *Forcipomyia* spp. on their hosts is closely similar.

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