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RESEARCH ARTICLE

Traction force measurements on male Strepsiptera (Insecta) revealed higher forces on smooth compared with hairy substrates

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ABSTRACT

The aim of this study was to find out how strongly the parasitic insect *Stylops ovinae*, which has tarsi equipped with tenent hairs and lacking claws, attaches to different substrates. We investigated adhesion of male *S. ovinae* to the abdomen of its hymenopteran host (*Andrena vaga*), the hairier abdomen of a *Bombus* sp. and two artificial smooth reference surfaces with different degrees of hydrophilicity. In our experiments, the male *S. ovinae* developed significantly higher forces on smooth surfaces. However, the forces were significantly lower on all the hymenopteran surfaces used in the experiment. The absence of anisotropy in the force grip in cranial/caudal direction relative to the host might indirectly indicate that *S. ovinae* generate forces by adhesion rather than mechanical interlocking with the host hairs. The tolerance of the attachment system of *S. ovinae* to the substrate chemistry might be explained by the primary contribution of van der Waals interactions and not capillary forces to adhesion in *S. ovinae*.

INTRODUCTION

Insects have evolved a number of adhesive structures on their tarsi to anchor themselves to different surfaces. In general, there are two different types of adhesive structures, smooth and hairy (Beutel and Gorb, 2001). They use different principles of contact mechanics to generate adhesive forces in noticeably different situations. By employing these structures, insects are able to walk vertically or even on the ceiling of smooth or slippery plant surfaces, to capture prey or to defend themselves against predators (Gorb, 2001). Furthermore, they may attach to their mating partners during copulation (Gorb, 2008). In the context of phoresy and parasitism, they also use their highly specialised attachment devices to attach themselves to the integument or integument derivates of other animals (Liu et al., 2019; Petersen et al., 2018). While attachment forces in various herbivorous insects on their specific host plants are comparably well studied (Eisner and Aneshansley, 2000; Gorb and Gorb, 2002; Friedemann et al., 2015; for review see Gorb and Gorb, 2017), the adhesion of phoretic or parasitic insects to their host has been measured only recently for the swift lousefly *Crataerina pallida* (Petersen et al., 2018).

Twisted-wing insects (Strepsiptera) present a small group of parasitic insects with about 600 described species worldwide

(Pohl and Beutel, 2005, 2008). The females of the vast majority of species (Stylopidia) are endoparasites of various insects, including cockroaches, praying mantises, crickets, bugs, cicadas, wasps, bees and ants. Modified forewings resembling halteres of flies, fan-shaped hindwings, and 'raspberry' compound eyes are striking features of the males (Pix et al., 1993; Buschbeck et al., 1999). Females are always wingless and free-living only in the most basal extant Mengenillidae. In contrast, females of Stylopidia (~97% of the species) are legless and extremely simplified morphologically: they expose only the sclerotized cephalothorax from the host's abdomen (Kinzelbach, 1971; Pohl and Beutel, 2005). Therefore, during mating, the males of the Stylopidia must anchor themselves to the host with their tarsi. This is the reason why males of the Stylopidia, in contrast to those of the Mengenillidae with free-living females, have specialized adhesive hairs on the ventral surface of their tarsi. The most elaborated adhesive structures are found in Stylopidae and Xenidae. Both groups are parasites of fast flying Hymenoptera (Aculeata). Stylopidae and Xenidae have four-segment tarsi. The tarsomers are distally elongated and their ventral side is occupied by a very dense layer of spatulate (*Paraxenos*, *Stylops*) or fork shaped (*Xenos*) microtrichia (Pohl and Beutel, 2004). The species studied in this paper, *Stylops ovinae* Noskiewicz and Poluszyński 1928 (Stylopidae) (Fig. 1), is a parasite of *Andrena vaga* Panzer 1799 (Hymenoptera, Andrenidae). Interestingly, stylopized individuals of *A. vaga* have a much denser coat on their abdomen than uninfested individuals (Brandenburg, 1953; Ulrich, 1956) (Fig. 2).

The aim of this study was to find out how strongly male *Stylops* adhere to different substrates. In particular, we were interested to find out whether a higher degree of hairiness leads to a lower or higher adhesion of the *Stylops* males to the substrate. We investigated the adhesion of *S. ovinae* to the host abdomen (*A. vaga*), the very hairy abdomen of a *Bombus* sp. and two artificial reference surfaces.

MATERIALS AND METHODS
Study insects

Andrena vaga parasitized by males of *S. ovinae* were collected near Osnabrück in the sand pit Niedringhaussee (Germany), in February 2015 by H.P. Until the dissection of the male puparia, the bees were kept dark at ~5°C in glass vessels (0.5 liters) closed with gauze and half filled with moist sand. In order to document the different degrees of the hairiness on the abdomen of uninfested and stylopized *A. vaga*, 10 well-preserved female individuals were photographed for each. The insects were photographed as described in Tröger et al. (2019). The images were used to measure the distance between the hairs of abdominal tergites II to IV of 10 stylopized and 10 uninfested individuals. These are the main areas where the *Stylops* males attach to the host during mating. Ten measurements were taken in the middle of each tergite using Adobe Photoshop 2020 (Adobe Systems Incorporated, San Jose, CA, USA). Uninfested *A. vaga* were collected at the Dümmer near

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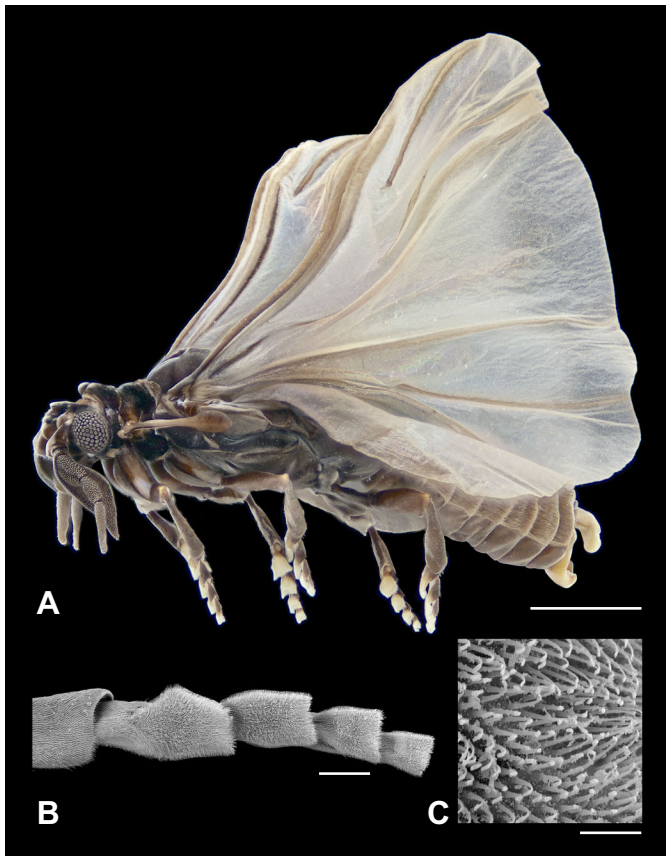H.P., 0000-0002-7090-6612; S.N.G., 0000-0001-9712-7953

Fig. 1. Adult male *Stylops ovinae*. (A) Lateral view. SEM of (B) prothoracic leg in ventral view and (C) adhesive microtrichia on tarsomere 2. (A modified from Pohl and Beutel, 2013; B,C modified from Pohl and Beutel, 2004). Scale bars: 1 mm (A), 100 µm (B), 10 µm (C).

Osnabrück between 16 April and 18 May 1994 by G. Hündorf. The stylopized individuals were collected near Osnabrück in the sand pit Niedringhaussee in February 2009, 2012 and 2015 by H.P.

To compare the density of hair on the abdomen of a stylopized *A. vaga* with the size of the tarsi of male *S. ovinae*, one air-dried

Fig. 2. Hairiness of the tergites of the abdomen of uninfested and stylopized female *Andrena vaga*. (A) uninfested and (B) stylopized host insect. Scale bar: 1 mm.

abdomen of a stylopized *A. vaga* was cut off with a razor blade and then mounted on a stub. The abdomen was sputter-coated with gold with an Emitech K 500 (Sample preparation division, Quorum Technologies Ltd., Ashford, UK). SEM micrographs were taken with a Philips ESEM XL30 (Philips, Amsterdam, The Netherlands).

Measurements of contact angles of an uninfested female *A. vaga* were conducted on an individual collected at the Heidesee in Halle Neustadt (Germany) on 29 April 2020 of a stylopized female of *A. vaga* collected near Osnabrück in the sand pit Niedringhaussee in February 2015 and of a *Bombus* sp. collected near Jena (Germany) in May 2020, all by H.P. The adhesion of *S. ovinae* to the host abdomen was measured on an uninfested female collected near Osnabrück in the sand pit Niedringhaussee in February 2015 and on a *Bombus* sp. collected near Jena in June 2014 by H.P.

Contact angle measurements

The two reference surfaces were a glass plate and a compact disc (Pioneer CD-R, Pioneer Optical Disc Europe S.A., Barcelona, Spain) and test insect surfaces were the posterior region of the dorsal side of abdomen in *Bombus* sp. and uninfested and stylopized *A. vaga*. Measurements of contact angles of double-distilled water (density=1.000 kg m^{-3}, surface tension=72.1 mN m^{-1}, dispersion component=19.9 mN m^{-1}, polar component=52.2mN m^{-1}; Busscher et al., 1984) on the two reference smooth surfaces and the insect surfaces were conducted by applying a high-speed optical contact angle measuring device OCAH 200 (DataPhysics Instruments GmbH, Filderstadt, Germany) according to the sessile or sessile needle-in drop methods (see Gorb and Gorb, 2006 for a detailed description of the method). We used 1 µl droplets and circle/ellipse fitting for evaluation of apparent contact angles. On each reference surface, the contact angles of 10 droplets were measured and four droplets were placed on each insect sample. In all, 32 contact angle measurements were carried out.

Traction experiments with insects

Traction experiments with tethered walking male insects were carried out to measure their attachment forces on different substrates. Force tests were performed using a force transducer MP 100 (Biopac Systems, Goleta, CA, USA) equipped with a 10 g force sensor FORT-10 (World Precision Instruments, Sarasota, FL, USA) as described in Gorb et al. (2010). Freshly hatched adults were used in experiments. For this, puparia were carefully dissected out from host *A. vaga* maintained in the refrigerator (5°C) and kept in a Petri dish laid out with a paper towel at room temperature (~23°C) until new males hatched. The further preparation of test insects was performed on a cool plate (5°C). The hind wings were cut off with a razor blade. The insects were attached to the force sensor through a thin polymer thread (5–7 cm long, 0.1 mm in diameter), produced by heating and pulling out a pipette tip (Pasteur-Plast pipet 3.0 ml Macro, 158 mm, Ratiolab GmbH, Dreiech, Germany). The thread was glued to the dorsal surface of the metathorax with a droplet of super glue (5925 Elastomer, Kisling AG, Bad Mergentheim, Germany).

Experiments were performed at room conditions (23°C temperature and 26–29% relative humidity). The experimental design included six successive force tests with each insect individual: (1) on glass plate, (2) on CD surface, (3) on the dorsal side of the *A. vaga* abdomen in the caudal direction, (4) on the latter surface in the cranial direction, (5) on the dorsal side of the bumblebee *Bombus* sp. abdomen (used as a reference insect surface) in the caudal direction, and (6) on the latter surface in the cranial direction. The order of substrates/directions was randomised. The force generated by the insect walking horizontally on

test substrates was measured. Force–time curves, where the insect stretched the polymer thread for ~5–10 s, were used to estimate the maximal traction force. We tested 22 males and conducted 132 traction tests in total.

The experimental males were individually weighed using Ultra Microbalance UMX2 and software Balance Link (Mettler-Toledo GmbH, Greifensee, Switzerland). The average mass was 1.96 mg (s.d.=0.38, N=22, min=1.5 mg, max=2.8 mg).

Statistical analysis

First, the possible effects of the insect weight on traction forces were tested for each substrate using linear regression. Second, we examined whether individual insects performed differently and whether the traction force generated by an insect depended on the surface/walking direction, by applying two-way ANOVA. Then, *post hoc* Holm–Šídák method was used in order to pairwise compare the surfaces/directions. Statistical analyses were carried out using SigmaStat 3.5 (Systat Software Inc., Point Richmond, CA, USA). If not stated otherwise, values are given as means±s.d.

RESULTS

The distance between hairs of abdominal tergite II is slightly different in uninfested and stylopized *A. vaga* individuals. In contrast, the hairiness of tergites III and IV is strongly increased in stylopized compared with uninfested females (Fig. 2, Figs S1 and S2). The distance between the hairs on tergites III and IV of stylopized *A. vaga* is on average ~44 µm (tergite III) and ~30 µm (tergite IV) in comparison to uninfested *A. vaga* with an average of ~98 µm (tergite III) and ~111 µm (tergite IV) (for detailed statistical analysis, see Appendix). The distance between the hairs on tergites III–IV in stylopized *A. vaga* is much smaller than the width of the tarsi of the *Stylops* males (86–149 µm) (Fig. S3, Table S1). The tarsi can therefore only come into contact with the hair and not with the smooth cuticle surface of the tergites. The hairiness is asymmetrical on tergite IV, as the hairs are considerably longer in the areas of the tergite under which the female cephalothorax is exposed (Fig. 1, Fig. S1). The hair of the bumblebee abdomen is so dense that the individual hairs overlap and the cuticle surface of the tergites is completely covered with hairs.

Both artificial smooth surfaces (glass and CD) showed hydrophilic properties, with the contact angles of water being 33.95±3.38 deg and 76.05±2.62 deg (n=10 for each surface), respectively. In all three insect abdomen samples, water contact angles exceeded 100 deg, indicating hydrophobic surface properties in both uninfested (101.52±10.86 deg) and stylopized (111.41 ±6.35 deg) *A. vaga* and superhydrophobic properties in the case of *Bombus* sp. (153.52±9.73 deg) (n=4 for each sample).

Stylops ovinae males generated relatively weak traction forces on the tested substrates, with mean values lower than 0.5 mN (Fig. 3). There were no correlations between the forces and weights of insects on either substrate/walking direction (ANOVA for linear regressions: P>0.05; Table 1). Although the force values seemed to be rather similar, a highly significant statistical influence of both factors (insect individual and substrate/direction) on the force values was detected (two-way ANOVA: $H_{21,131}$=10.815 for insect individuals and $H_{5,131}$=5.480 for substrates/directions, P<0.001 for both). The statistical comparison of traction forces obtained in different tests (Table 2) showed that on both artificial smooth substrates (glass and CD) insects performed better than on the abdomens of *A. vaga* and *Bombus* sp. (P<0.05). Although the glass and CD surfaces showed divergent contact angles of water, no significant difference between the force values was found here
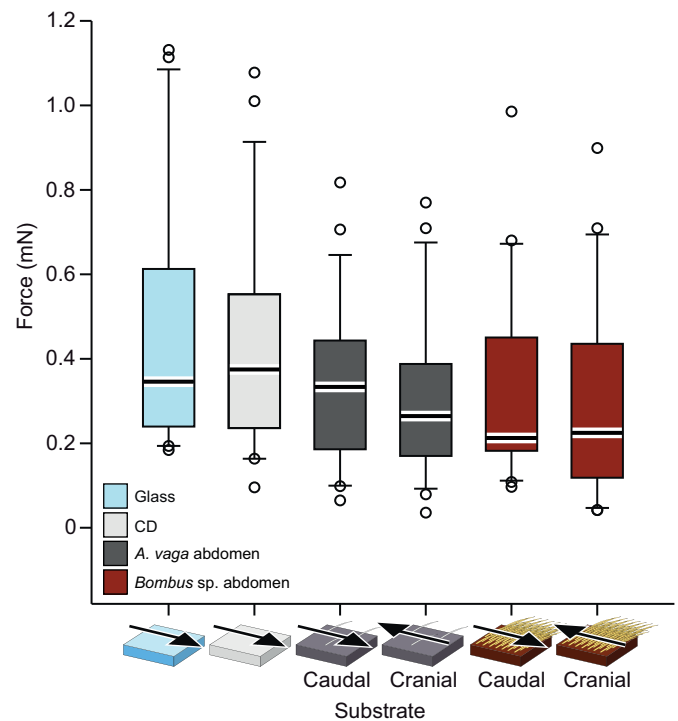

Fig. 3. Traction forces of *S. ovinae* males. Traction force was measured on a smooth glass plate (glass), compact disc surface (CD), dorsal side of *A. vaga* abdomen in the caudal and cranial directions, and dorsal side of *Bombus* sp. abdomen in the caudal and cranial directions.

(P>0.05). Also, the forces generated on different insect substrates and in different walking directions were similar (P>0.05).

DISCUSSION

The males of the Strepsiptera must either hold on to the free-living females during mating (Mengenillidae and very probably Bahiaxenidae) or to the host's abdomen where their permanent endoparasitic females are located and only protrude with their cephalothorax (Parker and Smith, 1934; Silvestri, 1941, 1943; Pohl and Beutel, 2004). Attachment to other surfaces does not play a significant role, since the males move almost only by flying (Pohl and Beutel, 2004).

During the evolution of this group of insects, the acquisition and modification of their tarsal adhesion structures played a crucial role. The five-segment tarsi of Mengenillidae and Bahiaxenidae have strong pre-tarsal claws. Specialized adhesive hairs, arolium or pulvilli are missing (Pohl and Beutel, 2004; Pohl et al., 2012) (Fig. S4). The absence of tarsal adhesive structures in the males of

Table 1. Results of ANOVA for dependence of the traction force (in mN) on insect mass (in mg) in different tests

Linear regression	Substrate	R	R^2	P
F=0.425+0.0240*M*	Glass	0.0303	0.0009	0.893
F=0.485−0.0258*M*	CD	0.0379	0.0014	0.867
F=0.465−0.0650*M*	Host caudal	0.1310	0.1310	0.562
F=0.210+0.0492*M*	Host cranial	0.0965	0.0093	0.669
F=0.292+0.0127*M*	*Bombus* caudal	0.0210	0.0004	0.926
F=0.336−0.0187*M*	*Bombus* cranial	0.0306	0.0009	0.892

Traction force was measured on insects on the dorsal side of the *Andrena vaga* or *Bombus* sp. abdomen in the caudal and cranial directions. **F**, force; *M*, mass; *P*, probability value; *R*, correlation coefficient; R^2, coefficient of determination.

Table 2. Results of pairwise comparisons (*post hoc* Holm–Šídák method) of means for the traction forces obtained in experiments with different surfaces/directions

	Glass	CD	Host caudal	Host cranial	*Bombus* caudal
CD	$t=-0.859$ $P=0.392$	–	–	–	–
Host caudal	$t=-3.036$ $P=0.003$	$t=-2.177$ $P=0.032$	–	–	–
Host cranial	$t=-3.745$ $P<0.001$	$t=-2.886$ $P=0.005$	$t=-0.709$ $P=0.480$	–	–
Bombus caudal	$t=-3.489$ $P=0.003$	$t=-2.630$ $P=0.001$	$t=-0.453$ $P=0.652$	$t=0.256$ $P=0.799$	–
Bombus cranial	$t=-3.893$ $P<0.001$	$t=-3.034$ $P=0.003$	$t=-0.857$ $P=0.393$	$t=-0.148$ $P=0.882$	$t=-0.404$ $P=0.687$

P, probability value; t, Holm–Šídák method statistics.

Mengenillidae and Bahiaxenidae is easily explained by the slow moving, ground living females of these families. To adhere to the female during mating, the unspecialized tarsi are sufficient, especially taking into account the small size of these animals. Most likely the specialized tarsal adhesive hairs on the ventral surface of the tarsi evolved with the transition to permanent endoparasitism of the females of the Stylopidia. The claws of the males were reduced, and specialized adhesive hairs evolved on the ventral surface of the tarsi. These hairs are differently shaped, ranging from spatulate (Corioxenidae, Stylopidae, Xenidae), forked (Xenidae partim) to mushroom-shaped (Elenchidae, Halictophagidae) (Pohl and Beutel, 2004). It can be assumed that these adhesive structures are adapted to the specific surface structures of their hosts.

In individuals of *A. vaga* stylopized by *S. ovinae*, the hairiness of the tergites of the abdomen is modified. This affects both male and female hosts. In both sexes, the hairiness of the tergites of the host is clearly increased (Brandenburg, 1953, see above). If the males of *S. ovinae* are better able to hold on to a hairy surface than to a smooth surface, a higher reproductive success could also be achieved. Conversely, the increased hairiness of the host abdomen could be a counter-adaptation of the host to the stylopization. We decided not to measure the forces on a stylopized bee, because of the very inhomogeneous hairiness of the abdominal tergites and to rule out the possibility of the males attaching to the smooth female cephalothorax. We used instead a homogeneously hairy abdomen of a bumblebee for our measurements.

According to field and laboratory observations, the males of *S. ovinae* walk the last few centimeters to the stylopized host bee. Climbing onto the host abdomen is always done from behind (Peinert et al., 2016). The hook-shaped penis is then firmly anchored in the female's paragenital organ. The anchorage is so firm that the male does not fall down even when the host bee is flying (personal observations of H.P., 2016). However, the initial phase of contact with the host, as long as the male's penis is not firmly anchored in the female, is a critical point before mating (Fig. 4). Video footage of *S. ovinae* mating shows that the male is better able to hold on to the smoother surface of the tergite in front of the protruded female cephalothorax. Middle and hind legs do not find support in the dense hair of the host abdomen and are therefore in constant motion (see movie 1 of Peinert et al., 2016).

In our experiments, the males of *S. ovinae* developed significantly higher forces on smooth surfaces, such as glass or a compact disc. However, the forces were significantly lower on all the hymenopteran surfaces used in the experiment. In contrast, these reduced forces were not significantly different on either the unstylopized host abdomen or
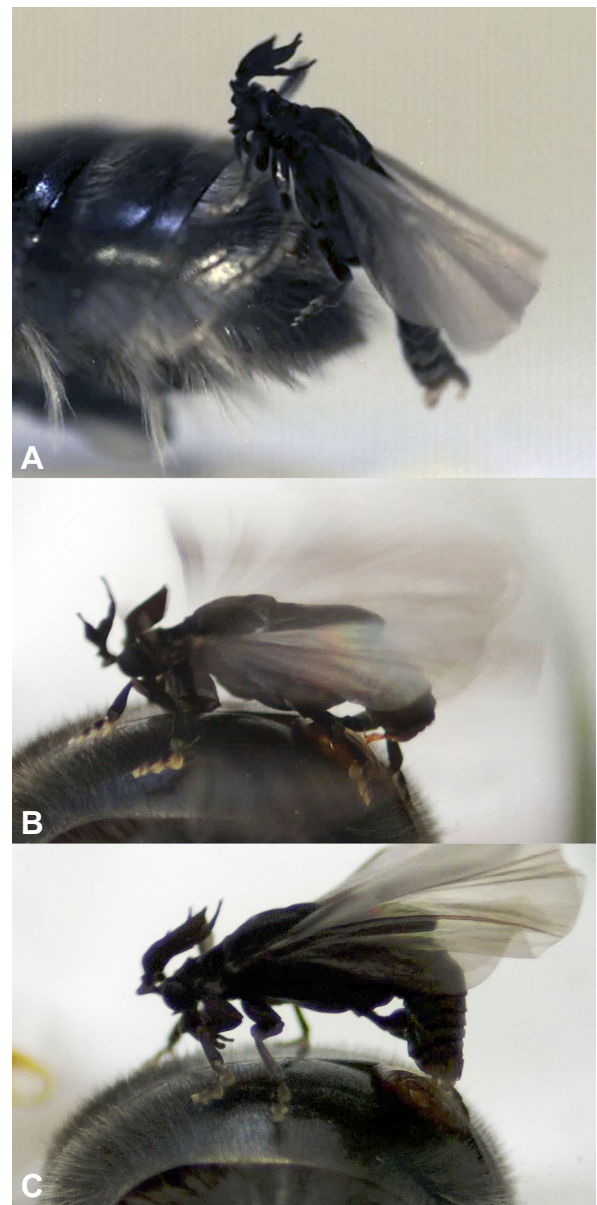
Fig. 4. Mating of *S. ovinae* (film stills). (A) Mounting the host. (B) Unfolding the penis. (C) Penetration (modified from Peinert et al., 2016).

the heavily hairy abdomen of the bumblebee. On the other hand, this result may indicate certain universality of the attachment system of the males of *S. ovinae* that may adhere equally well to the rather smooth surface of the bare cuticle of the host and to the host hairs, whose diameters are much larger than the diameter of tenent setae of *S. ovinae* males (Fig. S3). This tolerance of tenent hairs to the substrate geometry might be explained by the very small size of their terminal tips (about 500 nm). These tips are among the smallest ones found in insects: similar dimensions have been previously reported from representatives of Mantophasmatodea (Beutel and Gorb, 2006). It is also known from the comparison of the tolerance of tenent hairs to the critical roughness that the smaller the dimension of terminal contact elements, the higher the tolerance, which means that attachment structures of these animals are less sensitive to the fine roughness of the substrate (Wolff and Gorb, 2012; Kovalev et al., 2018). Among the most tolerant adhesive systems are those of geckos and spiders (Huber et al., 2007), whereas insects are usually very

strongly sensitive to the substrate roughness (Gorb, 2001; Gorb and Gorb, 2002, 2017; Voigt et al., 2008; Al Bitar et al., 2010). The absence of anisotropy in the force grip in the cranial/caudal direction relative to the host might indirectly indicate that *S. ovinae* generate forces rather by adhesion and not by mechanical interlocking with the host hairs.

The tolerance of the attachment system of *S. ovinae* to the substrate chemistry was quite surprising: animals adhered equally well to the more or less hydrophilic/hydrophobic substrates, which might be potentially explained by the primary contribution of van der Waals interactions and not capillary forces to adhesion in *S. ovinae* (Autumn et al., 2002). Adhesion of the majority of other insects is to some degree sensitive to the hydrophobicity of the substrate (Hosoda and Gorb, 2012; Grohmann et al., 2014). However, scattering of the force data on the same substrate was very high, which might be explained by the fact that animals moved too much during the experiment, because they had a varying number of legs in simultaneous contact with the substrate. On the host, this effect might be additionally enhanced by the curvature of the substrate.

The traction forces of the males of *S. ovinae* are approximately in the range of the traction forces of the slightly smaller pea aphid *Acyrthosiphon pisum* on glass with average values of less than 0.5 mN (Friedemann et al., 2015). On the other hand, the traction forces of the much larger and permanently ectoparasitic avian louse fly *Crataerina pallida* on glass are much higher and are ~15 mN (Petersen et al., 2018). The pulvilli of the louse fly are the only structures responsible for the forces on glass. In the feathers of their host, the common swift *Apus apus*, however, isolated legs of the louse fly develop a force of up to 324 mN. The modified claws of the louse fly are primarily responsible for the high forces (Petersen et al., 2018). In *S. ovinae*, such a mechanical grip would be not possible owing to the absence of claws.

Strepsiptera can manipulate the behavior and morphology of their hosts and as far as we know, always with a positive effect on the strepsipterans. Females of European paper wasps *Polistes dominula* stylopized by female *Xenos vesparum* lose their ovaries and have a prolonged lifespan. They form overwintering clusters with uninfected gynes. In the next spring, the stylopized paper wasps do not found nests, but transmit primary larvae to other newly founded *Polistes* nests (Hughes et al., 2004a; Manfredini et al., 2010). Furthermore, stylopized *Polistes* desert the colony and form extranidal aggregations in summer. This behavior is thought to facilitate mating of the strepsipterans (Hughes et al., 2004b). Stylopized *Andrena* bees emerge earlier in comparison to uninfected individuals (Brandenburg, 1953; Straka et al., 2011). By manipulating its host, the parasite can gain more time for the development or spread of its primary larvae, which are present when uninfected bees emerge (Kinzelbach, 1978; Straka et al., 2011).

The increased hairiness of the stylopized bees has no effect on the adhesion of the *Stylops* males to the host abdomen and thus no effect on the reproductive success of the parasite. The function of the denser hairiness of the stylopized bees therefore remains unclear. However, it is possible that the primary larvae are better able to hold on to the denser hairs and are thus distributed to more flowers and thus can reach more host bees.

Appendix
Details of statistical analysis of distance measurements between hairs of abdominal tergites II–IV of stylopized and uninfested females of *A. vaga*
Stylopized individuals showed smaller distance values between hairs than unstylopized ones. In the series of tergites T2–T3–T4 of

stylopized individuals, the values slightly decreased, whereas in unstylopized individuals, they increased. More detailed statistical analysis supports these statements. Comparison of different tergites in stylopized individuals showed that all tergites are statistically significantly different (Table A1).

Table A1. Kruskal–Wallis one way analysis of variance on ranks for stylopized insects

Group	N	Missing	Median	25%	75%
STY_T2	100	0	0.0548	0.0454	0.0653
STY_T3	100	0	0.0417	0.0343	0.0509
STY_T4	100	0	0.0265	0.0191	0.0372

$H=119.366$ with 2 degrees of freedom ($P \leq 0.001$).

The differences in the median values among the treatment groups were greater than would be expected by chance; there is a statistically significant difference ($P \leq 0.001$). To isolate the group or groups that differ from the others we used a multiple comparison procedure (Table A2).

Table A2. All pairwise multiple comparison procedures (Tukey test) for stylopized insects

Comparison	Difference of ranks	Q	P<0.05
STY_T2 vs STY_T4	13394.000	15.440	Yes
STY_T2 vs STY_T3	6274.000	7.233	Yes
STY_T3 vs STY_T4	7120.000	8.208	Yes

Comparison of different tergites in unstylopized individuals revealed that T2 is statistically significantly different from T3 and from T4. T3 was not statistically significantly different from T4 (Table A3).

Table A3. Kruskal–Wallis one way analysis of variance on ranks for unstylopized insects

Group	N	Missing	Median	25%	75%
USTY_T2	99	0	0.0623	0.0516	0.0707
USTY_T3	99	1	0.0807	0.0633	0.124
USTY_T4	100	0	0.0943	0.0743	0.133

$H=68.974$ with 2 degrees of freedom ($P \leq 0.001$).

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P \leq 0.001$). To isolate the group or groups that differed from the others we used a multiple comparison procedure (Table A4).

Table A4. All pairwise multiple comparison procedures (Dunn's method) for unstylopized insects

Comparison	Difference of ranks	Q	P<0.05
USTY_T4 vs USTY_T2	96.477	7.924	Yes
USTY_T4 vs USTY_T3	21.627	1.772	No
USTY_T3 vs USTY_T2	74.850	6.116	Yes

Comparison of T2, T3 and T4 between stylopized and unstylopized individuals showed that this was statistically significantly different in each case (Table A5). The differences in the median values between the two groups is greater than would be expected by chance.

Table A5. Mann–Whitney Rank Sum Tests for comparison of stylopized and unstylopized insects

Group	N	Missing	Median	25%	75%	U	T	P
USTY_T2	99	0	0.0623	0.0516	0.0707			
STY_T2	100	0	0.0548	0.0454	0.0653	3803.00	11047.000	0.005
USTY_T3	99	1	0.0807	0.0633	0.124			
STY_T3	100	0	0.0417	0.0343	0.0509	696.500	13954.500	≤0.001
USTY_T4	100	0	0.0943	0.0743	0.133			
STY_T4	100	0	0.0265	0.0191	0.0372	287.000	14763.000	≤0.001

U, Mann–Whitney U statistic.

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.223784.supplemental

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Figure S1. Related to Figure 2. Comparison of hairiness of the tergites of the abdomen of uninfested (left) and stylopized females of *Andrena vaga* (right). Scale bar: 1 mm.
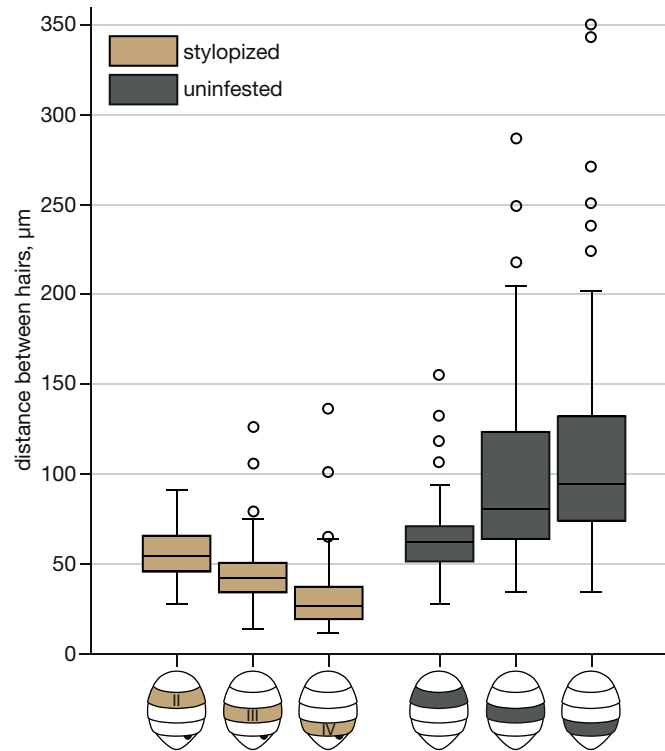
Figure S2. Distance between hairs of the abdominal tergites II–IV of the abdomen of stylopized and uninfested females of *Andrena vaga*.
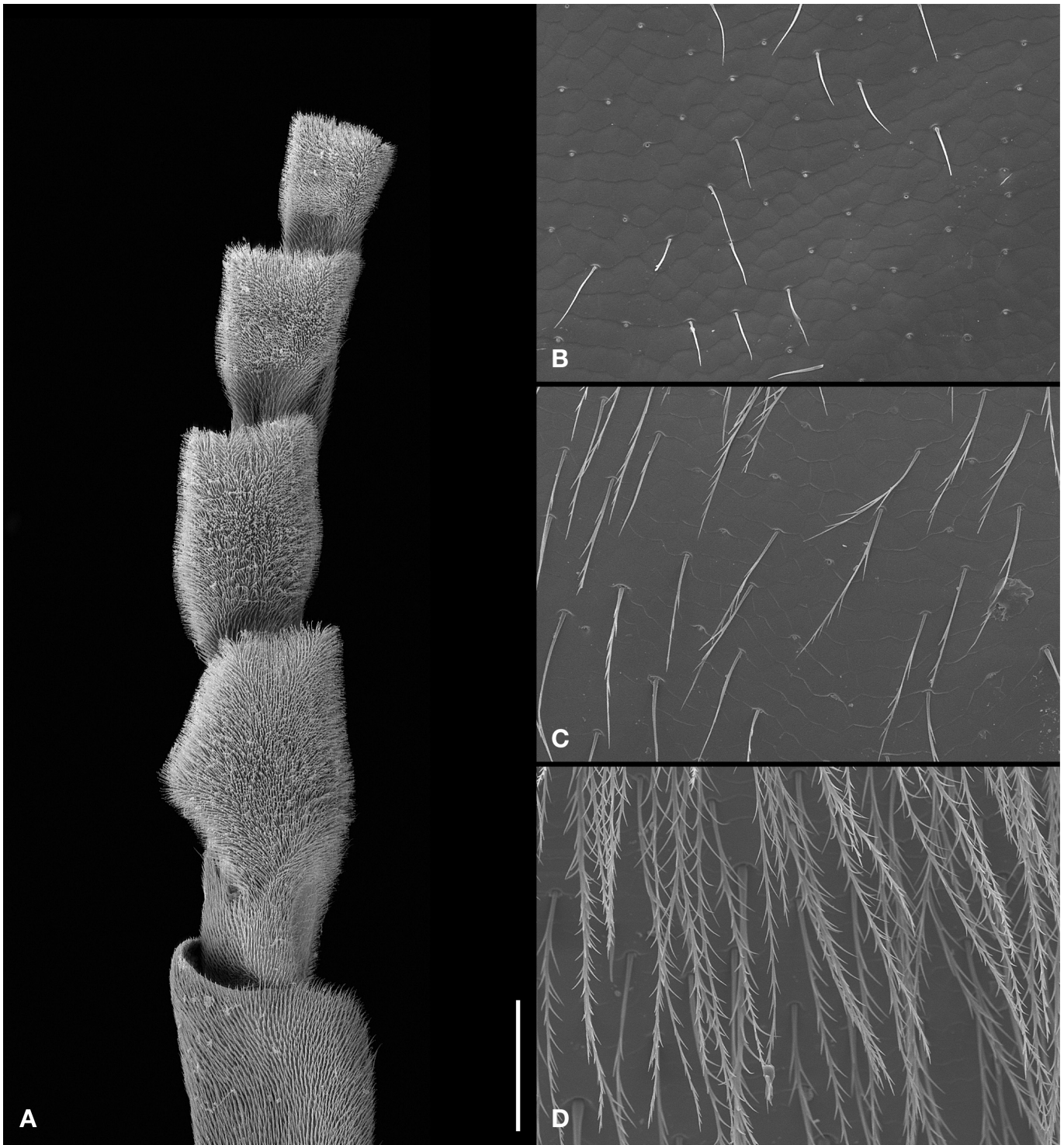
Figure S3. Tarsus of *Stylops ovinae* (A) and hairiness of the abdominal tergites II–IV of a stylopized *Andrena vaga* (B–D), SEM-micrographs. (A) Prothoracic leg, ventral view (modified from Pohl & Beutel, 2004). (B) Abdominal tergite II. (C) Abdominal tergite III. (D) Abdominal tergite IV. Scale bar: 100 μm.
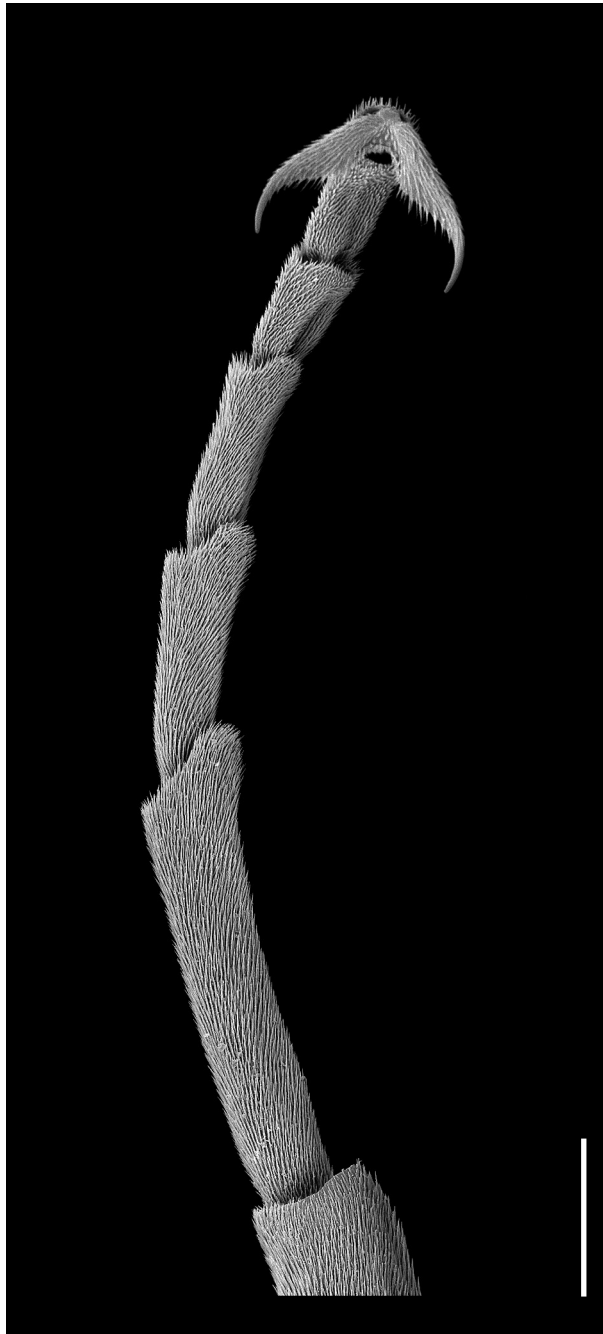
Figure S4. *Mengenilla moldrzyki* (Mengenillidae), male, tarsus of metathoracic leg, ventral view, SEM-micrograph (modified from Pohl & Beutel, 2004). Scale bar: 100 µm.

Table S1. Distance (µm) between hairs of abdominal tergites II–IV of stylopized and uninfested *Andrena vaga*.

	Stylopized					Uninfested			
	average	med.	max.	min.		average	med.	max.	min.
Tergite II	55.6	54.8	91.3	27.7	Tergite II	63.1	62.3	155.2	28.3
Tergite III	43.9	41.7	126.5	13.4	Tergite III	98.5	80.7	286.9	34.3
Tergite IV	30.3	26.5	136.7	11.4	Tergite IV	111.1	94.3	350.5	34.3