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RESEARCH ARTICLE

Gluing the 'unwettable': soil-dwelling harvestmen use viscoelastic fluids for capturing springtails

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ABSTRACT

Gluing can be a highly efficient mechanism of prey capture, as it should require less complex sensory–muscular feedback. Whereas it is well known in insects, this mechanism is much less studied in arachnids, except spiders. Soil-dwelling harvestmen (Opiliones, Nemastomatidae) bear drumstick-like glandular hairs (clavate setae) at their pedipalps, which were previously hypothesized to be sticky and used in prey capture. However, clear evidence for this was lacking to date. Using high-speed videography, we found that the harvestman *Mitostoma chrysomelas* was able to capture fast-moving springtails (Collembola) just by a slight touch of the pedipalp. Adhesion of single clavate setae increased proportionally with pull-off velocity, from 1 µN at 1 µm s^{-1} up to 7 µN at 1 mm s^{-1}, which corresponds to the typical weight of springtails. Stretched glue droplets exhibited characteristics of a viscoelastic fluid forming beads-on-a-string morphology over time, similar to spider capture threads and the sticky tentacles of carnivorous plants. These analogies indicate that viscoelasticity is a highly efficient mechanism for prey capture, as it holds stronger the faster the struggling prey moves. Cryo-scanning electron microscopy of snap-frozen harvestmen with glued springtails revealed that the gluey secretions have a high affinity to wet the microstructured cuticle of collembolans, which was previously reported to be barely wettable for both polar and non-polar liquids. Glue droplets can be contaminated with the detached scaly setae of collembolans, which may represent a counter-adaptation against entrapment by the glue, similar to the scaly surfaces of Lepidoptera and Trichoptera (Insecta) facilitating escape from spider webs.

KEY WORDS: Adhesion, Tensile test, Wetting, Microstructure, Opiliones, Collembola

INTRODUCTION

The interaction between prey and predator is one of the central aspects of life and is an important driving force for the evolution of complex morphological and behavioural traits. There is often an arms race including avoidance strategies or defence mechanisms in the prey and means of prey capture in the predator. The usage of glue has a great benefit in prey capture, as complex sensory–muscular feedback is less necessary to successfully catch and arrest the prey, compared with other mechanisms (Betz and Kölsch, 2004). In contrast, secretory products may be related to high synthesis costs, and structural or behavioural adaptations that prevent gluing of the predator's own body parts must co-evolve. Thus, this strategy might be especially useful for organisms with locomotion capabilities worse than those of the prey or those with a simple (or complete lack thereof) nervous system, such as carnivorous plants (Darwin, 1875) and cnidarians (Lewis and Price, 1975). In arthropods, a high diversity of structures associated with sticky secretions for prey entrapment has evolved (Betz and Kölsch, 2004), which may also act in combination with very fast movements (Betz, 1996). Whereas numerous cases of such mechanisms are known from insects, reports from the second most diverse group of terrestrial arthropods, the arachnids, concentrate nearly exclusively on the viscid capture threads of spider webs and their derivations (Betz and Kölsch, 2004; Sahni et al., 2011). Other arachnid orders are notoriously understudied, although they may represent a considerable portion of arthropod communities and may have evolved a similar high diversity of structures and behavioural mechanisms. Harvestmen (Opiliones) occur in high densities in litter habitats in temperate regions of the Northern Hemisphere (Pinto-da-Rocha et al., 2007). These are mainly species of the Phalangiidae and Nemastomatidae, belonging to the opilionid infra-order Palpatores. Their pedipalps (second pair of extremities) are often equipped with glandular hairs (setae), which carry droplets of a viscous secretion at their tips (Shultz, 1998).

In the Nemastomatidae, the glandular setae densely cover the whole pedipalps except the coxa and trochanter. These setae [formerly called 'Kugelhaare', clavate setae or drumstick-like setae (Pinto-da-Rocha et al., 2007)] exhibit a unique ultrastructure: they are equipped with an umbrella-like apical structure, which is surrounded by a spherical droplet (Schwangart, 1907; Rimsky-Korsakow, 1924). The 'umbrella' consists of various microtrichia (chetae), which highly increase the surface area covered by the secretion and thus adhesion of the droplet to the pedipalp (Wachmann, 1970). It was shown that the fluid is transported through channels in the cuticular shaft and released at the basis of the umbrella (Rimsky-Korsakow, 1924; Wachmann, 1970). These setae were first interpreted as sensory organs (Hansen, 1903). Later it was assumed that their secretions were sticky and used to capture prey, namely, small arthropods co-occurring in the litter habitats of nemastomatids, such as springtails (Collembola) and oribatid mites (Schwangart, 1907; Rimsky-Korsakow, 1924; Wachmann, 1970), which have been shown to be the major food source of these harvestmen (Adams, 1984). Yet, a study on the prey capture behaviour in *Paranemastoma quadripunctatum* came to the conclusion that these setae do not play any role in prey adherence as the prey is grasped by the chelicerae (Immel, 1955). However, the author reported that the prey attack was very fast, and thus there may be insufficient time to observe the contact development between the predator and prey. Another observation with nymphs of the related *Dicranolasma scabrum* (Dicranolasmatidae), carrying similar setae at their pedipalps, suggested an adhesive function of these setae, although springtails were able to escape in some cases (Gruber, 1993).

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Presumably, gluing a springtail or other small soil organism might not be a trivial feat, as they are equipped with low-wettable surfaces to avoid capillary adhesion by soil moisture. In springtails, low wettability is gained by waxes (low free surface energy), microstructures (low contact area) and macrostructures (setae that prevent wetting by larger water droplets) (Noble-Nesbitt, 1963; Ghiradella and Radigan, 1974; Helbig et al., 2011). Further, they are often densely covered with scale-like or fringed bristle-like setae, that easily detach when glued (Bauer and Pfeiffer, 1991). In oribatid mites, hydrocarbon-dominated secretions produce a highly water-repellent effect (Raspotnig and Leis, 2009). To overcome the repellent effect, the prey capture secretions should closely match the surface physics (low polarity) of the prey. Further, they may contain surfactants reducing surface tension and facilitating spreading over microstructures. Sticky secretions for catching arthropod prey can be based on chemically quite different substances, such as glycosaminoglycans (Heslop-Harrison and Knox, 1971), oils and resins (Lloyd, 1942) in carnivorous plants; proteins, waxes, oils or mixtures of proteins and lipids in onychophorans and insects (Betz and Kölsch, 2004); and glycoproteins in spider webs (Opell and Hendricks, 2010; Sahni et al., 2010). In early study, Rimsky-Korsakow (Rimsky-Korsakow, 1924) carried out solubility and staining tests, which revealed that the secretion of the harvestmen's clavate setae contains lipids. However, the physical properties and the contact behaviour of the secretion have not been studied to date.

The aim of the present study was to clarify the adhesive mechanism and the role of clavate setae of harvestmen [*Mitostoma chrysomelas* (Hermann, 1804), *Nemastoma lugubre* (Müller 1776) and *N. dentigerum* Canestrini 1873] in capturing springtails. Therefore, we observed the prey capture behaviour of nemastomatids with the help of high-speed videography, adhesive force measurements and estimations of the elastic modulus of the secretion droplets, and studied their rheological behaviour. Furthermore, using micromanipulation and cryo scanning electron microscopy (cryo-SEM), we examined whether and how the pedipalpal secretion is able to wet the complex micro-structured cuticular surface of different springtails to give insight into the microscale mechanism of gluing a surface showing an extremely low wettability.

RESULTS

Prey capture behaviour

Prey capture could not be observed in *Nemastoma* spp. because they exhibited a highly photophobic behaviour. *Mitostoma chrysomelas* was found not to be disturbed by light, and foraging was instantly triggered under the presence of collembolans when the harvestman had previously been starved for 1 day. In total, 38 prey capture events (of 18 harvestmen and including different species and sizes of springtails) were recorded, half of which were successful (prey was eaten) (Fig. 1K). In the other half of the trials, the prey could free itself from the pedipalps, often after a significant period of heavy struggle. Success rate decreased with prey size. Both entomobryomorph (elongated) and symphypleonan (globular) springtails were attacked and captured, but no attack attempts on *Neanura muscorum* (Poduromorpha, worm-like) were observed.

In most prey capture events recorded, the harvestmen searched actively for prey (except in three cases, where the springtail directly jumped onto the pedipalp of the resting or walking harvestman). In these cases, the harvestmen slowly moved around using their second leg pairs as feelers. In certain time intervals the pedipalps were simultaneously stretched forward (Fig. 1E), held closely above the ground, and retracted again. If one pedipalp touched a springtail, the springtail was immediately glued (Fig. 1D). The harvestmen immediately reacted by trying to get the second pedipalp into contact and pulling the springtail towards its mouthparts. A springtail that could not be caught (glued) was sometimes pursued with remarkably fast movements. Even springtails twice the size of the harvestmen were attacked (with one of three attacks being successful). In the high-speed video recordings we observed that the usually heavily struggling entomobryomorph springtails were repeatedly able to partly release the glue contact. However, if a springtail was not able to attach itself to other body parts of the harvestman or to the ground, it stuck again to other parts of the pedipalp. To prevent escape of the prey due to its attachment to the surrounding objects, the harvestman stretched its legs and elevated its body over the ground, while holding its pedipalps away from its body (Fig. 1G,H). When the springtail was securely fixed (decrease of mobility), the pedipalps were retracted, and the prey was grasped with the chelicerae and released from the pedipalps. Then the prey was intensively chewed with the chelicerae. Symphypleonan springtails are less agile because of their compact body shape, and they could only use their furca (jumping organ) to get free. In all six cases of capturing of Symphypleona springtails, the prey were immediately fixed and could not come free, not even by strong pushes of the furca. When catching prey, the harvestman's pedipalpal tarsus is often bent towards the tibia like a clasp (Fig. 1I,J). In some cases it was observed that an antenna of the prey was fixed in this clasp. This was seen in two cases during the capture of small collembolans, such as the globular Symphypleona, which were directly grasped at their distal antenna.

In some cases, harvestmen groomed their pedipalps after unsuccessful attacks by repeatedly clasping them with the chelicerae.

Morphology of clavate setae and their interaction with the springtail integument

The clavate setae are present on the femur, patella, tibia and tarsus of the pedipalp in all developmental stages of both *Nemastoma* spp. and *M. chrysomelas*. The setae on the dorsal side of the distal tibia carry the biggest droplets, with diameters between 11 and 17 µm (Fig. 1B, Fig. 2E). The main difference between these two genera is the length of the pedipalp, which is less (~80%) than the body length in *Nemastoma* spp. but approximately twice the body length in *M. chrysomelas*. The distribution of clavate setae is very sparse on the femur and patella in *Nemastoma* spp. (only few single setae), whereas in *M. chrysomelas* both segments are densely covered. No difference in size and microstructure of the clavate setae was found between studied representatives of these genera. The clavate setae emerge from round, slightly elevated sockets and consist of a thick shaft that narrows towards the pointed tip (Fig. 2C). The shaft appears rather rigid, as it bends only slightly before it breaks off at its socket when laterally pushed with a capillary tip. Two to three micrometres below the tip there is a dense globule (head) consisting of microtrichia [previously called 'chaetae' (Wachmann, 1970)] (Fig. 2D). The microtrichia are 1–2 µm long and carry three nano-hooklets (each ~200 nm long) at their distal tips (Fig. 2D, inset), which have not been described previously. The shaft consists of parallel filaments connected by a dense matrix. Underneath the head, the filaments are only loosely attached to each other, providing space for secretion delivery (Fig. 2D). Canals in the outer cuticle (Fig. 2F) are probably responsible for transporting the fluid to the head, as proposed by Wachmann (Wachmann, 1970) on the basis of his transmission electron microscopy images of serial transverse sections of clavate setae. The secretion droplets did not change their shape when the pedipalp was air dried during sample preparation for

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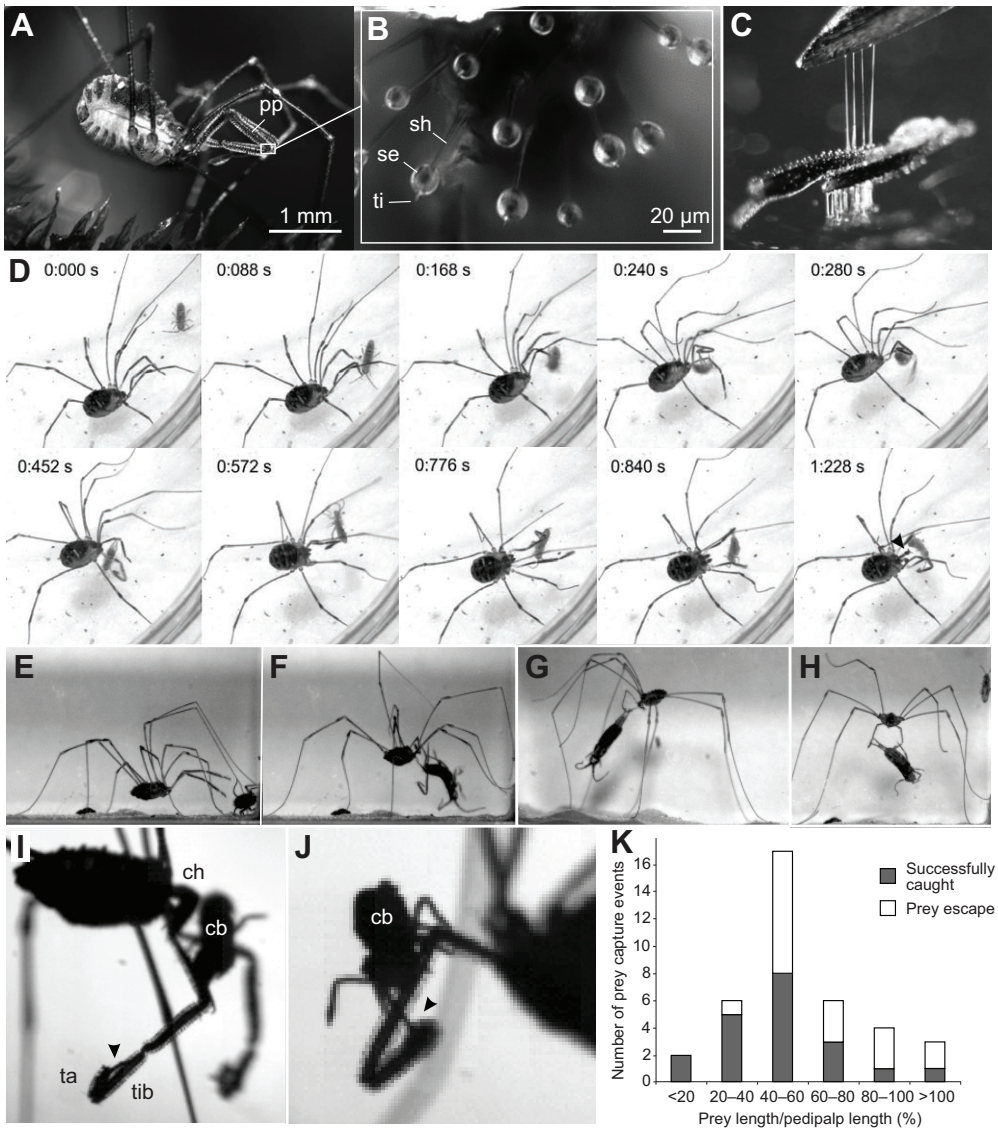

Fig. 1. Morphology of the sticky apparatus and prey capture behaviour of *Mitostoma chrysomelas*. (A) Adult individual in its natural habitat. The long pedipalps (pp) are normally held in a flexed position; the secretion droplets of the clavate setae are clearly visible against the dark background. (B) Light micrograph of clavate setae. The setae have a stiff narrowing shaft (sh) and bear spherical secretion droplets (se) closely beneath the tip (ti), which is usually free of secretion. (C) Stretched secretion droplets of a dried pedipalpal fragment, adhering to a Plexiglas Petri dish (bottom) and to an insect needle (top). (D) Frames of a high-speed video (HSV) recording (250 frames s^{-1}) of a successful catch of a juvenile *Orchesella flavescens* springtail. The springtail is touching the tip of the right tarsus. It tries to escape but is presumably held back by the secretion of the clavate setae. The harvestman tries to fix the prey, which is heavily struggling, and eventually attaches it at both the antenna and the furca. The prey is then grasped with the chelicerae (protracting chelicera is indicated by arrowhead). The whole video sequence is available upon request from the corresponding author. (E–I) Frames of HSV recordings (1000 frames s^{-1}). (E) Typical search-and-attack posture with stretched pedipalps. (F–H) Successful catch of a large *Tomocerus vulgaris* springtail. The harvestman elevates its body to prevent the prey from contacting the ground. Note that the prey is only in contact with the pedipalps. (I) Successful catch of a *Lepidocyrtus* sp. springtail (cb). The prey was initially grasped at its antenna, which was clasped between tibia (tib) and tarsus (ta) of the pedipalp and eventually broken off (arrowhead). Note that the clavate setae are contaminated by bristles and scales, which the springtail lost in struggle. The chelicerae (ch) are still retracted. (J) Successful catch of a symphypleonan springtail (cb). Its legs stick to the clavate setae of the pedipalp patella; additionally, one antenna is clasped between the tarsus and the tibia (arrowhead). (K) Number of single attacks and hunting success per prey size, analysed from 38 HSV-documented prey capture events, including 18 *M. chrysomelas* and different collembolan prey (see Table 2). These anecdotal reports depict the high variance of accepted and caught springtail prey.

SEM and did not evaporate even in the high vacuum of the SEM chamber. When large parts of the secretion droplet were mechanically removed, the shape of the remaining droplet was largely affected by the underlying microtrichia.

The surface of the cuticle of the large and agile epedaphic *Tomocerus vulgaris* and *Orchesella flavescens* springtails (Entomobryomorpha) exhibits primary granules arranged in regular hexagonal patterns (Fig. 3F) and dense covers of setal structures, which are scale-like in *T. vulgaris* (Fig. 3A) and fringed bristle-like in *O. flavescens* (Fig. 3C). The smaller entomobryomorph *Lepidocyrtus lignorum*, which was used in the adhesion measurements, exhibited similar hexagonally arranged primary granules and small scale- and bristle-like setae. Detached setae of these collembolans often contaminated large parts of the secretion droplets of the clavate setae after having been glued to them (Fig. 1I, Fig. 2B). The secretion of the clavate setae was found to completely

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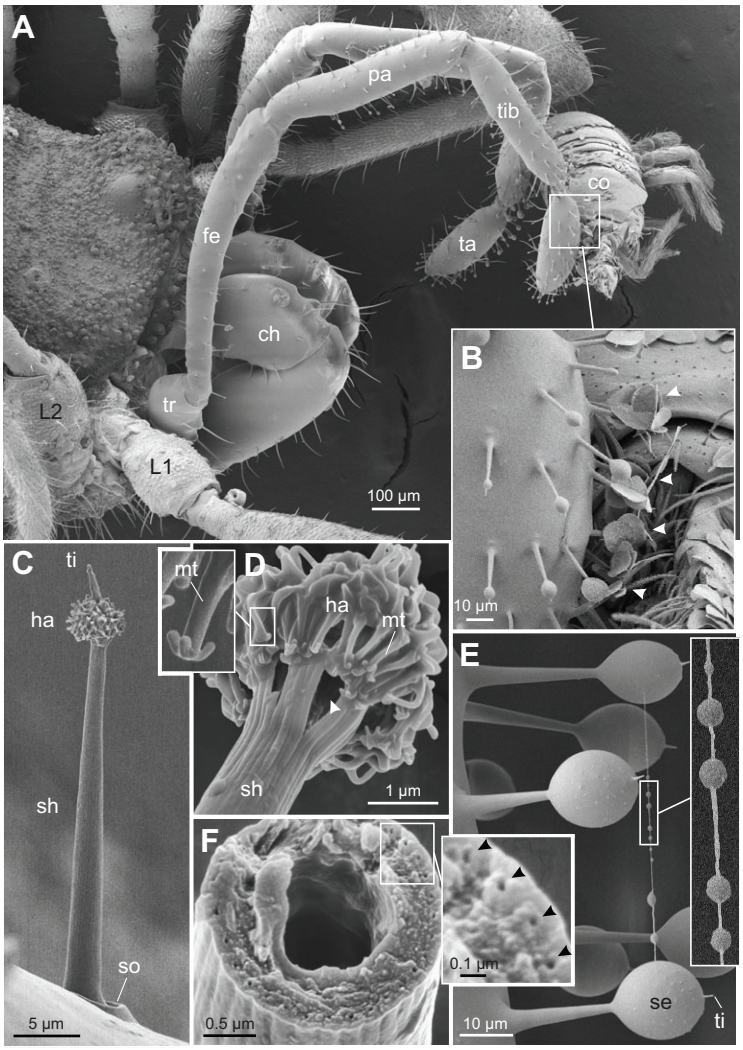

Fig. 2. Scanning electron microscopy of the clavate setae. (A) *Nemastoma dentigerum* with a glued juvenile *Tomocerus vulgaris* springtail (co). The pedipalp consists of coxa (not visible here), trochanter (tr), femur (fe), patella (pa), tibia (tib) and tarsus (ta). The clavate setae are primarily distributed in the last two segments. ch, chelicerae; L1,L2, anterior walking legs. (B) Detail of pedipalp-collembola interaction. Remark the detached scale-like setae of the springtail contaminating the clavate setae of the tarsus (arrowheads). (C,D) Clavate seta of *Nemastoma* sp. with secretions largely removed by 15 min treatment with acetone. (C) The seta emerges from a round socket (so) and consists of a rigid, narrowing shaft (sh), the head region (ha), where the secretion droplet emerges and a pointed tip (ti). (D) Detail of head region with microtrichia (mt), here partly glued by remains of the secretion. The inset shows one microtrichium with its three distal hooklets in detail. (E) Tibial clavate setae of a juvenile *Mitostoma chrysomelas* with large secretion droplets (se). The secretion forms beads-on-a-string (BOAS) structures, after being stretched (inset). (F) Broken shaft of a clavate seta after treatment with acetone. Canals presumably serving the secretion transport are visible in the outer cuticle (arrowheads). The inset shows the canals at higher magnification.

wet the microstructures on the springtail setae (Fig. 3B,D,E) as well as the micro-patterned cuticle. The formation of a capillary bridge happened in less than 100 µs and the secretion then quickly spread over the surface, pulling the seta towards it (Fig. 3G). When retracted, the secretion can be highly stretched before losing contact with the springtail cuticle, indicating significant adhesion and viscosity (Fig. 3B,H). In the small globular *Bourletiella viridescens* (Symphypleona), setal coverage is only sparse, and the cuticle is covered by primary granules comparable to those of *T. vulgaris* and *O. flavescens*, arranged in rhombic or hexagonal patterns, which are completely wetted by the clavate setal secretion (Fig. 3I–K). The worm-like and less mobile *Neanura muscorum* exhibits the cuticle structure of two distinct hierarchical levels (macrostructure and microstructure; Fig. 3L) with only few smooth setae. *Neanura muscorum* repeatedly pushed onto the pedipalps of *Nemastoma dentigerum* did not stay attached, and remains of clavate setal secretion hardly wetted the surface (Fig. 3N,O). When retracted, the capillary bridge shrank very fast (Fig. 3M). A capillary bridge comparable to that observed during the capture of above-mentioned collembolans was only observed after the clavate seta was deeply (20 µm) pressed into the cuticle (Fig. 3P), indicating better wetting under applied pressure. Interestingly, the clavate setal secretion exhibits comparable contact angles on glass and on entomobryomorph cuticle, but the contact angle was three times larger on *Neanura* cuticle (Fig. 3Q).

Adhesive properties

The pull-off forces of clavate setae contacting a smooth glass surface are comparable between *N. lugubre* and *M. chrysomelas* and increase linearly with an increased pull-off velocity within the measured range (Table 1). The forces range from 0.8 to 6.9 µN in *N. lugubre* and from 1.0 to 2.2 µN in *M. chrysomelas*. As the range was larger in *N. lugubre*, its secretion likely was more viscous. However, as only single specimens from each species were tested, this does not necessarily indicate an interspecific difference. Adhesion tests of *N. lugubre* setae on *L. lignorum* springtail cuticle showed forces in a range comparable to those measured on glass, but with large variation (Table 1). The mean forces were three to five times larger when contact was made with a scale-like seta of the springtail. In these cases, the pull-off forces were approximately double those on glass. On the bare springtail cuticle, forces reached less than one-half of those measured on glass at low pull-off velocities, but at high pull-off speeds the forces exceeded the values obtained on glass. These results indicate that the springtail cuticle microstructures may influence the flow of the adhering secretion.

The shape of the stretched droplet during the tensile test changed over time. At an elapsed time of 10–20 s, an initiation of the so-called beads-on-a-string (BOAS) formation was observed (Fig. 4A). A varying amount of fluid was left behind on the substrate in these cases, indicated by the flow of BOAS droplets along the central string in both directions, towards the seta and towards the substrate.

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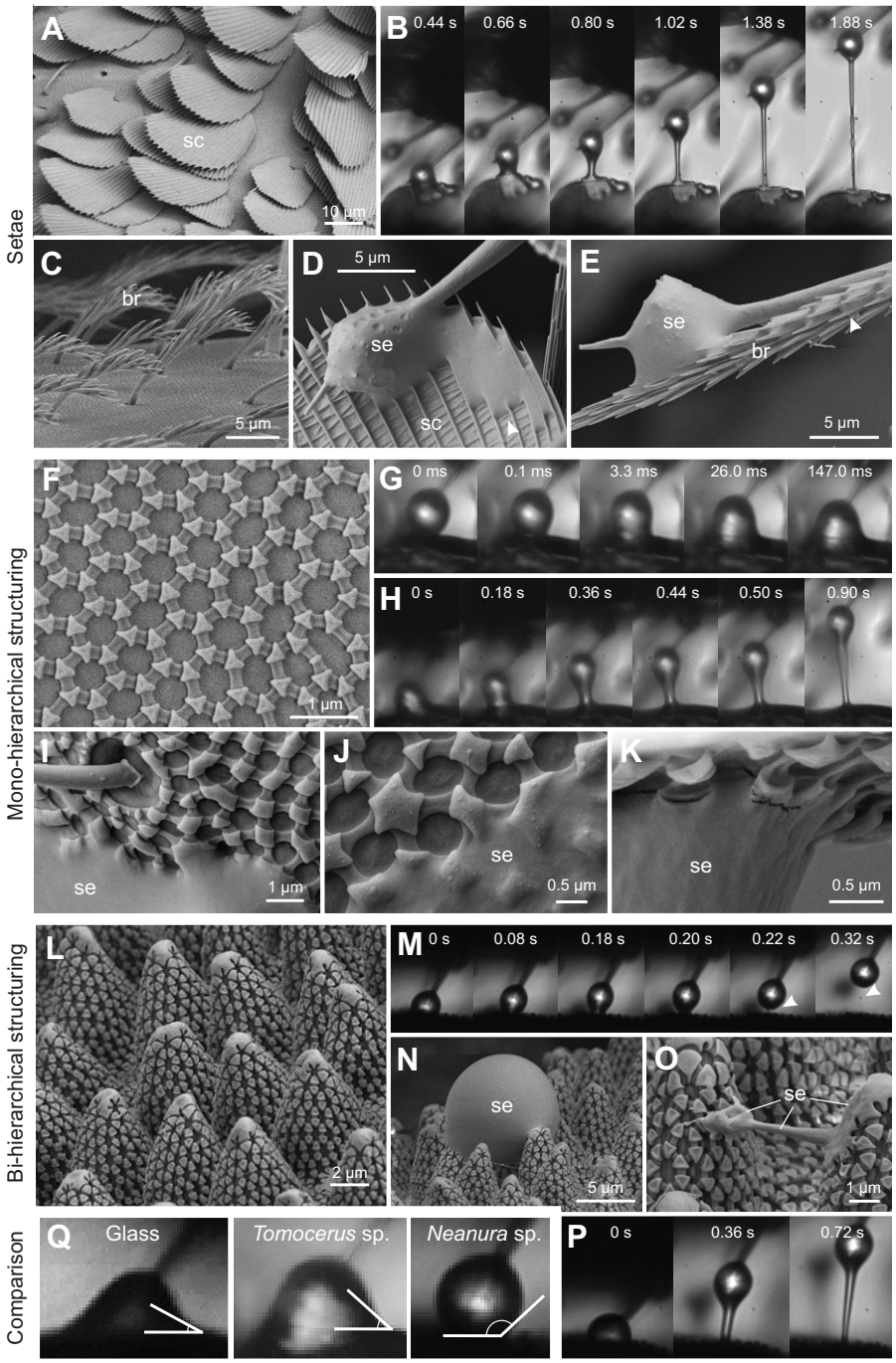

Fig. 3. Wetting of springtail cuticle. (A) Scale-like setae (sc) of *Tomocerus vulgaris*, covering the whole body including legs, antenna and furca. (B) Frames of a video sequence (60 frames s^{-1}) of a *Nemastoma dentigerum* clavate seta retracted from a *Tomocerus* scale. Note that the secretion is primarily floating along the micro-ridges on the scale surface (compare with D). (C) Bristle-like setae (br) of *Orchesella flavescens* covering the whole body including legs, antenna and furca. (D) Detail of a *Nemastoma* clavate seta with a glued scale-like seta of a *T. vulgaris* springtail (sc). The secretion (se) is spreading over the microstructures of the scale (arrowhead), guided by micro ridges. (E) *Nemastoma* clavate seta with adhering detached bristle-like seta of an *O. flavescens* springtail (br). The secretion (se) is spreading over the microstructures of the scale (arrowhead). (F) Cuticle with hexagonal patterns of primary granules in *Tomocerus vulgaris*. (G) Frames of a high-speed video sequence (10,000 frames s^{-1}) showing the fast wetting of the bare cuticle (scales removed) of a *T. vulgaris*. (H) Frames of a video sequence (60 frames s^{-1}) of a *Nemastoma* clavate seta retracted from a *T. vulgaris* bare cuticle. (I–K) Secretion of *Mitostoma* clavate setae spreading over the microstructures of the antennal cuticle of a *Bourletiella viridescens* springtail. (L) Cuticular structures of *Neanura muscorum*. (M) Frames of a video sequence (60 frames s^{-1}) of a *Nemastoma* clavate seta retracted from *N. muscorum* cuticle. Note the high initial contact angle and the fast shrinkage of the capillary bridge. A very thin capillary bridge remains at higher tension indicated by a tiny droplet between seta and springtail cuticle (arrowheads). (N,O) *Nemastoma* clavate seta secretion residuals on *N. muscorum* cuticle. Spreading of the fluid is prevented by the complex cuticle microstructure. (P) Frames of a video sequence (50 frames s^{-1}) of a *Nemastoma* clavate seta retracted from a *N. muscorum* cuticle. The setal droplet was initially driven 20 µm into the springtail cuticle, achieving higher wetting. (Q) Comparison of contact angles of attached *Nemastoma* clavate setae on glass and bare springtail cuticle.

At high-speed tension, the elongation of the droplet was too fast to allow fluid to flow and form BOAS. In these cases, the fluid was usually completely pulled off the substrate until the contact broke. Near the site of fluid–substrate contact, branching of the capillary bridge was often observed (Fig. 4B). Droplet elongation at high-speed tension was several times larger (30–40 times the droplet diameter) than that at low speed.

The stress–strain curves of a *N. lugubre* clavate seta show a short period of linear increase. When a constriction was formed between the seta and the substrate attachment site, there was a change in the slope of the curve, indicating plastic deformation. At low speed, this was accompanied by a drop of the pull-off force, whereas at high speed the pull-off force reached its peak later. The Young's modulus calculated from the initial part is 9–15 kPa.

Clavate setal droplets remained sticky when the pedipalp was air dried for a month, which was evaluated by slightly touching a few setae with the tip of an insect needle and then pulling them apart. However, the secretion seemed to become more viscous after this period of drying, and it could be pulled into long elastic threads (Fig. 1C) that were able to reshape to spherical droplet form after breaking apart.

DISCUSSION

Springtails, in addition to oribatid mites, are a dominant and ubiquitous component of soil communities (Hopkin, 1997). Thus, they are a reliable food source for predators in epi- and hypogaeic habitats (Bilde et al., 2000). However, they have evolved an effective escape mechanism: jumping using an elastic spring

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Table 1. Adhesive forces (µN) at different pull-off velocities of tibial clavate setae in *Nemastoma lugubre* and *Mitostoma chrysomelas*

		Pull-off velocity (µm s^{-1})						
Species	Substrate	1	5	10	50	100	500	1000
N. lugubre (female)	Glass	1.14±0.58^{a} (10)	1.33±0.63^{a,b} (8)	1.33±0.63^{a,b} (10)	1.44±0.36^{b} (10)	1.70±0.66^{b,c} (11)	2.53±1.00^{c,d} (9)	3.58±1.91^{d} (12)
	Springtail (bare cuticle)			0.49±0.25 (6)		0.97±0.52 (4)		4.53±2.26 (5)
	Springtail (setae)			2.45±2.87 (6)		3.79±4.16 (4)		
M. chrysomelas (juvenile)	Glass	1.23±0.14 (7)		1.21±0.20 (7)		1.43±0.20 (9)		1.69±0.27 (9)

Data are means ± s.d. (*n*). Different superscript letters indicate significant differences within measurement series of *N. lugubre* clavate setae on glass (Kruskal–Wallis rank sum test and pairwise Wilcoxon test with FDR correction in R). Shaft length and droplet size of the clavate setae are comparable in both species. Substrates were a smooth glass surface and different parts of the cuticle of a *Lepidocyrtus lignorum* springtail.

catapulting mechanism (Christian, 1979). Gluing seems to be the best way to capture prey with such escape capabilities, as it has been evolved in parallel in different springtail predators. For example, some carnivorous plants of the genus *Drosera* capture springtails with gluey mucilage, secreted by hair-like structures (Verbeek and Boasson, 1993). This glue exhibits rheological behaviour quite similar to that of the harvestman pedipalpal setae studied by us (Erni et al., 2011). In addition, *Stenus* beetles (Staphylinidae) quickly protrude their elongated labium, which distally bears microtrichious pads covered by a sticky, viscous fluid that adheres largely independent of surface topography and chemistry (Bauer and Pfeiffer, 1991; Betz, 1996; Koerner et al., 2012a; Koerner et al., 2012b). Rather similar to the harvestmen studied here, the presence of setae delivering adhesive secretions has been reported from the forelegs of epicriid mites, which also use them to capture springtails (Alberti, 2010). The role of the nemastomatid clavate setae in prey capture and retention is now evident as a result of the present study.

The effectivity of the clavate seta secretions in springtail capture is remarkable, as the cuticle of the collembolans has been repeatedly shown to be low-wettable and thus exceptionally anti-adhesive (Noble-Nesbitt, 1963; Ghiradella and Radigan, 1974; Helbig et al., 2011). Wetting of the complex micropatterned springtail cuticle must happen very fast. When the secretion droplet touched the substrate in our experiments, it spread on the surface within less than 1 ms. When pulling apart, capillarity and viscosity produced strong adhesion. Our force measurements show that only a few setae are necessary to securely arrest a springtail. The body mass of adult springtails ranges between 0.1 and 0.2 mg (Christian, 1979; Verhoef and Witteveen, 1980), which corresponds to a weight force of 1–2 µN. A struggling springtail may release forces largely exceeding its own body weight, especially when jumping (Christian, 1979). We found that adhesion of the secretion increases proportionally with pull-off velocity, reaching forces up to 7 µN at a speed of 1 mm s^{-1}. The take-off velocity of a jumping springtail is 1000 times higher [1.4 m s^{-1} (Christian, 1978; Christian, 1979)]. We observed that the pedipalp could stop a springtail during take-off, which means that the adhesion and the cohesion of the secretion must be high enough to withstand the thrust elicited by jump. At the highest pull-off velocity tested (1 mm s^{-1}), cohesion failure was only rarely observed, and a failure of adhesion between the secretion and the clavate seta was never observed. Instead, contact usually broke at the substrate. Similarly, it was shown that in spider capture threads, the tensile strength of a glue droplet exceeds its adhesion (Opell et al., 2011). Interestingly, the capillary bridge of the pulled droplet tended to branch at high velocities. This phenomenon might be comparable to the fibrillation observed in released pressure-sensitive adhesives, soft deformable solids that stick to various surfaces without a physical (i.e. solidifying) or chemical transformation (Creton, 2003). This indicates that the secretion behaves like a solid at high (>0.5 mm s^{-1}) pull-off velocities. For adhesion, the branching near the contact site may have an important enhancing effect: in elastic solids, contact splitting significantly enhances adhesion because of crack arresting and multiple peeling effects (Peressadko and Gorb, 2004; Pugno, 2011).

The observed velocity-dependent cohesion indicates that the secretion is viscoelastic, with an elastic modulus similar to what has been found for the droplets of spider capture threads (Torres et al., 2014). This could be further evaluated by the observation of the rheological behaviour of the secretion at low pull-off velocities. The formation of regular droplets of different size with ultrathin thread-like connections (BOAS morphology) is only known from

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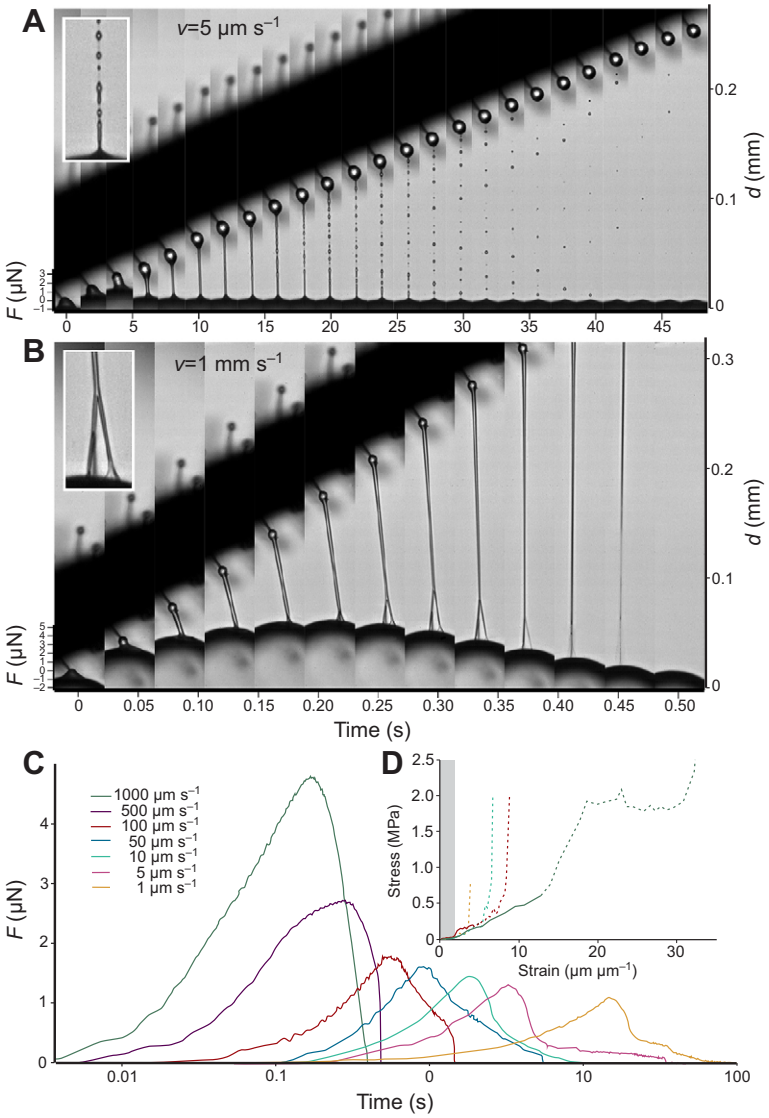

Fig. 4. Secretion adhesion and rheology. (A) High-speed video frames of a pull-off test at 5 µm s^{-1} showing the formation of BOAS-structures. The inset shows a detail of the secretion jet near its contact to the glass bead at *t*=23 s. The vertical shift of the glass bead surface indicates the pull-off force (scale at the left image side). The whole video sequence is available upon request from the corresponding author. (B) High-speed video frames of a pull-off test at 1 mm s^{-1}, same seta. The inset shows a detail of the branching secretion jet near to the contact to the glass sphere at *t*=0.25 s. The whole video sequence is available upon request from the corresponding author. (C) Exemplary time–force curves for one seta pulled off at different velocities. Note that the time scale is logarithmic. (D) Force–extension curves for one example droplet (same as in C) pulled off at different velocities. The grey area marks the initial part of the curves where the increase is linear (elastic deformation), giving the Young's modulus (stress unit per strain unit). At high strain, the measurement of stretched droplet diameter and thus the calculation of stress becomes imprecise; graph lines are dashed in these parts. *d*, deflection; *F*, force.

viscoelastic fluids (Bhat et al., 2010). This was analogously documented for the glue of protocarnivorous plants (Voigt and Gorb, 2008) and spider capture threads (Opell and Hendricks, 2010; Sahni et al., 2010). These examples depict that viscoelasticity is a highly efficient principle in mechanisms to capture fast-moving prey, because the adhesion increases with the intensity of escape movements of the prey. Interestingly, being the target of these analogous adhesive systems, prey evolved quite similar counter strategies against the viscoelastic glue entrapment. Moths (Lepidoptera) and caddisflies (Trichoptera) are rarely trapped in spider webs, because they carry a dense coverage of micro- and nanostructured scales or bristle-like setae that are only loosely attached and easily break off when trapped (Eisner et al., 1964; Nentwig, 1982). In collembolans, scale- and bristle-like setae may detach when glued, contaminating the secretion droplets and freeing the collembolan. This explains why entomobryomorph springtails were able to escape from pedipalps in half of the observed prey capture events, which is consistent with previous observations in other harvestman species (Immel, 1955; Gruber, 1993). In the case of *M. chrysomelas*, an increase in pedipalpal length and thus clavate seta number may have been an adaptation against prey loss.

It should be mentioned that nemastomatids do not consume solely collembolans and other small soil organisms, but also dead animals and plant material (Hvam and Toft, 2008). Perhaps the differences in length of the pedipalps and the number and size of clavate setae between *Mitostoma* and *Nemastoma* reflect a different degree of diet specialization. It should also be noted that springtails with secondary granules (microstructure of the macropatterned cuticle) can hardly be glued, which was shown here for the hemiedaphic *N. muscorum*. However, it is unlikely that those belong to the prey spectrum of nemastomatids, because most of these species live in the deeper litter and soil layers (Nickerl et al., 2013). *Neanura muscorum* springtails, which were offered to *M. chrysomelas*, were not attacked, probably because they move very slowly and thus may not be recognized as prey by the harvestmen.

The sticky pedipalps of nemastomatids are often remarkably clean, although these species live in a particle-rich environment. We found that adhesion of the setae was prevented when contact with the substrate was made perpendicularly. This is due to the terminal setal tip, which slightly protrudes from the secretion droplet and seems to be rather stiff, thus functioning like a spacer. Further, these harvestmen usually move very carefully, holding their pedipalps in a retracted position near the body and high above the ground. *Mitostoma chrysomelas* was also observed grooming its pedipalps with the chelicerae after unsuccessful attacks.

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Conclusions

The pedipalpal secretions of nemastomatids can easily wet the complex surface structures of most collembolans and elicit a high dynamic strength by viscoelasticity. The adhesive strength and elasticity of single droplets are comparable to those of viscid capture threads in spider orb webs (Sahni et al., 2010; Torres et al., 2014). Special microtrichia with distal nano-hooklets prevent the adhesive droplet from losing contact with the seta under high dynamic loads. Behavioural observations underline that this is a very successful way to capture very mobile prey, despite effective counter adaptations of the prey, such as setal discharge.

MATERIALS AND METHODS

Animals and behavioural studies

Nemastomatidae were collected between July and November 2013 and March and April 2014 in the southern suburban area of Kiel, Northern Germany, and in rural areas near Mainz, southwestern Germany. *Mitostoma chrysomelas* was found on the ground in open areas when the upper vegetation was removed. *Nemastoma* spp. (*N. lugubre* and *N. dentigerum*) could be found under rotten logs and by sieving leaf litter under shrubs and trees in hedgerows and woodland. Different microhabitat preferences are in accordance with previous studies (Meijer, 1971). Collembolans were collected directly before experiments nearby on the campus of the university of Kiel by the means of same methods and stored in plastic tubes equipped with humid tissues. Springtail identification followed Fjellberg (Fjellberg, 1998; Fjellberg, 2007) and Bellinger et al. (Bellinger et al., 2014). A summary of used springtail species and their cuticular structures (studied by SEM) is given in Table 2.

The harvestmen were kept in plastic tubes with humid tissues under cool conditions (10–15°C). For observations of prey capture, the animals were placed in plastic Petri dishes (diameter 6 cm) containing humid tissues and different species and sizes of living collembolans. Behaviour was observed and prey capture events were photo- or video-documented using an EOS 600D SLR (Canon Inc., Tokyo, Japan) equipped with a macro lens and an extension tube. Further prey capture events were additionally filmed with a high-speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA), equipped with a macro lens and using frame rates of 250–1000 frames s^{-1}. For high-speed videography (HSV), an additional light source was used (Storz Techno Light 217, Karl Storz GmbH & Co. KG, Tuttlingen, Germany). For lateral views, an arena 24×32×15 mm made from cover slides glued together with dental wax (Polyvinylsiloxane) was used.

We documented the success of each observed attack. Only attacks in which the prey was touched were taken into account. A prey capture event was considered successful when the prey was eventually taken by the chelicerae and the harvestman fed on it. If the prey escaped from the pedipalps after some time and stayed alive, the prey capture event was not considered successful. Each attacked prey was categorized by size (springtail body length relative to harvestman pedipalpal length).

Scanning electron microscopy

Pedipalps of *M. chrysomelas*, *N. dentigerum* and *N. lugubre* were ablated from individuals freshly killed with carbon dioxide. Some specimens were air dried and some were treated with acetone for 15 min and then air dried. Samples were glued on stubs using a carbon-rich tape and sputter coated with 10 nm Au-Pd. Specimens were studied with a Hitachi S 4800 scanning electron microscope (Hitachi Ltd, Tokyo, Japan) at an acceleration voltage of 3.0 kV.

Living collembolans collected in the same habitat as the harvestmen (see Table 2) were attached to a sample holder using Tissue-Tek® compound, shock frozen in liquid nitrogen, directly sputtered with 10 nm Au-Pd using the Gatan ALTO-2500 cryo system (Gatan Inc., Abingdon, UK) and viewed in the SEM with the stage cooled up to −120°C. The same Cryo-SEM setup was used to study the interaction between clavate seta secretion and collembolan cuticle. For this purpose, *N. dentigerum* and *M. chrysomelas* harvestmen and different springtails were anesthetized with carbon dioxide. Harvestmen were attached to the SEM sample holder with Tissue-Tek® compound and springtails were carefully laid onto the pedipalp.

Adhesion measurements and analysis

The adhesive forces of single clavate setae were measured at different pull-off rates using a calibrated glass pipette tip as a force sensor. The setup is displayed in Fig. 5A. Very fine pipette tips (tip diameter 5–10 µm, tip length 1.5–2 mm) were made from glass capillaries (diameter 1 mm, length 120 mm) using a micropipette puller (KE Pipetten-Puller horizontal, H. Saur Laborbedarf, Reutlingen, Germany) with the basic settings of pull left and pull right set to 000 and the current for the heating element to 6.5, corresponding to a current of ~16.7 A at pulling. Micro glass beads (diameter 100 µm) were glued laterally at the tip of the pipettes with a small amount of dental wax (Polyvinylsiloxane, Coltène/Whaledent AG, Altstätten, Switzerland). Onto the tip of one pipette a *Lepidocyrtus lignorum* springtail, freshly killed with carbon dioxide, was attached with dental wax and immediately used for measurements.

The pipettes were calibrated by stepwise pushing onto an ultra-microbalance (UMX2, Mettler-Toledo Inc., Columbus, OH, USA). For this purpose, a piece of the blunt side of a razor blade was glued perpendicularly onto a turned micro weight bowl. The capillary was placed horizontally, in such a way that the bead at the tip of the pipette was slightly above the razor blade edge (Fig. 5B). Then it was moved downwards at one step (step size 5 or 10 µm) per minute with a three-axis micromanipulator F-131.3SS (Physik Instrumente GmbH & Co. KG, Karlsruhe, Germany). Weight data were automatically collected into a text file at the frequency of 1 Hz. The whole process was carried out three times for each capillary using step lengths of 5 and (two times) 10 µm at a velocity of 50 µm s^{-1} and driving over a distance of 60 and 120 µm, respectively. Recorded weight data were processed with Excel 2007 (Microsoft Corporation, Redmond, WA, USA). From each step, the mean value of the data points between 28 and 58 s after the start of the load increase was calculated (usually the values were stable within this time frame) (Fig. 5D). The weight force per pipette deflection distance was then calculated for each of the three runs using a linear regression approach (Fig. 5E), and mean values of the slopes were taken as the calibration factor for each pipette tip. The calibration factors obtained ranged between 0.09 and 0.11 N m^{-1}.

For the force measurements, the pipette was attached with double-sided tape to the 2D movable microscope table of an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany), operated with transmission light and a 40×/0.65 lens. A freshly detached (by squeezing the coxa-trochanter region with fine forceps) pedipalp of an adult *Nemastoma lugubre* female was proximally embedded into dental wax (so that the

Table 2. Springtail species used in this study and their cuticular features

Order	Species	Primary granules	Secondary granules	Fringed bristles	Scales	Test
Entomobryomorpha	*Tomocerus vulgaris* (Tullberg 1871)	+	–	–	+	PC, WT
	Orchesella flavescens (Bourlet 1839)	+	–	+	–	PC, WT
	Lepidocyrtus lignorum (Fabricius 1793)	+	–	+	+	PC, AM
	Lepidocyrtus curvicollis Bourlet 1839	+	–	+	+	PC
Symphypleona	*Bourletiella viridescens* (Stach 1920)	+	–	–	–	PC, WT
	Indet. spp.	+	?	–	–	PC
Poduromorpha	*Neanura muscorum* (Templeton 1835)	+	+	–	–	PC, WT

The last column specifies in which tests the species were used: AM, adhesion measurement; PC, prey capture observation via high-speed video (HSV); WT, wetting tests via HSV and/or cryo-scanning electron microscopy.

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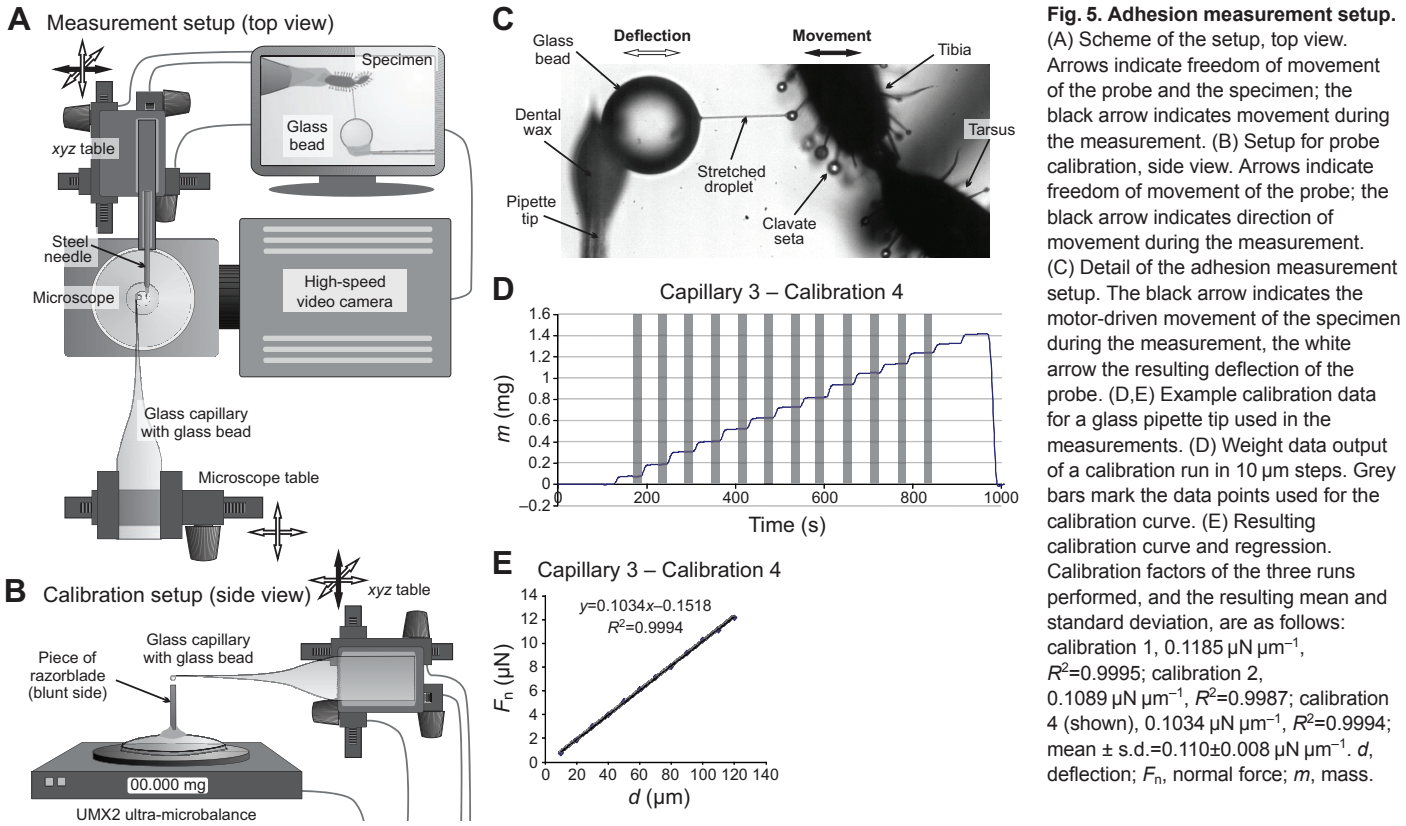

Fig. 5. Adhesion measurement setup. (A) Scheme of the setup, top view. Arrows indicate freedom of movement of the probe and the specimen; the black arrow indicates movement during the measurement. (B) Setup for probe calibration, side view. Arrows indicate freedom of movement of the probe; the black arrow indicates direction of movement during the measurement. (C) Detail of the adhesion measurement setup. The black arrow indicates the motor-driven movement of the specimen during the measurement, the white arrow the resulting deflection of the probe. (D,E) Example calibration data for a glass pipette tip used in the measurements. (D) Weight data output of a calibration run in 10 µm steps. Grey bars mark the data points used for the calibration curve. (E) Resulting calibration curve and regression. Calibration factors of the three runs performed, and the resulting mean and standard deviation, are as follows: calibration 1, 0.1185 µN µm^{-1}, R^2=0.9995; calibration 2, 0.1089 µN µm^{-1}, R^2=0.9987; calibration 4 (shown), 0.1034 µN µm^{-1}, R^2=0.9994; mean ± s.d.=0.110±0.008 µN µm^{-1}. *d*, deflection; F_n, normal force; *m*, mass.

patella-tibia joint was fixed) and glued onto the tip of a steel needle attached with double-sided tape to the mobile stage of a three-axis micromanipulator (F-131.3SS, Physik Instrumente GmbH & Co. KG, Karlsruhe, Germany) (Fig. 5A). The pedipalp was carefully driven towards the glass bead until the apical droplet of a seta made contact (Fig. 5C). Then it was pulled back at different velocities (1, 5, 10, 50, 100, 500, 1000 µm s^{-1}) until the contact broke. Pull-off tests were recorded with a high-speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA) mounted onto the microscope using frame rates of 50–1000 frames s^{-1} depending on pull-off velocity, and a shutter speed of 1/5000 s. Videos were saved as image sequences (tiff) and analyzed with ImageJ 1.47v (Wayne Rasband, National Institutes of Health, USA). Movement of the glass sphere near the contact were tracked using the MTrackJ plugin by Erik Meijering. To obtain force–time curves, *x*-coordinate shift data (the change in the *y*-coordinate was negligible) was imported into Excel and pipette deflection distances were calculated according to the scaling factor obtained by measuring the scale of an object micrometer (with Measure 2.1d software, DatInf® GmbH, Tübingen, Germany) in a image recorded using the same lens and camera. Adhesive forces causing the deflection could then be calculated using the previously obtained calibration factor of the pipette tip. With this method, forces can be measured at a resolution of 0.05 µN, limited by the 40× lens used (higher magnification lenses can produce sharp images only with immersion oil) and the resolution of the high-speed camera video chip (1048×1048 pixels, obtaining 0.485 µm pixel^{-1} in this setup).

Remains of the secretion were occasionally seen after testing at low (1–10 µm s^{-1}) pull-off rates. As this may affect consecutive measurements, each tested seta was driven to another contact point with the bead, and a new testing pipette was used for each specimen. We decided not to chemically clean the pipette tip as this affected the bead fixation and may further change the spring constant of the pipette tip and the surface chemistry of the glass bead. Attention was paid that the contact was pulled off perpendicularly, though at high pull-off velocities a slight lateral drift could not be prevented.

To estimate the elastic modulus of the secretion, stress–strain curves were calculated on the basis of the recorded pull-off test image sequences (three tests from three setae at pull off-speeds of 10, 100 and 1000 µm s^{-1}). For this purpose, the change of length (strain) was determined by measuring the initial diameter of the droplet in contact with the glass bead and the change of length as the difference of seta *x*-coordinate shift and glass bead *x*-coordinate shift both tracked with MTrackJ in ImageJ. To calculate the stress, the pull-off forces were divided by the cross-section of the stretched droplet at the narrowest point. A cylindrical geometry was assumed, and the diameter was measured in every 10th image of the sequence using DatInf® Measure software. When the diameter of the stretched droplet fell below 2 pixels (1 µm), the analysis was stopped. As the stress–strain curve was linear only in the initial phase, linear regression was performed only on this part of the curve, and the resulting scaling factor represents the elastic modulus of the secretion.

To observe the wetting of springtail cuticle by the secretion, the setup described was used in similar manner. In this case, a collembolan (*T. vulgaris* or *N. muscorum*) was glued onto the tip of a stiff steel needle. The pedipalp was carefully driven towards the springtail cuticle with a speed of 1 µm s^{-1} until contact was made. Contact formation was filmed at 10,000 frames s^{-1}. Droplet stretching at a pull-off velocity of 50 µm s^{-1} was recorded at 50–60 frames s^{-1}. In tests to find out whether the pressure applied on the clavate setae causes wettability enhancement, the pedipalp was driven 5, 10, 15 and 20 µm further towards the pipette tip after contact formation.

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Competing interests

The authors declare no competing financial interests.

Author contributions

J.O.W. and C.F.S. conceived and designed the experiments. J.O.W. performed the experiments and analyzed the data. J.O.W., A.L.S., C.F.S. and S.N.G. wrote the paper.

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