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Tarsal attachment structures of the biting midge *Forcipomyia paludis* (Diptera: Ceratopogonidae), a specialized ectoparasite of Odonata imagines

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Abstract

The female of the biting midge *Forcipomyia paludis* is a dipteran ectoparasite of West Palaearctic damselflies and dragonflies, sucking haemolymph mainly from wing veins of their hosts. This tiny midge remains firmly attached to the wings even during fast flight and aerial fight maneuvers as shown in the present paper by field studies of the large dragonfly, *Cordulegaster boltonii*. Since individuals of *F. paludis* firmly attach themselves to the challenging wing surface of their host and can successfully withstand drag and vibrations during flight, we assume that this midge species has specific microstructural adaptations on its legs for attaching to the wing surface. In our morphological study, we used scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), to study the structure of *F. paludis* tarsi, as well as the micro morphology of the wing surfaces of their host. Additionally, for the first time, we were able to show attachment devices of the midges dried out in contact with the host's surface. The spatulae of the plantar setae and especially the empodial setae, are capable of replicating nanoscale wax crystals of the super hydrophobic wing coverage of the dragonfly wing membrane, in order to increase an effective contact area and therefore adhesion. This ability requires extremely soft materials of the spatula, which seems to be rather unique even in comparison to the leg attachment devices of other dipterans and other insect taxa in general.

Keywords Biting midge · Parasitism · Zygoptera · Anisoptera · Attachment · Adhesion · Tarsus

Introduction

Female biting midges (Diptera: Ceratopogonidae) are well known to require blood/haemolymph from vertebrates and/or invertebrates to obtain protein for egg production. Among the more than 6000 known ceratopogonid species, a number of them parasitize arthropods such as millipedes, spiders and insects, in order to feed on their haemolymph (Borkent and Dominiak 2020). Their mouthparts are typically well-developed for cutting the integument of its host by saw-like protuberances (Krenn and Aspöck 2012). A few ceratopogonid species, representatives of the genus *Forcipomyia*, have specialized on damselflies and dragonflies (Odonata) generally attaching to the wings and most likely taking haemolymph from the wing veins (Wildermuth and Martens 2007; Büsse et al. companion). *Forcipomyia*-species are known from Odonata from both the Old and New World (e.g., Macfie 1932; Orr and Cranston 1997; Naraoka 1999; Martens et al. 2007; Guillermo-Ferreira and Vilela 2013). One of them, *Forcipomyia paludis* (Macfie 1936), is a widespread biting midge in the Western Palaearctic that occurs between the British Islands and Morocco in the west and the Caucasus in the east (Boudot et al. 2019; Wildermuth 2012; Wildermuth et al. 2019). Currently, more than 80 odonate species are known to host this parasite (Wildermuth 2021). The average parasite load on a single host is rather low (Martens et al. 2007; Vinko et al. 2017), however, in one exceptional case more than 170 midges were found attached to the wings of a teneral *Libellula quadrimaculata* (Clastrier et al. 1994). Most midges cling to the basal quarter of the wings, sucking from the lower veins of the corrugated wings (Manger 2021).

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In general, biting midges are able flyers and infest their host in an imaginal stage during the host's resting phases. There are documented cases that the female *F. paludis* infest their host during or shortly after emergence – i.e. ecdysis from larva to adult – when the cuticle is still soft (Wildermuth and Martens 2007; Cordero-Rivera et al. 2019; Wildermuth and Martens 2019: 229). In this situation the midges may also attach to other body parts such as the eyes or the abdomen. However, in adult dragonflies and damselflies, *F. paludis* cling almost exclusively to the wings (Martens et al. 2007) especially in mature odonates that are infested (e.g. Wildermuth and Martens 2007). However, there are accounts, that other *Forcipomyi*a species also infest parts of the thorax or head of odonates (Trapero-Quintana et al. 2019).

By direct behavioral observations (Wildermuth and Martens 2007) and morphological investigations (Büsse et al. companion) of female midges inserting their proboscis into the host's wing veins it became evident that *F. paludis* is an active ectoparasite, although phoresis may not be excluded completely as suggested by Dell'Anna et al. (1995) for *F. paludis* and Orr and Cranston (1997) for related species.

Highly specialized ectoparasitic species adapted to insect hosts in general, and for *F. paludis* Odonata imagines as powerful flyers in particular, often show strong morphological adaptations to such a parasitic lifestyle (cf. Jandausch et al. 2018; Petersen et al. 2018; Rebora et al. 2018; Büscher et al. 2021). The functional morphology of its feeding organs, sensory equipment and especially the attachment devices might help to shed light on the biology of this midge species. In this respect the micromorphology of the tarsi would be of special interest. Hitherto, corresponding structures have not been studied in detail and not in respect to the attachment of the parasite to its hosts (Macfie 1936; Cordero-Rivera et al. 2019). Since individuals of *F. paludis* are able to attach themselves strongly to the challenging wing surface of the host and can successfully withstand drag and vibrations during dragonfly flight, we assume that this midge species show specific microstructural adaptations for attachment on their legs. The surface of adult dragonflies, especially including their wings, is covered by nanoscale crystalline wax (Gorb et al. 2000, 2009), which usually makes surfaces super hydrophobic (Kuitunen et al. 2014; Šigutová et al. 2020) and rather 'unsticky' (Peressadko and Gorb 2004; Gorb et al. 2010; Purtov et al. 2013; Gorb and Gorb 2017; Rebora et al. 2020).

In the present paper, we used photographic documentation of field observations in order to provide evidence that the midges cling firmly to the host's wings during forward flight and intraspecific fights. As host species we chose *Cordulegaster boltonii* (Donovan, 1807), a large anisopteran species typically found in small running waters. We deployed scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) to study the tarsal structure of *F. paludis* and the micromorphology of wing surface of one of its hosts. We discuss the results in comparison to the morphology of attachment devices of other representatives of Diptera and in the context of the parasitic lifestyle of *F. paludis*. For the first time we show special attachment devices of the midges dried out in contact with the host's surface, enabling us to formulate functional hypotheses about the adhesion mechanism of this midge species. Therewith, we could underline the behavioral observations indicating a truly parasitic lifestyle to substantiate the role of *F. paludis* as an ectoparasite of Odonata.

Materials and methods

The field studies were conducted in July 2013 and 2019 at the Tüfenbach, a small forest brook near Uster, Switzerland (47°18′49′′N, 08°44′30′′E), and additionally on narrow fen ditches near Hinwil, Switzerland (47°17′53′′N, 08°48′48′′E). On sunny days the study site at the Tüfenbach was sunlit for about two hours in late forenoon. There, the male members of *Cordulegaster boltonii* patrolled regularly up and downstream, sometimes getting in intraspecific fights when two males encountered one another. In order to induce the males for a stopover we put a thin wooden stick 80 cm long at the sunlit water's edge. While some males just passed by when crossing the small forest clearing, others did stop and used the offered perch for a short rest. All perched individuals were photographed, whenever possible, from the dorsal side using a digital camera (Nikon D7100, Macro objective 105 mm f/2.8) with a special focus on biting midges present on their wings. All dragonfly individuals were identified by their wing vein pattern, especially by the anal triangle. This enabled us to compare the parasite load pattern of every individual documented two or more times; having been flying in between.

Specimens of *Forcipomyia paludis* (Macfie, 1936) were collected from the wings of *Cordulegaster boltonii* at the localities mentioned above. In general, the midges detach from the host's wing when the dragonflies are captured with a sweeping net. To prevent the midges from escaping, a fine-meshed net was used (cf. Schröter 2021). Occasionally single midges remain attached to the dragonfly's wings (Wildermuth and Martens 2007). Few samples of *F. paludis* specimens still attached to wing fragments of *C. boltonii* were preserved for microscopic studies. Samples of 'solo' *F. paludis* were stored in ethanol.

For scanning electron microscopy (SEM) midge specimens stored in 70% ethanol were dehydrated in an ascending ethanol series and critical-point-dried using an automatic Leica EM CPD300 (Leica, Wetzlar, Germany). Dry odonate wings with midges attached were carefully dissected
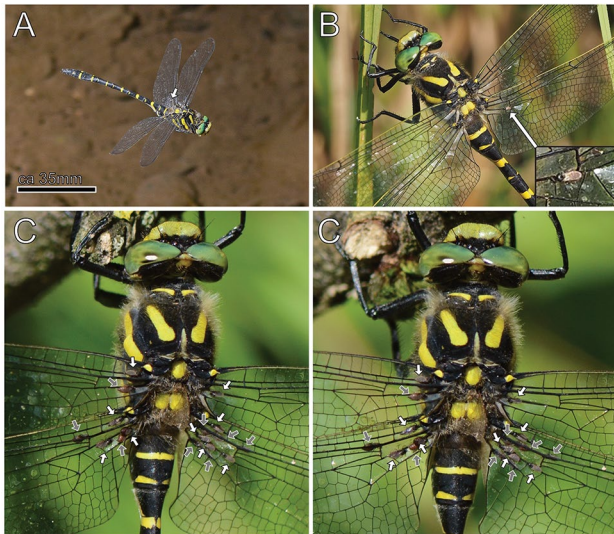
Fig. 1 Male *Cordulegaster boltonii* infested with female *Forcipomyia paludis* at the study site. **A** Patrolling male in flight with 1 midge on the left hindwing (arrow). **B** Perched male with 1 midge on the right hindwing (arrow and enlarged inset). **C–D** Male (M4) of *C. boltonii* perched on 18-vii-2013 at 11:13 h (**C**) and 11:16 h (**B**) with a ca 3 min patrol flight in between (solar noon at 13:40h). The dragonfly individual is identified by the vein pattern of the anal triangle on the left and right hind wing. In total, 17 midges are attached to the wings, 9 on the dorsal side (white arrows) and 6 on ventral side (grey arrows) of the wings. The infestation pattern of the midges remained unchanged during the flight

and mounted on the aluminum holders using double-sided adhesive conductive tape. The respective specimens were sputter-coated with a 10 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 confocal laser scanning microscopy (CLSM) midge specimens stored in 70% ethanol were dissected and their tarsi were embedded in glycerin (99.8%). The scans were conducted using a Zeiss LSM 700 (Carl Zeiss Microscopy GmbH, Jena, Germany), with the wavelengths 405, 488, 555 and 639 nm and emission filters BP420–480, LP490, LP560, LP640 nm (cf. Fig. 1 in Büsse and Gorb 2018). The resulting data was combined into a maximum intensity projection using the program ZEN 2008 (www.zeiss. de/mikroskopie). CLSM in this application makes use of the autofluorescence of the insect cuticle. The mixed fluorescence signals, detected in each pixel, are compiled and visualized in a maximum intensity projection. This allows determining regions of the cuticle with certain auto fluorescences corresponding to different cuticle components. The observed material compositions can generally be subdivided into three main groups (after Michels and Gorb 2012; Appel and Gorb 2014; Michels et al. 2016): (1) sclerotized cuticle showing red auto fluorescence; (2)

strong but relatively flexible, chitinous areas showing a range of color through red over green to blue; (3) mostly flexible materials, such as resilin-bearing cuticle, are characterized by a violet or blue color. In the latter case, we refer to the observed areas of blue auto fluorescence as "resilin-supplemented", leaving room for interpretation on other components of the cuticle.

Results

Photographical field study

Biting midges could only be photographically documented once on flying dragonflies (Fig. 1A), yet clearly detected when perched (Figs. 1B–D). They were mainly found on or in the vicinity of the longitudinal veins at the base of the wings, on both the upper and lower side. They clung mostly close to the wing base on the longitudinal wing veins in the valleys of the wing corrugations, usually with the head directed towards the dragonfly's thorax. The legs were generally spread out radially from the midge's thorax, the tarsal end is mostly in contact with the wing membrane, sometimes with the wing vein itself. However, due to insufficient digital resolution, this was not always clearly seen. Furthermore, the legs were sometimes covered by other body parts.

Within 300 min total observation time at the study site Tüfenbach on 18-vii-2013, 21-vii-2013 and 27-vii-2013 altogether 37 different male individuals of *Cordulegaster boltonii* stopped at the implanted perch. These individuals could be photographed and identified, while others just passing by were not registered. In total, 59 perch events were documented for these 37 individuals, i.e., 25 individuals perched only once while 12 males perched two or more times: 6 individuals twice, 4 individuals three times, 1 individual four times and 1 individual six times (Table 1). The period between the first and the last perch event per individual amounted to 3 min at the least and 112 min at the most, respectively. The parasite load varied between 4 and 17 midges per individual. In eight cases with two or more recorded perch events the number and the position of the midges remained unchanged (Fig. 1C, D and supplemental Fig. 1). In one case (M1 21-vii-2013) two midges came in addition between the second and the third perch event, and in two cases (M3 and M11 21-vii-2013) one additional midge settled on the dragonfly's wings. In only one case the dragonfly (M10 27-vii-2013) lost one midge during the patrol flight.

Table 1 Timespan between first and last perch event of 12 different parasitized individuals of male *Cordulegaster boltonii* that were recorded two or more times at the observation spot on three different days

Date, Nr	Maximal para-site load (n)	Perch events (n)	Timespan (min)	Change of para-site load	Change of position
18-vii.M4	17	2	3	No	No
21-vii.M1	10	3	112	+2	No
21-vii.M3	14	4	77	+1	No
21-vii.M9	9	6	34	No	No
21-vii.M10	11	2	16	No	No
21-vii.M11	11	2	3	+1	No
21-vii.M12	12	2	6	No	No
27-vii.M1	17	3	5	No	No
27-vii.M10	7	3	10	−1	No
27-vii.M11	17	2	34	No	No
27-vii.M13	6	2	38	No	No
27-vii.M15	4	3	38	No	No
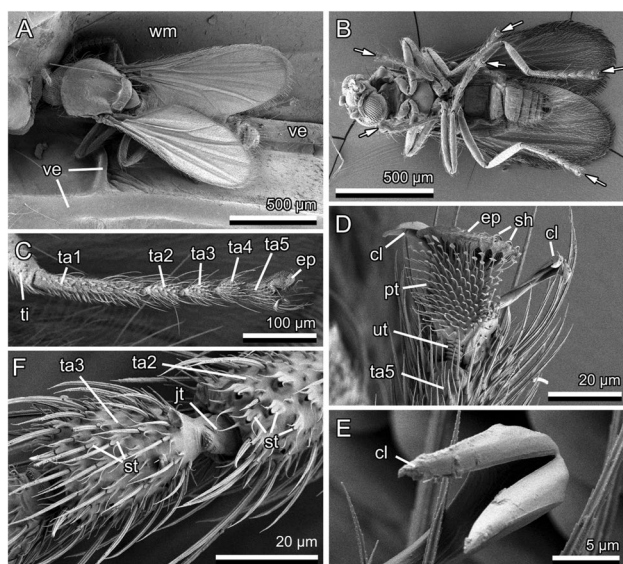
Fig. 2 *Forcipomyia paludis*, SEM images. **A–B** Dead dried out individuals that remained attached in contact with the wings of the dragonfly *Cordulegaster boltonii*. **A** Dorsal aspect of the midge attached to the wing (the base of the wing on the left side of the image). **B** Ventral aspect of the midge (removed from the wing) with the native position of the legs and tarsi (arrows). **C** Tarsus. **D** Pretarsus with attachment devices. **F** Ventral side of the second and third tarsomere. **E** Bifurcated claw. *cl* claw, *ep* empodium, *jt* joint between tarsomeres, *pt* planta, *sh* setal shafts; *st* stoppers of tarsal setae, *ta1–ta5* first to fifth tarsomeres, *ti* tibia, *ut* unguitractor plate, *ve* wing vein, *wm* wing membrane

(b) Morphological study

The tarsi of *F. paludis* consist of five tarsomeres. The longest one is the basitarsus (ta1) (150–200 μm), ta2–ta5 are much shorter having rather similar length (30–50 μm). The tarsomeres are strongly covered by cuticular outgrowths of three types. Rather thick spines are situated ventrally at the distal side of each tarsomere except from the terminal one (Fig. 5A–C). Smaller setae (cuticle outgrowths with mobile sockets at the connection to the tarsal cuticle) of varying length with longitudinal grooves and cuticular stoppers at the basis (Fig. 2F) densely cover all sides of all tarsomeres (Fig. 2C–F). Between these setae, short acantae (cuticle outgrowths without mobile sockets) are situated.

The pretarsus consists of: (i) an unguitractor, (ii) the claws, (iii) the plantae and (iv) the empodium (Fig. 2D). The two latter structures (iii and iv) are covered by tenent (adhesive) setae terminated with spatula-like compressed and widened tips. The claws are strongly curved and bifurcated; showing a narrow gap between these two parts (Figs. 2D, E; 3A, B). In lateral view, the plantae are laterally slightly widened towards the pulvilli, resembling lobes and the empodium is connected to the pretarsus via a joint (Fig. 3A–B, D).

The tenent setae on the planta and the empodium are similar in their length (7–10 μm), but show rather strong differences in the structure of their distal regions. Plantar setae are terminated with the spatula with a width of 0.7–1.0 μm (Fig. 3E), whereas empodial spatulae are much wider (5 μm) and are fused into a continuous adhesive surface (Fig. 3C, G). The setal shafts are slightly compressed at their bases with specific uniform orientation of compression planes (Fig. 3D, F). Such a compression of cross-sectional shapes of setae is responsible for an anisotropic bending stiffness of the setae; stiffer in lateral-medial direction and more flexible forwards-backwards. The surfaces of the empodial spatula are slightly corrugated, which is an indication of the soft material they consist of (Fig. 3G).

We were able to study the attachment devices of several specimens of *F. paludis* that remained attached to the wing surface of the dragonfly (*n* = 3) and dried out in contact with the wing surface (Fig. 4). The most distant empodial setae
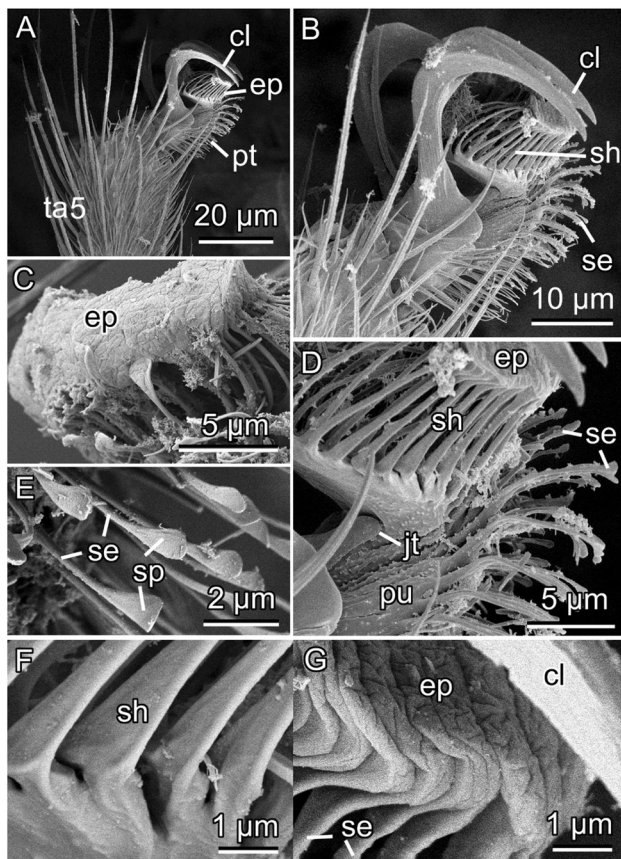
Fig. 3 *Forcipomyia paludis*, SEM images of the pretarsus and attachment devices. **A–B** Lateral view of the pretarsus. **C** Empodium, adhesive surface composed of enlarged spatula of the empodial pulvillus. **D** Transition between empodium and pretarsus. **E** Setae of the planta lobes (pulvilli). **F** Basal region of the shafts of empodial setae. **G** Distal region of empodial setae with spatula that are adhered together and compose adhesive surface. *cl* claw, *ep* empodium, *jt* joint between empodium and pretarsus, *pu* plantar lobe (pulvillus), *pt* planta, *se* setae, *sh* setal shafts, *sp* spatula, *ta5* fifth tarsomere

are fused together with the spatula (Fig. 4F). Their surface profile is showing strong differences (black and white arrows in Fig. 4): (i) spatulae with no contact to the surface of the wing (exposed to the air), showed a wavy corrugated pattern (Fig. 4E), (ii) spatulae with contact to the surface of the wing, showed an extremely corrugated fine grain pattern (Fig. 4B–D). The dimensions of fine depressions in the surface of the spatula correspond to the dimensions of crystalline wax-protuberances on the wing membrane/veins surface (Fig. 4G), which means that nanoscale depressions represent replicas of the wax crystals. The hidden parts of spatulae are rather smooth, because they are dried in contact with the back surface of neighbouring spatulae (Fig. 4C–D).

The CLSM results show details of the material composition of the tarsal cuticular structures of the midge (Fig. 5). The tarsomeres demonstrate rather stiff sclerotised cuticle, which is shown in an orange-reddish autofluorescence (Fig. 5A, C). A similar type of cuticle is observed in the ventral spines of tarsomeres ta1–ta4 and in the claws. Between the tarsomeres, in the joint regions, there are regions showing a bluish autofluorescence, corresponding to a soft resilin-enriched membranous cuticle (Fig. 5A, C).

The fused empodial spatulae are bluish and can therefore be considered as resilin-rich. The same holds true for the spatula of plantar setae (Fig. 5A, B, D). However, the spatular shafts in empodial and plantar regions are yellowish-greenish, which is an indication of less sclerotised chitinous material with lesser amount of resilin in comparison to that of the spatulae (Fig. 5B, D).

Discussion

The firm attachment of the biting midge *Forcipomyia paludis* during flight activities of its host has hitherto been presumed (Wildermuth and Martens 2019: 871) but not evidenced. In the present study on *Cordulegaster boltonii* as host species we could show for the first time that the ectoparasitic midges are not flung away even during different flight manoeuvres such as forward flight or vigorous intraspecific fights typical for this dragonfly when patrolling a linear water course (Kaiser 1982). Furthermore, it was documented in three cases that biting midges likely infest dragonflies also during the adult stage and not only during or after emergence as suggested by Cordero-Rivera et al. (2019). In only one case one single midge was lost within the timespan between the second and third perch event of the dragonfly. Presumably, this midge was saturated and therefore disengaged from the dragonfly's wing (cf. Wildermuth and Martens 2007).

The *F. paludis* individuals usually select sites near the thorax on or in very close proximity to the wing veins in the concave parts ('valley') of the wing corrugations as shown in different host species (Martens et al. 2007; Manger 2021). (Fig. 2A). This site selection together with head facing position to the wing base (Martens et al. 2007; Manger 2021; Büsse et al. companion) likely reduce drag caused by the flapping wing during the host's flight (Fig. 1). Tarsi of the midge are rather difficult to observe from above, because they are usually hidden below other body parts including the midges' wings, but are usually spread out radially from the center of the body mass (Fig. 2B). This posture may provide rather isotropic resistance to the shear forces that can potentially act from different directions especially during flight.

Presumably, this site selection and body posture also allows for easy piercing of the vein at the sites with softer cuticle (Appel and Gorb 2014; Büsse et al. companion). Attachment forces of midges must be very high, because *F. paludis* can easily resist forces caused by the flapping wings of the flying dragonflies (Fig. 1). We, therefore, assume that in addition to the action of tarsal attachment devices, an

Fig. 4 *Forcipomyia paludis,* SEM images of attachment devices that have been dried out in contact with the wing of the dragonfly *Cordulegaster boltonii.* **A** Overview of the pretarsus, ventral aspect. **B–E** Details of the empodium surface. **F** Empodium, dorsal aspect. **G** Surface of the wing membrane of the dragonfly *C. boltonii,* tiny wax crystals are visible. White arrows, spatula surface dried not in contact with the wing surface. Black arrows, spatula surface dried in contact with the wing surface. *cl* claw, *ep* empodium, *ex* exposed area of the spatula, *hd* hidden area of the spatula, *pt* planta, *sh* shaft of the seta, *sp* spatula
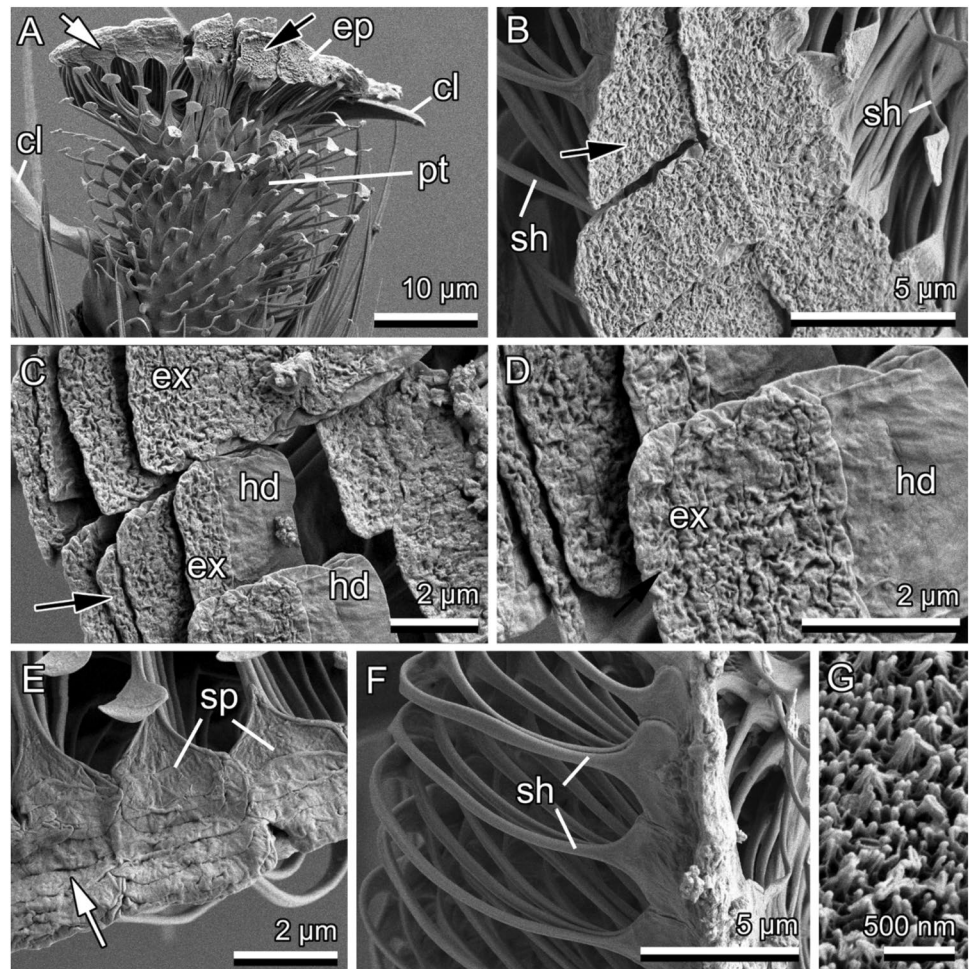
interlocking-like effect of the mouthparts with the vein cuticle might be involved (Büsse et al. companion).

The tarsal end of *F. paludis* with the deeply cleft claw is typical for this species, and the bottlecap-like empodium has been visualised by Macfie (1936) and Cordero-Rivera et al. (2019), however, without detailed morphological description and morpho-functional interpretations. This gap is filled now by the present study. Tarsal attachment devices of *F. paludis* consist of several parts working in concert: (1) five tarsomeres richly covered by the cuticular protuberances, (2) bifurcated claws, (3) plantae covered by tenent setae with flattened spatulate tips, and (4) empodium with setal spatulae fused together at their tips. On the tarsomeres, setae with stoppers at the setal base in the vicinity of sockets might be of major importance. The stoppers lead to an increase of the bending rigidity of setae and may contribute to the enhancement of friction/interlocking with the coarse roughness of the substrate. The same function may be expected from the rigid spines situated ventrally at distal margins of ta1–ta4.

The bifurcated claws of *F. paludis*, having a narrow gap between the two parts are rather similar to the comb-like claws of spiders using these structures for grasping of web threads or draglines (cf. Gorb and Barth 1994). Many plant-associated Coleoptera rely on bifurcated claws, in order to attach to the plant surfaces covered by trichomes (Salerno et al. 2020). Also parasitic flies from different dipteran lineages (Hippoboscidae and Braulidae) have similar solution for attachment on the hairy surface of their vertebrate or insect hosts (Liu et al. 2018; Petersen et al. 2018; Büscher et al. 2021). In general, this kind of hairy substrates, cannot be considered either smooth or rough due to its structural and mechanical specificity. They represent a rather specific kind of substrate that often requires strong specialisations from attachment devices to cope with.

The results of the present study show that in comparison to all other previously studied dipterans, *F. paludis* exhibit the most strongly specialised pretarsal structures for adhesion. First, the lateral plantar lobes (pulvilli) are strongly reduced in comparison to the majority of flies, but there is much stronger coverage of the plantae by tenent setae. Second, the empodium is strongly transformed from its original form, where the fusion of spatulae led to the appearance of arolium-like medial structure between claws with a continuous smooth adhesive surface. Still
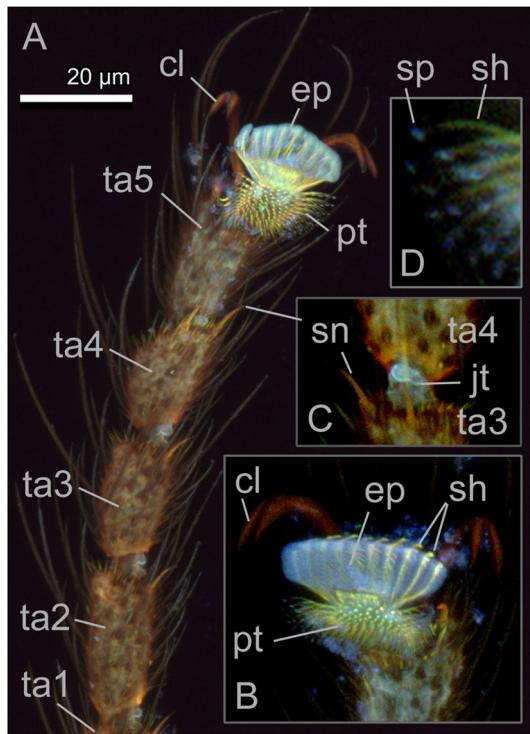
Fig. 5 *Forcipomyia paludis,* CLSM images of attachment devices. **A** Overview of the tarsus, ventral aspect. **B** Details of the pretarsus, ventral aspect. **C** Joint between the third and fourth tarsomeres. **D** Tenent setae of the planta. *cl* claw, *ep* empodium, *jt* joint, *pt* planta, *sh* shafts of the setae, *sn* spine, *sp* spatula, *ta1–ta5* tarsomeres one to five

this surface remains consisting of discrete partially overlapping islands (spatulae), which have extremely flexible membranous layer capable of intimate contact formation with nanoscale roughness of the host crystalline wax coverage.

In other dipterans, like tipulids (Nematocera), a smooth arolium is present (Rees and Ferris 1939; Hennig 1973; Gorb 2001) and hairy pulvilli have been described in adults of other dipteran groups (Bauchhenss and Renner 1977; Bauchhenss 1979; Walker et al. 1985; Niederegger et al. 2002; Niederegger and Gorb 2003; Gorb et al. 2012; Rebora et al. 2020; Salerno et al. 2020). A lobe like, pulvilliform empodium is present as an additional adhesive structure in tabanoids (Brachycera) and Nematocera excl. Tipulidae (Hennig 1973). Brachyceran and nematoceran pulvilli show soft lobes at the pretarsal plantae and are responsible for attachment to smooth surfaces (Walker et al. 1985). They are covered by tenent setae, sometimes termed tenent hairs, which serve to increase the actual area of contact to the surface. Proximal and distal tenent setae have often different ultrastructure as in some syrphid flies (Gorb 1998), and in some groups the sexual dimorphism is present (Gorb 2001). Both the shape and size of dipteran pulvilli correlate with the weight and biology of the species (Röder 1984a; b).

The tenent setae of *F. paludis* are relatively soft structures, as known for dipterans in general. In calliphorid flies, for example, their tips are usually compressed, widened, and bent at an angle of 60° to the hair shaft (Bauchhenss and Renner 1977; Bauchhenss 1979). When walking on smooth surfaces, these hairs produce fluid secretion, which is essential for attachment (Gorb 2001; Kovalev et al. 2012, 2013; Peisker and Gorb 2012; Peisker et al. 2014), especially on rough surfaces. Some authors have hypothesised that different forces may therefore contribute to the resulting attachment force: capillary adhesion and intermolecular van der Waals forces. The action of intermolecular forces is possible only at very close proximity between surfaces. The distance decreases and the contact force increases when the contacting surfaces slide against each other (Filippov et al. 2011). This may explain why flies sitting on a smooth ceiling always move their legs in a lateral-medial direction (Wigglesworth 1987). During these movements, pulvilli slide over the surface obtaining optimal intimate contact with the substrate. This is most likely one of the main reasons why *F. paludis* females attach firmly to odonate wings also during fast flight maneuvres of the host. Furthermore, the spatulated plantar setae of *F. paludis* resemble smooth adhesive organs of orthopterans, phasmatopterans, hymenopterans, etc. (Fig. 3C, G), also known for reliable attachment.

Even more, it was previously suggested that fly setae are composed of flexible cuticle, and are able to replicate a surface profile (Gorb et al. 2001; Gorb et al. 2012). The results of freezing-substitution experiments show that the area of the setae tips becomes larger when the pulvillus is in contact with the surface. This deformation is best seen in the middle of the pulvillus, whereas on the side parts, the setae are often not in contact. The whole pulvillus becomes compressed and elongated, while contact is made (Gorb 2001). Setal spatulae of *Calliphora* are highly flexible structures that form contact with the surface by bending their tips in the distal direction. The mid-part of the spatulae is filled with a secretion that is released into the contact area in the region of contact. After initial wet contact is formed, the thin membranous central region of the endplate presumably comes into contact with the substrate due to attractive forces, acting similar to the wet thin foil, so that a solid–solid contact is presumably formed in the centre of the plate (Gorb et al. 2012). This fact might be the most important factor for the strong adhesion in *F. paludis* as the most distant empodial setae are fused together, making firm contact with the hosts' wing.

It is well known that large scale roughness affects animal adhesion less, due to the good mechanical interaction with the claws (Dai et al. 2002). In contrast to the situation on fine-scale roughness in the range of 0.1–2.0 μm (Peressadko and Gorb 2004; Voigt et al. 2008; Bullock and Federle 2011), where claws do not interlock and adhesive pads do not adhere (Wolff and Gorb 2012). Many plant surfaces,

covered with crystalline waxes having similar dimensions, can effectively reduce insect adhesion (Gorb and Gorb 2002; Gorb et al. 2014a, b; Rebora et al. 2020; Salerno et al. 2020).

A similar situation is present in *F. paludis*, here, the attachment capability must be highly reduced due to the nanostructured superhydrophobic surface of the odonate wing (Kuitunen et al. 2014; Šigutová et al. 2020). We assume that the solution for adhesion enhancement on the fine-scale roughness of the dragonfly wing is a very soft and adaptable membranous surface of the midges' spatulae. This functional adaptation in *F. paludis* partially relies on the gradient of resilin concentration in the setae, with its stronger concentration on the setal tip (spatula). Similar gradients have been previously reported in beetles and interpreted as an adaptation to contact enhancement with various substrate profiles and to the reduction of setal clusterisation (Peisker et al 2013; Gorb and Filippov 2014; Heepe et al. 2018).

We do not know much about the possible role of the fluid in setal adhesion of *F. paludis* yet. More experimental studies with living midge individuals are required. Also, the adhesion force measurements in the future will shed more light on the functionality of different parts of the midge pretarsus.

Some Ceratopogonidae spp. suck haemolymph also from other insects such as lacewings, beetles or butterflies (e.g. Lane 1977; Havelka 1979; Dobosz 1991). However, to our knowledge, no micromorphological studies on the tarsal adaptation to the attachment on the host's surface are available. Only in two African *Forcipomyia* species specific structural modifications are known, enabling the claws to attach on the butterfly's wing scales (Lane 1977). It would be worth to investigate and compare the tarsal devices of various host specific Ceratopogonidae that parasitize arthropods.

Declarations

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