



Morphological adaptations of the mouthparts to the ectoparasitic lifestyle of the biting midge *Forcipomyia paludis* (Diptera: Ceratopogonidae), specialized in Odonata

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Abstract

Damselflies and dragonflies are well-known hosts of the West Palaearctic biting midge *Forcipomyia paludis*. Females of this ectoparasitic dipteran mainly cling to the host's wings, sucking hemolymph from the wing veins. The midges are firmly attached to the wing surface with specialized tarsi, thus not being flung away during the host's flight maneuvers. As for another ceratopogonid—*F. odonatophila* from New Guinea—had been suggested, we assumed that in *F. paludis*, the attachment would be reinforced by the mouthparts during the suction action. In the present study, we used behavioral field observations, scanning electron microscopy (SEM) and high-resolution micro-computed tomography (μ CT), to study the mouthparts of *F. paludis*. We focused on the mouthpart configuration post sucking and thus on the contact with the host's wing as well as on the piercing process into the wing veins. We foster our understanding of *F. paludis* being a parasite of Odonata by showing proof of the piercing and therefore the sucking of hemolymph from the wings. Additionally, the mouthparts clearly show contamination with odonate wing wax after the sucking procedure. Furthermore, we discuss probable additional functions of the piercing process for the firm attachment to the flying host of *F. paludis*.

Keywords Midge · Parasitism · Anisoptera · Zygoptera · Attachment · Feeding · Mouthparts

Introduction

Biting midges (Diptera: Ceratopogonidae) are well-known ectoparasites with a wide spectrum of different hosts, such as mammals, birds and various arthropods including damselflies and dragonflies (Odonata) (Macfie 1932; Borkent and Dominiak 2020). One of the worldwide more than 6000 described species, females of the West Palaearctic *Forcipomyia* (*Pterobosca*) *paludis* (Macfie 1936), have exclusively specialized in odonates as hosts (e.g., Martens et al. 2007). This tiny fly is generally attached to the odonate's wings, clinging firmly to the wing membrane with the tarsi and inserting the short proboscis into the veins or the membranes of the wing bases (Wildermuth and Martens 2007). Direct

behavioral observations of active females revealed that these midges very likely suck hemolymph from the veins, as indicated by their rhythmical head nodding, regularly releasing air bubbles from the anus and their abdomen continuously swelling over time (Wildermuth and Martens 2007).

In biting midges in general, the saw-like movements from the serrated mandibles open the skin/cuticle of the host (Krenn and Aspöck 2012; Krenn 2019). The specialized labella is placed on the outside of the hosts' opened body cavity and provides a guiding function for the piercing mouthpart structures, as well as sensing for an appropriate feeding site (Krenn 2019). Interestingly, biting midges (Ceratopogonidae) are among the smallest known hematophagous insects (Krenn 2019).

Field studies on infested males of the large dragonfly *Cordulegaster boltonii* (Donovan, 1807) have shown that the midges remain firmly attached to the host's wings during various flight maneuvers, obviously due to highly specialized micromorphological attachment devices on the tarsi, especially on the empodium (Gorb et al. 2022). The position of the midges near the wing base and on the lower veins of the corrugated wing may further help to reduce

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the drag during the host's flight (Martens et al. 2007; Manger 2021). In addition, we suppose that, during suction feeding, the mouthparts would reinforce the attachment of the midge to its host.

In the present study, we used photographs of Odonata imagines that were infested with *Forcipomyia paludis*, taken in the field, as well as scanning electron microscopy (SEM) and micro-computed tomography (μ CT) analysis to describe the position of the midges on the wing in regard to the corresponding wing vein and the piercing of the wing vein itself. Furthermore, we depict the mouthparts during and after contact with the host and discuss their structure in the context of parasitism. Thus, we present evidence for the piercing of the host's wing veins by the midges' mouthparts. Therefore, we could underline the observations on behavior indicating the parasitic lifestyle of *F. paludis* as an ectoparasite of Odonata, although simultaneous phoresis as suggested by Dell'Anna et al. (1995) and Orr and Cranston (1997) would not be completely excluded.

Materials and methods

Field observations and in situ photography, focused on various odonate species infested with *Forcipomyia paludis* (Macfie 1936), were conducted in southern France (Saint-Martin-de-Crau), Switzerland (Uster, Hinwil) and Georgia (Sartichala), at localities where infested odonates had been known (Martens et al. 2007; Wildermuth 2012; Wildermuth et al. 2019). Samples of *F. paludis* specimens attached to wing fragments of *Cordulegaster boltonii* (Donovan, 1807) were air-dried. Specimens of *F. paludis*, detached from their hosts, originating from Switzerland and Georgia were preserved in ethanol.

For binocular photography, a Leica M205A (Leica, Wetzlar, Germany) with a motorized stage was used. Stacks of 10–20 individual images were taken at different focus levels and afterward processed into a focus-stack image using binocular specific Leica software. For scanning electron microscopy (SEM) and micro-computed tomography (μ CT), we critically point dried the used 'solo' specimens using an automatic Leica EM CPD300 (Leica, Wetzlar, Germany). For SEM, the respective specimens were sputter-coated with a 20 nm layer of Au–Pd (Leica Bal-TEC SCD500). SEM images were taken with a Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) at an acceleration voltage of 3 kV. For μ CT, a SkyScan 1172 desktop μ CT scanner (Bruker micro-CT, Kontich, Belgium) was used (40 kV, 250 μ A; 360° rotation (step size: 0.25°)). The segmentation and visualization of the data were done in Amira 6.2 (Thermo Fisher Scientific, Waltham, USA).

Results

Based on photographs taken in the field in various regions of the western Palaearctic, both teneral (Fig. 1B) and adult odonates (Fig. 1C–E) are infested by *Forcipomyia paludis*. Judged from the wear of the host's wings, even old dragonflies are parasitized (Fig. 1F). The midges are attached to the clefts of the corrugated wing membrane on the basal half of the wings, the body axis almost exclusively directed toward the host's thorax (Fig. 1C). The same is found in dried midge individuals that are still attached to the wings of one of the host's species, *Cordulegaster boltonii* (Fig. 2). They cling on both wing sides, dorsally and ventrally (Figs. 1D–F). Most midges were positioned above, rarely parallel to the wing veins (Figs. 1, 2, 3). During piercing, the midges' head is often tilted downwards and sideways toward the lateral base of the wing vein when positioned above the vein (Figs. 1D, 3). The proboscis of the midge is inserted into the wing vein of its host laterally at the vein's basis (Fig. 4).

The mandibles show saw-like protuberances on the ventral side (Figs. 5B, C). Remains of the wax crystals of the wing surface, mixed with some solidified fluid residuals (presumably dragonfly hemolymph or/and midge saliva), can be found rosette-shaped around the proboscis (Figs. 5A, B, E).

Discussion

On photographs taken under natural conditions most female individuals of *Forcipomyia paludis* are located and positioned on the dragonflies' wings as described in earlier studies (e.g., Martens et al. 2007; Manger 2021). Obviously, the body posture and the slightly tilted head position allow an easier piercing of the vein at its lateral base (cf. Wildermuth and Martens 2007). Here, the wing vein shows a weak point: the cuticle seems to be thinner at the lateral base, especially on the convex side of the vein (cf. Appel et al. 2015). Furthermore, soft resilin pads might be present in some areas of the lateral base of the vein, especially at the intersections between the veins—the vein joints (Gorb 1999; Appel and Gorb 2014; Appel et al. 2015; Rajabi et al. 2018).

The SEM data show the saw-like teeth on the mandibles, typical for biting midges in general (cf. Krenn and Aspöck 2012) and also specifically in *Forcipomyia paludis* enabling piercing the cuticle of the wing. However, the general morphology of the mouthparts, as briefly described in Macfie (1936) and Cordero-Rivera et al. (2019) is not in focus here. More interestingly, on SEM

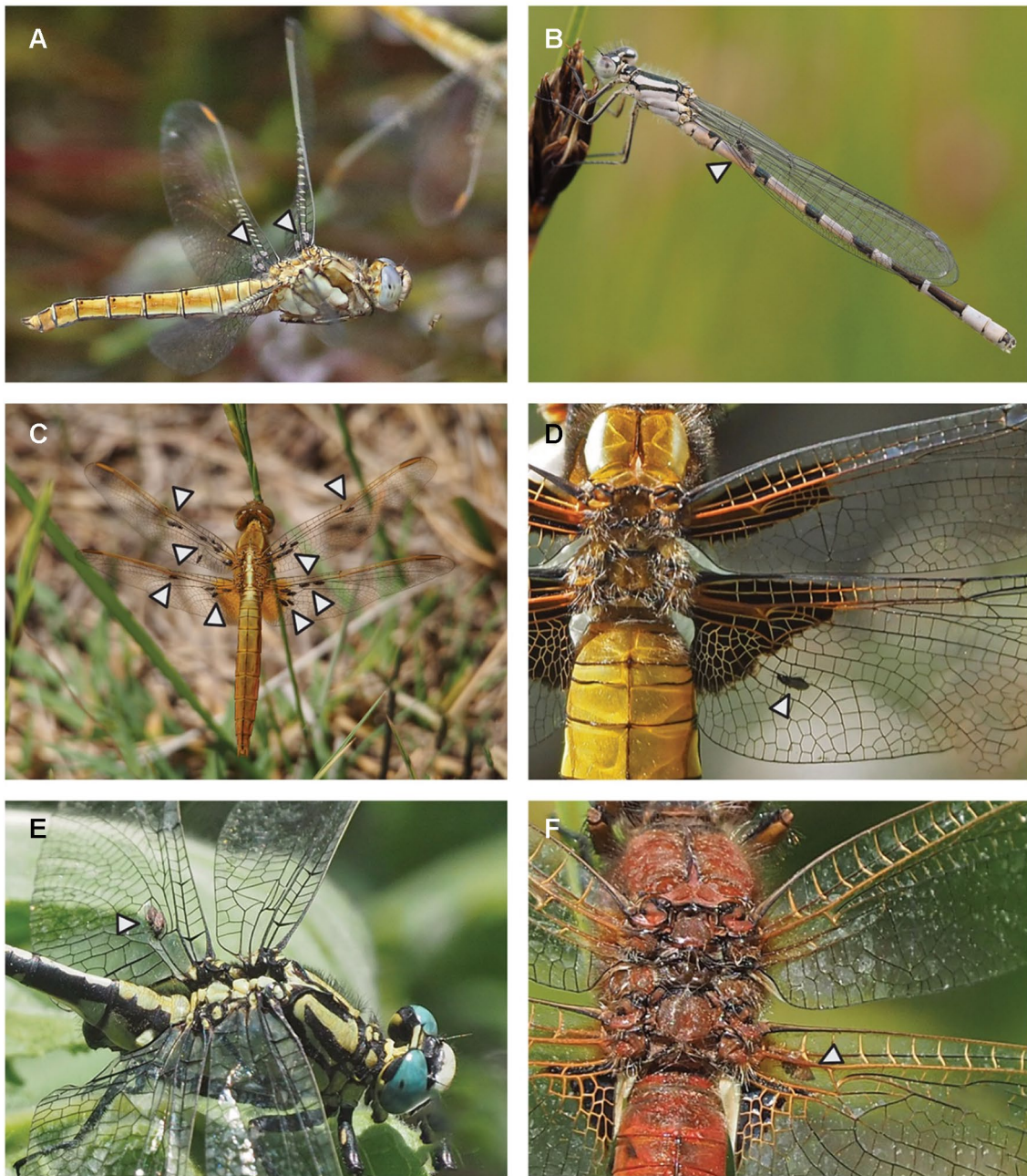


Fig. 1 Biting midges (*Forcipomyia paludis*) on (arrows) the wings of different species of Odonata. **A** Adult female of *Orthetrum brunneum* (Fonscolombe, 1837) in flight with midges on the wing bases. **B** Teneral male of *Enallagma cyathigerum* (Charpentier, 1840) with one midge on the wing. **C** Adult female of *Crocothemis erythraea* (Brullé, 1832) infested with an exceptionally high parasite load. **D** Adult female of *Libellula depressa* (L., 1758) with one biting midge,

the mouthparts of it attached to a longitudinal vein of the anal loop of the right hindwing. **E** Adult male of *Gomphus schneiderii* (Selys, 1850) with one biting midge on the cubital vein of the dorsal wing side. **F** Adult male of *Libellula pontica* (Selys, 1887) with one midge parallel to the R+M-vein of the ventral wing side. Photo credit: Hanns-Jürgen Roland (1A), Hans Jörg Müller (1C) and HW (1B, D-F)

images (Fig. 5A, B, E), crystal-like remains of the wax coating of the wing cuticle, mixed together with some solidified fluid (presumably dragonfly hemolymph or/and midge saliva), can be found rosette-shaped around the proboscis. The wings of Odonata are covered with wax

to, for example, decrease wettability, allow for coloration or maybe influence the wing mechanics (Gorb et al. 2000, 2009). This crystalline wax layer is removed from the wing during the retraction of the midges' proboscis as a leftover after sucking hemolymph from the vein. The

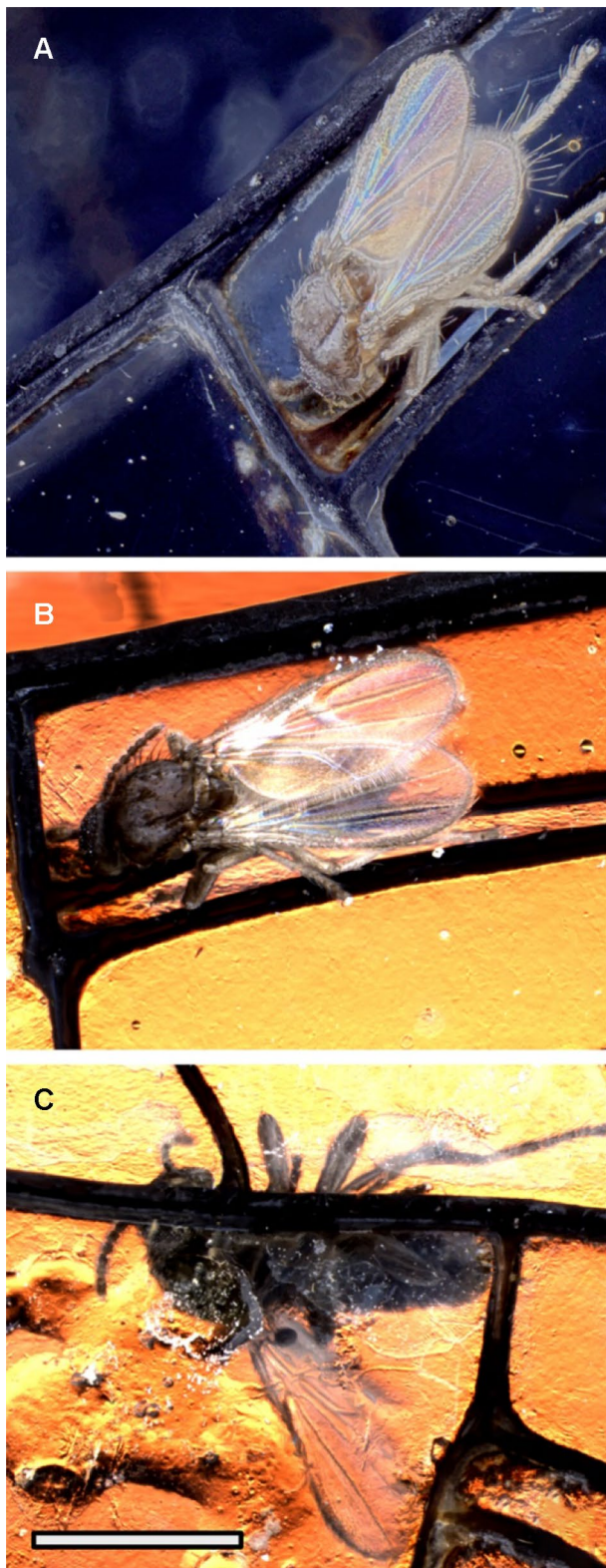


Fig. 2 *Forcipomyia paludis*, dead dried individuals that remained attached in contact with the wings of *Cordulegaster boltonii* (images taken in binocular microscope at different illuminations). Scale bar: 1 mm

μ CT-data show the proboscis of *F. paludis* inserted into the wing vein to suck hemolymph (Fig. 4). The proboscis has presumably not only the function of sawing the odonate cuticle and sucking hemolymph, but additionally reinforces the attachment of the midge to the dragonfly's wing. The parasites can stably stay on the wing during the dragonfly's flight (Gorb et al. 2022), when not only strong turbulences may generate drag on the midge body, but also wing vibration may potentially cause a strong challenge to stay attached just by the action of the tarsal attachment devices. Especially, the attachment must be difficult on the nanostructured super-hydrophobic surface of the odonate wing (Kuitunen et al. 2014; Šigutová et al. 2020) as it was previously shown for representatives of other Diptera and Coleoptera (Niederegger et al. 2002; Peressadko and Gorb 2004; Voigt et al. 2008). Notably, already Mayer (1936), having studied the mouthparts of *F. odonatophila* (Macfie 1936) from New Guinea, supposed that dried hemolymph would cement the midges' mouthparts to the odonate's wing. In general, the attachment forces must be very high, since *F. paludis* can easily resist forces caused by the flapping wings of the flying dragonflies (Fig. 1C); even during turbulent intraspecific aerial fights (Gorb et al. 2022). If there is an interlocking-like effect of the mouthparts involved—because of the piercing itself—or if the attachment forces are generated by the foot morphology remains unclear.

It is not evident yet at what stages of the adult life the hosts are infested. Some photos with attached biting midges show teneral dragonflies (e.g., Fig. 1D; Vinko et al. 2017; Fig. 2; Cordero-Rivera et al. 2019; Fig. 2). On most of the photos, however, the dragonflies are mature. Even aged individuals are documented to be parasitized (Fig. 1F, Vinko et al. 2017; Fig. 2). As the midges generally detach from their host when they are saturated (Wildermuth and Martens 2007), it is supposed that also old dragonflies may be infested. In one case it was documented that a biting midge inserted its mouthparts into the still soft integument of a teneral *Cordulia aenea* (Linnaeus, 1758) (Wildermuth and Martens 2007, plate IV), and in another case an individual of *Onychogomphus uncatatus* (Charpentier, 1840) was infested by several biting midges at emergence (Cordero-Rivera et al. 2019). The midges were seen to sting the abdomen and the eyes, but not the wings as these are not unfolded yet. However, after emergence, the wings would be ideal for hemolymph sucking, as at other body parts, such as head and abdomen, the midges would be more likely wiped off by the cleaning movements of the host's legs. However, there are records that other *Forcipomyia* species are able to suck hemolymph from parts of the thorax or head of odonates (Trapero-Quintana et al. 2019).

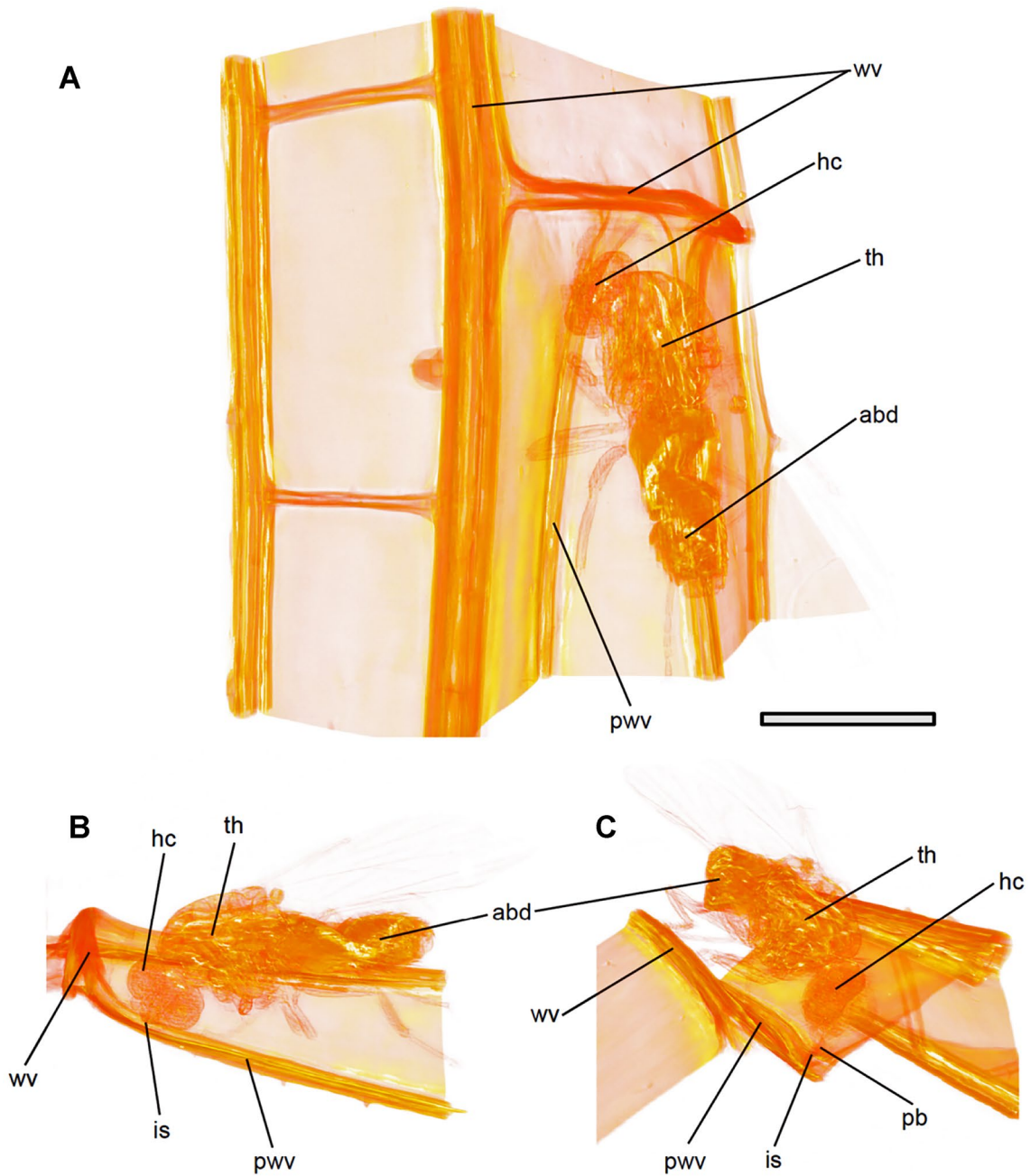


Fig. 3 3D-Volume rendering of *Forcipomyia paludis* from X-ray microtomography data. **A** Dorso-lateral overview. **B** Lateral view with penetrated wing vein. **C** Frontal view with penetrated wing vein.

abd abdomen, *hc* head capsule, *is* injection site, *pb* proboscis, *pwv* penetrated wing vein, *th* thorax, *wv* wing vein. Scale bar: 0.7 mm.

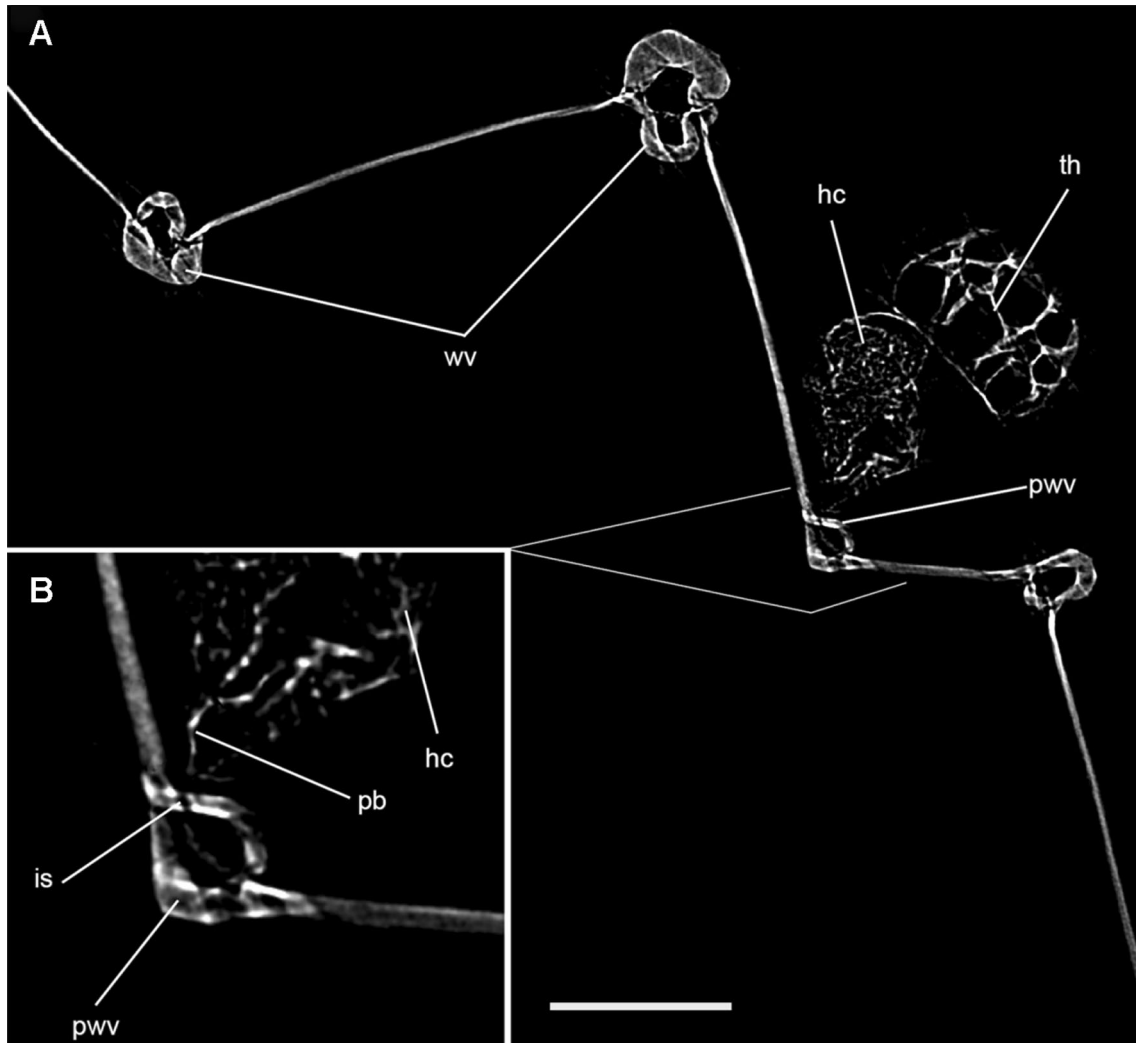


Fig. 4 2D–Cross section of *Forcipomyia paludis* that has penetrated the wing vein of the dragonfly *Cordulegaster boltonii* (X-ray tomography data). **A** Overview. **B** Detail with injection site. *hc* head cap-

sule, *is* injection site, *pb* proboscis, *pwv* penetrated wing vein, *th* thorax, *wv* wing vein. Scale bar: 0.35 mm.

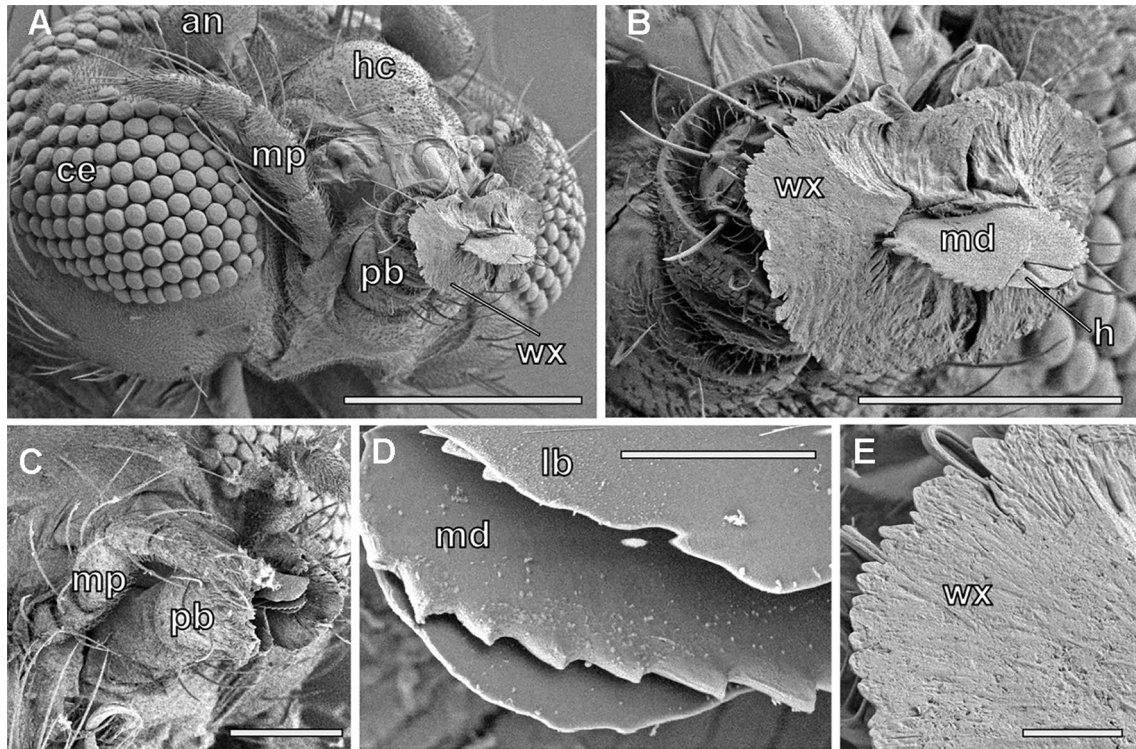


Fig. 5 *Forcipomyia paludis*, head with some details of the mouthparts (SEM images). **A** A general view, with wax from an odonate's wing. **B** Details of the proboscis with wax 'rosette'. **C** Proboscis without wax. **D** Details of the mandible **E** Details of the wax. *an* antenna,

ce compound eye, *h* hypopharynx, *hc* head capsule, *lb* labium, *md* mandible, *mp* maxillary palp, *pb* proboscis, *wx* wax mixed together with some solidified fluid (from the odonate's wing). Scale bars: A 100 μ m; B, C 50 μ m; D, E 5 μ m

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Author contributions SB, HW and SNG: designed the project and developed the concept of the study. SNG: did the SEM analysis and post-processing. SB: carried out the CT: analysis and post-processing. HW: did the field photography. SB: did the post-processing of the field studies photographs. SNG, HW and SB: wrote the original manuscript. All authors edited the manuscript as well as read and approved the final version.

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Data availability All data supporting our findings are presented in the paper and the supplementary material respectively. The raw data can be made available on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest We declare that we have no known competing interests.

Consent to participate and publication We all agree(ed).

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