Comparative functional morphology of attachment devices in Arachnida

Vergleichende Funktionsmorphologie der Haftstrukturen bei Spinnentieren (Arthropoda: Arachnida)

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Abstract

Attachment is one of the major interactions between an organism and its environment. An enormous diversity of organs or secretory products has been evolved to enhance adhesion and friction with various substrates. The biological functions comprise maintenance of position, locomotion, prey capture, defence, reproduction or dispersal. There are numerous studies that deal with this issue in lizards, frogs, insects, barnacles, mussels and echinoderms, but the second largest class of arthropods, the Arachnida, are highly neglected. This work surveys the attachment organs and structures, and adhesive secretions occurring in this class and discusses the relationship between morphology and function, evolutionary trends, and biomimetic potential. The found diversity comprises interlocking and clamping devices (like claws, spines, hooks, pincer, locking piercer and raptorial legs), smooth and hairy adhesive pads, suckers, and hardening and viscid glue. Mechanical attachment is found in every arachnid order. The membrane based smooth adhesive pads occur in pseudoscorpions (Pseudoscorpiones), camel spiders (Solifugae), ticks (Ixodida and Holothyrida), mites (Opilioacarida, Mesostigmata, Sarcoptiformes and Trombidiformes), harvestmen nymphs (Opiliones), and prenymphs of scorpions (Scorpiones), whip spiders (Amblypygi) and whip scorpions (Thelyphonida). Hairy adhesive pads are found in spiders (Araneae), harvestmen (Opiliones), mites (Trombidiformes), and hooded tickspiders (Ricinulei). A unique micro-patterned adhesive pad (‘smooth’ pad with spatulae) occurs in whip spiders (Amblypygi). Suckers are present in mites (Sarcoptiformes and Trombidiformes). Hardening glue is produced by spiders (Araneae), whip spiders (Amblypygi), whip scorpions (Thelyphonida), scorpions (Scorpiones), harvestmen (Opiliones), pseudoscorpions (Pseudoscorpiones), ticks (Ixodida), and mites (Mesostigmata, Sarcoptiformes and Trombidiformes). Viscid secretions for attachment purposes are produced by spiders (Araneae), harvestmen (Opiliones), and mites (Mesostigmata and Trombidiformes). The mechanical function and fine structure of selected attachment devices was studied, namely the arolia of whip spiders, whip scorpions, scorpions, pseudoscorpions and ticks, the tenent setae of some mites, the glandular setae and secretion of harvestmen, and the silken attachment discs of spiders. Further morphological examinations were performed on the scopulae of harvestmen and ricinuleids, the arolia of harvestmen and the secretions serving camouflage with soil particles in harvestmen and mites. Most structures and secretion properties have remarkable analogies among insects, lizards and tree frogs, which illustrate the optimal biological solutions for universal or specific attachment. This holds for the shape of claws, the spatulate or (rarer) mushroom-like shape of contact elements in adhesive hairs, the inner directed fibrillation of smooth adhesive pads, and the viscoelastic properties of prey capture adhesives. However, some of the described attachment devices are rather unique. In scorpion prenymphs the perhaps most simple type of an adhesive foot pad was found, represented by the non-sclerotized sac-like tip of the pretarsus that is hold in shape by the internal fluid pressure and can be invaginated by a muscle. An aroliar shape building a narrow, transverse contact has evolved in three different orders of arachnids. This structure presumably enhances the ability to switch quickly between a distribution and concentration of stress, which is of high importance for dynamic adhesion. In whip-spiders an arolium was found that exhibits hexagonal microstructures with spatulate tips, which have previously only known from hairy adhesive pads. These pads can generate a high adhesive strength, comparable to spiders and geckoes, even in the absence of fluids. The hexagonal order presumably permits a rapid drainage, enhancing the adhesion on wet surfaces, as known from tree frogs. The prehensile tarsi of harvestmen have dense pads of thin pointed setae, permitting a secure grip without the necessity of adhesive structures. The glandular setae of harvestmen exhibit special microstructures that arrest a droplet of viscid glue even at high pulling stresses. The silken attachment discs of spiders are secretory glue products with a unique hierarchical structure enhancing the flaw tolerance and attachment performance on anti-adhesive surfaces. The results may be of high relevance for our understanding of the function and evolution of attachment devices, as well as for the life history, behaviour, ecology and phylogeny of arachnids. The findings may also be a source of inspiration for biomimetic approaches and demonstrate that the high neglecting of arachnids in biomechanic research is unjustified.

Kurzzusammenfassung

Befestigung ist eine der wichtigsten Interaktionen zwischen einem Organismus und seiner Umwelt. Zu diesem Zweck entstand im Laufe der Evolution eine enorme Vielfalt an Klammer- und Haftorganen sowie klebrigen Sekreten. Ihre biologischen Funktionen umfassen Positions-Beibehaltung, Fortbewegung, Beutefang, Verteidigung, Reproduktion und Verbreitung. Zahlreiche Studien befassen sich mit dem Aufbau, der mechanischen Funktion und der stofflichen Zusammensetzung solcher Organe oder Sekrete bei Echsen, Fröschen, Insekten, Seepocken, Muscheln oder Stachelhäutern, aber die zweitgrößte Klasse der Gliederfüßer (Arthropoda), die Spinnentiere (Arachnida), sind bei dieser Forschung weitgehend ignoriert. Diese Arbeit gibt einen Überblick über Haftorgane, -strukturen, und -sekrete in dieser Tierklasse und diskutiert den Zusammenhang zwischen deren Morphologie und Funktion, evolutionäre Trends und ihr Potenzial für die Bionik.

Die Haftorgane der Arachniden umfassen Verhakung- und Klammer-Vorrichtungen (Krallen, Stacheln, Haken, Zangen, Riegel, Klammer- und Fangbeine), glatte und haarige Haftpolster, Saugnäpfe, sowie aushärtende oder schleimartige Klebstoffe. Vorrichtungen zur mechanischen Haftung, wie Krallen, treten in jeder Arachniden-Ordnung auf. Die membran-basierten glatten Haftpolster treten bei den Pseudoskorpionen (Pseudoscorpiones), Walzenspinnen (Solifugae), Zecken (Ixodida und Holothyrida), Milben (Opilioacarida, Mesostigmata, Sarcoptiformes und Trombidiformes), Weberknecht-Nymphen (Opiliones), sowie den Pränymphen von Skorpionen (Scorpiones), Geißelspinnen (Amblypygi) und Geißelskorpionen (Thelyphonida) auf. Hafthaare (mit spatelförmigen Terminalstrukturen) kommen bei Spinnen (Araneae), Weberknechten (Opiliones), Milben (Trombidiformes) und Kapuzenspinnen (Ricinulei) vor. Eine einzigartige Form eines ‚glatten' Haftpolsters mit spatulären Strukturen wurde in Geißelspinnen (Amblypygi) gefunden. Saugnäpfe finden sich bei einigen Milben (Sarcoptiformes und Trombidiformes). Aushärtender Klebstoff wird produziert von Spinnen (Araneae), Geißelspinnen (Amblypygi), Geißelskorpionen (Thelyphonida), Skorpionen (Scorpiones), Weberknechten (Opiliones), Pseudoskorpionen (Pseudoscorpiones), Zecken (Ixodida) und Milben (Mesostigmata, Sarcoptiformes und Trombidiformes). Schleimartige Klebstoffe gibt es bei Spinnen (Araneae), Weberknechten (Opiliones) und einigen Milben (Mesostigmata und Trombidiformes). Die mechanische Funktion und Feinstruktur wurde an ausgewählten Haftstrukturen näher untersucht: die Arolien der Geißelspinnen, Geißelskorpione, Skorpione, Pseudoskorpione und Zecken, die Hafthaare einiger Milben, die Drüsenhaare und Haftsekrete von Weberknechten, sowie die seidenen Haftscheiben der Spinnen. Desweiteren wurden morphologische Untersuchungen durchgeführt an den Scopulae von Weberknechten und Kapuzenspinnen, den Arolien bei Weberknechten, sowie den Sekreten zur Anhaftung von Bodenpartikeln zur Tarnung bei Weberknechten und Milben.

Für die meisten Strukturen und Sekrete gibt es erstaunlich ähnliche Analogien bei Insekten, Echsen und Fröschen, was die optimalen biologischen Lösungen für universelle oder spezifische Haftung aufzeigt. Dies gilt für die Form und Struktur von Krallen, die spatuläre oder (seltener) pilzkopfartige Form von adhäsiven Kontaktelementen, die faserige innere Struktur glatter Haftpolster, sowie die Viskoelastizität schleimartiger Klebstoffe zum Beutefang.

Einige der beschriebenen Haftstrukturen sind dagegen Besonderheiten der Spinnentiere. Bei den Pränymphen der Skorpione wurde die vielleicht basalste Art eines adhäsiven Fußpolsters gefunden, gebildet von der nicht-sklerotisierten sack-artigen Spitze des Prätarsus. Diese wird in Form gehalten durch den inneren Flüssigkeitsdruck und lässt sich durch einen Muskel zurück ziehen. Eine besondere Form des Aroliums, welche mit dem Substrat eine schmale Kontaktfläche quer zur Beinachse bildet, ist in drei Arachniden-Ordnungen unabhängig voneinander entstanden. Dies verbessert wahrscheinlich die Fähigkeit schnell zwischen Stressverteilung und Stresskonzentration zu wechseln, was für dynamisches Haften von großer Bedeutung ist. In Geißelspinnen wurde ein Arolium gefunden das hexagonale Mikrostrukturen mit apikalen Spatulae aufweist. Spatulae waren zuvor nur von Hafthaaren bekannt. Die Haftpolster der Geißelspinnen können auch ohne die Kapillarkraft durch Sekrete eine große Haftstärke generieren,

vergleichbar mit den haarigen Haftpolstern von Spinnen und Geckos. Die hexagonalen Strukturen begünstigen wahrscheinlich den Ausfluss von Flüssigkeiten aus dem Kontakt, was den Halt auf nassen Oberflächen verbessert, ein Prinzip das von den Haftzehen von Baumfröschen bekannt ist. Die vielgliedrigen Greiffüße von Weberknechten sind mit dichten Polstern feiner Häärchen besetzt, was vermutlich einen sicheren Griff auf zahlreichen Oberflächen ermöglicht, ganz ohne die Notwendigkeit von adhäsiven Strukturen. Die Drüsenhaare von Weberknechten zeichnen sich durch den Besatz mit speziellen Mikrotrichien aus, wodurch sich der anhaftende klebrige Sekret-Tropfen selbst bei starkem Zug nicht ablöst. Die seidenen Haftscheiben von Spinnen sind sekretorische Klebstoff-Produkte mit einer einzigartigen hierarchischen Struktur, die die Haftung auf mikrostrukturierten und anti-adhäsiven Oberflächen verbessert.

Die Ergebnisse können von hoher Relevanz für das Verständnis über die Funktion und Evolution von Haftstrukturen, sowie der Biologie, der Ökologie, dem Verhalten und der Systematik der Arachniden sein. Sie können auch eine Inspirationsquelle für bionische Ansätze zur Lösung technischer Probleme oder der Verbesserung von Produkten sein. Die Studie zeigt, dass bei Arachniden eine Fülle spannender Mechanismen zu finden sind, und sie damit wertvolle Studienobjekte der Biomechanik sind.

Content

1. Introduction

Attachment is a fundamental part of the interaction between an organism and its environment. In dependence of the phylogenetic origin and the ecological demands a huge variety of attachment organs and structures evolved including different physical principles like friction, dry and wet adhesion, cohesion, mechanical interlocking, suction and penetration (Nachtigall, 1974, Gorb, 2001). Recent efforts in resolving the functional principles of attachment structures in insects and geckoes at the macroscopical, microscopical and molecular scale led to intense interdisciplinary research and, not at least, technical innovations, like the recently developed Gecko® Tape (Binder GmbH) (for review see (Gorb, 2011)). However, the second largest class of arthropods, the Arachnida, has been highly neglected in this research. A comprehensive overview on attachment devices in arachnids has never been undertaken, and there are only few studies that deal with the mechanical function of some of these.

Beside the structure based sticking devices a large diversity of gluey secretions can be found among all kinds of organisms, basing on various substances from polysaccharides and lipids to polypeptides and proteins (von Byern and Grunwald, 2010, Betz and Kölsch, 2004). Depending on their composition and biological function the bonding can be temporary or permanent. Its functions include substrate attachment, prey capture, nest and cocoon construction, defense, camouflage and reproduction. Studying the mechanical function of such secretions, their efficacy and limitations, may help to understand the biology and ecology of these animals. Further, lots of these natural glues exhibit a set of properties that surpasses artificial adhesives in several aspects: with often small amounts necessary they can create strong bonds even on repellent or wet surfaces, under water, or with living tissue; they are bio-degradable, use non-toxic solvents and might be bio-compatible (Smith and Callow, 2007). Hence, they might have very innovative potential for the development of custom adhesives.

This work aims to explore the structural and mechanical principles of cuticular attachment devices and secretion products in the different orders of Arachnida LAMARCK 1815. It focuses on the morphology, mechanical and biological function of those structures, sets them in relation to life history, ecology and phylogeny of the studied animals, and discusses their potential for biomimetic approaches.

The first chapter presents a comprehensive overview on the occurrence, morphological diversity and functional anatomy of attachment organs and structures, and adhesive secretions in arachnids combing a literature survey with a variety of imaging methods. The results of comparative force measurements are presented and briefly discussed.

The subsequent chapters focus on some of the found structures, namely the smooth adhesive food pads of arachnid prenymphs (***3***), the arolium of whip spiders (***4***), the glue carrying pedipalpal setae of harvestmen (***5-7***), and the silken attachment discs of araneomorph spiders (***8-9***). These chapters represent research papers resulting from this PhD project that have already been published (papers no.1, 4, 6 and 7) or are drafts ready for submission (papers no.2, 3 and 5).

The last chapter sets the findings in context with the state of knowledge on biological adhesion and discusses the mechanisms that influence the evolution of attachment devices. It depicts the noteworthy features that are uniquely found in this class and of potential interest for biomimetic and bioengineering approaches, especially the transverse-strip shape of smooth adhesive food pads that evolved three times among arachnids, the micro-patterned cuticle of whip spider adhesive food pads and the nano-composite structure of silken attachment discs.

2. Attachment devices and adhesive secretions in Arachnida: a survey

2.1. Introduction in the structural and functional principles of attachment devices

Various types of attachment devices can be categorized based on different functional principles and structural properties. Fig.2.1. presents a schematic overview on the types of attachment devices, which have been found in the Arachnida in this study. In the following the functional principles are introduced and the state of knowledge is summarized.

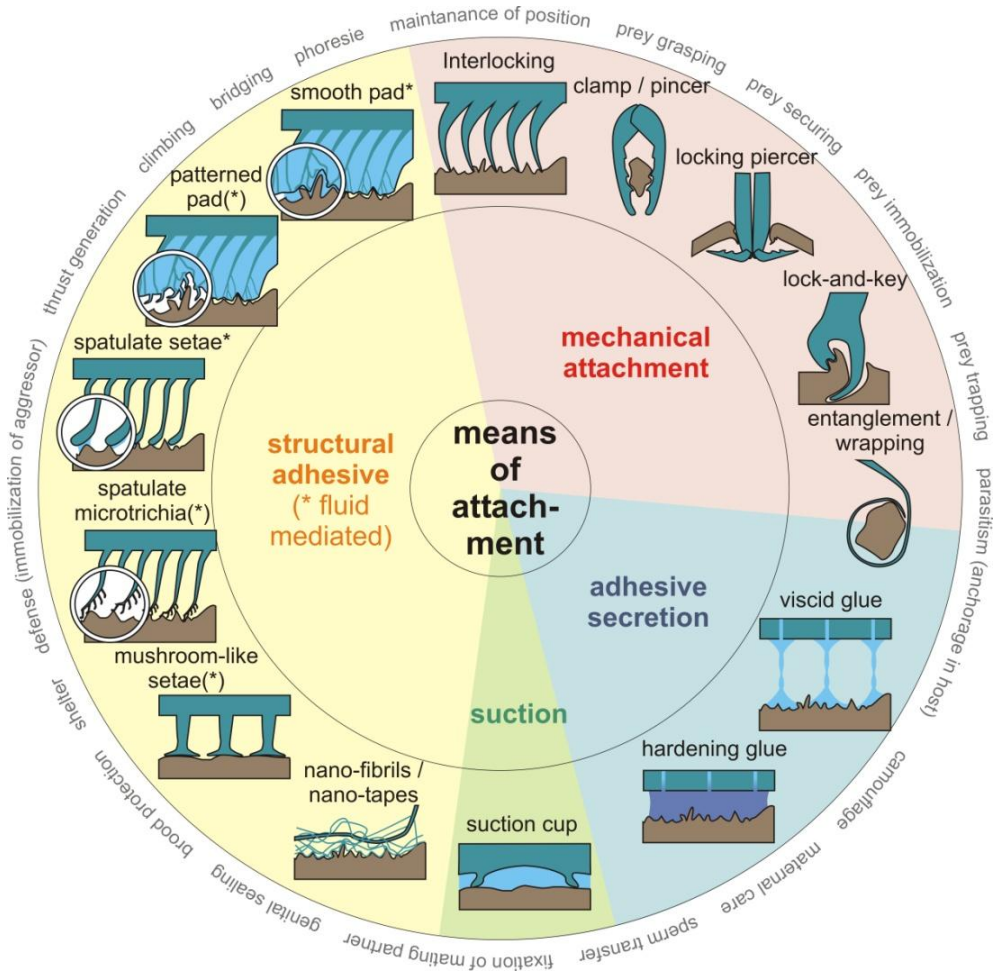


Fig.2.1. Schematic overview on the different mechanisms and biological functions of attachment occurring among arachnids.

2.1.1. Mechanical attachment

Most attachment structures use the principle of mechanical interlocking with substrate structures. This principle is associated with low costs as no material discharge (like secretions) is necessary and the structures are relatively simple. Further these structures or organs often exhibit a high universality of usage. This may explain why it is the most widespread principle. Ubiquitous attachment devices are claws at the foot, which are characterized by a broad base, a curvature towards the substrate and a tapering tip. The tip diameter is crucial for the interlocking ability on a broad range of substrates and should be preferably small (Dai et al., 2002, Ditsche-Kuru et al.,

2012, Labonte and Federle, 2015). The efficacy is dependent on the resistance of the structure, because deformation under load would lead to a loss of the interlocking mechanism. Hence, the material must be very stiff at the tip the sharper it is, in order to prevent deformation. However, a rigid claw exhibits a high stress concentration in the tip, which increases the risk of breakage the more pointed the tip is (Labonte and Federle, 2015). Counter-measures are an underlying elastic material layer or an elastic hinge that absorbs some of the stress, and the increase of material strength, e.g. by the incorporation of metal ions (Fontaine et al., 1991, Schofield et al., 2003) or a specific material structure (Bar-On et al., 2014).

Spines (stiff, pointed setae) are frequently found and may act similarly to claws, but usually on smaller substrate structures, because of a smaller tip diameter. Hence they might be very effective supporting the claws in order to generate high friction on rough surfaces. Arrays of spines may also improve the foothold in a complex microenvironment (Spagna et al., 2007) (i.e. herbal layer with cylindrical plant stems in short distances and different angles), or as a prey capture basket (building a barrier for struggling prey) (Fig.2.2.E-H).

Other structures are pierced and locked within a substrate, like the fangs of spiders, or evolved to interlock with a highly specific structure as in spider genitals or some spermatophores (lock-and-key mechanism). Clamps like the chelae of some pedipalps or chelicerae (Fig.2.2.A-D), raptorial pedipalps (Fig.2.2.E-H) or some modified legs in mites, work by the flexion of a moveable part against an immobile part, often with function enhancement by leverage. The closure is performed with strong muscles, the opening may be muscular driven or by the release of stored elastic energy (Sensenig and Shultz, 2004).

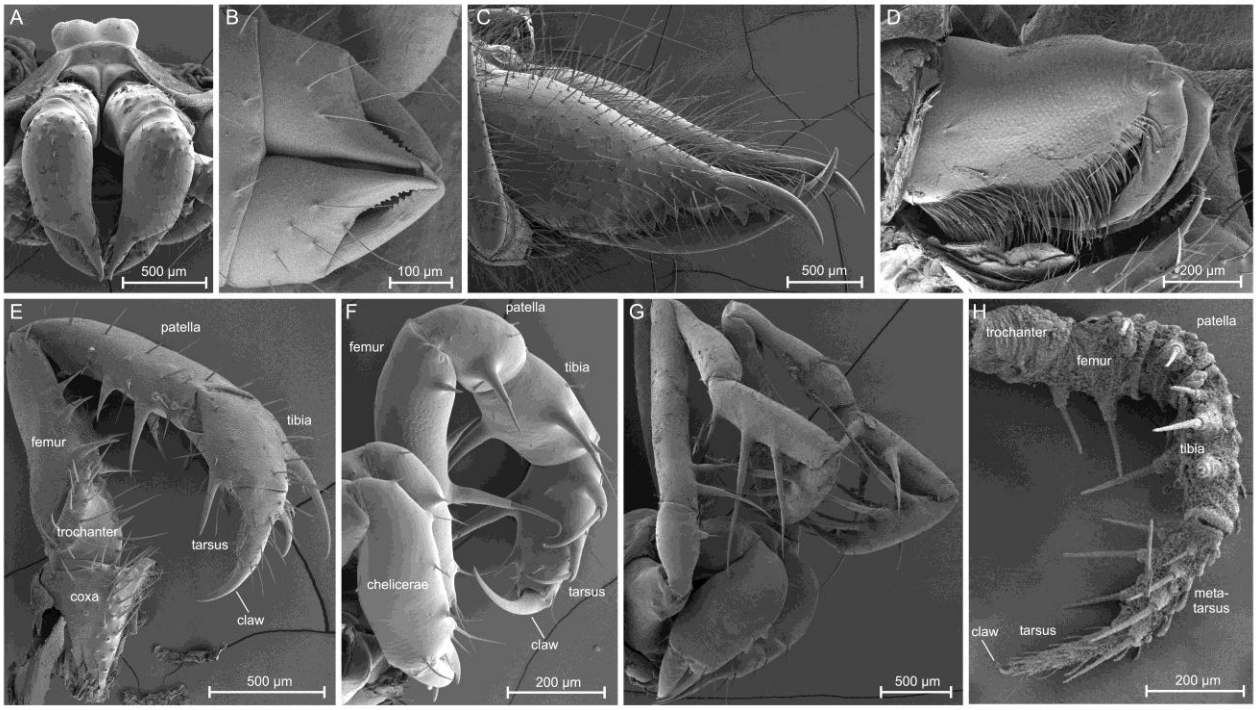


Fig.2.2. Clamping devices in arachnids. (A-D) Chelicerae. (A) Harvestman *Dicranopalpus ramosus* (Opiliones: Phalangiidae), frontal view. (B) Pseudoscorpion *Neobisum* sp. (Pseudoscorpiones: Neobisiidae), dorsal view. (C) Juvenile Solpugidae (Solifugae), unidentified species, lateral view. (D) Whip spider *Charinus cubensis* (Amblypygi: Charinidae), lateral view. Jackknife-like type, as typical for the Tetrapulmonata. (E-G) Raptorial pedipalps. Please note the elongated patella, the high flexion of the femur-patella joint and the enlarged claw. (E) Whip spider *Charinus cubensis* (Amblypygi: Charinidae). (F) Harvestman *Holoscotolemon naturae* (Opiliones: Cladonychiidae). (G) Nymph of the harvestman *Dibunus similis* (Opiliones: Epedanidae). (H) Raptorial leg 1 of the predatory mite *Caeculus* sp. (Trombidiformes: Caeculidae).

2.1.2. Smooth adhesive pads

Many, especially small, animals have pad-like adhesive organs, which are usually located at the tip of appendages and used for substrate attachment during locomotion, prey capture or copulation. The main principle of adhesive pads is high compliancy (Gorb, 2001). Such pads are preferably soft to highly adapt to the topography of the substrate and create a high contact area. In so-called smooth pads the outer-most layer is very thin and thus deformable very locally (Jiao et al., 2000), and at the same time stiff and tough to resist high shear forces (Bennemann et al., 2014). A mechanical stability at maintenance of compliancy is usually achieved by a foam-like or fibrillar structure of the underlying material layer (Gorb et al., 2002). Such arrays of fibrils are usually not perpendicular to the outer membrane, which causes an anisotropic friction of the pad (usually high friction when dragged towards the body and low friction when pushed away from the body) (Gorb, 2000, Gorb, 2007, Bullock et al., 2008). This is a frequent means of controlling the attachment by leg positioning. Another means of adapting the attachment strength is the control of the pad contact area by pretarsal muscles (Federle and Endlein, 2004). Substantial for a good adhesion of smooth pads is the secretion of a thin, continuous fluid film into the contact, which produces high capillary and viscous forces (Drechsler and Federle, 2006, Barnes, 2007).

2.1.3. Hairy adhesive pads

The other major type of adhesive foot pads are so-called hairy or fibrillar pads. These are dense arrays of hair-like structures (setae) that are characterized by broad, flattened tips (spatulae). The physical principles of such hairy adhesive pads have been reviewed by (Gorb, 2001) and (Federle, 2006). The spatula is the terminal contact element which generates an intimate contact with the substrate. It can be modeled as a soft, elastic and thin tape (Varenberg et al., 2010). If it is very thin and soft, the force necessary to detach such a tape from the surface depends primarily on the width of the tape, because the stress is then acting locally in the delamination zone (peeling edge) (Kendall, 1975). Due to the close contact with the substrate the spatula can adhere by short ranging intermolecular forces, like van-der-Waals forces (dry adhesion), or the capillary and/or viscous forces of thin films of fluid secretions (wet adhesion). Because the single setae are elastic and can deform rather independently from the neighboring ones the pad can deform very locally and adapt well to the surface topography of a rough substrate. Further, the adhesion failure of a single spatula does barely affect the bonding of the neighboring setae. As the initiation of delamination demands more energy than its propagation, it is beneficial to split the contact area into several sub-contacts, such that a crack is stopped at each gap and has to be repeatedly initiated at each sub-contact (Peressadko and Gorb, 2004, Chung and Chaudhury, 2005). This observation lead to the theory that the adhesion of a fibrillar pad does less depend on its contact area, but primarily on the sum of the width of all contacting spatula (peeling line length) (Arzt et al., 2003). The contact splitting theory is also applicable if the spatulae are fluid mediated, because then the capillary and viscous forces depend on the circumference of the contacts (Qian and Gao, 2006). An overall tendency is an increased degree of contact splitting with increasing body mass, with the largest animals, the geckoes, exhibiting the smallest spatulae (width 0.1-0.4 µm), standing in the highest density (about 4 000 000 spatulae per mm^2) (Arzt et al., 2003). With an increasing density the spatulae tend to stick to each other (self-matting), which highly reduces the functionality of the pad (Federle, 2006). Hence, such high densities as in geckoes can only be achieved by setal branching: Each seta splits up at its tip or bears numerous microtrichia which end in a spatula. Whereas the relation between body mass and spatular density holds between different lineages of

animals that analogously evolved hairy adhesive pads, this does not necessarily hold for animals within the same lineage, and is thus controversially discussed (Peattie and Full, 2007). Further the finding may in part depend on the choice of included taxa, as some tiny mites exhibit spatulae very similar to large spiders (see ***2.11.4.***).

The adhesion by spatulate adhesive setae needs an initial directed shear movement (activation), by which the spatula is spread onto the substrate (Filippov et al., 2011, Autumn et al., 2006). The setae are usually orientated in such a way that this movement is proximal (towards the body) (Niederegger and Gorb, 2006, Autumn et al., 2006, Wolff and Gorb, 2013). By distal movement the spatula turns over due to its flexibility, and looses the contact (deactivation). The rear part is often corrugated or equipped with nubs or short protuberances that prevent the generation of a high contact area (Niederegger et al., 2002, Gorb, 2001). By this anisotropy the animal can quickly and effortlessly switch between high and low adhesion, which is crucial for fast locomotion.

2.1.4. Glue

Glue (an adhesive) is a substance that wets a surface and builds chemical or physical bonds with it, resulting in strong adhesive forces (Kinloch, 1987). Efficient glue shows a good wetting behavior on the target surface, strong adhesion (i.e. by building hydrogen or covalent bonds with the substrate surface) and a similarly strong cohesion (inner strength). The initial cohesion should be preferably much lower than the adhesion to the target surface to provide a good wetting behavior, but should then quickly increase to a value at least as high as the adhesion to provide a strong bonding. In glue for permanent adhesion, as for the substrate attachment of sessile organisms, this is usually achieved by hardening after application. Hardening may be due to polymerization or the evaporation of a solvent (in biological adhesives usually water) due to the air exposition after secretion (Nachtigall, 1974). If the target surface is rough also the bonding is increased after hardening due to mechanical interlocking. Types of permanent glue are primarily based on self-assembling proteins or peptides that may cross-link and build a large polymer complex after application (Voigt and Gorb, 2010, Naldrett, 1993, Dickinson et al., 2009, Silverman and Roberto, 2007, Hennebert et al., 2008).

The disadvantage of permanent glue is that formation and break up usually take some time. Hence they are barely applicable for locomotion, prey capture and defense, which demand a quick action (but see polymeric glue of Onychophorans (Röper, 1977)). For that purposes often viscous or visco-elastic substances are used that may create strong bonding over a short period of time without hardening (Betz and Kölsch, 2004). In fluids cohesion is enhanced by capillary and/or viscous forces. A capillary effect is most efficient in thin films between two surfaces (e.g. an attachment organ and a substrate) (Betz and Kölsch, 2004, Dirks and Federle, 2011). In thicker films, high capillarity demands a high surface tension, which, however, reduces the spreading behavior on micro rough surfaces. A high viscosity leads to a slow flow, which reduces the ability to separate two conglutinated surfaces when pulled apart quickly (Betz and Kölsch, 2004). If the target surface has to be wetted quickly certain impact might be necessary. Visco-elastic adhesives show a shear thickening behavior which temporarily highly enhances the cohesion under quick load (Sahni et al., 2010, Wolff et al., 2014b, Dirks et al., 2009). In the case of the sundew, a carnivorous plant, this is based on a hygroscopic polysaccharide network that is highly compliant under pressure, but stretches to long tough nano-fibers under tension (Huang et al., 2015). Adhesive secretions are based on a large variety of substances, like mucopolysaccharides (Rost

and Schauer, 1977), glycoproteins (Vollrath and Tillinghast, 1991), emulsions of lipoids in water or protein solutions (Betz and Kölsch, 2004, Dirks et al., 2009), and waxes or resins (Hermann and Blum, 1981). They may contain surfactants that highly reduce the surface tension, which may permit spreading on surfaces with complex microstructures that usually act highly repellent on lots of liquids and are present in both arthropods (Helbig et al., 2011) and plants (Neinhuis and Barthlott, 1997).

2.1.5. Suction cups

Suction cups are devices that produce a pressure gradient of the medium between a formed cavity and the surrounding. The higher the pressure difference the higher the attraction between the sucker and the substrate. To produce a stable pressure gradient the inner cavity must be sealed as good as possible, which means that the surrounding lip-like structure should be highly compliant (Tramacere et al., 2014b). The secretion of viscous fluids or mucous can improve the sealing (Tramacere et al., 2014a). The pressure in the cavity is lowered by a retraction of the median part, which increases the inner volume. If the cavity is properly sealed the medium flow to the inside is hampered and the density and hence the pressure of the medium within is reduced. The retraction can be performed by muscles and/or or the elastic energy of the initially deformed cup (Gorb, 2008, Kier and Smith, 1990, Frutiger, 2002). Suction cups are much more effective in water than in air, because of the higher viscosity (which hampers the flow). Further, in water very small volume changes of the cavity are sufficient to produce high attachment forces due to the lower compressibility of water than air (Kier and Smith, 1990). That's why suction cups are nearly restricted to aquatic or endo-parasitic organisms. Another restriction of suction cups is given by their size. In contrast to adhesive pads the adhesive strength of suction cups does not scale with area or length, but with volume, because the force is based on the generated pressure gradient. This means, while adhesive pads are more effective at smaller body size, it is vice versa for suction cups. Hence, with deceasing diameter the adhesive strength of suction cups decreases much faster than that of flat, elastic contacts of equal size (Spolenak et al., 2005). In very small suction cups (diameter <10µm) the volume, and hence the achievable pressure gradient, is assumed to be too small to generate sufficient adhesion (Spolenak et al., 2005).

2.2. Material and methods

2.2.1. Literature survey and material sourcing

Morphological and functional studies on attachment devices in arachnids are rare. To find hints on the distribution of such organs, structures or the use of adhesive secretions, zoological monographs and descriptive taxonomical literature was assessed (see citations in the text).

During visits of the arachnological collections of the Senckenberg Research Institute Frankfurt and the Zoological Museum at the University of Greifswald conserved material of different arachnid orders was studied by means of a stereo microscope to find specific attachment organs.

On the basis of this preliminary survey the taxa of interest were selected and both living individuals and specimens conserved in ethanol were acquired for morphological and functional studies. The material for these studies was collected in the surroundings of Kiel, during a conference travel to Taiwan and a collection trip to the South-Western Alps. Living individuals were also obtained from breeding stocks from the Institute of Ecology and Evolution at the University of Bern, the Ethology group at the University of Hamburg and private persons. Further specimens were obtained from private collections and the arachnological collections of the Senckenberg Research Institute Frankfurt and the Zoological Museum at the University of Greifswald.

I studied living individuals of spiders (Araneae: Mygalomorphae and Araneomorphae), whip spiders (Amblypygi: Pulvillata and Apulvillata), whip scorpions (Thelyphonida and Schizomida), harvestmen (Opiliones: Laniatores, Eupnoi and Dyspnoi), scorpions (Scorpiones), pseudoscorpions (Pseudoscorpiones), ticks (Ixodida) and mites (Mesostigmata, Trombidiformes and Sarcoptiformes). Conserved material was obtained for all arachnid orders except Holothyrida and Palpigradi.

2.2.2. Light microscopy

Light microscopical images were recorded with a multifocus stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420), and an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany) with a mounted B/W high speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA).

2.2.3. Scanning electron microscopy

Standard SEM: For Standard SEM material stored in 70% ethanol was used. The specimens or parts of them were dehydrated in a series of increasing ethanol concentrations (80%, 90%, 100% and 100% on molecular sieve), followed by critical point drying. 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., Tokio, Japan) at an acceleration voltage of 3.0 kV.

Cryo-SEM: Living individuals (most of investigated mites, some pseudoscorpions, scorpion prenymphs and whip scorpion prenymphs) were attached to a sample holder using Tissue-Tek® compound and shock frozen in liquid nitrogen. The frozen samples were sputter-coated with 10 nm Au-Pd using the Gatan ALTO-2500 cryo system (Gatan Inc., Abingdon, UK) and viewed in the Hitachi S 4800 SEM with the stage cooled down to -120°C. Some specimens

were slightly touched with a frozen scalpel mounted in the prechamber in order to produce freeze fractures.

Histological sections: Living individuals were anaesthetized with carbon dioxide and legs were clipped off using micro scissors. Legs were immediately fixed with gluteraldehyde and osmium tetroxide, dehydrated in a series of increasing ethanol concentrations and mounted in Epon. Epon blocks were trimmed with a Leica EM TRIM2 and sections of 1 and 1.5 µm were made using a Leica EM UC7 ultra-microtome with a Diatome MC2391 diamond knife (Diatome AG, Biel, Switzerland) to about a half of the embedded specimen foot. The rest was cut from the block with a fine circular saw mounted onto a drilling machine and further processed like the sections. Some sections were placed on super frost glass slides for light microscopical investigations, the others were placed on cover slides, previously cleaned with absolute ethanol and 70% acetone and bathed in pioloform solution. Sections were bathed for 45 min in a concentrated KOH solution in two parts methanol and 1 part propylene oxide (Maxwell, 1978), the half cut block samples for 1.5 h. Sections were air dried; the block samples were put in absolute ethanol and critical point dried. Following treatment and microscopy as described above (Standard SEM).

2.2.4. High speed videography (HSV)

Standard HSV: For observations of pretarsal kinematics, living individuals running on horizontal and vertical glass slides were filmed. High speed videos were taken using a color high speed video camera (Fastcam 1024 PCI, Photron Inc., San Diego, CA, USA) mounted onto a stereo microscope, which could be positioned vertically, horizontally and upside-down. To obtain high frame rates, a bright light source was focused onto the foot and a white sheet of paper was held behind. Videos were taken with post-action trigger and rates of 250-3000 frames per second.

Reflection-interference contrast microscopic HSV (RICM-HSV): For investigation of the actual contact area of adhesive foot pads, its change during locomotion and the deposition of fluid secretions living individuals running on thin glass slides were studied by means of an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany) with a mounted B/W high speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA) and operated in reflection interference contrast mode (coaxial light). Videos of detaching feet were taken at rates of 1000-8000 frames per second and a shutter speed of 1/10000 second, using post-action trigger.

2.2.5. Pull-off force measurements

Adhesive forces to smooth glass slides were measured with living individuals of whip spiders (*Sarax brachydactylus*, *Charinus cubanus*, and different stages of *Charon grayi*), pseudoscorpions (*Neobisum* sp.), scorpion prenymphs (*Liocheles australasiae*) and spiders (*Zoropsis spinimana*, females). With the means of non-toxic two compound Polyvinylsiloxane (dental wax) the animals were glued onto a thin wire mounted onto the cantilever of a micro force transducer (FORT-10 with 10 g force range, World Precision Instruments Inc., Sarasota, FL, USA). The force transducer could be moved vertically by a micromanipulator at a speed of 0.25-0.5 mm per second. The signal of the force transducer was amplified and processed by a Biopac MP-100 acquisition system (Biopac Systems Ltd, Goleta, CA, USA). Force curves were recorded using the AcqKnowledge 3.7.0 software (Biopac Systems Ltd). Each animal was tested at least at three different days with 10-25 measurements in total. Peak forces were divided by animal weight

(measured with an AG 204 Delta Range scale, Mettler Toledo GmbH, Greifensee, Switzerland) to calculate the safety factor (sf), and by the sum of contact areas of all legs in contact (measured from coaxial light micrographs) to calculate the adhesive strength. In order to estimate the effective contact area single feet were filmed with means of RICM-HSV (see above) during the test. The contact area at highest forces (usually before contact breakage) was used. The contact area was measured as the proportion of the pad being in direct contact (indicated by dark contrast in the image), not the actual contact area given by microstructures and fluids. Otherwise the adhesive strength could hardly be compared between animals. Further, to get a better estimate of the effective contact area under natural load, we measured the contact area of each foot in each individual when hanging upside down on a glass slide. For that purpose images were recorded with a stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420), operated with coaxial light.

2.3. Terminology

In arachnids no consistent terminology is present for adhesive organs and pretarsal structures. Hence I first summarize the termini used in arachnological literature and uniform them following the definitions established in entomology by Holway (1935) and Dashman (1953).

Ambulacrum: (also *ambulacral apparatus*) From Latin = ambulare = to walk (Maggenti et al., 2005). The 'foot' of ticks and mites (Acari). It usually refers to all structures distal of the tarsus. It is usually the last eudesmatic segment with all its appendages, and hence may be homologous with the pretarsus. The basal pretarsus is often subdivided and can be highly elongated and equipped with enclosing sclerites (*ambulacral stalk*). Distally there is the section that makes the effective contact with the ground, often including a ventral arolium or empodium and up to three claws (*ambulacral claws*). In ectoparasitic mites the claws may be reduced and the arolium greatly enlarged, forming a round disc (*ambulacral disc*).

Apotele: From Greek *apo* = away from + *telos* = end (Maggenti et al., 2005). In mites the distal most appendages, including the tarsal claws, empodium and arolium. Some authors, in contrast, apply this term to the ambulacral stalk. It is presumably an appendage or partition of the pretarsus, however, some authors call the part distally to the ambulacral stalk the pretarsus, hence comparative approaches are necessary to homologize this structure. Dunlop (2002) applied this term more general to all chelicerates, naming the pretarsus with all its apical structures, although he was unsure about the homology. In these cases, the term *apotele* should be avoided in favour of the traditional term *pretarsus*.

Arolium: (pl.: *arolia*) From Greek *arole* = protection (Maggenti et al., 2005). Median unpaired lobe- or cushion-like attachment organs situated at the pretarsus are called *arolia*. They arise from the median pretarsal section and may contain sclerotized elements. They may occasionally be apically sub-divided.

Caruncle: From Latin *caro* = flesh (Maggenti et al., 2005). Soft, bloated pretarsus in acariform mites, forming a 'fleshly pad' for substrate attachment. This term is synonymous to the term *arolium* and should be replaced accordingly.

Claw tuft: Hairy adhesive pads in the distal leg of spiders (Araneae). The structure is very similar to the *scopula* (see below), but the term is well-established to clearly distinguish between the leg pads and the foot pads.

Empodium: From Greek *en* = in + *pous* = foot (Maggenti et al., 2005). In insects the *empodium* is an outgrowth of the *unguitractor* (a sclerite between the claw retractor tendon and the claws). It can be claw-, bristle- or pad-like. We use this term for an unpaired sclerotized protuberance of the ventral pretarsus, usually in the shape of a little claw. In trombidiform mites it can also be a hairy adhesive foot pad in the shape of a flat lobe covered with spatulate microtrichia ('*tenent empodium*'), or it may be modified into an arolium.

Onychium: From Greek *onyx* = little claw, nail (Maggenti et al., 2005). In insects occasionally used synonymous with the *arolium*. In spiders occasionally used synonymous with the *empodium*; or to name a distal sub-segmentation of the tarsus, as in Scytodidae, Drymusidae, Oonopidae and Orsolobidae. Due to its very different usage the term is confusing and imprecise and should be replaced by respective synonyms.

Pretarsus: From Latin *prae* = before + Greek *tarsos* = foot (Maggenti et al., 2005). The pretarsus is the most distal leg segment which is controlled by muscles. In insects there is only a claw depressor muscle whose tendon inserts at a ventral sclerite, which bears the claws (the *unguitractor plate*). In arachnids the (synapomorphic) pretarsus is reduced to a thick ring- or bow-like sclerite (*pretarsal sclerite*), controlled by a ventral *depressor (protractor)* and a dorsal *levator (retractor)* muscle (De Meijere, 1901).

Pulvillus: (pl.: *pulvilli*) From Latin *pulvillus* = little cushion (Maggenti et al., 2005). In insects the term traditionally describes paired lobes underneath the claws. They are often outgrowths of the claw articulation membranes and not the median pretarsal membranes. This term is divergently applied on the median foot pads of ticks and amblypygids and should consequently be replaced by the term *arolium* (see above). In mites this term is also occasionally used for large tenent setae underneath the claws (Vitzthum, 1931), which is misleading.

Scopula: From Latin *scopa* = broom (Homann, 1957). This term is traditionally used for a dense brush of modified setae in arachnids. The term has been established for pads of spatulate (*tenent*) setae on the legs of Araneae and Opiliones. In spiders a distally separated section of the scopula underneath the claws is called *claw tuft* (see above). The occasional application of the term *scopula* for hairy pads at the chelicerae or pedipalps is misleading, because the structure and function of these are different.

Spatula: From Latin *spatula* = spoon. A flattened and broadened apical tip of a *tenent seta* or of microtrichia on a tenent seta.

Tenent plate: *Tenent* from Latin *tenere* = to hold (Maggenti et al., 2005). The basal sclerite of a *claw tuft* (see above), also *claw tuft plate*. It is highly sclerotized and carries a dense array of sockets, in which the tenent setae are based. The tenent plate is part of the tarsus.

Tenent seta: Latin *seta* = bristle. Setae are hair- or bristle-like protuberances of the cuticle built by a single cell and usually based in an articulated socket (Beutel et al., 2013). Tenent setae, also *adhesive setae*, *scopula setae* or *aliform setae*, serve attachment purposes. They are characterized by a spatulate tip or the possession of spatulate microtrichia (see *spatula*).

Unguis: (pl. *ungues*) Latin = claw. The *pretarsal claw*, often erroneously called *tarsal claw*. The claw is a usually paired hook-like structure apically on the pretarsus, articulated by basal arthrodial membranes. The claws can be smooth or pectinate. One or both claws can be reduced. The median often claw like *empodium* is no unguis, as it is an outgrowth of the pretarsus sclerite and not articulated with it. In some trombidiform mites the claws can be equipped with spatulate microtrichia (‘*tenent claws*’).

2.4. Spiders (Araneae CLERCK 1757)

2.4.1. Claws, chelicerae and the pedipalp-epigyne interlocking system

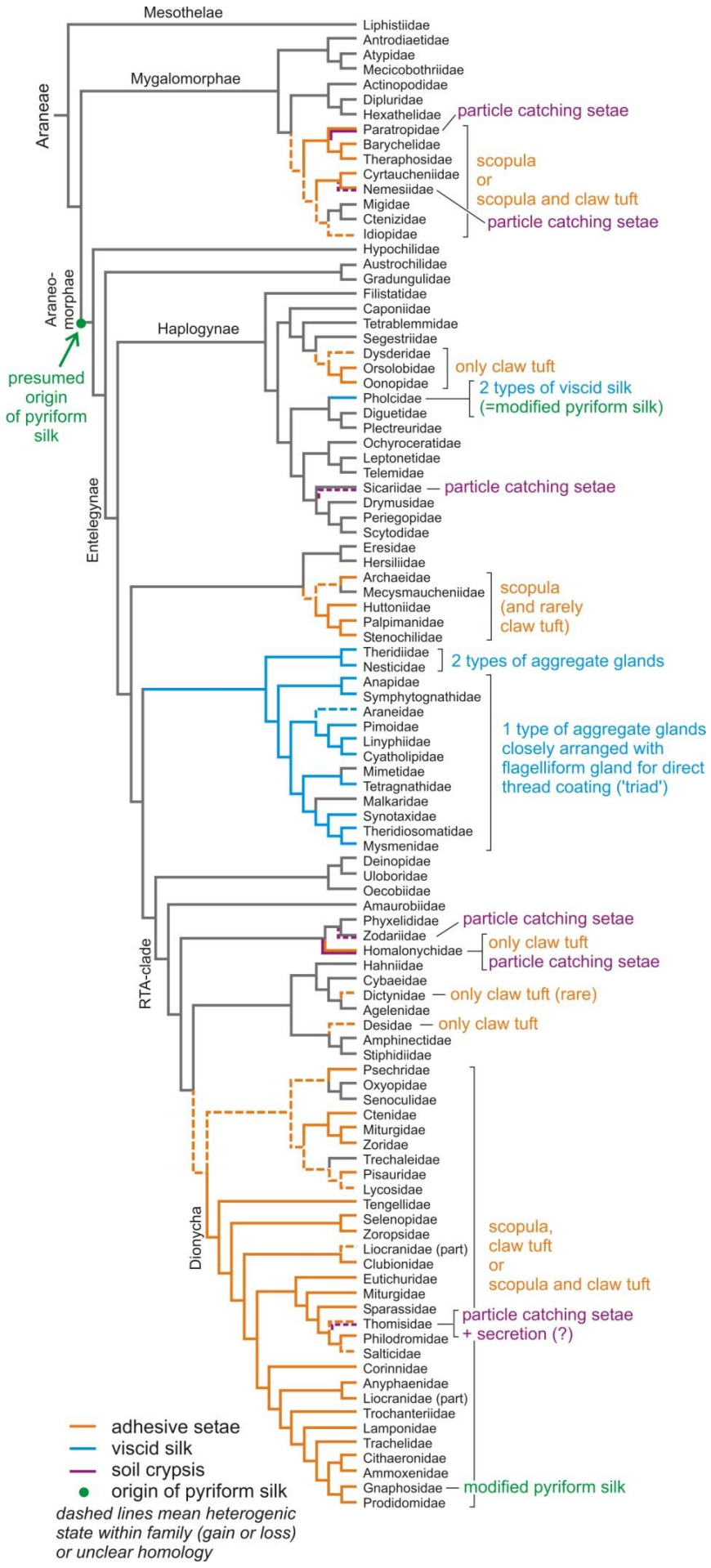
The chelicerae of spiders consist of two segments, of which the distal one, the fang, is blade like and highly pointed in order to be pierced into the prey's body. To be able to penetrate the cuticle of arthropod prey the fang cuticle must be stiffer and tougher, which is achieved by a specific material structure and the incorporation of zinc, calcium and chloride ions (Politi et al., 2012, Bar-On et al., 2014). When pierced into the prey's body and flexed against the basal segment, the fang is locked and the prey is unable to escape without severe injury. It is thus the most important means of prey attachment after successful overwhelming (own observation). The basal segment of the chelicerae usually carries tooth like denticles enhancing the friction with a grasped prey item (Foelix, 2011). In the basal lineages of spiders, the Mesothelae and the Mygalomorphae the chelicerae are oriented forwards (orthognath). In the derived spiders, the Araneomorphae, the chelicerae are facing each other. This apomorphic trait improves the grasping ability with both chelicerae acting together in a clamp like manner (Foelix, 2011). In the Mecysmaucheniidae and Pararchaeidae the chelicerae can be widely opened and arrested, and the sudden release of the stored elastic energy is then used in a rapid snap (Wood et al., 2012, Vellard, 1957).

In male spiders the pedipalpal tarsi are modified into copulatory organs, the bulbs. These contain a reservoir into which the sperm is absorbed and the embolus, a tapering pipe-like protuberance to inject it into the female genital tract. In the higher Araneomorphae, the Entelegynae, which include the majority of spider species, the copulatory structures are highly complex and work in a lock-and-key principle with the female sclerotized genital plate, the epigyne. The interaction is complex and highly specific, and involves, besides mechanical interlocking, a hydraulic inflation mechanism (Eberhard and Huber, 2010).

The four pairs of walking legs are equipped with two large claws, and, in the plesiomorphic state, a median hook-like empodium. Both claws and empodium can be smooth or pectinate. In most spiders that exhibit claw tufts the empodium is highly or totally reduced. In orb-web spiders and relatives (Araneidae, Theridiidae, Linyphiidae) it is elongated and forms a carabiner mechanism together with serrated bristles (see below). In the Gradungulidae and Trogloraptoridae the claws are highly asymmetric, with one claw highly enlarged (Griswold et al., 2012). In *Trogloraptor* the tarsus exhibits a sub-segmentation, such that it forms a raptorial prey capture apparatus (Griswold et al., 2012).

2.4.2. Spines and frictional setae

Spines are thick, stiff setae that work as interlocking devices or a physical barrier. They may occur at the ventral site of the walking leg tarsi. These are short and immobile and orientated distally. They are often more distributed in the hind legs and may generate friction on rough substrates during forward thrust, especially when the claws are already elevated (the claws usually get elevated before leg movement). Other spines are much longer and usually lay flat on the leg. The get erected by an increased hemolymph pressure like in attack situations (Rovner, 1980; Eggs et al., submitted; Ott and Wolff, unpublished data) or during jumps (Ott and Wolff, unpublished data). They are presumed to assist prey capture by building a switchable physical barrier in the leg capture basket (Rovner, 1980).


Mesothelae
Araneae
Mygalomorphae
Araneo-morphae
Haplogynae
Entelegynae
RTA-clade
Dionycha
presumed origin of pyriform silk
Liphistiidae
Antrodiaetidae
Atypidae
Mecicobothriidae
Actinopodidae
Dipluridae
Hexathelidae
Paratropidae
Barychelidae
Theraphosidae
Cyrtaucheniidae
Nemesiidae
Migidae
Ctenizidae
Idiopidae
particle catching setae
scopula or scopula and claw tuft
particle catching setae
Hypochilidae
Austrochilidae
Gradungulidae
Filistatidae
Caponiidae
Tetrablemmidae
Segestriidae
Dysderidae
Orsolobidae
Oonopidae
only claw tuft
Pholcidae
Diguetidae
Plectreuridae
Ochyroceratidae
Leptonetidae
Telemidae
Sicariidae
2 types of viscid silk (=modified pyriform silk)
particle catching setae
Drymusidae
Periegopidae
Scytodidae
Eresidae
Hersiliidae
Archaeidae
Mecysmaucheniidae
Huttoniidae
Palpimanidae
Stenochilidae
scopula (and rarely claw tuft)
Theridiidae
Nesticidae
2 types of aggregate glands
Anapidae
Symphytognathidae
Araneidae
Pimoidae
Linyphiidae
Cyatholipidae
Mimetidae
Tetragnathidae
Malkaridae
Synotaxidae
Theridiosomatidae
Mysmenidae
1 type of aggregate glands closely arranged with flagelliform gland for direct thread coating ('triad')
Deinopidae
Uloboridae
Oecobiidae
Amaurobiidae
Phyxelididae
Zodariidae
Homalonychidae
particle catching setae
only claw tuft
particle catching setae
Hahniidae
Cybaeidae
Dictynidae
only claw tuft (rare)
Agelenidae
Desidae
only claw tuft
Amphinectidae
Stiphidiidae
Psechridae
Oxyopidae
Senoculidae
Ctenidae
Miturgidae
Zoridae
Trechaleidae
Pisauridae
Lycosidae
Tengellidae
Selenopidae
Zoropsidae
Liocranidae (part)
Clubionidae
Euctichuridae
Miturgidae
Sparassidae
Thomisidae
Philodromidae
Salticidae
Corinnidae
Anyphaenidae
Liocranidae (part)
Trochanteriidae
Lamponidae
Trachelidae
Cithaeronidae
Ammoxenidae
Gnaphosidae
Prodidomidae
scopula, claw tuft or scopula and claw tuft
particle catching setae + secretion (?)
modified pyriform silk
adhesive setae
viscid silk
soil crypsis
origin of pyriform silk
dashed lines mean heterogenic state within family (gain or loss) or unclear homology

In many sheet-web building and hunting spiders dense brushes of microstructured setae are present on the distal tarsus (Fig.2.4.A). They are flattened and densely covered with tapered microtrichia on the substrate facing side. These may produce high friction forces on micro rough surfaces due to the interlocking with micro asperities. These 'frictional setae' may thus support the claws and, if present, claw tufts to cover a broad range of attachable substrates. These setal brushes also increase the manoeuvrability on sheet webs, which might have been their original function (Wolff et al., 2013).

In orb web-spiders bent serrated bristles are present on the distal tarsus underneath the claws (Fig.2.4.B). In interaction with the elongated empodium these work like a carabiner, used to grasp silk threads (Foelix, 2011).

2.4.3. Tenent setae

Tenent setae (adhesive setae) evolved for multiple times among spiders (Wolff et al., 2013). They consist of a cylindrical shaft and a highly flattened and broadened tip, which is densely covered with spatula bearing microtrichia on the substrate facing side (Homann, 1957, Foelix and Chu-Wang, 1975, Hill, 1977) (Fig.2.4.I,J). The spatulae are triangular shaped in araneomorphs (Fig.2.4.K), circular in mygalomorphs, and oval in the scopulae (but not claw tufts) of Palpimanidae (Fig.2.4.L) (Wolff et al., 2013). The elaborate microstructure of the tenent setae was found to be responsible for remarkable climbing abilities, even on very smooth surfaces, like glass slides. Since Homann (1957), different hypothesis were raised regarding the function, including suction (Hill, 1977), interlocking with minute asperities (Hill, 1977), capillary forces of a thin water layer on the substrate (Homann, 1957, Roscoe and Walker, 1991) or a fluid secretion (Peattie et al., 2011), electrostatic forces (Homann, 1957) and van-der-Waals forces (Kesel et al., 2003). Adhesion (Kesel et al., 2003) and friction (Niederegger and Gorb, 2006) measurements revealed that the tenent setae are basically structural, dry adhesives. Because only one side of the flexible distal setal section is covered with the spatulate microtrichia, the friction is highly anisotropic (Niederegger and Gorb, 2006, Wolff and Gorb, 2013). This mechanism permits a precise control of the attachment (Wolff and Gorb, 2013).

The evolution of tenent setae in spiders is highly correlated with the abandoning of building prey capture webs (Wolff et al., 2013). They obviously represent a considerable ecological benefit, because the majority of free hunting spiders is equipped with them. The tenent setae are hypothesized being evolved from frictional setae (Wolff et al., 2013).

2.4.3.1. Scopula

Tenent setae often occur in thin strips or dense brushes at the pro- and retrolateral sides of the distal leg segments (Fig.2.4.C,E). These, so-called, scopulae primarily assist prey capture (Rovner, 1978, Pekár et al., 2011, Foelix et al., 1984). The setae are based in oval sockets that permit a directed movement. In rest they lay flat on the leg and get erected by the increased hemolymph pressure during attack, similar to the spines (see above) (Rovner, 1978, Rovner, 1980). In comparison to the erectable spines, which usually lack in the tarsus the scopulae are most developed in the tarsi, which speaks for a different function, namely the seizing of the prey.

Fig.2.3. Phylogenetic distribution of adhesive structures and secretions in spiders (Araneae). Compiled cladogram based on (Ramírez, 2014, Coddington and Levi, 1991, Coddington, 2005, Bond et al., 2014, Griswold, 1993).

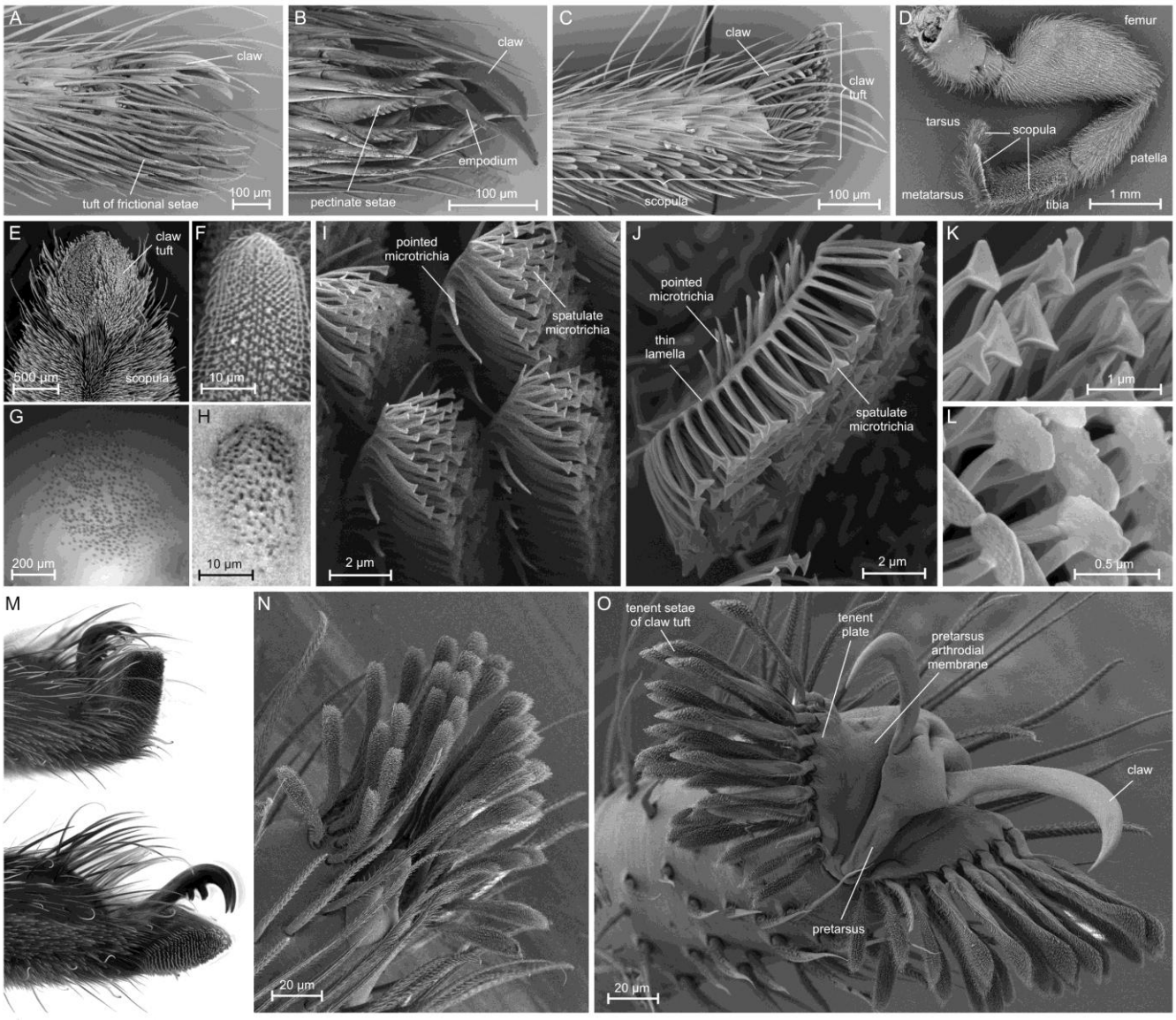


Fig.2.4. Attachment devices of spider feet. (A) Distal tarsus of *Malthonica ferruginea* (Agelenidae) with a dense tuft of brush like setae, presumably assisting friction generation on substrates and locomotion in sheet webs by interlocking with threads. (B) Distal tarsus of *Araneus quadratus* (Araneidae), bearing robust pectinate bristles, which are used to manipulate and move on threads. (C) Distal tarsus of *Clubiona terrestris* (Clubionidae) with brushes of tenent setae, forming the scopula and claw tuft. (D) Modified first leg of *Palpimanus gibbulus* (Palpimanidae), used to grasp and arrest prey (other spiders). A claw is lacking, and the dense scopula on the distal segments generates a strong grip. (E-H) Adhesive pads and tenent setae of *Cupiennius salei* (Ctenidae), (E) SEM micrograph, ventral view, showing the lateral scopulae and the distal claw tuft, (F) Detail of the tip of a claw tuft seta, (G) claw tuft in contact with a Plexiglas slide (spider hanging upside down), visualized with coaxial lighting, making the contact area of the single setae visible (dark spots), (H) Contact area of a single seta (compare with (F)). (I) Claw tuft setae of *Zoropsis spinimana* (Zoropsidae), apical view. In this species the setae are not distally broadened, presumably a plesiomorphic state. (J) Claw tuft seta of *Euophrys fronalis* (Salticidae), apical view. The tip is broadened and highly flexible. (K) Spatulate tips of the tenent microtrichia on a claw tuft seta of *E. frontalis*. The triangular shape is typical for araneomorph spiders, but very similar structures are found in trombidiform mites (see Fig.2.16). (L) Oval spatulate tips of the tenent microtrichia on a scopula seta of *P. gibbulus*. (M) Pretarsal and correlated claw tuft movement in *Phoneutria fera* (Ctenidae), above retracted, below protracted. The movement is possible due to a membranous delimitation of the tenent plates from the rest of the tarsus. (N-O) Hydraulic movement of the claw tuft in *E. frontalis*. The spreading of the tenent plates and the tenent setae is caused by a rise of the internal hemolymph pressure (here simulated by slightly squeezing the leg).

In spiders that use the front legs during attack (e.g. Gnaphosidae) the scopula is restricted to these legs, in others, which use a full capture basket (e.g. Philodromidae) the scopula is equally evolved in all legs (Wolff and Gorb, 2012a). The Palpimanidae only use the first pair of legs, which is highly modified in this family, lacking claws and spines, and hence totally relying on the adhesive capacity of the scopula (Pekár et al., 2011) (Fig.2.4.D).

2.4.3.2. Claw tuft

The claw tufts are dense brushes of tenent setae at the distal tip of the tarsus (Fig.2.4.C,E,M,N). They primarily serve friction generation and attachment on smooth and micro rough surfaces during locomotion. The claw tuft setae show some modifications if compared to the scopula. They are twistable and bendable in different directions, which presumably lowers the stress on the attachment in turning movements (Wolff et al., 2013). The basal shaft can be highly elongated, especially in large spiders and in the distal part of the pad. It often exhibits an S-like shape, which may work like a spring in order to absorb mechanical stress (Gasparetto et al., 2009). The claw tuft setae are based in a sclerite, the tenent plate, which is partly or totally separated from the tarsus by flexible non-sclerotized cuticle (Hill, 2006). The sockets are packed very densily, to provide space for as much tenent setae as possible. This is because in spiders normally only the distal tarsal tip and the pretarsal structures contact the ground during locomotion. A considerable pad area is achieved by the distal broadening of the tenent setae and an increase of the length of setal shafts from proximal to distal. This results in a pad area that is much higher than the tenent plate area. In rare cases there are only one or two single enlarged tenent setae beneath the claws, like in *Goyenia* (Desidae) and *Apostenus* (Liocranidae) (Ubick and Vetter, 2005). The tenent plates may be indirectly moveable by a mechanical coupling with the pretarsus or changes in hemolymph pressure (Wolff et al., 2013, Dunlop, 1994, Hill, 2006) (Fig.2.4.M-O). In other instances there are interlocking structures or hooks (‘claw tuft claspers’) that are assumed to manipulate the position and angle of the setae (Ramírez, 2014).

The performance of the adhesive foot pads is highest on very smooth surfaces and lowest at surfaces with roughness at the micron scale, where the asperity size is similar to the width of the contacting spatula elements of the tenent setae (Wolff and Gorb, 2012c). The performance of the adhesive pads is also highly influenced by humidity. It increases with relative humidity and drops at water saturation of the air followed by condensation which leads to aquaplaning effects (Wolff and Gorb, 2012b). This indicates that the function of this ‘dry’ adhesion system is supported by capillary effects of an adsorbed molecular water layer, as it was previously hypothesized by Homann (1957). The friction of the claw tuft with smooth glass is highly anisotropic (Wolff and Gorb, 2013). When the leg is pulled towards the body the setae of the adhesive pad align and get in contact, which results in high friction. When pushed away from the body, spatular contacts break and friction drops. This gives the opportunity to both generate high attachment forces and detach very quickly and effortlessly.

Claw tufts convergently evolved in mygolomorphs, haplogynes and for multiple times in entelegyne spiders (Wolff et al., 2013). They are nearly restricted to hunting spiders with the exception of the small family Psechridae, with web-building species that presumably evolved from free hunting ones. Hunting spiders with claw tufts are significantly more abundant in the higher vegetation strata than at the ground and herbal stratum, whereas the possession of claw tufts did not as obviously correlate with the microhabitat specialization of species (Wolff and Gorb, 2014). This indicates that claw tuft bearing species are ecologically more successful in

above-ground habitats and less competitive at the ground. The benefit should be primarily due to a higher safety factor and increased maneuverability than an advantage in the attachment ability, because the demand of climbing might also be high in highly structured ground habitats, like deciduous leaf litter.

2.4.3.3. Particle catching setae

Other setal structures in spiders serve camouflage: they attach soil particles, which blends the spider in its microhabitat (soil-crypsis). In *Sicarius* (Sicariidae) and *Homalonychus* (Homalonychiidae) a rather similar setal morphology evolved constituted by a thick and stiff shaft covered with very thin microtrichia, which have been hypothesized to adhere to sand particles by van-der-Waals forces (Roth, 1984, Duncan et al., 2007). In spiders of the genus *Cryptothele* (Zodariidae) soil particles are mechanically held in place by curved setae covered by long barbs (Ramírez et al., 2014). In some crab spiders (Thomisidae) environmental particles, detritus and growing fungi cover the body, attached by barbed club-like setae and papillate microtrichia; an adhesive secretion may also be involved (Ramírez, 2014, Gawryszewski, 2014). Soil crypsis is also known from some burrowing mygalomorphs (Paratropidae, some Nemesiidae). The soil is encrusted at scale-like setae (Raven, 1985).

2.4.4. Adhesive silk

The diversified silk of spiders is regarded as a key innovation of this order. It is a protein-based fibrous secretion that is produced by abdominal silk glands. The glands are differentiated by their morphology and open in seta-like tubes, the spigots, located on modified legs, the spinnerets. Silk is used for various purposes, like prey capture, locomotion, shelter and reproduction. These functions can only be fulfilled if there are proper means of attachment to substrates or with other threads. The attachment can be achieved by mechanical interlocking or entangling, agglutination, or van-der-Waals, viscous or viscoelastic forces.

2.4.4.1. Cribellate and aciniform silk

In some spiders the anterior median spinnerets are reduced to flat plates, which bear numerous minute spigots. This, so called, cribellum produces bundles of nano-fibres (cribellar silk), which are brushed out with a setal comb in the hind legs, the calamistrum (Foelix, 2011). The cribellar silk is used for prey capture in webs or for defense in shelters. It immobilizes an arthropod prey or predator by entangling with setal structures (Opell, 1994). However, it can also produce a significant adhesion on smooth substrates by a combination of van-der-Waals and hygroscopic forces (Hawthorn and Opell, 2002, 2003). This is presumably due to the thin diameter of the cribellar threads resulting in a high compliancy of the thread bundle, and hydrophilic properties.

A comparable adhesive mechanism might be present in the bridging lines of Araneidae and Linyphiidae. These are used to initiate webs or reach distant places by wind drift. The blown thread affixes at a distant structure by entanglement and mechanical interlocking, but can also stick to smooth surfaces (Wolff et al., 2014a). The bridging lines are composed of minor ampullate silk and bundles of aciniform silk (Peters, 1990, Wolff et al., 2014a). Aciniform silk fibers have a diameter comparable to cribellar silk and are thus very compliant. The minor ampullate silk is very stretchable (Blackledge and Hayashi, 2006) and can thus partly absorb

mechanical stress. Adhesion may be achieved by van-der-Waals forces and the enhancing effect by adsorbed water.

Aciniform silk is also used for prey wrapping (Blackledge and Hayashi, 2006). A tight casing is produced in order to restrain the prey. This specific type of attachment presumably works without the intake of any glue. High friction forces may act between single aciniform fibrils due to van der Waals forces in the locally very close contact. Prey wrapping behavior has been evolved at least four times independently and may include other types of silk threads and also viscid glue in some taxa (Barrantes and Eberhard, 2007).

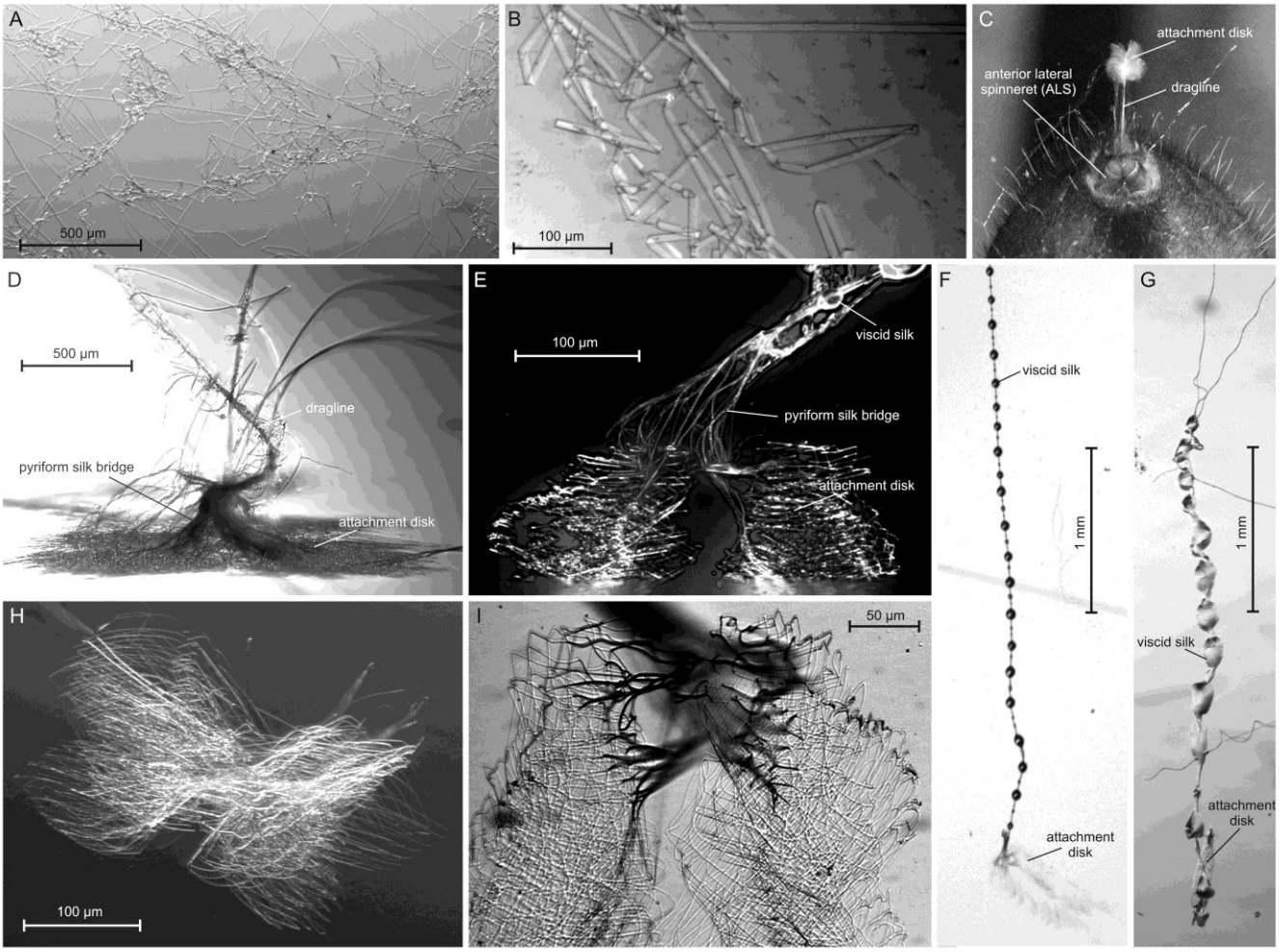


Fig.2.5. Adhesive silk of spiders. (A-B) Tape-like draglines of *Loxosceles rufenscens* (Sicariidae). The usually cylindrical major ampullate silk thread is highly flattened in this genus due to a modification of the spigot. By this means the thread becomes highly flexible and adheres to substrates by intermolecular forces. (C) Detail of distal opisthosomal part of *Cupiennius salei* (Ctenidae) with a spun security line fastened to a glass slide by means of an attachment disk. (D) Dragline attachment disk of *Nephila senegalensis* (Nephilidae) attached to a glass slide, side view. (E) Attachment disk of a gumfoot thread of *Parasteatoda tepidariorum* (Theridiidae). Note the weakened bridge that permits controlled breakage. (F) Gumfoot thread of *P. tepidariorum*. Note the coverage with viscid aggregate silk. (G) Gumfoot thread of *Pholcus phalangoides* (Pholcidae) with a coverage of viscid pyriform silk. (H) Dragline attachment disk of *Oxyopes heterophthalmus* (Oxyopidae), as seen from below the glass slide. The typical 'butterfly'-like structure is a result of two lateral sweeps of the spigot array carrying ALS. (I) Detail of a gumfoot attachment disk of *P. tepidariorum*, as seen from below the glass slide. The weakened bridge is well discernible.

2.4.4.2. Tape-like major ampullate silk

In the Araneomorphae the silk of the major ampullate glands is the most important and the toughest fiber (Blackledge and Hayashi, 2006). It exhibits a thin coat of glygoproteins (Sponner et al., 2007) that may produce some adhesion with substrates. However, the adhesion is usually weak, because of a small contact area, given by the cylindrical shape and comparably low compliancy (Wolff et al., 2014a). Brown recluse spiders (Sicariidae: *Loxosceles*) have evolved modified spigots (gland openings) that are slit-like and consequently extrude a ribbon-like fiber (Knight and Vollrath, 2002). This one is highly compliable and can generate a high contact area (Fig.2.5.A,B). It is barely removable from a smooth glass slide although the cementing pyriform silk (see below) is absent in these spiders (personal observation). The mechanism is probably based on dry adhesion and can be compared with the contact mechanics of thin elastic polymer films (see ***2.1.3.***).

2.4.4.3. Aggregate gland secretion

Cribellar silk is regarded as a thread which enhances the adhesion in order to capture prey (Hawthorn and Opell, 2002). Another lineage, comprising the Araneidae, Theridiidae and Linyphiidae, evolved viscid glue for the same purpose, but with a much higher efficacy (Opell and Schwend, 2009). Because representatives of both lineages build orb webs it was believed until recently that the cribellar silk was an ancient state of the capture threads (Opell et al., 2011b). However, recent broad phylogenomic approaches revealed that the orb web independently evolved in cribellate and ecribellate spiders, explaining the totally different structure and adhesive mechanism of the capture threads (Bond et al., 2014, Fernández et al., 2014). The viscid glue is a secretion of the aggregate glands containing hygroscopic glycoproteins (Vollrath and Tillinghast, 1991) and salts. The glygoproteins bind atmospheric water, forming viscoelastic mucilage (Sahni et al., 2010, Sahni et al., 2011a, Edmonds and Vollrath, 1992). The salts enhance the solvation of the glycoprotein thus permit good wetting ability even at low humidity (Sahni et al., 2014). The aggregate gland secretion coats a simultaneously spun fiber of flagelliform silk, which is extremely extensible (Blackledge and Hayashi, 2006). The coating is achieved by a very close location of the aggregate and the flagelliform spigots (Eberhard, 2010). The coat quickly breaks up into regularly arranged droplets (so-called beads-on-a-string morphology), due to the capillarity driven Raleigh instability (Torres et al., 2014). By the high extensibility of both the flagelliform fiber and the viscid glue a flying prey can effectively be stopped without a failure of the glue bondage. The stickiness of the glue is assumed to be constrained by the strength of the flagelliform fiber, because an excessive stickiness would not allow the spider to take the prey item without destroying its web (Agnarsson and Blackledge, 2009). It is further constrained by the inner strength of the glue, which is always significantly higher, presumably to avoid glue loss and keeping the functionality of the capture thread (Opell et al., 2011a). Nonetheless, the viscid glue is so effective that in some lineages the web has been highly reduced. In bolas spiders (Araneidae: *Mastophora*) only a single thread is spun carrying a single glue droplet, which is actively thrown onto moths flying by (Eberhard, 1980). Theridiidae use aggregate glue in so-called gumfoot traps (see below) and also throw threads with a viscid glue coating directly onto the prey (Hajer and Hrubá, 2007, Coddington, 1989). These spiders also use the glue in defense against aggressors and predators (Vetter, 1980). In both gumfoot and attack/defense threads the viscid glue comes from a specific aggregate gland (Coddington, 1989), and may thus differ in its drying and adhesion properties. However this has not been studied to date. Similar gumfoot traps and viscid silk attacks

have also been reported from Pholcidae, although these do not have aggregate glands (Japyassú and Macagnan, 2004) (Fig.2.5.G). Hence, it is a convergent development, presumably on the basis of a derived pyriform gland (Japyassú and Macagnan, 2004) (see below).

2.4.4.4. Pyriform, cylindrical gland and mygalomorph silk

Whereas the prey capture threads are adapted to stick quickly and reversibly, others have to ensure stable bonding. In the Araneomorphae this is achieved by pyriform silk. This silk is composed of a tough central fiber and a viscoelastic coat that quickly hardens after application, cementing the thread with a substrate and/or other threads (see ***chapter 9***). Both the fiber and the cement are produced by histochemically distinct gland tissues and do not mix within the gland lumen (Kovoor and Zylberberg, 1980). The cement consists of numerous aligned nano-fibrils, lipid enclosures and an isotropic presumed mono-layer of macromolecules assembling at the interface to substrates and the air (see ***chapter 9***). The exact composition and physical function of the glue is not understood yet. Pyriform fibers are usually not spun separately but in dense clusters, so called attachment discs (Fig.2.5.C,D,H). Experimental and theoretical studies on its structure have found that load distribution, multiple elastic peeling and crack arresting synergistically work to enhance the attachment strength (Sahni et al., 2012, Grawe et al., 2014, Pugno et al., 2013), which may partly reduce the demand of glue strength. This might be essential when primary gluing on plants, because strong adhesive bonds can hardly generated on many plant surfaces due to fluid repellent properties or layers of loose wax crystals (see ***chapter 8***). Consequently the attachment discs can generate high pull-off forces even on surfaces with a highly reduced free surface energy, like PTFE (see ***chapter 8***). The specific architecture of the attachment disc may be controlled by the spider in order to achieve strong or weak anchors (Sahni et al., 2012). The latter is used in the so called gumfoot-traps of Theridiidae. These are pre-stressed threads with a viscid coating from the aggregate glands in a short section above the ground (Blackledge et al., 2005) (Fig.2.5.F). The anchorage on the ground must be so weak that it fails when a prey walks against the trap, which eventually leads to an elevation from the ground and a reduced possibility to struggle free. It was assumed that the arrangement of the fibers within the disc is used to tune the attachment strength (Sahni et al., 2012). However, my observations indicate that the bridge between the substrate cementation and the dragline is modified rather than the structure (Fig.2.5.E,I). Gumfoot traps convergently evolved in the Pholcidae (Japyassú and Macagnan, 2004) (Fig.2.5.G). The included viscid glue may come from a modified pyriform gland (Japyassú and Macagnan, 2004), which implicates that the secretion is derived such that it is not hardening as usual. Highly enlarged pyriform glands occur in the Gnaphosidae, which do not use the secretion for attachment discs, but to tie down dangerous prey (e.g. other spiders) (Grimm, 1985) (and pers. observation).

In cocoons cylindrical gland silk forms a dense protective cover (Foelix, 2011). These threads are composed of numerous fibers within a matrix that appears electron dense in the transmission electron microscope (Stubbs et al., 1992), similar to pyriform silk. The matrix may be still fluid during extrusion and conglutinate different threads, resulting in a tough meshwork. However, this has not yet been studied in detail.

Mygalomorphs lack the high functional diversity of silk glands; however, some lineages, especially the Atypidae, Dipluridae, Hexathelidae and some Theraphosidae may construct large webs or silken tubes. This implicates that the threads are somehow conglutinated and fastened at the ground. Histochemical studies on some ‘primitive’ glands show a separate staining of two

gland tissues similar to the pyriform glands of araneomorphs (Glatz, 1973). This indicates that some mygalomorph fibers may also exhibit a cement coat; however, studies on the adhesion of mygalomorph silk are not available.

2.4.4.5. Spitting spider

Spitting spiders (Scytodidae) uniquely eject a contractile gluey substance directly on the prey (McAlister, 1960). The spit is a viscous fluid containing proteinaceous fibers and fibrous glycoproteins, originating from the highly enlarged venom glands (Kovoor and Zylberberg, 1972). It is sprayed in a zig-zag pattern onto the prey, which is a result of an oscillating movement of the chelicerae produced by the hydrostatic pressure of the ejected fluid (Suter and Stratton, 2009). Shortly after ejection (after 300 ms) the secretion highly contracts (shortening of about 60%), which strongly ties the prey down (Suter and Stratton, 2009). The physical effect causing the adhesion and contraction is not fully understood yet.

2.4.4.6. Tarsal 'silk'

In climbing bird spiders (Theraphosidae) thread-like traces originating from the feet have been reported (Gorb et al., 2006, Rind et al., 2011, Peattie et al., 2011). Those were first interpreted as primitive silk threads which should enhance the attachment ability (Gorb et al., 2006). This was rebutted by the finding that the secretions emerge from chemosensory setae and remain fluid (Foelix et al., 2012). The thread-like appearance comes from the dragging movement. Tarsal chemosensors with a distinct distal pore are ubiquitous in spiders. I made similar observations of thread-like tarsal secretions with cob web spiders (Theridiidae) and sac spiders (Clubionidae), showing that this is not restricted to mygalomorphs. I presume that the secretion does not serve adhesion, but functions as a solvent for gustatory substances. Nonetheless this observation shows that chemosensors can be secretory and may have played a role in the evolution of spigots and silk.

2.4.5. Mating plugs

In many spiders males seal the female genital opening after copulation in order to avoid sperm concurrence and secure their paternity (Uhl et al., 2010). One mechanism is the controlled breakage of the embolus tip (Schneider et al., 2001, Kuntner et al., 2009) or the autotomy of the whole pedipalp (Knoflach and Van Harten, 2001), which then stays attached to the epigyne. The embolus tip might stay attached because it is pressed and locked in the narrow duct due to the elasticity of both embolus and duct wall material. In single cases the whole body of the spontaneously dying male serves as a plug against subsequent matings (Knoflach and Benjamin, 2003). Its stable attachment might be due to the strong interlocking of the pedipalps with the epigyne. Another mechanism is the secretion of an amorphous substance that hardens forming a robust plug that is very hard to remove (Lopez, 1987). The secretion may come from a gland in the pedipalpal bulb (Uhl et al., 2014), or, in some cases, from glands of the female genital tract (Aisenberg and Barrantes, 2011). In *Leucauge argyra* (Tetragnathidae) the female secretes the fluid during copulation, which cements the embolus of the copulating male in the genital opening (Aisenberg and Barrantes, 2011). The composition and material properties of such plug secretions are not known yet.

2.5. Whip spiders (Amblypygi THORELL 1833)

2.5.1. Claws, spiny soles, raptorial pedipalps and chelicerae

Whip spiders have jack-knife like chelicerae (Fig.2.2.D) like spiders, which are equipped with sharp denticles and used to dismember prey. They are orthognath as in basal spiders. Whip spiders do not have venom glands and thus have to secure the still struggling prey while feeding (Weygoldt, 2000). In order to catch and arrest the prey whip spiders have evolved strong raptorial pedipalps, which are equipped with long curved spines pro-dorsally and pro-ventrally, forming a capture basket (Fig.2.2.E). The pedipalpal patella is highly elongated and can be highly flexed against the femur, clamping the prey in between.

The front leg is modified in extremely long and thin pseudo-antenna; hence, there are only six walking legs. The walking legs are equipped with two claws and, in the basal lineages, with a median attachment pad (see below) (Dunlop, 2000). Since the loss of this pad is a derived state the median claw (empodium), which is present in the closely related whip scorpions and basal spiders, is absent. The sharpness of the claw seems to differ between amblypygids that have adhesive pads and those that lack it (see ***chapter 3*** and ***4***). Basally thick, highly pointed spines are standing in two lateral rows on the ventral sides of the tarsal segments. The spines are directed distally and seem to play an important role in the generation of high friction and forward thrust during locomotion and resting (see ***chapter 4***).

2.5.1. Arolium ('Pulvillus')

In amblypygids the first instar emerging from the egg, the prenymph, is not yet fully developed and stays on the mother until it moulds again. For the attachment onto the mother it bears lobe-like adhesive pads between the claws (Quintero, 1975) (see ***chapter 3***). The basal lineages of whip spiders, including the Paracharontidae, Charontidae and Charinidae retain the pad in the subsequent instars (Weygoldt, 2000). Developmentally and functionally this organ is an arolium (see definitions in ***2.3.***), although it has originally and consistently been named *pulvillus* in the taxonomic literature. The arolium has a unique structure, combining features of smooth and hairy adhesive pads (see ***chapter 4***). The contact is made by a distal transverse-oval lip, which is lacking in prenymphs (in these the contact is presumably made by the entire pad). In the lip the cuticle is bloated and consists of numerous parallel fibers that are branching for multiple times towards the epiculticle. In the distal part the fine branches are merging again, forming hollow hexagonal tubes, which are apically keeled and equipped with a spatula-like tip. This specific microstructure leads to a discontinuous peel-off behavior, which highly enhances the adhesive strength (see ***chapter 4***). This kind of contact behavior has never been observed in any other membrane based adhesive foot pad. The branching fibers originate from parallel rigid ribs in the endo-cuticle. Their orientation stiffens the pad in a longitudinal direction, while permitting high bending in the transverse direction. This configuration permits a mechanism of switching between stress distribution (high adhesion) and local stress concentration (quick, effortless detachment) (see ***chapter 4***).

2.5.2. Spermatophores and egg sacs

Amblypygids use highly elaborate spermatophores for sperm transfer (Fig.2.6.A). These are produced in a complex glandular organ associated with the gonads and glued onto the substrate during courtship (Weygoldt, 2000). The adhesive plaque is disc-like and composed of an

amorphous and a granular material (Fig.2.6.D,E). It sticks to various substrates: smooth, rough, dry and wet ones. There are no information on the included substances and adhesive mechanisms. The actual spermatophore is composed of a loose fibrous material, covered by a thin layer of an isotropic material (Fig.2.6.B,C). The straight robust stalk has a constriction underneath the apical part, which permits a bending of the latter. The apical part bears the sperm packages and various protuberances, which might be tanned and hence being rather stiff (Fig.2.6.A). These horn-, wing- or hook-like protuberances are primarily assumed to help the female to orientate and find the sperm masses (Weygoldt, 2000). They may also interlock with the female's genital tract. The sperm masses are actively ripped out of the structure by hook- or sucker-like appendages of the genital operculum, the gonopods. The exact mechanism of this complex interaction is not entirely clear and remains to be investigated.

In amblypygids the eggs are not deposited on substrates but carried at the underside of the opisthosoma. They emerge embedded into a viscous filamentous fluid that hardens and gets tanned after being attached at the opisthosoma (Weygoldt, 2000). This, so-called, egg sac has been hypothesized to present a link to silk evolution in spiders (Pocock, 1895), which, however, was not supported by more recent authors, because the amblypygid egg sac glands are mesodermal whereas the spider silk glands are ectodermal (Shultz, 1987, Decae, 1984).

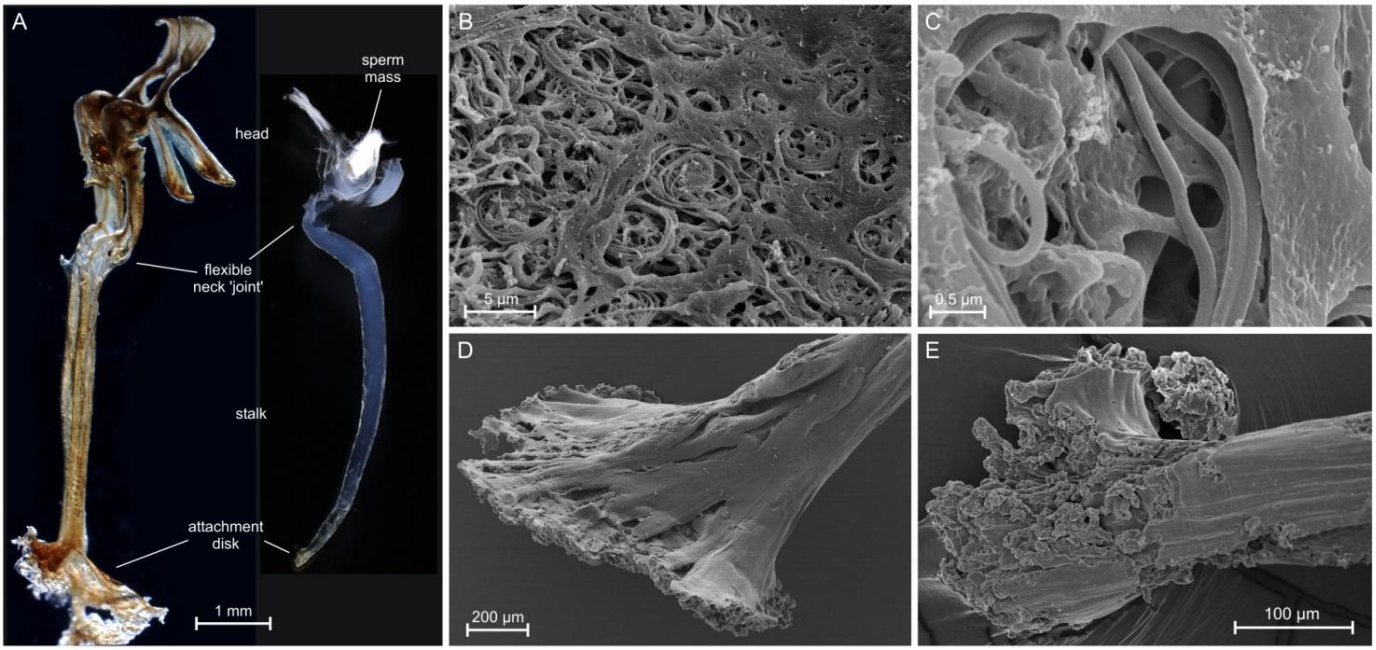


Fig.2.6. Spermatophores of whip-spiders (Amblypygi). (A) Left spermatophore of *Phrynus longipes* (Phrynidae), right *Charon grayi* (Charontidae), both lateral view. (B-C) Material of the head of the spermatophore of C. grayi, including a fibrous and an amorphous substance. (D-E) Attachment disk of the spermatophore, consisting of an amorphous and a granular material, (D) *P. longipes*, (E) *C. grayi*.

2.6. Whip scorpions (Uropygi THORELL 1883)

2.6.1. Claws, spiny soles, raptorial pedipalps and chelicerae

Whip scorpions (super-order Uropygi) comprise two orders, the large epigaeic Thelyphonida O. P-CAMBRIDGE 1872, and the small hypogaeic Schizomida PETRUNKEVITCH 1945, both of which are relatively similar in their morphology despite of their different size (Kästner, 1941). The chelicerae and pedipalps are basically comparable to those of whip spiders and used in a clamp-like manner. However, the pedipalps are much shorter, more robust and less equipped with spines. In the Schizomida the pedipalps are oriented forwards, which might be the basal configuration. In the Thelyphonida the pedipalps are oriented towards each other and can thus additionally used to clamp prey in between. The prey is often already squashed with the strong pedipalps, which is barely possible with the slender ones of amblypygids (pers. observation). It is barely possible for the prey to escape this strong grip.

The first leg pair is antenniform and lacks pretarsal structures, the subsequent walking legs bear two main claws and a claw-like empodium. The ventral soles of the tarsal segments bear strong spines, similar to amblypygids (see above). The spines may be slightly spread and elevated with increasing hemolymph pressure, as I observed in walking *Typopeltis crucifer*. As adhesive foot pads are absent in uropygids (with the exception of prenymphs, see below) both claws and spines are alone responsible for attachment. As these animals are restricted to the ground (in contrast to amblypygids), adhesion is less important for their locomotion than friction.

2.6.2. Prenymphal arolium

Similar to amblypygids the uropygids exhibit the pulli-carrying behavior and the prenymphs possess large disc-like arolia (see ***chapter 3***). In the Thelyphonida the prenymph pretarsus lacks all sclerotized structures including the claws and consists of the large arolium only. It is barely moveable and sticks passively by a high compliancy of the ventral cuticle and the viscous and capillary forces of a mucous-like secretion (see ***chapter 3***). Hence, the previous description of this organ being a sucker (Kästner, 1941) is erroneous. The high compliancy of the ventral cuticle is due to its sponge like structure with arrays of thin, branched fibers underneath the thin but robust epicuticle (see ***chapter 3***). In contrast the dorsal cuticle is rather stiff, with a much denser material structure and forming denticulate protuberances, which further reduce the potential contact area. In the Schizomida the morphology and function of this organ is not known, however, it is presumed to be comparable with that of thelyphonid prenymphs due to the close relationship and the similar pulli-carrying behavior.

2.6.3. Spermatophores and egg sacs

Like in amblypygids the uropygids use spermatophores for sperm transfer and egg sacs for clutch carriage. The spermatophores are equally elaborate like in whip spiders (Weygoldt, 1988, Weygoldt and Huber, 2013). The paired egg sacs exhibit only a weak dried and hardened protective shell and contain fewer fibers, and might thus be more primitive than those of amblypygids (Kästner, 1941, Weygoldt and Huber, 2013).

2.7. Harvestmen (Opiliones SUNDEVALL 1833)

2.7.1. Claws, chelicerae, raptorial pedipalps and penis

Harvestmen bear pincer like chelicerae which are composed of three segments (Fig.2.2A,G). The chelicerae are strong claspers and important means of holding prey items. This is especially important is the harvestmen have no venom glands in their mouth parts and may have to deal with a long time struggling prey. In various species the chelicerae are greatly enlarged and massive, and can reach multiple times of the body length. In some species of Ischyropsalis these may be used to grab and break the shells of snails (Verhoeff, 1900, Martens, 1965), but this was not observed in other species with enlarged chelicerae. In the Megalopsalidinae (Neopilionidae) (Taylor, 2011) and *Rhampsinitus* spp. (Phalangiidae) (Schönhofer, 2008) the chelicerae are highly elongated in males, which leads to the assumption that they might play a role in contest or in securing the female during copulation. However, this has not been observed to date.

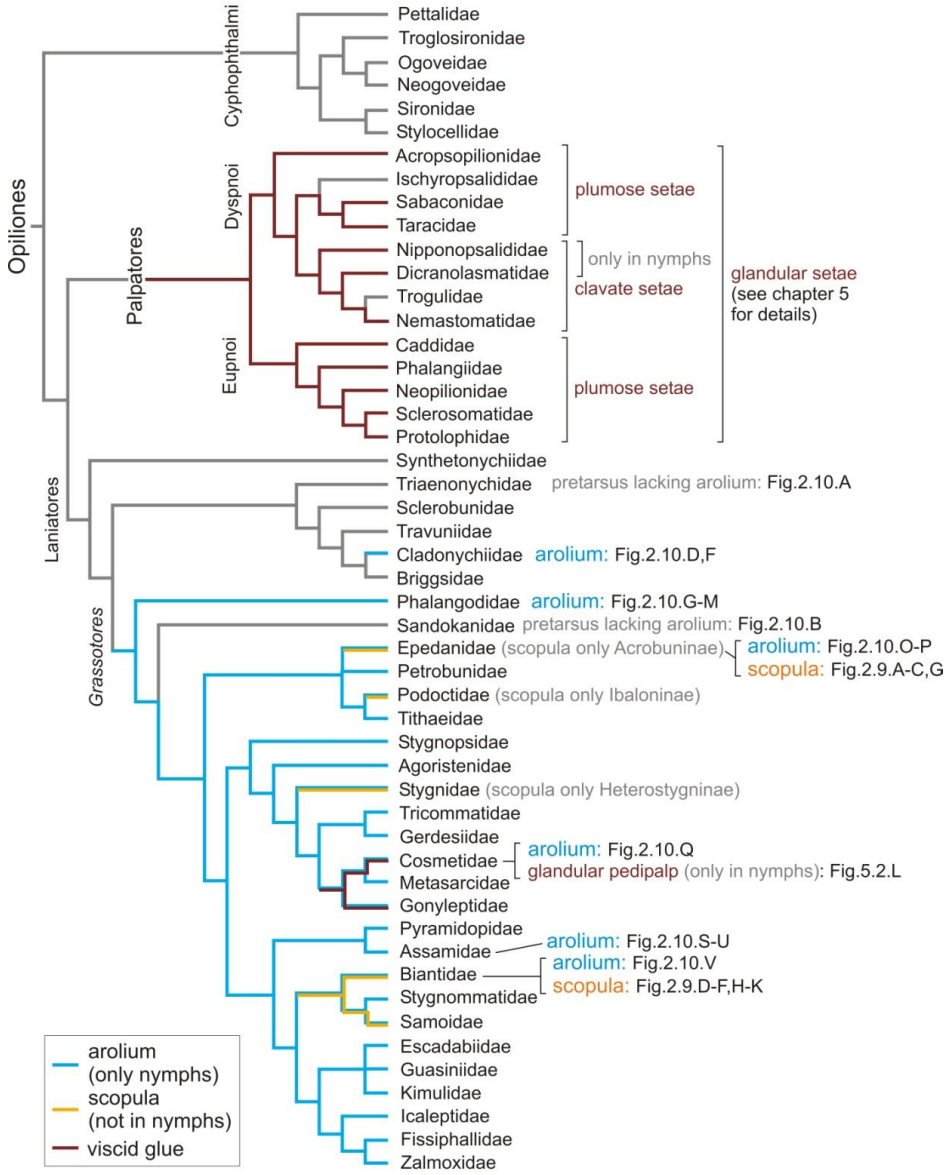


Fig.2.7. Phylogenetic distribution of attachment devices and adhesive secretions in harvestmen (Opiliones). Compiled cladogram based on (Hedin et al., 2012, Shultz and Regier, 2001, Schönhofer, 2013, Groh and Giribet, 2014, Giribet and Sharma, 2015, Sharma and Giribet, 2011). Characters may be inhomogeneously distributed with lineages (not displayed).

Most harvestmen bear a single claw in their pedipalps and walking legs. In most Dyspnoi and some Neopilionidae the pedipalpal claw has been totally reduced. In the Laniatores the palpal claw is highly elongated and can be highly flexed against the tarsus, which is an important means of prey capture (raptorial claw, see ***chapter 5***). The Laniatores bear two claws in the two posterior leg pairs, which are the primary (and often exclusive) locomotory appendages in this sub-order (Pinto-da-Rocha et al., 2007). In the Triaenonychidae and Travuniidae (Laniatores) the claws are strangely modified to a furcated or spoon like structure (Roewer, 1923, 1935), the function of this is unknown.

The pedipalps of most Laniatores, some Eupnoi and juveniles of the dyspnoid Dicranolasmatidae exhibit long spines (Fig.2.2.F,G), which are used to arrest prey (see ***chapter 5***). Spines may also present in the ventral tarsi, serving friction generation on rough substrates (see below).

Harvestmen perform direct sperm transfer via a penis. In several lineages its tip (the glans) is equipped with barbs or denticulate processes that may be spread by an increase of hemolymph pressure and retracted by muscles (Pinto-da-Rocha et al., 2007, Schönhofer, 2008). These presumably serve to anchor the genital into the female to prevent an interruption of sperm transfer (see also discussion in ***10.1.7.***). In *Sclerobunus* sp. (Travunioidea) it was observed that the attachment is so strong that it is not possible to separate a copulating male from the female without injuring (A.L. Schönhofer, pers. communication).

2.7.2. Prehensile tarsi

Many harvestmen of the Palpatores clade, especially of the Phalangiidae and Sclerosomatidae, have highly elongated legs with an excessive sub-segmentation of the tarsi. These are used as a prehensile organs to grasp corrugated surfaces, leaf edges or plant stems (Kästner, 1931, Guffey et al., 2000). The single tarsomeres have a trapeze-like shape, such that there is high freedom to bend ventrally, whereas the dorsal movement is blocked (Kästner, 1931) (Fig.2.8). None of these articles can actively be moved by a muscle, but passively by a contraction of the pretarsal muscle (Kästner, 1931). Harvestmen lack the depressor muscle, the extension of the tarsi may be driven by an increase of the internal hemolymph pressure, which is adjusted by prosomal muscles (Shultz, 2000). Kästner (1931) concluded from his observations that the prehensile tarsi are very effective to navigate through a complex terrain, because they always catch a structure without the necessity to search for foothold. A long-legged harvestman can thus step through the grass and herb vegetation very quickly and without having any adhesive pads. The ventral sides of the prehensile tarsi are often densely covered with setae (Fig.2.8.C,D), which may provide friction on natural surfaces, due to interlocking with minute asperities. This has not been studied up to now, but comparable structures have been found to enhance friction in the modified gripping feet of chameleons (Spinner et al., 2014), which might be a functional analogue. In both the harvestman prehensile tarsi and the chameleon foot the key to generate high attachment forces is the embracing of the substrate structure, which permits the application of high normal forces simoultaneously in different directions. The very fine and dense setae on the soles may generate high friction even on surfaces with very small roughness scales, at which the efficacy of spatulate setae is highly reduced (see previous chapters). In some species short but rigid spines are included in the dense hairy sole (Fig.2.8.A-C), which may enhance the efficacy on more planar surfaces, like tree stems. Some Laniatores also exhibit highly subsegmented tarsi, however, never in that extend like in Phalangiidae or Sclerosomatidae. In contrast these

harvestmen still possess both pretarsal muscles (Proud and Felgenhauer, 2013) and the tarsi are more flexible in the dorsal than in the ventral direction (Fig.2.8.A), hence they can hardly wrap around plant structures.

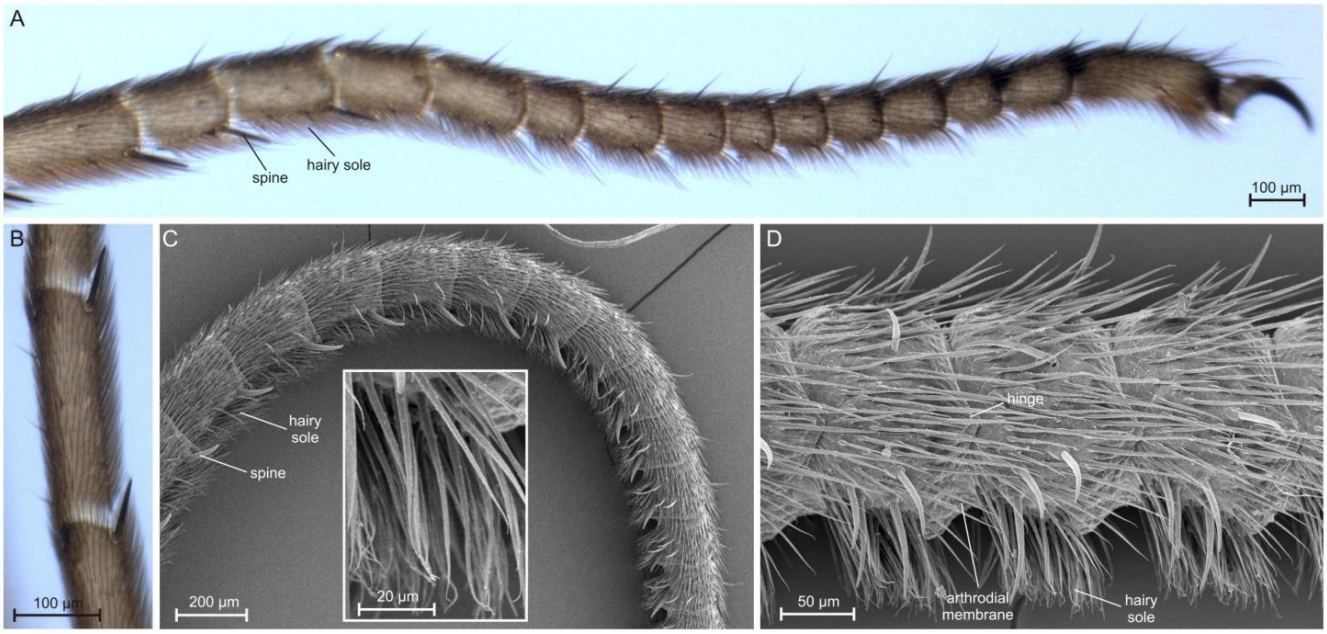


Fig.2.8. Prehensile feet of harvestmen (Opiliones: Eupnoi). Only the distal most parts of hind leg tarsi are shown. (A-B) *Metagagrella minax* (Sclerosomatidae), (C-D). *Zacheus crista* (Phalangiidae). Inset in (C) show detail of hairy sole. Note the trapeze-like shape of tarsomeres, permitting a ventral bending, whereas blocking in dorsal direction. This causes ventral bending of the tarsal chain (see (C)), when the pretarsal muscle is contracting. Note also the gradual decrease of segment length from proximal to distal, and the absence of spines in the distal most segments. The spines are present distally on each segment, near the segment, where they can act best as interlocking devices and lever.

2.7.3. Scopula

In some species of the tropical Laniatores the hairy soles of the tarsal segments of both posterior leg pairs are discernibly modified, and hence called scopulae (Roewer, 1923). This character has independently evolved in the Ibaloninae (Podoctidae), Acrobuninae (Epedanidae), Heterostygninae (Stygnidae), Biantidae and Samoidae (Pinto-da-Rocha et al., 2007, Roewer, 1923). In all cases the structure of the scopula setae is comparable. The setae have ribbon-like tips, which may be distally broadened (spatulae), and are assumed to work as adhesive devices (Rambla, 1990). However, it has never been tested if these harvestmen are capable to walk on smooth surfaces, and is unknown how these presumed attachment devices are used in life. Some of these species, like the Biantidae, are nearly exceptionally found at the ground, but knowledge on the biology of these harvestmen is very sparse. In *Hinzuanius flaviventris* (Biantidae) I studied both adults and nymphs and found that the scopula is not developed in the nymphs, which bear the arolium (see below). In the Biantidae the setae are spatulate only in the distal most tarsal segment, which is slightly broadened (Fig.2.9.D,E). There are no obvious morphological differences between the old world Biantinae (studied: *H. flaviventris*, Fig.2.9.E,H,I) and new world Stenostygninae (studied: *Galibrotus riedeli*, Fig.2.9.D,F,J,K). The setal rear is nubby and at the spatula equipped with a brim (Fig.2.9.K), presumably to prevent a substrate contact of these parts, as known from similar adhesive setae in beetles (Gorb, 2001). In *Metacrobunus* sp. (Epedanidae) spatulate setae occur on the two distal segments, which are not significantly broadened

(Fig.2.9.A,C). The scopula is less dense than in the Biantidae, and the setae are much longer and bear much larger spatulae (Fig.2.9.B). The setae are band like flattened, get gradually broadened towards the tip, but end more or less pointed, like a leaf (Fig.2.9.G). The back is not nubby. These differences underline the independent evolution of the scopula. In *Dibunus similis*, belonging to the same family (Epedanidae), the scopula is absent.

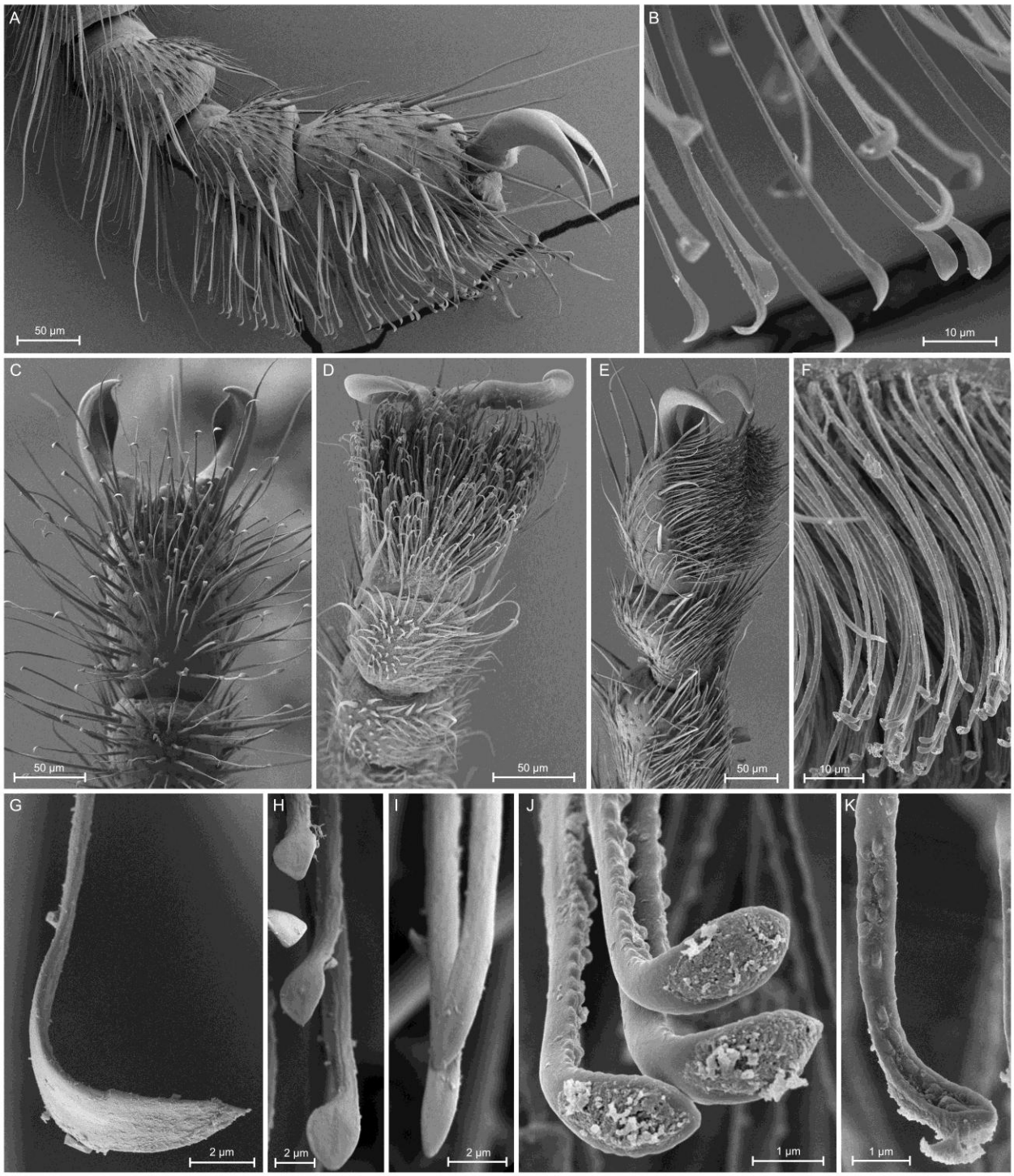


Fig.2.9. Scopula in Laniatores. Distal tarsi of hind legs. (A-C,G) *Metacrobunus* sp. (Epedanidae), spatulate setae are present on the two distal most tarsomeres. The brush of these modified setae is called scopula. (D-F,H-K) Biantidae. The scopula is only present on the distal most segment, which is slightly broadened. The spatulate setae are much shorter, have smaller tips and stand much denser than in *Metacrobunus*. The scopula of Epedanidae and Biantidae is not homologous (see tree in Fig. 2.7). (D,F,J,K) *Galibrotus riedeli* (Stenostygniynae). (E,H,I) *Hinzuanius flaviventris* (Biantinae).

In *G. riedeli* there were often clumps of an amorphous substance on the ventral side of the spatulate tips (Fig.2.9.J-K), which might be remains of a coagulated secretion. How the secretion is delivered so locally is unclear, because no pores or channels were discernable.

2.7.4. Nymphal arolium

An arolium is present in the two posterior leg pairs of nymphs in the Grassatores, which comprise the majority of Laniatores (Gnaspini, 2007). It is present in all nymphal instars, but absent in the larva, the subadult and adult (Gnaspini et al., 2004, Townsend et al., 2009) (Fig.2.10.E,N,R). This organ is used to attach to overhanging surfaces during the molting process (Juberthie, 1972). Many harvestmen molt in an overhanging position (Gnaspini, 2007). It presumably helps them to pull the long legs out of the exuvia. Further the risk of predation might be reduced by this means. With the help of the arolium the nymph can even attach to smooth surfaces like glass panes (Juberthie, 1972). Roewer (1935) compares the arolia with the pedipalpal organ of Solifugae (see ***2.10.3.***) and assumes a similar adhesive function. I studied the ultrastructure of the nymphal arolium in *Scotolemon doriae* (Fig.2.10,K-M), *S. lucasi* (Fig.2.10.G,H,J), *Sitalcina californica* (Fig.2.10.I) (Phalangodidae), *Dibunus similis* (Epedanidae) (Fig.2.10.O-P), *Cynortellana quadrimaculata* (Cosmetidae) (Fig.2.10.Q-R), *Hinzuanius flaviventris* (Biantidae) (Fig.2.10.V) and an unidentified species of Assamidae (Fig.2.10.S-U). Basically the structure is rather similar. It is a membranous dorso-median outgrowth of the elongated pretarsus, laterally flanked by the claws. A median claw (empodium) may also be present at the basis (seen in Assamidae (Fig.2.10.S), in others obviously absent). The surface of the arolium is longitudinally wrinkled, which permits a large expansion of the pad. The folding morphology differs between some lineages. The ventral cuticle internally exhibits branched fibrils as seen in a fractured arolium of *D. similis* (Fig.2.10.P). In *H. flaviventris* the arolium is rather small (Fig.2.10.V). In the Phalangodidae, which are the sister group to all other Grassatores (Sharma and Giribet, 2011), the arolium is not bubble-like as in the others, but more flat and pad like (Fig.2.10.G,K). The ventral cuticle appears to be rather stiff; it is smooth in *S. californica*, corrugated in *S. doriae* and nubby in *S. lucasi*. In contrast the distal and apical part is wrinkled and highly inflatable (in some specimens it is expanded, see Fig.2.10.H,I,L). In the other Grassatores the membranous area extends more to the ventral part, such that it may make a contact during normal locomotion. In the Sandokanidae the arolium has totally been lost (Gnaspini, 2007) (Fig.2.10.B). An arolium like modification is also present in nymphs of *Holoscotolemon* spp. (Cladonychiidae), but the structure totally differs from the above described, and is presumably not homologous. The pad is rather slender and distally tapered (Fig.2.10.D,F). It seems to be apically attached to the large empodium, and additionally bears numerous claw like sclerites (auxiliary claws) on the lateral sides. I could not observe an adhesive function in nymphs of *H. naturae* – the contact area is very small on smooth glass and the nymphs were not capable to scale smooth surfaces. Hence, I assume that the pad has to get somehow activated by inflation, which is not performed during usual locomotion. Unfortunately I could not observe a molting process to verify this. If the more pad like arolia of the Grassatores are used during locomotion remains also unknown, as it was not possible to obtain living nymphs of this clade for this study.


A
10 µm
B
claw
empodium
pretarsus (elongated)
20 µm
C
10 µm
D
auxiliary claw
arolium
claw
10 µm
E
20 µm
F
claw
arolium
empodium
5 µm
G
10 µm
H
10 µm
I
10 µm
J
2 µm
K
5 µm
L
5 µm
M
stiff ventral membrane
expandable dorsal membrane
1 µm
N
claw membrane
10 µm
O
25 µm
P
2 µm
epicuticle
Q
50 µm
R
50 µm
S
claw
pretarsus
arolium
empodium
T
20 µm
U
2 µm
V
20 µm

2.7.5. Pedipalpal glandular setae

Specialized glandular hairs (‘Kugelhaare’, (Rimsky-Korsakow, 1924, Wachmann, 1970)) on the pedipalps are known from the Dyspnoi and Eupnoi (Shear, 1986, Giribet et al., 2002, Gruber, 1970, Lopez et al., 1980, Wijnhoven, 2013). They exhibit a rather stiff stalk and a flexible apical section which is non-slerotized and equipped with numerous hooked microtrichia that hold a droplet of viscid secretion (*plumose setae*, see ***chapter 5*** and ***6***). In the higher Dyspnoi (Nemastomatidae, Dicranolasmatidae and Nipponopsalididae) the plumose section is modified: The microtrichia are radially arranged, which permits the attachment of a regularly shaped droplet. Another feature that evolved among Dyspnoi are slit like openings of the secretion delivery channels, which presumably permits a quicker extrusion of a more viscous fluid (see ***chapter 5***). The glandular setae are used to capture small fast moving prey like springtails or leaf hoppers (see ***chapters 5-7***). The fluid has viscoelastic properties and can produce high adhesive forces both on glass and springtail cuticle (see ***chapter 6***).

2.7.6. Nymphal glandular pedipalps

Nymphs of Cosmetidae, Cranaidae and Gonyleptidae (Laniatores) have modified pedipalps which lack any spines and exhibit a bulbous pretarsus that lacks the claw (see ***chapter 5***). Ultrastructural examination of the pedipalp in a nymph of *Cynortellana quadrimaculata* (Cosmetidae) revealed that the distal patella, the tibia, tarsus and the modified pretarsus are covered by tube-like gland openings with remains of some amorphous secretion. Some recent observations indicated that the pedipalps in these nymphs are covered with a sticky mucous, which is presumably used for prey capture (S. G. Hernández and G. Machado, pers. communication).

2.7.7. Soil crypsis

Some harvestmen can blend in their environment that they are nearly not discernable, because of a cover with soil particles. This is particularly known from the Trogulidae (Schwangart, 1907), but also from Dicranolasmatidae, some Nemastomatidae (*Ortholasma*,

Fig.2.10. Nymphal arolium in Laniatores. (A) Nymph of *Paranonychus* sp. (Triaenonychidae), leg 4, ventral view. In this rather basal family of Laniatores the arolium is absent. There is only a single claw in the hind legs exhibiting lateral denticles. (B) Nymph of *Gnomulus* sp. (Sandokanidae), leg 4, ventral view. The arolium is also absent in this family, presumably a secondary reduction. As typical for nymphs of the Grassatores the pretarsi of legs 3 and 4 are stalk-like elongated and bear two claws and a claw-like empodium. (C-F) *Holoscotolemon querilhaci* (Cladonychiidae). (C) Leg 1 of nymph, ventral view. Legs 1 and 2 bear single hook-like claws in both nymphs and adults in Laniatores. (D) Leg 4 of nymph with auxiliary claws and dorso-median arolium. (E) Leg 4 of adult. Note the totally different morphology of the pretarsus. (F) Leg 3 of nymph, ventral view. The median empodium is visible. (G-N) Phalangodidae. This is presumably the basal most lineage of the arolium bearing Grassatores. In contrast to *Holoscotolemon* the empodium is lacking and the median part between the claws is broadened. The arolium dorsally exhibits regular folds, which permit an inflation of the pad. In contrast, the ventral cuticle appears to be rather stiff. (G,H,J) *Scotolemon lucasi*, leg 4 of nymph, (G) ventral view, (H) lateral-ventral view with arolium partly inflated, (J) detail of arolium folds. (I) *Sitalcina californica*, leg 4 of nymph. (K-M) *Scotolemon doriae*, leg 4 of nymph, (K) in normal state, (L) arolium inflated, (M) detail of non-inflated arolium, distal edge. (N) *S. lucasi*, leg 4 of adult. (O-P) *Dibunus similis* (Epedanidae), leg 3 of nymph, (O) lateral-dorsal view, (P) fractured arolium with inner fibrillation visible. (Q-R) *Cynortellana quadrimaculata* (Cosmetidae), (Q) leg 4 of nymph, lateral view, (R) leg 4 of adult. (S-U) Nymph of Assamiidae, unidentified species, leg 4 with inflated arolium, lateral view, (S) Light microscope image with the empodium visible, (U) Detail of unfolded dorsal cuticle of arolium. (V) Nymph of *Hinzuanius flaviventris* (Biantidae), leg 3 with folded arolium, lateral view.

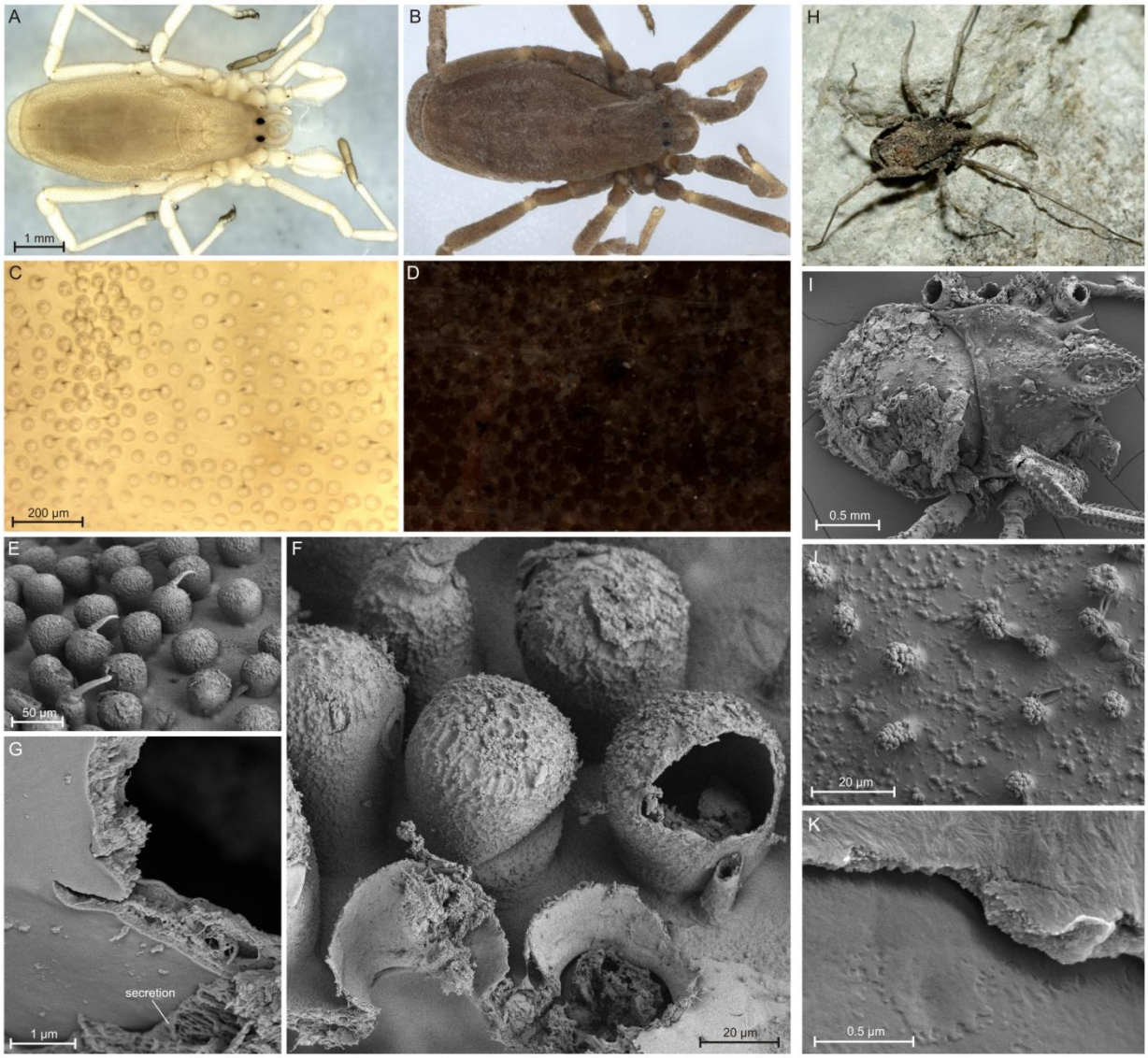


Fig.2.11. Soil crypsis in Dyspnoi. (A-F) *Trogulus martensi* (Trogulidae), (A,C,E-F) freshly molted, (B,D) usual habitus with dried crust of secretion and soil particles. (C-F) Detail of dorsal integument with glandular warts. (C,E) After molting the warts are still exposed and clearly visible. (D) A secretion emerges and assembles between the warts, catches soil particles and dries forming a crust that is hardly removable. The short, curved setae on some warts assist in the capture and retention of soil particles. (F) Cuticle, partly cut with a scalpel. The warts are hollow and build secretion reservoirs (see also Schwangart, 1907). (G) The cuticle is smooth; hence the roughness seen in (G) is already emerged secretion. There are no discernable pores; hence the secretion may emerge by diffusion. (H-K) *Dicranolasma* spp. (Dicranolasmatidae). (H) *D. scabrum*, habitus of living adult individual. (I-K) *D. pauper*, nymph. (I) Body, dorsal view. Note the inhomogeneous distribution of dirt. Glandular warts are absent in this family. (J) Crust of dried secretion on the dorsal integument. The nubs are clumps of secretion adhering to setae. (K) Fracture of the secretion crust. The underlying smooth cuticle is visible. The secretion seems to contain nano-fibrils.

Dendrolasma, *Vestiferum*) (Schönhofer, 2013) and some Phalangodidae (Roewer, 1923). The particles are attached via a hardening (presumably tanning) secretion (Fig.2.11). In *Trogulus* spp. the secretion is secreted and stored in bubble-like cuticular protuberances (*'Drüsenwärzchen'* = glandular warts) (Schwangart, 1907) (Fig.2.11.Q,E,F). I examined a freshly molted individual by means of scanning electron microscopy. There were no discernable pores in the warts nor between them, hence the secretion is presumably released by diffusion trough their thinned cuticular walls. The hardened secretion forms a very robust crust with embedded sand grains and detritus, which

can hardly be removed. Short, hook-like curved setae on some warts (Fig.2.11.C,E) additional help to catch and retain soil particles. The only clean body parts are the tarsi, eyes and mouth parts, the latter are remarkable free of dirt. In *Dicranolasma* spp. no glandular warts are present. The secretion assembles as a film on the cuticle and forms clumps at the short body setae (Fig.2.11.J). The secretion seems to be a fibrous material (Fig.2.11.K). In nymphs the crust is comparably easy to remove, indicating a lower amount or divergent composition of the secretion. Nonetheless, large clumps of soil material assemble on the dorsum (Fig.2.11.I). The composition and properties of these secretions are not known.

2.8. Scorpions (Scorpiones C. L. KOCH 1837)

2.8.1. Claws, chelicerae and pedipalp chelae

The scorpion chelicerae consist of three segments which is regarded as the plesiomorphic state in arachnids (Shultz, 1990). The chelate segments are equipped with denticles and primarily used to dismember the prey which is held with the much larger chelae of the pedipalps. The pedipalpal tarsus articulates against a tibial outgrowth. The basal section of the tibia is often thickened to provide room for thick flexor muscles. Since extensor muscles are lacking the opening of the chelae is driven by elastic energy stored in a joint sclerite (Sensenig and Shultz, 2004). The shape of the chelae can vary from slender and forceps-like to robust and pincer-like, which is correlated with prey capture behavior and the use of venom (Van der Meijden et al., 2010). The walking legs bear two articulated claws and a claw-like empodium, which is an outgrowth of the pretarsus sclerite. The cuticle of the tarsal claws have been shown to contain zinc ions, which is assumed to increase the material strength and reduce wear (Schofield et al., 2003).

2.8.2. Prenymphal arolium

Scorpions are viviparous and show maternal care. The born instar, the prenymph, is not fully developed yet and climbs onto the back of the mother, where it attaches (Millot and Vachon, 1949). For this purpose the distal leg segment, the pretarsus of this stage exhibits a large arolium (Millot and Vachon, 1949). The ventral cuticle of the arolium is highly compliant due to an internal disaggregation into parallel fibers, like in the arolium of whip scorpions (see ***2.6.2.***). Its adhesion is enhanced by the capillary forces of a secreted fluid film, which appears to be hydrophobic (see ***chapter 4***). Detachment is achieved by the invagination of the arolium by the pretarsus retractor muscle (see ***chapter 4***).

2.8.3. Spermatophore and mating plugs

The sperm is transferred by a spermatophore which is glued onto the ground during courtship (Lourenço, 2002). The apically projecting lobes interlock with the female genital tract, when the female is guided over the spermatophore by the male (Jacob et al., 2004). This is less an lock-and-key mechanism than a mechanical fixation which reduces the ability of the female to interrupt the sperm transfer (Peretti, 2004). Amorphous mating plugs have been reported from some species of scorpions (Polis and Sissom, 1990). In *Euscorpius italicus* (Euscorpiidae) the plug consist of dried sperm (Althaus et al., 2010).

2.9. Pseudoscorpions (Pseudoscorpiones DE GEER 1778)

2.9.1. Claws, chelicerae and pedipalp chelae

The small primary soil-dwelling pseudoscorpions have pincer-like chelicerae (Fig.2.2.B). Like in scorpions the tibia and tarsus of the pedipalps form a chela, which is used to grasp and manipulate prey items (Beier, 1932). In many species the chela holds a venom gland that opens into the moveable finger (tarsus) and is used to anaesthetize the prey (Weygoldt, 1969). The chela is also used to attach to phoretic hosts in order to disperse (Zeh and Zeh, 1992). Species that are frequent phorets bear special pointed accessory denticles on the chela (Carl, 1994). To withstand the drag forces during flight and grooming, high clamping forces are exerted, which may cause discernible damages to the cuticle of the phoretic host (Carl, 1994).

The tarsi of eight walking legs are distally slanted, giving room for the claws during pretarsal flexion. There are two smooth claws that are usually long and slender, but in some genera small in favor of the arolium.

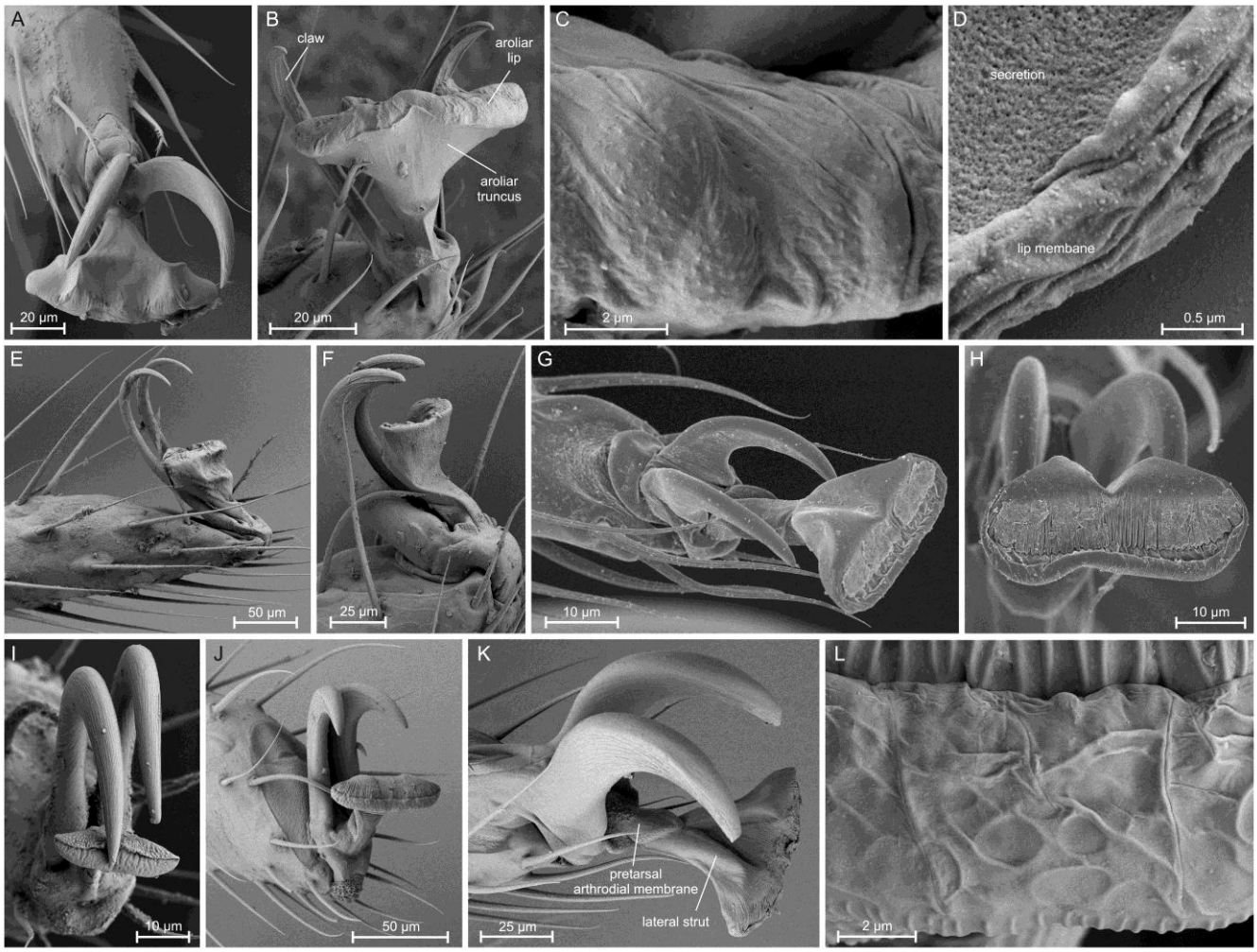


Fig.2.12. Pretarsus of Pseudoscorpions. (A-C,J-L) Cryo-SEM, (D-I) Standard SEM micrograph. (A-D) *Neobisum* sp. (Neobisiidae). (A) Leg 2, pretarsus protracted, dorsal view. (B) Same, pretarsus partly retracted, ventral view. (C) Detail of aroliar lip, frontal view. Note the nubby surface, and the minute folds indicating extreme thinness and high compliancy of the outer membrane. (D) Section of aroliar lip. The interior of the arolium is filled with a dense viscous fluid. The outer membrane consists just of the epicuticle. (E) *N. spelaeum*, leg 2, lateral view. (F) *Megachernes himalayensis* (Chernetidae), leg 4, lateral view. (G-H) Garypidae, unidentified species. (G) Leg 2, dorsal-lateral view. (H) Leg 4, frontal view. (I) *Pselaphochernes scorpioides* (Chernetidae), leg 4, frontal view. (J-L) *Atemnus* sp. (Atemnidae). (J) Leg 2, frontal view. (K) Leg 3, dorsal-lateral view. (L) Detail of aroliar lip, frontal view.

2.9.2. Arolium

Underneath the claws all pseudoscorpions bear an adhesive pad, the arolium (also ‘empodium’) (to the best of my knowledge no pseudoscorpion species lacking the arolium has ever been described). It is already present in the fossil *Dracochela deprehendor* from Devonian, which has been considered as a stem-group pseudoscorpion (Judson, 2012). The arolium has formerly been interpreted as a funnel-like suction cup (Beier, 1932), which is erroneous. My observations show that no cavity is present in the contact (Fig.2.13). The arolium has a narrow base and widens distally to a broad lip (Fig.2.12.A,B,G,K). All parts except the distal lip exhibit a rigid cuticle, which however is not tanned but translucent, and may be micro-structured. In the proximal and lateral parts the cuticle is rather thick (1-3 µm) and dense, in the median dorsal part it is thinner. The pretarsal retractor tendon extends into the arolium and is attached to its dorsal median part. The lip cuticle is very thin and hence highly compliant (Fig.2.12.C,D,L). The underlying material may be fibrous; however, it was not entirely clear from the sections, because dense fluid was present in the inner cavity of the arolium, which was fixed during sample preparation (Fig.2.12.D). In *Neobisum* the lip cuticle externally exhibits nubs (Fig.2.12.C), which might be the bulges of underlying fibers. It may be partly folded at the lateral sides and expanded by an increase of hemolymph pressure. In contrast the arolia of the Cheliferoidea (Fig.2.12.I-K) and Garypoidea (Fig.2.12.G,H) seem to be rather stiff and stable in shape, with the exception of the distal lip. The arolium is usually slightly shorter than the claws, but can also be much longer, as in many Olpiidae and Garypiidae (Fig.2.12.G,H) (Beier, 1932, Harvey, 1992). In the Garypinidae the arolium is divided in two halves (Harvey and Šťáhlavský, 2010). In some genera of Ideoroncidae the arolium bears a median hook-like protuberance (Harvey and Mahnert, 2006). If it is stiff or soft and how it affects substrate attachment remains unknown. In the Parahyidae the arolium bears two lateral pointed protuberances (Harvey, 1992).

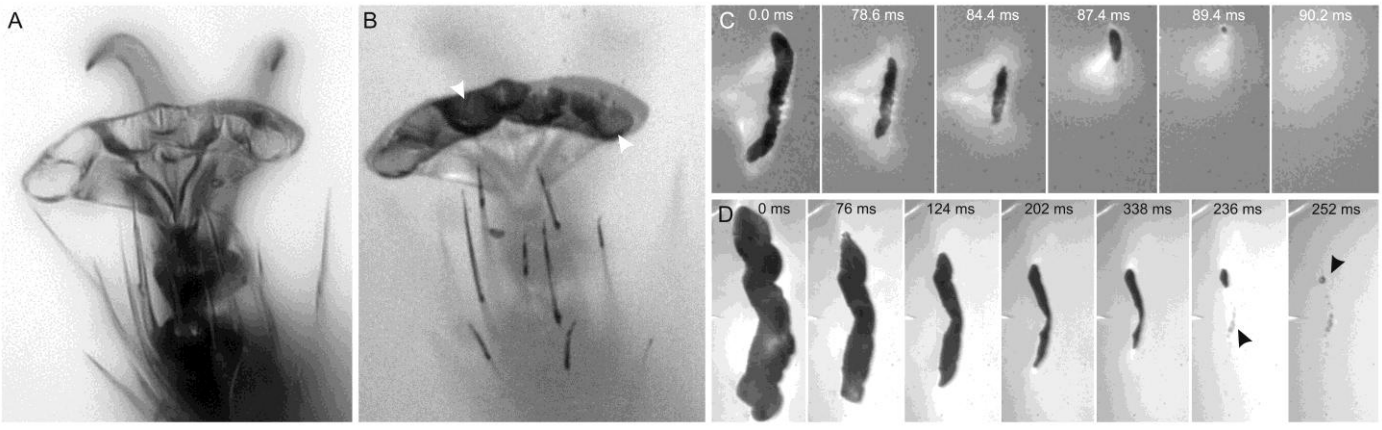


Fig.2.13. Contact mechanics of the pseudoscorpion arolium. (A,B) *Neobisum* sp. (Neobisidae). Light micrographs of pretarsus in contact with a smooth glass slide, view from below. (A) Transmission light micrograph. Note the two lateral struts embedded in the aroliar truncus. (B) Same, with coaxial lighting. The contact area appears darken. Note the local presence of secretion in the contact, indicated by a darker contrast (arrowheads). (C) *Chthonius* sp. (Chthoniidae), leg 4, detachment process, recorded by RICM-HSV (frame rate 5000 fps). Note the lateral peel-off of the pad. (D) *Neobisum* sp., leg 4, detachment process, recorded by RICM-HSV (500 fps, shutter speed 1/5000). Note the minute droplets of secretion remaining after detachment (arrowheads).

The contact of the arolium is only made by the distal lip (Fig.2.13.B). The contact area resembles a transverse, slightly convex narrow strip. During detachment the delamination zone propagates from lateral to median (Fig.2.13.C,D), like in the transverse-oval arolium of

amblypygids (see ***chapter 4***). This is presumably facilitated by the higher rigidity of the lateral parts, which better transmit the tensile stress, when the pretarsal retractor contracts, whereas the more flexible median section partly absorbs the stress and stays longer in contact. Adhesive fluids are secreted into the contact and a thin film may be left behind after detachment (Fig.2.13.D). These footprints shrink to droplets on the (hydrophilic) glass and do not evaporate, which indicates a lipidoid nature. As no pores were visible in the lip cuticle, the fluids probably diffuse through it, which may only be possible if the fluid has a low viscosity.

During pull-off tests *Neobisum* sp. individuals could resist tensile forces of 100-700 µN, which corresponds to a safety factor of 8-45 times of body weight.

2.9.3. Spermatophore, brood sac and silk

Male pseudoscorpions produce spermatophores in the genital atrium (Legg, 1973). It may consist of simply a sperm droplet onto a thin stalk, which is glued onto the substrate, like in the Olpiidae, or the sperm is enclosed in a complex capsule with wing- and horn-like protuberances similar to those in amblypygids (Legg, 1973, Kew, 1930, Weygoldt, 1969). In the latter case the protuberances do not necessarily play a role in a lock-and-key mechanism, as only the insemination duct enters the female genital opening (Weygoldt, 1966a). The spermatophore contains fibroin (but not the same as the silk) and a lipidoid pheromon (Legg, 1973, Hunt and Legg, 1971). There is no data on the ultrastructure and adhesive mechanism of the spermatophore stalk.

The first instars develop in the so-called brood-sac, which is a pad of mucous attached at the underside of the opisthosoma and covered by a membrane of dried secretion (Weygoldt, 1968). The mother secretes nutrients into the brood sac that are absorbed by the larvae (Weygoldt, 1968).

Pseudoscorpions are able to produce silk. The silk glands are located in the prosoma and open into spinnerets onto the chelicerae (Weygoldt, 1969). The silk is used to produce protective retreats while breeding, molting or hibernating (Kovoor, 1987). Males of *Serianus carolinensis* (Olpiidae) also use silk to mark deposited spermatophores for females (Weygoldt, 1966b). It is unknown how the silk is attached to substrates or conglutinated.

2.10. Camel spiders (Solifugae SUNDEVALL 1833)

2.10.1. Claws, chelicerae and spines

The forwards oriented chelicerae of solifuges are large and scissor-like (Fig.2.2.C). They are equipped with sharp denticles and used to grasp and dismember prey, and in males also to overwhelm the female and insert the spermatophore (Kästner, 1933). The proximal part is massive providing space for large, strong flexor muscles, which can produce biting forces over 900 kPa (van der Meijden et al., 2012). Prey which has been grasped with the chelicerae is barely able to escape this strong clamp. Males exhibit a dorsal cuticular protuberance on the chelicerae, the flagellum, which has been hypothesized to serve as an attachment device for sperm transfer (Kästner, 1933). However, its exact function is ambiguous (Lamoral, 1975, Punzo, 1998b).

The walking legs bear two very long claws that are uniquely sub-segmented in the apical part (Fig.2.14) (Kästner, 1933). This flexible hinge of the claw tip may have the function to reduce the stress working on the tip to prevent breakage and wear. The first leg pair is rather thin and weak and in many species not used for locomotion but sensing. Accordingly the pretarsus and claws are often reduced or totally lacking in the first leg pair, only in the Rhagodidae they are still fully developed (Klann, 2009, Kästner, 1933). The tarsus of the walking legs can be bend towards the substrate due to sub-segmentation (Fig.2.14.B). Its ventral sides are equipped with strong spines that play an important role in friction generation during locomotion (Kästner, 1933). Some spines may be based in articulated sockets (Fig.2.14.B) and change their angle by the internal hemolymph pressure. Since most solifuges are epigaeic, and live in stony desert habitats, the spines may provide sufficient attachment devices. Despite of their often large size solifuges are able to run very fast (Punzo, 1998a), which underlines the importance of a proper grip on the ground substrate. In some species the males may also exhibit dense brushes of setae on the metatarsi, and in the Galeodidae also in the soles of the tarsi of the fourth walking legs (Kästner, 1933). If these setae have a specific structure and serve attachment purposes to fixate the female during copulation is not known. Long spines are also often present in the pedipalps, in which they may serve as a prey capture basket.

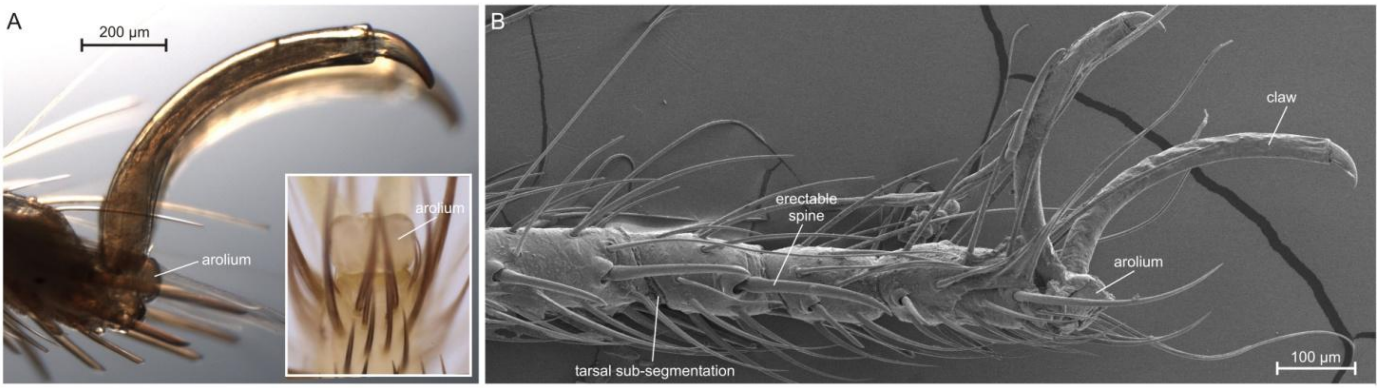


Fig.2.14. The solifuge foot. (A) *Eremobates* sp. (Eremobatidae), lateral view. Note the long, sub-segmented claws. Inset detail of pretarsus, ventral view. (B) Juvenile Solpugidae, unidentified species. Note the sub-segmented tarsus with dorsal articulations, similar to the prehensile tarsi of harvestmen, and the erectable spines.

2.10.2. Arolium

The pretarsus of solifuges exhibits a pro-ventral pad-like arolium (Fig.2.14) (Kästner, 1933), which may be devided in several lobes (Klann, 2009). These pads have been shown to build a contact on smooth surfaces and secrete a lipidoid fluid (Peattie et al., 2011). However, it is not known in which extend these are of importance for attachment or friction generation. Usually they are too small to generate sufficient friction and adhesive forces and permit climbing on smooth surfaces; that is why most solifuges use the pedipalpal adhesive pads (see below) for this purpose (Cushing et al., 2005; Klann et al., 2009, and pers. comm.). However, at least one solifuge species (Ammotrichidae: *Oltacola chacoensis*) is able to scale smooth glass panes with remarkable speed (Klann et al., 2009, and pers. comm.). This species is relatively small and dwells bush-like vegetation, in contrast to most other species that are barely observed climbing. The arolium in this species exhibits two large lateral lobes that may be inflatable; however the exact function has not been studied in vivo and thus remains unclear.

2.10.3. Pedipalpal 'suctorial organ'

Solifugids are known to bear smooth adhesive pads on their pedipalps, with which they can scale smooth surfaces (Cushing et al., 2005, Kästner, 1933), capture prey (Willemart et al., 2011, Kästner, 1933) and take up water to bring it to the mouth (Kästner, 1933). In order to climb smooth vertical surfaces both pedipalps are alternately anchored on the substrate (Cushing et al., 2005). Hence, the whole body weight is supported by one pad alone, which is quite remarkable as solifuges can reach a body length up to 15 cm. Insects flying by can be caught just by the touch of the pedipalp tip (Willemart et al., 2011, Kästner, 1933), which underlines its adhesive strength. The pedipalpal adhesive organ originates from the pretarsus like the arolia of the walking legs; however, it might be an independent development due to its rather different structure. Although the pedipalpal pads have been called 'suctorial organs' (Cushing et al., 2005) it is unlikely that a suction effect is involved in its adhesive mechanism. Ultrastructural analysis revealed that the cuticle of the contacting pad exhibits parallel, apically branched fibrils, very similar to those of smooth adhesive foot pads (Klann et al., 2008). This indicates a comparable function. Already Kästner (1933) proposed that the high compliancy of the pad may permit a close contact generation, which, together with a presumed secretion, may produce high adhesive forces. Previous authors argued that secretions may unlikely be involved, because they did not find according glands in the distal pedipalp and pores in the epicuticle of the pad (Klann et al., 2008, Cushing et al., 2005). However, visible pores are not necessarily needed for fluid emergence, like my data on the pseudoscorpion arolium show (see ***2.9.2.***). Since smooth adhesive pads are proposed to be dependent on the capillarity of a thin fluid film to generate considerable adhesive forces (Drechsler and Federle, 2006, Barnes, 2007), and secretion emergence was observed in the arolia of solifuges (Peattie et al., 2011), the same is very probable for the pedipalpal pad. The pedipalpal pad is usually retracted into the tarsus, apically covered by three lid-like sclerites and activated by inflation, presumably by internal hemolymph pressure (Klann et al., 2008, Kästner, 1933, Cushing et al., 2005). For that purpose the ventral apical sclerite is opened by the attached pretarsal protractor muscle and the dorsal ones presumably by a rise of the internal hemolymph pressure (Klann et al., 2008). Their closing is presumably driven by stored elastic energy of the hinge membranes and the retraction of the attached pad. The pad is retracted by the medially attached pretarsal retractor muscle (Klann et al., 2008).

2.11. Mites and ticks (Acari)

The Acari is the most diverse group of arachnids, both in terms of ecology and morphology. It is a presumably paraphyletic super-group comprised of two super-orders, the Parasitiformes REUTER 1909 (=Anactinotrichida ZAKHVATKIN 1952), and the Acariformes ZAKHVATKIN 1952 (=Actinotrichida VAN DER HAMMEN 1972). Despite of the name both super-orders contain both free living and parasitic lineages. The Parasitiformes comprise the orders Opilioacarida WITH 1902 (=Notostigmata WITH 1903), Holothyrida WITH 1902 (=Tetrastigmata EVANS ET AL. 1961), Mesostigmata CANESTRINI 1891 (=Gamasida VAN DER HAMMEN 1968) and Ixodida LEACH 1815 (=Metastigmata CANESTRINI 1891). The Acariformes comprise the Trombidiformes KRAMER 1877 (=Actinedida VAN DER HAMMEN 1968) and Sarcoptiformes REUTER 1909. I deal with all these eight orders in one chapter, as usual in comparative studies on arachnids (e.g. Dunlop, 2000; Shultz, 1990). Due to the large diversity of these animals and the estimated great number of un-described species this compilation is far from being complete, but will summarize the significant diversity of attachment organs and glues of this group.

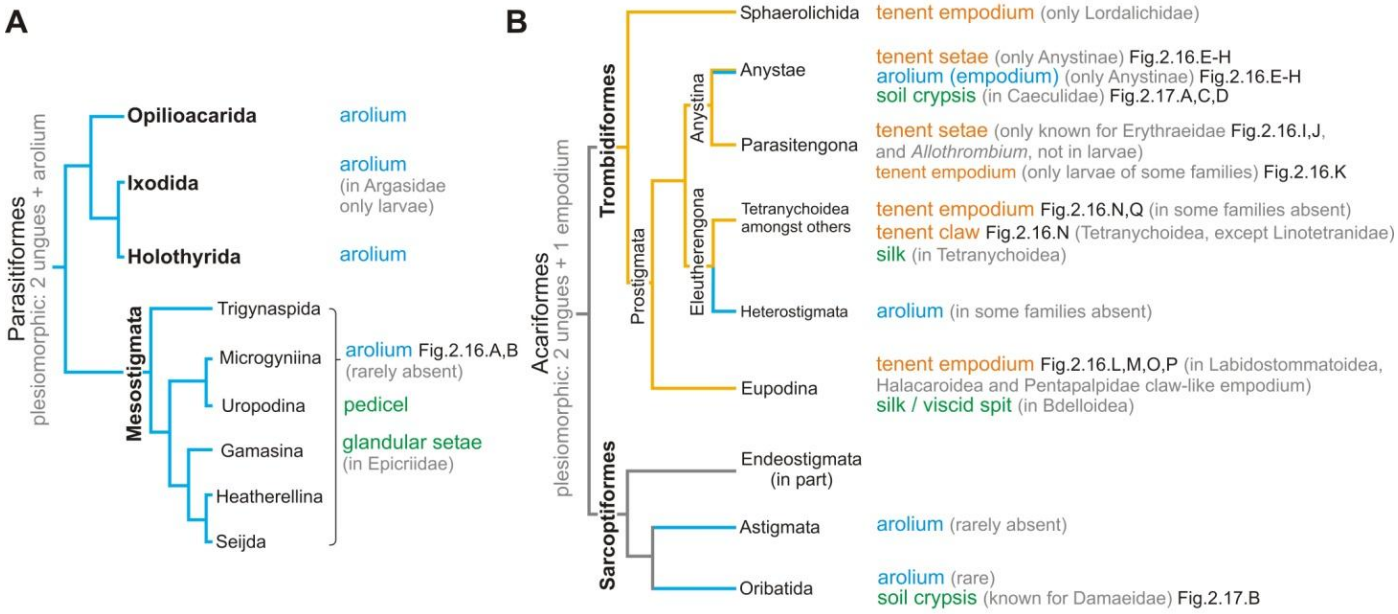


Fig.2.15. Phylogenetic distribution of adhesive pads in ticks and mites (orange: hairy; blue: smooth). The mapping marks lineages, in which the adhesive pads occur, but they are not necessarily autapomorphic for the particular lineage. Please note that the marked homology of the structures is a hypothesis, but since some clades are nested within the marked lineages that lack the pads, the organs may also have multiple origin. The occurrence of other noteworthy attachment devices or secretory products is indicated, printed in green. Links to according figures are given. (A) Parasitiformes, compiled tree based on (Lekveishvili and Klompen, 2004, Klompen et al., 2007, Dunlop and Alberti, 2008), orders printed in bold font. Opilioacarida, Ixodida and Holothyrida are comparably small orders, and hence not further resolved. (B) Acariformes, compiled tree based on (Norton et al., 1993, Dunlop and Alberti, 2008, Dabert et al., 2010, OConnor, 1984), orders printed in bold font.

2.11.1. Claws, chelicerae and clamping legs

The plesiomorphic mite chelicerae are pincer-like, as in Mesostigmata (Alberti and Coons, 1999). However, in many parasitic or plant sucking, but also predatory and saprophagous species the chelicerae are highly modified. There are various kinds of pincers, clamps, stilets and scissors (Alberti and Coons, 1999). Most of the parasitic forms attach to their hosts with some structures in the mouth parts. These are often structures that are pierced in the tissue and anchored there with means of hooklets, like in the hypostome and chelicera sheath of ticks (Ixodida) (Krantz and Walter, 2009, Latif et al., 2012, van der Hammen, 1983b, Kemp et al., 1982).

Other parasites or commensals attach via clamping legs especially to hairs, feathers or gills, as *Ewingia coenobitae* (Sarcoptiformes: Acaridae) (Alberti and Coons, 1999) or *Myobia* spp. (Trombidiformes: Myobiidae) (Paran, 1989). In *Chirodiscoides caviae* (Sarcoptiformes: Atopomelidae), a parasite of guinea pigs, The whole body and legs are ventrally concave and exhibit tansverse ridges for friction generation in order to attach and move along hairs (Alberti and Coons, 1999). Additionally, this species exhibits large disc like arolia, and the males have large, hook-like structures in the hind legs in order to attach to females during copulation (Alberti and Coons, 1999). In water mite larvae (Hydrachnellae: *Arrenurus* spp.) long claws on the pedipalps cooperate with the penetrating sabre-like chelicerae to attach to arthrodial membranes of the damselfly larva host (Åbro, 1979). The predatory Caeculidae (Trombidiformes) have raptorial front legs that are equipped with two ventral-lateral rows of long spines, which may serve as a capture basket (Figs.2.2.H and 2.17.A).

Various states of the pretarsal claws occur among mites: three, two, one or no claws. The three-clawed state (2 claws + empodium) is assumed to be plesiomorphic for the Acariformes, whereas it is the two-clawed state in the Parasitiformes (Dunlop, 2000). The claws and/or the empodium may also be modified in an adhesive apparatus carrying spatulate microtrichia, as in the Tetranychidae (see ***2.11.3.***). In Anystinae (Trombidiformes: Anystidae) the empodium is modified into an arolium (see ***2.11.2.***), in the other anystids it is claw-like (Meyer and Ueckermann, 1987). In the related Parasitengona-clade the empodium is only present in larvae and may be an adhesive pad (at least in Erythraeidae, see below) or a claw (presumably secondary). The claws are usually smooth in the parasitiform orders and the Sarcoptiformes, but may be highly pectinate in the Trombidiformes. In aquatic mites (Trombidiformes: Hydracarina and Halacaroidea) claws are the exclusive foot attachment organs and often enlarged and equipped with denticles (Alberti and Coons, 1999, Vitzthum, 1943). It was proposed that the claw structure may reflect the ecological niche, with the claws being longer in mites of the intertidal zone than in those of the supralittoral (Pugh et al., 1987). In the large soil mite sub-order Oribatida (Sarcoptiformes), adhesive pads are very rare and the claws are the dominant means of attachment (Fig.2.16.C). Due to the small tip diameter, they can generate high friction even on surfaces with a very small roughness, where the claws of larger arthropods fail, as found in the oribatid *Archegozetes longisetosus* (Trhypochthoniidae) (Heethoff and Koerner, 2007). The claw protractor muscle is very strong (about 1200 kN/m²), and the attachment apparatus can withstand loads over 1100 times of the mite body weight without damage (Heethoff and Koerner, 2007).

In the worm-like Nematalycidae (Endeostigmata), which live in the interstitial, palette like microstructures on the cuticle are assumed to support the peristaltic movements through the spaces between sand grains, by interlocking with surface irregularities of the grains in the contraction phase (Bolton et al., 2015).

2.11.2. Arolium

Smooth adhesive pads are widespread among ticks and mites and inconsistently called pulvillus, sucker, adhesive disc, ambulacral disc or caruncle. These pads arise from the membranes associated with the pretarsus or a modification (softening) of the entire pretarsus. According to the terms defined in ***2.3.*** we call these organs arolia. Previous authors often presumed a sucker function of these pads (Evans, 1992), however in most cases this is unlikely. The pads presumably function like smooth adhesive pads (see ***2.1.2.***) and are usually fluid mediated, as found in *Androlaelaps schaeferi* (Mesostigmata: Laelapidae) (Peattie et al., 2011), *Ixodes* spp. (Ixodida: Ixodidae) (Baker, 1997), and *Anystis* sp. (Trombidiformes: Anystidae) (this study). In the following we summarize the distribution, structure and function of the arolia for each of both super-orders.

Parasitiformes: Arolia are found among most parasitiform mites and might be homologous in most cases. The arolia of Opilioacarida and Ixodida are traditionally called *'pulvilli'*, however, I do not use this term since the pads obviously arise from the median pretarsus (see definitions in ***2.3.***).

In the Opilioacarida the arolium is expandable and pad-like (Alberti and Coons, 1999, van der Hammen, 1966). In the first leg pair, which is feeler-like elongated, the arolium is lacking (pers. observation).

In ticks (Ixodida) all stages possess arolia in Ixodidae (Baker, 1997) and Nuttalliellidae (Latif et al., 2012); but in the Argasidae they are only present in the larvae (van der Hammen, 1983b). The arolium is very similar between these families. In *Ixodes* spp. the arolium is a large inflatable pad, with a pair of dorsal sclerites that enclose the pad in the retracted state (Baker, 1997). The inflation is likely driven by an internal rise of hemolymph pressure, and the retraction by the pulling of the pretarsal retractor muscle and the elasticity of the dorsal closing sclerites. The arolia in the first leg pair are much larger than those of the other legs. The inner cuticle of the contacting pad forms thin walled hollow tubes, which apically disintegrate into branched fibers (S.N. Gorb, unpublished data). The enclosing epicuticle is very thin and due to the underlying structure highly compliable. In between there is a lipid fluid, which also emerges into the contact through pores in the epicuticle, leaving discernable footprints when walking on a glass slide (Baker, 1997) (and pers. observation).

In the Holothyrida the arolium is of a shape comparable to that of Ixodida, but it is lacking in the first leg pair (van der Hammen, 1983a).

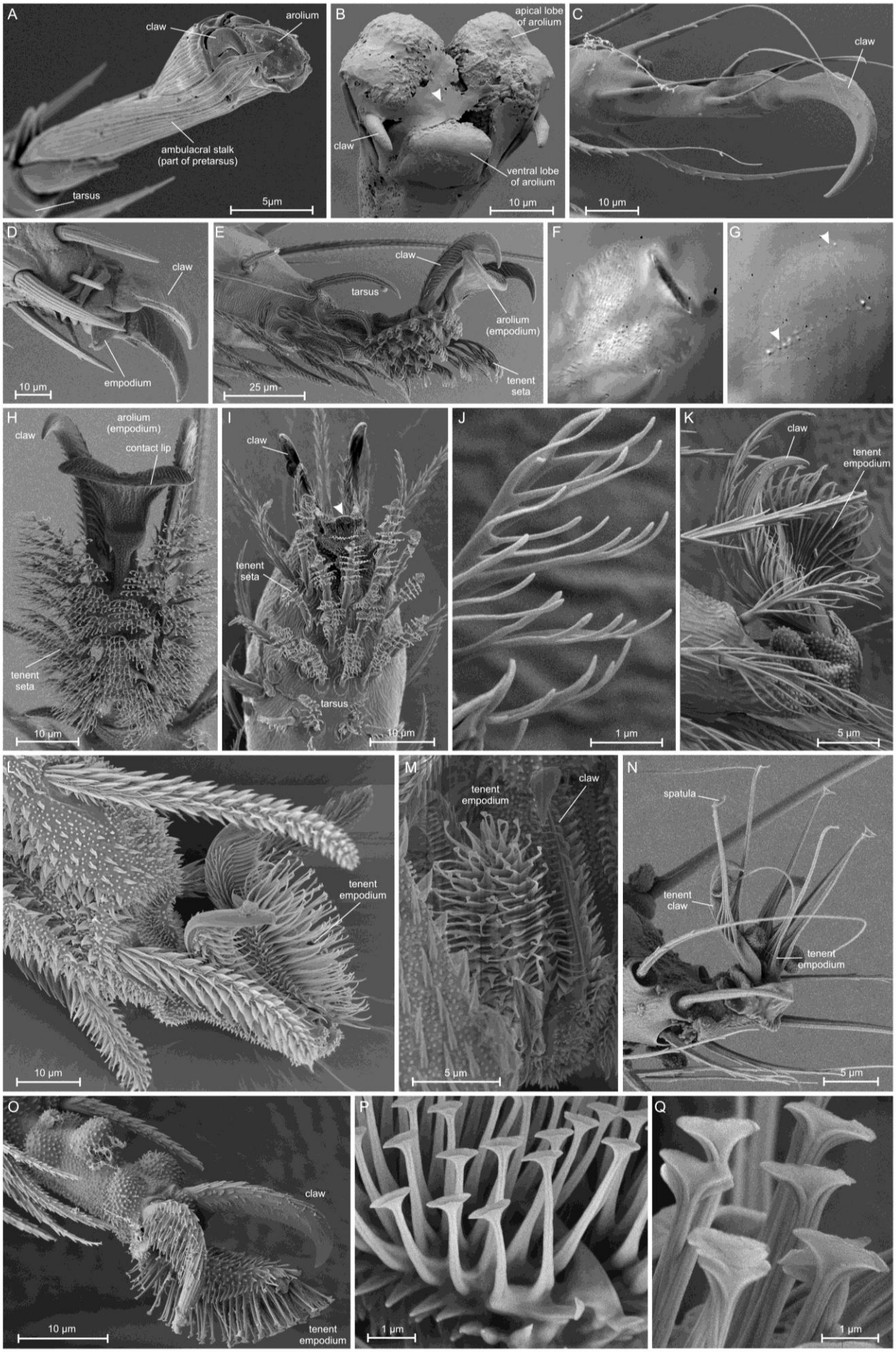
The diverse Mesostigmata contain mites with different ecology, including predatory and parasitic mites. Pad-like inflatable arolia are widespread, usually the whole distal section of the ambulacrum is membraneous, except the, usually small, dorsally attached pair of claws (Alberti and Coons, 1999). The basal ambulacrum often forms a long tube-like stalk, in which the arolium can partly be retracted (Evans and Till, 1965) (Fig.2.16.A). The expanded arolium forms a small median and a pair of large apical lobes (Fig.2.16.B). The common bee parasite *Varroa* spp. exhibits large disc-like arolia (*'caruncle'*), which also can be fully retracted into the ambulacral stalk (Liu and Peng, 1990). However, in adult females the pad bears claw-like sclerites, which were proposed to be used to grasp the setae of bee hosts (Ramirez and Malavasi, 1991). This shift from a smooth pad to an interlocking device reflects the adaptation to a change in the mode of life: The immature primarily move on the smooth walls of the bee's nest, whereas the adult females are phoretic in order to disperse (Ramirez and Malavasi, 1991).

Acariformes: Among Trombidiformes there are lineages with hairy adhesive pads (see below), smooth adhesive pads (Heterostigmata), with both (Anystinae), or which lack both and have enlarged claws instead (e.g. Hydracarina, Halacaroidea). In the Anystidae the arolium is only present in the sub-family Anystinae (Meyer and Ueckermann, 1987). It is presumably derived from the plesiomorphic empodium, like the hairy pads in the Eupodina. The arolium is very similar to that of pseudoscorpions: It is triangular shaped, highly broadening distally and of rather stiff cuticle, except in the distal most edge (Fig.2.16.E,H). Hence, the contact area is a narrow transverse strip (Fig.2.16.F). The arolium adheres via the capillary forces of a lipidoid secretion (Fig.2.16.G). Interestingly, these mites additionally bear a pair of large branched tenent setae, which further support the adhesion. The contact area of the arolium is located more distally than that of the tenent setae (Fig.2.16.F). During attachment the tenent setae make first contact, then the arolium, and during detachment vice versa. Hence, the tenent setae may primarily serve friction for secure the steps and generate forward thrust, whereas the arolium may securely attach the animal between the steps. *Anystis* sp. can run very fast: 42±14 body lengths per second (mean±s.d., N=16). This implicates that the contacts must be generated and loosened very quickly, which is quite remarkable. In many Heterostigmata (e.g. Tarsonemidae and Heterocheylidae) the basal articulation membrane of the single claw is highly inflated and form a large arolium, which is used to attach to hosts (Lindquist, 1986). In some families the arolium is built by a modified empodium and both claws are still present, e.g. in Pygmephoridae (Khaustov and Ermilov, 2011).

In the Sarcoptiformes, the large clade of Astigmata, which include many parasitic mites, have evolved several types of arolia (Alberti and Coons, 1999, Krantz and Walter, 2009). Often the whole distal section of the ambulacrum forms a soft cushion or large disc (often called *'caruncle'*, *'ambulacral disc'* or *'sucker'*) with a small embedded single claw and dorsal sclerites, both of which can also be totally reduced. Such discs may represent a mushroom-like contact element since the ambulacral stalk is dorso-medially attached. In lineages that live in the hair or gills of their hosts, arolia are usually absent and highly modified clamping legs have evolved (OConnor, 1982, Atyeo, 1979). In the Oribatida adhesive pads are very rare, but some arboreal species exhibit a pad like extension of the ventral claw articulation membrane (called *'pulvillus'*), as in *Adhaesozetes polyphyllos* (Adhaesozetidae) (Walter and Behan-Pelletier, 1993), *Phylleremus* spp. (Licneremaeoidea) (Behan-Pelletier and Walter, 2007), *Symbioribates aokii* (Oripodoidea) (Karasawa and Behan-Pelletier, 2007) or *Zetorchestes falzonii* (Zetorchestidae) (Alberti and Coons, 1999). In these cases the size of the arolium increases from front to hind legs, whereas the size of the single claw decreases, which speaks for a division of labor between the different legs in attaching to different surfaces. However, in which extend the rather small arolium generates adhesion has not been studied. For Zetochestidae, which are capable of leaps, Aberti and Coons (1999) argue that the arolium may help to generate the necessary thrust on smooth leaf surfaces.

2.11.3. Other smooth adhesive pads

Other smooth pads occur in the anal region of Heterozerconidae (Mesostigmata). These have been described as suckers (Baker et al., 1987), however, they do not exhibit an inner cavity, and hence I interpret these as smooth adhesive pads. These pads are found in the parasitic adults and used to attach to hosts, like millipedes (Farfan and Klompen, 2012). They are two soft protuberances lateral to the anal opening and can be retracted by muscles (Baker et al., 1987). Details on their internal ultrastructure and possible secretions are lacking. Another anal pad is the sucker of Eriophoridae, which may also function as a smooth adhesive pad (see ***2.11.4.***).


A
claw
arolium
ambulacral stalk
(part of pretarsus)
tarsus
5µm
B
apical lobe
of arolium
claw
ventral lobe
of arolium
10 µm
C
claw
10 µm
D
claw
empodium
10 µm
E
claw
tarsus
arolium
(empodium)
tenent
seta
25 µm
F
G
H
arolium
(empodium)
claw
contact lip
tenent
seta
10 µm
I
claw
tenent
seta
tarsus
10 µm
J
1 µm
K
claw
tenent
empodium
5 µm
L
tenent
empodium
10 µm
M
tenent
empodium
claw
5 µm
N
spatula
tenent
claw
tenent
empodium
5 µm
O
claw
10 µm
tenent
empodium
P
1 µm
Q
1 µm

2.11.4. Tenent setae

Spatulate microtrichia (tenent 'setae') are widespread among Trombidiformes, but are absent from the Sarcoptiformes and all parasitiform orders. These microtrichia may arise from tarsal setae (Anystidae: Anystinae, and Trombidiidae: *Allothrombium*), modified claws (some Tetranychoidea) (Fig.2.16.N), or the (often broadened) empodium (some Tetranychoidea, Cheyletoidea, and Raphignathoidea, Bdelloidea, some Eupodoidea, Tydeoidea and Eriophyoidea) (Fig.2.16.K-P) (Alberti and Coons, 1999, Krantz and Walter, 2009). The tenent empodium seems to be the plesiomorphic state, since it is found in all sub-lineages (in Eupodina and Eleutherengona widespread, in Anystina to my knowledge only in larvae of Erythraeidae, and in the basal Sphaerolichida among Lordalichidae (Krantz and Walter, 2009) but unclear if microtrichia are spatulate in the latter). Basically, the structure of the spatulate microtrichia is strikingly similar to those of spider tenent setae: the shaft consists of two fibrils that apically bifurcate, spanning up a thin membrane between them (Fig.2.16.P,Q). In gall mites (Eriophyoidea) the terminal elements of the microtrichia may be spatula-, band- or disc-like (Krantz and Walter, 2009). The Erythraeidae possess branched setae in the ventral tarsi exhibiting band-like microtrichia that lack spatula (Fig.2.16.I,J) (Alberti and Coons, 1999). I studied these setae in *Abrolophus* sp. and found that they are only present in nymphs and adults. The three-legged larva lacks them, but bears an empodium with spatulate microtrichia instead (Fig.2.16.K). Both larvae and adults are capable to scale smooth surfaces with high speed despite the differences in the tenent setae. The fast running *Anystis* spp. (Anystidae) possess a pair of large multiple branched tenent setae, but also an arolium in each of the eight walking legs (Fig.2.16.E,H). This is a unique configuration among arachnids. I found that both the arolium and the tenent setae are brought into contact during locomotion on smooth surfaces (Fig.2.16.F), but it remains unclear in which situation which of the pads (hairy or smooth) are dominating the attachment function.

Lipidoid fluid footprints were found in the tenent setae of *Tetranychus urticae* (Tetranychidae) (Mizutani et al., 2006), *Balaustium murorum* (Erythraeidae) (Peattie et al., 2011), *Abrolophus* sp. (Erythraeidae) and *Anystis* sp. (this study, Fig.2.16.G). Hence, capillary forces may play a role in the adhesive function of these setae. However, theoretical models and measurements on substrates with different free surface energy showed that at this small scale the

Fig.2.16. Pretarsal conditions in mites. (A,D,L,M,N) Standard-SEM, (B,C,E,H-K,O-Q) Cryo-SEM micrograph, (F,G) RICM image with additional transmission lighting. (A-B) Mesostigmata, (C) Sarcoptiformes, (D-Q) Trombidiformes. (A) *Amblyseius swirskii* (Phytoseiidae), distal portion of leg 4, lateral view. Arolium retracted. Note the elongated pretarsus (ambulacral stalk). (B) Unidentified mesostigmatid, leg 3, frontal-ventral view. Arolium expanded. Arrowhead indicates secretion. (C) Unidentified oribatid, leg 4, lateral view. As in many oribatids only a single claw is present. (D) *Caeculus* sp. (Caeculidae), leg 1, lateral view. This represents the presumed plesiomorphic condition of the pretarsus in the Acariformes, with two claws and a claw-like empodium, similar to other arachnids. (E-H) *Anystis* sp. (Anystidae). In the Anystinae the pretarsal empodium is modified into a triangular arolium with a distal soft lip. Additionally a pair of large, branched tenent setae is present on the distal tarsus. (E) Leg 2, lateral view. (F) Pretarsus in contact with a glass slide, contact area appears dark. (G) Same site shortly after foot detachment. Note the deposition of secretions both by the arolium and the tenent setae (arrowheads). (H) Leg 4, ventral view. (I-K) *Abrolophus* sp. (Erythraeidae). (I) Adult, leg 1, ventral view. Note absence of empodium (arrowhead). (J) Detail of tenent seta. Note absence of spatulate tips. (K) Larva, leg 1, lateral view. Tenent empodium present, tenent setae absent. (L-M) *Linopodes* sp. (Cocceupodidae). (L) Leg 4, lateral view. (M) Leg 1, frontal view. (N) *Bryobia* sp. (Tetranychidae), leg 1, lateral view. Note the presence of spatulate microtrichia at the claws and the slender empodium (both with 1 row at each lateral side). (O-P) *Cyta* sp. (Bdellidae). (O) Leg 1, lateral-dorsal view. (P) Detail of tenent empodium with spatulate microtrichia. (Q) *Bryobia* sp., leg 3, detail of spatulate microtrichia.

intermolecular attractive forces between spatula and substrate play the dominant role (Mizutani et al., 2006). The adhesive forces were measured in spider mites (*Tetranychus urticae*), which bear four spatulate microtrichia in each ambulacrum (Mizutani et al., 2006). The adhesion is about 8 µN on glass, which corresponds to a safety factor of 37 (Mizutani et al., 2006). The adhesion is reduced by high humidity and surface roughness (Mizutani et al., 2006). The tenent setae are the only attachment devices on the legs of these animals and hence play the dominant role in attachment and locomotion on the inhabited leaf surfaces.

Other adhesive setae with a totally different structure are 'sucker-like' discs dorsally on the hind legs of male Acaroidea (Evans, 1992), e.g. flour mites (Acaridae: *Acarus siro*), used to attach to females during copulation (Alberti and Coons, 1999). They are discoid, but lack a median depression and hence are considered as mushroom like contact elements, which have been shown to generate high adhesive forces on smooth surfaces, even without a mediating fluid (Carbone et al., 2011, Heepe and Gorb, 2014, Gorb et al., 2007). In some extend this may also count for some of the adanal suckers of acaridid mites, which are disc-like modified setae, but exhibit some features typical for suction cups (see below).

2.11.5. Suction cups

Suckers independently evolved in the anal region of Acaridida (Sarcoptiformes) and Eriophyidae (Trombidiformes) (Alberti and Coons, 1999, Witaliński, 1990, Baker et al., 1987, Popp, 1967). This is quite remarkable as suction cups usually are restricted to aquatic animals and are assumed to be less functional in air and at small scale (see ***2.1.5.***).

In the Acaridida the suckers occur in phoretic nymphs (so-called *hypopi*) (Alberti and Coons, 1999), and in males (Witaliński, 1990, Popp, 1967). In the first case they serve the attachment to arthropod phoretic hosts in order to disperse, in the latter case they fulfill the function of partner fixation, which plays a role for mate guarding (Witaliński, 1990, Witaliński et al., 1992). These structures have a concave shape and medially attached retractor muscles, which indicates a sucker function (Baker et al., 1987, Witaliński, 1990). Suction cups of different sizes and structure are grouped on the adanal sucker plate (Baker et al., 1987). This plate is sclerotized and slightly convex, like a sucker itself. The suckers are usually slightly depressed in a button-like fashion, but may also be tube like with a piston-like moveable internal structure, which can highly change the inner volume (Witaliński, 1990, Witaliński et al., 1992). The structure of the suction cups is described as relatively stiff and stable in shape (Witaliński, 1990), hence the alteration of the inner volume may also be passively performed by the elasticity of the cup material. It might be probable that a secretion is extruded into the contact, as this would greatly enhance the efficacy of the suckers. Glands have been found near the sucker plate (Popp, 1967), but if and how a secretion emerges into the suckers remains unknown. If the sucker is very flat the adhesive mechanism might be more related to mushroom-shaped contact elements (see above). Hence the exact mechanism of these structures demands closer investigations. In the case of female-male interaction the target surfaces on the female are often conspicuously smooth, in contrast to the often microsculptured cuticle on the rest of the body (Witaliński et al., 1992). This is obviously to ensure a proper function of the suction cups. In feather mites (Proctophyllodidae) the female even possesses tubercles that fit into the modified tube-like suckers of the males (Witaliński et al., 1992). These tubercles can be slightly inflated be an increase of the internal hemolymph pressure, which presumably enhances the sealing of the sucker and hence increases the efficacy of the attachment (Witaliński et al., 1992).

In gall mites (Eriophyidae) the sucker is used to attach to plant surfaces, to phoretic hosts (usually insects), to each other in order to build chains that are drifted by wind currents, but also for looper-like locomotion and even leaping (Baker et al., 1987). The sucker is simply a softening of the cuticle around the anus by a lack of sclerotization and the reduction of epi- and exocuticle (Baker et al., 1987). It is inflated by the internal hemolymph pressure. Due to the high compliancy of the membranous cuticle the inner cavity of the anus is sealed, when pressed against the substrate. The anus then can be retracted by attached muscles, which produces a drop of pressure (Baker et al., 1987). During molting the attachment is enhanced by the secretion of a viscous fluid emerging from the anal opening (Baker et al., 1987). It remains unclear in which extend a suction effect plays a role in the adhesion of this structure. The soft margin may already function as a smooth adhesive pad, especially if fluids are involved.

Another type of suckers occurs in some water miter like *Limnochares aquatica* (Limnocharidae), whose larvae are ectoparasites of water insects: The gnathosomal tip has a round suction cup like depression with the mouthparts in its middle (Alberti and Coons, 1999). The margin consists of non-slerotized cuticle with fine microtrichia, between which a mucous might be secreted. Due to the limnic environment a significant sucker function to attach to the host's cuticle is probable.

2.11.6. Pedicels, silk and other adhesive secretions

The deutonymphs of Uropodina (Mesostigmata) show a unique adaptation to phoresy: They secrete a stalk of dried secretion with a mushroom-like cement disc to attach to the cuticle of phoretic hosts (usually beetles) (Bajerlein and Bloszyk, 2004). This so-called pedicel emerges from anal glands and is strongly attached to the anus after drying (Bajerlein and Witaliński, 2012). The stalk of the pedicel consists of parallel fibers that are surrounded by a layer of a homogeneous material, which also forms the adhesive disc (Bajerlein et al., 2013). The length of the pedicel is adjusted to the attachment site: at more exposed sites longer and thinner (hence, more flexible) pedicels are produced, in order to reduce the stress on the adhesive disc (Bajerlein et al., 2013).

Spider mites (Thrombidiformes: Tetranychidae) bear spigots on their pedipalps (Clotuche et al., 2012). The silk is used to build silken covers as a protection against predators, rain and wind, and to exclude interspecific competitors (Gerson, 1979). In the presence of predatory mites eggs are laid suspended in the webbing, where they are less imperiled by predation (Lemos et al., 2010). As these mites live in large colonies the silken sheets of individuals are connected to form large cover sheets. The amino acid composition of the spider mite silk is comparable to that of pseudoscorpions (Hazan et al., 1975). With a diameter of 50-100 nm it is much thinner than the silk of other spinning arthropods (Clotuche et al., 2012). Hence, it might significantly adhere to substrates or between each other by van der Waals forces or the interlocking with minute surface asperities. However, it was also observed that spinning mites shortly remain still with the pedipalps pressed onto the substrates in order to attach the silk (Clotuche et al., 2012, Kanazawa et al., 2011). This may indicate that the silk is still fluid when emerging and is cemented onto the substrate. The threads seem to stay adhesive for up to one hour, which may indicate that some slowly drying fluid coating is present on the threads (Kanazawa et al., 2011). The mites use this property to remove dirt particle from their nest (Kanazawa et al., 2011).

Bdellidae and Cunaxidae (Thrombidiformes) use a viscoelastic glue for prey capture, and a comparable, more silk like, secretion for retreat building and egg protection (Alberti, 1973, Alberti and Ehrnsberger, 1977). In the Bdellidae the glue is squirted onto the prey, which is then tethered

to the substrate (Alberti, 1973), quite similar to the glue use of spitting spiders (see ***2.4.4.5.***). Cunaxidae use the secretion to build capture webs, which seem to contain droplets of viscous glue (Alberti and Ehrnsberger, 1977), like the capture threads of orb web spiders (see ***2.4.4.3.***).

The predatory Epicriidae (Mesostigmata) have glandular setae in their front legs carrying droplets of a viscous secretion used for prey capture (Alberti, 2010). Comparable to the glandular setae of harvestmen, which have a similar function (see ***2.7.5.***), these setae have internal secretion channels and bear microtrichia at the tip, where the secretion droplet is attached (Alberti, 2010).

Ticks (Ixodida) secrete a proteinaceous cement cone with carbohydrate and lipid additions to anchor their mouthparts in the host's skin (Bishop et al., 2002, Kemp et al., 1982, Gregson, 1960). The hardening is presumably an enzymatic tanning process (Kemp et al., 1982). Together with denticles on the chelicerae sheath this permits an extremely robust attachment. Interestingly, it is not entirely known, how the ticks lose their attachment, when detaching from their hosts. It was observed, that the cement cone is left in the skin, and it was assumed that the ticks retract their mouthparts from the anchorage (Gregson, 1960). A violently removed tick still has cement on its chelicerae, but is able to remove it within a few minutes (Gregson, 1960). It cannot be excluded that an enzymatic fluid is used in this process. Adult Argasidae do not secrete the cement anymore and also lack the hooklets on the chelicerae sheath (Kemp et al., 1982). Such cement cones, anchoring the mouth parts additionally to hook-like structures have also independently evolved in other mites, like in larvae of *Leptus* spp. (Trombidiformes: Erythraeidae) parasiting on harvestmen (Åbro, 1988).

Spermatophores including an adhesive disc, a stalk and a elaborate apical part occur in several free living acariform mite families, like Anystidae, Erythraeidae, Eriophyidae, Bdellidae (Trombidiformes), and Galumnidae (Sarcoptiformes) (Krantz and Walter, 2009). Their stalk may contain a fibrous protein and is cemented onto the substrate (Kovoor, 1987).

2.11.7. Soil crypsis

Like some harvestmen, some mites secrete a hardening fluid in order to cover their body with soil particles for camouflage. In particular I found this in *Caeculus* sp. (Trombidiformes: Caeculidae) (Fig.2.17.A). The secretion coagulates on the site of extrusion (Fig.2.17.C) and appears dark brown and tanned. On parts of the ventral side and at the arthrodial membranes no secretion is present. The underlying cuticle exhibits elaborate microstructures, forming hexagonal combs and globular granules (Fig.2.17.D). These parts are totally free of any dirt, which is consistent with the observation that very similar microstructures in springtails work fluid-repellent and self-cleaning (Helbig et al., 2011). In *Caeculus* there seems to be a dual function of the structures: it may provide good hold for the coagulating secretion, but prevent the adhesion of soil particles and fluids at sites, where it would disturb. Soil crypsis also occurs in larvae of some Oribatida, like *Belba corynopus* (Damaeidae) (Seniczak and Seniczak, 2013) (Fig.2.17.B). In some species the exuvia (in some cases with soil particles) stays attached for camouflage (Seniczak and Seniczak, 2013). In all these cases nothing is known on the composition and properties of the secretions.

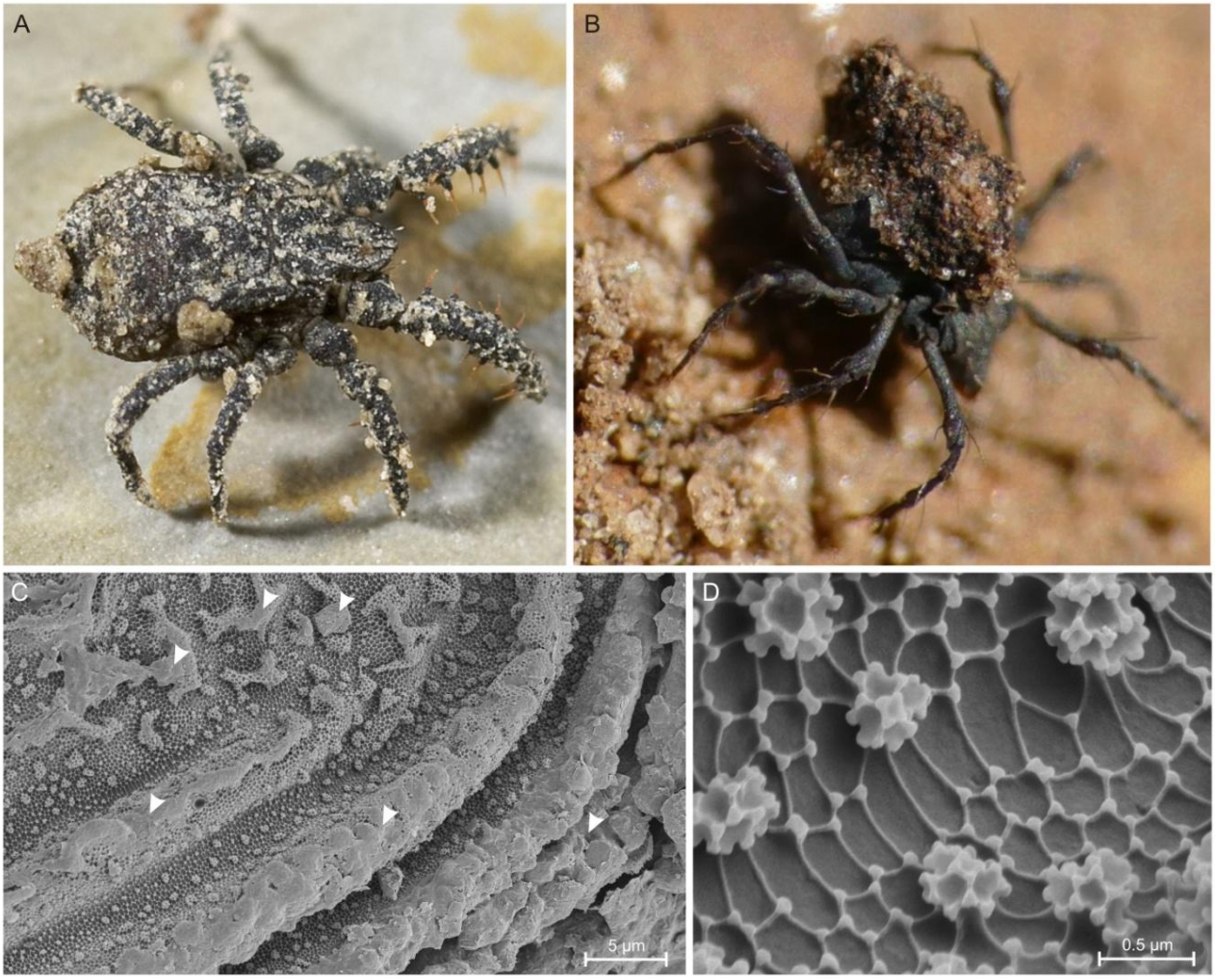


Fig.2.17. Soil crypsis in Acariformes. (A) Habitus of *Microcaeculus* sp. (Trombidiformes: Caeculidae). Note sand grains adhering to the body, and spiny front legs. Photograph by Arno Grabolle, with kind permission. (B) Unidentified Damaeidae (Oribatida) carrying its exuvia with a crust of dried secretion and soil particles. Photograph by Lennart Bendixen, with kind permission. (C) Ventral cuticle of *Caeculus* sp. Note the clumps of dried secretion (arrowheads) and the clean ornamented cuticle in between. (D) Detail of the micro-ornamentation of the cuticle. The 'Lotus-like' microstructures are presumed both to fasten the dried secretion coat and keep the non-coated sites free from contamination with soil particles.

2.12. **Ricinulei** THORELL 1976

The small cryptic arachnid order Ricinulei (‘hooded tickspiders’) is restricted to the tropics, comprising soil-, litter and cave-dwelling species. They exhibit clamps in both chelicerae and pedipalps and two tarsal claws. A unique structure is the cucullus (‘hood’) a dorsal projection, which covers the mouth parts. It can be raised and lowered by muscles and thus also be used in a clamp like manner. This is used to capture prey or carry the single eggs together with the pedipalps (Cooke, 1967). The males exhibit secondary copulatory structures in the third leg pair, which may work as a lock-and-key mechanism like the pedipalps of entelegyne spiders (Legg, 1977).

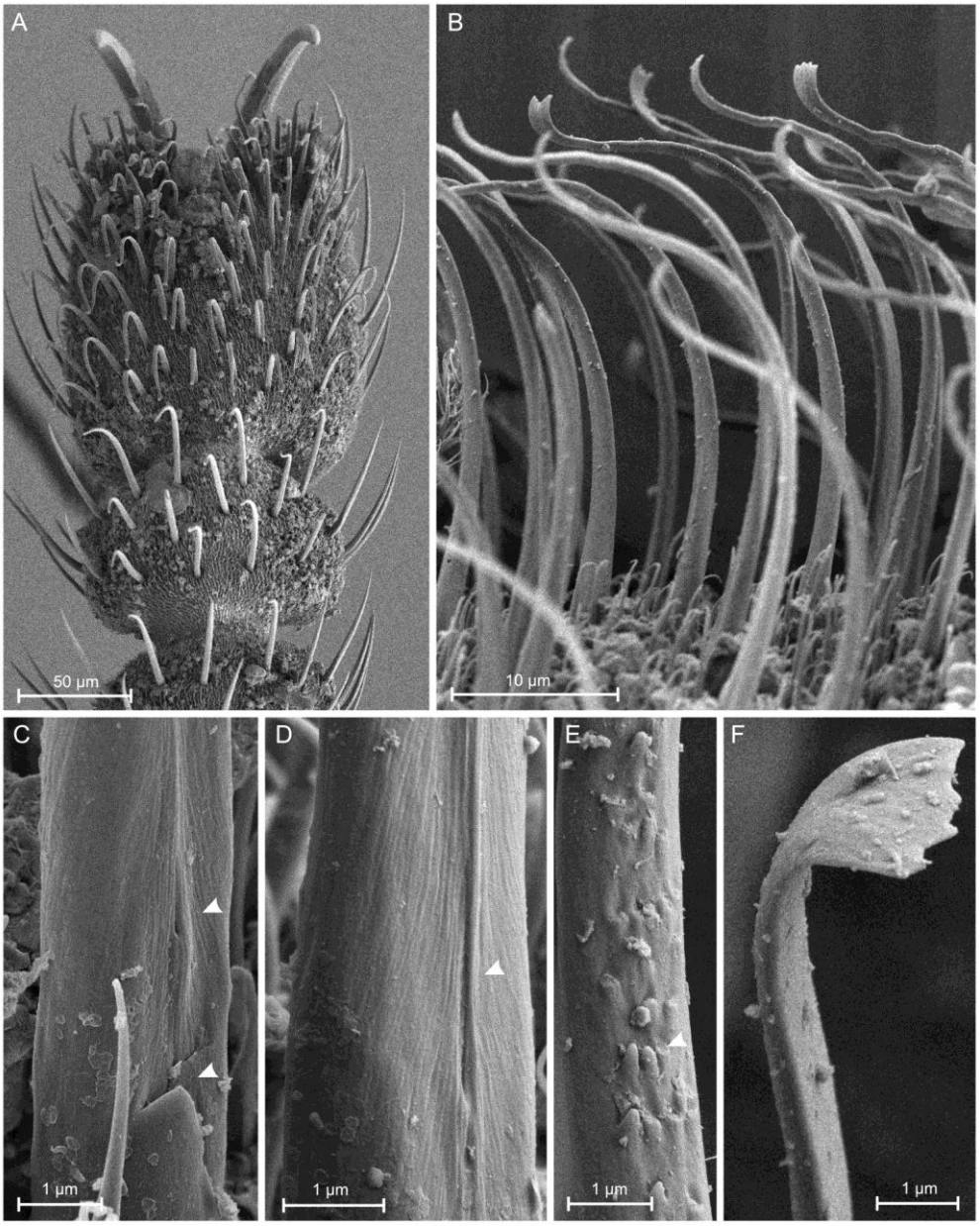


Fig.2.18. Scopula of Ricinulei. *Pseudocellus pachysoma* (Ricinoididae), nymph. (A) Tarsus of leg 4, ventral view. Spatulate setae are present only on the distal most tarsomere. (B) Detail of scopula, lateral view. (C-F) Details of spatulate setae. (C) Basis with slit-like channel openings (arrowheads). (D) Setal shaft with groove (arrowhead), presumably supporting a secretion transport (see also Talarico et al., 2006). (E) Setal back, with nubs (arrowhead). (F) Apical part with spatulate tip.

In *Pseudocellus* spp. the ventral sides of the distal tarsal segment is flattened and covered with a scopula (Fig.2.18.A,B), with the exception of the elongated second leg pair, which is used as feelers. The spatulate setae exhibit a rear fold (Fig.2.18.C,D), which stands in connection with secretion channels (Talarico et al., 2006). The rear side of both shaft and spatulae are irregularly covered by small nubs (Fig. 2.18.E), similar to the scopula setae of some harvestmen (see ***2.7.3.***). The similarity of the structure to hairy adhesive pads of insects, the location on the tarsal sole of the walking legs and the presence of secretion channels indicate an adhesive function. However, this could not be tested due to the lack of living individuals for this study. The ecological benefit is quite unclear. The species examined in this study (*P. pachysoma*) lives deeply inside a cave, where it was exclusively found under stones buried in a thick layer of guano (Teruel and Schramm, 2014). A need of climbing seems ambiguous under these circumstances. It is unknown if the scopula is also present in other ricinuleid genera than *Pseudocellus*.

2.13. Palpigradi THORELL 1888

The most enigmatic group of arachnids is the Palpigradi (‘microwhip scorpions’). The tiny palpigrades are found deeply in the soil or caves. The chelicerae are slender and pincer-like, and less used for attachment purposes, but for scratching biofilms from substrates for feeding (Smrž et al., 2013). The walking legs bear two large and a small claw-like empodium (Börner, 1901). Adhesive pads are lacking. Nothing is known about reproduction and sperm transfer.

2.14. Comparative adhesive strength of adhesive foot pads

The data of pull-off force and adhesive strength are summarized in Fig.2.19. In all cases the safety factor was high enough to support multiple times of the body weight, and was comparable between taxa with smooth and those with hairy adhesive pads. However, in arachnids with smooth pads in general a higher contact area (and presumably a higher amount of fluid) is nessecary for the generation of this force. As a general rule the efficacy of the pads depends both on the body size and the structure of the pad. The efficacy decreases with increasing body weight, due to the decreasing surface to volume ratio and presumably the decreasing evenness of load distribution (see Labonte and Federle, 2015). As a general trend the animals with spatulate microstructures gained a much higher adhesive strength, whereupon it is comparable between spiders (setal pad) and whip-spiders (flat spatulate arolium). However, in whip spiders the performance highly differed between individuals, which might be due to different amounts or composition of aroliar secretion or differences in the physical condition of the animals. The *Sarax brachydactylus* individual generated an adhesive strength lower than the *Charon grayi* individuals. *S. brachydactylus* lacks spatulae (see ***chapter 4***), which may have an effect on the adhesive performance. However, as only a single individual of this species was available for this study it is not clear if this generally holds true. For a final conclusion on the comparison of the performance of smooth, patterned and hairy pads, more individuals and/or species have to be tested to separate the structural from the allometric effect and allow an appropriate statistical analysis.

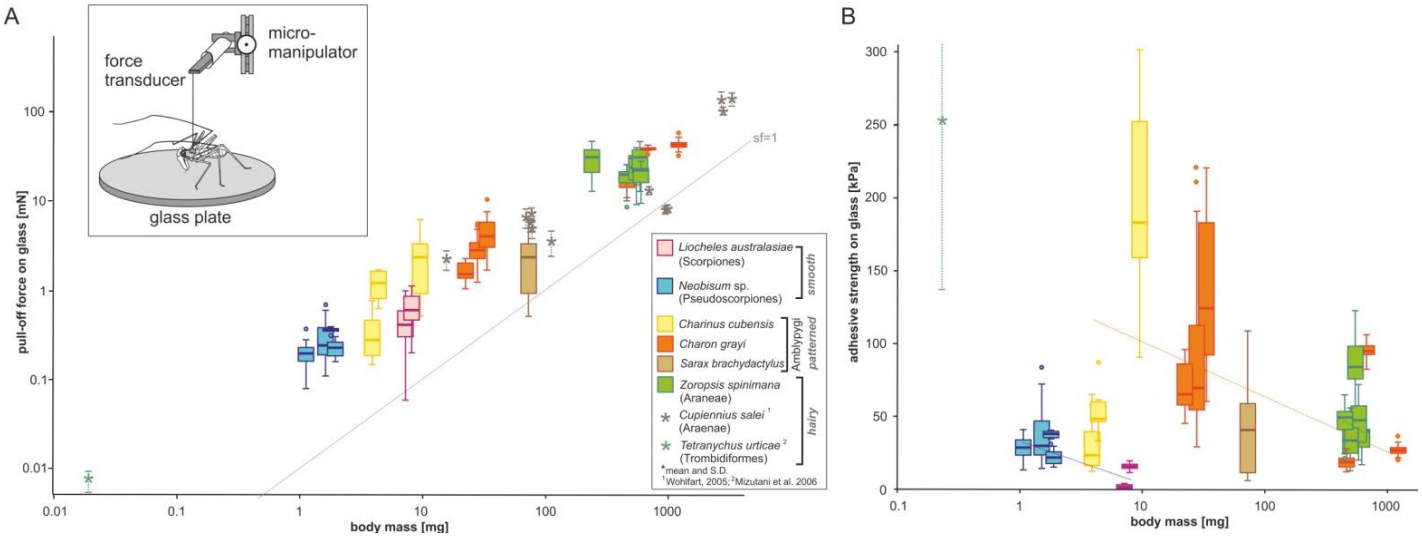


Fig.2.19. Results of comparative force measurements on adhesive foot pads. Boxplots give the median value, interquartile range and the extreme values, outliers are indicated by circles. Each box plot illustrates the data of a single individual (each 10-25 measurements). Of *C. salei* (different individuals) and *T. urticae* (individuals pooled) the mean and standard deviation are illustrated, data taken from (Wohlfart, 2005) and (Mizutani et al., 2006). (A) Peak pull-off forces of animals attached to a smooth glass slide. The inset illustrates the measurement setup. (B) Adhesive strength (pull-off forces divided by the contact area). For better comparison the macroscopic contact area was taken (part of the pad in contact), not the effective one given by microstructures and secretions. Data of *C. salei* is not included here, because the data on the contact area of the experimental animals is lacking. The adhesive strength decreases with increasing mass, because of the allometric relationship between surface and volume, and the increasing difficulty to prevent stress concentrations. Note the much lower values of smooth adhesive pads, indicating that a much higher proportion of the pad has to be used to generate similar adhesive forces as hairy and patterned adhesive pads.

2.15. Summary and distribution of attachment principles among arachnids

The diversity of found attachment devices and functional principles of Arachnida are summarized in Tab.1.2. Fig.2.20. presents the distribution of adhesive foot pads in arachnids. In every arachnid order, except Palpigradi, adhesive foot pads have evolved, either smooth or hairy. Due to the uncertainty of the phylogenetic relationships (see conflicting hypotheses and discussion in (Garwood and Dunlop, 2014, Pepato et al., 2010, Shultz, 2007)), it is hard to evaluate which of these structures are homologous. The relationships among the Tetrapulmonata and Parasitiformes are well supported and a homology of the arolia within these clades is likely. The hairy pads of spiders, harvestmen and Trombidiformes have multiple origins within the orders, as well as the smooth pads of Acariformes.

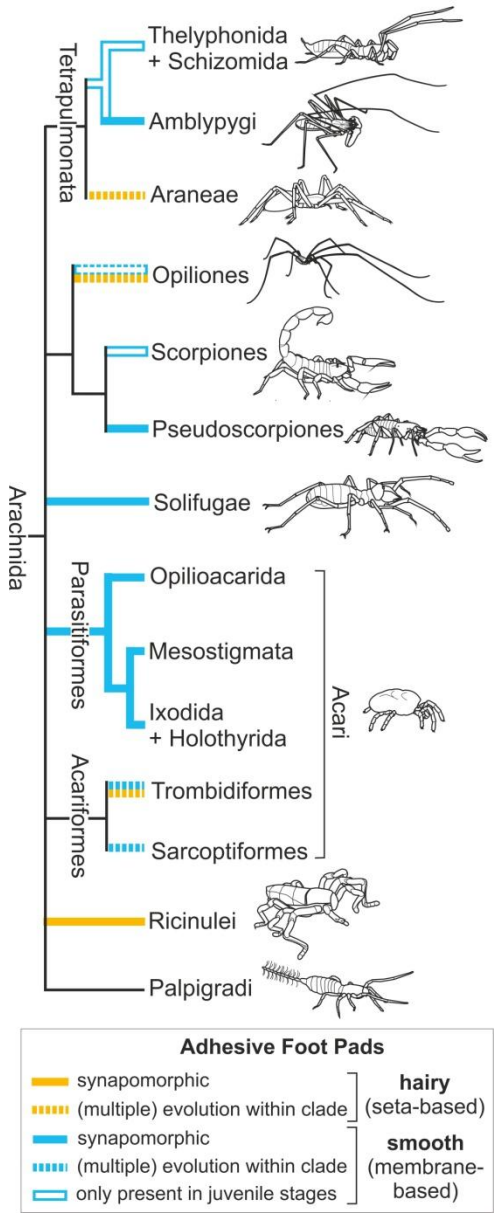


Fig.2.20. Phylogenetic distribution of adhesive foot pads in Arachnida. Characters are mapped on a compiled tree based on (Shultz, 2007, Garwood and Dunlop, 2014, Pepato et al., 2010, Regier et al., 2010). Please note, that the smooth pads of nymphal and adult Amblypygi are functional divergent ('patterned pad', see discussion).

Tab.2.1. Summary on attachment devices in arachnids. Remark that the particular devices may be restricted to some lineage within the clade (for details see main text). In Palpigradi no attachment devices are known besides the pincer-like chelicerae, claws and empodium, hence this order is not listed here. [1] may be supported by fluid secretions; [2] applied in a mushroom-like structure

		Araneae	Amblypygi	Uropygi	Opiliones	Scorpiones	Pseudo-scorpiones	Solifugae	Acari	Ricinulei
mechanical attachment	*interlocking*	2 claws (+ empodium); serrated bristles; frictional setae; spines; some mating plugs	2 claws; spines; spermato-phore head	2 claws + empo-dium; spines; spermatophore head	1-2 claws (+ empodium); spines; setal sole; penal barbs	2 claws + empodium; spines	2 claws; spermato-phore head	2 claws; spines	1-3 claws; spines (rare); body microstructures of peristaltic Nematalycidae	2 claws; copulatory legs
	clamp / pincer	chelicerae; whole-body capture basket; raptorial leg in *Trogloraptor*	chelicerae; raptorial pedipalps	chelicerae; raptorial pedipalps	chelicerae; (raptorial) pedipalps	chelicerae; pedipalp chelae	chelicerae; pedipalp chelae	chelicerae	some chelicerae; clamping legs; raptorial legs; whole body	chelicerae; pedipalp pincer; cucullus
	locking piercer	chelicerae	-	-	-	-	-	-	tick hypostome; some chelicerae	-
	lock-and-key	male pedipalp + epigyne	-	-	?	-	-	-	sucker + stick in some Acarida	?
	entanglement / wrapping	cribellate silk; aciniform silk (bridging line and prey wrapping); Scytodidae spit	-	-	prehensile tarsi	-	-	-	silk; Bdellidae spit	-
structural adhesive[1]	*smooth pad*	-	prenymph arolium	prenymph arolium	nymphal arolium	prenymph arolium	arolium	pedipalp organ; arolium	arolium; anal pads of Heterozerconidae	-
	patterned pad	-	arolium	-	-	-	-	-	-	-
	spatulate setae	-	-	-	scopula	-	-	-	-	scopula
	spatulate microtrichia	scopulae; claw tufts	-	-	-	-	-	-	tenent setae, empodium and/or claws	-
	mushroom setae	-	-	-	-	-	-	-	'sucker'- setae of Acaroidea; some arolia	-
	ribbon-like contact elements	tape-like ampullate silk of *Loxosceles*	-	-	scopula	-	-	-	tenent setae of Erythraeidae	-
	nano-fibrils	cribellate silk; aciniform silk; particle catching setae	-	-		-	silk	-	silk	-
suction	*sucker*	-	-	-		-	-	-	adanal suckers; gnathosomal sucker plate	-
adhesive secretion	*viscid glue*	aggregate gland secretion; Scytodidae spit; pyriform gland secretion in Pholcidae	-	-	glandular setae; glandular pedipalps	-	brood sac	-	Bdellidae spit; Cunaxidae capture silk; glandular setae in Epicriidae	-
	hardening glue	pyriform gland secretion([2]); cylindrical gland secretion; mygalomorph silk; mating plug	egg sac; spermato-phore pad[2]	egg sac; spermatophore pad[2]	body secretion for soil crypsis	spermato-phore pad[2]; mating plug	silk; spermato-phore pad[2]	?	pedicels in Uropodina deutonymphs[2]; cement cone in ticks; silk; spermatophore pad[2]; body secretion for soil crypsis	?

3. Paper no.1*:

Morphological and functional changes of the pretarsus in arachnid postembryonic stages

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Abstract

A specific type of maternal care occurs in several groups of Arachnida: mothers carry their offspring on their back (pulli-carrying behaviour). In scorpions, whip scorpions and whip spiders it is the prenymphal stage that settles on the mother. The prenymph is not yet fully developed for a free life and very limited in its mobility, but its feet are equipped with special adhesive organs (arolia) that become lost at the nymphal stage. Here we study the morphology, ultrastructure and mechanical function of the arolia. In scorpions (Scorpiones) the contact area between arolia and substrate and thus adhesion of the pad is controlled by the antagonistic work of hydrostatic pressure and muscular retraction. Arolia of whip scorpions (Thelyphonida) do not require muscular action for strong attachment. Arrays of long, branching fibres in the mesocuticle lead to high compliancy of the pad. In whip spiders (Amblypygi) the prenymphal pretarsus is already equipped with sclerites and claws. Its arolium is retained in nymphs and adults in some taxa, but acquires a more complex structure. These results contribute to our knowledge on the postembryonic development of arachnids and to the understanding of attachment pad evolution among arthropods. Some of the described developmental, structural, and mechanical phenomena are not known from other animals and might be of potential interest for further biomimetic developments.

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3.1. Introduction

From hatching until maturity the growth and development of arthropods is not continuous but subdivided by several moulting events. These can be accompanied by a radical change in morphology and ecology, as during the metamorphosis in holometabolous insects and in some mites (Minelli et al., 2006, Andre and Jocqué, 1986). In most arachnids, however, morphological changes between two instars (stages between two moults) appear to be minimal. Yet, postembryonic development in Arachnida can be sub-divided in three different phases (Canard and Stockmann, 1993): 1. The postembryo is not fully developed for a free life, because cuticular structures, such as the mouthparts or sensory structures, are not fully developed. It feeds from yolk and its locomotion capabilities are rudimentary. This stage usually contains three instars: the immobile first and the second postembryo and the prenymph, also called pulli (singular: pullus) (Pavlovsky, 1924), larva (Millot and Vachon, 1949), pre- or pro-juvenile (Lourenço, 2002). 2. The following instars (nymphs) are free living, actively feeding and usually of a rather similar morphology like the adults. 3. The adult stage is reached with sexual maturity (usually accompanied by the full development of copulatory organs). In some groups of Arachnida adults continue to grow and moult (female mygalomorph spiders, both sexes of Amblypygi (Weygoldt, 2000)).

Fig.3.1. Maternal care in arachnids. Species of the Scorpiones ((A) *Liocheles australasiae* FABRICIUS 1775), Thelyphonida ((B) *Typopeltis crucifer* POCOCK 1894), Schizomida ((C) *Zomus bagnallii* JACKSON 1908) and Amblypygi ((D) *Phrynus marginemaculatus* C.L. KOCH 1840) carry prenymphs of their offspring on the opisthosoma. In the whip-spider *Sarax* sp. also the protonymphs stay on the mother occasionally (E). So-called pulli-carrying behaviour is also found among lycosid spiders ((F) *Hogna radiata* LATREILLE 1817), with the difference, that here the prenymphs stay in the egg sac and settle on the back of their mother as protonymphs. Photographs A., B. and E. by Siegfried Huber, C. by Heiko Bellmann with the kind permission of Tobias Bellmann, D. by Michael Seiter and F. by Arno Grabolle, with kind permission.

As both the developing eggs and the (barely movable) postembryonic stages are at a high risk of predation and parasitism, parental care is widespread among arachnids. Many spiders, harvestmen (males of some Laniatores) and solifuges guard and defend their clutch and young offspring. In some spiders, the mother even self sacrifices and is consumed by its offspring (matryphagy), such as in Eresidae (Schneider, 2002), Amaurobiidae (Kim and Horel, 1998) and Eutichuridae (Toyama, 2001). Mothers of scorpions (Scorpiones, Fig.3.1.A), whip scorpions (Uropygi: Thelyphonida, Fig.3.1.B, and Schizomida, Fig.3.1.C), whip spiders (Amblypygi, Fig.3.1.D) and wolf spiders (Araneae: Lycosidae, Fig.3.1.F) carry their eggs with them and the hatching offspring on the opisthosomal back. This behaviour is called *pulli-carrying* and the stage that attaches to its mother is often called the *pullus* (Canard and Stockmann, 1993).

Scorpions are viviparous: their eggs as well as the first and second postembryo develop within the genital tract of the mother (Lourenço, 2002). 'Birth' occurs during the moult into the prenymph instar, which then actively climbs on the back of the mother. The protonymph leaves the mother's back, but is still closely associated with her. It feeds from the prey captured by the mother (Kästner, 1940).

In whip-scorpions and whip-spiders the carried eggs are attached to the ventral opisthosoma. In whip-scorpions the eggs are surrounded and clotted by a jellylike substance, forming two clusters (pers. obs.). In whip-spiders the eggs are surrounded by a filamentous viscous substance that hardens and forms a protective shell ('egg sac') (Weygoldt, 2000). The prenymph emerges from the egg sac and climbs onto the dorsal opisthosoma of its mother. After the moult into the protonymph it leaves the mother. However, in single cases the protonymph stays on the mother for a longer period of time, as reported for the whip scorpion *Thelyphonus* cf. *caudatus* (Weygoldt and Huber, 2013) and the whip spider *Sarax* spec. (Fig.3.1.E, pers. obs.).

While scorpions and whip scorpions usually stay rather inactive within a burrow during this period of maternal care, whip spiders and spiders maintain their free living lifestyle. In lycosid and trechaleid spiders the eggs develop in a silken cocoon that is attached to the anterior spinnerets by the means of glue-like piriform silk (Townley and Tillinghast, 2003). The postembryo stays within this cocoon and it is the protonymph that climbs onto the back of its mother (Fig.3.1.F). In Trechaleidae and some Lycosidae, the hatching protonymphs (pulli) stay on the cocoon for some time, until they disperse (Dolejš, 2013). This may be an ancestral state of this brood care behaviour, from which pulli-carrying may have evolved, although the author states, that it is an adaptation to humid environments. Thus, pulli-carrying behaviour in wolf spiders differs fundamentally by its time point and mechanism to that of scorpions, whip scorpions and whip spiders.

In pseudoscorpions the mother also carries the first instars, but this mechanism is significantly different from pulli-carrying behaviour: The postembryo does not attach actively, but is embedded into a secretion pad ('brood sac') attached to the ventral side of the opisthosoma (Weygoldt, 1968). During this stage the postembryo absorbs nutrients from the brood sac (Weygoldt, 1968).

Pulli-carrying behaviour includes the demand of active, strong and durable attachment in the prenymph, in high contrast to the subsequent free living instars, which must be able to attach and detach quickly and to environmental substrates. Therefore there should be a radical change in foot morphology, including the development of special prenymphal attachment organs. The scorpion prenymph lacks tarsal claws, but instead bears cushion-like adhesive pads on its feet,

called *arolia* (sing.: *arolium*) (Millot and Vachon, 1949). The thelyphonid prenymph is still in a very embryo-like state and bears large 'suction discs' on its feet (Kästner, 1941). The foot of the amblypygid prenymph is equipped with two tarsal claws and a lobe-like pad, called the *pulvillus*, which is either retained ('Pulvillata') or becomes lost ('Apulvillata') in the subsequent instars (Quintero, 1975). In contrast, the riding protonymphs of wolf spiders do not possess adhesive pads and attach by interlocking theirs claws and spun silk with the hook-like microstructures of specialized opisthosomal setae of the mother: if the mother is shaved, the nymphs are unable to settle on their mother and stay on the egg sac (Rovner et al., 1973). Beside these scattered observations nothing is known about the morphology, function and mechanics of prenymphal attachment organs and the change in pretarsal structures in the protonymph. This work aims to fill this gap by providing morphological, ultrastructural, behavioural and biomechanical data on prenymphs of scorpions, whip scorpions and whip spiders.

3.2. Material and Methods

3.2.1. Animals and behavioural studies

Whip scorpions (Thelyphonidae: *Typopeltis crucifer* POCOCK 1894) were reared with animals originating from Kenting National Park, Taiwan; pulvillate whip spiders (Charontidae: *Charon grayi*) from Negros Island (Phillippines) and apulvillate whip spiders from Hispaniola (Haiti) (Phrynidae: *Phrynus longipes* POCOCK 1894) and Cuba (*P. marginemaculatus* C.L. KOCH 1841 and *P. damonidaensis* QUINTERO 1981). Scorpions were obtained from Cebu Island (Phillippines) (Hormuridae: *Liocheles australasiae* FABRICIUS 1775), Mandalay (Myanmar) (Euscorpiidae: *Scorpiops* sp.), and Southern France (*Euscorpius concinnus*). Animals were kept in standard plastic terraria equipped with a 5 cm thick turf-sand layer, which was kept humid, and pieces of cork bark for hiding at 25-28°C (*E. concinnus* at 20-25°C). All animals were fed with crickets (*Acheta domestica*).

For observations of attachment behavior scorpion prenymphs were placed on glass cover slips, which were then turned upside down. Attachment and movement of the prenymph were photo- or video-documented using an EOS 600D SLR (Canon Inc., Tokio, Japan) equipped with a macro lens and an extension tube. For investigation of arolium movements, contact area and the deposition of fluid secretions prenymphs were placed horizontally and vertically on cover slides and studied with the means of an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany) with a mounted B/W high speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA) and operated in phase contrast (transmission light) or reflection interference contrast mode (coaxial light). Light microscopical images of animals and their feet were made with a multifocus stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420).

3.2.2. Scanning electron microscopy

Standard SEM: Prenymphs, protonymphs and adult females were conserved and stored in 70% ethanol. For scanning electron microscopy the specimens or parts of them (legs of pre-/protonymphs, and cut off pieces of the dorsal opisthosomal cuticle of mother animals) were dehydrated in a series of increasing ethanol concentration (80%, 90%, 100% and 100% on molecular sieve), followed by critical point drying. 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., Tokio, Japan) at an acceleration voltage of 3.0 kV.

Cryo-SEM: Living prenymphs 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 equipped with the stage cooled up to -120°C. The feet of some prenymphs were slightly touched with a scalpel mounted in the prechamber in order to produce freeze fractures.

Histological sections: Prenymphs were anaesthetized with carbon dioxide and legs were clipped off using micro scissors. Legs were immediately fixed with gluteraldehyde and osmium tetroxide, dehydrated in a series of increasing ethanol concentration and mounted in Epon. Epon blocks were trimmed with a Leica EM TRIM2 and sections of 1 and 1.5 µm were made using a Diatome MC2391 diamond knife (Diatome AG, Biel, Switzerland) and a Leica EM UC7 microtome to about a half of the embedded specimen foot. The rest was cut from the block with a fine circular saw mounted onto a drilling machine. Some sections were placed on super frost glass slides for light microscopical investigations, the others were placed on cover slides, previously cleaned with absolute ethanol and 70% acetone and bathed in pioloform solution. Sections were bathed for 45 min in a concentrated KOH solution in two parts methanol and 1 part propylene oxide (Maxwell's solution (Maxwell, 1978)), the half cut block samples for 1.5 h. Sections were air dried, the clock samples put in absolute ethanol and critical point dried. Following treatment and microscopy as described above (standard SEM).

3.2.3. Micro-computed tomography

Legs of pre- and protonymphs, stored in 70% ethanol, were dehydrated in a series of increasing ethanol concentration and critical point dried. Dried samples were glued onto plastic pipette tips with cyanacrylate glue and scanned with a SkyScan 1172 HR micro-CT (Bruker microCT, Kontich, Belgium) with an acceleration voltage of 40 kV and a voxel size of 0.53 µm. 3D images were reconstructed using NRecon 1.6.6. software and processed with Amira 5.4.3.

3.2.4. Adhesion measurements

Adhesive forces to smooth glass slides were measured in two scorpion prenymphs (*L. australasiae*). Using non-toxic two compound Polyvinylsiloxane (PVS) silicone elastomer the animals were glued onto a thin wire mounted onto the cantilever of a micro force transducer (FORT-10 with 10 g force range, World Precision Instruments Inc., Sarasota, FL, USA). The force transducer was moved vertically by a micromanipulator (DC3001R with controller MS314, World Precision Instruments Inc.) at a speed of 0.2 mm s^{-1}. The signal of the force transducer was amplified and processed by a Biopac MP-100 acquisition system (Biopac Systems Ltd, Goleta, CA, USA). Force curves were recorded using the AcqKnowledge 3.7.0 software (Biopac Systems Ltd). Peak forces were divided by animal weight (measured with an AG 204 Delta Range scale, Mettler Toledo GmbH, Greifensee, Switzerland) to calculate the safety factor (sf) and by the sum of contact areas of all legs in contact measured from coaxial light micrographs to calculate the adhesive strength.

3.3. Results

The smooth adhesive foot pads studied here are derived from membranous parts of the pretarsus. In arachnids no consistent terminology is available for pretarsal structures, thus we follow the definitions established in entomology by Holway (1935) and Dashman (1953) and apply it to the structures observed, as below.

Pretarsus: The pretarsus is the most distal leg segment which is controlled by muscles. In insects there is only a claw depressor muscle whose tendon inserts on a ventral sclerite, which bears the claws (the *unguitractor plate*). In arachnids the (synapomorphic) pretarsus is reduced to a thick ring- or bow-like sclerite, controlled by a ventral *depressor (protractor)* and a dorsal *levator (retractor)* muscle (De Meijere, 1901).

Arolium: Median un-paired lobe- or cushion-like attachment organs situated at the pretarsus are called *arolia* (sing.: *arolium*). The term *pulvillus* (pl.: *pulvilli*) used for the median pad of amblypygids is misleading as this term usually refers to paired lobe-like attachment organs underneath the claws.

Condyle: The *condyle* is an inner bulge of the tarsus that articulates with a bowl-like cavity of the pretarsus.

Empodium: The *empodium* is an outgrowth of the *unguitractor*. It can be claw-, bristle- or pad-like. We use this term for an unpaired sclerotized protuberance of the ventral pretarsus, usually with the shape of a little claw.

Unguis: The pretarsus usually bears two large claws, which are traditionally called *unguis* (pl.: *ungues*). They are not fused with the pretarsus sclerite but articulated by flexible membranes (*unguial membrane*). The membrane might be attached to a pin-like sclerite in the pro-ventral site, connecting the distal unguis basis and the lateral pretarsus (*unguial sclerite*).

Pretarsal membrane: The pretarsus is attached to the tarsus by a large dorsal and a small ventral membrane. The dorsal membrane is partially fused with the unguial membrane and may extend to the median pretarsal parts.

3.3.1. Scorpiones

The pretarsus of scorpion prenymphs is present as two lateral bow-like sclerites that are slightly sclerotized and fused with the tarsus. These were already found previously (Millot and Vachon, 1949) and called *'épaissemens chitineux'* (sclerotized bulge). Between the pretarsal sclerites, a large membrane, which is distally expanded and bubble-like, forms the arolium (Fig.3.2.B,D). In the ventral part of this cushion the cuticle is thickened and possesses a fibrillar structure, including arrays of aligned fibrils, running parallel at a steep angle towards the epicuticle. At the basis, between the pretarsal sclerites a depressor tendon attaches. In the upper two thirds of the dorsal aroliar membrane the retractor tendon is attached (Fig.3.3.H). It is much thicker than the protractor tendon, which is hard to find at all. The attachment site of the tendon can be recognized externally as a funnel-like invagination of the cuticle (Fig.3.2.D arrowhead, Fig.3.3.G). Claws are lacking totally. A few setae are present on the distal tarsus that may serve as mechanoreceptors for the sensory control of attachment. The developing cuticle of the protonymph is already visible within the tarsus, including the large ungues (Fig.3.3.G,H).

The distal prenymph leg lacks pigmentation and therefore the action of the inner structures during movement could be easily observed. Pad detachment is induced by a pull of the retractor tendon, which leads to an invagination of the arolium and a circular shrinkage of the contact area

to the substrate (Fig.3.3.D). No action of the protractor tendon could be observed. Instead, a protraction of the arolium was induced by the relaxation of the retractor muscle and a passive inflation due to internal hemolymph pressure. When a foot was detached, a small film of fluid was left behind (Fig.3.3.F). The fluid layer quickly shrank to droplets on the (hydrophilic) glass and did not evaporate, indicating a lipid-based composition.

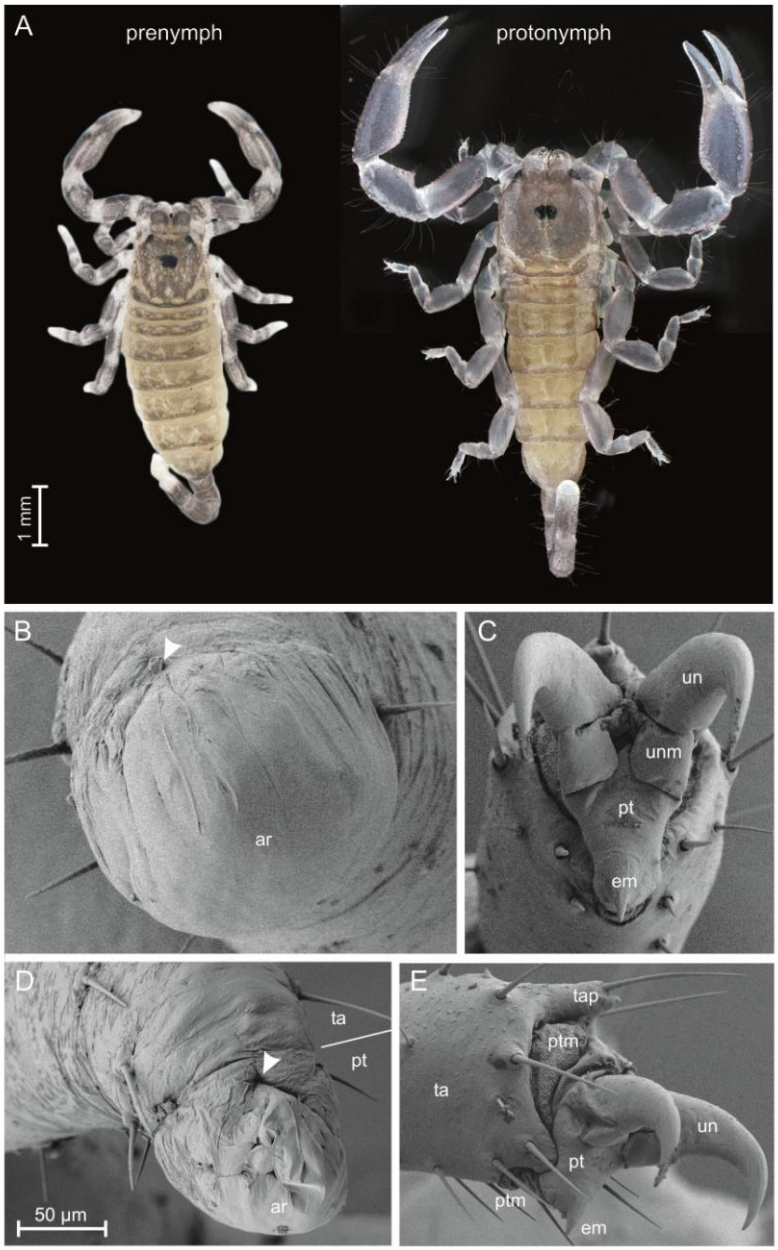

Fig.3.2. Pretarsal metamorphosis in *Liocheles australasiae* (Scorpiones). Although the prenymph is less sclerotized and many cuticular structures are not fully developed yet, it is rather similar in morphology and pigmentation, if compared with the protonymph (A). However, the morphology of the pretarsi (pt) of walking legs differs fundamentally: In the prenymphs (Cryo SEM micrographs B and D) it is a bubble-like cushion (ar, arolium) with a thin, flexible membrane, which can be invaginated by the pretarsal levator muscle (arrowheads point to the tendon attachment site). After the moulting (SEM micrographs C and E) the pretarsus resembles that of an adult scorpion with two claws (un, unguis) connected to the pretarsal plate (pt) by flexible membranes (unm). The pretarsus bears a small median claw (em, empodium) and is connected to the tarsus (ta) by two joint membranes (ptm) with nubby surfaces. Its movement is controlled by two muscles, the depressor and the levator.

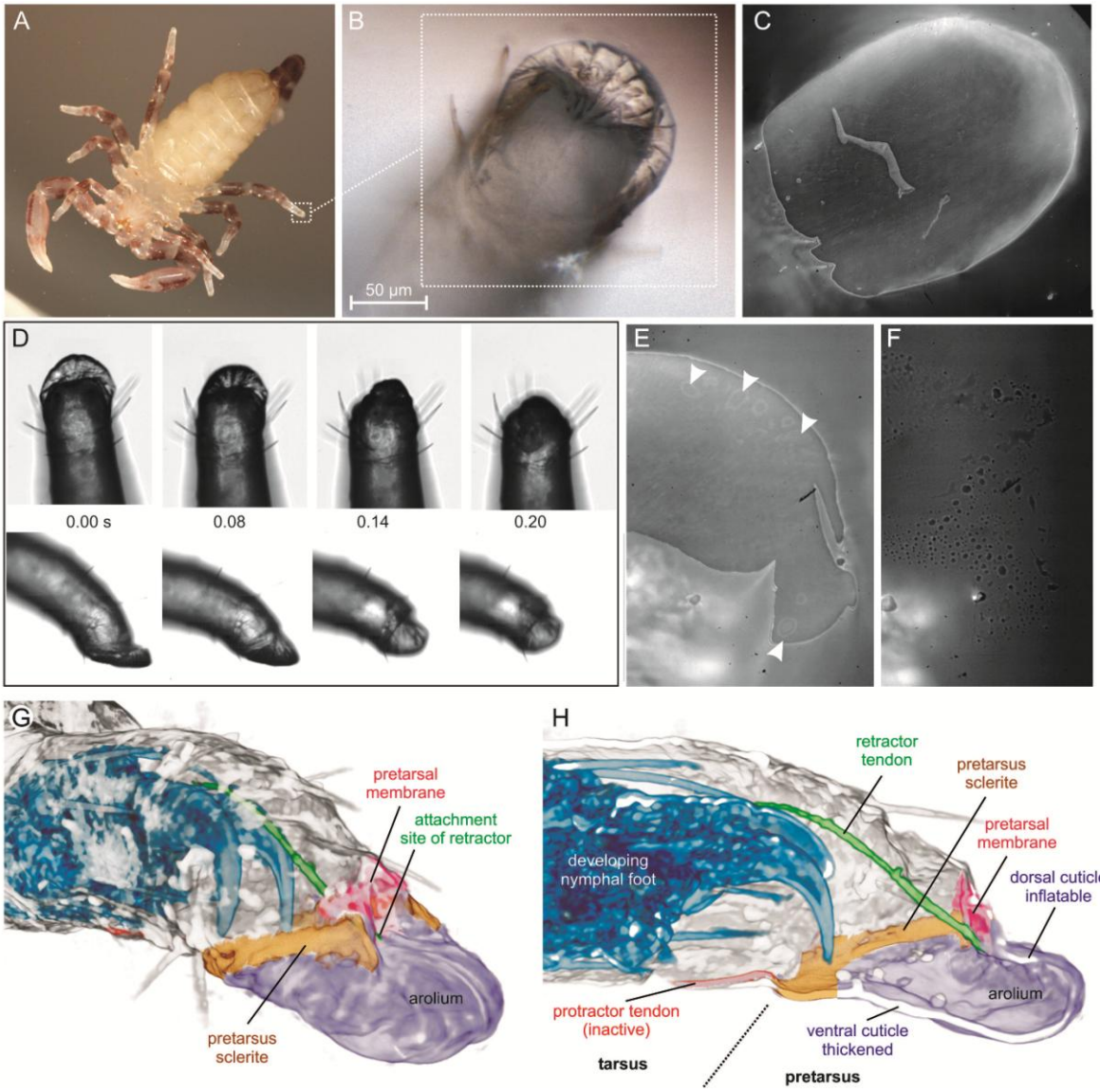

Fig.3.3. Functional morphology of the scorpion prenymph foot. The large arolia help the prenymph cling to its mother. It can easily walk upside down on a glass slide (A). The arolium is pressed flat against the substrate (B.). Images C, E and F show reflection interference contrast microscopical (RICM) snapshots, visualizing the oval contact area of the arolium (C) and fluid secretion (arrowheads in E, and footprint left after foot detachment in F, similar area). Foot detachment is achieved by the pull of the dorsal retractor tendon, leading to partial invagination of the arolium (high speed video frame sequences D, above - dorsal view, below - lateral view). Images G and H show 3D reconstructions of the foot based on micro-computed tomographical imaging: pro-lateral view (G) and lateral view (H) with transverse section.

Scorpion prenymphs could easily hold their body weight when placed upside down on a glass slide, even when moving forward (Fig.3.3.A). During movement the foot detachment was relatively slow (about half a second) and seemed to demand a considerable force. We measured the force necessary to pull off the attached prenymph from smooth glass slides in two *L. australasiae* prenymphs. The attachment forces reached 0.66 ± 0.26 mN (n=23) (mean ± standart deviation) in individual 1 (8.0 mg) and 0.47 ± 0.27 mN (n=21) in individual 2 (7.3 mg), corresponding to a safety factor of 8.4 ± 3.4 and 6.6 ± 3.7, respectively, and an adhesive strength of 15.8 ± 2.3 kPa and 9.3 ± 1.8 kPa.

The protonymph pretarsus is radically different from the prenymph one (Fig.3.2.C,E). The arolium is absent and median pretarsal membranes are reduced. Instead, the pretarsus is heavily

sclerotized in the lateral and the ventromedian parts. The latter one bears a small hook-like empodium. The pretarsus is articulated with tarsus condyles in the lower part. It is controlled by both protractor and retractor tendons, which are developed to a similar extent. The retractor tendon branches distally, with one part attaching on the pretarsal sclerite, and the other on the claw base. The claws are proximally articulated with the pretarsus and at a more distal site by a pin-like sclerite (*unguial sclerite*). The distal tarsus bears a median *dorsal processus*, which protrudes between the claws, when the pretarsus is retracted.

We did not find differences in foot morphology between the three scorpion species studied.

3.3.2. Thelyphonida

The prenymphs of *T. crucifer* were totally immobile when studied (about three days after moulting), although they were still alive and moulted into the protonymph after some weeks. When detached, it keeps its legs in a flexed state, with a ventrally bent body and legs. This may lead to a passive cramping to the body of the mother (Fig.3.4.A). The tarsus bears a pair of (presumably) mechanosensory setae, which are directed ventrally, and the large arolium at the distal tip (Fig.3.4.C). The pretarsal sclerite is not developed at all. A retractor tendon is attached to the dorsal cuticle of the arolium. The much thinner protractor tendon attaches to the ventral cuticle of the arolium. Ventrally, the arolium is delimitated from the tarsus by a small trench. A blunt tarsal processus slightly protrudes above the arolium.

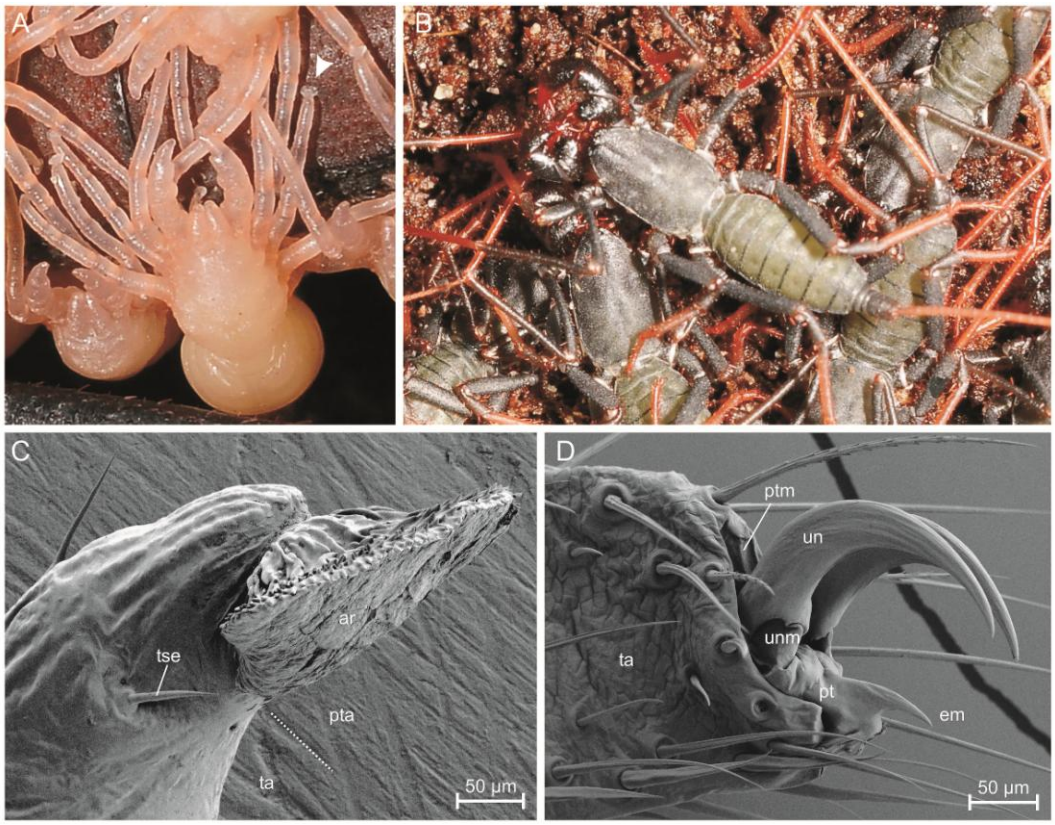

Fig.3.4. Pretarsal metamorphosis in *Typopeltis crucifer* (Uropygi). The whip-scorpion prenymph (A) is still in a embryonic state and immobile most of the time. Its feet bear large arolia (ar) (C and arrowhead in A) and a pair of tactile setae (tse) controlling ground contact. The protonymph (B) is free-living and self feeding and resembles the adult animal. Its feet (D) bear two large (un, unguis) and a small median claw (em, empodium). The latter is an outgrowth of the pretarsal sclerite (pt). The ungues are flexibly attached to the pretarsal sclerite by thin cuticle (unm, unguial membrane). The pretarsus articulates against the tarsus (ta), connected by the joint cuticle (ptm, pretarsal membrane).

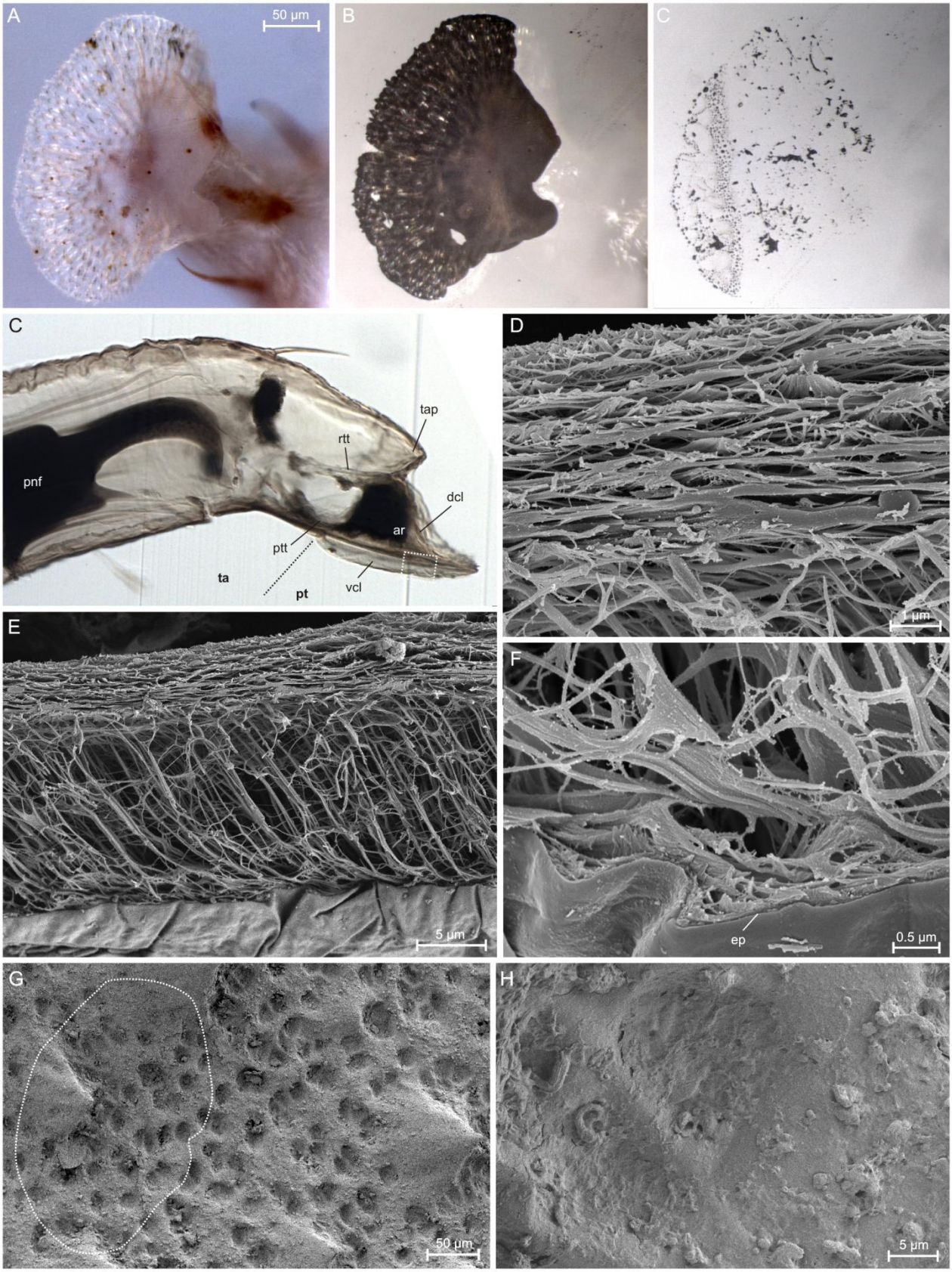

Fig.3.5. Functional morphology and ultrastructure of the whip-scorpion prenymph foot. A single foot (passively) attached to a glass slide (A.) holds the body weight of the animal (B. contact area of the same, visualized by coaxial illumination). After detachment, remains of lipid- and mucous-like secretions are visible on the glass slide, with highest concentration on the former borders. Image C shows an osmium tetroxide stained and Epon-embedded prenymph foot, transversally cut. The developing protonymph foot (pnf) is visible. Above the arolium (ar) a short tarsal processus (tap) protrudes. The pretarsus, which just consists of a large arolium, is controlled by the dorsal ...

The dorsal cuticle of the arolium is rigid, dense and forms denticulate protuberances. The ventral cuticle is highly thickened and sponge-like (Fig.3.5.E). The thickening shows a smooth transition from the thin and dense cuticle of the ventral tarsus. It is already present before the trench between the tarsus and pretarsus and reaches the highest thickness in the median portion of the arolium (about 20 µm). Its inner part consists of several layers of fibres that are only loosely connected (Fig.3.5.D). The median part is the thickest and the less dense one, and consists of parallel fibres running at an angle of 60-80° towards the outer cuticle (Fig.3.5.E). The fibres are partly connected by crossing fibrils and branches close to the epicuticle. The terminal epicuticle is very dense and only 10-20 nm thick (Fig.3.5.F). It is very compliant, indicated by occasional infolds, which are very local and lead to slight deformations of the underlying fibrils.

Due to the flexed state of the prenymph's legs, it could not be placed onto the surface of a planar glass slide. Therefore, we slightly touched an arolium with a thin, cleaned glass rod, which lead to immediate attachment. The whole animal could hang on the arolium of one leg. When touching other parts of the animal, including the dorsal side of the arolium, no attachment took place. A foot placed near the edge of a glass slide (such that the animal could hang down at the side) lead to attachment (Fig.3.5.A,B). When pulled off, a lipid footprint was left behind, with the largest amount of fluid present at the margins of the previous contact area (Fig.3.5.C).

The structure of the dorsal opisthosomal cuticle of an adult female was found not to be smooth, but highly corrugated, including deep dimples, denticles, short bristles and a crust of a hardened secretion (Fig.3.5.G,H).

The pretarsus of the protonymph is basically similar to that of scorpions (Fig.3.4.D). The claws and the empodium are larger and more pointed. The pretarsal sclerite is more massive than that of scorpions. The protractor tendon runs through a cuticular sheath in the distal part of the tarsus. The tarsal processus is lost in the protonymph.

3.3.3. Amblypygi

The prenymphs of both pulvillate (*C. grayi*; Fig.3.6.A,B) and apulvillate (*P. marginemaculatus*; Fig.3.6.D) species bear pretarsi of similar morphology. The pretarsus already includes most structures of the nymphal one. The pretarsal sclerite is of a U-like shape with the tips of the lateral flanks bearing the fully developed ungues, connected by the unguial membrane and unguial sclerite. Between the flanks the arolium emerges as a median lobe-like extension of the dorsal pretarsus membrane. At its dorsal membrane the retractor tendon is attached. Here the dorsal aroliar membrane is slightly infolded. The protractor tendon is attached to the ventral part of the pretarsus sclerite, which is slightly thickened and protruded. The pretarsus articulates slightly above the attachment site of the protractor tendon with the condyles of the tarsus. The condyles lay underneath a denticle-like protrusion of the tarsal margin. The tarsus is equipped with long setae and on its ventral side with stiff pointed bristles. It is dorsally subdivided by a

... retractor tendon (rtt). The ventral protractor tendon (ptt) is presumably inactive. The arolium has a rigid dorsal and a highly thickened, sponge-like ventral cuticle (frame marks detail view shown in E). In the inner part of the ventral cuticle staples of filamentous layers occur, that are only loosely attached to each other (D). In the thicker outer part the filaments run in parallel towards the ultra-thin but dense epicuticle (ep) (E and F). The configuration leads to high compliancy, necessary to generate a sufficient contact area on the highly corrugated cuticle of the mother animal (G, dotted frame marks size of a prenymph arolium; H shows detail).

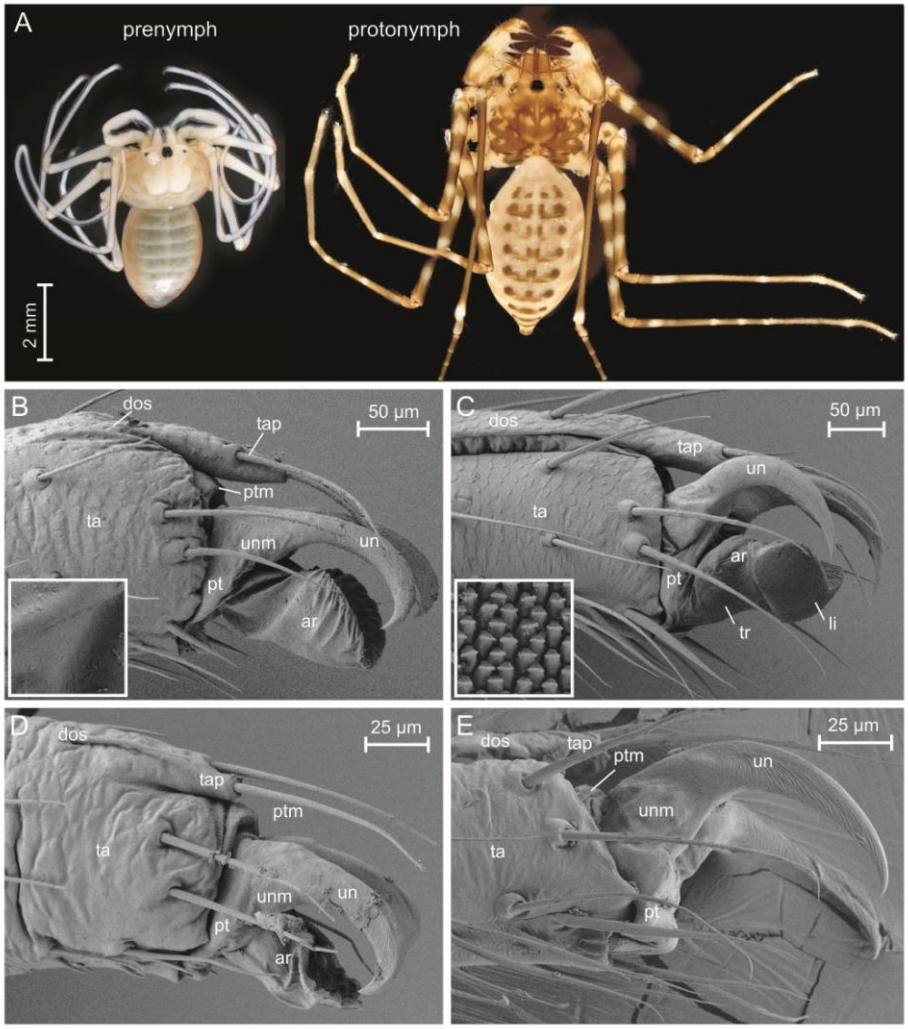

Fig.3.6. Pretarsal metamorphosis in whip-spiders (Amblypygi). In whip-spiders the prenymph (A left) still bears all features of the protonymph (A right) in its feet, including the large claws (un), based flexibly on the pretarsus sclerite (pt) by membranes (unm), and the dorsally separated tarsal sclerite (dos) with the protruding tarsal processus (tap). The arolium (ar) is present in prenymphs of all taxa (B *Charon grayi*; D *Phrynus marginemaculatus*). It is retained in the subsequent instars of the basal lineages (C protonymph of *C. grayi*), but acquires a more elaborate structure, including the division into a stiff truncus (tr) and a compliant lip (li). While its contacting surface is smooth in the prenymph, it exhibits hexagonal outgrowths bearing spatula-shaped structures in the following instars (compare details in the insets, same magnification). However, in most whip-spider species the arolium becomes lost in the protonymph (E *P. damonidaensis*). In its place, there is only a stiff membrane spanned between the claws and the protruding ventral part of the pretarsal sclerite.

transverse membrane, which separates a dorsal sclerite that distally bears a long tarsal processus equipped with long setae.

The ventral cuticle of the arolium is not thickened like in the scorpion and thelyphonid prenymphs, and does not contain perpendicular fibres. However, it is of similarly low density and consists of a small number of cuticular layers that are only loosely connected. Its epicuticle has a smooth surface (Fig.3.6.B inset). The dorsal cuticle is denser and forms nubs, which are typical for joint membranes in most arachnids (pers. obs.). Besides the slight thickening of the dorsal and marginal cuticle the arolium does not include hardened structures and thus it is often deformed in dried specimens. We did no experimental investigations with living amblypygid prenymphs.

The protonymph pretarsus of the apulvillate *P. damonidaensis* is basically similar to that of the *P. marginemaculatus* prenymph, however, with the most important difference that it lacks the

arolium (Fig.3.6.E). The pretarsus is more massive and exhibits a median sclerite that slightly protrudes between the claws. An empodium is lacking. The claws are much larger than in the prenymph. In protonymphs of the pulvillate *C. grayi,* the arolium is still present, but exhibits a more elaborate structure (Fig.3.6.C). It is subdivided into a stiff truncus and a broad bulged lip. The ventral cuticle of the truncus is dense and rigid and bears short hook-like microtrichia. The dorsal cuticle is also dense, but half as thick and with a nubby surface. The dorsal membrane is attached to a T-shaped median sclerite that is fused to the lateral flanks of the pretarsus sclerite and connected with the retractor tendon. In the lip, the ventral cuticle is thickened like in the thelyphonid arolium and forms thick longitudinal ribs, from which branches emerge, that narrow, and again branch towards the epicuticle. The epicuticle forms hexagonal shaped protuberances bearing spatula-like tips (Fig.3.6.C inset). The complex structure and function of the amblypygid arolium is presented in detail in another study (***chapter 4***).

The foot anatomy of taxa studied is comparatively illustrated in Fig.3.7 (based on our results of both micro computed tomographical and light microscopical analysis).

3.4. Discussion

The cuticle of arthropods is a composite material consisting of aligned chitin-fibres connected by a proteinous matrix, which may include additional compounds (Vincent and Wegst, 2004, Krishnakumaran, 1961). It is composed of layers which differ by the orientation and density of chitin fibres and the composition of the matrix. The outermost layer is a very thin and dense epicuticle made of proteins and lipids. A common phenomenon in smooth adhesive pads is the reduction of the matrix in the outer cuticle (Gorb et al., 2000). This leads to regular arrays of fibrils (often branching towards the epicuticle) that permit a high and very local deformation of the epicuticle by maintaining an overall stability and strength of the cuticle (Gorb et al., 2000). Thus the epicuticle can closely adapt to rough surfaces (like the cuticle of the mother animal) and generate a high contact area. The continuous secretion of a thin fluid film serves as a bonding agent between pad and substrate and produces high capillary and viscous adhesive forces. This principle seems to be rather simple from a developmental point of view as it has evolved numerous times in insects (Gorb and Beutel, 2001). Arachnid prenymphs are barely equipped with cuticular structures, such as setae, as they are in a relatively early state of postembryonic development. Thus it might not be surprising to find simple smooth adhesive pads with rectangular chitin fibre arrays in the ventral cuticle in both scorpions and thelyphonids. Lipid footprints were found, which is consistent with their occurrence in smooth attachment pads of solifuges and mites (Peattie et al., 2011).

From the three arachnid orders investigated here, scorpions have traditionally considered as the most basal one (Fig.3.8) (Shultz, 1990, Wheeler and Hayashi, 1998, Regier et al., 2010). The pad of their prenymphs might resemble the simplest (but not necessarily ancestral) type of attachment organs in arthropods, a soft membranous extension filled with fluid. The attachment-detachment mechanism is relatively slow and might not be useful for the free-living instars that must be able to move suddenly and quickly, i.e. to capture prey or to escape from a predator. From the ontogenetic point of view the arolium is an expansion of the pretarsal membranes and the lateral bows might resemble the pretarsus. In the scorpion arolium the contact area with the

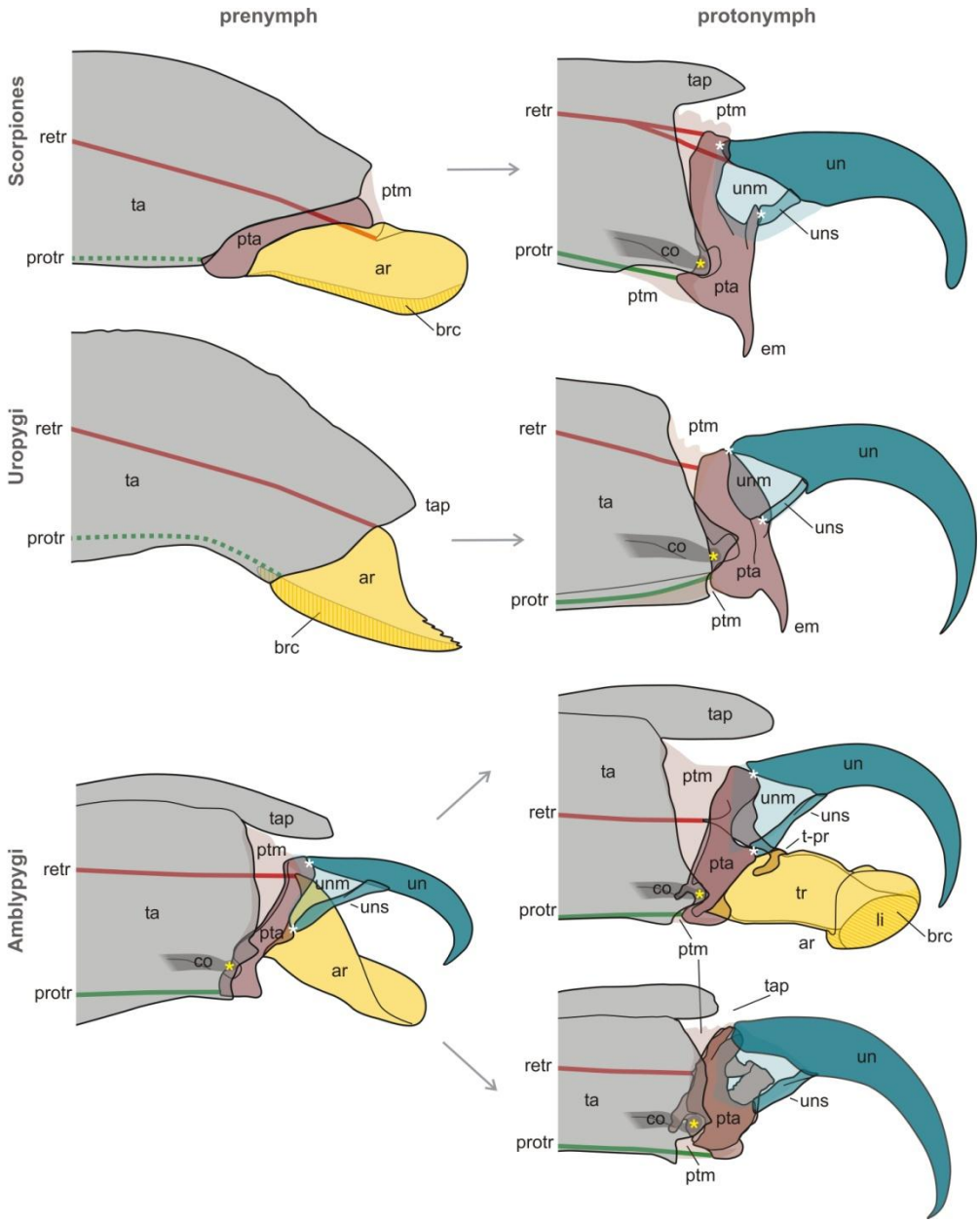

Fig.3.7. Schematic illustration of comparative foot morphology in the arachnid taxa studied. Based on the light microscopical and micro-computed tomographical analysis. ar, arolium; brc, thickened cuticle with branching filaments; co, condyle; em, empodium; li, arolium lip; pta, pretarsus; ptm, pretarsus-tarsus-membrane; pro, protractor tendon; retr, retractor tendon; ta, tarsus; tap, tarsal processus; t-pr, pretarsal t-shaped processus; tr, arolium truncus; un, unguis (claw); unm, unguial membrane; uns, unguial sclerite. Yellow asterisks mark pivot point of pretarsus (vertical movement), whereas white asterisks mark pivot of claws (lateral movement).

substrate is uniquely controlled by the antagonistic interaction between internal hydro pressure and a retractor muscle. Recent experiments with air pumped elastic membranes analogously demonstrate how internal pressure can effectively be used to control adhesion (Dening et al., 2014). The ventral protractor tendon seems not to play a role in the movement, and we could not verify whether the protractor muscle is developed at all.

The thelyphonid prenymph is the most embryo-like and sclerotization is lacking in any part of the pretarsus. The only elaborate cuticular structures of the prenymph are connected with the attachment function: the paired ground receptor and the large arolium. This instar is probably only mobile shortly after leaving the egg sac and then remains in an immobile pre-moulting state for a

long period. This is consistent with our observation that the arolium is very sticky without any action of the animal. The arolium is not a sucker, as suggested by earlier authors (Kästner, 1941), but attaches by the compliancy of the fibrillar cuticle and the capillary forces of a secreted lipid film. Whether prenymphs of Schizomida (micro whip-scorpions) bear similar adhesive organs remains unknown, as no material was available for this group (and breeding attempts failed).

Finally, the amblypygid prenymphs are in the most advanced state and are equipped with comparably small arolia with low fibrillation of the ventral cuticle. The claws might significantly contribute to the attachment to the mother's cuticle, which is highly corrugated in these animals. Interestingly, the arolium is retained in the subsequent instars in the basal amblypygid lineages. The pad, however, is much more elaborate in the nymphs and adults, and produces a high adhesive strength (***chapter 4***), assisting locomotion on vertical and upside down substrates (Weygoldt, 2000). Many whip-spiders live on vertical or overhanging substrates, such as cave walls or rocks, and barely move on the ground. This might explain why this organ remains functional also in adults.

These observations indicate that, at least in whip spiders, pulli-carrying behaviour has been the primary selective pressure for the evolution of arolia in these groups. Its loss in the nymphs of the more basal scorpions and whip scorpions is related to the epi- and hypogaeic lifestyle of those taxa. The reason for arolium loss in apulvillate amblypygids is ambiguous, as these show no obvious differences to the pulvillate ones in climbing behaviour and preferred microhabitats.

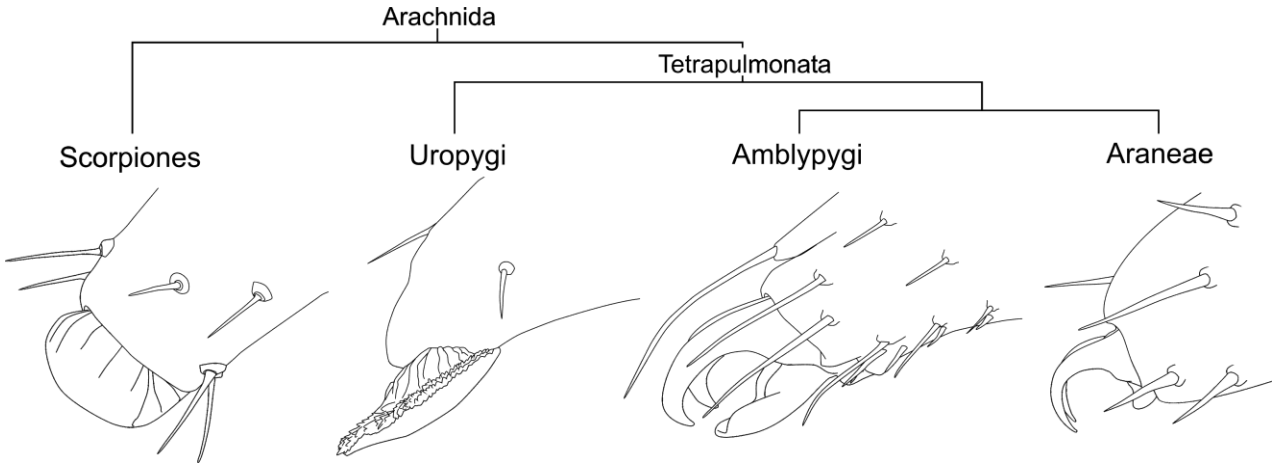

Fig.3.8. Phylogenetic relationships and schematic illustration of the prenymph foot in pulli-carrying arachnids. Phylogenetic tree modified after (Wheeler and Hayashi, 1998, Shultz, 1990, Regier et al., 2010). Please consider that the relationships to the other arachnids orders are not displayed (the exact position of Scorpiones is controversial, but it is consensus that it is not nested within the Tetrapulmonata), hence the homology of pulli-carrying behavior and the arolia is uncertain. The position of Amblypygi as the sister group of Araneae is debatable, but supported by our analysis, showing an advanced state of the pretarsus in both Amblypygi and Araneae. Spider (Araneae) prenymph foot after (Canard et al., 1988).

For the sake of completeness, it should be mentioned that juvenile adhesive pads also occur in harvestmen (Opiliones), yet with a totally different function. Nymphs of Laniatores harvestmen (with the exception of some ancient lineages) possess expandable pads (arolia) on the two posterior leg pairs (Roewer, 1935, Gnaspini, 2007). The arolia are not developed yet in the prenymph and become lost in the subadult instar. They are assumed to play their major role in moulting: Nymphs attach upside down on smooth surfaces during this process, which presumably

reduces the risk of predation (Juberthie, 1972). Further, in hunting spiders, which possess the hairy adhesive foot pads (claw tufts), those are present from the nymphal instars on and are lacking in prenymphs (Hill, 1977, Canard et al., 1988). This is reflected in the habit of staying within the egg sac, until moulting to the protonymph.

Conclusion

The presented data on prenymphal adhesive pads increase our knowledge on postembryonic development in arachnids and might contribute to our understanding of adhesive pad evolution in this successful group of terrestrial arthropods. The semi-hydraulic adhesion control revealed in scorpion prenymphal arolia might be of interest for engineering approaches seeking precise and low-impact attachment-detachment devices in robotics and manufacturing systems.

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4. Paper no.2:

Functional anatomy of the pretarsus in whip spiders (Arachnida, Amblypygi)

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Abstract

Whip spiders (Amblypygi) are a small, cryptic order of arachnids mainly distributed in the tropics. Some basal lineages (families Charinidae and Charontidae) have large adhesive pads on the feet of their six walking legs. The present study describes the macro- and ultrastructure of these pads and investigates their contact mechanics and adhesive strength on smooth and rough substrates. Furthermore, the structure of the pretarsus and its kinematics are compared in *Charon* cf. *grayi* (with an adhesive pad) and *Phrynus longipes* (without an adhesive pad). Results show an elaborate structure of the adhesive pad, exhibiting a unique combination of morphological features including a long transversal contact zone performing lateral detachment, a thick internally-branched cuticle with longitudinal ribs and hexagonal surface microstructures with spatulate keels. The contact area of the pad on smooth glass is discontinuous due to the spatulate microstructures leading to specific discontinuous detachment, which could be observed *in vivo* by means of high speed videography at a rate of up to 10 000 fps. Adhesive strength was measured with vertical whole animal pull-off tests, obtaining mean values between 55 and 200 kPa. The emergence of viscous lipid secretions between microstructures was occasionally observed, which, however, seems not to be a necessity for a good foothold. The results are discussed in relation to the whip spider's ecology and evolution. Structure-function relationships of the adhesive pads are compared to those of insects and vertebrates.

4.1. Introduction

Whip spiders (Amblypygi) are a small, cryptic order of arachnids that are distributed in tropical and partly sub-tropical regions. They are closely related to spiders (Araneae) and exhibit several unique characters: The front legs are highly modified to extremely elongated, thin, sensors-equipped antenniform structures (Foelix and Hebets, 2001, Igelmund, 1987). Due to the great length of the antenniform front legs and the high amount of sensory input, signals are transported by giant neurons that permit sufficient propagation speed (Igelmund and Wendler, 1991). The pretarsus is reduced. Three unique outgrowths at the tip are innervated by several dendrites and were hypothesized to be the derived tarsal claws (Foelix and Hebets, 2001). The pedipalps are the primary means for prey capture. They are massive and equipped with thick spines and can be rather long. The pedipalp patella can be flexed against the femur, providing a strong hold of a struggling prey, which is dismembered by the orthogonal chelicerae. The body is highly flattened and inconspicuously coloured. Accordingly the three pairs of walking legs are latigrade as in running crab spiders (Araneae: Sparassidae). This enables the animal to squeeze into narrow crevices in rocks or tree bark where it rests during the day (Weygoldt, 2000). The walking legs are relatively long and slender. The patella is rather short and barely movable against the tibia. The patella-tibia joint, however, provides a breaking site for leg autotomy (Weygoldt, 2000). Tibia and tarsus are divided into sub-segments, which may provide elastic energy storage during locomotion as known from opiliones (Sensenig and Shultz, 2006). The disti-tarsus is uniquely longitudinally sub-divided, thus exhibiting a dorsal sclerite with a long distal protuberance (*tarsal processus*) bearing three long bristles. The pretarsus bears two smooth claws strongly curved ventrally and articulated by a membraneous base. Furthermore, there is a median lobe-like pad underneath the claws, called the *pulvillus* (Quintero, 1975). This is present in the barely moving and non-feeding prenymphal stage carried by the mother and helps it to attach to her (Weygoldt, 1970). The pulvillus is retained in the subsequent instars in the basal lineages of amblypygids, including the families Paracharontidae (genus *Paracharon*), Charontidae (genera *Charon* and *Stygophrynus*) and Charinidae (*Charinus* and *Sarax*) (Weygoldt, 2000, Dunlop, 2000) (Fig.4.1). This character has been classically used for the delimitation of the infra-orders Pulvillata and Apulvillata, which, however, are obsolete because of the paraphyly of Pulvillata found in cladistic analysis (Weygoldt, 1996). The adult pulvillus is thus the plesiomorphic character, whereas the lack of which must be regarded as an apomorphic state (Fig.4.1). The pulvillus permits a foothold onto smooth surfaces where claw attachment fails and enables those species to climb steep smooth surfaces such as the glass panes of a terrarium (Weygoldt, 2000).

Beside this rough description there is no other information, neither on the anatomy and kinematics of the amblypygid pretarsus nor the structure and adhesive function of the pulvillus. As the pretarsus is often the most important part of the leg in regard to substrate attachment, the associated structures are very important adaptive features, functionally acting at the interface between an animal and its environment. Presence or absence as well as differences in morphology and/or mechanical properties of foot structures may have a great influence on microhabitat access and niche occupation. Furthermore, biological attachment devices can be a valuable source of inspiration for technical solutions in a broad band of technical applications (Gorb, 2008). Keeping all this in mind, amblypygids are a very interesting study system for the following reasons. (1) They are morphologically highly-conserved and show no great differences in body and leg shapes as well as their proportions except for the presence or absence of the adhesive pad. Thus,

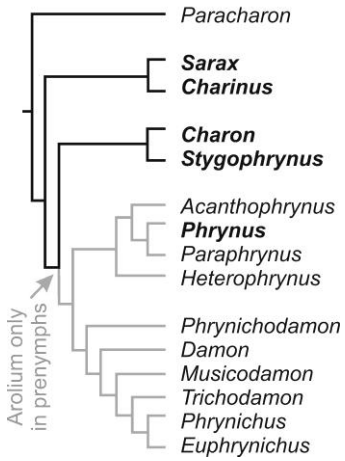


Fig.4.1. Cladogram of extant whip spider genera. Black lines mark the lineages which exhibit an arolium in all instars, grey lines, those in which it gets lost in the protonymph. Genera of species used in this study printed in bold font. Adapted after (Weygoldt, 1996).

they provide a unique opportunity to comparatively study the functional benefit or cost of an adhesive foot pad without experimental manipulation. (2) Whip spiders can reach significant sizes. The largest pulvillate species belongs to the genus *Charon* and reaches body lengths of 4 cm and a weight up to 4 g. As the feet are relatively small and only three leg pairs are involved in locomotion the pads must be rather efficient to hold the body of a whip spider onto a smooth glass slide. (3) Whip spiders can move very quickly. Attachment and detachment must thus happen very quickly. (4) The pad exhibits a unique mixture of characters of smooth and hairy adhesive pads, which will be described in this paper.

The aim of this study is thus to comparatively study the functional morphology of the pretarsus in pulvillate and apulvillate species of whip spiders and provide detailed experimental information on the adhesive mechanism of the pulvillus.

4.2. Material and Methods

4.2.1. Animals

The animals used in this study are bred from animals caught in the wild. Living individuals of the following species were included: Charinidae: *Charinus cubensis* QUINTERO 1983 from Cuba, *Sarax brachydactylus* SIMON 1892 from Phillippines; Charontidae: *Charon* cf. *grayi* GERVAIS 1842 from Phillippines (different populations from Luzon Island, unclear species status, see (Weygoldt, 2002)) (Fig.4.2.e,h); Phrynidae: *Phrynus longipes* POCOCK 1894 from Hispaniola (Samana, Dominican Republic). Animals were kept in standard plastic terraria equipped with a 5 cm thick turf-sand layer, which was kept humid, and pieces of cork bark for hiding at 25-28°C. All animals were fed with crickets (*Acheta domestica*).

Additional ethanol material from the following collections was included: Senckenberg Museum of Natural History Frankfurt, arachnological collection: Charinidae: *Charinus neocaledonicus* KRAEPELIN 1895 from New Caledonia. M. Seiter personal collection: Charontidae: *Stygophrynus* sp. from Sulawesi; Phrynidae: *Phrynus marginemaculatus* C.L. KOCH 1841 and *P. damonidaensis* QUINTERO 1981 from Cuba (Fig.4.2.l). Zoological Museum Greifswald, arachnological collection: Charinidae: *Sarax sarawakensis* THORELL 1888 from Indonesia.

Fig.4.2. Whip spider species in their natural habitat. (a) *Charinus acosta* on the underside of dead wood. (b) Microhabitat of *C. acosta* on Cuba. (c) Microhabitat of *C. cubensis* on Cuba. This species was found under stones embedded in the thick guano layer in this cave. (d) *Sarax brachydactylus* in the soil under a stone. (e) Juvenile *Charon* cf. *grayi* under tree bark. (f) Large tree in rainforest on Phillippines, one of the typical microhabitats of *C. grayi*. (g) Adult male *S. brachydactylus* on the rock wall of a cave. (h) Adult male of *C. grayi* on the rock wall in a cave, covered with biofilm. (i-k) Details of *C. grayi* legs in contact with the substrate in its natural microhabitat. (l) *Phrynus damonidaensis* on the underside of a stone. (m) Microhabitat of *P. damonidaensis* on Cuba. (n) *P. decoratus* on the underside of a stone.

4.2.2. High speed videography (HSV)

For observations of pretarsal kinematics, individuals of pulvillate whip spiders (*Charinus cubensis* and *Charon* cf. *grayi*) were placed on horizontal and vertical glass slides. High speed videos were taken using a color high speed video camera (Fastcam 1024 PCI, Photron Inc., San Diego, CA, USA) mounted onto a stereo microscope, which could be positioned vertically, horizontally and upside-down. Videos of single detaching feet were taken from dorsal, ventral and lateral views. To obtain high frame rates, a bright light source was focused onto the foot and a white sheet of paper was held behind. Opening the shutter of the light source usually induced a quick movement of the foot. Videos were taken with post-action trigger and rates of 500-3000 frames per second. The apulvillate *Phrynus longipes* was filmed on a white sheet of paper and thus only from dorsal and lateral views.

4.2.3. Reflection interference contrast microscopic high speed videography (RICM-HSV)

For investigating the actual contact area and the deposition of fluid secretions, single feet of whip spiders resting on a horizontal glass slide were studied by means of an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany) with a mounted B/W high speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA) and operated in reflection interference contrast mode (coaxial light). For the highest magnifications, an oil immersion lens in combination with cover slips, onto which the animal had placed its foot, was used. Videos of detaching feet were taken at rates of 6 250 - 10 000 fps and a shutter speed of 1 / 10 000 seconds, using post-action trigger.

4.2.4. Assessment of adhesion ability

Force measurements: Adhesive forces to smooth glass slides were measured in individuals of *Charinus cubensis*, *Charon* cf. *grayi* (different stages) and an adult female individual of *Sarax brachydactylus*. By means of a non-toxic, two compound Polyvinylsiloxane silicone mass (dental wax), the animals were glued onto a thin wire mounted onto the cantilever of a micro force transducer (FORT-10 with 10 g force range, World Precision Instruments Inc., Sarasota, FL, USA). The force transducer could be moved vertically by a manual micromanipulator at a speed of about 0.5 mm per second. The signal of the force transducer was amplified and processed using a Biopac MP-100 acquisition system (Biopac Systems Ltd, Goleta, CA, USA). Force curves were recorded using the AcqKnowledge 3.7.0 software (Biopac Systems Ltd). Peak forces were divided by animal weight (measured with an AG 204 Delta Range scale, Mettler Toledo GmbH, Greifensee, Switzerland) to calculate the safety factor (sf). Additionally, peak forces were divided by the sum of all leg areas in contact, measured from coaxial light micrographs, to calculate the adhesive strength. In order to estimate the effective contact area, single feet were filmed by means of RICM-HSV (see above) during the test. The contact area at highest forces (usually before contact breakage) was used. We measured the contact area as the proportion of the pad being in direct contact (indicated by dark contrast in the image), not the real contact area given by microstructures and fluids. Otherwise the adhesive strength could hardly be compared with values obtained in experiments with other animals. Further, to get a better estimate of the effective contact area under natural load, we measured the contact area of each foot in each individual, when hanging upside down on a glass slide. For this purpose, images were observed in a stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany), operated with coaxial light, and recorded with a camera (Leica DFC420). In some pull-off tests single legs were

filmed from a lateral view using an EOS 600D SLR (Canon Inc., Tokio, Japan) equipped with a macro lens and an extension tube in order to record changes in leg positioning and the angle between tarsus and substrate.

Vertical pull-off tests were further performed on epoxy resin surfaces, which were casts of a smooth glass surface and different fine-grained polishing papers with a defined degree of roughness (see Wolff and Gorb, 2012 for details on substrates).

Tilting tests: Juvenile to adult individuals of *Charon* cf. *grayi* were placed on the horizontal glass pane of a terrarium which then was successively tilted up to 180°. The angle at which the animal lost its foothold and began to slide was recorded and set in relation to the body weight (weighted using a Sartorius L 220).

4.2.5. Scanning electron microscopy (SEM)

Standard SEM: Prenymphs, protonymphs and adult females were conserved and stored in 70% ethanol. For scanning electron microscopy, legs were cut off and dehydrated in a series of increasing ethanol concentrations (80%, 90%, 100% and 100% on a molecular sieve), followed by critical point drying. Dried samples were glued onto 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., Tokio, Japan) at an acceleration voltage of 3.0 kV. Remains of secretions were imaged immediately after being produced by the animals during climbing trials, without prior drying. For this purpose, circular cover slips were sputter coated with 8 nm Au-Pd and used in experiments. Thus, the remains of secretion were easily found during the SEM imaging. In one case, a large footprint, produced in pull-off tests, was mounted with the (un-sputtered) glass slide onto a stub, sputter-coated and viewed in the SEM.

Cryo-SEM: Living prenymphs and freshly-ablated legs 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 down to -120°C. Some feet were slightly touched with a frozen scalpel mounted in the pre-chamber, in order to produce freeze fractures.

Histological sections: A female individual of *Charinus cubensis* was anaesthetized with carbon dioxide and the legs were clipped off using micro scissors. The legs were immediately fixed with 2.5% gluteraldehyde in PBS and after rinsing with aqua bidest, postfixed with 0.1% osmium tetroxide, dehydrated in a series of increasing ethanol concentrations and mounted in Epon resin. After polymerisation at 70°C for 24 h, Epon blocks were trimmed with a Leica EM TRIM2, and 1 and 1.5 µm thick sections were made using a Diatome MC2391 diamond knife (Diatome AG, Biel, Switzerland) and a Leica EM UC7 ultramicrotome to about a half of the embedded specimen foot. The rest was cut from the block with a fine circular saw mounted onto a drilling machine. Some sections were placed on super frost glass slides for light microscope investigations, the others were placed on cover slides, previously cleaned with absolute ethanol and 70% acetone and coated with pioloform film. Sections mounted to the glass slides in this manner were bathed for 45 min in a concentrated KOH solution of methanol and propylene oxide 2:1 (Maxwell, 1978), to remove Epoxy resin. The half cut block samples were treated in the solution for 1.5 h. The de-epoxynazed samples were put in absolute ethanol and critical point dried. The following treatment and microscopy are described above (see *Standard SEM*).

4.2.6. Micro-computed tomography

Legs stored in 70% ethanol were dehydrated in a series of increasing ethanol concentrations and critical point dried. Dried samples were glued onto plastic pipette tips with cyanacrylate glue and scanned with a SkyScan 1172 HR micro-CT (Bruker microCT, Kontich, Belgium) with an acceleration voltage of 40 kV and a voxel size of 0.53 µm. 3D images were reconstructed using NRecon 1.6.6. software and further processed with Amira 5.4.3.

4.3. Results

4.3.1. Terminology

In arachnids there is no consistent terminology for pretarsal structures, which impedes comparative studies. We follow the descriptions by DeMejere (De Meijere, 1901) and the terminology well established in entomology by Holway (Holway, 1935) and Dashman (Dashman, 1953) and apply it to the structures observed here, as follows.

Pretarsus: The pretarsus is the most distal leg segment controlled by muscles. In insects there is only a claw depressor muscle whose tendon inserts at a ventral sclerite, which bears the claws (the *unguitractor plate*). In arachnids the (synapomorphic) pretarsus is reduced to a thick ring- or bow-like sclerite, controlled by a ventral *depressor (protractor)* and a dorsal *levator (retractor)* muscle.

Arolium: Median un-paired lobe- or cushion-like attachment organs situated at the pretarsus are called *arolium*. The term *pulvillus*, used for the median pad of amblypygids, is misleading as this term usually refers to paired lobe-like attachment organs underneath the claws (see below).

Condyle: The *condyle* is an inner bulge of the tarsus that articulates with a bowl-like cavity of the pretarsus.

Unguis: The pretarsus usually bears two large claws, which are traditionally called *ungues* (sing.: *unguis*). They are not fused with the pretarsus sclerite, but rather articulated by the flexible membrane (*unguial membrane*). The membrane might be attached to a pin-like sclerite in the pro-ventral site, connecting the distal unguis base and the lateral pretarsus (*unguial sclerite*).

Pretarsal membrane: The pretarsus is attached to the tarsus by a large dorsal and a small ventral membrane. The dorsal membrane is partially fused with the unguial membrane and may extend to the median pretarsal parts.

Pulvillus (‘cushion’): The term traditionally describes paired lobes underneath the claws. They are often outgrowths of the claw articulation membranes and not the median pretarsal membranes. Consequently, this term is erroneously applied on the amblypygid foot pad and should be replaced by the term *arolium* (see above).

4.3.2. Anatomy of the pretarsus

In the first leg pair (antenniform legs) the pretarsus is totally reduced (Fig.4.3.a). In the walking legs it differs considerably between pulvillate and apulvillate species.

Charon: In the pulvillate *C.* cf. *grayi* the pretarsal sclerite is of a U-like shape with a median sclerite flexibly hinged between the lateral flanks (Fig.4.4.f,j,k). The median sclerite is built of thickened, sclerotized parts of the dorsal pretarsal membrane. In its basal part it is an infold of the membrane, internally connected to the tendon of the retractor muscle. Distally it

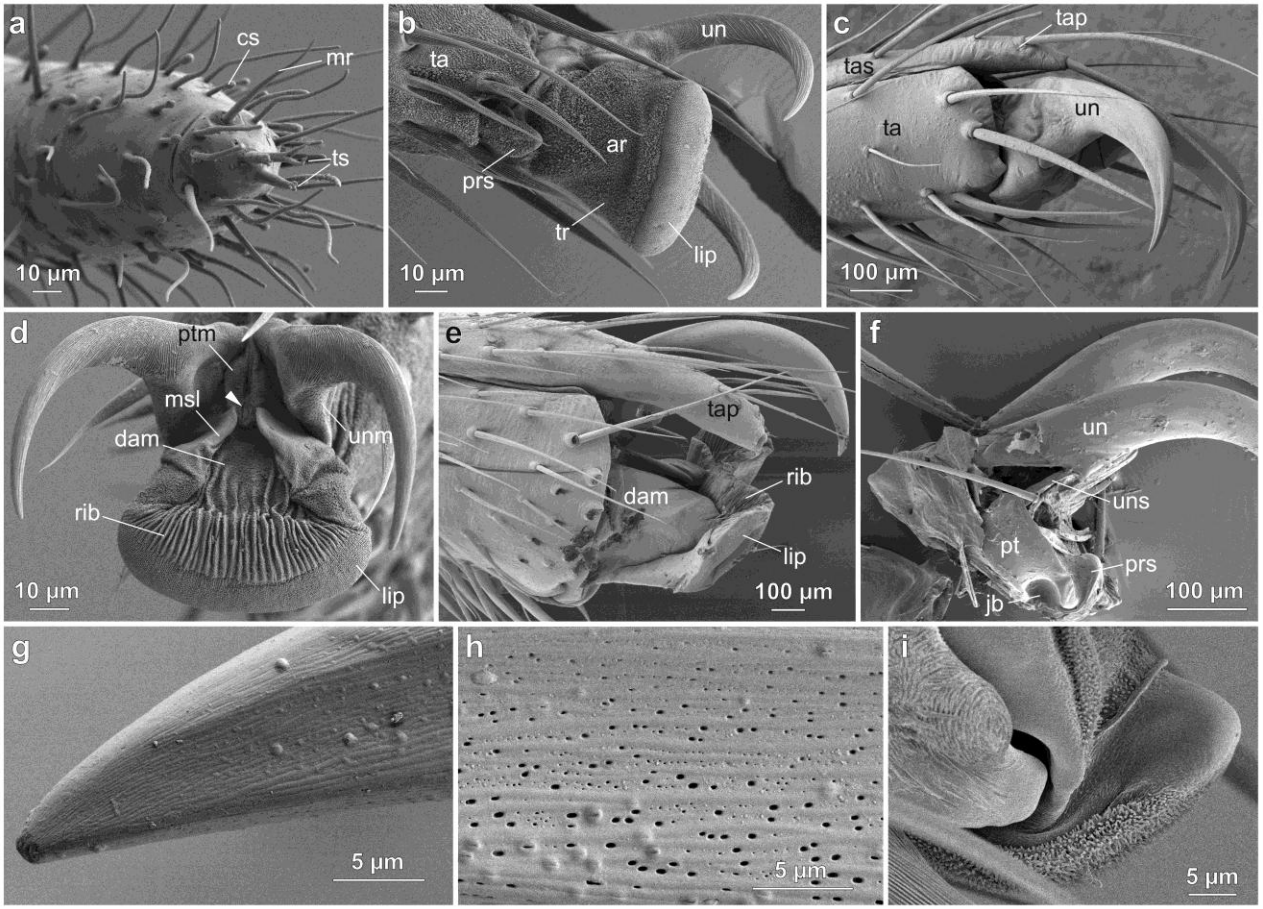
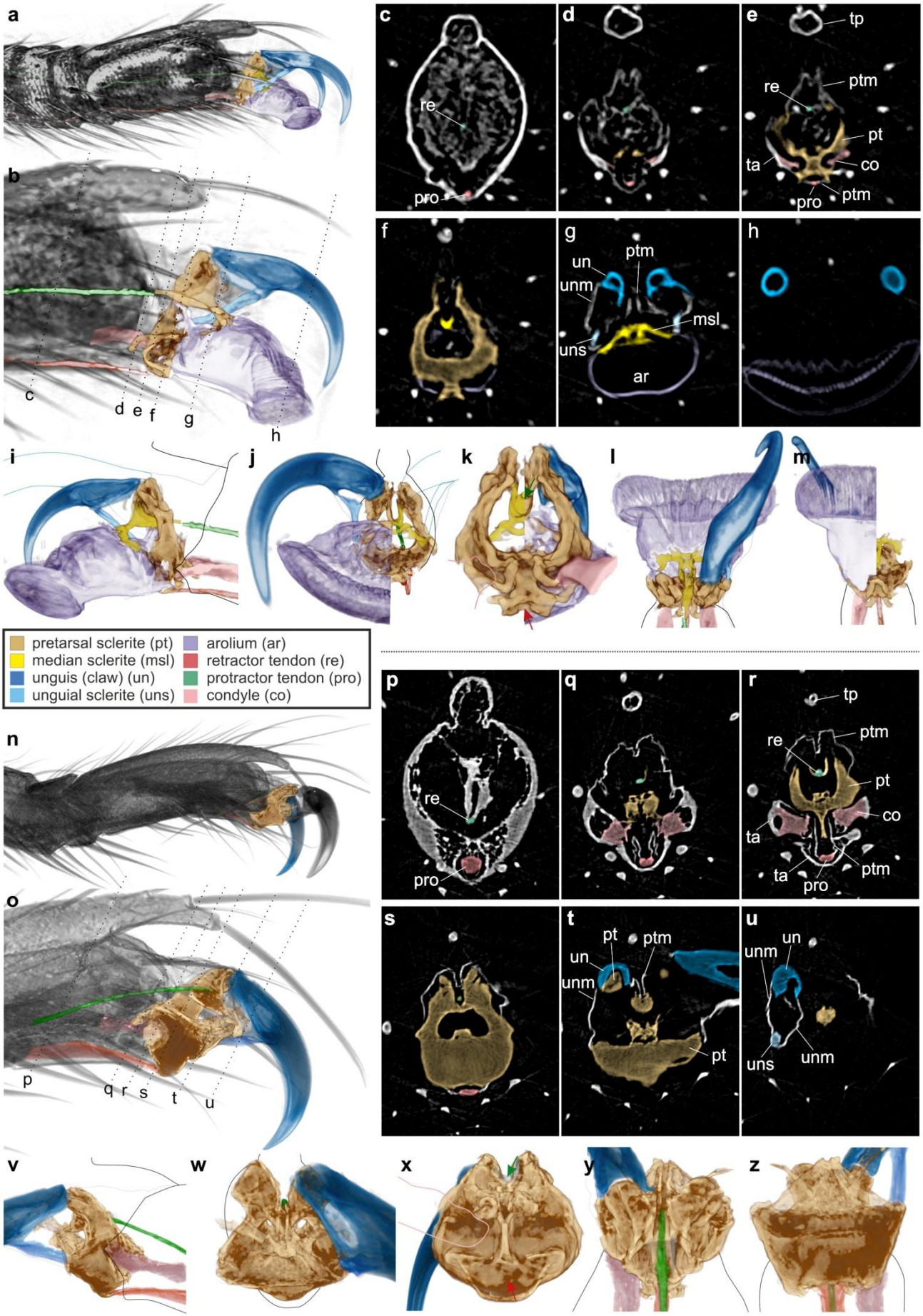


Fig.4.3. Morphology of whip spider feet. SEM and Cryo-SEM images of whip spider appendages. (a) Tip of antenniform leg in a protonymph of *Sarax brachydactylus*. (b) Walking leg foot of a *S. brachydactylus* protonymph, lateral view. (c) Walking leg foot of an adult *Phrynus longipes*, lateral view. (d) Walking leg foot of a *S. brachydactylus* protonymph, apical view. (e) Walking leg foot of an adult *Charon* cf. *grayi*, one claw is removed, dorso-lateral view. (f) Separated pretarsus of *C.* cf. *grayi*, arolium removed, lateral view. (g) Tip of the claw in *C.* cf. *grayi*. (h) Claw surface in *C.* cf. *grayi*. (i) Protractor sclerite and pretarsal joint in *Charinus cubensis*. Abbreviations: ar, arolium; cs, club-like sensory seta; dam, dorsal aroliar membrane; jb, joint bowl; lip, distal aroliar lip; mr, mechanoreceptor; prs, protractor sclerite; pt, pretarsus; rib, ribs of the inner cuticle in the distal aroliar lip; ta, tarsus; tap, tarsal processus; tas, tarsal dorsal sclerite; ti, tip sensors; tr, truncus; un, unguis (claw); uns, unguial sclerite.

bears a protuberance with a broad T-shaped ending (Fig.4.4.l). This is part of the dorsal aroliar membrane permitting a controlled movement of the pad. Ventrally, the pretarsal sclerite bears a blunt protruding hump (Fig.4.3.i), where the flexible ventral membrane and the depressor tendon are attached (protractor sclerite) (Fig.4.4.b,m). Slightly above, there are bowl-like cavities that enclose the condylar humps of the inner tarsal brim (Fig.4.3.i, 4.e,k). This is the pivot of the pretarsus rotation. The paired claws are massive and smooth and strongly curved ventrad. They are hollow and with a porous fine structure (Fig.4.3.h), which presumably permits high stability at low material volume and therefore reduced weight. The claws are articulated on one side with the dorsal most tip of the lateral flanks of the pretarsal sclerite (Fig.4.4.b,l). Additionally, they bear a ventral unguial sclerite articulated with a short median protuberance on each lateral flank (Fig.4.3.f, 4.a,b,j). Between the massive pretarsal sclerite, the claws, and the unguial sclerite there is a rather stiff but thin triangular membrane (unguial membrane). Between the claws and within the median cavity of the pretarsal sclerite the long truncus of the arolium arises. It is a sac-like membranous cushion with a rubber like consistency. The dorsal membrane is fused with the


a
b
c
d e f g
h
i
j
k
l
m
pretarsal sclerite (pt)
median sclerite (msl)
unguis (claw) (un)
unguial sclerite (uns)
arolium (ar)
retractor tendon (re)
protractor tendon (pro)
condyle (co)
n
o
p q r s t u
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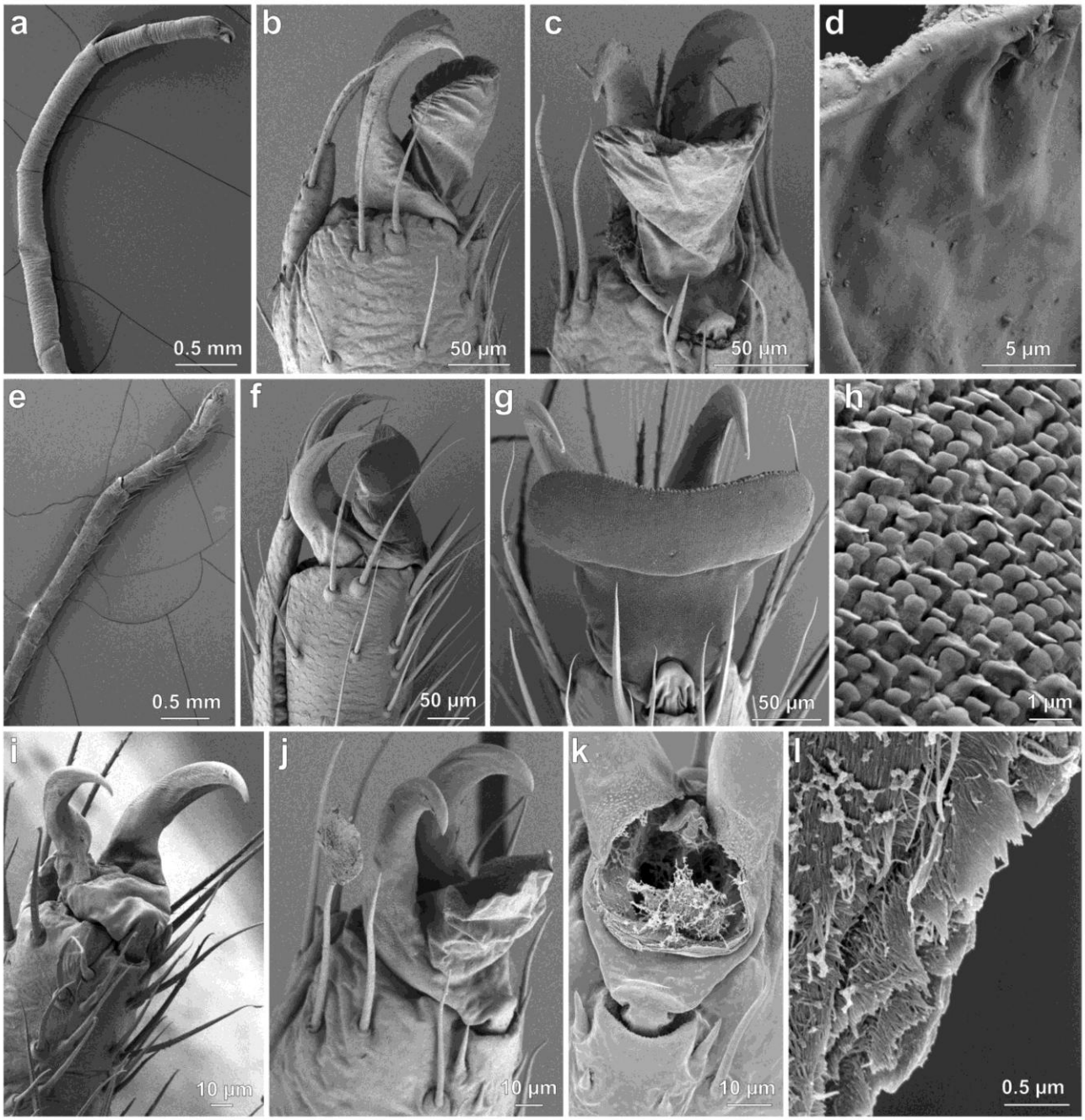

Fig.4.5. Developmental changes of the pretarsus. SEM and Cryo-SEM images of whip spider appendages. (a-h) *Charon* cf. *grayi*, (a-d) prenymph, (e-h) protonymph. (i) Protonymph of *Phrynus damonidaensis*. (j-l) Prenymph of *P. marginemaculatus*, (k-l) fractured arolium.

Fig.4.4. Anatomy of the pretarsus. Reconstructed micro computed tomography (µCT) images, structures are coloured (see the legend in the image). (a-m) *Charon* cf. *grayi*. (n-z) *Phrynus longipes*. (a,n) Lateral view of the distal tarsus. (b,o) Longitudinal section. (c-h, p-u) Single digital cross sections. (i,v) Pretarsus, lateral view, one claw is removed. (j, w) Frontal view, one claw and one half of the arolium are removed. (k, x) Back view, position of condyles and tendon attachment sites are marked. (l, y) Dorsal view, one claw is removed. (m, z) Ventral view, one claw and one half of the arolium are removed. Abbreviations: ar, arolium; co, condyle; msl, median sclerite; pro, protractor tendon; pt, pretarsal sclerite; ptm, pretarsal membrane; re, retractor tendon; ta, tarsus; tp, tarsal processus, un, unguis (claw); uns, unguial sclerite; unm, unguial membrane.

T-shaped protuberance of the median pretarsal sclerite and exhibits several regular folds permitting dorsal bending during retraction (Fig.4.3.e, 4.i,l and like in *Sarax* Fig.4.3.d). The ventral membrane is rather stiff and barely bendable. The truncus is hollow and filled with fluid. Distally it widens and bears an apical broad lip, which makes contact with the substrate. The lip is compliant, but of stable, bulging shape, presumably due to internal fluid pressure. The dorsal part bears thick longitudinal ribs; the ventral part is rather flat and exhibits a complex microstructure (see 3.4.).

In prenymphs most structures are already developed (Fig.4.5.a-c), in contrast to those in the related Thelyphonida (Wolff et al., 2015). However, the median sclerite is barely developed and the T-shaped protuberance is lacking. The arolium is simple, sac-like, and with a less stable structure because stabilizing sclerotized areas are lacking (similar to *Phrynus* prenymph Fig.4.5.k). The lip cuticle and microstructures are not yet developed (Fig.4.5.b-d).

Phrynus: In the apulvillate *P. longipes* the pretarsal sclerite is more massive and thicker than in *C.* cf. *grayi* (Fig.4.4.n-z). The material, however, is less densely packed and partly porous. The median sclerite is clumped and fuses with the lateral flanks. A slight protuberance is situated medially. The remaining holes are covered by the median pretarsal membrane, which has a smooth surface. The tarsal processus is shorter than in *C.* cf. *grayi* and the claws are longer, less curved and more pointed. The arolium is only present in prenymphs (Fig.4.5.j) and has a rather similar configuration than the prenymph of *Charon* cf. *grayi*.

4.3.3. Kinematics of the pretarsus

The movement of pretarsal structures during walking and climbing differs considerably between pulvillate and apulvillate species.

Charinus and *Charon*: The pretarsus has a horizontal freedom of movement of about 60°. Elevation and depression are conducted in 50-100 ms in the small *Charinus cubensis* and 100-400 ms in the large *Charon* cf. *grayi* (Fig.4.6.h-q). In the retracted state the dorsal part of the pretarsus, including the claw bases, is withdrawn into the tarsus and the claws are in a nearly parallel orientation to the median longitudinal axis of the tarsus (Fig.4.6.l). The dorsal membrane of the aroliar truncus is folded and the distal lip is concavely curved and tilted rectangular to the tarsus. In the protracted state the lip is tilted ventrally such that it comes in parallel to the substrate. The dorsal membrane of the truncus is spread and the claws are widely opened laterally, up to an angle of 90° towards the median longitudinal axis (Fig.4.6.f,m). The protractor sclerite is withdrawn into the tarsus (Fig.4.6.f). Movement of the arolium and spreading of the claws are presumably driven both by an increase in internal hemolymph pressure and the differences in cuticular stiffness in different parts of the arolium. Because the retractor tendon is directly connected to the dorsal foldable membrane of the arolium via the median sclerite, which itself is flexibly articulated with the pretarsal sclerite, it can be withdrawn to a greater extent than the pretarsal sclerite thus leading to the dorso-medially folding process. This mechanism permits the simultaneous control of both attachment systems, claws and adhesive pad, by the same muscles. It further permits a mechanism of quick, effortless detachment of the pad by a buckling of the contacting lip (see 3.5.). The function of the uniquely separated tarsal dorsal sclerite was assumed to assist hemolymph pressure regulation, especially during pretarsal retraction. However, we could not observe an obvious change in the width of the delimitating slit during pretarsal movement.

Phrynus: The pretarsus of *Phrynus longipes* has freedom of movement of about 90° (Fig.4.6.a-f). Elevation and depression of the claws are conducted in 50-100 ms. The lateral angle of the claws is about 20-30° from the median longitudinal axis. It barely changes during retraction.

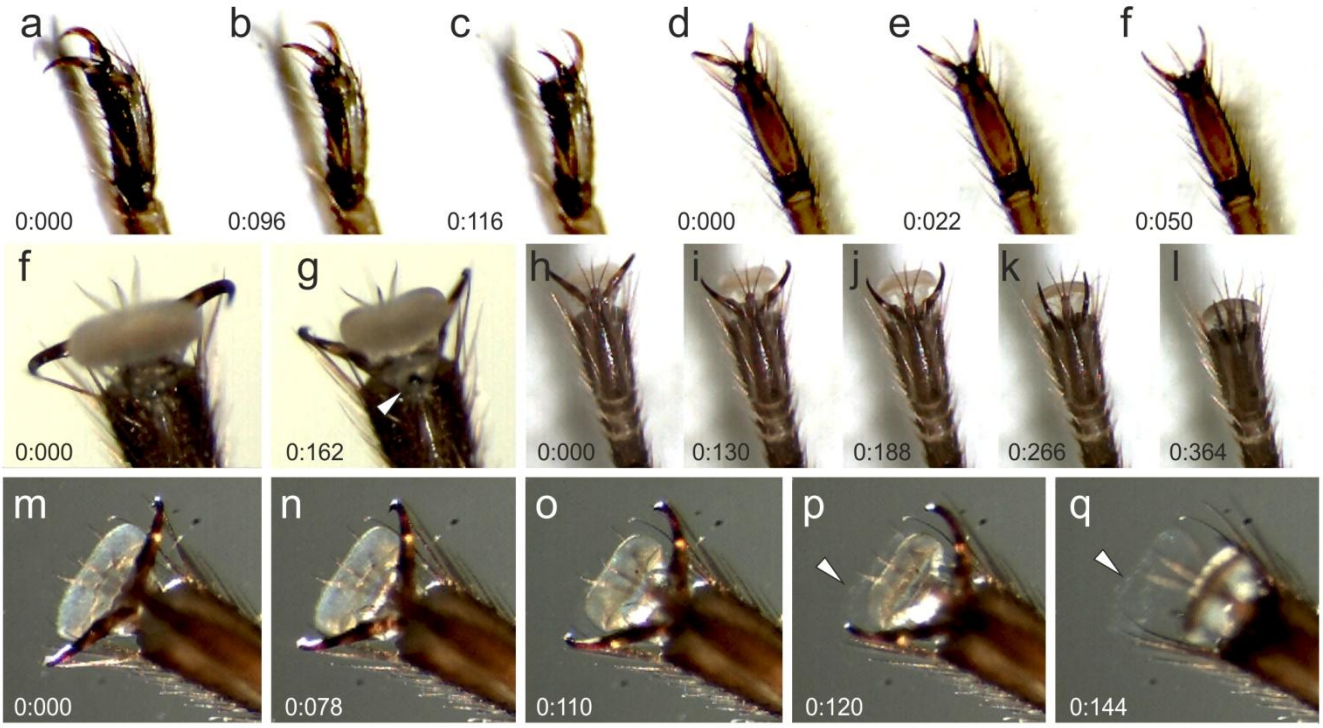

Fig.4.6. Kinematics of the pretarsus. High speed video frames (HSV) (500 frames per second) of recordings of foot detachment. (a-f) *Phrynus longipes*, (f-q) *Charon* cf. *grayi*. Arrowheads mark secretion remains (footprint) left behind.

4.3.4. Fine structure of the arolium

The arolium exhibits complex functional microstructured surfaces. Its lip additionally bears an elaborate inner structure. In the truncus, the dorsal membrane beside the median sclerite is smooth (Fig.4.3.e) or nubby (Fig.4.3.d, 4.7.e,f), which is typical for arthrodial membranes of arachnids (personal observation, see also Fig.4.8.i). A similar pattern was found in the membrane delimitating the tarsal dorsal sclerite. The cuticula of the dorsal membrane is dense and about 0.5 µm thick (Fig.4.7.e,f). The ventral cuticle can be smooth or densely covered with 1-2 µm long microtrichia (depending on species and stage, see below). This cuticle is about 1 µm thick and perforated by channels with a diameter of about 100 nm (Fig.4.7.g). These channels seem not to open to the outside as no pores were found in the epicuticle. The microtrichia can also be present on the protractor sclerite. Here, and in the proximal region, the microtrichia can also be reduced to nubs. The distal lip of the arolium is clearly delimited from the truncus by divergent microstructures (no transitional zone). The surface is composed of hexagonal structures that medially bear a spatula-like protuberance (Fig.4.7.a,b,h; 4.8.b,e,h). Individual hexagonal structures are loosely connected, such that there are slits between them, from which fluids may emerge (Fig.4.7.b). The hexagonal structures including the spatula are composed of the epicuticle alone, which is only 10-30 nm thick. These structures are thus hollow (Fig.4.7.b,h) and fluid filled. The underlying exocuticle accordingly forms hollow thin-walled tubes composed of branching fibres, more densely packed in the distal part. The mesocuticle forms long, 250 nm thick fibres branching

off the 1-2 µm thick longitudinal ribs in the dorsal most part (Fig.4.7.a). These ribs permit a lateral bending of the lip, but fix its shape in the distal direction. When the arolium is fully expanded there are large spaces between the ribs and the fluid in the aroliar cavity can flow into the bulged filamentous lip cuticle.

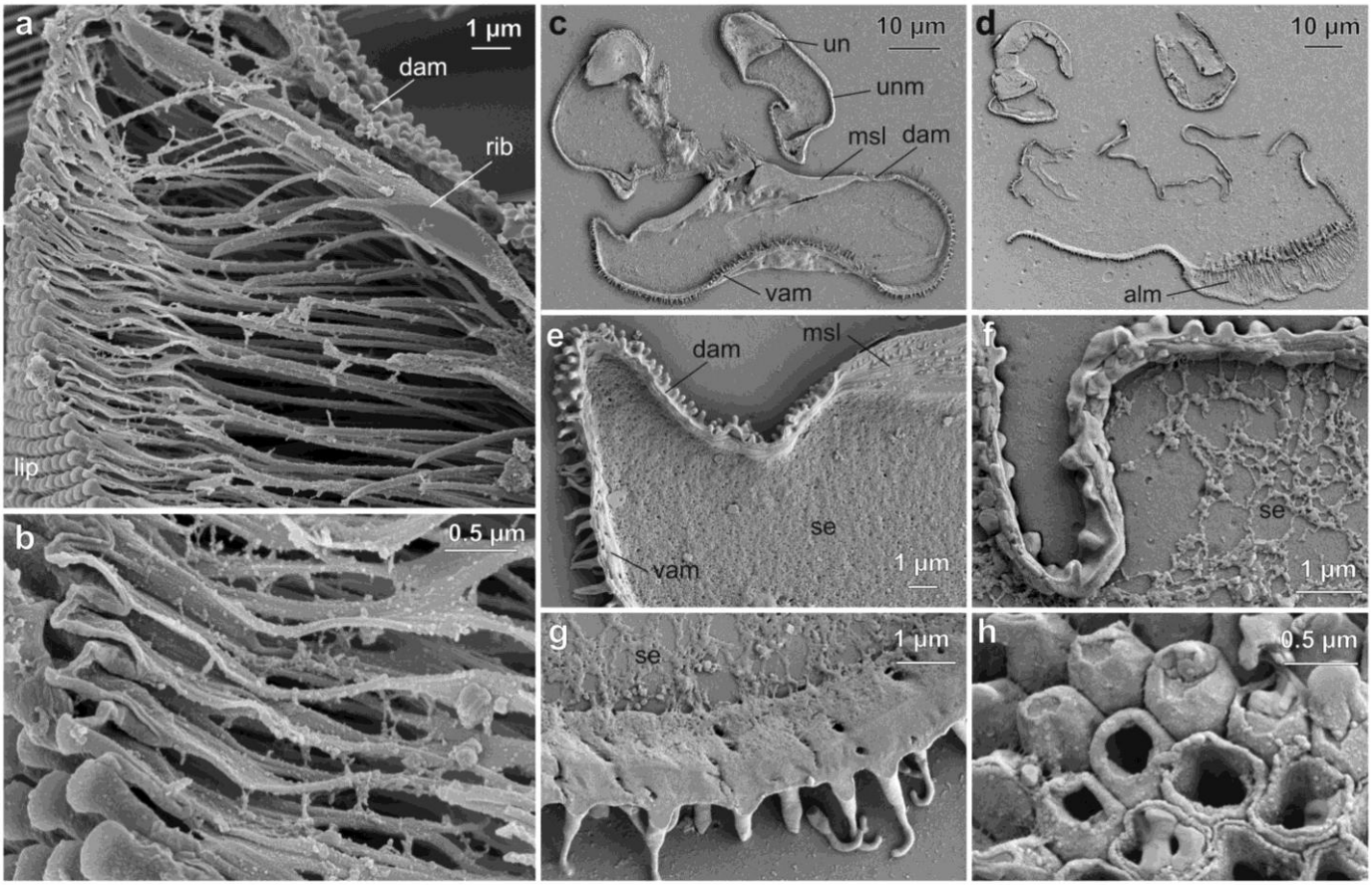

Fig.4.7. Inner fine structure of the arolium. SEM microsections of the arolium of *Charinus cubensis*. Abbreviations: alm, aroliar lip membrane; dam, dorsal aroliar membrane; msl, median sclerite; se, secretion fluid; un, unguis (claw); unm, unguial membrane; vam, ventral aroliar membrane.

The shape of the microstructures differs between representatives of different genera (Tab. 4.1) and stages. In prenymphs no microstructures are present in either ventral or distal parts of the arolium (Fig.4.5.d). There is also no elaborate branched structure of the contacting cuticle (Fig.4.5.l). The only microstructures are nubs of the dorsal cuticle, which are already present in prenymphs, indicating that the arolium is built from the pretarsal arthrodial membrane and the unguial membranes. Microstructures revealed in different genera are described below.

Charinus: Lip with spatulae (Fig.4.9.a). Two types of microtrichia are situated ventrally on the truncus: the first type with a broad base, bent hook-like, and with a slightly broadened, flattened tip; the second type is trichoid with varying lengths (0.5-1.5 µm) (Fig.4.9.b). The surface of the protractor sclerite is similar to the truncus, but smooth in its distal third (Fig.4.9.c). Dorsal membrane of the arolium is nubby.

Sarax: Lip lacks spatulae, hexagons are simply roof-shaped (Fig.4.9.d) and occasionally slightly keeled. Two types of microtrichia are situated ventrally on the truncus: the first one is hook-like as in *Charinus*, but with long filamentous, flattened, and tapered tips; the second type is

trichoid, with short regular length (0.5 µm) and regular arrangement (Fig.4.9.e). The surface of the protractor sclerite and dorsal aroliar membrane is as in *Charinus* (Fig.4.9.f).

Charon: Lip is with spatulae (Fig.4.8.e, 4.9.g). Two types of microtrichia are found ventrally on the truncus: (1) straight trichoid and (2) club-like (clavate) ones with blunt tips, both of similar length (Fig.4.8.d, 4.9.h). The microtrichial coverage of the ventral truncus varies between stages. In nymphs they cover the whole ventral membrane. With growth, the coverage is reduced to the proximal part, and eventually, in adults, they are found only in the basal most part beneath the protractor sclerite and partly reduced to blunt nubs. The surface of the protractor sclerite is smooth, but the basal attached pretarsal membrane is nubby (Fig.4.9.i). Dorsal membrane of the truncus is smooth (Fig.4.3.e), but the lip is wrinkled (Fig.4.8.f).

Stygophrynus: as in *Charon*, but nubs on the pretarsal membrane at the protractor sclerite are longer (trichoid).

Prenymphs: Lip is lacking. The ventral surface is smooth (Fig.4.5.d), the dorsal one is nubby. Protractor sclerite except for the pretarsal membrane is smooth.

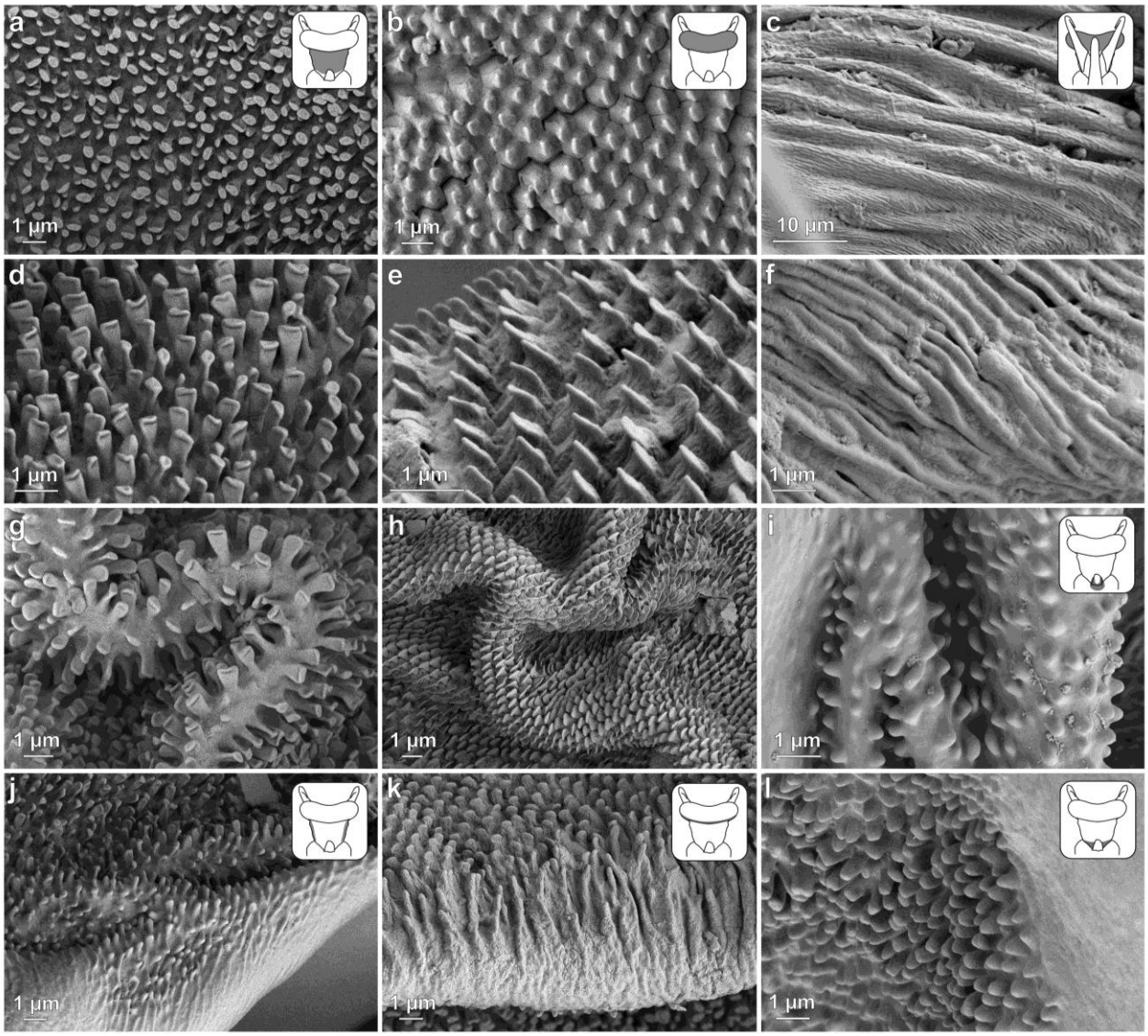

Fig.4.8. Diversity of cuticular microstructures in different parts of the arolium. SEM images of the arolium in *Charon* cf. *grayi*, pictograms indicate location of image detail (including the images in the same column, if no new pictogram is presented).

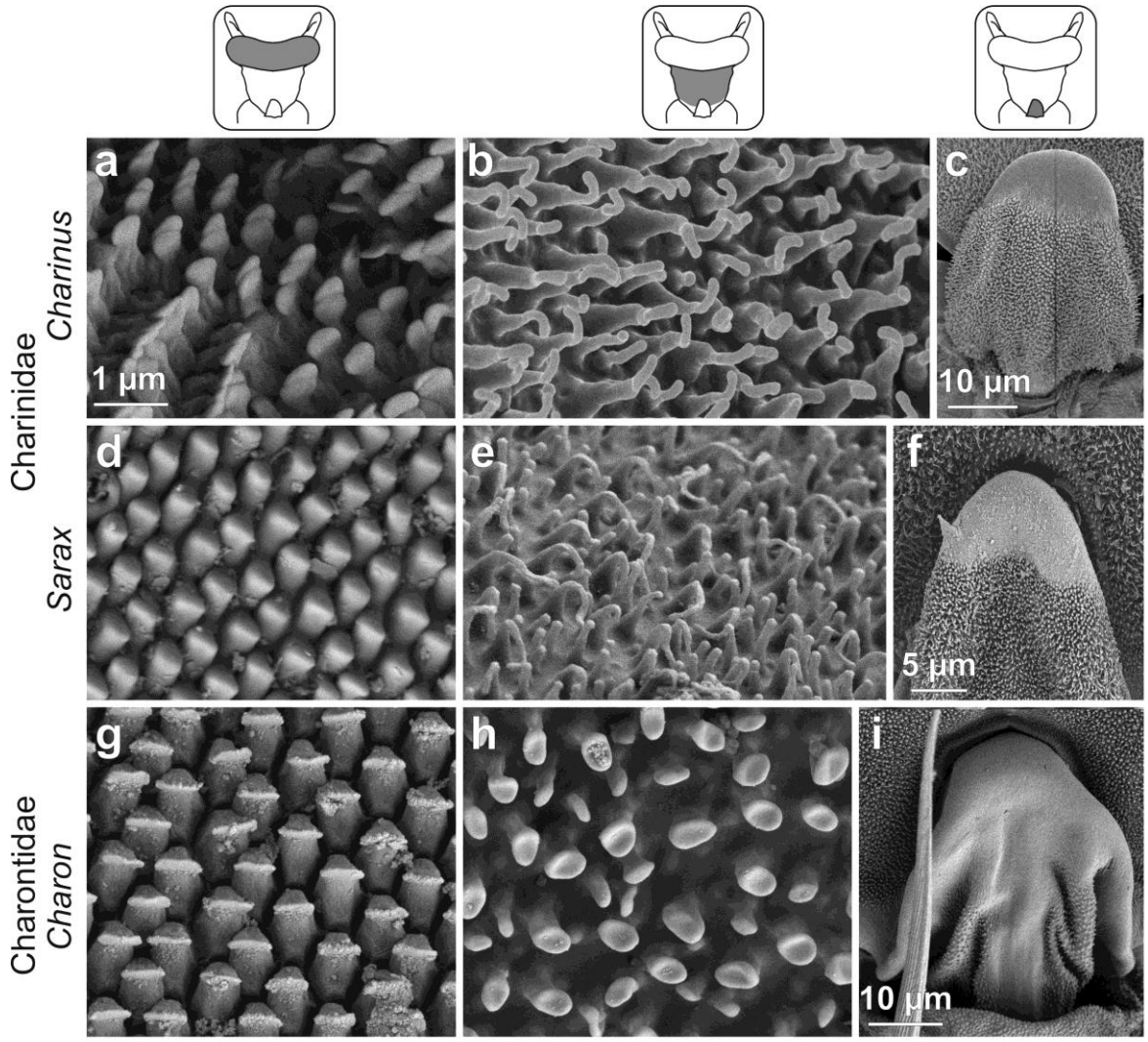

Fig.4.9. Differences in aroliar microstructures in different genera.

Tab.4.1. Morphology of aroliar microstructures in the different genera of pulvillate whip-spiders. See also Fig. 4.9.

	dorsal			ventral	
	lip	truncus	lip	truncus	protractor
Charinidae					
Charinus	nubby	nubby	spatulate	variable trichoid + short hooks	like truncus, distal 1/3 smooth
Sarax	nubby	nubby	not spatulate	short trichoid + long hooks	like truncus, distal 1/3 smooth
Charontidae					
Charon	wrinkled	smooth	spatulate	long trichoid + blunt clavate	smooth, basal membrane nubby
Stygophrynus	wrinkled	smooth	spatulate	long trichoid + blunt clavate	smooth, basal membrane trichoid

4.3.5. Contact mechanics of the arolium

Only the aroliar lip is involved in substrate contact (Fig.4.10.a, inset) (in prenymphs, which lack the lip, it is not known, as no experiments with living prenymphs were performed). The lip area is about one forth smaller in L2 than in L3 and L4. The contact area of the arolium (effectively used portion) varies considerably between animals of different size (Fig.4.11.c). In *Charinus cubensis* (4.0-6.5 mg) only 10-16 % of the lip area was in contact when resting upside down. In the *Sarax brachydactylus* individual (73 mg), 28-33% of the lip area was used. In juveniles of *Charon* cf. *grayi* (22-34 mg), 25-36 % of the total lip area of all feet was in contact

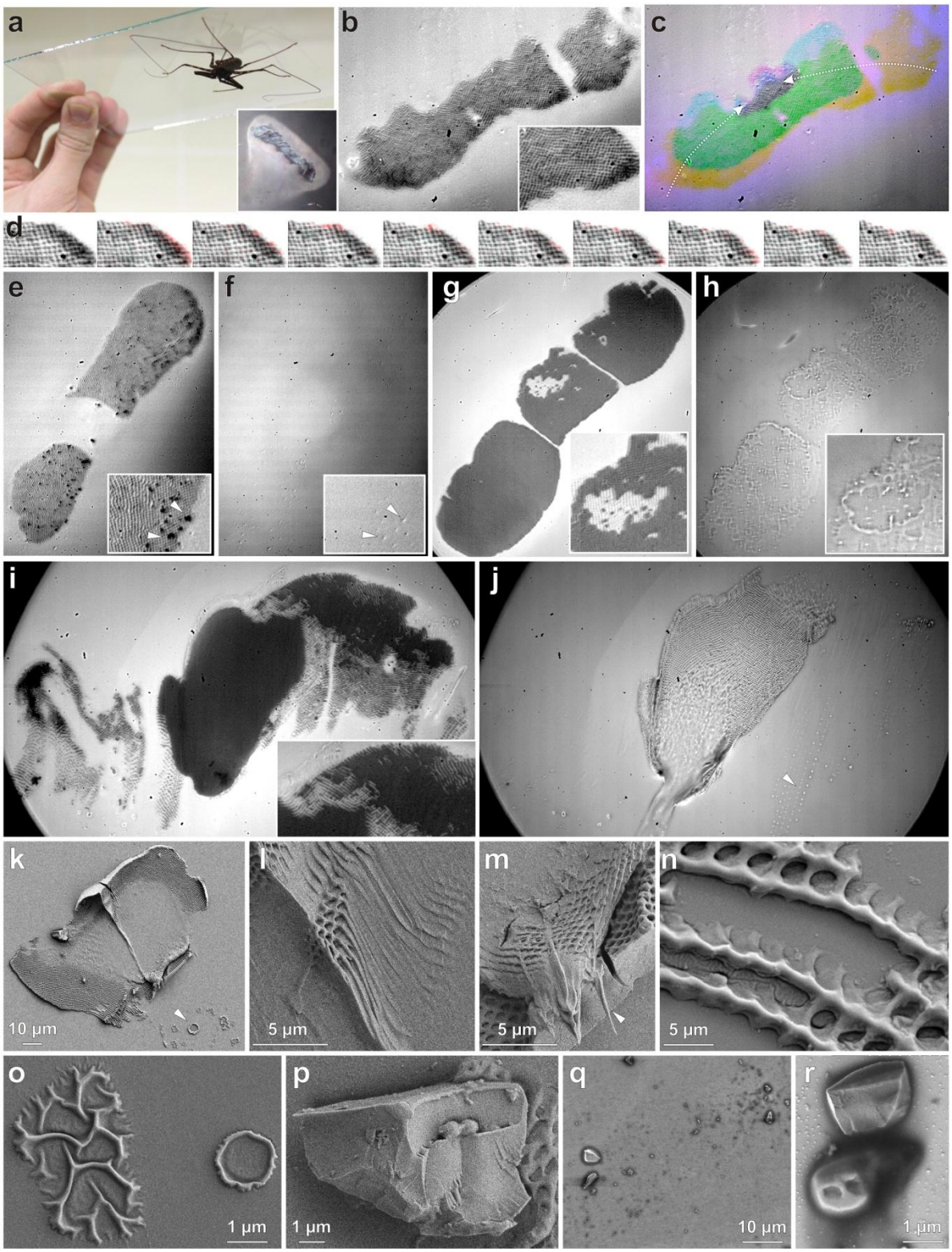

Fig.4.10. Contact mechanics of the arolium. (a-h, k-r) *Charon* cf. *grayi*. (i-j) *Sarax brachydactylus*. (a) Photograph of a subadult *C. grayi* walking upside down on a smooth glass pane. Inset shows stereo microscope image of the single arolium, illuminated by coaxial light; the contact area is visible as dark contrast. (b-j) Reflection interference contrast microscopy (RICM) images of single arolia in contact, insets show details at higher magnification. (b) Contact without fluid secretion. (c) Overlay of different frames of RICM-HSV (8000 fps) showing the detachment process (single frames are differentially coloured), arrows indicate the delamination direction. (d) Detail of RICM-HSV sequence (8000 fps), showing the independent detachment of single spatulae in the delamination zone (peeling edge). Overlay of two subsequent frames (dt=125 ns) each with the difference marked in red. (e-j) Emergence of fluids and their remains left behind on the glass slide directly after detachment. Arrowhead in (j) marks trail of droplets where the arolium slid over the surface after detachment. (k-r) Standard SEM images of fluid remains.

(may vary between 8 and 48 % in different legs, L2 is often used less than L3). In sub-adult *C. grayi* (0.5-1.2 g), 42-49 % of the total area was in contact (varied between 39 and 51% in different legs).

The detachment of the arolium is completed in 20-80 ms. It begins in the distal-lateral part and propagates to the proximal-median part (in the median part the contact area can occasionally increase in the proximal direction during the detachment process) (Fig.4.10.c). At highest magnification, single spatulae could easily be distinguished, indicating that only their surface areas are in real contact with the substrate (Fig.4.10.b). This led to a discontinuous propagation of the contact breaking zone (peeling-line). Single spatulae within this zone detach independently (in less than 200 ns), but these detachment events alternate with periods of time when the crack propagation is stopped for up to 2 ms (Fig.4.10.d).

Occasionally, the emergence of fluid was observed between spatulae. Often this occurred only in small amounts (Fig.4.10.e). Small droplets were then left behind after detachment (Fig.4.10.f). These droplets did not evaporate over time indicating that they contain lipids. However, in some cases the fluid spread over the total pad, but obviously only filling the spaces between the spatulae, as the spatulae were still distinguishable in the RICM image (Fig.4.10.g,i). In these cases a fluid film was left behind after detachment, which then partly shrank to larger individual droplets due to surface tension and dewetting (Fig.4.10.h). In three cases (two in *S. brachydactylus* and one in *C.* cf. *grayi*), the deposited fluids were highly-viscous such that imprints of the spatulae were visible (Fig.4.10.j). This caused stick-slip behaviour of arolium, visible by propagated parallel waves of alternating contact and non-contact regions prior to detachment. Such secretion remains were stable in shape, even under the high vacuum of the Standard-SEM and could be imaged accordingly (Fig.4.10.k-n). SEM images show that the secretion had been located almost exclusively between the spatulae, but may occasionally also wet them with a very thin layer (Fig.4.10.n). Remains of fluid droplets, almost totally evaporated under the high vacuum of the SEM and occasionally left behind wrinkled remains (Fig.4.10.o), indicating that the fluid is composed of both short (fluid) and long-chained (very viscous or even solid) substances. Furthermore, irregular crystals were found at the sites where droplets were previously situated (Fig.4.10.p-r), perhaps indicating that salts are also present in the fluid, presumably acting as wetting agents.

The occurrence of fluid secretions was observed more often in larger whip spiders, but dry state (no secretions observed between spatulae and no discernable droplets left behind) occurred in all individuals as did wet state, with measurable adhesion force in both states. The fluids did not affect the discontinuity of the peel-off behaviour of the arolium, but occasionally a lubricating effect was observed when the pad slid, leaving behind a trail of droplets.

4.3.6. Adhesive strength

When pulled, whip spiders could withstand the pulling force over a long distance of vertical movement, due to their relatively long legs. Pull off force increased approximately linearly with the increase in distance (Fig.4.11.e). During horizontal rest the distitarsus was situated parallel to the surface, thus its ventral bristles were in contact with the ground (Fig.4.11.e). Contacts of the arolia usually broke at an angle of about 30° between the distitarsus and the surface (the basitarsus and tibia were then at an angle of about 45° towards the substrate) (Fig.4.11.e). When the contact broke in one leg, the breakage of others usually followed immediately. The contact breakage seemed not to be induced by the animal as no pretarsal

movement was observed during contact breakage. It occurred abruptly and violently, leading to the animal jerking. The protracted arolium usually took hold again somewhere more proximally, but not for long and eventually the animal was unable to re-attach, struggling wildly with its legs. Forces, after successful re-attachment, never reached the previous maximum, presumably due to the steep angle between the feet and substrate.

The measured pull-off forces (attachment forces) and calculated values of safety factor as well as the adhesive strength are summarized in Tables 4.2 and 4.3 and in Figure 4.11. The safety factor decreases with the body size (with a factor of -0.0068 calculated for *C.* cf. *grayi*). The calculated critical mass, where animal weight and its attachment force are similar, is 1.55 g. This is in accordance with the observation that individuals of *C.* cf. *grayi* larger than 1.55 grams (adult females) began to slide at a tilting angle under 180° (ceiling situation) (Fig.4.12). The mean adhesive strength of single individuals ranged between 28 and 200 kPa decreasing with the body weight. However, some small individuals of *C. cubensis* and *C.* cf. *grayi* also achieved comparably small values, this being why an allometric effect cannot be concluded for sure. On epoxy resin surfaces the attachment forces were significantly lower than on glass (Fig.4.11.g). At a mean substrate asperity size of 9 µm, the force was significantly lower than on other tested epoxy resin surfaces (Fig.4.11.f).

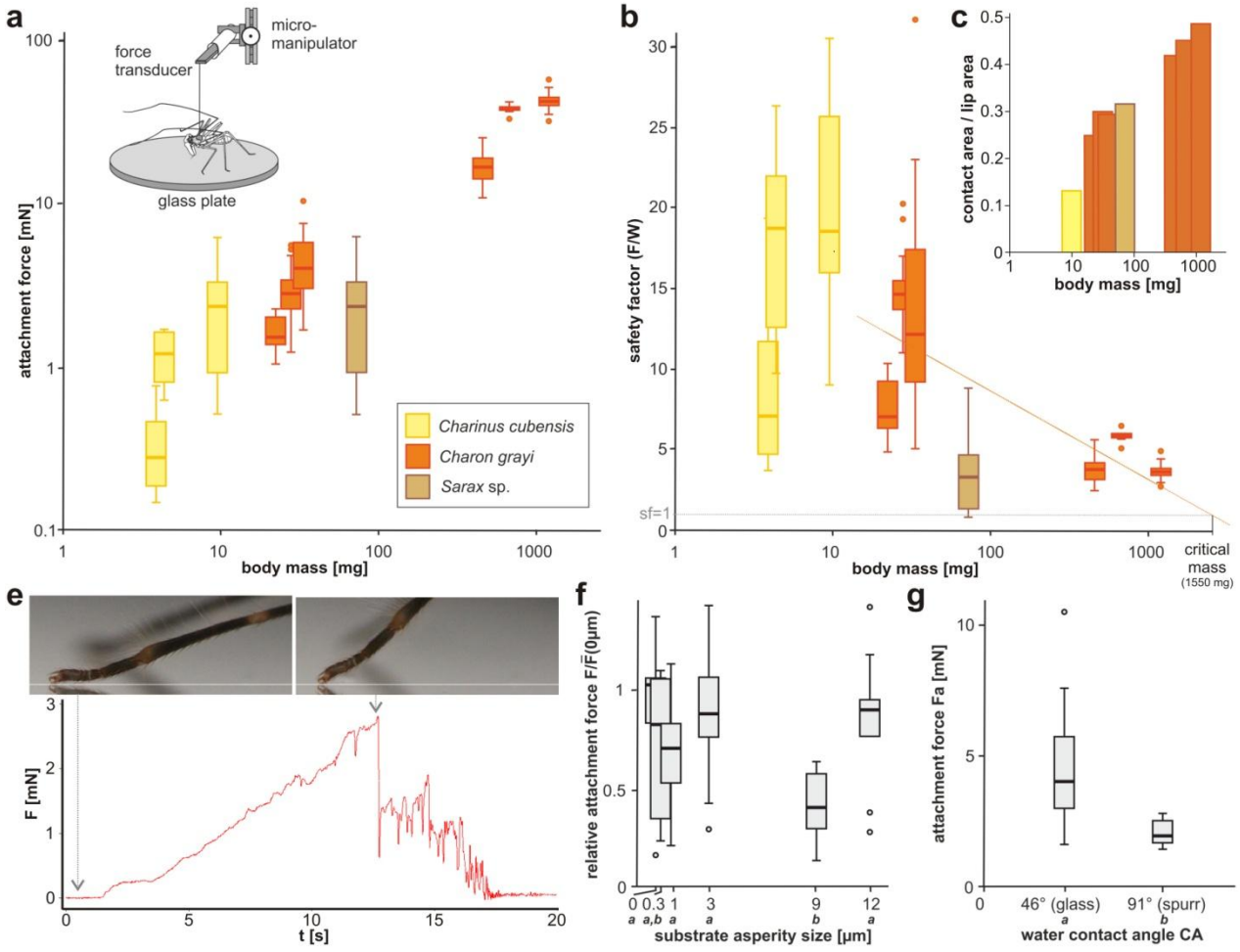

Fig.4.11. Results of measurements of attachment strength.

Tab.4.2. Summary of measurement data obtained from vertical pull-off test on smooth glass. Mean values ± standard deviations are given.

Individual	mass [mg]	contact area [mm²]	% of total pad	attachment force [mN]	safety factor	adhesive strength [kPa]
Charinus cubensis juvenile 1	4.3	0.003	NA	0.17 ± 0.05 (N=7)	4.0 ± 1.3	54.3 ± 17.7
C. cubensis juvenile 2	4.0	0.002	NA	0.33 ± 0.18 (N=16)	8.4 ± 4.6	28.3 ± 15.4
C. cubensis adult 2	9.7	0.010	13.5	1.92 ± 0.64 (N=13)	20.25 ± 6.75	200.4 ± 66.8
Sarax brachydactylus adult	73.3	0.057	31.4	3.22 ± 1.29 (N=23)	4.5 ± 1.8	56.4 ± 22.7
Charon cf. *grayi* juvenile 1	33.8	0.032	30.2	4.41 ± 2.02 (N=29)	13.3 ± 6.1	145.2 ± 68.9
C. grayi juvenile 2	22.2	0.023	25.1	1.64 ± 0.41 (N=12)	7.5 ± 1.9	71.1 ± 17.6
C. grayi juvenile 3	28.0	0.025	29.9	3.37 ± 1.39 (N=14)	12.3 ± 5.1	135 ± 55.8
C. grayi subadult 1	680.0	0.401	45.7	38.55 ± 2.76 (N=7)	5.8 ± 0.4	96.1 ± 6.9
C. grayi subadult 2	469.8	0.915	42.2	19.11 ± 3.12 (N=31)	4.15 ± 0.68	20.9 ± 3.4
C. grayi adult (male)	1227.7	1.610	49.1	41.02 ± 4.24 (N=25)	3.41 ± 0.35	25.5 ± 2.6

Tab.4.3. Summary of force measurement data obtained from vertical pull-off tests on different substrates (water contact angle CA and mean asperity size of rough surfaces given). Mean attachment force values in mN ± standart deviations are shown.

Individual	glass (CA=46°)	epoxy resin (Spurr) (CA=91°)					
	smooth	smooth	0.3 µm	1 µm	3 µm	9 µm	12 µm
C. grayi juvenile 1	4.41 ± 2.02 (N=29)	2.10 ± 0.52 (N=6)	1.65 ± 0.77 (N=3)	1.92 ± 0.69 (N=3)	2.10 ± 0.63 (N=4)	0.92 ± 0.44 (N=4)	2.28 ± 0.52 (N=4)
C. grayi juvenile 3	3.37 ± 1.39 (N=14)	1.82 ± 0.21 (N=4)	1.14 ± 0.64 (N=5)	1.24 ± 0.18 (N=5)	1.81 ± 0.29 (N=2)	0.85 ± 0.24 (N=5)	1.59 ± 0.17 (N=3)

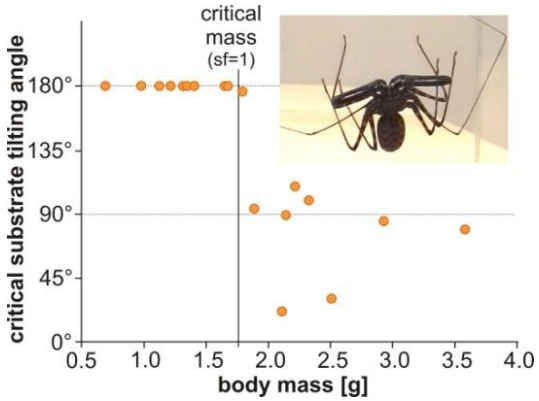

Fig.4.12. Results of tilting tests. Inset image shows large individual sliding down the vertical glass pane.

4.4. Discussion

4.4.1. Evolutionary trends in the amblypygid pretarsus and functional significance of the arolium

In the antenniform legs the pretarsus is totally reduced, similar to the condition in the front legs of whip scorpions (Wolff, unpublished), solifuges (Klann, 2009) and the pedipalps of some opiliones (***chapter 5***). Previous authors (Foelix and Hebets, 2001) proposed the group of three most distal, strongly deviant sensors to be the claw remnants. However, the pretarsal claws of the other walking legs are two in number and not innervated, as is the usual case for arthropods, thus this interpretation seems implausible.

The walking legs (L2-L4) exhibit a well-developed pretarsus with two claws and, in the plesiomorphic state, an arolium. An arolium is also found in prenymphs of the sister group of Amblypygi, the Uropygi (whip-scorpions). In both lineages the development of this prenymphal attachment organ correlates with so-called pulli-carrying behaviour, a type of maternal care, in which the mother carries its not fully developed instars on its body (Wolff et al., 2015). This indicates that the initial selective pressure that influenced arolium evolution might have been related to this specific kind of behaviour at earlier stages of development. The plesiomorphic state of the pretarsus in chelicerates is the tridactyle one, in which a (shorter) median claw (empodium) occurs beneath the major pair of claws (Dunlop, 2000). This state is found in nymphal to adult uropygids, but not in amblypygids, which stands in relation with the presence of the arolium in the basal lineages. Also in the Apulvillata an empodium is not present, which shows that the lack of the arolium is indeed a derived state within amblypygids.

As the highly complex and secretory arolium might be associated with significant costs, it must exhibit a significant benefit, when present. However, the arolium was lost in all developmental stages except the prenymph in the monophyletic Apulvillata, which comprise most of the known extant whip spider species. Although lacking the arolium the apulvillate species are good climbers and equally often found on steep rock surfaces. In *P. longipes* the claw tip diameter is much smaller than in *C.* cf. *grayi*, which may assist interlocking with micro asperities. The stiff, highly pointed bristles situated ventrally on the tarsus may also play an important role in both pulvillate and apulvillate species. These bristles are directed distally and are usually in contact with the substrate in the resting leg (Fig.4.2.j, 4.11.e). Hence, they might generate strong friction on vertical micro rough surfaces in the downward directed legs. Thus, the benefit of the arolium during climbing might be marginal on most surfaces. Especially in large whip spiders, the effectiveness of the arolium decreases, unable to provide a sufficient safety factor in individuals heavier than 1.7 g. This may explain why it was lost in the dominant lineage of whip spiders. We can only speculate why the arolium was retained in other lineages. It might be important, at least in the following three instances. (1) On surfaces with very fine roughness the claws cannot get sufficient foothold. This is especially the case for many plant surfaces, and might play some role in leaf litter. Mineral surfaces (especially crystalline ones) can be very smooth, however, it is not known, if those occur in the natural habitats (caves) of these species. (2) We often found amblypygids resting on highly wet surfaces or such covered with biofilms (Fig.4.2.a,e,h,i). The claws might slip on such lubricious films, whereas the complex microstructure of the arolium could have de-wetting properties (see 4.2. below), permitting generation of high friction forces. (3) The arolium, especially in combination with the viscous, hardening secretion could lead to a strong and effortless attachment on surfaces with an angle above 90°, where the attachment increasingly depends on adhesion or interlocking with micro-cavities. This could be beneficial as large whip spiders often rest, quiescent, on overhanging surfaces for a long period of time.

Charinidae are primarily found on the ground (Fig.4.2.a-d), making the benefit of an arolium implausible, at first sight. However, especially the litter habitats of rain forests are highly structured (Fig.4.2.b), and as most charinids are rather small, there might be a significant demand for climbing, especially if large, not yet decayed leaves are present (for surface characteristics of aged leaves i.e. see (Neinhuis and Barthlott, 1998)). The legs are neither involved in prey capture nor in mating of these animals, thus their arolium exceptionally serves locomotion purposes.

4.4.2. Unique characteristics and strength of the amblypygid arolium

The structure of the whip spider arolium has various characters that are in this configuration unique among animals. It is, above all, a smooth (membranous) adhesive pad. Adhesive pads in general gain their adhesive strength by high local compliancy permitting generation of an intimate contact with a substrate surface, and on the same time an overall structural stability which keeps the pad in shape (Gorb, 2001). This is often achieved by multi-hierarchical branching of fibres in the inner cuticle, as previously found in insects (Jiao et al., 2000), but also other arachnids, like solifuges (Klann et al., 2008) and the prenymphal arolia of both scorpions and whip-scorpions (Wolff et al., 2015). We found such fibrous inner architecture in the lip region of the amblypygid arolium, however with a unique elaboration. The fibres originate from stiff parallel rib-like structures, which permit highly directional bending properties of the lip. Further, the most apical branches situated underneath the epicuticle merge again forming a 3D architecture resembling hexagonal tubes.

Hexagonal structures are also known from the surface of smooth adhesive pads of bush crickets (Jiao et al., 2000) and tree frogs (which, however, are composed of a different material) (Scholz et al., 2009). The frogs often rest on highly wet surfaces, which would normally lead to slipping. The hexagonal structures permit de-wetting of the substrate surface in the contact area due to a drainage effect (Federle et al., 2006). This feature has been carried over into an important design in car tires to prevent aquaplaning (Roberts, 1971, Persson, 2007). This is achieved by the regular arrangement of forming straight crevices between the microstructures, permitting an unhindered flow of excessive water to the margins of the pad. In amblypygids the arolium surface pattern might have a similar function, as the microenvironment of these is also often very humid.

In amblypygids the hexagons are not flat, as in bush crickets or tree frogs, but rather roof-like with a spatula-like keel (except in *Sarax*). Such thin, spatulate structures are a universal feature of hairy adhesive pads, permitting close contact generation and adhesion as well as fast detachment due to elastic peeling (Varenberg et al., 2010). The peeling theory assumes that the work necessary to detach a thin film from a smooth surface depends (among other factors, such as thickness and elasticity modulus) on the width of the film, because the detachment force works primarily within the delamination zone (peeling line) (Kendall, 1975). Delamination demands the highest energy at the start of peeling, because the initial adhesion at the edge has to be overcome (beginning of the crack formation). The propagation of the delamination zone (crack propagation) demands comparatively less work. The introduction of discontinuities effectively stops the crack propagation, which has been shown in theory and in experiments with artificial models (Peressadko and Gorb, 2004, Chung and Chaudhury, 2005). Here, we could observe this effect directly *in vivo* in the whip spider arolium through the discontinuous delamination velocity. To the best of our knowledge, this is the first documented observation of this kind of contact behaviour in biological attachment devices. It may be an important effect leading to the observed high adhesive strength, comparable to hairy adhesive pads (see below).

Another interesting feature of the amblypygid arolium is the shape of the broad aroliar lip. From the functional point of view, this specific shape makes a longitudinal delamination more difficult due to the much longer effective peeling line than a transversal (lateral) delamination. This apparent anisotropy in the peeling off behaviour of the arolium may be important for both strong attachment (when pulling the legs towards the body) and for comparative ease in detachment (by lateral bending) simultaneously. This function is further supported by the strong anisotropic bending properties of the lip given by the internal thick longitudinal ribs. Altogether

this may provide a mechanism to quickly switch from load distribution over a large area to very local stress concentration. Fast detachment is of high importance, as these animals are capable of very sudden and quick movements, when disturbed or fleeing from a predator.

Although the adhesive strength of the whip spider arolium is comparatively high, large individuals are constrained by the allometric effects. This is mainly because the legs of whip spiders are very slender and the feet areas accordingly small. Such scaling effects are an overall constraint of adhesive pads in animals (Arzt et al., 2003). We found that in whip spiders the loss of effectiveness is partly compensated for by an increase of the contact area proportion, and further, presumably by an increased amount of viscous fluid secretion. In the ethanol material of adult Charontidae, the arolia are often covered by a thick hardened crust which may further support this latter assumption. The presence of higher amounts of the hardening fluids, on the other hand, may bring a higher risk of pad contamination.

Fluids play an important role in the functionality of adhesive pads and are assumed to be essential in the smooth type of pads (Barnes, 2007, Drechsler and Federle, 2006), whereas the hairy pads of geckoes and spiders may also generate high adhesive forces in a dry contact (Autumn et al., 2002, Kesel et al., 2003). In arachnids, including spiders (hairy pads), mites (smooth and hairy pads) and solifuges (smooth pads) occasional fluid secretion has been observed (Peattie et al., 2011), similar to our observations in amblypygids. However, it remains unclear whether the fluid is, indeed, to assist adhesion, to clean the elaborate microstructures or is a part of chemoreceptive function (Foelix et al., 2012). We could not observe a direct correlation between achieved attachment force and fluid secretion in pull-off tests because we could only observe the contact area of one foot at a time, and because this effect is normally much more strongly expressed on rough substrata (Kovalev et al., 2013), this question remains unresolved.

In comparison with smooth adhesive pads of other animals, the whip spider arolium exhibits much higher adhesive strength, which might be the result of its unique structure. The adhesive strength of the foot pads in the great green bush cricket *Tettigonia viridissima* is about 2 kPa (Jiao et al., 2000). However, these values were obtained from measurements with single euplantula (pad) in immobilized animals. In whole animal pull-off tests, higher values may be achieved by the counter action of opposing feet (Wohlfart et al., 2014). However, this does not explain the values up to 50 magnitudes higher in whip spiders, hence concluding a higher effectiveness of their pads.

In hairy adhesive pads usually higher adhesive strength is achieved, i.e about 30 kPa in the blowfly *Calliphora vomitoria* and about 80 kPa in the leaf beetle *Hemisphaerota cyanea* (Kesel et al., 2003). In the dry adhesive pads of spiders and geckoes, composed of multiple branched setae with very fine spatulae values between 400 and 600 kPa are reported (Kesel et al., 2003, Autumn et al., 2000). These studies, however, calculate the adhesive strength from measurements with single setae or single spatulae on the basis of the estimated actual contact area, resulting in an strong overestimation (see also (Wolff and Gorb, 2013)). Our own calculations on the basis of whole animal pull-off tests with the hunting spider *Cupiennius salei* (measurement data published in (Wohlfart et al., 2014)) resulted in a mean adhesive strength of 135 kPa, which is comparable to the value achieved by whip spiders. Hence, the adhesive strength of the whip spider arolium is outstanding for a membraneous pad and underlines that whip spiders are a valuable source of inspiration for the design of micro structured adhesive tapes. Further, the pad seems to be less affected by surface micro roughness than hairy adhesive pads (see (Wolff and Gorb, 2012c)), however, the sample size might be too low to generalize this observation. The lowest forces were

achieved on a comparably rough surface, which may indicate a limit in compliancy, presumably due to the hexagonal structure of the exocuticle (see also image of folding in Fig.4.7.h).

In conclusion, we found the arolium of whip spiders to be an elaborate attachment organ with high effectiveness. Furthermore, the structure and kinematics of the pretarsus is affected by the presence of an arolium. These results might potentially be interesting, not only for arachnologists and entomologists, but also for physicists, materials scientists and biomimetic engineers.

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5. Paper no.3:

The evolution of pedipalps and glandular hairs as predatory devices in harvestmen (Arachnida, Opiliones)

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Abstract

Pedipalps are the most versatile appendages of arachnids. They can be equipped with spines (Amblypygi), chelae (Scorpiones) or adhesive pads (Solifugae), modifications to grasp and handle fast moving prey. Harvestmen (Opiliones) show a high diversity of pedipalpal morphologies. Some are obviously related to prey capture, as in the enlargement and heavy spination of Laniatores pedipalps. Many Dyspnoi on the other hand exhibit thin thread-like pedipalps that are covered with complex glandular setae (clavate setae). These extrude viscoelastic glue that is used to arrest prey items. Comparable setae (plumose setae) have previously been found in representatives of both Eupnoi and Dyspnoi, yet comprehensive data on their distribution is lacking. This study examines the distribution and ultrastructure of glandular setae in harvestmen and relates it to pedipalpal morphology. Pedipalpal and setal characters are analysed in a phylogenetic framework to retrace character evolution. We found that glandular setae are synapomorphic for and widespread in the Palpatores clade (Eupnoi plus Dyspnoi). Their occurrence correlates with pedipalp morphology and feeding habit. Remnants of arthropod cuticular structures or secretions frequently found attached to glandular setae, and behavioural observations, underline their importance for capturing and securing prey. We hypothesize that glandular setae evolved as an adaptation to capture small and agile prey, which is hard to catch with a capture basket. Ultrastructural aspects indicate that they are derived sensilla chaetica, with both a secretory and sensory function. Derived ultrastructural characters of the glandular setae, which occur in some lineages, may increase their effectiveness. The functional role of further pedipalpal modifications, such as apophyses, stalked hyper-bendable joints and curved segments, as well as sexual and ontogenetic dimorphism, are discussed. Some implications of the results for the taxonomic treatment of Phalangiidae are also discussed. These results shed new light on the biology and evolutionary history of this fascinating group of arthropods.

5.1. Introduction

With about 6,500 described species (Kury, 2012), harvestmen (Opiliones) are the third most diverse order of arachnid arthropods, following mites (Acari) and spiders (Araneae). They can be found worldwide in damp and humid to semi-arid areas from subarctic regions to the wet tropics (Curtis and Machado, 2007), and up to elevations of 5540 m in the Himalayas (Martens, 1973, Swan, 1961). In both temperate and tropical regions harvestmen are an important part of the litter fauna, however, some species are also frequently found arboreally (Curtis and Machado, 2007, Burns et al., 2007). Harvestmen also utilise a broad range of food sources, from arthropods, gastropods, annelids and even small vertebrates to plant material, fungi and even detritus (reviewed in Acosta and Machado (2007)). Harvestmen are frequently encountered carrying prey; however, there are few reports on prey capture, and it was long believed that they only feed on already dead animals. Scavenging, and even kleptoparasitism, has indeed been demonstrated being part of foraging in certain harvestmen (Morse, 2001, Sabino and Gnaspini, 1999, Bristowe, 1949, Wijnhoven, 2011). However, they are also able to grasp and overcome highly mobile prey, such as collembolans, lepidopterans, hymenopterans, and dipterans, using the front legs, pedipalps and chelicerae (Gruber, 1993, Wolff et al., 2014b, Santos and Gnaspini, 2002, Immel, 1955a, Phillipson, 1960, Rühm, 1926, Bristowe, 1949, Frank, 1937).

The legs are highly elongated in numerous harvestmen, hence their alternative vernacular name of ‘daddy-long-legs’. This assists locomotion (Guffey et al., 2000, Kästner, 1931, Sensenig and Shultz, 2006), but may also help in prey capture, as they are used as feelers (second leg pair) or as an ‘aerial web’ stopping flying prey (Santos and Gnaspini, 2002). However, besides bearing a high density of sensory organs (Willemart et al., 2009, Wijnhoven, 2013, Barth and Stagl, 1976), the legs usually do not show special adaptations for prey capture.

The chelicerae are the first pair of appendages in arachnids. In harvestmen they are scissor-like and often equipped with sharp denticles. They are used to retain and dismember prey. The chelicerae can be extremely enlarged, i.e. in the genera *Ischyropsalis* (Ischyropsalididae), *Taracus* (Taracidae) and male Megalopsalidinae (Neopilionidae). At least one *Ischyropsalis* species feeds on snails and the massive chelicerae are used to break their shells (Verhoeff, 1900, Martens, 1965). However, this food source is not exploited by other species of this genus and thus cheliceral enlargement may not always be a direct adaptation for this mode of feeding (Martens, 1975), likewise in *Taracus* (Shear, 1975).

The most versatile appendages in arachnids are the pedipalps. As part of the mouth parts, they are equipped with a high number of chemosensors and used as feelers in most groups. They can be equipped with large chelae, as in scorpions and pseudoscorpions; with large and massive spines, as in amblypygids and uropygids; or with adhesive pads, as in solifuges - all modifications that assist prey capture. They can also be modified into highly complex secondary copulatory organs as in spiders (Araneae). In harvestmen the pedipalps are basically leg-like but often equipped with modifications such as apophyses or spines. The pedipalps can also be distinctly derived, as already indicated by taxonomic names such as *Nemastoma* (“thread mouth”) or *Dicranopalpus* (“two headed palp”). They can be sexually dimorphic (with male pedipalps being elongate, thickened or equipped with spines), in which case also male dimorphism may frequently occur (Buzatto and Machado, 2014). Dimorphism is related to mating, in which the pedipalp is used to grasp and secure the female at the front leg coxae (Willemart et al., 2006, Burns et al.,

2013). The pedipalps can further be differentiated between juveniles and adults (Martens, 1978, Gruber, 1993, Dahl, 1903).

Fig.5.1. Morphological variation of harvestmen pedipalps. All macro photographs taken in the field. A. *Siro acaroides* (Sironidae) of the basal most suborder Cyphopthalmi, exhibiting small, non-modified (leg-like) pedipalps. B. *Holoscotolemon querilhaci* (Cladonychiidae), a more basal lineage of the sub-order Laniatores, exhibiting massive raptorial pedipalps. C. An unidentified species of Epedanidae, a more derived lineage of Laniatores, exhibiting an extreme elongation of femur and patella, shifting the raptorial segments distally. D. *Caddo agilis* (Caddidae) the most basal lineage in the sub-order Eupnoi, exhibiting thick femoral spines and dense field of glandular setae on the pro-lateral sides of pedipalps (secretion droplets appear like whitish dew-drops between both pedipalps). E. *Platybunus* sp. (Phalangiidae) with thick femoral spines and both glandular seta bearing patellar and tibial apophyses. F. Male *Phalangium opilio* (Phalangiidae), exhibiting pedipalps with highly increased length, and all spines, apophyses and glandular setae reduced. G. Juvenile gagrellinae harvestman (Sclerosomatidae) with well-developed patellar and tibial apophyses, bearing glandular setae. H. *Sabacon viscayanus* (Sabaconidae), belonging to the sub-order Dyspnoi, showing a highly modified pedipalp with dense coverage of glandular setae and hyper-flexible patellar-tibia and tibia-tarsus joints. I. Juvenile *Mitostoma chrysomelas* (Nemastomatidae), exhibiting the typical dyspnoid 'tentacle'-pedipalp, densely covered in glandular setae. Photos C. and G. by Melvyn Yeo and I. by Jörg Pageler, with kind permission, all others by Axel Schönhofer.

Pedipalpal morphology differs between the four major clades of harvestmen. The Cyphophthalmi include close to 200 species found in disparate regions around the world (Kury, 2012). They represent the sister group of all other harvestmen (Shultz and Regier, 2001, Giribet et al., 2010) and exhibit a mite-like appearance and a leg-like pedipalp (Fig.5.1.A). The Laniatores include over 4100 species, with the highest diversity in the tropics (Kury, 2012). These harvestmen are often armoured with a thick integument and bear (often enlarged) raptorial pedipalps (Fig.5.1.B–C), which are used in prey capture (Pereira et al., 2004, Costa and Willemart, 2013) and fighting (Nazareth and Machado, 2009, Buzatto and Machado, 2008). The sister group

of Laniatores are the Palpatores (Shultz and Regier, 2001) which include two major clades, the Eupnoi (close to 1800 species found worldwide (Kury, 2012)) and the Dyspnoi (close to 300 species) which are mostly restricted to the Northern Hemisphere with the exception of the basal Acropsopilionidae (Schönhofer, 2013, Groh and Giribet, 2014). In the Eupnoi the pedipalp is often described as 'leg-like'; however, it differs distinctly from walking legs in the proportions of segments and joint positions, leading to a cramped position in rest (Fig.5.1.D,F). It may bear large apophyses, spines or denticles (Fig.5.1.D-E,G), or be highly elongated and thread-like. The Dyspnoi bear thread-like pedipalps lacking the terminal claw (with the exception of some *Sabacon* species) and with the distal segments being occasionally swollen (Fig.5.1.H-I).

A remarkable character in the Nemastomatidae and Dicranolasmatidae (Dyspnoi) is the presence of clavate ('drumstick-like') setae ('Kugelhaare') that densely cover the pedipalps. These exhibit a complex ultrastructure that supports the secretion and attachment of a glue droplet (Rimsky-Korsakow, 1924, Wachmann, 1970). This secretion shows high wetting properties and a shear thickening behaviour and is used to capture small arthropods like springtails (see ***chapter 6***). Dense fields of branching setae (so-called plumose setae or 'bottle-brush' setae) also occur in other dyspnoid harvestmen, such as Sabaconidae and Taracidae, and in many Eupnoi (Giribet et al., 2002, Gruber, 1970, Lopez et al., 1980, Wijnhoven, 2013, Shear, 1986). These may fill a similar function to the clavate setae of Nemastomatidae, though behavioural data is lacking. In *Sabacon* (Sabaconidae) the setae are also reported to be supported by both dendrites and glandular cells and to carry glue droplets (Juberthie et al., 1981). Plumose setae are also found in the eupnoid family Neopilionidae, especially the sub-family Ballarrinae, in which the pedipalps are modified in a similar manner to Dyspnoi (Hunt and Cokendolpher, 1991). The structure of plumose setae in the widespread Phalangiidae (Eupnoi) is mostly unstudied although their occurrence is repeatedly documented (Giribet et al., 2002, Shear, 1986). There are no reports of glandular setae in the eupnoid family Sclerosomatidae. Plumose setae are also reported from the Caddidae (Shear, 1996, Gruber, 1974), which have recently been found being polyphyletic with the genus *Caddo* being the sister group of all other Eupnoi (Caddidae *sensu* Groh & Giribet 2014), and *Acropsopilio* and related genera forming the sister group of Dyspnoi (Acropsopilionidae) (Groh and Giribet, 2014). Accordingly, symplesiomorphy of plumose setae was suggested for the Palpatores (Shear, 1986, Groh and Giribet, 2014, Hunt and Cokendolpher, 1991).

Shear (1986) remarked that plumose glandular setae are so wide-spread in harvestmen that their occurrence might be the plesiomorphic state of at least the Palpatores, if not of all Opiliones. However, he later described differences between plumose and clavate setae and questioned their homology (Shear, 2010). No comprehensive study on this issue has been conducted.

The scope of this study is thus to present a comparative analysis of pedipalp morphology in harvestmen with a focus on the occurrence, position and ultrastructure of clavate and plumose glandular setae. The results are mapped onto a phylogenetic framework to retrace the evolution of pedipalpal and setal characters. Sexual and ontogenetic differences in glandular seta distribution have previously been reported (Dahl, 1903, Wijnhoven, 2013, Gruber, 1993, Martens, 1978). Thus, we included pedipalps of both sexes and juveniles in the analysis where possible. Behavioural observations and high speed video recordings are performed to elucidate the use of pedipalps during prey capture and mating. We hypothesize that (1) glandular setae are widespread in the Palpatores and (2) their occurrence and position correlates with pedipalp morphology and diet, indicating an important role in capturing prey.

5.2. Material and Methods

5.2.1. Animals

Material was obtained from private and institutional collections. Abbreviations: AXLS – private collection A. Schönhofer; CAS – arachnological collection at Californian Academy of Sciences; CJM – private collection J. Martens; HW – private collection H. Wijnhoven; MS – private collection M. Seiter; SH – private collection S. Huber; WAM—Western Australian Museum; coll. – freshly collected in purpose of this study

Cyphophthalmi: **Stylocellidae**: *Meghalaya* sp. GIRIBET ET AL. 2007, Northern Myanmar, 2013 (SH); **Laniatores**: **Triaenonychidae**: *Paranonychus* sp. BRIGGS 1971, CA, USA, 2011 (AXLS); **Cladonychiidae**: *Holoscotolemon naturae* TEDESCHI & SCIAKY 1994, Trentino-Alto, Italy, 2014 (coll.); **Phalangodidae**: *Scotolemon doriae* PAVESI 1878, Genoa, Italy, 2009 (AXLS); *Sitalcina californica* BANKS 1893, Sonoma Co., CA, USA, 2011 (AXLS); **Sandokanidae**: *Gnomulus* sp., Luzon, Phillippines, 2014 (SH); **Epedanidae**: *Dibunus similis* ROEWER 1912, Cebu, Phillippines, 2011 (AXLS); **Gonyleptidae**: *Acanthopachylus aculeateus* KIRBY 1818, Salinas, Uruguay, 2012 (MS); *Pachyloides thorelli* HOLMBERG 1878, Uruguay, 2012 (MS); **Cosmetidae**: *Cynortellana quadrimaculata* GERVAIS 1844, Artemisa, Cuba, 2013 (MS); **Biantidae**: *Galibrotus* cf. *riedeli* ŠILHAVÝ 1973, Cienfuegos, Cuba, 2013 (MS); *Hinzuanius flaviventris* POCOCK 1903, Jemen, Socotra, 2010 (AXLS); **Eupnoi**: **Caddidae**: *Caddo agilis* BANKS 1892 (AXLS); **Phalangiidae**: *Amilenus aurantiacus* SIMON 1881, Postojna, Slovenia, 2006 (CJM); *Dicranopalpus ramosus* SIMON 1909, Kiel, Germany, 2013 (coll.) and Nijmegen, Netherlands, 2014 (HW); *D.* cf. *pyrenaeus* DRESCO 1948, Valle Ara, Spain, 2014 (HW); *Gyas annulatus* OLIVIER 1791, Trento, Italy, 2009 (AXLS); *Lophopilio palpinalis* HERBST 1799, Fulderaue, Germany, 2002 (AXLS); *Megabunus rhinoceros* CANESTRINI 1872, Monte Camino, Italy, 2009 (AXLS); *M. vignai* MARTENS 1978, Matto, Italy, 2011 (AXLS); *Metaphalangium cirtanum* C.L.KOCH 1839, Lesbos, Greece (HW); *Metaplatybunus* cf. *rhodiensis* ROEWER 1924, Lesbos, Greece (HW); *Mitopus morio* FABRICIUS 1779, Kiel, Germany, 2013 (coll.); *Odiellus troguloides* LUCAS 1847, Alpi Marittime, Italy, 2014 (coll.); *Oligolophus tridens* C. L. KOCH 1836, Kiel, Germany, 2014 (coll.); *Paroligolophus agrestis* MEADE 1855, Kiel, Germany, 2013 (coll.); *Phalangium opilio* LINNAEUS 1761, Mainz, Germany, 2007 (AXLS); *Rilaena triangularis* HERBST 1799, Kiel, Germany, 2013 (coll.); **Protolophidae**: *Protolophus singularis* BANKS 1893, Costa Co., CA, USA, 2011 (AXLS); **Sclerosomatidae**: *Astrobunus laevipes* CANESTRINI 1872, Mainz, Germany, 2014 (coll.); *Bullobunus bakeri* ROEWER 1926, Luzon, Phillippines, 2011 (AXLS); *Cosmobunus granarius* LUCAS 1846, Tarragona, Spain, 2010 (AXLS); *Gagrella bakeri* ROEWER 1926, Luzon, Phillippines, 1945 (CAS); *G. crassitarsis* SUZUKI 1969 cf., Champasak, Laos, 2012 (AXLS); *G. disticta* THORELL 1889 cf., Kbal Spean, Cambodia, 2012 (AXLS); *Homalenotus quadridentatus* CUVIER 1795, Hautes-Pyrénées, France, 2009; *Hypsibunus* sp., Bago, Myanmar, 2014 (AXLS); *Leiobunum limbatum* L. KOCH 1861, Mainz, Germany (AXLS); *L. rotundum* LATREILLE 1798, Kiel, Germany, 2013 (coll.); *L. socialissimum* C. KOCH 1873, Haha Mountains, Morocco, 2014 (AXLS); *L. tisciae* AVRAM 1968, Bug River Valley, Poland, 2010 (AXLS); *Metagagrella formosa* ROEWER 1911 cf., Champasak, Laos, 2012 (AXLS); *M. minax* THORELL 1889 cf., Bago, Myanmar, 2014 (AXLS); *M. nigra* ROEWER 1912, Kbal Spean, Cambodia, 2012 (AXLS); *M. sordidata* THORELL 1889 cf., Kbal Spean, Cambodia, 2012 (AXLS); *Nelima doriae* CANESTRINI 1871, Sassari, Sardinia, 2012 (AXLS); *Verpulus marginatus* ROEWER 1912, Champasak, Laos, 2012 (AXLS); *Zaleptus spinosus* ROEWER 1910 cf., Kbal Spean, Cambodia, 2012 (AXLS); **Neopilionidae**: *Ballarra longipalpis* HUNT & COKENDOLPHER 1991, Western Australia, 2013 (coll.); *Tercentenarium linnaei* TAYLOR 2008, Western Australia (WAM); *Thrasychirus gulosus* SIMON 1884, Chile (CAS); **Dyspnoi**: **Acropsopilionidae**: *Acropsopilio neozealandiae* FORSTER 1948, South-Island, New Zealand, 1990 (CJM); **Ischyropsalididae**: *Ischyropsalis carli* C. L. KOCH 1865, Valle d'Aosta, Italy, 2014 (coll.); *Ischyropsalis luteipes* SIMON 1872, Cataluña, Spain, 2009 (AXLS); **Taracidae**: *Hesperonemastoma modestum* BANKS 1894, CA, USA, 2011 (AXLS); *Taracus* sp. Modoc Co., CA, USA, 2012 (AXLS); **Sabaconidae**: *Sabacon simoni* DRESCO 1952, Alpes-Maritimes, France, 2008 (AXLS) and 2014 (coll.); *Sabacon* sp., Massif Central, France, 2014 (coll.); **Nipponopsalididae**: *Nipponopsalis yezoensis* SUZUKI 1965, Iturup Is., Russia (CJM); **Dicranolasmatidae**: *Dicranolasma pauper* DAHL 1903, Trentino-Alto, Italy, 2014 (coll.); **Trogulidae**: *Trogulus martensi* CHEMINI 1983, Ilmenaue, Germany, 2002 (AXLS); *Trogulus nepaeformis* SCOPOLI 1763, Lago di Garda, Italy, 2014 (coll.); *Trogulus tricarinatus* LINNAEUS 1767, Schwentinental, Germany, 2013 (coll.); **Nemastomatidae**: *Acromitostoma rhinoceros* ROEWER 1917, Sierro de Gredos, Spain, 1981 (CJM); *Carinostoma elegans* SØRENSEN 1894, Ossa Mountains, Greece, 2009 (AXLS); *Centetostoma dubium* MELLO-LEITÃO 1936, Languedoc-Roussellion, France, 2009 (AXLS); *C. ventalloi* MELLO-LEITÃO 1936, Catalonia,

Spain, 2009 (AXLS); *Dendrolasma mirabile* BANKS 1894, WA, USA, 2012 (AXLS); *Giljarovia tenebricosa* REDIKORZEV 1936, Zakatali, Azerbaijan, 1981 (CJM); *Histricostoma dentipalpe* AUSSERER 1867, Trentino-Alto, Italy, 2014 (coll.); *Mediostoma humerale* C.L. KOCH 1839, Petrovista, Greece, 2009 (AXLS); *M. stussineri* SIMON 1885, Nestos Canyon, Greece, 2009 (AXLS); *Mitostoma chrysomelas* HERMANN 1804, Kiel, Germany 2013, 2014 and Mainz, Germany, 2014 (coll.); *Nemastoma dentigerum* CANESTRINI 1873, Kiel, Germany 2013,2014 (coll.); *N. lugubre* MÜLLER 1776, Holstein, Germany 2013, 2014 (coll.), *Ortholasma coronadense* COCKERELL 1916, CA, USA, 2011 (AXLS); *Paranemastoma quadripunctatum* PERTY 1833, Brenner, Austria, 2014 (coll.); *P. thessalum* SIMON 1885, Ossa Mountains, Greece, 2009 (AXLS); *Pyza bosnica* ROEWER 1916, Pelister Mountain, Macedonia, 2006 (CJM); *Vestiferum alatum* MARTENS 2006, Turkey (CJM).

Light microscopical images of animals and pedipalps were made with a multifocus stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420).

5.2.2. Scanning electron microscopy

For the ultrastructural analysis specimens stored in 70% ethanol were primarily used. Pedipalps were removed using fine forceps and dehydrated in a series of increasing ethanol concentration (80%, 90%, 100% and 100% on molecular sieve), followed by critical point drying. The secretion found on clavate setae in Nemastomatidae and Dicranolasmatidae hardly dissolves in ethanol and cannot be removed by polar fluids. Thus some pedipalps of representatives of these families were ablated from individuals freshly killed with carbon dioxide and treated with acetone for 30 minutes to totally remove the lipiduous secretion. The samples were then transferred to 70% ethanol, dehydrated and critical point dried as above. All 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. In some cases samples were slightly tipped with needles to partly destroy the glandular setae, re-coated and investigated to get information on inner structure (in most cases, however, some broken setae could yet be found without treatment).

The pro-lateral sides of pedipalps were screened for glandular setae and their position documented. Certain characters of pedipalps such as spination, occurrence of apophyses, size of the tarsal claw and the shape of distal segments were recorded (see results for details). Ultrastructural details of glandular setae (proportion of plumose part, distribution and tip morphology of microtrichia, setal tip structure, inner structure of setae broken off at different parts), as well as attached remnants of arthropods were recorded (see results for details).

5.2.3. Microcomputed tomography

Pedipalps of female *Sabacon simoni* (expanded and flexed tarsus) and male *Dicranopalpus ramosus* that had been stored in 70% ethanol were dehydrated in a series of increasing ethanol concentrations and critical point dried. Dried samples were glued onto plastic pipette tips with cyanacrylate glue and scanned with a SkyScan 1172 HR micro-CT (Bruker microCT, Kontich, Belgium) with an acceleration voltage of 40 kV and a voxel size of 1 µm. 3D images were reconstructed using NRecon 1.6.6 software and processed with Amira 6.0.0.

5.2.4. Behavioural observation and High speed videography

Harvestmen collected alive 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 (*Neanura* cf. *muscorum, Tomocerus* spp., representatives of Entomobryidae and Symphypleona) collected on the campus of Kiel University. Behavior 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 500-1000 fps. For high speed videography 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.

Further, a literature survey on pedipalp usage and feeding biology was conducted and images by macro photographers recorded in the field were assessed (Melvyn Yeo, Singapore, photo-stream: https://www.flickr.com/photos/melvynyeo/sets/72157633538372937/; Jörg Pageler, Oldenburg, kindly provided; Jan van Duinen, Odoorn, kindly provided; Hans-Jürgen Thorns, Deggendorf, kindly provided). Further, image sequences of copulations recorded in captivity by Jörg Pageler (kindly provided) were analysed according to pedipalp usage.

5.2.5. Phylogenetic analysis

Pedipalpal and setal characters were mapped onto a compiled phylogenetic tree following (Hedin et al., 2012, Shultz and Regier, 2001, Schönhofer, 2013, Groh and Giribet, 2014, Giribet and Sharma, 2015). No robust phylogenetic studies exist for the Phalangiidae; the included species were grouped into current sub-families with their relationships following the suggestion by Buzatto et al. (2013). For few rare but morphologically and phylogenetically interesting taxa no material was available. To cover these for the analysis of characters, figures and descriptions additional references were assessed:

Synthetonychidae: *Synthetonychia cornua* FORSTER 1954 (Forster, 1954); **Neopilionidae**: *Americovibone lanfrancoae*, *Ballarra drosera*, *B. clancyi* and *Plesioballarra crinis* HUNT & COKENDOLPHER 1991: (Hunt and Cokendolpher, 1991); **Ischyropsalididae**: *Acuclavella merickeli* SHEAR 1986: images by Casey Richards, published on morphbank (http://www.morphbank.net); **Nipponopsalididae**: *Nipponopsalis abei* SATO & SUZUKI 1939: (Sato and Suzuki, 1939, Miyoshi, 1942), **Nemastomatidae**: *Paranemastoma bureschi* ROEWER 1926: (Mitov, 2011).

The pedipalpal characters traced were: (1) pedipalpal claw: (1a) enlargement, (1b) reduction, (1c) loss; (2) possession of spines (reinforced setae with highly elevated sockets); (3) possession of glandular setae; (4) patellar apophysis: (4a) hump-like, (4b) finger-like (elongated); (5) miniaturization of the tibia-tarsus joint; (6) tibia and tarsus significantly swollen; (7) glandular seta lacking in males; (8) glandular setae only present in juveniles.

The characters of glandular setae traced were: (9) plumose part: (9a) none (sensilla chaetica), (9b) only at tip (about 1/7 of setal length), (9c) at distal 1/4-1/3 of setal length, (9d) about half of setal length, (9e) significantly more than half of setal length, (9f) sub-apical cluster with radial arrangement (clavate setae); (10) anisotropy: (10a) none (microtrichia equally distributed around the shaft), (10b) slight, (10c) pronounced (setal 'back' free of microtrichia except at distal most part); (11) terminal lobes of microtrichia: (11a) irregular branching, (11b) primarily bifid, (11c) primarily trifid; (12) tips of terminal lobes knob-like swollen; (13) apical pore of seta with bulged brim; (14) lobes flanking the apical pore (in some specimens this character could not be evaluated because of contamination of the pore with remains of secretion): (14a) four, (14b) more than four (5-7); (15) 'backing' of seta (side directed towards the

harvestman body) with significant depression or deep trench: (15a) depression, (15b) depression with lateral bulges, (15c) trench-like invagination; (16) slit like openings of the cuticular channels to the outside.

5.3. Results

5.3.1. Pedipalp morphotypes

Pedipalpal characters of analysed species are summarized in Tab.5.1. Pedipalp morphology highly differs between taxa and may exhibit various modifications that influence its function and use. Accordingly we define the following morphotypes.

Leg-like: The pedipalp of the basal-most Cyphophthalmi (Fig.5.2.A) exhibits the lowest degree of modification. The shape and proportions of segments are rather similar to those of the walking legs (with the exception that the metatarsus is missing like in all remaining opilionids). Spines, apophyses or glandular setae are missing. The claw might be miniaturized with a knob-like tip instead of the usual hook-like shape (Fig.5.2.J). The cyphophthalmid pedipalp is rather small, but equipped with various sensory organs.

Raptorial: The raptorial morphotype is typical for the Laniatores. It is characterized by an enlarged, elongated and highly pointed claw that lacks denticles and is highly flexible against the tarsus (Fig.5.2.K). Further, the tarsus is highly flexible against the tibia by a bending in proximal portion, flattening (*Holoscotolemon*) or joint miniaturization (*Dibunus*). The pedipalp is heavily sclerotized and equipped with large spines, built by stiff pointed bristles in highly elevated sockets, forming a capture basket. The spines are most prominent on the broadened tibia and tarsus, standing in two longitudinal rows. In some cases the spines may be partially or totally reduced, as in Cosmetidae and Sandokanidae. In adult cosmetids the femur and tibia are flattened and broadened. The tibia is concave on the ventral side. The prolateral margin of the femur bears a row of strong denticles. In some laniatorean families the femur and patella may be highly elongated, shifting the raptorial clamp of tibia, tarsus and claw more distantly (Stygnidae, Biantidae, Epedanidae) (Fig.5.2.B). In some species the front legs may be involved in the capture apparatus, as indicated by equal equipment with dense, long spines (many Podoctidae, some Triaenonychidae).

Synthetonychia: This genus is considered as the basal-most lineage of Laniatores (Sharma and Giribet, 2011). Its pedipalp still lacks some characters of the raptorial morphotype. Spines are lacking (except for a tarsal spur, which is restricted to males). The claw is not as pronounced and bendable. The tibia-tarsus joint is not flattened or miniaturized, but the tarsus can be flexed against the tibia by a bent in the proximal part.

Clamp: In pedipalps of Eupnoi (except Neopilionidae: Ballarrinae), both patella and tibia are shortened and thickened. The pivot angle of the joint between them is tilted sideways, such that flexion occurs laterally, not ventrally as usual (Fig.5.3.C). There are uniquely two pairs of muscles that attach at opposite sides of the hinge, thus permitting adduction and abduction (Fig.5.3.B and Shultz, 2000). This helps to secure a food item between the pedipalps. The proximal part of the patella is bent in such a way that the joint stands in a flat angle to the tibia. The corresponding part on the femur is slanted and shifted ventrally, thus the joint stands in an angle over 100° towards the femur. This leads to a cramped position of the pedipalp in rest and a

high degree of freedom of the femur-patella joint. Thick bundles of flexor muscle fibres are present in femur and tibia (Fig.5.3.A). Extension of the femur-patella and the tibia-tarsus joint may be driven by elastic deflation of the joint membranes (Sensenig and Shultz, 2003). The tarsus is often elongated and bent ventrally (sickle-like). The femur may be equipped with spines (*raptorial-clamp*) (Fig.5.2.D, 5.6.E) or an apophysis (*Dicranopalpus* spp.) (Fig.5.2.E, 5.6.E-F) facing the tarsus in the flexed pedipalp forming a capture basket. Apophyses (hump- or finger-like outgrowths) are often present at the prolateral-dorsal side of patella, and occasionally distally on the tibia and femur, contributing to the formation of a median capture basket (Fig.5.2.D-E). The apophyses are usually densely covered in glandular setae. The modifications in segment proportions and joint peculiarities may help in generating a strong grip by the large flexor muscles in femur patella and tibia.

Tentacle: This morphotype is characterized by a lack (or at least strong reduction) of the pedipalpal claw, an elongation of the patella and a rounded distal tip of the tarsus (Fig.5.2.F,H-I,O-P). The distal segments (tibia and tarsus) may be swollen and/or curved dorsally (especially in *Sabacon*, Fig.5.1.H). The tentacle has evolved twice, in the Dyspnoi (Fig.5.2.H-I) and Eupnoi (Neopilionidae: Ballarrinae) (Fig.5.2.F), in accordance with a dense coverage in glandular setae. At least on tibia and tarsus these setae occur around the whole segment, in contrast to the clamp, where they are usually restricted to the prolateral and ventral sides. These pedipalps may be highly elongated and used as sticky whips. Glandular seta coverage may be partially (in adults) or totally reduced (Trogulidae, Ischyropsalididae), but the other mentioned characters are still developed. Very rarely spines are present on femur (*raptorial tentacle*; only in juvenile *Dicranolasma*). The tentacle in Dyspnoi and Ballarrinae differs in some points, indicating their phylogenetic burden. The patella-tibia and tibia-tarsus joint is bicondylic in the Ballarrinae with the patella-tibia joint articulating lateral, as in the clamp morphotype. The tarsus is much longer than the tibia (as for all Eupnoi), whereas proportions are reversed in the Dyspnoi. The dyspnoid tentacle exhibits unique derivations of both distal-most joints (miniaturization). The proximal, articulating part of both tibia and tarsus is shrunk to a thin stalk, which leads to a high degree of freedom of the joint. Since both joints are derived from the dicondylic to a monocondylic state, additionally to dorsal-ventral movement also torsonial movements are possible due to hyper-flexion (see ***chapter 7***). In *Sabacon* the large tarsal flexor muscle is attached to a sclerite that is embedded in the joint membrane. Its retraction leads to a rapid inversion of the membrane and flexion of the tarsus in less than 10 milli-seconds, which is used as a snap-trap during prey capture (see ***chapter 7***).

Tab.5.1. Characters of pedipalps in harvestmen. All characters refer to adult females (except sexual dimorphism characters) and characters of Dicranolasma pauper juvenile. For detailed description and analysis of characters, see main text. Abbreviations: rel. len., relative pedipalp length (pedipalp length divided by body length); cx, coxa; tr, trochanter; fe, femur; pa, patella; ti, tibia; ta, tarsus; prox, proximal part; dist, distal part; dist. seg. thick., distal segments considerably thickened; elo., considerable elongation of pedipalps in males; g.s.-, lack of glandular setae in males (replaced by sensilla chaetica). [1] based on microscopical images, drawings and descriptions from Forster, 1954; [2] based on microscopical images, drawings and descriptions from Hunt & Cokendolpher, 1975; [3] based on microscopical images by Casey Richards, published on morphbank :: biological imaging; [4] based on drawings and descriptions by Sato & Suzuki, 1939; [5] based on drawings and descriptions by Miyoshi, 1942; [6] based on microscopical images from Mitov, 2011.

	rel. len.	type	spines						Glandular setae						apophysis			claw	stalked		Segment bent					dist. seg. thick	Sexual dimorphism	
			cx	tr	fe	pa	ti	ta	tr	fe	pa	ti	ta prox	ta dist	fe	pa	ti		ti	ta	ventrally fe	ventrally ti	ventrally ta	dorsally ti	dorsally ta		elo	g.s.-
Cyphophthalmi																												
Stylocellidae																												
Meghalaya sp.	0.6	leg-like	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	?	
Laniatores																												
Synthetonychiidae																												
Synthetonychia ramosa	1.6	(raptorial)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	+	
Triaenonychidae																												
Paranonychus sp.	0.7	raptorial	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	
Cladonychiidae																												
Holoscotolemon naturae	1.2	raptorial	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	+	-	
Phalangodidae																												
Scotolemon doriae	1.2	raptorial	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	+	-	
Sitalcina californica	1.3	raptorial	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	+	-	
Sandokanidae																												
Gnomulus sp.	0.5	(raptorial)	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	L	-	-	-	-	-	-	-	-	-	
Epedanidae																												
Dibunus similis	2.3	raptorial	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	L	+	+	+	-	-	-	-	+	?	
Gonyleptidae																												
Acanthopachylus aculeateus	0.5	raptorial	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	+	?	
Pachyloides thorelli	0.9	raptorial	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	L	-	-	-	+	-	-	-	+	?	
Cosmetidae																												
Cynortella quadrimaculata	0.8	(raptorial)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	+	-	-	-	-	?	
C. quadrimaculata juvenile	1.6		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mod	-	-	-	-	+	-	-	-		
Biantidae																												
Hinzuanius flaviventris	1.3	raptorial	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	L	+	+	-	-	-	-	-	+	?	
Galibrotus cf. *riedeli*	1.6	raptorial	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	L	+	+	-	-	-	-	-	+		
Eupnoi																												
Caddidae																												
Caddo agilis	0.8	rapt. clamp	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	M	-	-	-	+	+	-	-	-	-	-
Phalangiidae																												
Lophopilio palpinalis	0.7	rapt. clamp	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	M	-	-	+	-	-	-	-	-	-	-
Megabunus rhinoceros	0.8	rapt. clamp	+	+	+	-	+	+	-	-	+	+	+	+	(+)	+	+	M	-	-	-	-	-	-	-	-	-	-
Metaplatybunus cf. rhodiensis	0.7	clamp	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	M	-	-	+	-	+	-	-	-	-	-
Mitopus morio	1.1	clamp	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	M	-	-	-	-	+	-	-	-	-	-
Oligolophus tridens	0.8	clamp	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	M	-	-	-	-	-	-	-	-	-	-
Paroligolophus agrestis	0.8	clamp	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	M	-	-	-	-	-	-	-	-	-	-
Odiellus troguloides	0.5	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	M	-	-	-	-	-	-	-	-	-	
Metaphalangium cirtanum	0.7	clamp	-	-	-	-	-	-	-	+	+	+	+	-	(+)	+	-	M	-	-	+	-	+	-	-	-	-	-
Phalangium opilio	0.7	clamp	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	M	-	-	-	-	+	-	-	-	+	+
Rilaena triangularis	0.7	rapt. clamp	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	M	-	-	-	-	+	-	-	-	-	+
Amilenus aurantiaticus	0.9	clamp	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	M	-	-	-	-	-	-	-	-	-	+
Dicranopalpus ramosus	1.6	clamp	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	M	-	-	-	-	+	-	-	-	-	+
D. cf. *pyrenaeus*		clamp	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	M	-	-	-	-	-	-	-	-	-	+
D. cf. *pyrenaeus* juvenile	1.4	rapt. clamp	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	M	-	-	-	-	-	-	-	-		
Gyas annulatus	0.8	clamp	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	M	-	-	-	-	+	-	-	-	-	+
Protolophidae																												
Protolophus singularis	0.9	clamp	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	M	-	-	+	-	-	-	-	-	+	+
Sclerosomatidae																												
Astrobunus laevipes	0.8	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-		
Homalenotus quadridentatus	0.3	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-
Leiobunum limbatum	0.7	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	+	-	-	-	-	(+)
L. rotundum	0.7	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	+	-	-	-	-	(+)
Nelima semproni	0.8	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	(+)
Cosmobunus granarius	0.7	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	+	-	-	-	-	-
Gagrella bakeri	0.6	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	

G. crassitarsus cf.	0.8	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	
G. disticta cf.	0.8	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	M	-	-	-	-	+	-	-	-	-	
G. disticta cf. juvenile	0.8	clamp	-	-	-	-	-	-	-	+	+	+	-	-	-	(+)	-	M	-	-	-	-	-	-	-	-		
Bullobunus bakeri	0.7	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	M	-	-	-	-	-	-	-	-	?	
Hypsibunus sp.	0.9	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	+	-	-	-	?	
Metagagrella formosa cf.	0.5	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	
M. minax cf.	0.9	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	M	-	-	-	-	+	-	-	-	-	
M. minax cf. juvenile	1.1	clamp	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	M	-	-	-	-	-	-	-	-		
M. sordidata cf.	0.9	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	M	-	-	-	-	+	-	-	-	-	
Verpulus marginatus cf.	0.9	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	M	-	-	-	-	-	-	-	-	-	
Zaleptus spinosus cf.	1.3	clamp	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	M	-	-	-	-	-	-	-	-	-	
Neopilionidae																												
Tercentenarium linnaei	1.4	clamp	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	M	-	-	-	-	+	-	-	-	-	-
Thrasychirus gulosus	1.3	clamp	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	M	-	-	-	-	-	-	-	-	?	?
Americovibone lanfrancoae [2]	3.4	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Plesioballarra crinis [2]	1.5	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	M	-	-	-	-	-	-	-	+	-	-
Ballarra drosera [2]	1.5	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	S	-	-	-	-	-	-	-	-	-	-
B. clancyi [2]	2.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
B. longipalpis	2.7	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-
Dyspnoi																												
Acropsopilionidae																												
Acropsopilio neozealandiae	1.2	rapt. clamp	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	S	-	-	-	-	-	-	-	-	-	-
Ischyropsalididae																												
Acuclavella merickeli [3]	0.6	tentacle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
Ischyropsalis luteipes	1.1	tentacle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
Taracidae																												
Hesperonemastoma modestum	1.5	tentacle	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Taracus sp.	1.7	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Sabaconidae																												
Sabacon simoni	1.3	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	+	-	+	-	-
Nipponsalididae																												
Nipponopsalis abei [4]		tentacle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	
N. abei juvenile [5]		tentacle	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-		
Dicranolasmatidae																												
Dicranolasma pauper	0.7	tentacle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	
D. pauper juvenile	1.2	rapt. tenta.	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-		
Trogulidae																												
Trogulus martensi	0.3	tentacle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Nemastomatidae																												
Ortholasma coronadense	0.8	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	(+)	-	-
Dendrolasma mirabele	0.7	tentacle	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Mitostoma chrysomelas	1.8	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Paranemastoma quadripunctata	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
P. thessalum		tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-
P. bureschi [6]		tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	+	-	-	-	?	-
Carinostoma elegans	1.3	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Histricostoma dentipalpe	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Acromitostoma rhinoceros	1.0	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	(+)	-	-
Centetostoma ventalloi	0.8	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
C. dubium	1.7	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Pyza bosnica	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-
Vestiferum alatum	1.0	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Mediostoma humerale	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Nemastoma lugubre	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	(+)	-	-	-	-
N. dentigerum	1.1	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Giljarovia tenebricosa	1.2	tentacle	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-

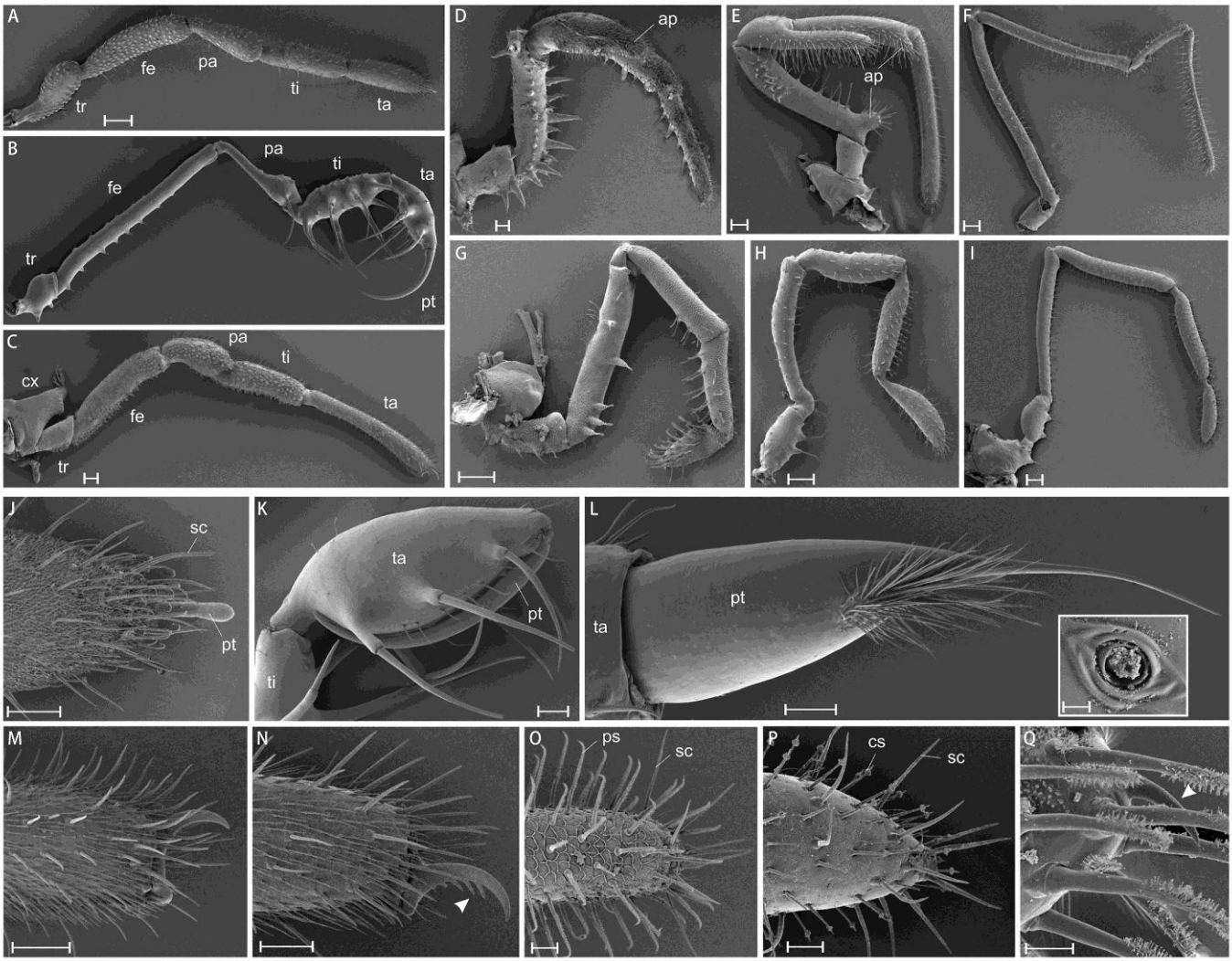


Fig.5.2. Pedipalpal characters. Scanning electron micrograph of harvestmen pedipalps. Cyphophthalmi: A. *Meghalaya* sp. (Stylocellidae). Laniatores: B. *Galibrotus* cf. *riedeli* (Biantidae). Eupnoi: C. *Gagrella* cf. *disticta* (Sclerosomatidae). D. *Megabunus rhinoceros* (Phalangiidae); E. *Dicranopalpus* cf. *pyrenaeus* (Phalangiidae), juvenile; F. *Ballarra longipalpis* (Neopilionidae). Dyspnoi: G. *Acropsopilio neozealandiae* (Acropsopilionidae); H. *Dendrolasma mirabile* (Nemastomatidae); I. *Mediostoma stussineri* (Nemastomatidae). Condition of tarsal tip and pretarsus (claw): J. *Meghalaya* sp., claw reduced, knob-like tip. K. *Dibunus similis* (Dibunidae), enlarged raptorial claw (here flexed condition). L. *Cynortellana quadrimaculata* (Cosmetidae), juvenile, modified pretarsus, inset shows one of numerous pores at the bulbous region. M. *Phalangium opilio* (Phalangiidae), smooth claw. N. *Amilenus aurantiacus* (Phalangiidae), pectinate claw (arrowhead). O. *Ballarra longipalpis*, claw totally reduced, tarsal tip rounded. P. *Mediostoma stussineri*, claw totally reduced, tarsal tip rounded. Q. *A. neozealandiae*, claw highly but not totally reduced (arrowhead). Abbreviations: ap, apophysis; cs, clavate seta; cx, coxa; fe, femur; pa, patella; pt, pretarsus (claw); sc, sensilla chaetica; ta, tarsus; ti, tibia; tr, trochanter. Scale bar A-I,K,M 100 µm, J,N-P 30 µm, L 50 µm (inset 1µm),Q 10 µm

Acropsopilio (Acropsopilionidae): This genus is considered sister group to the Dyspnoi (Groh and Giribet, 2014), exhibits an intermediate form between *clamp* and *tentacle* (Fig.5.2.G). The claw is already reduced, but not totally absent as in Dyspnoi *sensu stricto* (Fig.5.2.Q). The tarsus is shortened and the patella elongated, but the patella-tibia joint still exhibits a low degree of freedom. At the femur there are some spines, which– unlike the basal Eupnoi – are modified glandular setae.

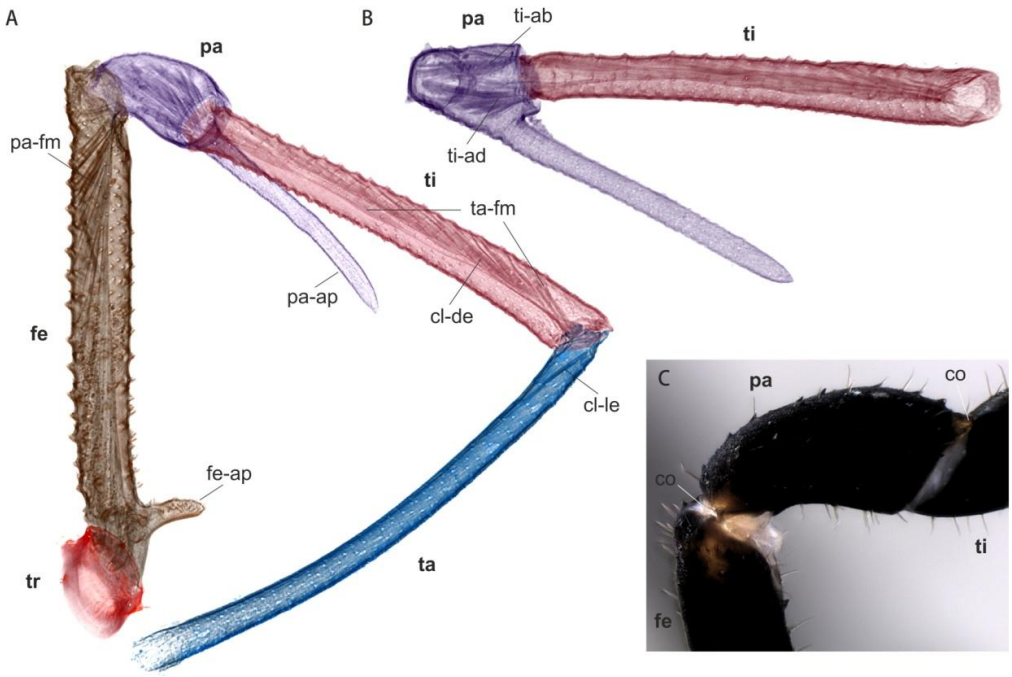


Fig.5.3. Joint and muscle modifications in the eupnoid clamp morphotype. A-B. 3D reconstruction from µCT images, with different segments differentially coloured. Joints in arachnid appendages are usually only operated by flexor muscles and extension is achieved by elastic trans-articular sclerites or internal hemolymph pressure. The pivot of patella-tibia joint is tilted, such that it articulates laterally. This joint is controlled by two pairs of opposing muscles. C. Patellar joints in *Metagagrella formosa* cf. (Sclerosomatidae). cl-de, claw depressor muscle, cl-le, claw levator muscle, co, condyle, fe, femur, pa, patella, fe-ap, femoral apophysis, pa-ap, patellar apophysis, pa-fl, patella flexor muscle, ta, tarsus, ta-fm, tarsus flexor muscle, ti, tibia, ti-ab, tibia abductor muscle, ti-ad, tibia adductor muscle, tr, trochanter.

5.3.2. Relative pedipalp length

There is a negative allometry between body length and relative pedipalp length (pedipalp length per body length) in Palpatores (Fig.5.4). This relationship was found both between species and different instars of the same species. Relative pedipalp length is significantly higher in pedipalps with glandular setae than in those lacking glandular setae.

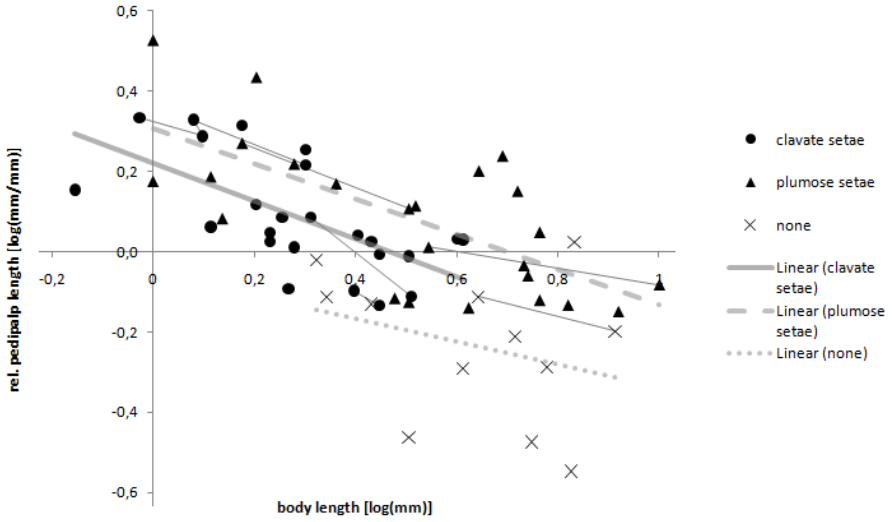


Fig.5.4. Negative allometry of pedipalpal length. Plot of relative pedipalp length in relation to body length in Palpatores for species lacking glandular setae (none), with plumose setae and with clavate setae. Thin lines between symbols mark different stages of the same species.

5.3.3. Ontogenetic variation

Laniatores: In Cosmetidae femur and tibia are flattened in adults (Fig.5.5.B). In juveniles, the pedipalps are relatively longer and cylindrical (Fig.5.5.A). The pretarsus (claw) is modified, and is soft, bulbous and equipped with a ventral dense brush of smooth setae and numerous pores (Fig.5.2.L). The pores are present all over distal patella, tarsus and prestarsus and often contain residuals of an amorphous secretion. Accordingly, in living cosmetid nymphs a sticky mucous was observed covering the distal pedipalp (G. Machado, pers. comm.). The apical tip is long and filamentous, and a sclerotized claw is lacking.

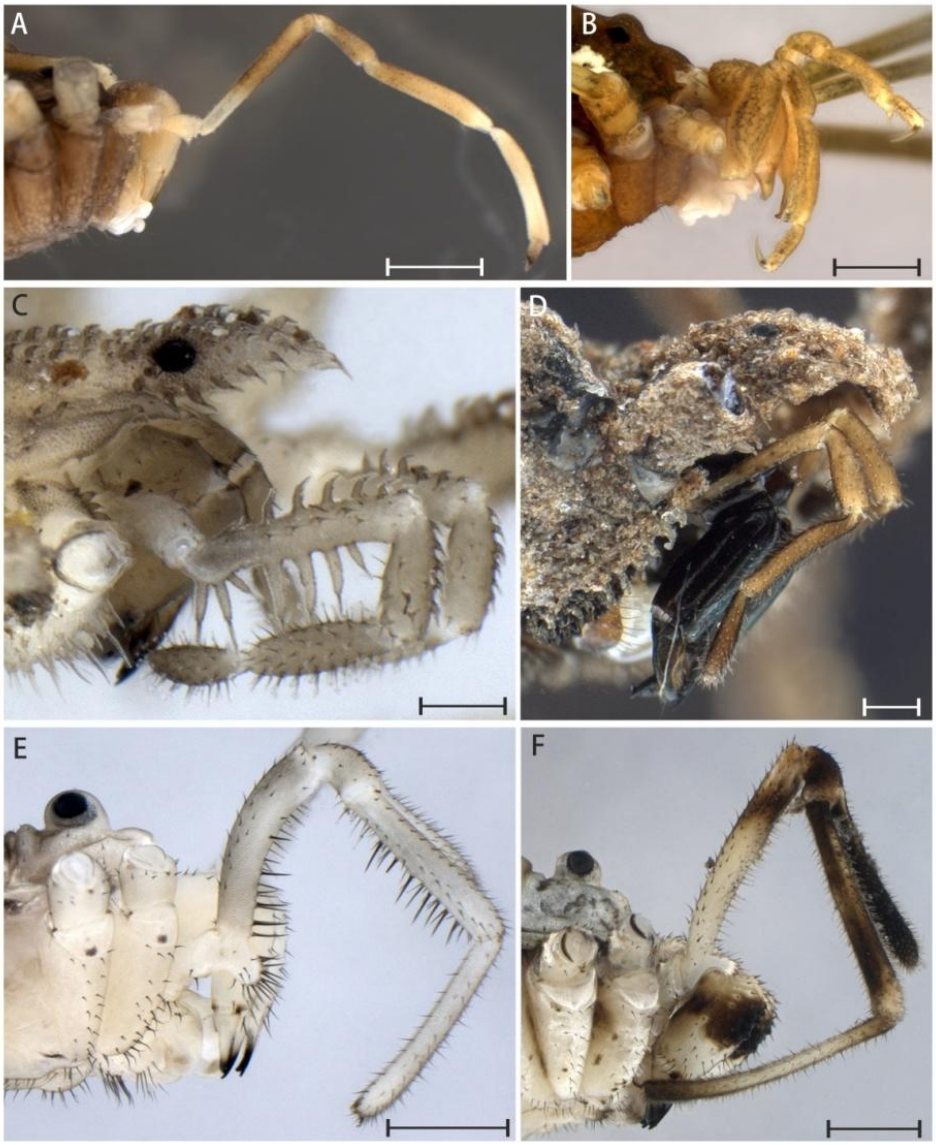


Fig.5.5. Ontogenetic dimorphism of pedipalps. A-B. *Cynortella quadrimaculata* (Cosmetidae), A. juvenile exhibiting elongated cylindrical pedipalps with modified claw, B. adult female, exhibiting shortened pedipalps with flattened tibia and raptorial claw. C-D. *Dicranolasma pauper* (Dicranolasmatidae), C. juvenile, exhibiting spines on femur and clavate setae on patella, tibia and tarsus, D. adult female, exhibiting a relatively shorter pedipalp, lacking spines and clavate setae. E-F. *Dicranopalpus* spp. (Phalangiidae), E. juvenile of *D.* cf. *pyrenaeus*, with spine-like elongated plumose setae on femur and tibia (black color), F. adult female of *D. ramosus*, lacking the spine-like plumose setae.

Eupnoi: In Gagrellinae (Sclerosomatidae) the prolateral and ventral sides of pedipalps in adults are armed with thick denticles which are absent in juveniles (Fig.5.6.B,D). In some species (*Gagrella disticta* cf., Fig.5.6.C and *Metagagrella* cf. *minax*, Fig.5.6.A) the juveniles bear

glandular setae in similar places which are absent in adults (partly they are substituted by sensilla chaetica). In *M.* cf. *minax* the juvenile pedipalp is equipped with an elongate patellar apophysis and a hump-like tibial apophysis. The latter is totally absent in the adult and the patellar apophysis is reduced in size (much shorter and thinner). Similar apophyses have previously been reported from juvenile *Bastioides coxopunctatus* (Tourinho, 2003), but also adult *Rongsharia* spp. (Martens, 1982). Glandular setae were not found in juveniles of *Gagrella crassitarsis* and *Hypsibunus* sp. In the latter species the sensilla chaetica on the patella differed in juveniles, having a flattened shaft.

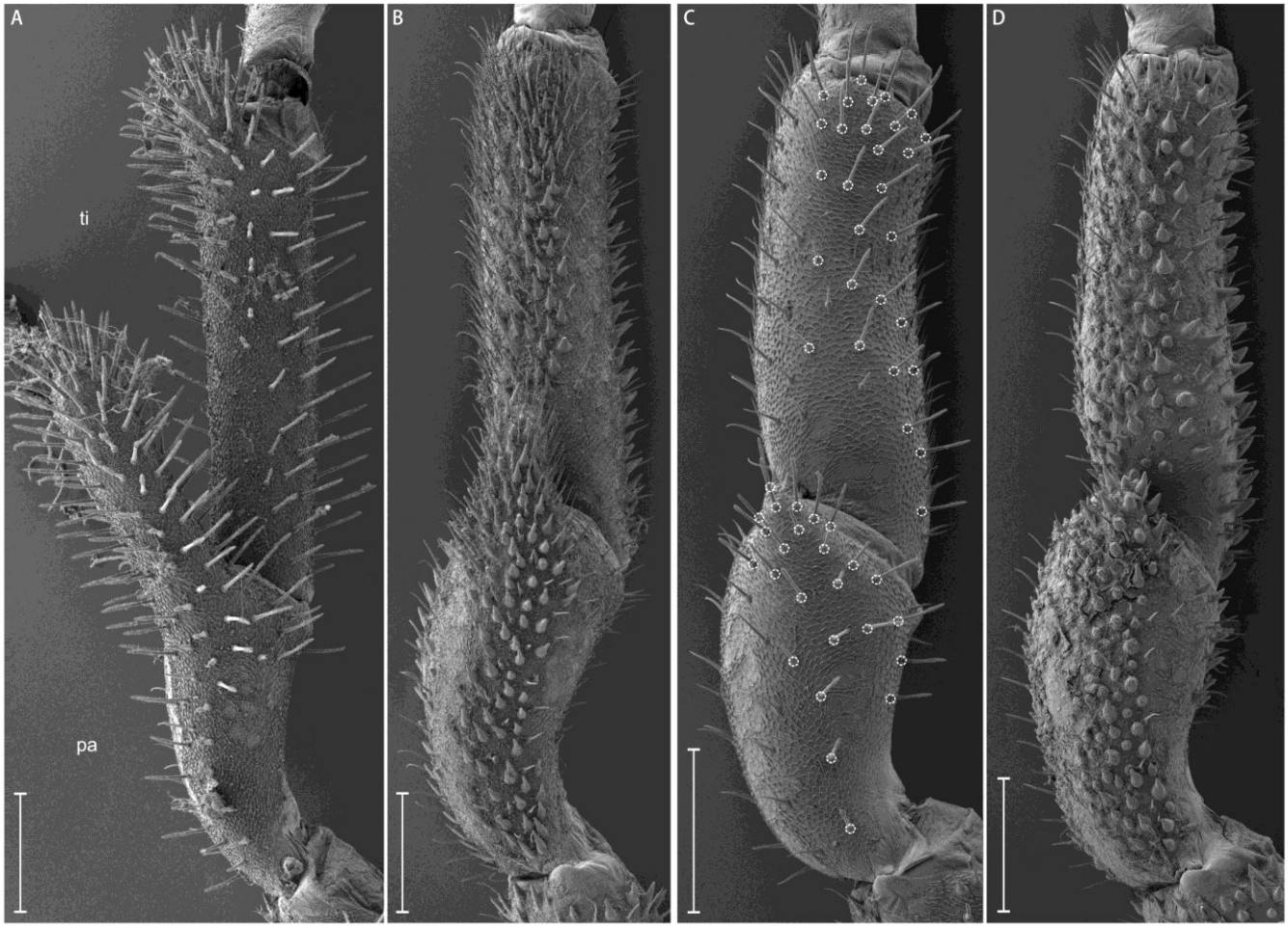


Fig.5.6. Ontogenetic dimorphism in Gagrellinae. Scanning electron micrographs of pedipalp patella and tibia. A-B. *Metagagrella minax* cf., A. juvenile with a large patellar and a small tibial apophysis and plumose setae, B. adult female with reduced patellar apophysis and lacking tibial apophysis, glandular setae lacking, strong denticles present. C-D. *Gagrella disticta* cf., C. juvenile, with plumose setae (marked with circles), D. adult female, with denticles. Abbreviations: pa, patella; ti, tibia. Scale bars: 200 µm.

In *Phalangium opilio* (Phalangiidae) the glandular setal coverage is highly reduced in adults (only a few on the patella in females and totally absent in males). In juveniles of *Dicranopalpus* some glandular setae are modified as "spines": The sclerotized shaft is highly elongated and the plumose part is reduced. Those spine-like glandular setae are present on the venter of the femur and tibia, standing in a longitudinal row, and on the proximal femoral apophysis (Fig.5.5.E). In *D. ramosus* the hump-like apophysis on the distal tibia is reduced in adults.

Dyspnoi: Juvenile stages in the Dicranolasmatidae have glandular clavate setae that are lost in adults (Fig.5.5.D). The juveniles further bear strong spine-tipped tubercles on the femur (Fig.5.5.C). A comparable ontogenetic variation has been reported for *Nipponopsalis abei* (Nipponopsalididae), with clavate setae only present in juveniles (Miyoshi, 1942). The ontogenetic dimorphism of glandular seta possession may be synapomorphic for the lineage including Nipponopsalididae, Dicranolasmatidae and Nemastomatidae. In *Ortholasma* and *Dendrolasma* (Ortholasmatinae), which show a head capsule like Dicranolasmatidae and Trogulidae, the number of clavate setae is reduced (but not totally) in adults (Shear and Gruber, 1983).

5.3.4. Sexual dimorphism

Laniatores: Sexual dimorphism of the pedipalp is present in most Laniatores. In males the pedipalpal segments are often thicker and equipped with a higher number of spines (Kury, 2007). In the basal Synthetonychidae male pedipalps are much longer and bear a thick spine in the distal tarsus forming pliers together with the claw (Forster, 1954). In Sandokanidae, which lack spines in their pedipalps, nipple-like apophyses are present in the trochanter and the proximal part of femur, which are more developed in males.

Eupnoi: In some Phalangiidae (see Tab.5.1) males lack the glandular setae, which are then replaced by sensilla chaetica (Fig.5.7.B,E,H). In *Dicranopalpus* spp. the patellar apophysis is also much thinner in males (Fig.5.7.H). In *Phalangium opilio* males the pedipalps are also highly elongated (up to three times female pedipalp length).

The Protolophidae exhibit the most extreme sexual dimorphism. The female exhibits a typical, slender clamp-pedipalp with a finger-like elongated patellar apophysis and dense coverage of glandular setae. The male pedipalp is massive and swollen, and equipped with longitudinal rows of heavy denticles at trochanter, femur and tarsus. Femur and tibia are curved ventrally and form a strong clasp against each other.

In Sclerosomatidae, the pedipalps of males are often more massive and more strongly denticulate. Some species of *Leiobunum* also have curved segments or heavy apophyses, forming a clasp against a flattened part of an opposing segment, a trait that is associated with antagonistic mating behaviour (Hedin et al., 2012, Burns et al., 2013). In some Gagrellinae the tarsi of male pedipalps are swollen and bear a ventral row of denticles.

Dyspnoi: We found a marked sexual dimorphism in pedipalpal length in *Paranemastoma thessalum* (Nemastomatidae) with males having longer pedipalps. This elongation, however, was highly variable within the specimen assemblage and in some males the pedipalp was not elongated at all. Such variation has previously been reported for *P. superbum* (Martens, 2006), but in other congeneric species this sexual dimorphism was not found. In highly elongate pedipalps, glandular setae were less densely distributed but never absent.

In many Asian *Sabacon*-species (Sabaconidae), the pedipalp of males bears medio-distal femoral denticles and is less swollen than in juveniles and females, thus being more nemastomatid-like (Suzuki, 1974, Martens, 2015).

In *Nipponopsalis yezoensis* (Nipponopsalididae), the tibia and tarsus are slightly swollen and flattened in males, forming a clasper. This sexual dimorphism is only known from this particular species of this genus (Suzuki, 1958). Glandular setae are absent in both males and females.

In males of *Nemastoma dentigerum* (Nemastomatidae), the femur is swollen and curved ventrally and distally bears a thorn, which, however, has also occasionally been found in 'andromorphic' females (Wijnhoven, 2014). A medio-distal femoral thorn, in several species movable, is present in *Centetostoma ventalloi*, single *Nemastomella* and all *Histricostoma* species.

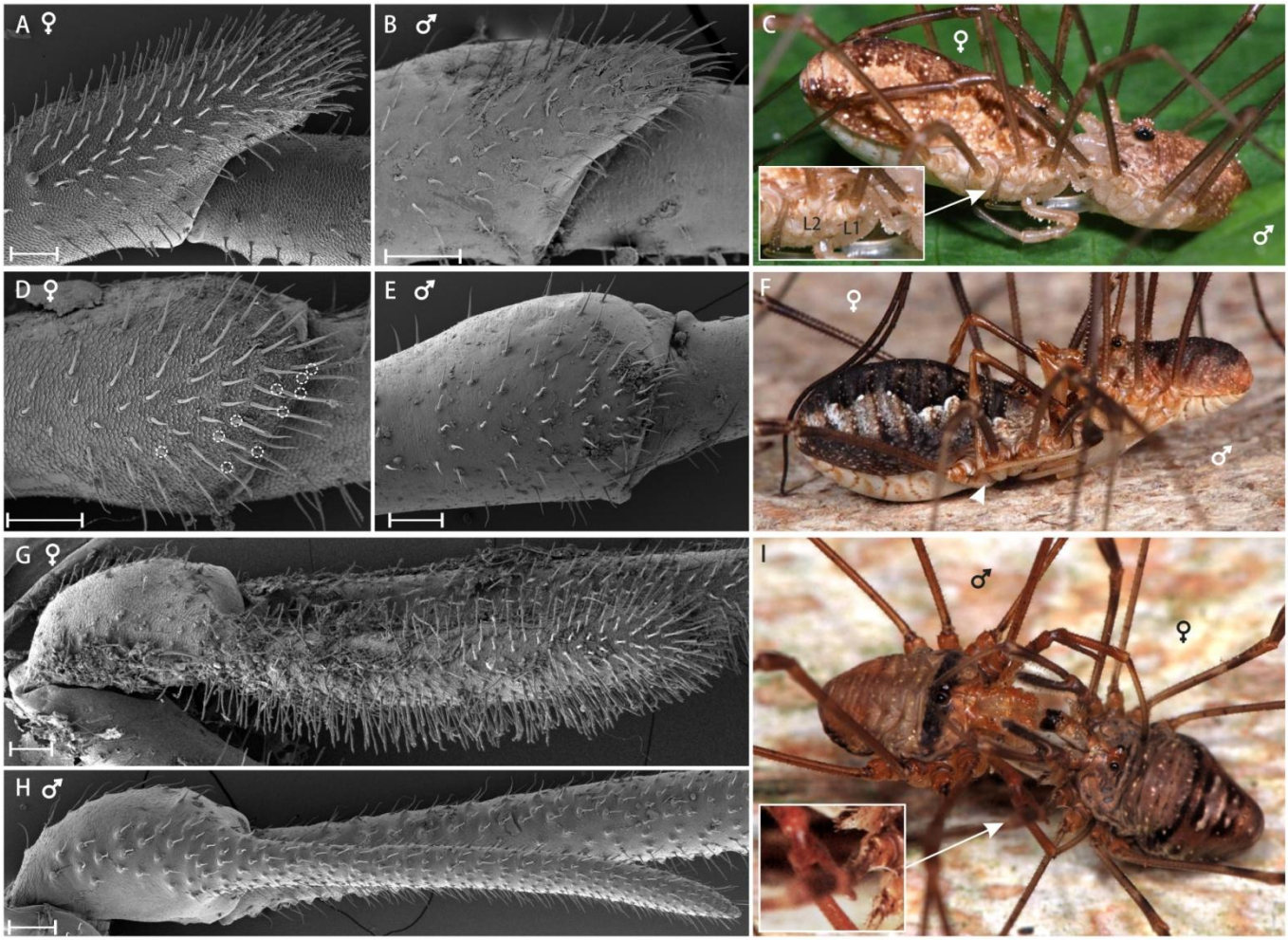
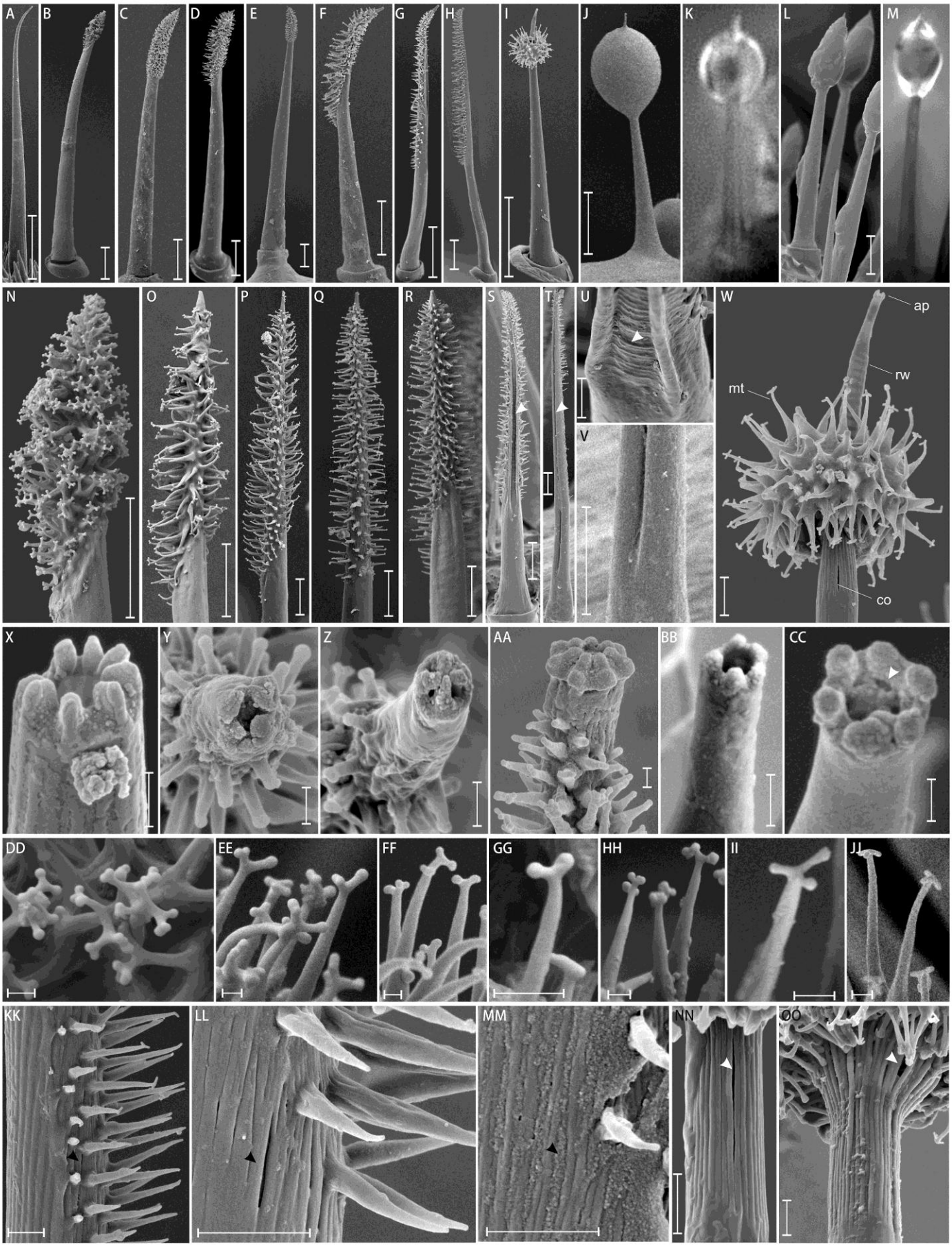


Fig.5.7. Sexual dimorphism of glandular seta possession and mating posture in Phalangiidae. SEM images show the prolateral part of distal tibia, left female (basic), right male (modified). Photographs from copulations in captivity, insets show magnified detail of use of male pedipalp in female leg grasping. A-C. *Rilaena triangularis*. D-F. *Phalangium opilio*. Female plumose setae marked by circles. G-I. *Dicranopalpus ramosus*. Scale bars 100 µm. Photographs by Jörg Pageler, with kind permission.

5.3.5. Ultrastructure and classification of glandular setae

Glandular setae: Setae that extrude a viscous secretion which accumulates at the distal end, forming a droplet (Fig.5.8.J-M). The distal end (plumose section) is provided with microtrichia (plumes), with a broad basis and tapering towards the tip, which branches rectangularly. The shaft is sclerotized (dark coloured), rigid and slightly corrugated (the ridges get more distinct towards the plumose part and may separate, as being the case in clavate setae). It contains thin tubular channels situated in the interface between exo- and epicuticle (Fig.5.9.C,D,H). The channels have been shown to contain the secretory fluid and may have pores to the setal lumen or the outside (Wachmann, 1970). The plumose section is less sclerotized and appears white or translucent in the light microscope. The cuticle is rather thin in this part, but equipped with parallel thick rings (Fig.5.9.D,E,G) which allow a certain degree of bending. The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plumose section begins rather suddenly and is marked by a slight depression of the cuticle below the developing microtrichia. The setal tip is free of microtrichia and shows a distinct pore, which is often flanked by lobe-like protuberances of the epicuticle. The tip usually protrudes from the secretion droplet. Glandular setae are accompanied by secretory and dendritic cells (this was not proven for every type studied here, but presumed, as shown for both plumose (Juberthie et al., 1981) and clavate setae (Rimsky-Korsakow, 1924, Wachmann, 1970) before). Dendrites extend into the seta up to the tip, surrounded by a thin cuticular shell (Fig.5.9.B,J). All characters except the possession of microtrichia are shared with *sensilla chaetica* (Figs.5.8.A,X, 5.9.A, 5.15). These are sensory setae that are easily recognized as they are usually much longer than the surrounding setae. Sensilla chaetica were previously hypothesized to be both contact chemoreceptors and mechanoreceptors (Spicer, 1987).

Glandular setal characters of analysed species are summarized in Tab.5.2 and visualised in Figs.5.8, 5.9 and 5.13. The glandular setae can be classified as follows by distinct microstructural characters that may determine their functional and mechanical properties, particularly the dimension and isotropy of the plumose part, the structure of microtrichial tips and the presence of channel openings.

Plumose setae Type 1 (ps1): In the ps1-type the plumose section is restricted to the distal seventh of the setal length, with its broadest width in the middle (Fig.5.8.B,N). It shows slight anisotropy. The microtrichia branch irregularly and repeatedly (Figs.5.8.DD, 5.9.K). The branches end in a knob-like swollen tip. The apical pore is flanked by four lobes (Fig.5.8.Y). Channel

Fig.5.8. Characters of glandular setae. Scanning electron micrographs (of critical point dried ethanol conserved material, unless stated otherwise). A. Sensilla chaetica, Stylocellidae. Plumose setae: B. *Caddo agilis* (Caddidae); C. *Lophopilio palpinalis* (Phalangiidae); D. *Metaplatybunus* cf. *rhodiensis* (Phalangiidae); E. *Dicranopalpus* cf. *pyrenaeus* (Phalangiidae), juvenile, spine-like plumose seta; F. *Thrasychirus gulosus* (Neopilionidae); G. *Ballarra longipalpis* (Neopilionidae); H. *Sabacon* sp. (Sabaconidae). Clavate setae: I. *Mitostoma chrysomelas* (Nemastomatidae), secretion removed with acetone; J. Same as in I, but with secretion, Cryo-SEM image; K. *Nemastoma lugubre*, fresh, stereo microscope image. L. Air dried plumose seta of *Oligolophus tridens* (Phalangiidae) with secretion droplet. M. Plumose seta of *Dicranopalpus ramosus*, fresh, stereo microscope image. Plumose head of setae showing slight anisotropy, lateral view: N. *C. agilis*; O. *O. tridens*; P. *D. ramosus*. Q. *Protolophus singularis* (Protolophidae), seta showing no anisotropy. Backing of anisotropic setae: R. *M.* cf. *rhodiensis*, with slight basal depression; S. *B. longipalpis*, with deep depression (arrowhead) along nearly the whole plumose part; T. *Sabacon* sp., with deep trench (arrowhead) along nearly the whole plumose part; U. Detail of the depression in its basal part, in *B. longipalpis*, inner ringed walls visible (arrowhead); V. Detail of the trench in its basal part, in *Sabacon* sp. W. Plumose head of clavate seta, in *M. chrysomelas*, basal channel opening slits, inner ringed walls in the tip and tip lobes visible. States of apical tip opening: X. Sensillum chaeticum, *M. chrysomelas*, 8 lobes; Y. Plumose seta, *C. agilis*, with 4 short lobes; Z. *B. longipalpis*, with 4 long lobes; AA. *Sabacon* sp., with 7 lobes and swollen brim; BB-CC. Clavate seta, *M. chrysomelas*, BB. with 5 lobes, CC. with 6 lobes, radial pore openings (arrowhead) and median dendrite visible. States of microtrichial tips: DD. *C. agilis*, multiple irregularly branched tips with knob-like endings; EE. *L. palpinalis*, irregularly branched tips with knob-like endings; FF. *Mitopus morio* (Phalangiidae), predominantly bifid tips with knob-like endings; GG. *O. tridens*, bifid tip with knob-like endings; HH, Juvenile *Metagagrella minax* cf. (Sclerosomatidae), short irregular branches with knob-like endings; II. *Hesperonemastoma modestum* (Taracidae), trifid tip with slightly swollen endings; JJ. *M. chrysomelas*, trifid tips with smooth endings. Lateral slit-like channel openings: KK-LL. Detail of anisotropic plumose seta in *Sabacon* sp., channel openings (arrowhead) only on microtrichious side; MM. same as in LL, but with secretion remnant in the grooves (arrowhead); NN-OO. Detail of plumose seta, channel openings (arrowhead) radial around the shaft, widening towards the microtrichious part, NN. Juvenile *Dicranolasma pauper* (Dicranolasmatidae), OO. *Paranemastoma quadripunctatum* (Nemastomatidae). Abbreviations: ap, apical pore; co, slit-like channel openings; mt, microtrichium; rw, inner ringed walls. Scale bars: A-L. 10 µm; N-T,V. 5 µm; U,W,KK-OO. 1 µm; X-BB,DD-JJ. 250 nm; CC. 100 nm.

openings were not found. This type is only found in *Caddo* (Eupnoi: Caddidae) and considered the basal-most type of glandular setae.

Plumose setae Type 2 (ps2): The ps2-type is rather similar to the ps1-type, but the plumose section is longer and more slender (Fig.5.8.C,D), and the microtrichial tips are less extensively branched. The plumose section occupies one fourth to one third of the setal length and has its longest microtrichia in the proximal third (Fig.5.8.O,P). It shows slight anisotropy. The short backing at the basal part, which is free of microtrichia, shows a slight depression where the cuticle is thinner, such that the inner rings become apparent (Fig.5.8.R). The depression begins gradually below the plumose section. The microtrichia tips are usually bifid (Fig.5.8.GG) but may also be irregularly branched (Fig.5.8.EE,FF) or, in rare cases, trifid. The apical pore is flanked by four lobes. Channel openings were not found. This type is typical for the Phalangiidae but also found within Neopilionidae (*Ballarra drosera*).

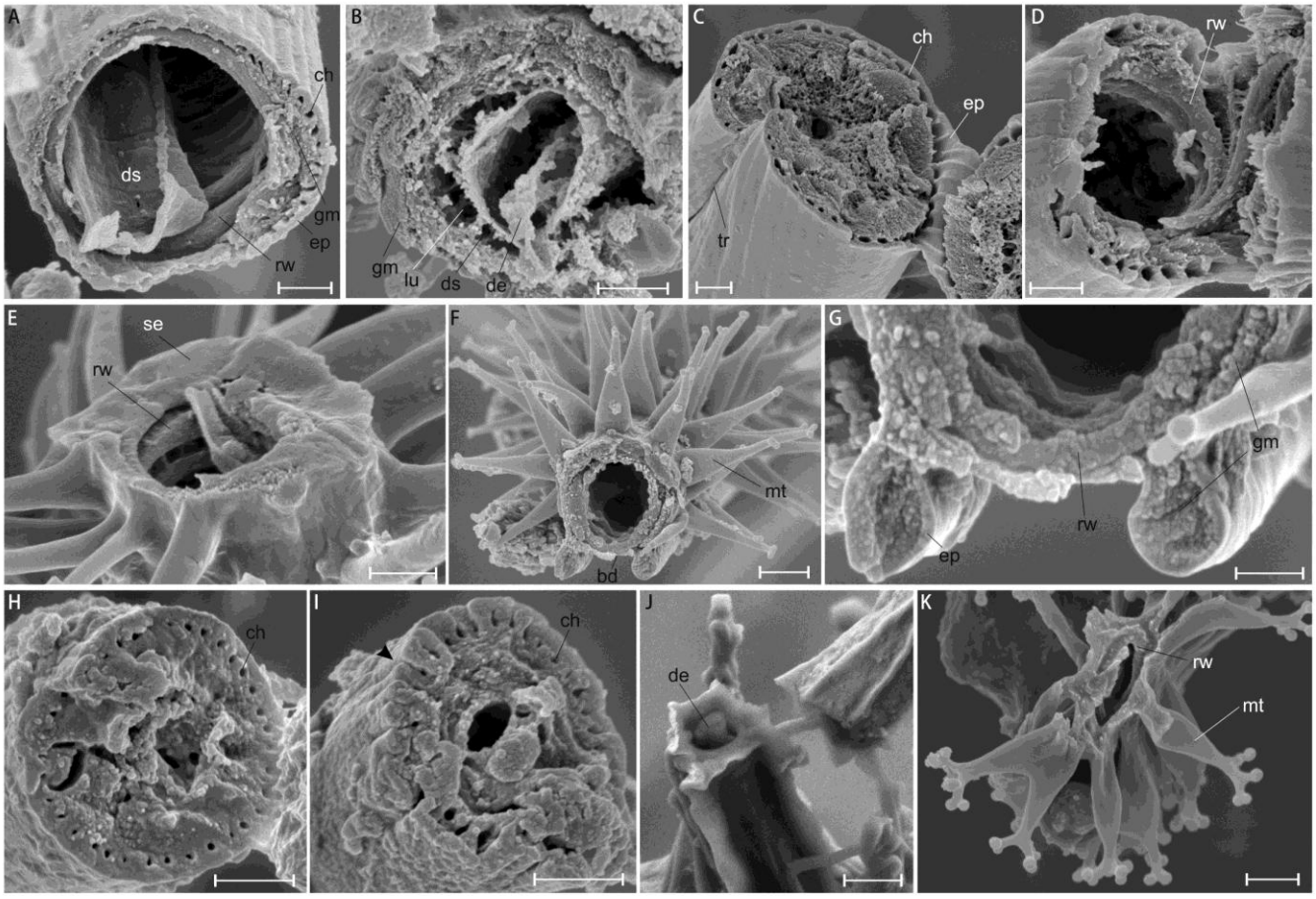


Fig.5.9. Inner structure of glandular setae. Scanning electron micrographs of broken setae. A. Broken shaft of sensillum chaeticum, *Hesperonemastoma modestum* (Taracidae), radial cuticular channels, inner ring walls and dendritic sheath visible. B. Broken plumose part of glandular seta, *Thrasychirus gulosus* (Neopilionidae), dendritic sheath still in native shape. C. Broken shaft of plumose seta, *Sabacon* sp. D. Broken shaft of plumose seta, *H. modestum*. E. Broken plumose part, *Dicranopalpus ramosus*. F-G. Broken plumose part, *Ballarra longipalpis*. H-I. Broken shaft of clavate seta, *Dendrolasma mirabile*, I. close to the microtrichious part, channels open to the outside (arrowhead). J. Broken tip of clavate seta, inner dendrite visible, *Paranemastoma quadripunctatum* (Nemastomatidae). K. Broken plumose part, *Caddo agilis*, microtrichia are only built by the epicuticle and obviously filled with secretion. Abbreviations: ep, epicuticle; ch, secretion channel; de, dendrite; ds, dendrite sheath; gm, granular material; lu, setal lumen; mt, microtrichia; rw, ringed walls; tr, backing trench (invagination). Scale bars: A-F,H-K. 0.5 µm; G. 250 nm.

Plumose setae Type 3 (ps3): In the ps3-type the plumose section occupies one third to one half of the setal length. The microtrichia are equally distributed around the cylindric shaft (no anisotropy) (Fig.5.8.Q). The shaft is often highly sclerotized (dark colour) and the cuticular depression in the plumose section is less distinct. This gives the setae a spine-like appearance in the light microscope. The microtrichial tips are irregular (Fig.5.8.HH), bifid or trifid. The apical pore is flanked by four lobes. Channel openings were not found. This type is found in Sclerosomatidae and Protolophidae.

Plumose setae Type 4 (ps4): In the ps4-type the plumose section occupies one half to two third of the setal length (Fig.5.8.F,G). The basal shaft can be slightly curved forwards (especially in *Ballarra*). The anisotropy is extreme (few microtrichia at the back only in the distal-most part). At the back of the plumose part there is a depression in which the cuticle is thinner, such that the inner rings are visible (as in ps2 but much more pronounced) (Figs.5.8.S,U, 5.9.F,G). The depression is flanked by longitudinal ridges (most developed in *Ballarra*). The microtrichia are bifid or branch irregularly, with knob-like tips. The apical pore is flanked by four long lobes (Fig.5.8.Z). Channel openings were not found. This type is found in the Neopilionidae.

Plumose setae Type 5 (ps5): In the ps5-type the plumose section occupies one third to half of the setal length. There is a pronounced anisotropy of microtrichial coverage. A rear depression or trench is not present. There is no constriction below the plumose section as in ps1 or ps2, but the shaft gradually tapers towards the middle. The longitudinal ridges of the shaft get more pronounced in the plumose section. The epicuticle in the grooves within seems to be thinner as it is occasionally cracked in dried specimens. However, true slits like those in ps6 and cs are lacking. The microtrichia are bifid or trifid and the branch tips not knob-like swollen. The apical pore has a smooth margin. This type is found in *Acropsopilio* (Acropsopilionidae).

Plumose setae Type 6 (ps6): The overall morphology of the ps6-type is rather similar to the ps4-type. The plumose section occupies one half to two thirds of the setal length (Fig.5.8.H). There is a slight bend below the plumose part but no distinct constriction. The anisotropy is extreme (a few rear microtrichia in the distal-most part only). At the back of the plumose part there is a trench-like invagination (Fig.5.8.T,V). In the basal portion of the plumose part there are slit-like openings of the secretion channels below and between the microtrichia (Fig.5.8.KK,LL). The microtrichial tips are bifid or trifid, the branch tips might be slightly swollen (Fig.5.8.II) (but not in *Sabacon*). The apical pore is flanked by four (*Taracus*) or six to seven (*Hesperonemastoma*, *Sabacon*) (Fig.5.8.AA) lobes. The brim of the pore might be significantly swollen (but not in *Taracus*). This type is found in Taracidae and Sabaconidae.

Clavate setae (cs): These are the most derived glandular setae with a set of unique characters. Cs are comparably shorter (30-40 µm) than plumose setae (40-100 µm). The plumose part is restricted to a globular body at the distal three quarter mark of the shaft (Fig.5.8.I,W). Thus the microtrichia are arranged radial symmetrically, and the secretion droplet is of a globular shape (Fig.5.8.K) in contrast to the plumose setae, where it is oval or irregular (Fig.5.8.M). The microtrichia are of variable length (longest usually in setae in the distal dorsal part of the tibia, accompanied with the largest droplets). The microtrichia are trifid (in very rare cases, i.e. *Centetostoma dubium*, bifid), and without knob-like tips (Fig.5.8.JJ). The protruding tip is long (at least twice as long as the apical microtrichia) and flexible, because of the thin epicuticle, under which the inner rings slightly protrude (Fig.5.13). The apical pore is flanked by five to six lobes

(Fig.5.8.BB,CC). Below the plumose part there are parallel slit-like openings of the secretion channels (Fig.5.8.NN,OO) (widening and opening of the grooves between the longitudinal ripples in the distal shaft, visible in (Fig.5.9.I)). This type is found in Nemastomatidae and juvenile Dicranolasmatidae, and presumably in juvenile Nipponopsalididae.

Pseudo-clavate seta (p-cs): Hunt and Cokendolpher (1991) recorded a type of plumose setae in one *Ballarra* species (Neopilionidae: *B. clancyi*), which resembles the clavate setae in following characters: plumose part restricted to a (nearly) globular body, trifid microtrichia and long protruding tip. On the basis of their SEM images it is not possible to evaluate the fine structure of the tip pore or whether channel openings occur. As we did not find channel openings in the related *B. longipalpis*, it is presumed that they are also absent in the pseudo-clavate seta type.

Tab.5.2. Characters of pedipalpal glandular setae in harvestmen. Character states in brackets means less pronounced, '?' unknown character state, [1] based on microscopical images and descriptions in Hunt & Cokendolpher, 1975; [2] based on drawings and descriptions by Sato & Suzuki, 1939.

	type	plumose part		terminal element of microtrichia		apical pore		backing		channel opening slits
		range	anisotropy	branching	knobbed	brim	lobes	depress-sion	trench	
Eupnoi										
Caddidae										
Caddo agilis	ps1	1/7	+	irregular	+	-	4	-	-	-
Phalangiidae										
Lophopilio palpinalis	ps2	1/3	+	irregular	+	-	?	(+)	-	-
Megabunus rhinoceros	ps2	1/3	+	irregular	+	-	?	(+)	-	-
Metaplatybunus cf. rhodiensis	ps2	1/3	+	2	+	-	?	(+)	-	-
Mitopus morio	ps2	1/3	+	2 / irreg.	+	-	4	(+)	-	-
Oligolophus tridens	ps2	1/3	+	2 / irreg.	+	-	?	(+)	-	-
Paroligolophus agrestis	ps2	1/4	+	2 / irreg.	+	-	?	(+)	-	-
Metaphalangium cirtanum	ps2	1/4	+	2	+	-	?	(+)	-	-
Phalangium opilio	ps2	1/4	+	2-3	+	-	?	(+)	-	-
Rilaena triangularis	ps2	1/3	+	2 / irreg.	+	-	?	(+)	-	-
Amilenus aurantiaticus	ps2	1/3	+	2 / irreg.	+	-	4	(+)	-	-
Dicranopalpus ramosus	ps2	1/3	+	2	+	-	4	(+)	-	-
Gyas annulatus	ps2	1/3	+	2	+	-	?	(+)	-	-
Protolophidae										
Protolophus singularis	ps3	1/2	-	2-3	+	-	4	-	-	-
Sclerosomatidae										
Gagrella disticta cf. juvenile	ps3	1/3	-	2 / irreg.	+	-	4	-	-	-
Metagagrella minax cf. juvenile	ps3	1/2	-	2 / irreg.	+	-	4	-	-	-
Neopilionidae										
Tercentenarium linnaei	ps4	1/2	++	?	?	-	?	+	-	?
Thrasychirus gulosus	ps4	1/2	++	irregular	+	-	4 (?)	+	-	-
Americovibone lanfrancoae [1]	ps4	2/3	?	irregular	?	?	?	?	?	?
Plesioballarra crinis [1]	ps4	1/2	++	?	?	-	?	?	?	?
Ballarra longipalpis	ps4	2/3	++	2	+	-	4	+	-	-
B. drosera [1]	ps2	1/3	+	3	?	-	?	-	-	?
B. clancyi [1]	p-cs	clav.	-	3	+	-	?	-	-	?
Dyspnoi										
Acropsopilionidae										
Acropsopilio neozealandiae	ps5	1/2	++	2-3	-	-	- (?)	-	-	-
Taracidae										
Hesperonemastoma modestum	ps6	2/3	++	2-3	(+)	+	6-7	-	+	(+)
Taracus sp.	ps6	2/3	++	2	(+)	-	4	-	+	+
Sabaconidae										
Sabacon simoni	ps6	2/3	++	3	-	+	6-7	-	+	+
Nipponopsalididae										
Nipponopsalis abei [2]	cs	clav.	-	?	?	?	?	?	?	?
Dicranolasmatidae										
Dicranolasma pauper	cs	clav.	-	3	-	-	5-6	-	-	+
Nemastomatidae										
spp.	cs	clav.	-	3	-	-	5-6	-	-	+

5.3.6. Use of pedipalps and glandular setae in prey capture

Literature data on feeding biology of Palpatores is summarized in Tab.5.3. Agile prey (requiring a capture and securing mechanism) is taken by the majority of harvestmen, all of which carry glandular setae. However, agile prey is occasionally also caught by species lacking such setae such as *Leiobunum* spp. or adult *Dicranolasma pauper*. These are reported to use both legs and chelicerae to grasp the prey (Gruber, 1993). Agile prey only constitutes a small proportion of food in these harvestmen.

The usage of the pedipalps during prey capture was observed in a number of species. Prey capture could not be induced in lab trials with *Dicranopalpus ramosus*, *Paranemastoma quadripunctatum*, *Dicranolasma pauper*, *Ischyropsalis carli* and *Holoscotolemon naturae*, presumably because these photophobic species were disturbed by the strong light source necessary to achieve sufficient video frame rates at sufficient magnification. In contrast, the observation of prey capture was comparably easy in *Mitostoma chrysomelas* and *Sabacon* spp., which seemed less affected by the light (although these species also usually hide at daytime, and presumably are nocturnal). Single observations were made of *Rilaena triangularis* and *Oligolophus* sp. Recordings of previously unpublished observations of harvestmen encountered with food items in the field are added.

5.3.6.1. Eupnoi

We observed a juvenile *Rilaena triangularis* (Phalangiidae) successfully capturing a springtail. The capture movement was very sudden, occurring when the springtail touched a front leg of the resting harvestman. The harvestman quickly jumped forwards onto the springtail, grasping it with its spine-armed pedipalps.

We also recorded an attack by an *Oligolophus* nymph on a springtail. The harvestman seemed to recognize the springtail from a distance, initiating a careful approach, stopping closely in front of the springtail. The harvestman then stretched its hind legs and elevated its body, slightly tilted forwards, facing the springtail. In that posture it did some stealthy movements forwards and then rested for a moment. It then suddenly jumped forwards onto the springtail with its pedipalps extended. It slightly missed the springtail, which jumped away. Further capture attempts were not observed. Additionally, an adult *O. tridens* was observed taking a poduromorph springtail. These caterpillar-like and slow-moving springtails were refused as prey items by the dyspnoid harvestmen tested.

When collecting material, we observed an *Odiellus troguloides* carrying a dead wasp three times larger than itself. We assume that it was taken already dead as it seems unlikely that this harvestman with its short legs and small pedipalps and chelicerae can overwhelm a living wasp.

Photos by M. Yeo taken in the field show several unidentified Gagrellinae with food items (n=33), most frequently fungi (n=11) (Fig.5.10.D), further, fruit (n=3), freshly moulted arthropods (a moth and an ant), a butterfly pupae, a beetle larva and a mouldy salticid spider, but also moths (n=3), stick insects (n=2), ants (n=3), wasps (n=2), a crab spider, a spider leg, a scorpion and a centipede, unclear if caught alive. The pedipalps hold the (often large) prey items, primarily with the claws. One of the moths was obviously spun in spider silk, indicating that these harvestmen are also kleptoparasitic. A photo of a species of Sandokanidae eating a snail was also taken. Photos by J. Pageler show individuals of *Rilaena triangularis* eating wasps (Symphyta, n=2) and a mosquito (Fig.5.10.B), a *Phalangium opilio* eating a beetle, a juvenile *Opilio canestrinii* eating a psocopteran (Fig.5.10.C) and a *Dicranopalpus ramosus* eating a fly. J. Pageler successfully fed

Fig.5.10. Use of pedipalps during feeding. All macro photographs, except E. and F., taken in the field, E. and F. taken in captivity. A-B. *Rilaena triangularis* (Phalangiidae), the prey is held between the pedipalps, glandular setae partly in contact and contaminated with prey setae, A. Juvenile feeding on a captured springtail, B. Adult feeding on a mosquito. C. Juvenile *Opilio canestrinii* (Phalangiidae) feeding on a psocoptera. D. Unidentified Gagrellinae feeding on fungi, which is held both with pedipalpal claws and chelicerae. E. *Ischyropsalis kollari* (Ischyropsalididae) feeding on snail, the pedipalps are only used as feelers. F. *Trogulus martensi* (Trogulidae) feeding on snail, the pedipalps are highly reduced and enclosed in tergal processus, thus not visible. G. *Sabacon cavicolens* feeding on captured springtail. Photo A. by Jan van Duinen, B-C. and E-F. by Jörg Pageler and D. by Melvyn Yeo, with kind permission, G. by Axel Schönhofer.

and photo documented individuals of *Odiellus spinosus* with dead dipterans. J. van Duinen observed and photo documented the prey capture of a springtail by a juvenile *R. triangularis* in the field (Fig.5.10.A).

5.3.6.2. Dyspnoi

We successfully filmed 38 prey capture events in adult and juvenile *Mitostoma chrysomelas* (Nemastomatidae) and 12 prey capture events by juvenile *Sabacon* spp. (Sabaconidae). The harvestmen caught different species of agile entomobryomorph and symphypleonan springtails, whereas the plump poduromorph springtails were refused. In most cases the harvestmen actively searched for prey when they recognized their presence in the tank. In *Mitostoma* this searching behaviour included waving and tapping movements of the second leg pair and a simultaneous extension and retraction of both pedipalps, but without touching the substrate. In *Sabacon* the pedipalps were held forwards with tibiae flexed (such that their concave sides faced forwards) and tarsi extended. Sooner or later the harvestman touched a springtail, which then was immediately affixed to the pedipalps, despite a heavy struggle. The harvestmen immediately reacted by flexing the pedipalp tarsi (clasping the legs, furca or antenna of the prey) and stretching the legs and elevating the body to prevent ground contact by the springtail. When the springtail was secured between both pedipalps it was grabbed with the chelicerae and dismembered. With this strategy the harvestmen were able to overwhelm prey even larger than themselves. In *Mitostoma* it was obvious that the predatory technique totally relies on the stickiness of the glandular setae. In some cases the springtails were able to escape due to discharge of their scale like setae (see ***chapters 6*** and ***7*** for more details, statistics and discussion of predator-prey interaction and counter-adaptations of springtails). *Sabacon* more extensively used grasping and clamping movements and the prey was more quickly transferred to the chelicerae. It seems to not totally rely on the sticky secretion of the glandular setae, although it is obvious that the prey is partly affixed by the secretion. Both species were able to pursue previously detected prey with surprisingly fast and directed movements.

H.-J. Thorns also photo documented the capture of different springtails by *Mitostoma chrysomelas*, and A. Schönhofer a juvenile *Sabacon cavicolens* eating a symphypleonan springtail in the field (Fig.5.10.G). J. Pageler successfully fed and photo documented individuals of *Trogulus martensi* (Fig.5.10.F) and an *Ischyropsalis kollari* (Fig.5.10.E) with snails.

5.3.6.3. Food remnants on glandular setae

We frequently found remnants of small arthropods between or attached to the microtrichia of glandular setae. Those remnants were rarely found at other locations on the pedipalp. A variety of large unidentified setae (scale-like, bristle-like, pinnate and plumose setae) may originate from pterygote insects or other arthropods, such as spiders. These remnants were found especially in larger species like female *Dicranopalpus ramosus* (Phalangiidae) and *Protolophus singularis* (Protolophidae) (Fig.5.11.E), and *Gyas annulatus* (Phalangiidae), but also the smaller *Ortholasma coronadense* and *Pyza bosnica* (Nemastomatidae) (Tab.5.3).

The most frequent remnants found were detached scale-setae of epigaeic springtails (Collembola: Entomobryomorpha). Scales have independently evolved multiple times among springtails and may cover the whole body, including legs and furca (Zhang et al., 2014). They easily detach enabling the animal to escape when glued (Bauer and Pfeiffer, 1991, Wolff et al., 2014b). Springtail scales were frequently found on the small soil dwelling *Caddo agilis*

(Caddidae) (Fig.5.11.B), *Nemastoma lugubre* and *Mitostoma chrysomelas* (Nemastomatidae), and in smaller amounts in the larger *Megabunus rhinoceros*, *Dicranopalpus* cf. *pyrenaeus* (Phalangiidae), *Thrasychirus gulosus*, *Ballarra longipalpis* (Neopilionidae), *Taracus* sp. (Taracidae) and *Mediostoma humerale* (Nemastomatidae).

Other remnants frequently found were so-called *brochosomes*. These are "football-like" microbodies which have been shown to be secretory products of leafhoppers (Hemiptera: Cicadellidae) (Day and Briggs, 1958). This large family of herbivorous insects is distributed worldwide, with its hotspot in tropical regions (Oman et al., 1990, Nielson and Knight, 2000). Bristowe (Bristowe, 1949) previously reported the consumption of leafhoppers by *Leiobunum blackwalli* (Sclerosomatidae) and *Mitopus morio* (Phalangiidae). Brochosomes were found in high densities on glandular setae of *Thrasychirus gulosus* (Neopilionidae) (Fig.5.11.D) and *Dicranopalpus* ssp. (Phalangiidae) (Fig.5.11.C) (of which the latter at least is arboricolous), but also on *Phalangium opilio* (Phalangiidae) and, in traces, on *Nemastoma lugubre* (Nemastomatidae).

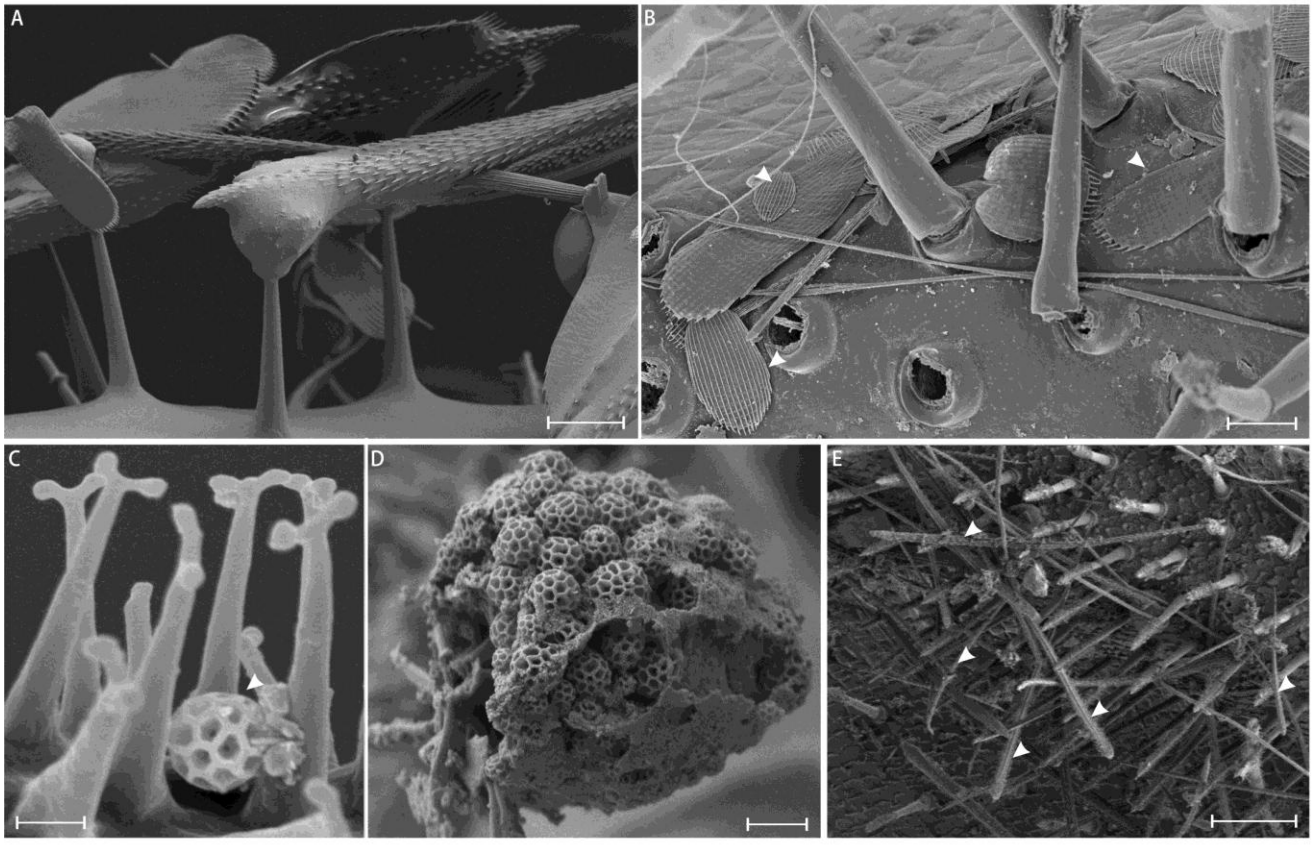


Fig.5.11. Prey remnants on glandular setae. Scanning electron micrographs. A. Cryo-SEM image of collembolan setae adhering to clavate setae of *Mitostoma chrysomelas* (Nemastomatidae) after touching the pedipalp with an entomobryomorph springtail (see ***chapter 6*** for details of experimental procedure). B. Scale like collembolan setae (arrowheads) between plumose setae, as frequently found in conserved material of *Caddo agilis* (Caddidae). C. Leaf hopper brochosome (arrowhead) between microtrichia of a plumose seta of a juvenile *Dicranopalpus* cf. *pyrenaeus* (Phalangiidae). D. High amount of brochosomes adhering to the remnants of the secretion at a plumose seta in *Thrasychirus gulosus* (Neopilionidae). E. Plumose setae on the patellar apophysis of *Protolophus singularis* (Protolophidae), highly contaminated with foreign setae (arrowheads). Scale bars: A-B. 10 µm; C. 250 nm; D. 1 µm; E. 50 µm.

Tab.5.3. Summary of data available on feeding behaviour in relation to pedipalp morphology in Palpatores. Observation records: '+' means food uptake / prey capture observed (printed in bold font means confirmed by other studies); '(+)' item taken only after longer period of starvation; '-' item refused even after longer period of starvation; [1] observation in nature; [2] feeding tests in captivity; [3] gut content analysis.

	pedipalp			observation records									prey remnants at glandular setae		
	rel. len.	type	gland. setae	mobile prey	appendages used in capt.	eggs, larvae	gastro pods	anne llids	scavan ging	fruits	fungi	references	Collem -bola	Cicade- llidae	unident. arthrop.
Eupnoi															
Caddidae															
Caddo agilis	0.8	rapt. clamp	+										+		
Phalangiidae															
Lophopilio palpinalis	0.7	rapt. clamp	+	+			+	+				Adams, 1984[3]			
Mitopus morio	1.1	clamp	+	**+**	L1+2, pedipalps / pedipalps + cheli-cerae	+	- / +	(+)	**+**			Kästner, 1931[1]; Bristowe, 1949[1] Phillipson, 1960[2]; Frank, 1937[2] Adams, 1984[2,3]			
Oligolophus tridens	0.8	clamp	+	**+**	pedipalps + chelicerae	+		+	+	+		own observation[2] Hvam and Toft, 2008[2] Bristowe, 1949[1,2]			
Paroligolophus agrestis	0.8	clamp	+	+		+	+		+			Bristowe, 1949[1,2]			
Odiellus spp.	0.5	clamp	-						**+**			own observation[1] Pageler, photo doc.[1]			
Phalangium opilio	0.7	clamp	(+)	**+**		**+**			+			Rühm, 1926 [1] Bristowe, 1949[1,2] Allard and Yeargan, 2005[2]		+	
Rilaena triangularis	0.7	rapt. clamp	+	**+**	pedipalps			+	(+)	(+)		Pageler, photo doc.[1] Hvam and Toft, 2008[2]			
Megabunus spp.		rapt. clamp	+	+								Bristowe, 1949[1]	+		
Platybunus bucephalus		rapt. clamp	+	+	pedipalps + L1				(+)	-		Immel, 1955[1,2]			
Dicranopalpus ramosus	1.6	clamp	+	+								Pageler, photo doc.[1]		+	+
D. cf. *pyrenaeus* juvenile	1.4	rapt. clamp	+										+	+	
Gyas annulatus	0.8	clamp	(+)												+
Protolophidae															
Protolophus singularis	0.9	clamp	+												+
Sclerosomatidae															
Leiobunum spp.	0.7	clamp	-	**+**		+	+	**+**	**+**	**+**		Bristowe, 1949[1]; Wijnhoven, 2011[1]; Halaj and Cady, 2000[1]			
Gagrellinae unid.		clamp	-	?		+			+	+	**+**	Yeo, photo doc.[1]			
Neopilionidae															
Thrasychirus gulosus	1.3	clamp	+										+	+	
Ballarra longipalpis	2.7	tentacle	+										+		

Dyspnoi															
Ischyropsalididae															
Ischyropsalis hellwigi		tentacle	-				**+**					Verhoeff, 1900[2]; Martens, 1965[2]			
I. kollari		tentacle	-	+	chelicerae							Martens, 1975[2]			
Taracidae															
Taracus sp.	1.7	tentacle	+										+		
Sabaconidae															
Sabacon spp.	1.3	tentacle	+	**+**	pedipalps							own observation[1,2]			
Dicranolasmatidae															
Dicranolasma pauper	0.7	tentacle	-	+	L1+2 + chelicerae	+	-		+			Gruber, 1993[1,2]			
D. pauper juvenile	1.2	rapt. tenta.	+	+	pedipalps		-		(+)			Gruber, 1993[2]			
Trogulidae															
Trogulus spp.	0.3	tentacle	-	-			+	(+)	(+)			Pabst, 1953[2]			
Nemastomatidae															
Ortholasma coronadense	0.8	tentacle	+												+
Mitostoma chrysomelas	1.8	tentacle	+	**+**	pedipalps							own observation[2] Thorns, photo doc.[2]	+		
Paranemastoma quadripunctata	1.1	tentacle	+	+	chelicerae (?)		-	(+)	(+)			Immel, 1954[2]			
Pyza bosnica	1.1	tentacle	+												+
Mediostoma humerale	1.1	tentacle	+										+		
Nemastoma spp.		tentacle	+	**+**	pedipalps		+	**+**	+	+		own observation[2] Adams, 1984[2,3] Hvam and Toft, 2008[2]	+	+	

5.3.7. Use of pedipalps in copulation and grooming

In both Phalangiidae and Sclerosomatidae the males were found to use their pedipalps to secure the partner during copulation (Tab.5.4). The extent of pedipalpal usage varies considerably. In *Mitopus morio* and *Paroligolophus agrestis* (Oligolophinae), the pedipalp is slightly flexed over the femur of the female front leg, but not used in every case. In *Rilaena triangularis* and *Phalangium opilio* (Phalangiinae), the pedipalps are tightly twined around the bases of legs 1-2 or 1-3 respectively (Fig.5.7.C,F). In *Leiobunum* spp. (Leiobuninae), the males likewise grab the bases of female front legs. In *Dicranopalpus ramosus* (Dicranopalpinae), the male clasps the female front leg using its pedipalpal patellar apophysis (Fig.5.7.I). A copulation of Phalangodidae photo documented by M. Yeo indicates that in Laniatores males secure females by grabbing their pedipalps.

Observations on *Leiobunum blackwalli* (Sclerosomatidae) and *Opilio canestrinii* (Phalangiidae) show that the pedipalps play an important role in grabbing and securing the walking leg tarsi when groomed with the chelicerae.

Tab.5.4. Summary of data available on pedipalp usage during copulation in relation to sexual dimorphistic characters in Eupnoi.

	pedipalp type	sex. dim. elo.	sex. dim. g.s.-	male pedipalp usage during copulation	references
Phalangiidae					
Mitopus morio	clamp	-	-	grabbing female L1 femur	Pageler, photo doc.
Paroligolophus agrestis	clamp	-	-	grabbing female L1 femur	Wijnhoven, 2008
Phalangium opilio	clamp	+	+	grabbing female L1-3 bases	Pageler, photo doc.; Willemart et al., 2006
Rilaena triangularis	rapt. clamp	-	+	grabbing female L1+2 bases	Pageler, photo doc.
Dicranopalpus ramosus	clamp	-	+	clasping female at front leg femur using patellar apophysis and tibia	Pageler, photo doc.
Sclerosomatidae					
Leiobunum spp.	clamp	-	no g.s.	grabbing female L1 or L1+2 bases	Pageler, photo doc.; Burns et al., 2013

5.3.8. Phylogenetic mapping

The events of character gain are marked in Fig.5.12 and Fig.5.13 (events of character loss/reversion are not depicted). The pedipalpal claw has been lost for multiple times within Ballarrinae (*Americovibone* and *Ballarra clancyi* + *B. longipalpis*) and in Dyspnoi *sensu stricto* (excluding Acropsopilionidae). Spines of Laniatores and Palpatores are considered as homoplasic, because of their different position (Laniatores: most prominent spines at tibia and tarsus, in two lateral rows; Palpatores most prominent spines at femur, in single median row). A further argument is their absence in the basal-most Laniatores (*Synthetonychia*). Spines are further homoplasic for *Dicranopalpus* (juvenile) and *Dicranolasma* (juvenile). Glandular setae are apomorphic to Palpatores. Plumose setae Type 1 (ps1) are restricted to Caddidae, but might represent a conserved ancestral state of all glandular setae (for arguments see discussion). Highly anisotropic plumose setae with an extended plumose part and a rear depression or trench have independently evolved in Neopilionidae (ps4) and Sabaconidae + Taracidae (ps6). The clavate setae of Nemastomatidae and juvenile Dicranolasmatidae and Nipponopsalididae are considered homologous, because of a similar ultrastructure. In the Trogulidae the clavate setae have been lost. Slit-like openings of the secretion channels to the outside are apomorphic to Dyspnoi *sensu stricto*.

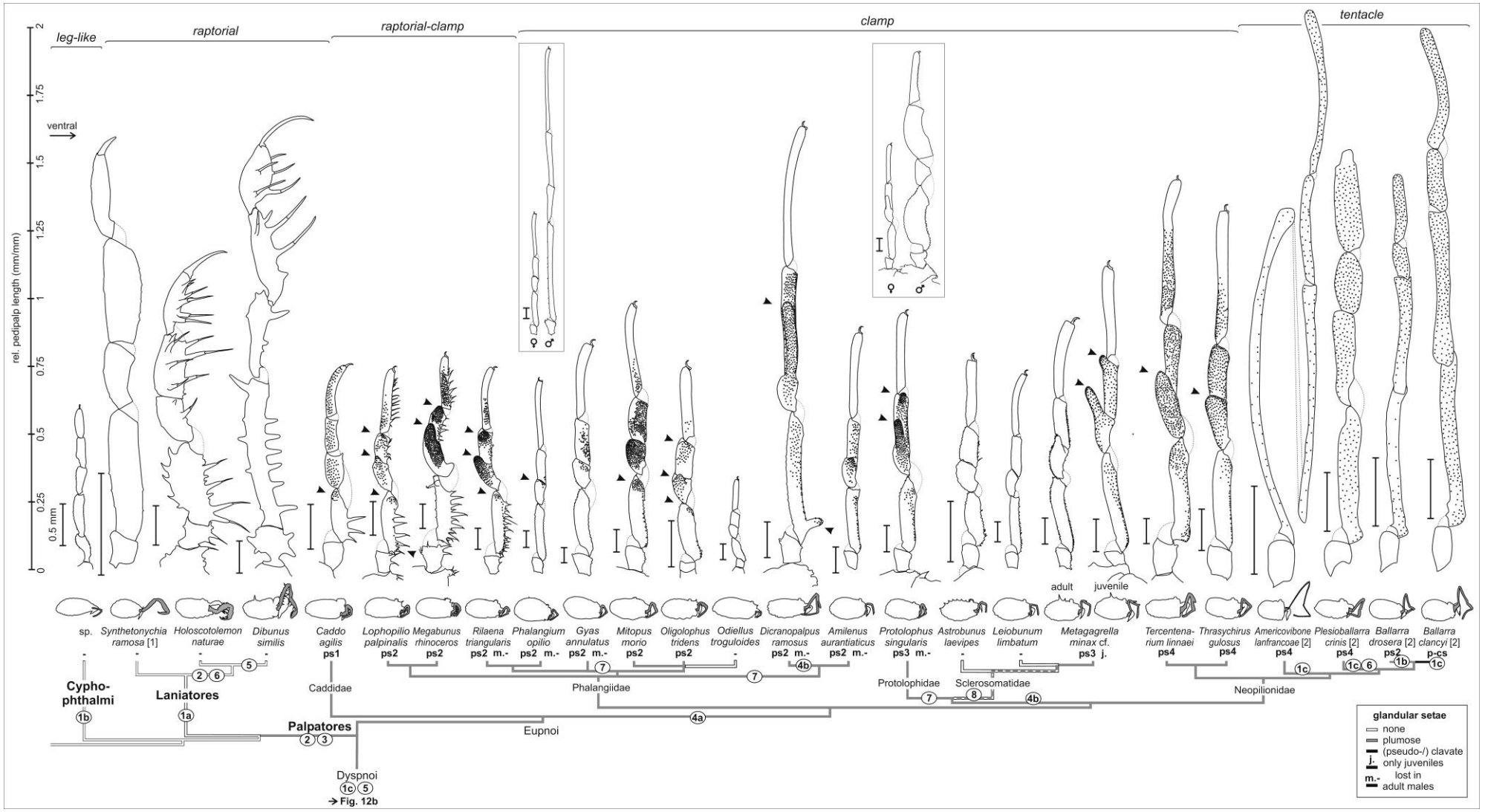
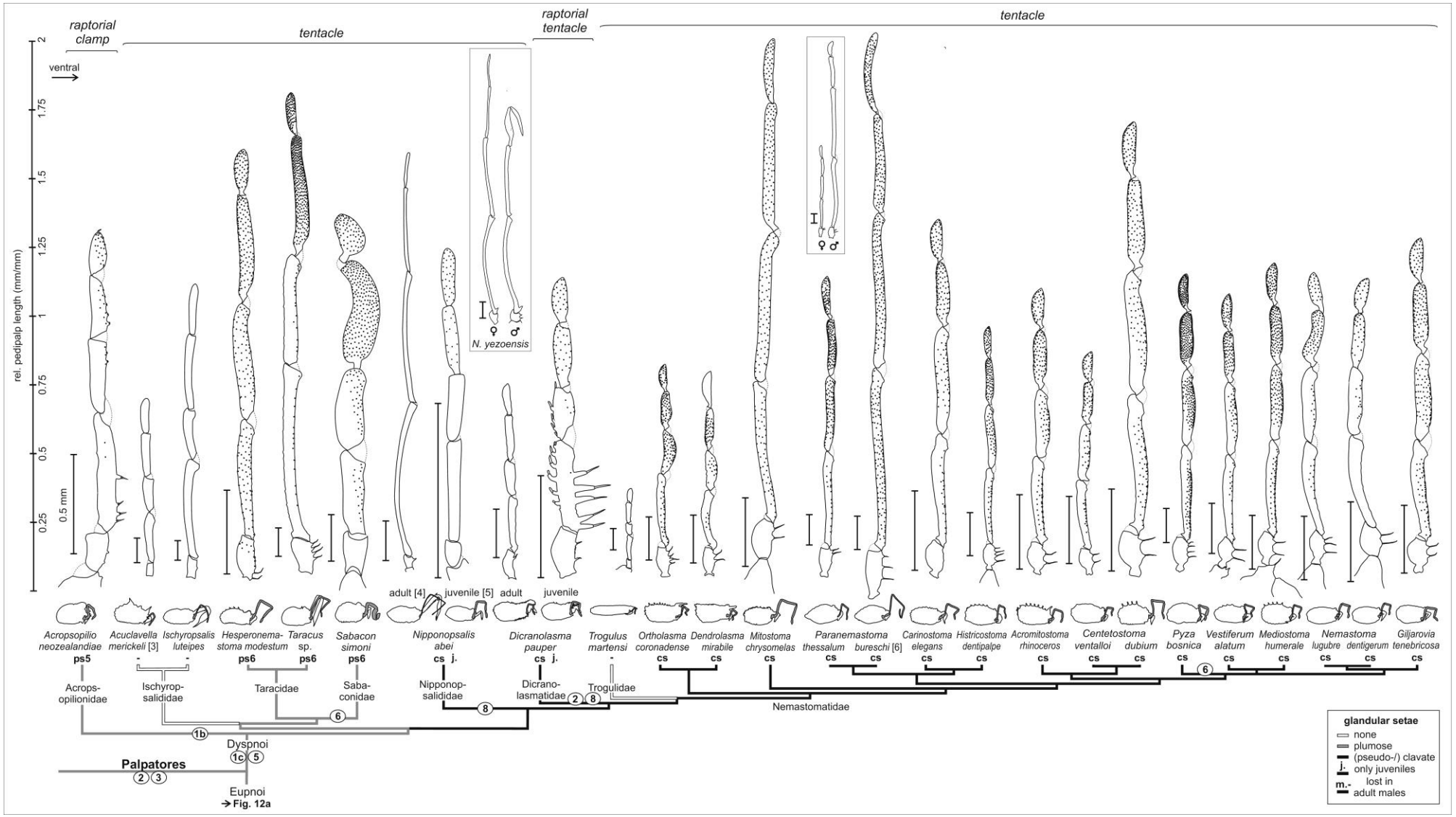
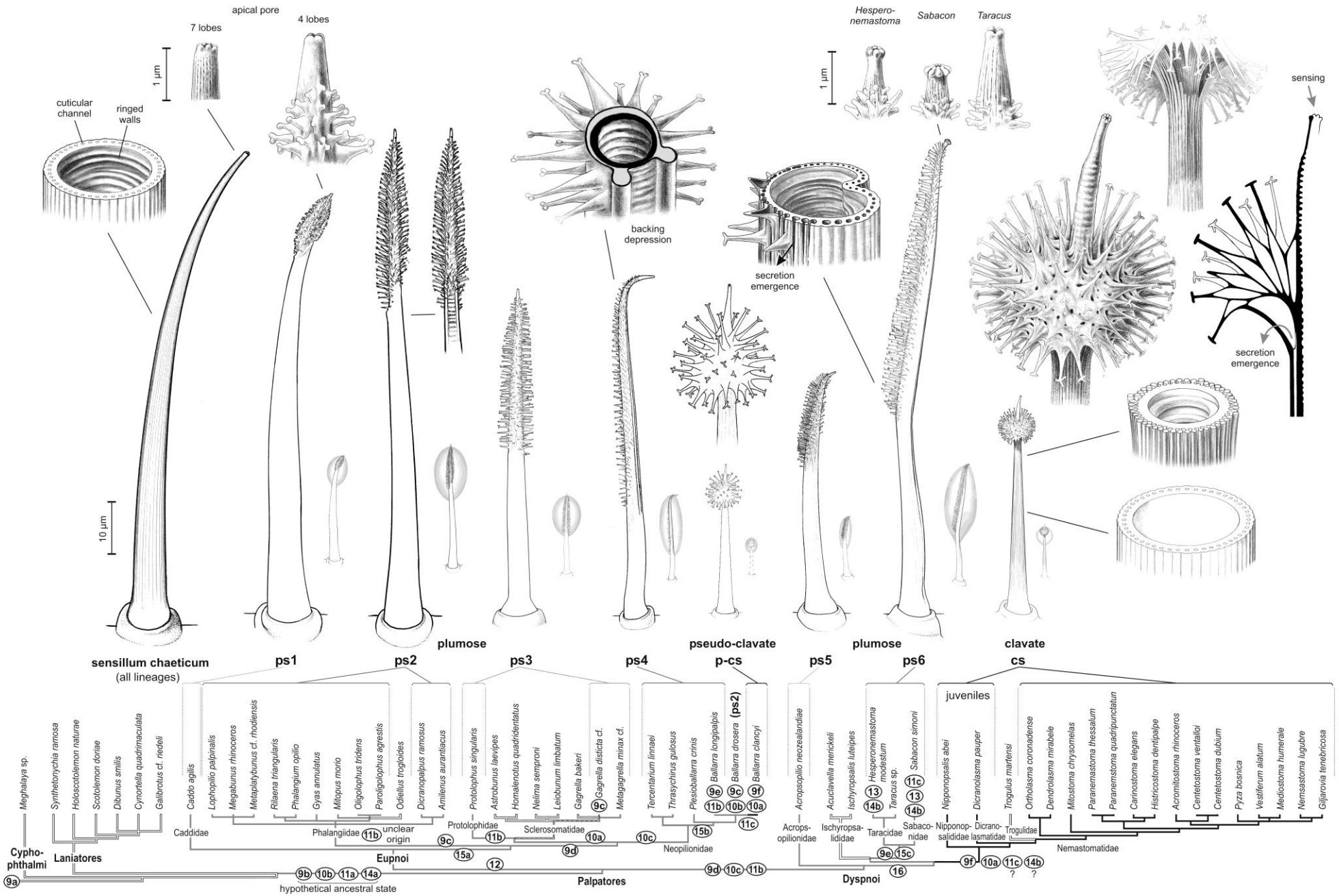


Fig.5.12. Phylogeny of pedipalp morphotypes and distribution of glandular setae. Schematic illustration of pedipalps, prolateral side; only prominent bristles and spines displayed, all other setae and microsculpture neglected; black dots mark the position of glandular setae; arrowhead points to apophysis bearing glandular setae. Inset frames show sexual dimorphism of pedipalps (glandular setae are not marked here). 12a. (this page) Cyphophthalmi, Laniatores and Eupnoi, 12b. (next page) Dyspnoi. [1] adapted from Forster, 1954; [2] adapted from Hunt & Cokendolpher, 1975; [3] based on microscopical images by Casey Richards, published on morphbank; [4] adapted from Sato & Suzuki, 1939; [5] adapted from Miyoshi, 1942; [6] adapted from Mitov, 2011; cs, clavate setae; p-cs, pseudo-clavate setae; ps1-5, plumose setae type 1-5. Phylogenetic tree based on (Hedin et al., 2012, Shultz and Regier, 2001, Schönhofer, 2013, Groh and Giribet, 2014, Giribet and Sharma, 2015); internal topology of the Phalangiidae follows the suggestion by (Buzatto et al., 2013). Apomorphic

characters marked (for details, see main text): (1) pedipalpal claw: (1a) enlargement, (1b) reduction, (1c) loss; (2) possession of spines (reinforced setae with highly elevated sockets); (3) possession of glandular setae; (4) patellar apophysis: (4a) hump-like, (4b) finger-like (elongated); (5) miniaturization of the tibia-tarsus joint; (6) tibia and tarsus significantly swollen; (7) glandular seta lacking in males; (8) glandular setae only present in juveniles.




apical pore
7 lobes
4 lobes
1 µm
cuticular channel
ringed walls
10 µm
backing depression
secretion emergence
Hespero-nemastoma
Sabacon
Taracus
1 µm
sensing
secretion emergence
sensillum chaeticum
(all lineages)
ps1
ps2
plumose
ps3
ps4
pseudo-clavate
p-cs
ps5
plumose
ps6
clavate
cs
juveniles
Meghalaya sp.
Synthetonychia ramosa
Holoscotolemon naturae
Scotolemon doriae
Dibunus smilis
Cynortella quadrimaculata
Galibrotus cf. riedeli
Caddo agilis
Lophopilio palpinalis
Megabunus rhinoceros
Metaplatybunus cf. rhodiensis
Rilaena triangularis
Phalangium opilio
Gyas annulatus
Mitopus morio
Oligolophus tridens
Paroligolophus agrestis
Odiellus troglodes
Dicranopalpus ramosus
Amilenus aurantiacus
Protolophus singularis
Astrobunus laevipes
Homalenotus quadridentatus
Nelima semproni
Leiobunum limbatum
Gagrella bakeri
Gagrella disticta cf.
Metagagrella minax cf.
Tercentarius linnaei
Thrasychirus gulosus
Plesiobaldarra crinis
Ballarra longipalpis
Ballarra drosera (ps2)
Ballarra clancyi
Acropsopilio neozealandiae
Aculacvella merickeli
Ischyropsalis luteipes
Hesperonemastoma modestum
Taracus sp.
Sabacon simoni
Nipponopsalis abei
Dicranolasma pauper
Trogulus martensi
Ortholasma coronadense
Dendrolasma mirabele
Mitostoma chrysomelas
Paranemastoma thessalum
Paranemstoma quadripunctatum
Carinostoma elegans
Histricostoma dentipalpe
Acromitostoma rhinoceros
Centetostoma ventalioi
Centetostoma dubium
Pyza bosnica
Vestiferum alatum
Mediostoma humerale
Nemsastoma lugubre
Giljarovia tenebricosa
Caddidae
Phalangiidae
unclear origin
Protolophidae
Sclerosomatidae
Neopilionidae
Acrops-opilionidae
Ischyropsa-lididae
Taracidae
Sabaco-nidae
Nipponop-salididae
Dicrano-lasmatidae
Trogulidae
Nemastomatidae
Cypho-phthalmi
Laniatores
Eupnoi
Palpatores
Dyspnoi
hypothetical ancestral state

5.4. Discussion

5.4.1. How important is active predation for harvestman feeding?

Many harvestmen species are regarded as omnivorous. This would implicate that predation on arthropods is not obligate. However, food quality experiments with *Rilaena triangularis* (Phalangiidae) show a high mortality rate when fed exclusively on annelids or gastropods (despite successful food uptake), whereas it was significantly lower when fed on living flies, aphids or fresh meat (Hvam and Toft, 2008). A similar experiment with *Oligolophus tridens* showed a significantly higher survival rate when fed with epigaeic springtails (Entomobryidae) or *Drosophila melanogaster* than when fed with hypogaeic springtails (Isotomidae) or aphids (Hvam and Toft, 2005). That shows that reports of harvestmen refusing mobile prey must be handled with care if they are obtained from feeding trials in captivity. It further shows that small agile insects are of important nutritional value. Indeed, the capture of agile prey (in smaller species mainly springtails or fruit flies; in larger species flies or hymenopterans) is recorded for numerous harvestmen (Tab.5.3). Its refusal is only reported for Trogulidae (which instead prey on living snails). This suggests that most harvestmen are excellent predators that depend on freshly killed prey for development and survival. Other food sources frequently exploited might provide supplemental nourishment.

Harvestmen have evolved adaptations to overcome agile prey. Both our observations and literature data underline the importance of pedipalps in prey capture (Tab.5.3). The contradictory report by Immel (1955b), who reported that the pedipalps play no role in prey capture in *Paranemastoma quadripunctatum* (Nemastomatidae), is doubtful because it was observed in a low-light situation and happened very fast.

5.4.2. Pedipalps – multifunctional tools

The opilionid pedipalp is an appendage typically located at the frontal side of the body, in close proximity of the first two leg pairs, the chelicerae, mouthparts as well as the genital operculum/reproductive organs. The pedipalps can physically interact with all these structures, resulting in different behavioural traits like grooming and assisting the chelicerae in prey-handling. Above all, the multifunctionality of the pedipalp is demonstrated by the evolution of numerous additional adaptations performing in e.g. courtship and mating behaviour, male-male fights and prey-capture.

Fig.5.13. Structure and evolution of pedipalpal glandular setae. Drawings reconstructed from scanning electron images (this study and (Hunt and Cokendolpher, 1991)) and transmission electron images (Guffey et al., 2000, Juberthie et al., 1981, Wachmann, 1970); schematic illustration of droplet shape and dimension based on stereo microscope images of living harvestmen and macro photographs. Origin of setal characters marked (for details, see main text): (9a) sensilla chaetica (no plumose parts); (9b) plumose part restricted to tip; (9c) plumose part distal 1/4-1/3 of setal length; (9d) plumose part about half of setal length; (9e) plumose part significantly more than half of setal length; (9f) clavate; (10a) no anisotropy; (10b) slight anisotropy; (10c) high anisotropy; (11a) terminal lobes of microtrichia irregularly branched; (11b) microtrichia bifid (origin within Phalangiidae unclear, because intra-familiar phylogenetic relationships highly unresolved); (11c) microtrichia trifid (origin in Dyspnoi unclear, because state in Nipponopsalididae unknown); (12) tips of terminal lobes knob-like swollen; (13) brim of apical pore swollen; (14a) apical pore flanked by 4 lobes; (14b) more than 4 lobes (origin in Troguloidea unclear, because state in Nipponopsalididae unknown); (15a) depression at setal backing; (15b) backing depression confined by thick bulges; (15c) backing with trench-like invagination; (16) channel openings. Drawings by Hay Wijnhoven.

(1) ***Sensing***: To enable the pedipalps to perform their many tasks they are equipped with a variety of sensory structures. In Laniatores the pedipalps seem to be of minor importance as sensorial tools (Costa and Willemart, 2013). This is reflected by the sparse equipment with sensilla. However in most opilionid species of the other sub-orders studied thus far, the pedipalpal tarsus is the site with the largest numbers and the highest densities of sensilla. Sensilla chaetica, which likely are chemo- and mechanoreceptors, occur scattered over the whole tarsal surface (Willemart et al., 2009, Wijnhoven, 2013). They may perform in assessing potential food items and sensing (moving) objects at close range. In *Mitostoma chrysomelas* and *Sabacon* sp. we frequently found a reaction of the harvestman after a collembolan had touched the pedipalp. Females *Leiobunum* sp. in search for microsites for egg deposition have have been observed to crawl into crevices and holes and to explore them with the pedipalps (Wijnhoven, 2011). The first and second pair of legs also have many types of sensory structures that are used by harvestmen to investigate their wider surroundings and possibly to locate potential prey. An experiment with *Paranemastoma quadripunctatum* showed that if the pedipalps were removed it was still capable to find prey (Immel, 1954). When studying in detail the SEM-micrographs of the pedipalps we observed that apart from the sensilla chaetica, most other sensilla (like sensilla basiconica and solenidia) were concentrated at the posterodorsal side of the pedipalpal tarsus, while the majority of plumose setae were located on the dorsal to pro-lateral side. These different distributions likely reflect their different functions (Wijnhoven, 2013).

(2) ***Prey capture***: Agile prey is caught with the help of front legs, pedipalps and/or chelicerae. Prey capture might be one of the key functions of pedipalps in most harvestmen and the evolutionary driving force that lead to the observed diversity in pedipalpal morphotypes. This will be discussed in detail in the following sections.

(3) ***Food handling / securing***: When dismembering and chewing a food item, it is held and moved about by the pedipalps, especially in the Eupnoi (Fig.5.10.A-D). This might especially play a role if a living prey item is eaten. As harvestmen lack venom glands to immobilize their prey it can remain struggling over a long period. Prey loss is avoided by using the chelicerae alternately, in such a way that one chews and the other one stays grasping, and by a strong grip with the pedipalps.

(4) ***Defense***: The raptorial pedipalps of Laniatores are occasionally used as defensive weapons (Pereira et al., 2004). Males of *Chavesincola inexpectabilis* (Gonyleptidae) guard their females' clutches and chase off aggressive males with strikes of their pedipalps (Nazareth and Machado, 2009).

(5) ***Contest***: A fight for territory or females occurs in some neotropical Laniatores (Buzatto and Machado, 2008). Males attack each other using their massive raptorial pedipalps. In *Phalangium opilio* (Eupnoi: Phalangiidae) the elongated pedipalps are not only used to secure the female during copulation, but also to clamp opponent males in contests (Willemart et al., 2006).

(6) ***Mating***: Most harvestmen exhibit a sexual dimorphism of the pedipalps indicating a role in mating. In Eupnoi the male pedipalp is frequently used to secure the female during copulation by grasping the basis of its front legs (see section 5.3.7. and Tab.5.4). Sexual dimorphism is also present in some Dyspnoi (i.e. male 'claspers' in *Nipponopsalis yezoensis*, spines and denticles in males of some *Sabacon*, *Nemastoma* and *Histricostoma*), indicating that the pedipalps may also play a role in female securing in this group. In *Paranemastoma*

quadripunctatum it was observed that both partners intertwine their pedipalps during copulation (Immel, 1955a). In Laniatores both partners often interlock their pedipalps at the partner's legs, and females may also use their pedipalps to manipulate and guide the penis of the partner (Machado and Macías-Ordóñez, 2007).

(7) ***Substrate attachment***: The pedipalp may have a supporting function in climbing locomotion and resting on vertical or overhanging surfaces. Aggregating individuals of *Leiobunum paessleri* (Eupnoi: Sclerosomatidae) attach themselves mainly with their pedipalpal claws to rough substrates (hanging from cave ceilings) or to each other (Cockerill, 1988, Wijnhoven, 2011).

(8) ***Grooming***: In Eupnoi the pedipalp is used to hold the leg when grooming the tarsi with the chelicerae. Also, the ovipositor or penis may be groomed with assistance of the pedipalps.

5.4.3. Foraging – the primary adaptive function for pedipalpal evolution?

The results of our analysis strongly indicate that certain pedipalpal characters are primarily related to prey capture, or at least prey retention, resulting in the observed high diversity of morphotypes.

(i) Types and distribution of sensory organs: A high number of sensilla are contact chemoreceptors and mechanoreceptors, with their highest densities at the tarsal tip and often the ventral parts of distal segments. These come into play when something is grasped and manipulated with the pedipalps. The quality and agility of food items is assessed in this fashion. Campaniform sensilla were frequently found at the narrowed femoral base of dyspnoid pedipalps. These may provide information on cuticular stresses working in relation to the size and agility of hold prey items.

(ii) Spines: Harvestmen whose pedipalps are equipped with spines use them in a raptorial way. This was observed in *Discocyrtus pectinifemur* (Costa and Willemart, 2013) (Laniatores: Gonyleptidae), *Platybunus bucephalus* (Immel, 1955a), *Rilaena triangularis* (this study) (Eupnoi: Phalangiidae) and juvenile *Dicranolasma pauper* (Dyspnoi: Dicranolasmatidae) (Gruber, 1993, Dahl, 1903). In Laniatores contest may also play a role, but usually in juveniles and females the spination is developed likewise, thus this does not play the dominant role for spine evolution.

(iii) Glandular setae: When discovered the glandular setae were believed to be sensory organs (Hansen, 1893). Later it was proposed that they are used as a prey capture device, supported by the observation that their secretion is sticky (Schwangart, 1907, Rimsky-Korsakow, 1924, Wachmann, 1970). Our observations confirm this hypothesis, although we assume that these setae still have an important sensory function which comes into play in prey capture.

(iv) Segment modifications: The pedipalpal segments often show modifications, such as apophyses, shifts in joint angles, bendings or swellings. Most are related to a clamp mechanism or the formation of a capture basket between both pedipalps, which we will discuss in the following.

5.4.4. Snapping and clasping mechanisms in modified pedipalps

Hyper-bendable joints: A high degree of freedom of the tibia-tarsus-joint evolved in both the raptorial morphotype of Laniatores and the tentacle morphotype of Dyspnoi. By this means the tarsus can be flexed against the tibia and parts of the prey can be clamped within. This increases the effectiveness of spines or sticky setae, respectively. In Laniatores there is a trend of femur and patellar elongation, which increases the action radius of the raptorial clamp. This might be beneficial for the capture of large, vigilant or dangerous prey.

The clamp morphotype: The clamp-morphotype of eupnoid pedipalps might be an adaptation to secure agile prey or large prey items as it permits the execution of high clasping forces. Thick femoral spines (basal Eupnoi), strong denticles (Sclerosomatidae) or sticky glandular setae help to get a good grip of the item. The modification of the patella-tibia joint may help to secure the prey between the pedipalps.

The role of apophyses: Apophyses may increase the area covered in glandular setae and may form a capture basket together with the flexed tibia and tarsus. They are a barrier for a prey held between the pedipalps. This may help in restraining and holding down struggling prey. Apophyses always occur on the prolateral side of the pedipalp, which may support this argument. The extreme elongation of the patellar apophysis in *Dicranopalpus* functionally leads to a pincer-like clamp similar to the chelae of scorpion pedipalps. Although we observed that males may use it to grasp the female front legs during copulation, we don't know how significant this grasping is, as the male chelicerae too are used in precopulatory behaviour to stabilize both bodies. It remains unknown whether it is also used in prey capture as we could not observe hunting behaviour in this nocturnal and arboricolous species. High densities of foreign setae and brochosomes adhering to the glandular setae of the apophysis indicate that these harvestmen are predators. However, the articulation between patella and tibia only allows a low movement angle.

5.4.5. The role of glandular setae in feeding biology

Spinose capture baskets as represented by the laniatorid raptorial pedipalp are an effective means of prey capture. This is reflected by widespread occurrence of similar appendage modifications, e.g. in pedipalps of whip-spiders (Amblypygi) or raptorial legs of certain insects such as praying mantids (Neoptera: Mantodea) or mantisflies (Neuroptera: Mantispidae). These capture baskets, however, might not be very effective in capturing and retaining small prey, as it demands a very coordinated grabbing movement and small spacing between the spines (which in turn limits the effectiveness in capturing large prey). Sticky secretions, on the other hand, do not exhibit these limits (but for larger prey, see below). The most ancient lineages carrying glandular setae (Caddidae and Acropsopilionidae) also possess femoral spines. The glandular setae are distributed mainly on the prolateral sides, indicating a supporting role in prey capture and securing the prey between both pedipalps when dismembering. That these setae already have an adhesive function is supported by the finding of collembolan scales attached to them.

Phalangiids with a high density of glandular setae are reported to be able to capture fast moving prey such as flies or springtails (see Tab.5.3). In those that additionally bear spines the pedipalps (raptorial-clamp morphotype) are the primary prey capture tools, although they might be relatively short. In phalangiids lacking the spines other appendages are also used for prey capture, and other food sources are also frequently exploited. In *Mitopus morio* the prey (fly) is either first caught with the legs and then secured with the pedipalps (Phillipson, 1960) or directly grasped

with both pedipalps and chelicerae (Frank, 1937). In *Oligolophus tridens* the prey is grasped with both pedipalps and chelicerae. In most Eupnoi the number of glandular setae is reduced on the tarsus. Glandular setae are related to costs such as the production of the adhesive fluid or an increased demand of grooming. Thus they might only be beneficial in harvestmen that are primarily predators of small, agile animals. The absence of glandular setae in the distal tarsal parts of Phalangiidae and Neopilionidae (excluding Ballarrinae) may reflect a trade-off-effect: food items can be handled without contaminating the glandular setae, but if needed, an agile prey can be captured and secured by pulling it between the median parts of the pedipalp.

Further, in Eupnoi, glandular setae might be important in exploiting an abundant food source (especially in tropical regions): leafhoppers, as indicated by the frequent finding of brochosomes attached to the plumose parts of the setae. In epiphytic microhabitats leafhoppers and aphids might play a similar role as food source to springtails in soil habitats. Leafhoppers and springtails are capable of large escape jumps and may be hard to catch without special adaptations of the pedipalp. Both springtails (Noble-Nesbitt, 1963) and leafhoppers (Rakitov and Gorb, 2013) have evolved hardly wettable body surfaces which may have represented a high selective pressure on the function of harvestmen glandular setae and their secretions.

In the Sclerosomatidae glandular setae are restricted to juveniles or are (in most cases) totally absent. Sclerosomatids are reported to be omnivorous, feeding on living and dead animals, fruits and seeds, as well as fungi (see Tab.5.3). This indicates that these are not obligate predators, and glandular setae are thus not beneficial. However, in their place (prolateral and ventral sides and apophysis, if present) there are often thick, highly sclerotized denticles that may generate strong friction with the surface of a prey item, assisting in retention. Also the claws, which are serrated in this family, might play an important role. We found that, at least during feeding, prey items are often held with the claws.

In many Dyspnoi, and analogously in the Ballarrinae (Neopilionidae), the tentacle morphotype evolved. These harvestmen are rather small and epi- or hypogaeic or cavernicolous. They are presumably obligate predators: Gut content analysis showed that springtails are the dominant component of the nemastomatid diet (Adams, 1984). Springtails are a dominant and ubiquitous food source in soil habitats worldwide (Hopkin, 1997), and of high relevance for arthropod predators (Bilde et al., 2000). Both harvestmen and collembolans evolved very early, with the oldest fossils known from Devonian (400 MYA) (Whalley and Jarzembowski, 1981, Dunlop et al., 2003). However, springtails may be hard to catch due to their jumping capabilities. We think that the tentacle morphotype is an adaptation to exploit this food source. Its evolution is correlated with modifications of the glandular setae, which are presumed to enhance their function.

In most harvestmen exhibiting the tentacle morphotype (Nemastomatidae, Sabaconidae, Taracidae and Ballarrinae) glandular setae have an outstanding importance for prey capture. This is reflected by their presence on the distal parts (here on both pro- and retrolateral as well as on dorsal and ventral sides). When capturing springtails usually no other appendages are used (see also ***chapter 6***). Thus the animal totally relies on the sticky secretion of its glandular setae.

However, in some harvestmen with the tentacle type glandular setae are absent. Our phylogenetic character mapping indicates that this is an apomorphic state. It is obviously related to a change in the feeding habit, like in the Trogulidae or some Ischyropsalididae, which are specialized on snails (see part 4.1.). Preying on (slow) snails does not demand a grasp and attachment mechanism. In the Dicranolasmatidae there is a change in food spectrum and prey

capture behaviour throughout ontogeny, reflected by a pedipalpal dimorphism (Gruber, 1993). Juveniles capture prey with the help of their pedipalps, which are armed with both glandular setae and spines. The pedipalp can thereby be used both in a tentacle or a raptorial mode. Adults in contrast use both legs and chelicerae to capture the prey. Besides living prey, dead matter is also taken. Trogulidae, Dicranolasmatidae and Ceratolasmatinae (Nemastomatidae) further exhibit a unique camouflage mechanism: soil crypsis. A glue-like substance is secreted to catch soil particles, blending the animal into its micro-environment (Schwangart, 1907). This occurs in the later instars and is accompanied by the growth of a hood-like protuberance of the dorsal integument, totally covering the mouthparts to keep them clean. Long pedipalps with glandular setae might interfere with this mechanism.

Another reason for the ontogenetic shift might be that the glandular setae are most effective in small harvestmen. This could be due to the relationship between surface and mass scaling. Because the volume (and accordingly mass) of a body increases by a power higher than surface area the relative area covered in glandular setae decreases with increasing body size. Further, because setae do not scale with body size, the size and amount of the sticky droplets are restricted. These restrictions are well known from attachment devices of arthropod feet, used in climbing, and can only be overcome by an increase in effectiveness (strength) of the attachment (Labonte and Federle, 2015, Peattie and Full, 2007, Arzt et al., 2003). This means that the glandular setae are primarily useful for small prey animals, but in the capture of large prey they may only play a supporting function. Accordingly the only reliance on glandular setae is especially found in rather small species, whereas larger ones often exhibit additional adaptations such as spines, additionally use their legs and chelicerae for prey capture or feed on dead or slow moving animals. Further, we found that the relative length of the pedipalp decreases with increasing body size (Fig.5.4), which means that the loss of effectiveness cannot be compensated by a relative increase of setal number, as space is limited. These relationships might indicate that predation (using the pedipalps for prey capture) is especially important in nymphs and small species.

Finally, in nymphs of some Laniatores (Cosmetidae) a sticking mechanism of the pedipalps could have been evolved independently, as the unique modification of the pedipalpal pretarsus indicates. A sticky mucous is secreted from numerous gland openings (G. Machado and S. García, unpublished data), which may be an effective trap for prey. However, behavioural observational data is lacking.

5.4.6. Evolutionary trends of glandular setae

We found that glandular setae are synapomorphic for the Palpatores clade. They may have their origin in a soil-dwelling caddid-like small harvestman, being ancestor of both Eupnoi and Dyspnoi, and not an ancestor of both Laniatores and Palpatores, as suggested by previous authors (Groh and Giribet, 2014, Shear, 1986).

The results of our ultrastructural analysis suggest that the glandular setae are modified sensilla chaetica. This hypothesis is supported by both a similar inner structure (stapled rings, dendrite bundle with sheath, channels in outer cuticle, pore at tip flanked by lobe-like protuberances) and their replacement with sensilla chaetica in males of sexually dimorphic Phalangiidae as well as in adults of species with an ontogenetic dimorphism (i.e. Gagrellinae). Sensilla chaetica are presumed both mechano- and chemosensors (Spicer, 1987). If the sensilla chaetica are already secretory is not totally clear. In TEM images shown in Guffey et al. (2000) an

electron dense material is present in the channels and cells near the setal basis, flanking the dendrites, appear to be glandular. For a chemosensor a secretion may have the function as a solvent for gustatory substances. In spiders chemosensors have been shown to extrude small amounts of fluids, leaving thread like traces behind when sliding the foot over a smooth glass slide (Foelix et al., 2012) (and own observation).

The presumably most primitive type of glandular setae (ps1) might be found in *Caddo agilis* (Caddidae). The seta is rather stiff, its plumose part is small, the microtrichia are short and multiply branched (no regular rectangular branches). These characters may limit the capability of the seta to keep a large droplet, especially under load. We found that this is an important function of the plumose part, as high pulling forces work on the secretion when a struggling prey is glued (see ***chapter 6***). Rectangular surface structures are necessary to prevent roll-off of the droplet from the pointed seta (and pointed microtrichia). Both the microtrichia and the short branches at the tip of the microtrichia are presumed to play an important role in flow stopping on two hierarchical levels. The setal tip should also exhibit a certain degree of flexibility to buffer some of the dynamic load. The same principle applies to the microtrichia on the micro scale which should then preferably be long. The ps1-type might therefore be less effective than the following setal types in several regards.

The ps2-type of the Phalangiidae shows a longer plumose part and longer microtrichia with more regular tip branching, which may increase the capability to retain the droplet under tensile load. An innovation might also be represented by the backing depression. The cuticle is much thinner and thus presumably more flexible at the base of the plumose section, permitting a bending in a guided direction (towards the body), whereas it stiffens in the other direction (away from the body). This might be possible due to the unique inner cuticular structure, with staples of thick cuticular rings of dense material, connected by thin epicuticle and occasionally a less dense inner material. The cuticle between the rings might be easy to compress, permitting a buckling of the rear of the seta at the depression below the plumose part. Besides a possible function as a mechanoreceptor this might also be important to withstand the high dynamic load of the struggling prey and might also function as a trap, because the seta is less compliant in the other direction (away from the body).

This principle might be very effective as it is even enhanced in the ps4-type of Neopilionidae and the ps6-type of Taracidae and Sabaconidae. In the ps4-type the depression is even deeper and longer, thus the compliance is enhanced along the most part of the seta. In the analogue ps6-type there is a trench-like invagination, which presumably has a comparable function. Both depression and trench may further assist the secretion flow in the longitudinal direction and help its spreading, as the plumose part is rather large in these setae (however, for the prevention of secretion loss during a vertical pull-off of the droplet this should be disadvantageous).

In Protolophidae and Sclerosomatidae the seta (ps3-type) is stiffened, thus more spine-like. This might be related to the more capture-basket like pedipalps, with long apophyses protruding medially. As a sticking device this type might be less effective and got lost for multiple times partially (in adults) or totally (all instars).

The tentacle pedipalp-morphotype totally relies on the function of the glandular setae. Thus there might have been a stronger selective pressure on both setal and secretion effectiveness than in the plesiomorphic types, where the setae have only supportive function. Accordingly, these groups exhibit a morphological derivation of glandular setae, presumably related to an increase in

effectiveness. The tentacle convergently evolved in two lineages and independently on the Northern and the Southern hemisphere (Fig.5.14). Interestingly, this is correlated with the evolution of rather similar setal morphotypes in both lineages: The highly anisotropic ps4 vs. ps6 and the clavate vs. pseudo-clavate seta (Fig.5.13). The dyspnoid morphotype was already evolved at least in Cretaceous as represented by an amber fossil clearly showing the thread-like pedipalp and the secretion bearing clavate setae (Giribet and Dunlop, 2005).

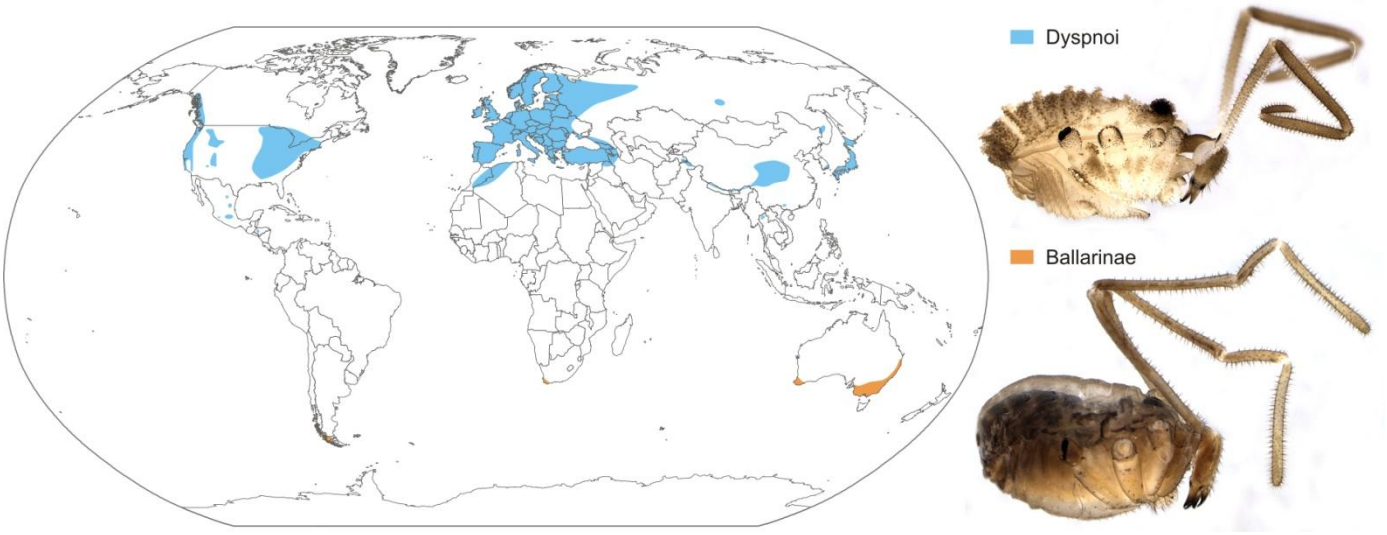


Fig.5.14. Convergent pedipalpal modification in Dyspnoi and Ballarrinae (Eupnoi: Neopilionidae). Known distribution of Dyspnoi after (Shear and Gruber, 1983, Shear, 1986, Schönhofer et al., 2013, Shear, 1975, Zhang and Zhang, 2013), of Ballarrinae after (Hunt and Cokendolpher, 1991). Habitus of *Mitostoma chrysomelas* (above) and *Ballarra longipalpis* (below), showing similarities and differences of the convergent tentacle pedipalp morphotype.

The globular arrangement of microtrichia in cs and p-cs is perhaps the most complex and effective modification. It permits an equal pull-off resistance in various directions and a relatively large droplet volume, because of the stabilization of a globular droplet shape. Further, the setal tip is free of secretion and microtrichia and highly flexible and thus can be independently function as both a mechanoreceptor and chemoreceptor, which gives direct feedback about prey contact. The swollen brim of ps6 in *Sabacon* and *Hesperonemastoma* may similarly have the function to prevent secretion flow into the opening of the apical pore. Accordingly the pores of those setae and the clavate setae often look clean with the flanking lobes distinctly visible, whereas the pores of ps1-5 setae are frequently highly contaminated with secretion remains. Further, we found in both *Mitostoma* (Nemastomatidae) and *Sabacon* (Sabaconidae) an immediate reaction to prey touching the glandular setae, indicating a direct sensory function.

Another important character leading to the high effectiveness of the dyspnoid glandular setae is the occurrence of channel openings. In the less derived ps1-5 no channel openings are apparent. Thus there are two possibilities of the secretion emergence: (1) The secretion channels open to the inside and the secretion emerges from the setal tip. (2) The secretion diffuses through the thin epicuticle. We think the second option is the most likely, because of the following reasons: We found that the cuticular material between the channels vanishes in the plumose part, thus the channels fuse and a secretion reservoir is formed between the thin epicuticle and the inner ringed wall. We found (presumed) secretion remains (an irregular granular substance) within this reservoir, whereas the inner tube was often hollow (sometimes the dendritic sheath was still present within the tube). As the microtrichia are built of only epicuticle this reservoir spreads into

the basal parts of the microtrichia. It therefore has a highly enlarged surface area, which may support diffusion of the secretion to the outside. Further, an emergence of the secretion from the apical pore seems to be disadvantageous, as the droplet would hardly get between the microtrichia and be in a high risk of loss and contaminating other body parts.

The major dyspnoid lineage has evolved slit-like openings of the secretion channels. This might be very beneficial especially for two reasons: (1) Larger volumes of the secretion can be extruded faster. This might permit a quick recovery of the droplet after moulting or is fast renewal after contamination. (2) The openings allow a higher viscosity of the fluid. Tensile tests with clavate seta secretion showed a high shear thickening behaviour, which indicates the interaction of long-chained macromolecules (see ***chapter 6***). Such a fluid may not be able to diffuse through the epicuticle and demand openings. Indeed there seems to be a derivation of the secretion, as the one of clavate setae does hardly dissolve in polar liquids, whereas the one of plumose setae can be removed to most parts by ethanol. Comparing the wetting and tensile properties of secretions of different lineages may shed light on the evolution of this adhesive secretion and be a valuable subject for future studies.

5.4.7. The functional role of sexual dimorphism in glandular seta possession

Many Phalangiidae show a sexual dimorphism in glandular seta possession. The reason for their loss and replacement by sensilla chaetica in males is correlated with the behaviour to grasp and secure the female during copulation with the help of the pedipalps. Sticky setae would probably hinder a detachment after copulation leading to death of both sexes in worst case. A reduction of prey capture capability is less costly in this case, especially as those species are omniphaguous (see above).

5.4.8. Implications of the results for the phylogenetic treatment of Phalangiidae

In general, our results are consistent with the recent hypotheses of inter-familiar relationships. Some findings may be valuable for the evaluation of the (largely unresolved) relationships within Eupnoi.

Some Phalangiidae share several characters with the basal Caddidae: The correlated occurrence of a pro-lateral hump-like apophysis on the distal femur (bearing glandular setae) as well as femoral spines and glandular setae at the tarsus might be synapomorphic for the Eupnoi and mark the most basal lineages. This includes the Platybuninae (*Lophopilio palpinalis* and *Megabunus rhinoceros* (in the latter species the femoral apophysis does not bear glandular setae, but spines)) and *Rilaena triangularis* (part of Phalangiinae). Both platybunin species also show very similar ultrastructure of the setal plumes like in *Caddo* (multiple branching, see part 4.4. for evolutionary trends in glandular setae). *Mitopus morio* (part of Oligolophinae) shares the mentioned characters but the spines. In *Oligolophus tridens* and *Paroligolophus agrestis* (Oligolophinae) the number of glandular setae in the tarsus is greatly reduced, but the femoral apophysis is still present. In analysed species of both Platybuninae and Oligolophinae sexual dimorphism in glandular seta possession is not present, which is regarded as an ancestral state. Behavioural observations in the latter subfamily show that pedipalps are used in a female-grabbing behaviour, but not as excessively as in the species with sexual dimorphism.

Whereas femoral spination and the density of glandular setae at the tarsus could potentially be homoplasic characters, it seems unlikely in case of the femoral apophysis (which is actually too small to play a significant role), the irregular terminal elements of setal plumes (where an

adaptational benefit is implausible) and sexual dimorphism (correlated with excessive female-grabbing behaviour). Thus, some characters presented in this work could potentially be valuable for future cladistic analysis, combining both morphological and genetic data.

The most derived pedipalp among the Phalangiidae is represented by *Dicranopalpus*, including elongation of femur, tibia and tarsus, the occurrence of a sharp apophysis on proximal femur, a serrated claw (untypical for Phalangiidae, but shared with *Amilenus*), the lack of glandular setae on the tarsus, the finger-like elongation of the patellar apophysis (forming a clasper with the prolateral curved tibia as counterpart) and a hump-like apophysis on distal tibia (in *D. ramosus* only in juveniles). The latter four characters are shared with some Gagrellinae (juveniles of *M. minax*, in *Rongsharia* spp. also in adults (Martens, 1982)), the latter three with *Protolophus*, which is the sister lineage to the Sclerosomatidae, however, it is hard to evaluate if these character states are homologous. Though, it is possible that the Phalangiidae are paraphyletic and that Sclerosomatidae and Neopilionidae are nested within this clade. Future approaches on this issue may resolve this open question.

Conclusion

Our comprehensive survey depicts that pedipalps are the primary predatory device in harvestmen, and that pedipalps have been highly shaped by the predatory activity of this omnivorous group of arachnids throughout evolution. Key innovations are glandular setae releasing a sticky fluid, spines and hyper-flexible joints permitting the fast capture and securing of agile prey. The glandular setae play an important role in the capture of small, agile prey, such as collembolans and cicadellidae, and thus are especially important in small-bodied predators or juveniles. A comparative chemical and physical analysis of the sticky fluid in different lineages seems to be a valuable issue for future studies. Further, field studies on prey capture behaviour and diet analysis are important to evaluate the importance and adaptive influence of prey capture in harvestmen with different pedipalp morphotypes. The micro-morphological analysis of certain pedipalpal (and setal) characters may be valuable for future taxonomic and phylogenetic studies.

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6. Paper no.4*:

Gluing the ‘un-wettable’: 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 well known in insects, this mechanism is much less studied in arachnids, besides 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 the harvestman Mitostoma chrysomelas being able to capture fast moving springtails (Collembola) just by slight touch of the pedipalp. Adhesion of single clavate setae increased proportionally with pull-off velocity, from 1 µN at 1 µm/s up to 7 µN at 1 mm/s, 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 sticky tentacles of carnivorous plants. These analogies depict that viscoelasticity is a highly efficient mechanism for prey capture, as it holds the 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 hardly 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.

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6.1. Introduction

The interaction between prey and predator is one of the central aspects of life and 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, since complex sensory-muscular feedback is less necessary to successfully catch and arrest the prey, if compared to other mechanisms (Betz and Kölsch, 2004). On the other hand, secretory products may be related to high synthesis costs, and structural or behavioural adaptations that prevent gluing of own body parts must co-evolve. Thus, this strategy might be especially useful for organisms with locomotion capabilities worse than the prey or those with a simple or totally lacking nervous system, like carnivorous plants (Darwin, 1875) or 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 exceptionally on the viscid capture threads of spider webs and their derivations (Sahni et al., 2011b, Betz and Kölsch, 2004). Other arachnid orders are notoriously understudied, although they may represent a considerable part in 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 (Curtis and Machado, 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), that carry droplets of a viscous secretion at their tips (Shultz, 1998).

In the Nemastomatidae the glandular setae densely cover the whole pedipalps except coxa and trochanter. These setae (formerly called ‘Kugelhaare’, clavate setae or drumstick-like setae (Pinto-da-Rocha et al., 2007)) exhibit an 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 (Wachmann, 1970, Rimsky-Korsakow, 1924). These setae were first interpreted as sensory organs (Hansen, 1893). Later it was assumed that their secretions are sticky and used to capture prey, namely small arthropods co-occurring in the litter habitats of nemastomatids, like 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 *Nemastoma quadripunctatum* came to the conclusion that those setae do not play any role in prey adherence as the prey is grasped by the chelicerae (Immel, 1955b). However, the author reported that the prey attack was very fast, thus, she may hardly 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, although springtails were able to escape in some cases (Gruber, 1993).

Presumably, glueing a springtail or other small soil organisms might not be trivial, as they are equipped with hardly 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, Helbig et al., 2011, Ghiradel and Radigan, 1974). 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, like 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 (Sahni et al., 2010, Opell and Hendricks, 2010). 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 this study is to clarify the adhesive mechanism and the role of clavate setae of harvestmen in capturing springtails. This is done by observing 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 studying their rheological behaviour. Furthermore, using micromanipulation and cryo scanning electron microscopy (Cryo-SEM), it is examined if 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.

6.2. Results

6.2.1. Prey capture behaviour

Prey capture could not be observed in *Nemastoma* spp., because it 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 one 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.6.1.L). In the other half of the trials, the prey could free itself from the pedipalps, often after a significant time of heavy struggle. Success rate decreased with prey size. Both entomobryomorph (elongated) and sympypleonan (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.6.1.e), held closely above the ground, and retracted again. If one pedipalp touched a springtail, the latter

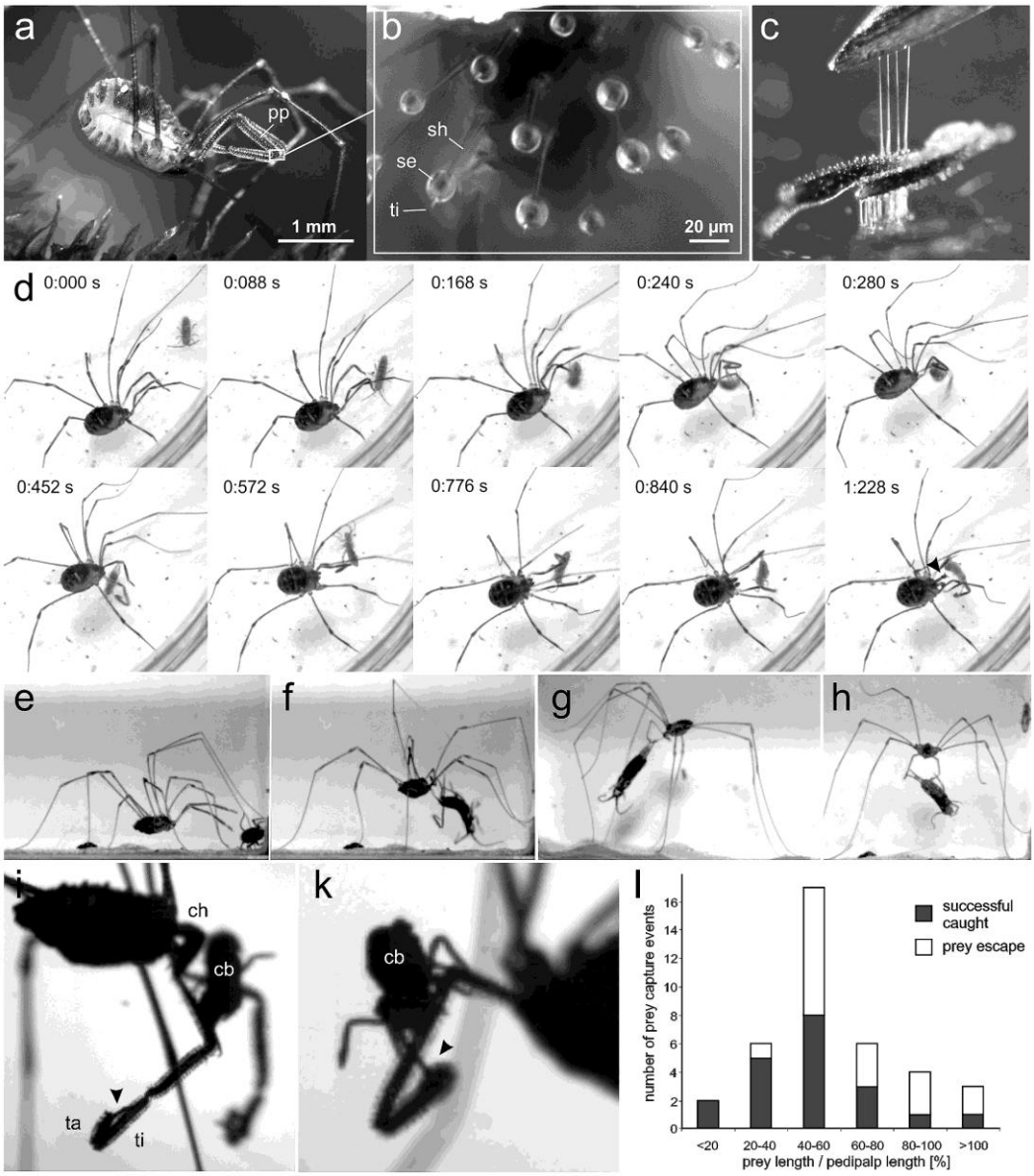


Fig.6.1. Morphology of the sticky apparatus and prey capture behaviour of *Mitostoma chrysomelas*. (a.) Adult individual in its natural habitat. The long pedipalps (pp) are normally held in a flexed position; the secretion droplets of the clavate setae are clearly visible against the dark background. (b.) Light micrograph of clavate setae. The setae have a stiff narrowing shaft 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 recording (250 fps) 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 attaching it at both the antenna and furca. Then the prey is grasped with the chelicerae (protracting chelicera is indicated by arrowhead). The whole video sequence can be found in the electronic supplemental material (S1). (e.-i.) Frames of high speed video recordings (HSV) (1000 fps). (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 (ti) 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. (k.) Successful catch of a symphypleonan springtail (cb). Its legs stick to the clavate setae of the pedipalp patella; additionally one antenna is clasped between tarsus and tibia (arrowhead). (l.) 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 Tab.2). These anecdotal reports depict the high variance of accepted and caught springtail prey.

one was immediately glued (Fig.6.1.d). Then 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 of twice the size than the harvestmen were attacked (with 1 of 3 attacks being successful). In the high speed video recordings it could be 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.6.1.g,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 was immediately fixed and could not come free, not even by strong pushes of the furca. When catching prey, the harvestmen´s pedipalpal tarsus is often bent towards the tibia like a clasp (Fig.6.1.i,k). 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, like 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.

6.2.2. 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 *Mitostoma chrysomelas*. The setae on the dorsal side of the distal tibia carry the biggest droplets with diameters between 11 and 17 µm (Figs.6.1.b, 6.2.e). The main difference between both genera is the length of the pedipalp, which is less (about 80%) than the body length in *Nemastoma* but about twice the body length in *Mitostoma*. The distribution of clavate setae is very sparse on femur and patella in *Nemastoma* (only few single setae), whereas in *Mitostoma* 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.6.2.c). The shaft appears rather rigid, as it bends only slightly before it breaks off at its socket when laterally pushed with a capillary tip. 2-3 µm below the tip there is a dense globule (head) consisting of microtrichia (previously called “chaetae” (Wachmann, 1970)) (Fig.6.2.d). The chaetae are 1-2 µm long and carry three nano-hooklets (each about 200 nm long) at their distal tips (Fig.6.2.d-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, giving space for secretion delivery (Fig.6.2.d). Canals in the outer cuticle (Fig.6.2.f) are probably responsible for transporting the fluid to the head, as proposed by Wachmann (1970) on the basis of his TEM 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 SEM and even did not evaporate 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.

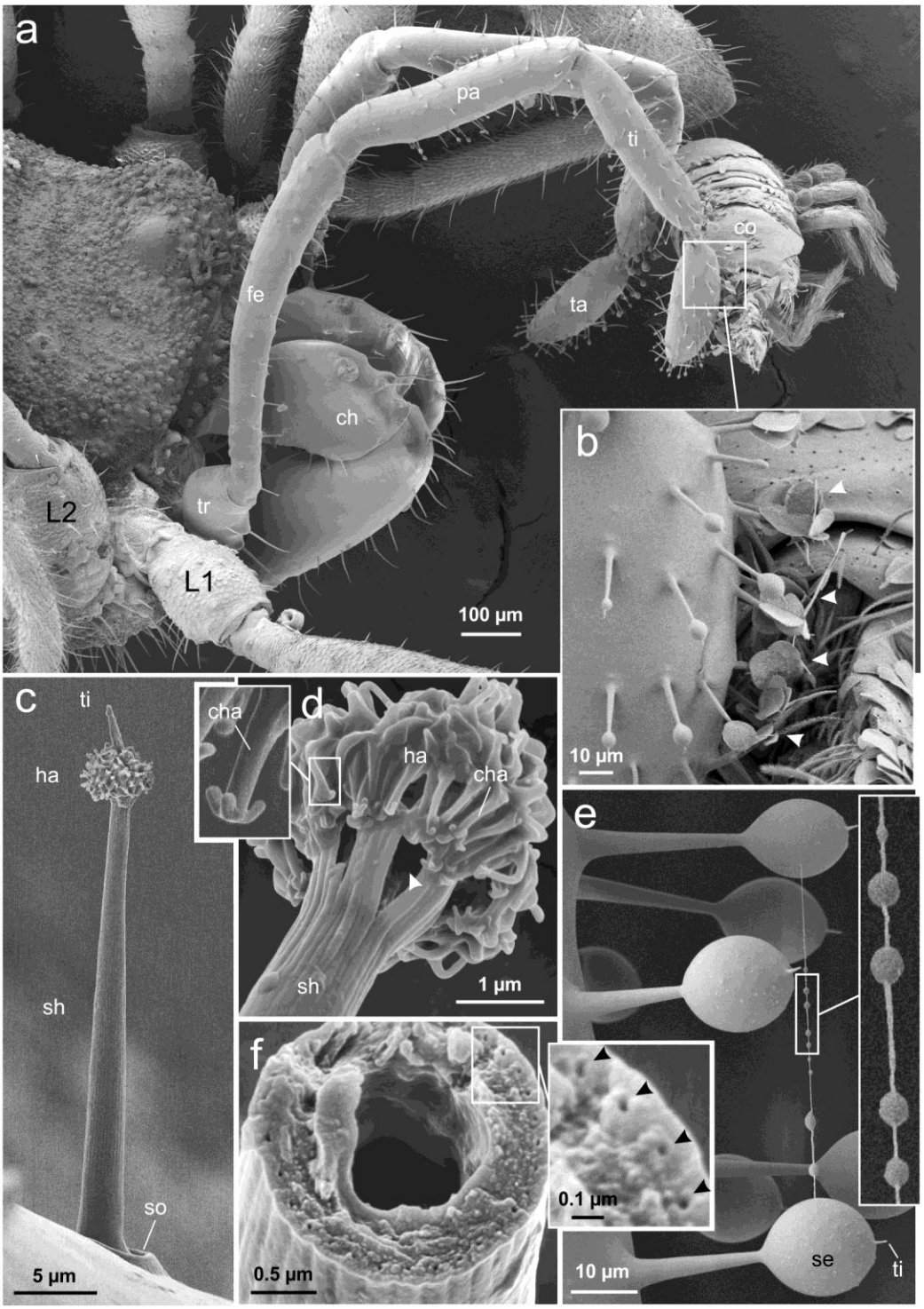
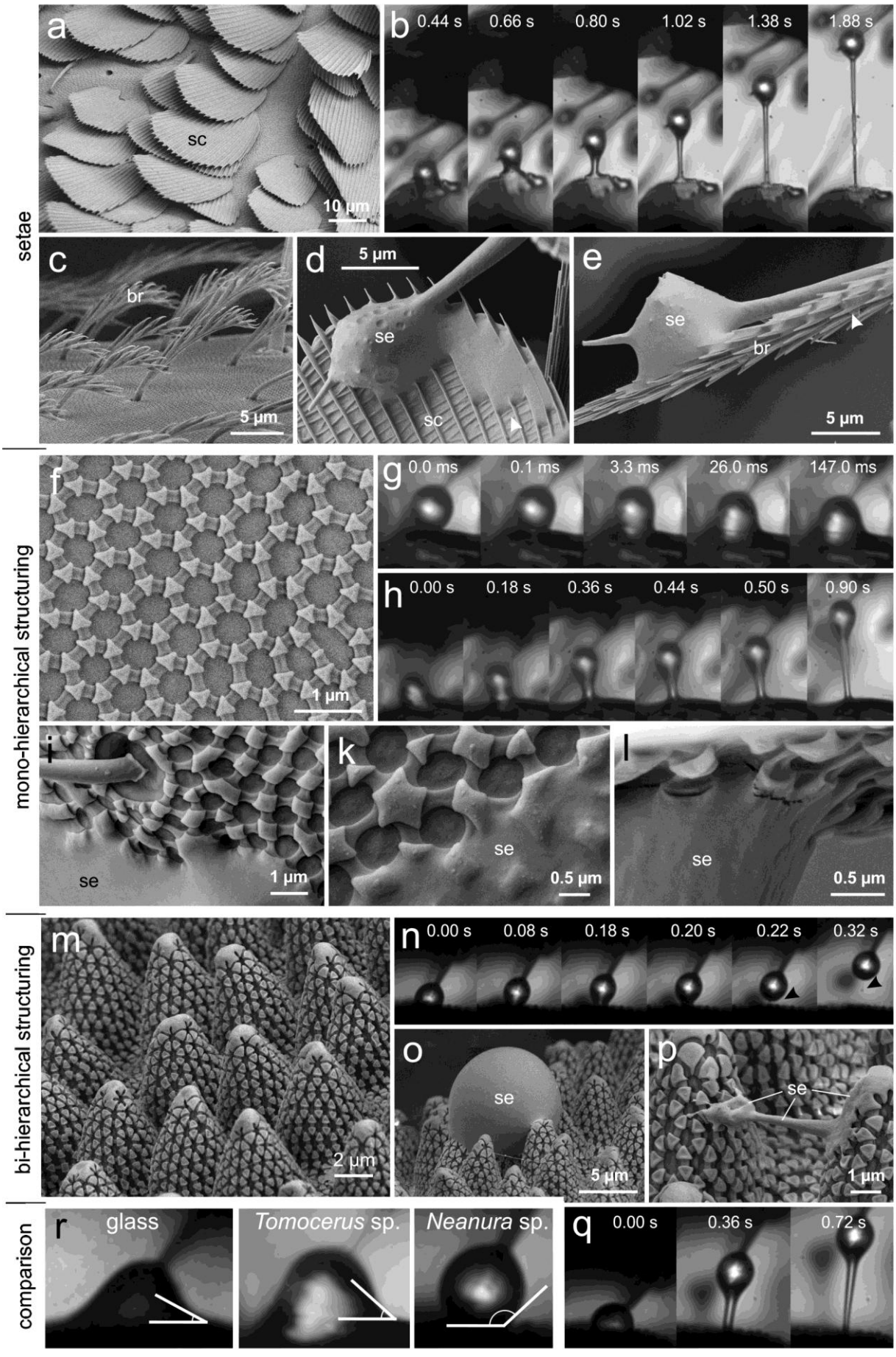


Fig.6.2. Scanning electron microscopy of the clavate setae. (a.) *Nemastoma dentigerum* with a glued juvenile *Tomocerus vulgaris* springtail (co). The pedipalp consists of coxa (not visible here), trochanter (tr), femur (fe), patella (pa), tibia (ti) and tarsus (ta). The clavate setae are primarily distributed in the last two segments. ch, chelicerae; L(1,2), 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 chaetae (cha), here partly glued by remains of the secretion. The inset shows one chaeta with its three distal hooklets in detail. (e.) Tibial clavate setae of a juvenile *Mitostoma chrysomelas* with large secretion droplets. 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.


setae
a
sc
10 µm
b
0.44 s
0.66 s
0.80 s
1.02 s
1.38 s
1.88 s
c
br
5 µm
d
5 µm
se
sc
e
se
br
5 µm
mono-hierarchical structuring
f
1 µm
g
0.0 ms
0.1 ms
3.3 ms
26.0 ms
147.0 ms
h
0.00 s
0.18 s
0.36 s
0.44 s
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se
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r
glass
Tomocerus sp.
Neanura sp.
q
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0.72 s

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.6.3.f) and dense covers of setal structures, which are scale-like in *T. vulgaris* (Fig.6.3.a) and fringed bristle-like in *O. flavescens* (Fig.6.3.c). 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 (Figs.6.1.i, 6.2.b). The secretion of the clavate setae was found to totally wet the microstructures on the springtail setae (Fig.6.3.b,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.6.3.g). When retracted, the secretion can be highly stretched before losing its contact with the springtail cuticle, indicating significant adhesion and viscosity (Fig.6.3.b,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.6.3.i-l). The worm-like and less mobile *Neanura muscorum* exhibits the cuticle structure of two distinct hierarchical levels (macrostructure and microstructure; Fig.6.3.m) with only few smooth setae. *N. muscorum* repeatedly pushed onto the pedipalps of *Nemastoma dentigerum* did not stay attached, and remains of clavate setal secretion could hardly wet the surface (Fig.6.3.o,p). When retracted, the capillary bridge shrank very fast (Fig.6.3.n). A capillary bridge comparable to the that observed during the capture of above mentioned collembolans could only be observed after the clavate seta was deeply (20 µm) pressed into the cuticle (Fig.6.3.q), 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.6.3.r).

Fig.6.3. Wetting of springtail cuticle. (a.) Scale-like setae of *Tomocerus vulgaris*, covering the whole body including legs, antenna and furca. (b.) Frames of a video sequence (60 fps) 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 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 (10000 fps) showing the fast wetting of the bare cuticle (scales removed) of a *T. vulgaris*. (h.) Frames of a video sequence (60 fps) of a *Nemastoma* clavate seta retracted from a *T. vulgaris* bare cuticle. (i.-l.) Secretion of *Mitostoma* clavate setae spreading over the microstructures of the antennal cuticle of a *Bourletiella viridescens* springtail. (m.) Cuticular structures of *Neanura muscorum*. (n.) Frames of a video sequence (60 fps) 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). (o.-p.) *Nemastoma* clavate seta secretion residuals on *N. muscorum* cuticle. Spreading of the fluid is prevented by the complex cuticle microstructure. (q.) Frames of a video sequence (50 fps) 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. (r.) Comparison of contact angles of attached *Nemastoma* clavate setae on glass and bare springtail cuticle.

6.2.3. Adhesive properties

The pull-off forces of clavate setae contacting a smooth glass surface are comparable between *Nemastoma lugubre* and *Mitostoma chrysomelas* and increase linearly with an increased pull-off velocity within the measured range (Tab.6.1). 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 comparable range to those measured on glass, but exhibited large variation (Tab.6.1). 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 about double as large as 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 exceeded the values obtained on glass. These results may indicate that the springtail cuticle microstructures influence the flow of the adhering secretion.

Tab.6.1. Adhesive forces [µN] at different pull-off velocities [µm/s] of tibial clavate setae in *Nemastoma lugubre*, female, and *Mitostoma chrysomelas*, juvenile; mean ± standard deviation, (n=sample size); 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 is comparable in both species. Substrates were a smooth glass surface and different parts of the cuticle of a *Lepidocyrtus lignorum* springtail, respectively.

Species	**Substrate**	**Pull-off velocity [µm/s]**						
		1	**5**	**10**	**50**	**100**	**500**	**1000**
N. lugubre	glass	1.14±0.58 [a] (n=10)	1.33±0.63 [a, b] (n=8)	1.33±0.63 [a, b] (n=10)	1.44±0.36 [b] (n=10)	1.70±0.66 [b, c] (n=11)	2.53±1.00 [c, d] (n=9)	3.58±1.91 [d] (n=12)
	springtail (bare cuticle)			0.49±0.25 (n=6)		0.97±0.52 (n=4)		4.53±2.26 (n=5)
	springtail (setae)			2.45±2.87 (n=6)		3.79±4.16 (n=4)		
M. chryso-melas	glass	1.23±0.14 (n=7)		1.21±0.20 (n=7)		1.43±0.20 (n=9)		1.69±0.27 (n=9)

The shape of the stretched droplet during the tensile test changed over time. At elapsed time of 10-20 s, an initiation of the so-called beads-on-a-string (BOAS) formation was observed (Fig.6.4.a). 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. 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 totally pulled off the substrate until the contact broke. Near the site of the fluid-substrate contact, a branching of the capillary bridge was often observed (Fig.6.4.b). Droplet elongation at high speed tension was several times larger (30-40 times of 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 is 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 few setae with the tip of an insect needle and then pulling

apart. However, the secretion seemed to become more viscous after this period of drying, and it could be pulled into long elastic threads (Fig.6.1.c) that were able to reshape to spherical droplet after breaking apart.

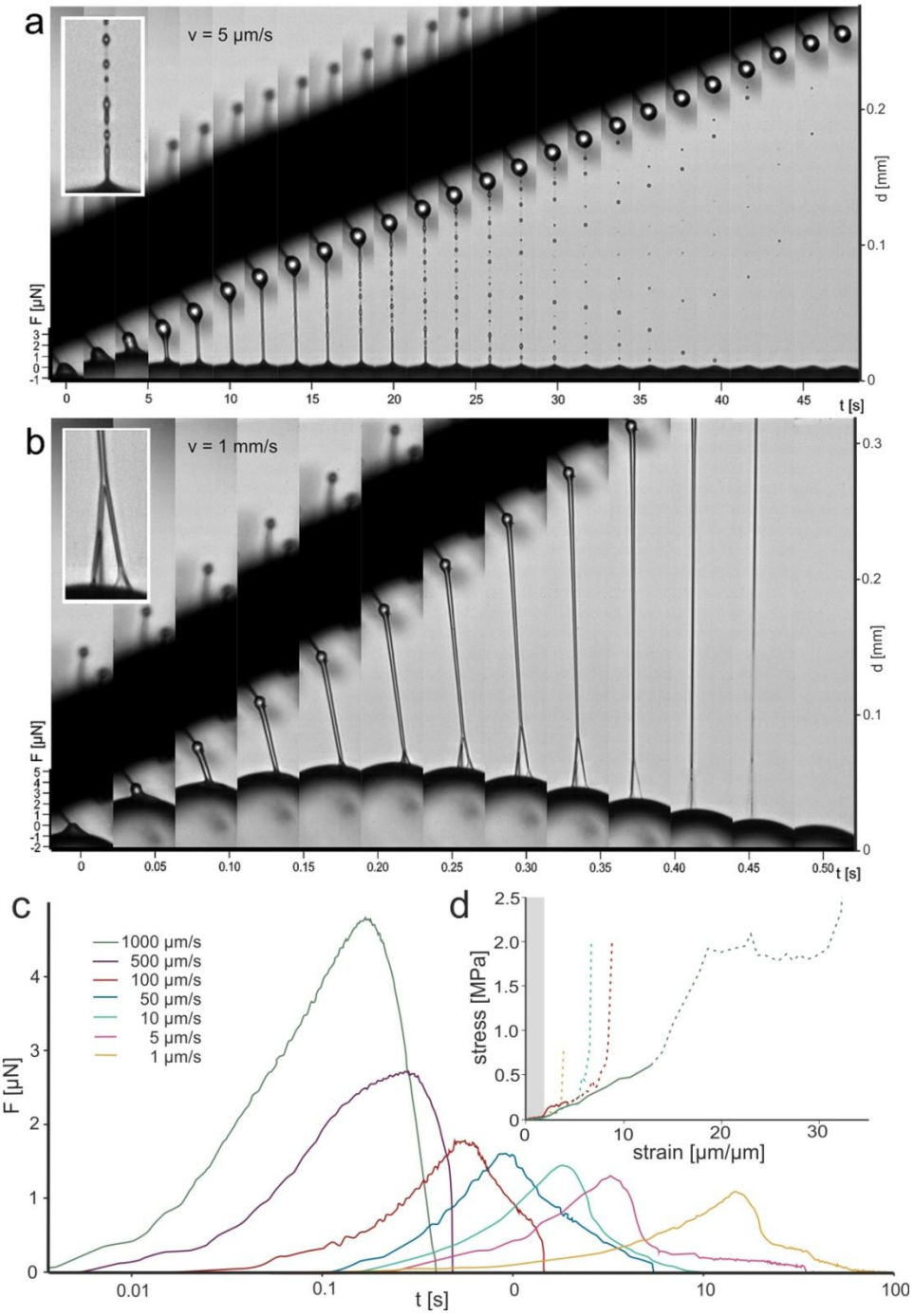

Fig.6.4. Secretion adhesion and rheology. (a.) High speed video frames of a pull-off test with 5 µm/s showing the formation of BOAS-structures. The inset shows a detail of the secretion jet near its contact to the glass bead at t = 23 s. The vertical shift of the glass bead surface indicates the pull-off force (scale at the left image side). The whole video sequence can be found in the electronic supplemental material (S6). (b.) High speed video frames of a pull-off test at 1 mm/s, 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 can be found in the electronic supplemental material (S7). (c.) Exemplary time-force curves for one seta pulled off with different velocities. Remark that the time-scale is logarithmical. (d.) Force-extension curves for one exemplary 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 gets imprecise; graph lines are dashed in these parts.

6.3. Discussion

Springtails are, besides oribatid mites, 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 by using an elastic spring 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: Some carnivorous plant of the genus *Drosera* capture springtails with a gluey mucilage, secreted by hair-like structures (Verbeek and Boasson, 1993). This glue exhibits quite similar rheological behaviour as the one of the harvestmen studied by us (Erni et al., 2011). *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 (Koerner et al., 2012a, Betz, 1996, Bauer and Pfeiffer, 1991, 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 due to 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 hardly wettable and thus exceptionally anti-adhesive (Helbig et al., 2011, Ghiradel and Radigan, 1974, Noble-Nesbitt, 1963). 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 a millisecond. 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 the 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. The take-off velocity of a jumping springtail is even one thousand times higher (1.4 m/s (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), cohesion failure was only rarely observed, and never a failure of adhesion between the secretion and the clavate seta. 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., 2011a). 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 (PSA), 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 solid-like at high (> 0.5 mm/s) pull-off velocities. For its adhesion, the branching near the contact site may have an important enhancing effect: in elastic solids, contact splitting significantly enhances adhesion due to 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 further be 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 (beads-on-a-string morphology, BOAS) is only known from viscoelastic fluids (Bhat et al., 2010). This was analogously documented for the glue of protocarnivorous plants (Voigt and Gorb, 2008) and spider capture threads (Sahni et al., 2010, Opell and Hendricks, 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, prey being the target of these analogous adhesive systems 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 (Nentwig, 1982, Eisner et al., 1964). 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, 1955b, 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 solely consume collembolans and other small soil organisms, but also dead animals and plant material (Hvam and Toft, 2008). Maybe 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). *N. 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 highly above the ground. *M. chrysomelas* was also observed grooming its pedipalps with the chelicerae after unsuccessful attacks.

6.4. Conclusion

The pedipalpal secretions of nemastomatids can easily wet the complex unwettable surface structures of most collembolans and elicit a high dynamic strength by viscoelasticity. The adhesive strength and elasticity of single droplets are comparable to 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 load. Behavioural observations underline that it is a very successful way to capture very mobile prey despite effective counter adaptations of the prey, like setal discharge.

6.5. Methods

6.5.1. 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, South-Western Germany. *Mitostoma chrysomelas* HERMANN 1804 was found on the ground in open areas when the upper vegetation was removed. *Nemastoma* spp. (*N. lugubre* MÜLLER 1776, *N. dentigerum* CANESTRINI 1873) 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, 1972). Collembolans were collected directly before experiments nearby the university by the means of same methods and stored in plastic tubes equipped with humid tissues. Springtail identification followed Fjellberg (1998) and Bellinger et al. (2014). A summary of used springtail species and their cuticular structures (studied by SEM) is given in Tab.6.2.

Tab.6.2. Springtail species used in this study and their cuticular features. Last column specifies in which tests those were used: AM, adhesion measurement; PC, prey capture observation via HSV; WT, wetting tests via HSV and/or Cryo-SEM.

	species	primary granules	secondary granules	fringed bristles	scales	tests
Entomobryomorpha	*Tomocerus vulgaris* TULLBERG 1871	+	-	-	+	PC, WT
Entomobryomorpha	*Orchesella flavescens* BOURLET 1839	+	-	+	-	PC, WT
Entomobryomorpha	*Lepidocyrtus lignorum* FABRICIUS 1793	+	-	+	+	PC, AM
Entomobryomorpha	*Lepidocyrtus curvicollis* BOURLET 1839	+	-	+	+	PC
Symphypleona	*Bourletiella viridescens* STACH 1920	+	-	-	-	PC, WT
Symphypleona	spp.	+	?	-	-	PC
Poduromorpha	*Neanura muscorum* TEMPLETON 1835	+	+	-	-	PC, WT

The harvestmen were kept in plastic tubes with humid tissues under cool conditions (10-15°C). For the 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. Behavior was observed and prey capture events were photo- or video-documented using an EOS 600D SLR (Canon Inc., Tokio, 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 fps. 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.

For each observed attack the success was documented. Only attacks were taken into account, in which the prey was touched. A prey capture event was considered as 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 as successful. The attacked prey was categorized by size (the springtail body length relative the to harvestmen pedipalpal length).

6.5.2. 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., Tokio, Japan) at an acceleration voltage of 3.0 kV.

Living collembolans collected in the same habitat as the harvestmen (see Tab.6.2) 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.

6.5.3. 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.6.5.a. 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 about 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.6.5.b). Then it was moved downwards with one step (step size 5 µm 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 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.6.5.d). The weight force per pipette deflection distance was then calculated for each of the three runs using linear regression approach (Fig.6.5.e), and mean values of the slopes were taken as the calibration factor for each pipette tip (Fig.6.5.f). The calibration factors obtained ranged between 0.09 and 0.11 N/m.

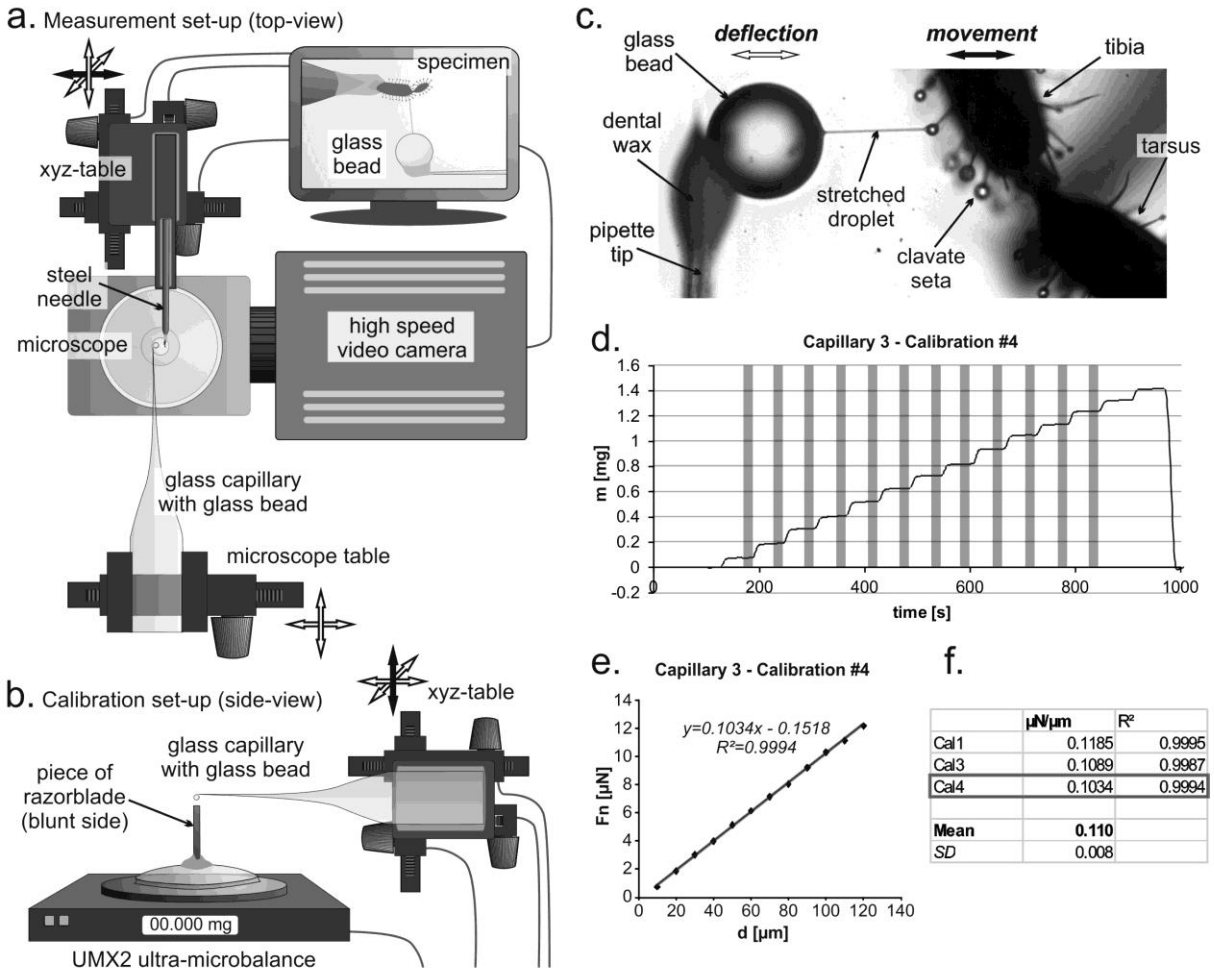

Fig.6.5. Adhesion measurement setup. (a.) Scheme of the setup, top view. Arrows indicate freedom of movement of probe and specimen, of which the black arrow indicates the movement during the measurement. (b.) Setup for probe calibration, side view. Arrows indicate freedom of movement of the probe, of which the black arrow indicates direction of the 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.-f.) Exemplary 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. (f.) Calibration factors of the three runs performed, and the resulting mean and standard deviation (SD). The thick frame marks the factor obtained from the curve shown in (e.). d, deflection; Fn, normal force; m, mass; R^2, coefficient of determination.

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 40x/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 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.6.5.a). The pedipalp was carefully driven towards the glass bead until the apical droplet of a seta made contact (Fig.6.5.c). Then it was pulled back with different velocities (1, 5, 10, 50, 100, 500, 1000 µm/s) 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 fps 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 was tracked using the MTrackJ plugin by Erik Meijering. To obtain force time-curves, x-coordinate shift data (change in 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 by 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 an immersion oil) and the resolution of the high speed camera video chip (1048x1048, obtaining 0.485 µm/pix in this setup).

Remains of the secretion were occasionally seen after testing at low (1-10 µm/s) 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 (3 tests from 3 setae at pull off-speeds of 10, 100 and 1000 µm/s). 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 most narrow point. A cylindrical geometry was assumed, and the diameter was measured in every 10^{th} image of the sequence using DatInf® Measure software. When the diameter of the stretched droplet fell below 2 pixel (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*; *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 until contact was made. Contact formation was filmed with 10000 fps. Droplet stretching at a pull-off velocity of 50 µm/s was recorded at 50-60 fps. 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.

Acknowledgements

Dr. Hans-Jürgen Thorns (Deggendorf) communicated his own observations on the prey capture of *Mitostoma chrysomelas* and provided unpublished photographs with the kind permission to use for this study. Dr. Simon Poppinga (University of Freiburg) gave some worthy remarks regarding this study. Two anonymous reviewers are acknowledged for their constructive criticism and valuable proposals.

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7. Paper no.5:

Joint miniaturization permits fast snapping and twisting in the sticky pedipalps of Sabaconidae (Arachnida, Opiliones)

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Abstract

The co-evolution between predator and prey often results in an 'arms race' in speediness, means of prey capture and defensive mechanisms. Springtails (Collembola) are a ubiquitous source of food in soil communities, but hard to catch because of their catapult mechanism and setal discharge. Harvestmen of the genus *Sabacon* (Sabaconidae) exhibit a unique set of modifications in their pedipalps that are related to capture springtails. The distal segments (tibia and tarsus) are inflated and bent and densely covered with setae that extrude sticky mucus. The basal most section of both segments is miniaturized to a thin stalk with the articulation derived from a di-condylic to a mono-condylic condition. This permits torsional movements beyond the basic flexion-extension movements. The large tarsal flexor muscle is attached to a sclerite that is embedded in the joint membrane. Due to this configuration the contraction of the muscle leads to a rapid inversion of the membrane and flexion of the tarsus in less than 10 milli-seconds, which is used during prey capture. A subsequent inward torsion propels the glued prey between the pedipalps and towards the chelicerae that quickly grasp and dismember it. As gluing does not demand a sensory feedback *Sabacon* can get a grip to the springtail before it can take-off, although the collembolan catapulting is faster than the *Sabacon* snapping movement. Snapping is regarded as a counter-measure against prey loss by the defensive setal discharge of springtails, which limits the efficacy of the gluey setae. The differences in pedipalpal morphology in some Chinese species may represent different steps in the evolution of this unique prey capture apparatus.

7.1. Introduction

The co-evolution of predator and prey frequently leads to an increase in speed and efficacy of means of prey capture or defence (Abrams, 2000, Vermeij, 1994, Dawkins and Krebs, 1979). In arthropod predators there are numerous examples of limb modifications that enhance the ability to overwhelm and/or secure prey, including raptorial clamps (e.g. mantids and amblypygids) (Ass, 1973, Weygoldt, 2000), capture baskets (e.g. dragonflies and asilids) (Gorb, 1999), sticky pads (e.g. solifuges) (Willemart et al., 2011), snap-traps (trap-jaw ants) (Gronenberg et al., 1993), harpoons (mantis shrimps) (Murphy and Patek, 2012), smashing clubs (mantis shrimps) (Patek and Caldwell, 2005) or even hydraulic pistols (snapping shrimps) (Versluis et al., 2000). The fastest striking movements are enabled by a catapulting mechanism, in which a high amount of energy is accumulated and suddenly released, as in trap-jaw ants which strike in less than 1 milli-second (Gronenberg et al., 1993), and likewise in prey rescue leaps as in springtails (Collembola) which can reach a take-off speed of 1.4 m/s (Christian, 1978). Springtails are, besides mites, a ubiquitous and dominant part of soil arthropod communities and an important food source for arthropod predators (Hopkin, 1997). Springtail hunters have evolved a variety of catching devices like sticky appendages (Alberti, 2010, Wolff et al., 2014b) and harpoons (Bauer and Pfeiffer, 1991), and capture baskets (Hintzpeter and Bauer, 1986). In some soil-dwelling harvestmen (Opiliones) belonging to the Nemastomatidae complex gland-associated setae (clavate setae) cover the pedipalps (the second pair of appendages in arachnids) that have been shown to secrete a viscoelastic glue that traps springtails (see ***chapter 6***). Similar setae (plumose setae) are wide-spread in harvestmen and frequently involved in the capture of small agile insects like springtails and leafhoppers (Cicadellidae) (see ***chapter 5***).

Harvestmen of the monogeneric family Sabaconidae inhabit cool, humid montane regions around the northern hemisphere (Schönhofer et al., 2013). They show a unique morphology of the pedipalps (Fig.7.1). The tibia and tarsus are inflated and densely covered with plumose setae (Shear, 1986). Similar to the nemastomatid clavate setae these are associated with glands and dendrites and extrude a sticky mucilage (Juberthie et al., 1981). It was assumed that they are involved in prey capture (Shear, 1986), but there have been no observational reports. In conserved specimens we often found the tibia and tarsus bent and twisted in an unusual manner. In the nemastomatid *Mitostoma chrysomelas* we observed that the tarsus could be flexed highly towards the tibia, which was used to clasp appendages of the prey (Fig.7.2.L,M) (see ***chapter 6***). Also we occasionally observed torsional movements, but the underlying morphological modifications permitting such movements remained unclear.

To reveal the biological and mechanical function of the modified pedipalp we studied (1) the prey capture mechanism and pedipalp movements of *Sabacon simoni* on the basis of high speed video recordings, (2) the anatomy and joint structure of the distal pedipalp using micro-computed tomography and light microscopy and (3) compared the pedipalps of different species to find evolutionary trends and variations in this unique prey capture apparatus.

7.2. Material and methods

7.2.1. Animals

Individuals of *Sabacon simoni* DRESCO 1952 were collected during a field trip in the south-western Alps August-September 2014. The animals were collected by hand (turning logs and stones). For comparing different species conserved material was assessed held in the private collections of A.L. Schönhofer and J. Martens. For details on material see (Martens, 1972, 1983, 1989, 2015). Further, drawings and descriptions of taxonomic papers were assessed (Suzuki, 1974, Shear, 1975).

Light microscopical images of animals and pedipalps were made with a multifocus stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420).

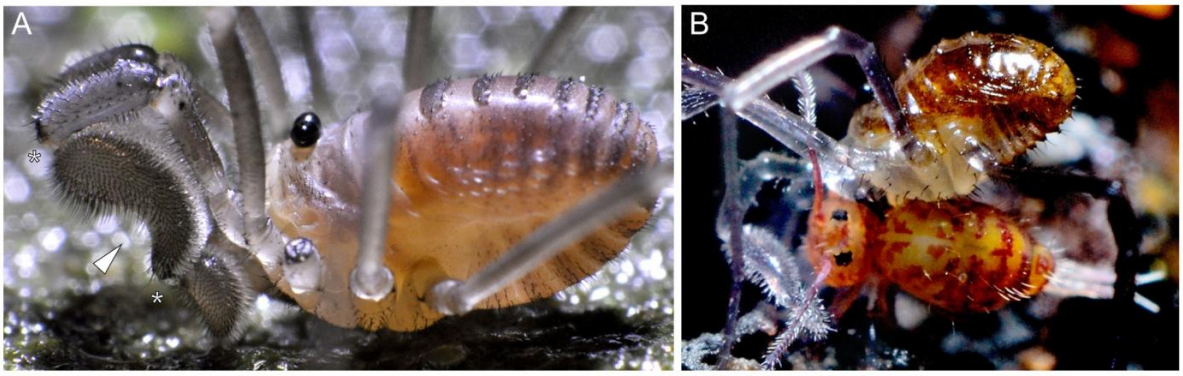

Fig.7.1. Habitus of Sabaconidae. A. *S. viscayanus*, resting female, with expanded tarsus. Arrowhead points to dorsal depression of the tibia, asterisks mark the miniaturized joints (basal stalk-like constriction of tibia and tarsus). B. *S. cavicolens* nymph with captured symphypleonan springtail, photographed in the field. In both pictures the high density of clavate setae on the palpal tibia and tarsus well to be seen. Photographs by A. L. Schönhofer.

7.2.2. Microcomputed tomography

To study the joint and muscle configuration we used pedipalps of female *Sabacon simoni*, of which one had the tarsus in an expanded and one in a flexed condition. Specimens were stored in 70% ethanol and dehydrated in a series of increasing ethanol concentrations and critical-point dried. Dried samples were glued onto plastic pipette tips with cyanacrylate glue and scanned with a SkyScan 1172 HR micro-CT (Bruker microCT, Kontich, Belgium) with an acceleration voltage of 40 kV and a voxel size of 1 µm. 3D images were reconstructed using NRecon 1.6.6 software and processed with Amira 6.0.0.

7.2.3. Behavioural observation and High speed videography

Living *S. simoni* nymphs were kept in plastic tubes with humid tissues under cool conditions (10-15°C). For observations of prey capture the animals were placed in an arena 24×32×15 mm made from cover slides glued together with dental wax (Polyvinylsiloxane) containing humid tissues and different species and sizes of living collembolans (representatives of Entomobryidae) collected on the campus of Kiel University. Prey capture events were 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 500-1000 fps. To obtain such frame rates at sufficient magnification the usage of an additional light source (Storz Techno Light 217, Karl Storz GmbH & Co. KG, Tuttlingen, Germany) was necessary.

7.3. Results

7.3.1. Pedipalp Morphology

The pedipalp of *Sabacon* exhibits unique derivations of both distal-most joints (miniaturization). The proximal, articulating part of both tibia and tarsus is shrunk to a thin,

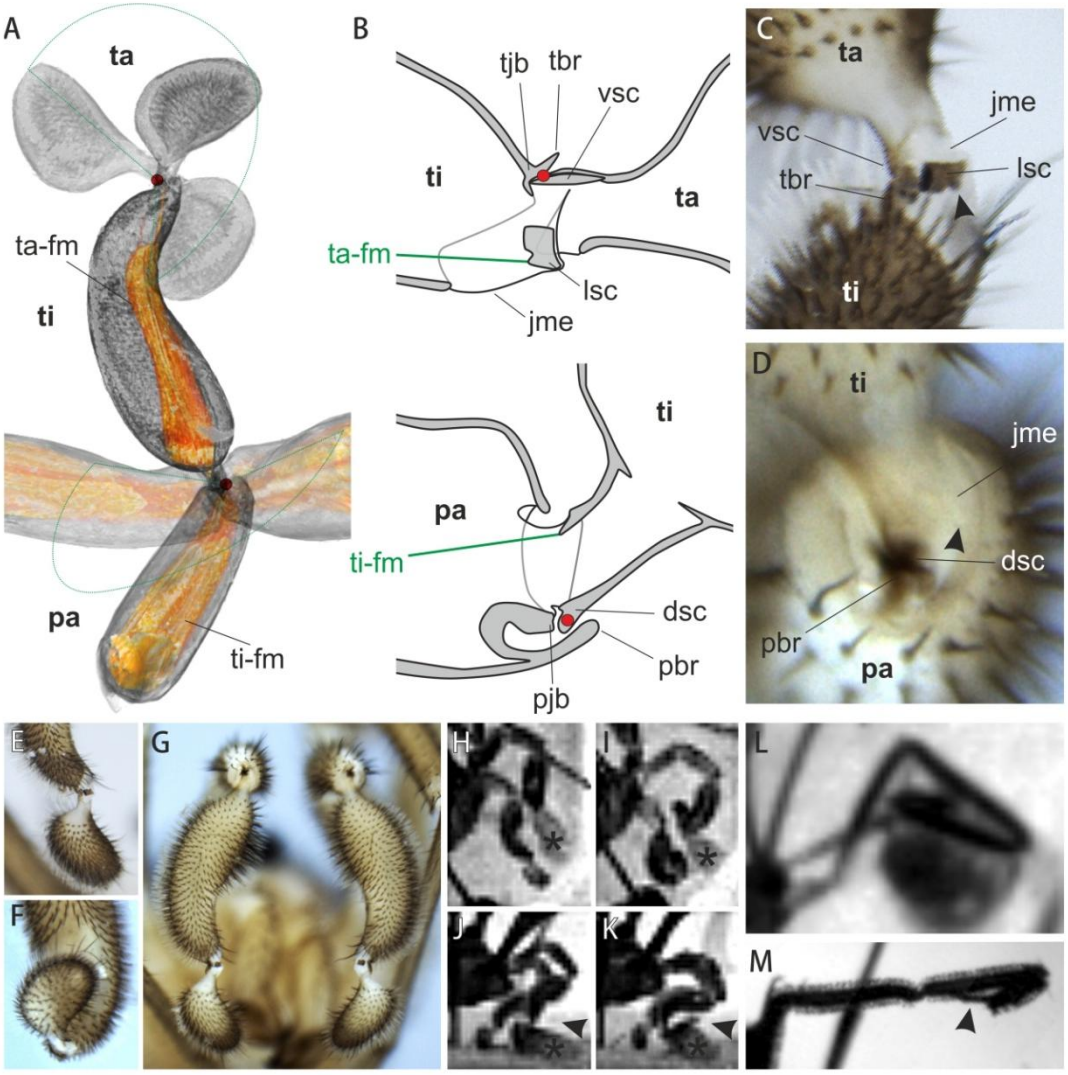

Fig.7.2. Functional morphology of the *Sabacon* pedipalp. A-K. Joint kinematics in the distal pedipalp of *Sabacon simoni* (Sabaconidae). L-M. *Mitostoma chrysomelas* (Nemastomatidae), for comparison. A. 3D reconstruction from µCT images and degrees of freedom of patella-tibia and tibia-tarsus joint. Muscles are orange-coloured. B. Schematic illustration of the joints (transverse section), above tibia-tarsus joint, below patella-tibia joint. The red dot marks the pivot point of these mono-condylar joints, the green lines the muscle tendons. C. Light microscopical image of the tibia-tarsus joint. D. Light microscopical image of the patella-tibia joint. E. Expanded state of the tibia-tarsus joint. F. Flexed state of the tibia tarsus joint. G. Flexed state of the patella-tibia joint with the tibiae twisted inwards, forming a capture basket between them. H-M. High speed video frames of prey capture sequences, prey marked with asterisk. H. Expanded state of both joints, the prey is glued to the tarsus of the right pedipalp. I. Flexed state of both joints. Some appendages of the prey are clamped in between tibia and tarsus of the right pedipalp. J-K. ‚Snap-attack' with a rapid flexion of the tarsus (about 5 milli-seconds passed between both frames). L. Hyper-flexed state of patella-tibia and tibia-tarsus joint in *Mitostoma*. Some appendages of the prey are clamped between the segments. M. Expanded state of the patella-tibia joint and hyper-flexed state of the tibia-tarsus joint. A detached antenna of the prey is clamped between tibia and tarsus (arrowhead). dsc, dorsal sclerite (pin-like); lsc, lateral sclerite; pa, patella; pbr, pjb, protruding brim of patellar joint bowl; patellar joint bowl; jme, joint membrane; ta, tarsus; ta, tarsus flexor muscle; tbr, protruding brim of tibial joint bowl; ti, tibia; ti-fm, tibia flexor muscle; tjb, tibial joint bowl; tsp, tibial joint spur; vsc, ventral sclerite.

cylindrical stalk, which leads to a high degree of freedom of the joint (Fig.7.2.A,H-I,L-M). The tarsus can be highly flexed against the tibia, which exhibits a dorsal depression that is free of glandular or any other setae. The tarsus is of a globular shape and fits perfectly in this depression. As both distal joints are derived from the dicondylic to a monocondylic state (Fig.7.2.B), additionally to dorsal-ventral movement also torsonial movements are possible due to hyper-flexion (frequently observed in high speed videos of hunting *Sabacon*) (Fig.7.2.E-F,H-I). In the tibia-tarsus joint the ventral joint membrane is sclerotized in great part (ventral sclerite), including a pin-like outgrowth, which is pivoted in an invagination of the distal margin of the tibial cuticle (tibial joint bowl) (Fig.7.2.C). Further, there is a square sclerite at the pro-lateral joint membrane (lateral sclerite), which carries an internal apodeme, where the flexor muscle is attached. During muscle tension the pro-lateral sclerite is pulled into the tibia, causing an infolding of the pro-ventral membrane. This leads to both a quick tilting and torsional movement of the tarsus, because the pivot point is located laterally to the muscle attachment site. Because of the leverage, this 'snapping' movement is extremely fast (less than 10 milliseconds as observed in high speed video recordings) (Fig.7.2.J-K). In the flexed state the ventral sclerite is buckled and therefore presumably stressed. It may release its stored elastic energy, when the flexor muscle relaxes and the joint expands passively (see also (Sensenig and Shultz, 2003, 2004)). Thus in rest the tarsus is expanded (Fig.7.2.G). In contrast the patella-tibia joint lacks a ventral sclerite and usually is flexed in rest. Its expansion might be driven by hemolymph pressure.

7.3.2. Prey capture

We observed and high-speed-video recorded 12 prey capture events by juvenile *Sabacon* on entomobryid springtails, of which 11 were successful (in the remaining case the prey was lost due to its large size). The harvestmen preferred springtails half of to similar to its own body length, but in one case a springtail of twice its own body length was successfully overwhelmed. In most cases the harvestmen actively searched for prey. When doing so they used the second leg pair as feelers and held the pedipalps forwards with tibiae flexed (such that their concave sides faced forwards) and tarsi extended (Fig.7.3.A,B). The pedipalps were slightly tapped on the ground alternately. When a pedipalp touched a springtail (usually at the frontally-held side of tibia or tarsus), the harvestmen immediately reacted by flexion of the tarsus against the tibia and elevation of the body (Fig.7.3.E-G). The flexion movement of the tarsus was very quick (less than 10 milliseconds) and usually led to at least one appendage of the springtail being clamped between tibia and tarsus (Fig.7.2.J-K). The tibiae frequently then performed a quick torsional movement inwards, such that the prey was pulled between the pedipalps and in reach of the chelicerae (Figs.7.2.H-I, 7.3.H-I). The prey was then pulled closely towards the body and grasped with the chelicerae. The harvestmen were able to pursue previously detected prey with surprisingly fast and directed movements (i.e. Fig.7.3.C-E).

7.3.3. Interspecific variation and sexual dimorphism

The degree of tibial and tarsal inflation and tarsal shape varies considerably between some species and also between sexes. In many species, the pedipalp of males is less swollen than in juveniles and females, thus being more nemastomatid-like (Suzuki, 1974, Martens, 2015). Among the Central Asian clade *S. kangding* represents a hypothetical plesiomorphic state with a barely inflated tibia and an unmodified tarsus in both sexes (Fig.7.4.A) similar to the pedipalps of Taracidae being the sister family of Sabaconidae (Schönhofer, 2013). The pedipalp of *S. kangding*

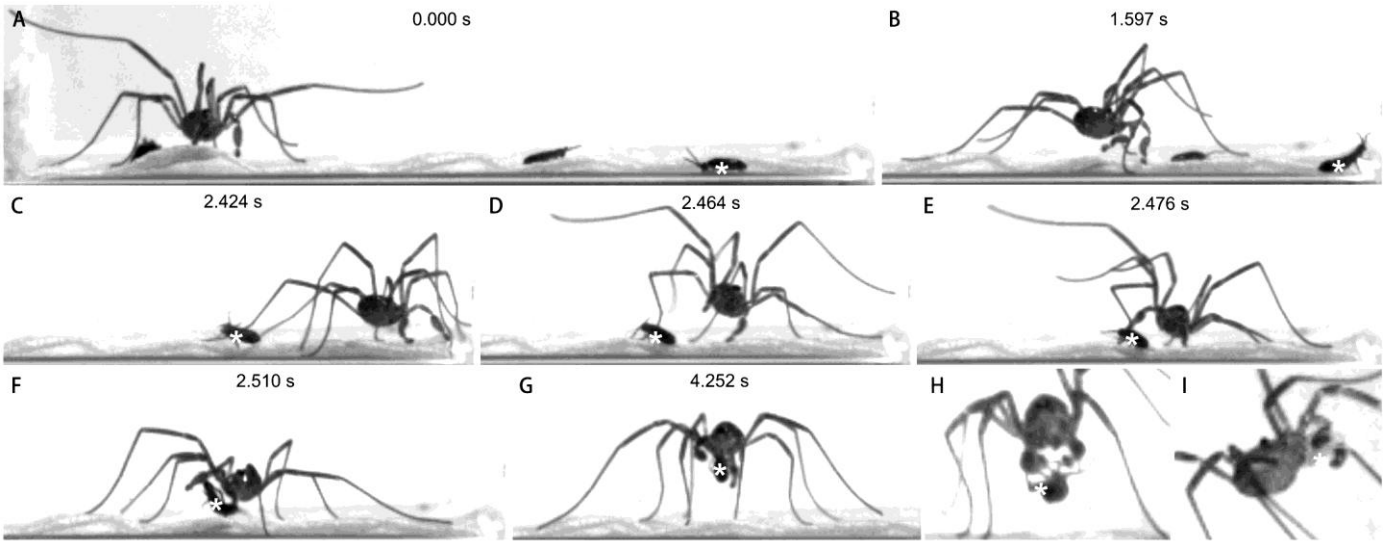

Fig.7.3. Prey capture behaviour of *Sabacon*. Frames of high speed video recordings (1000 frames per second) of *S. simoni* nymphs capturing entomobryomorph springtails. Prey marked by asterisks. A. Resting posture with the second leg pair extended as feelers. B. Active search for prey, the pedipalps probe the substrate while walking. C-D. The springtail touches a leg of the harvestman, leading to a quick turn and heading for the prey. E. The pedipalps reach for the springtail, which gets obviously stuck. F. The glued springtail is elevated from the ground, tibia and tarsi of the pedipalp get flexed. G. The harvestman stretches its leg to prevent ground contact and escape of the prey. Eventually the prey is grasped with the chelicerae and dismembered. H. Tibial and tarsal flexion securing the legs of the springtail. I. Flexed tibiae and tarsi may form a capture basket to secure small prey items.

is still exhibiting a rudimentary claw, which is usually totally reduced in Dyspnoi besides the basal-most Acropsopilionidae (see ***chapter 5***). The genital morphology of *S. kangding* shows several differences from the other species of the Central Asian clade, indicating that it is a pretty old basal species and probably the sister taxon to all the other species within this lineage (Martens, 2015). In *S. schawalleri*, patella and tibia are slightly inflated, but not the tarsus, which might represent an intermediate step (Fig.7.4.B). The tarsal claw is lacking. *S. thakkolanus* shows the most common state with patella, tibia and tarsus inflated (Fig.7.4.C). The tibia is dorsally curved and the resulting depression is free of setae. The tarsus is asymmetrically inflated, such that the basal stalk is shifted sideways. *S. beishanensis* and *S. minshanensis* (Fig.7.4.D) show the highest degree of inflation with tibial width half of length. Other palpal variation shows an overall reduced density of plumose setae in comparably small species as *S. petarberoni* and *S. chomolongmae* (for details see Martens, 2015).

Glandular setae are wide spread among harvestmen and considered apomorphic to the Palpatores clade (Eupnoi+Dyspnoi) (see ***chapter 5***). Joint miniaturization and high tarsal flexion is also present in other Dyspnoi, such as the Taracidae, being the sister group to the Sabaconidae, as well as in Nemastomatidae (see ***chapter 6***). It is considered an apomorphy of the higher Dyspnoi (see ***chapter 5***). The specific joint sclerites might be an apomorphy to Sabaconidae. The data presented above indicate that the inflation of distal segments evolved solely within Sabaconidae as well as the globular shape of the tarsus and the according tibial depression.

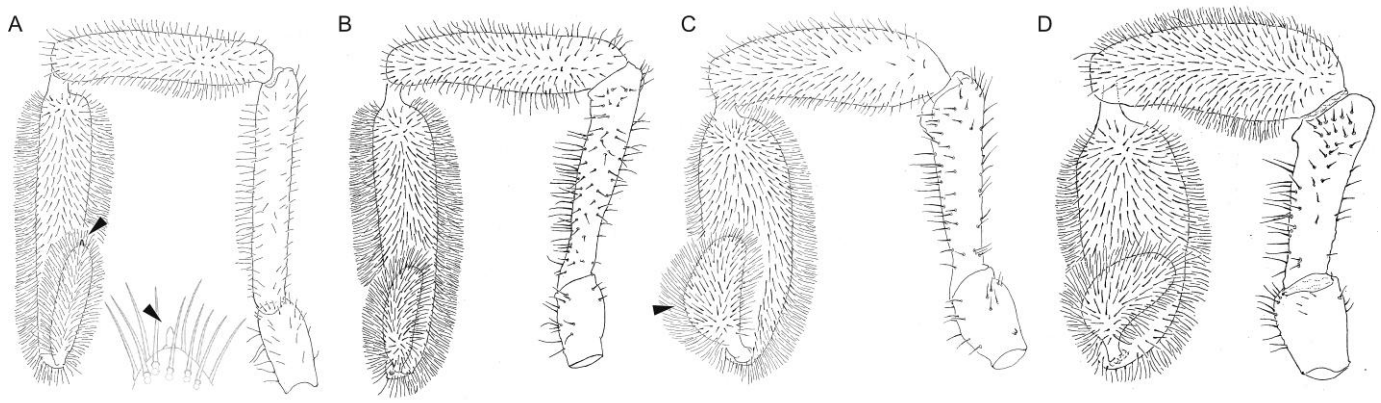

Fig.7.4. Comparative morphology of pedipalps in the Central Asian *Sabacon* clade. Prolateral view of female pedipalps, flexed condition (tibia twisted and tarsus flexed). The depicted species may represent an evolutionary trend from filiform (non-bloated) pedipalps with a rudimentary tarsal claw to highly inflated pedipalps with modified tarsus. This is not based on a comprehensive phylogenetic analysis, but the basal position of *S. kangding* is supported by a divergent probably plesiomorphic genital morphology (see Martens, 2015, for details). A. *S. kangding*, below detail of tarsal tip, arrowheads point to rudimentary claw. B. *S. schawalleri*. C. *S. thakkolanus*, arrowhead indicates the asymmetrically inflated tarsus. D. *S. minshanensis*. Adapted from Martens, 2015, not to scale.

7.4. Discussion

An 'arms race' in speediness is often observed in predator-prey co-evolution (Dawkins and Krebs, 1979). The collembolan catapult mechanism is based on elastic energy storage, which allows movements quicker than by muscular contraction (Christian, 1978). A springtail predator can hardly be quicker, even if using a similar mechanism, because the speed of neuronal feedback is limited. This limit can only be escalated by an increase of the efficacy of neural signal transport or increase of dendrite diameter as in trap-jaw ants (Gronenberg et al., 1993). Another, more frequent, strategy is the usage of a mechanism that does not rely on sensory feedback (Betz and Kölsch, 2004). Sticky secretions act directly on the prey without any reaction necessary. Those have independently evolved in various springtail hunters such as in rove beetles (Bauer and Pfeiffer, 1991), epicriid mites (Alberti, 2010) and harvestmen (see ***chapter 6***). However, the efficacy of gluing is reduced by setal discharge in springtails, which is regarded as a counter-adaptation during the 'arms-race' of springtails and springtail-predators (Bauer and Pfeiffer, 1991, Wolff et al., 2014b). Several lineages of epigaeic collembolans have evolved broad, flattened setae ('scales') that densely cover both body and appendages and easily detach (Zhang et al., 2014). In *Mitostoma* harvestmen counter-adaptations are an elongation of the pedipalps, an extended degree of tarsal flexion that allows the clasping of prey appendages (Fig.7.2.L,M) and a leg-stretching behaviour that prevents ground contact by the springtail prey (see ***chapter 6***). In *Sabacon* a rapid snapping movements clamps parts of the prey and an inward torsion quickly transfers the prey towards the chelicerae. This limits the risk of prey loss and increases the efficacy of the sticky setae (Fig.7.5). The speed of tarsal flexion is caused by a leverage effect, similar to the raptorial legs in mantis shrimps which reach a comparable striking speed (Burrows, 1969). The high inflation of the distal pedipalpal segments increases the surface area covered in sticky setae and gives room for both glands and muscles. The efficacy of this derived capture apparatus is very high as no springtail of appropriate size was able to escape in our trials.

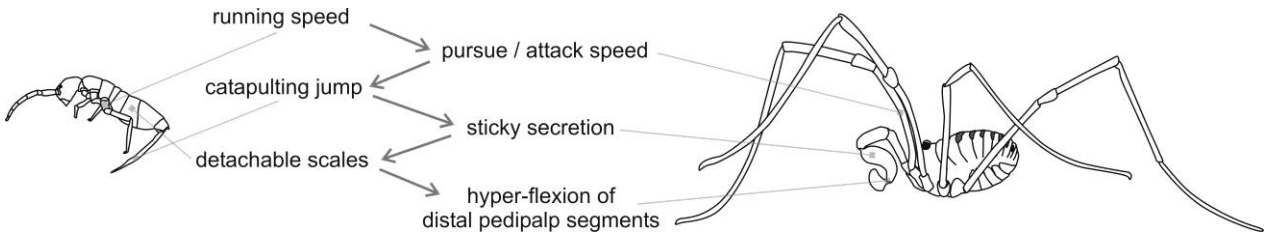

Fig.7.5. Summary of adaptations and counter-measures in *Sabacon* harvestmen and springtails. Please remark that *Sabacon* is an example for an arthropod springtail hunter. Speediness and sticky secretions can also be found in other springtail-predators, such as rove-beetles, hence the evolution of the illustrated defensive strategies of entomobryomorph springtails is not necessarily driven by the predation of *Sabacon*.

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8. Paper no.6*:

Composition and substrate-dependent strength of the silken attachment discs in spiders

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Abstract

Araneomorph spiders have evolved different silks with dissimilar material properties, serving different purposes. The two-compound pyriform secretion is used to glue silk threads to substrates or to other threads. It is applied in distinct patterns, called attachment discs. Although ubiquitously found in spider silk applications and hypothesized to be strong and versatile at low material consumption, the performance of attachment discs on different substrates remains unknown. Here we analyze the detachment forces and fracture mechanics of the attachment discs spun by five different species on three different substrates, by pulling on the upstream part of the attached thread. Results show that although the adhesion of the pyriform glue is heavily affected by the substrate, even on Teflon it is frequently strong enough to hold the spider weight. Since plant surfaces are often difficult to wet, they are hypothesized to be the major driving force for evolution of the pyriform secretion.

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8.1. Introduction

Natural silks are biofibers that fascinate through their outstanding material properties and versatility. Araneomorph spiders have evolved different kind of silks with altered material properties serving different purposes – they are equipped with a 'silken toolkit' (Blackledge and Hayashi, 2006, Apstein, 1889, Peakall, 1964, Vollrath, 1992). The pyriform gland secretion is used to glue silk threads to substrates (Apstein, 1889, Schütt, 1996, Saffre et al., 1999) or with each other (Gorb et al., 1998, Townley and Tillinghast, 2003). It is applied in a distinct pattern, called attachment disc, and was formerly hypothesized to be a two compound material including spidroins (silk proteins) as the first phase and an amorphous hydrocarbon-rich cement-like substance as the second phase (Kovoor and Zylberberg, 1980, Blasingame et al., 2009). Attachment discs are fundamental for locomotion, prey capture and reproduction: They secure climbing spiders in the case of a fall or enable them to reach distant places by descending (Apstein, 1889, Ortlepp and Gosline, 2008), provide robust anchorage for webs (Pugno et al., 2013, Schütt, 1996, Sahni et al., 2012), retreats and egg sacs (Hajer and Rehakova, 2003), stabilize jumping (Chen et al., 2013) and on-water movements (Gorb and Barth, 1994) and facilitate navigation (Gorb et al., 1998) and brood care (Townley and Tillinghast, 2003). Through an adapted spinning behaviour they can even be used to produce special traps like trapdoors, stumbling threads (Stern and Kullmann, 1975) and catapulting lines (Sahni et al., 2012). As there is such a broad and frequent use of attachment discs in a spider's life, they must provide a strong attachment capability on various surfaces, with a broad bandwidth of surface chemistry and topography (universality) at the minimum amount of the secretion applied (economy). This makes them also very interesting for potential biomimetic applications. Yet, first attempts were made to use this principle for technical applications, including electro spun artificial attachment discs (Jain et al., 2014) and 'dragline attachment' in robots (Wang et al., 2014). Nonetheless, the pyriform secretion and its use in the generation of attachment discs are remarkably scarcely studied.

The morphology of attachment discs spun by different spiders including representatives of Araneidae, Linyphiidae, Filistatidae, Salticidae and Pholcidae was studied by Schütt (Schütt, 1996). She found differences in the architecture depending on phylogeny and hunting style of the spider. Basically the structure includes a bilateral symmetric network of pyriform threads firmly attached to the ground and bundled in a central part, where it forms an envelope around the dragline, which is composed of a paired (or more) major ampullate silk threads. The close location of the major ampullate spigot to the numerous piriform spigots on the anterior lateral spinnerets provides an easy and fast way to spin such rather complex structure (Eberhard, 2010). Ultrastructural analyzes of the pyriform glands showed that two distinct types of secretory cells are involved, which produce at least two different substances and which are extruded by the same spigot (Kovoor and Zylberberg, 1980, Kovoor and Zylberberg, 1982). It was proposed that the pyriform spidroins (silk proteins) produced by these glands self-assemble into fibers in a liquid environment (Blasingame et al., 2009). This could be shown for biotechnologically produced pyriform spidroins (Geurts et al., 2010). Evaporation of a solvent, like water, may lead to quick solidifying of the matrix cementing the threads to the substrate and to each other.

The first biomechanical study on attachment discs was recently published by Sahni and colleagues (Sahni et al., 2012). They reported that the attachment discs adhere very strongly to smooth surfaces like glass. However, during their measurements they did not directly pull on the

dragline, but on thin underlying nylon threads, which does not reflect the natural way how the structure performs. They explained the function of the attachment disc by a geometrical staple-pin model with parallel peeling membranes (Sahni et al., 2012). Pugno and co-workers proposed an alternative model, which is based on the simultaneous peeling of multiple membranes (Pugno et al., 2013). Their theoretical study, however, is not based on experimental data. Despite this, nothing is known about the function of attachment discs, especially how strong they adhere to natural substrates, for which these secretion products have been presumably optimized. It is likely that the discs behave very differently on different substrates, because the strength of a glue-based attachment highly depends on surface properties, such as surface free energy and microtopography (Kinloch, 1987). Nevertheless, in models on spider web biomechanics (Tarakanova and Buehler, 2012, Harmer et al., 2011, Ko and Jovicic, 2004, Lin et al., 1995, Cranford et al., 2012, Sensenig et al., 2011, Sensenig et al., 2012, Aoyanagi and Okumura, 2010) and dragline security during falling-spider events (Brandwood, 1985, Ortlepp and Gosline, 2008, Osaki, 1996, Osaki, 2003), thread anchoring has always been presumed as robust enough to keep the spider body weight and therefore neglected for biomechanical models. Furthermore, spider species of different ecology and size may have different demands on thread anchorage or their discs may have different phylogenetic constraints. In order to understand the functional morphology of the disc, and to evaluate, whether its structure is universal or shows specializations, it is important to structurally and experimentally compare discs of different species.

The aim of this study is thus to compare the disc attachment performance on (a) different substrates and (b) between different spider species with different ecology. We used three different substrates: (1) glass, which is hydrophilic and provides a high surface free energy (this control surface was also used in previous experiments (Sahni et al., 2012)), (2) Teflon, which has a very low free surface energy and is thus highly anti-adhesive and (3) upper surface of leaves of sycamore maple (*Acer pseudoplatanus*) as a wide spread generic plant surface. We used the large orb web spiders *Argiope trifasciata* (Araneae) and *Nephila senegalensis* (Nephilidae) and the cob-web weaver *Parasteatoda tepidariorum* (Theridiidae), which was used in the previous study by Sahni et al. (2012), the large wandering spider *Cupiennius salei* (Ctenidae) and the flower associated ambush predator *Thomisus onustus* (Thomisidae). We performed tensile tests, in which we measured forces resisting pulling onto the dragline. To evaluate which part of the disc structure is in direct contact to the substrate and estimate where the forces are exactly applied and energy dissipated within the structure, we studied attachment discs with means of transmission (TLM) and coaxial light microscopy (CLM) and scanning electron microscopy (SEM), and analyzed structure deformation and fracture behaviour in pull-off tests combined with video recording.

8.2. Methods

8.2.1. Animals and harvesting of attachment discs

Adult females of the species *Argiope trifasciata* FORSSKÅL 1775 (Araneidae), *Nephila senegalensis* WALCKENAER 1842 (Nephilidae) and *Cupiennius salei* KEYSERLING 1877 (Ctenidae) were obtained from laboratory stocks, *Parasteatoda tepidariorum* C. L. KOCH 1841 (Theridiidae) was collected in the greenhouses of the botanical garden of Kiel University and *Thomisus onustus* WALCKENAER 1805 (Thomisidae) was collected during a field trip on Sardinia (Italy) in May 2013. Spiders were kept at 23-26°C and sprinkled daily with water. The ground substrate (turf-

sand) was constantly kept humid. Spiders were weekly fed. *A. trifasciata* and *N. senegalensis* were kept in terraria (40 × 40 × 40 cm) equipped with wooden frames and fed with flies (*Musca domestica*) and juvenile grasshoppers (*Locusta migratoria*). *C. salei* were kept in cylindrical glasses (diameter: 10 cm, height: 25 cm) and fed with house crickets (*Acheta domestica*). *P. tepidariorum* and *T. onustus* were kept in plastic tubes (diameter: 5 cm, height: 10 cm) and fed with flies (*Drosophila melanogaster* and *M. domestica*) and cricket larvae (*A. domestica*). Spiders were weighted on an AG 204 Delta Range scale (Mettler Toledo GmbH, Greifensee, Switzerland) one day after feeding.

Attachment discs were collected directly before experiments. For this purpose, three different substrates were used: glass (microscopy glass slide), the upper surface of sycamore (*Acer pseudoplatanus*) leaves and Teflon (PTFE) foil. Tree leafs and Teflon foils were cut to the size of 76 × 26 mm and mounted on microscopy glass slides. To harvest attachment discs, spiders were stimulated to walk and climb on the prepared substrate, which was held by tweezers. Spiders usually spun attachment discs instantly, when brought in an upside-down position. In the large *C. salei*, the substrate was carefully put under the opisthosoma of the resting spider and the existing dragline was cut with a fine pair of scissors, which usually triggered the spider to renew the attachment. After the spider has spun an attachment disc and a sufficient length of the dragline, the dragline was grasped with a pair of tweezers and cut 3 cm above the attachment disc with a fine pair of scissors.

8.2.2. Microscopy

The morphology of attachment discs spun on glass was investigated with the means of a stereo microscope (Leica M205 A, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a camera (Leica DFC420), using a reflected and a coaxial light source. Using coaxial light, the area of direct contact was made visible as a dark contrast. Contact area was measured from the obtained still images with ImageJ 1.47v (Wayne Rasband, National Institutes of Health, USA).

For scanning electron microscopy (SEM), small pieces of the substrates including attachment discs were cut out and mounted on stubs in the fresh condition. Teflon and glass samples were sputter coated with 10 nm Au-Pd and viewed at 3.0 kV in a Hitachi S 4800 scanning electron microscope (Hitachi Ltd., Tokio, Japan). Leaf samples were immediately shock frozen in liquid nitrogen using Gatan ALTO-2500 cryo system (Gatan Inc., Abingdon, UK). Samples were then sputter coated in a frozen state at -140°C with 10 nm Au-Pd and viewed at 3.0 kV and -120°C in the Hitachi S 4800 scanning electron microscope.

8.2.3. Tensile tests

The upstream dragline (the one spun last and leading to the animal) of freshly harvested attachment discs was glued to the cantilever of a load cell force transducer (FORT-10 with 10 g force range, World Precision Instruments Inc., Sarasota, FL, USA) with a molten droplet of beeswax at a length of about 20 mm. The force transducer could be moved vertically by a micromanipulator (DC3001R with controller MS314, World Precision Instruments Inc., Sarasota, FL, USA) at a speed of 0.2 mm/s. The signal of the force transducer was amplified and processed by a Biopac MP-100 acquisition system (Biopac Systems Ltd, Goleta, CA, USA). Force curves were recorded using the AcqKnowledge 3.7.0 software (Biopac Systems Ltd.). Tension tests were simultaneously filmed using a Firefly pro GT 800 camera (Firefly Global, Belmont, USA).

Pull-off forces were taken as the highest forces occurring during attachment disc pulling. Attachment strength was calculated as the quotient of pull-off force and the mean contact area of attachment discs by the species measured previously. Safety factor was calculated as the quotient of pull-off force and the weight of the spider. Data were statistically analyzed using Kruskal Wallis rank sum test with α=0.05 and Mann Whitney U test with FDR alpha adjusting for pairwise comparison in SigmaStat 12.5 (Systat Software GmbH, Erkrath, Germany). We compared the attachment strength on the three different substrates for each species separately, with pooled individual data. To exclude an effect of individuals on the analysis, we previously performed a two-way ANOVA (F1=individual, F2=substrate) on the pull-off force data of each species in R (R Core Development team, http://www.r-project.org/) (SM.8.2). We found, that the substrate type has a much more significant effect on the detachment force than the individual in the large species *Argiope trifasciata*, *Nephila senegalensis* and *Cupiennius salei*. We conclude that the bias by individuals is neglectable and maintain the procedure of pooling data from individuals for the Kruskal-Wallis tests. However, in both *Thomisus onustus* and *Parasteatoda tepidariorum*, the differences between pull-off forces from glass and leaf substrates are not significant in single individuals. This result was taken into account in the following analysis. Species were compared with pooled substrate data.

8.3. Results

8.3.1. Architecture of attachment discs

Attachment discs of the analyzed spider species basically consist of a network of pyriform glue coated fibres, which cement the dragline in the central part of the disc and are attached to the substrate in the lateral parts of the disc (Fig.8.1, SM.8.1.A,F). The dragline cementation is present as thick bundles of pyriform fibres and glue forming an envelope around the double stranded dragline (Fig.8.1.G,I, SM.8.1.A). We term this part *the conjunction*. It is not in direct contact with the substrate, which can easily recognized by comparing reflection and coaxial light micrographs (Fig.8.1.B-C,E-F). Substrate cementation consists of numerous parallel and crossing loops of pyriform glue coated fibres (SM.8.1.B) that are denser in the central part of the disc and more separated in its lateral parts (SM.8.1.F). We term this part of substrate anchorage *the baseplate*.

The glue of pyriform glands wets the three tested substrates differently (Fig.8.1.J-L, SM.8.1.C-D). On glass it spreads widely (Fig.8.1.J), whereas Teflon has a repellent effect (Fig.8.1.K). On the surface of the sycamore leaf wetting is hampered by cuticular micro ripples (Fig.8.1.L, SM.8.1.D) and (more obvious on the underside of the leaf) crystalline waxes (SM.8.1.C). Between the conjunction and the baseplate, a meshwork of pyriform glue-coated fibres is spread, which branches towards the substrate (Fig.8.1.G, SM.1.A,F). We call this part *the bridge*.

Comparison of attachment discs of different species shows that the disc morphology is relatively similar. However, in *T. onustus* the loops of the baseplate are more round, resulting in steeper angles of pyriform fibre crossings and a more radial shape of the attachment disc (Fig.8.1.D-F, compare with A-C). The upstream part (last spun, in the direction to the animal) often exhibits a larger width than the downstream part (most apparent in *T. onustus* and *C. salei*). Contact area of the attachment disc scales with species weight, ranging from 40 µm² in *P. tepidariorum* to 1,400 µm² in *C. salei* and *N. senegalensis* (Tab.8.1).

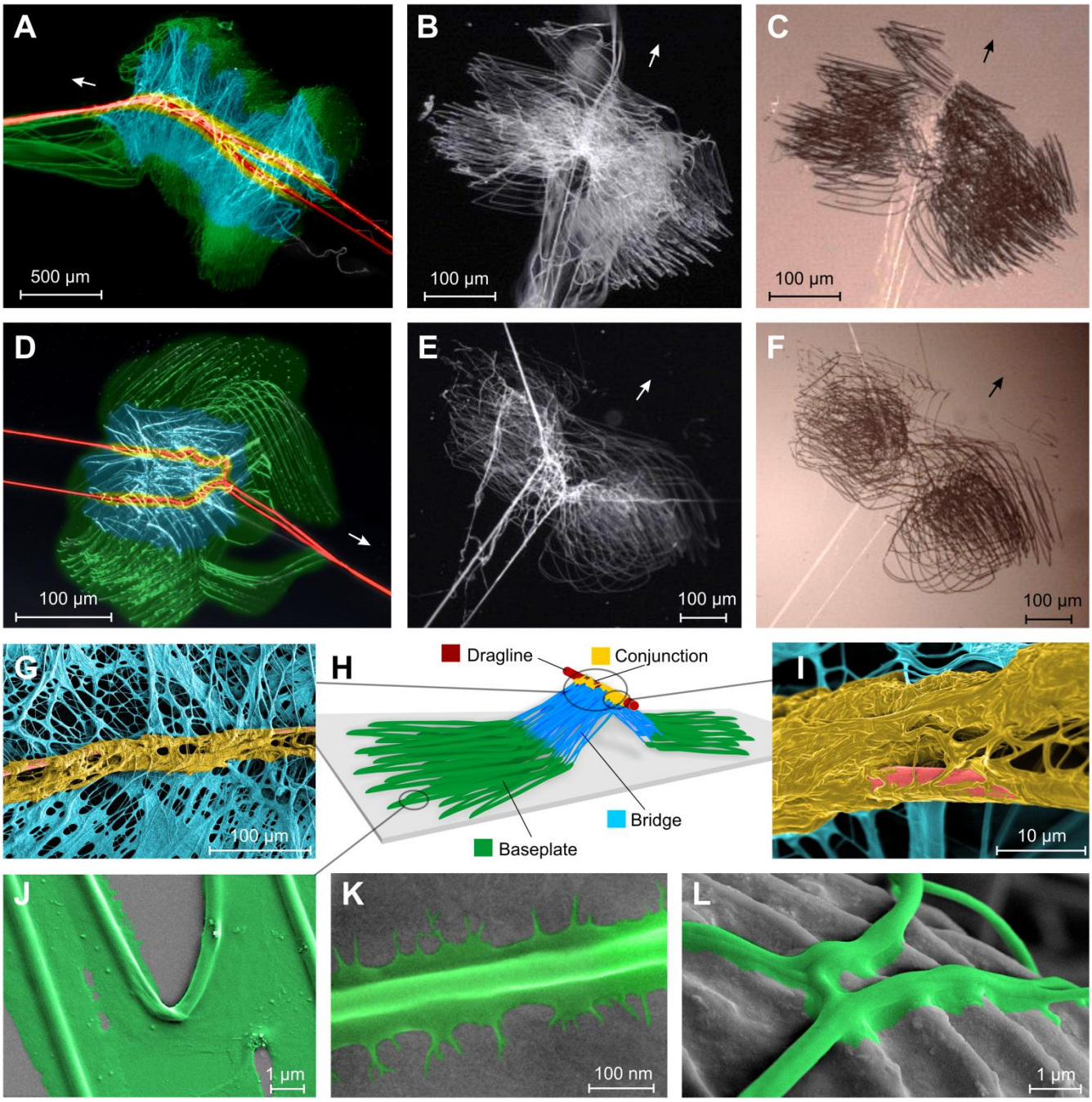

Fig.8.1. Morphology of spider attachment discs. (A-G) shows light microscopy images of attachment discs spun by different spider species on glass slides (arrows indicate the direction of spinning, upstream): (A) *Argiope trifasciata*, (B-C) *Parasteatoda tepidariorum*, (D-F) *Thomisus onustus*. In (A-B) and (D-E), reflected light was used. In (C) and (F), same specimens were used as in (B) and (F), respectively, are imaged using coaxial light, which displays the contact area appearing dark. (A) and (D) are colored to depict different functional regions within the attachment disc, as schematically illustrated in (H). In the central part, the pyriform secretions form an envelope around the dragline (red), which we term *the conjunction* (yellow). A network of pyriform glue coated fibres spreads to both lateral parts towards the substrate, which we call *the bridge* (blue). The direct substrate anchorage is called *the baseplate* (green). The coloured SEM micrographs shown in (G) and (I-L) reveal fine structural details of the silken disc structure. (G) and (I) show details of the central part, with the dragline, the conjunction and the bridge. (J-L) show single pyriform glue coated fibres of the baseplate, whereas in (J) those are attached to glass, in (K) to Teflon, and in (L) to the upper surface of an *Acer pseudoplatanus* leaf.

Tab.8.1. Body mass (m) and contact area (A) of attachment discs of different spider species used in experiments. Mean values are given ± standard deviations. N = number of spiders, n = number of measurements.

Species	**m [g]**	**A [× 1000 µm²]**
Argiope trifasciata (N=6)	0.59±0.15	0.98±0.13 (n=10)
Nephila kenianensis (N=3)	0.75±0.11	1.35±0.29 (n=11)
Cupiennius salei (N=4)	2.48±0.82	1.41±0.33 (n=20)
Thomisus onustus (N=2)	0.09±0.02	0.09±0.01 (n=10)
Parasteatoda tepidariorum (N=4)	0.03±0.01	0.04±0.01 (n=11)

8.3.2. Fracture mechanics

During tensile tests, four different failure modes were observed: (1) *dragline-mode*, (2) *conjunction-mode*, (3) *bridge-mode*, (4) *baseplate-mode*. The *dragline-mode* is due to the dragline breakage above or directly at the attachment disc. Force-time curves in those cases often show two peaks, as the dragline usually consists of a double strand (Fig.8.2.C). The *conjunction* failure occurred at the interface between the dragline and pyriform glue. In these cases, rupture could usually not be seen in the video, but was followed by a sliding of the dragline through the conjunction envelope, which caused considerable friction forces (Fig.8.2.D). When the *bridge* failed, the conjunction was totally removed from the attachment disc (Fig.8.2.A, SM.8.1.E). According to the meshwork character of the bridge, its rupture often caused multiple force peaks (Fig.8.2.E). When the failure occurred at the interface between the pyriform glue and the substrate

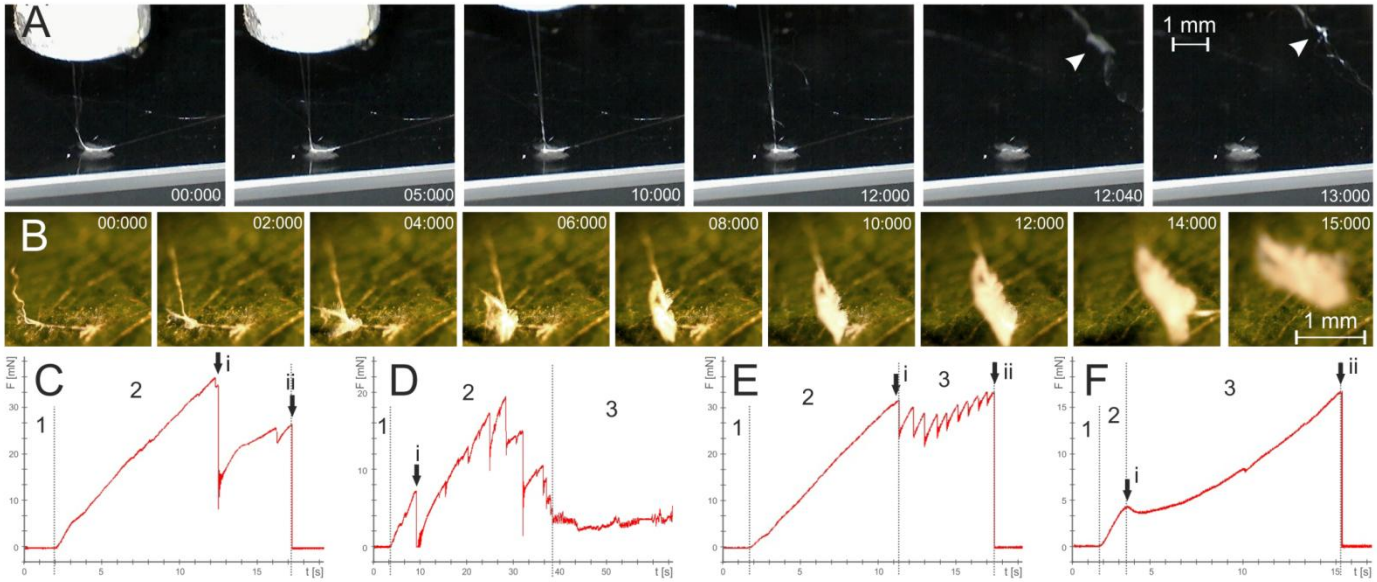

Fig.8.2. Results of tensile tests of spider attachment discs pulled at the upstream dragline. (A) and (B) show two video sequences recorded during individual tensile tests showing different failure modes. In (A) the tested attachment disc was spun on a smooth glass slide and failed at the bridge. Arrowheads point to the conjunction and bridge remnants on the dragline, broken off the attachment disc. In (B) the tested attachment disc was spun on the upper surface of an *Acer pseudoplatanus* leaf and failure occurred at the baseplate-substrate interface, leading to a total peel-off of the disc structure from the substrate. (C-F) show exemplary time-force curves recorded in the tensile tests, with different parts of the attachment disc failing. Arrows indicate the first crack (i) and final rupture (ii). In (C) dragline fails. After thread is tightened (1), it is strained (2), leading to a linear increase in force, until the material breaks. Two force peaks are typical, as the dragline is constituted of a double strand. If the conjunction fails (D), several rupture events are followed by sliding friction (3), caused by the dragline sliding through the envelope. Bridge failure (E) caused one or multiple similar force peaks (3), depending on the length of the attachment disc. The baseplate failure (total detachment) event included time-force curves with multiple peaks similar to those in (E), but with a higher variation in peak size, or a flattening of the slope after initial crack induction, similar to that in (F).

(*baseplate-mode*), a total peel-off (delamination) of the attachment disc from the substrate was observed (Fig.8.2.B). In those cases, the time-force curve usually did not show force peaks between the crack initiation event and completed peel off, just a smooth slope (Fig.8.2.F).

In which part the attachment disc breaks, heavily depends on its attachment strength to the substrate (Fig.8.3). On glass, where highest pull-off forces were recorded, the bridge failed in a majority of cases, from about a half in *A. trifasciata* to three fourth in *N. senegalensis* and *C. salei*. Second most frequent failure occurred at the conjunction in *A. trifasciata, N. senegalensis* and *P. tepidariorum*, as well as at the dragline in *C. salei* and *T. onustus*. On Teflon, where significantly lower pull-off forces were recorded than on glass, there was always a total delamination of the attachment disc from the underlying substrate. On the sycamore leaf, the majority of attachment discs also showed total delamination, however, in *N. senegalensis,* the conjunction failed in one fifth of the cases, and in *T. onustus,* the bridge failed in over one third of the cases (Fig.8.3).

8.3.3. Attachment strength and safety factor

Pull-off forces differed significantly between substrates in all spider species studied, except *Parasteatoda tepidariorum* (Kruskal-Wallis rank sum test with $p<0.05$, df=2) (Fig.8.3, Tab.8.2). Pull-off forces of attachment discs spun on glass reached mean values of 36 mN in *N. senegalensis*, 35 mN in *C. salei*, 28 mN in *A. trifascata*, 7 mN in *T. onustus* and 3 mN in *P. tepidariorum*. Pull-off forces on the sycamore leaf were significantly lower (about one third), except in *T. onustus*. Lowest forces were obtained on Teflon (always significantly different to both other substrates), with mean values about 5 mN in *A. trifasciata* and *N. senegalensis*, 8 mN in *C. salei* and 3 mN in *T. onustus*, which is only one sixth to one seventh of the force measured on glass in the first three species, and one half in *T. onustus* (attachment discs of *P. tepidariorum* were not tested on Teflon). Pull-off forces scale with body mass with the power law of 2/3, if individuals of all species are taken into account (Fig.8.4). This indicates that the forces are dependent on the contact area of the attachment disc. Thus, the attachment strength was calculated (pull-off force divided by average contact area measured for each species), to compare the performance of attachments discs spun by different species. The attachment strength did not differ significantly between *A. trifasciata*, *N. senegalensis* and *C. salei*, but was about three times higher

Tab.8.2. Pull-off force (F), attachment strength (τ), and safety factor (sf) of attachment discs measured in tensile tests. Mean values are given ± standard deviations. n = number of measurements.

Species	Substrate	F [mN]	τ [N/cm²]	sf	n
A. trifasciata	glass	28.3±9.1	2.8±0.9	4.3±1.5	54
	tree leaf	23.2±10.8	2.2±1.1	3.3±1.5	32
	Teflon	5.0±3.1	0.4±0.3	0.8±0.5	31
N. kenianensis	glass	35.7±8.9	2.5±0.7	4.6±1.2	32
	tree leaf	22.3±9.5	1.7±0.7	2.9±1.2	33
	Teflon	5.2±2.9	0.3±0.2	0.7±0.4	21
C. salei	glass	35.1±16.5	2.2±1.2	1.4±0.6	30
	tree leaf	26.0±11.8	1.7±0.8	1.0±0.4	33
	Teflon	8.0±3.6	0.5±0.3	0.4±0.2	15
T. onustus	glass	6.7±3.9	6.3±4.3	7.5±3.4	32
	tree leaf	6.5±3.2	7.3±3.5	7.1±2.8	30
	Teflon	3.3±1.4	3.4±1.6	3.6±1.6	20
P. *tepidariorum*	glass	2.9±1.4	7.8±3.6	13.0±4.5	33
	tree leaf	1.8±0.8	4.7±2.0	8.4±7.6	20

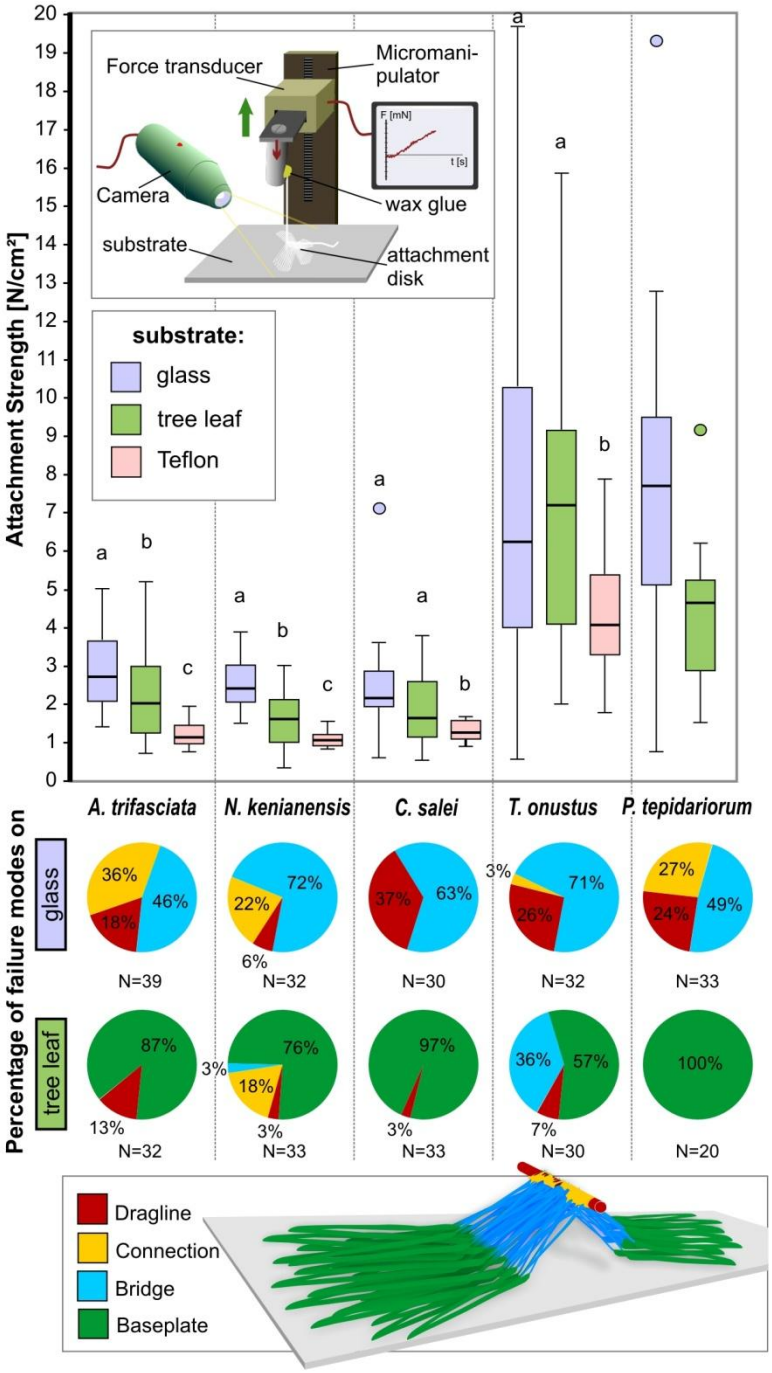

Fig.8.3. Attachment strength and failure analysis. The attachment strength was calculated as the quotient of the maximal force during the tensile test and the mean contact area of attachment discs typically spun by the corresponding species. Boxplots illustrate the median value, interquartile range and the extreme values, outliers are indicated by circles. Letters above indicate significant differences between the substrates (within species). Inset shows setup of the tensile tests. Pie diagrams below illustrate the percentage of failure modes on the on glass (upper row) and the upper surface of an *Acer pseudoplatanus* leaf (lower row). Which part failed first was analyzed from combined evaluations of video recordings and time-force curves. Failure modes on Teflon are not displayed, because there was only a total peel-off detachment of discs in all cases.

in the smaller *T. onustus* and *P. tepidariorum*, with mean values ranging from 3.4 to 7.8 N/cm² depending on the substrate (Fig.8.3).

Safety factor (pull-off forces divided the weight of the spider individual) did not differ between the arboreal web-builder *N. senegalensis* and the low vegetation dwelling web-builder *A.*

trifasciata (4 on glass, 3 on tree leaf and 0.8 on Teflon). In the large arboreal hunting spider *C. salei* safety factor of the attachment disc is significantly lower (about 1 on glass and tree leaf and 0.4 on Teflon). In small species, *T. onustus* and *P. tepidariorum*, safety factor is significantly higher than in the other species. On glass *P. tepidariorum* reached a higher safety factor (sf=13) than *T. onustus* (sf=8). On the tree leaf, both species did not differ, with means about 8 in *P. tepidariorum* and 7 in *T. onustus*. On Teflon, *T. onustus* had a reduction of the safety factor only about one half, if compared to glass. The safety factor decreases with an increasing body mass of the spider, if individuals of all species are taken into account (Fig.8.4).

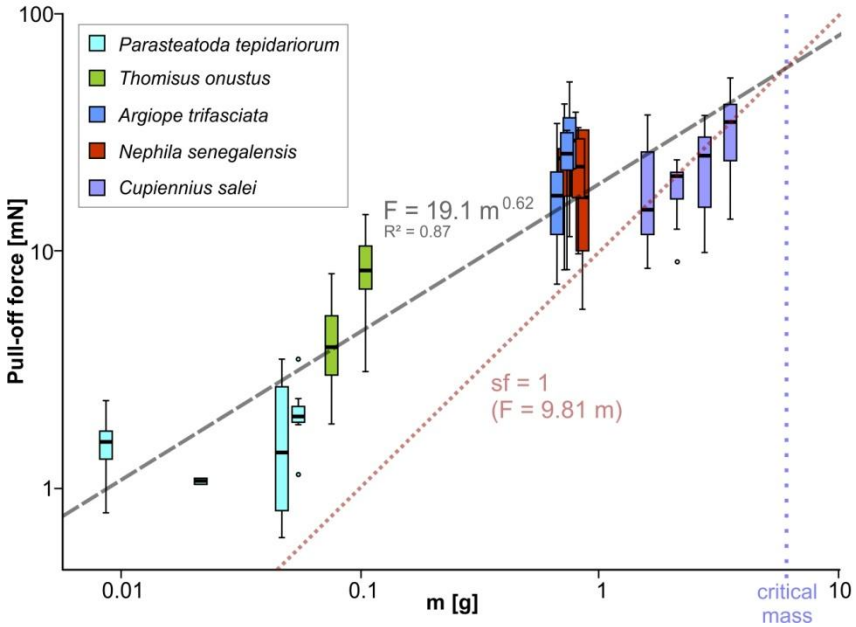

Fig.8.4. Scaling effect of the body mass on the attachment disk pull off force. The pull-off force of attachment discs spun on *Acer pseudoplatanus* leaf surface scales with body mass by the power lawof 2/3. This indicates that forces are dependent on contact area. As the surface area increases by a power lower than the power of the volume, the safety factor (detachment force divided by the weight force) decreases with increasing spider size. The critical mass indicates the limit of spider mass, at which the mean safety factor falls below 1, thus getting insufficient to hold the animal. Indeed, araneomorph spiders rarely exceed this size. Boxplots are defined as in Fig.8.3.

8.4. Discussion

Attachment discs represent an important part of the relationship of araneomorph spiders to their environment. Our data shows that the discs provide strong attachment to different substrates due to their specific hierarchical structure. Sahni et al. (Sahni et al., 2012) recently described two different architectures of attachment discs, spun by the cobweb spider *Parasteatoda tepidariorum* for different purposes. The usual 'staple-pin' architecture is used in the so-called scaffolding disc, which is for firm cementing of draglines to substrates. This one is comparable to the dragline anchors of other spiders (Schütt, 1996, Sahni et al., 2012). The other type with the dendritic architecture is used to attach so-called gumfoot threads. These 'gumfoot discs' are only loosely attached to the substrate and can be pulled off by much lower forces. They are connected to preloaded threads and easily detach, if walking prey touches the thread, causing the prey catapulting into the web. In contrast to the experimental data provided by Sahni et al. (Sahni et al., 2012), the multiple peeling theory by Pugno et al. (Pugno et al., 2013) predicts, that such dendritic architecture must be the optimal one for strong attachment at low material cost. The latter paper is

based on the so-called theory of multiple peeling suggesting that a simultaneous detachment of opposing adhesive tapes leads to much higher adhesion than the sum of detachment forces needed to peel off each tape separately (Pugno, 2011), a model that is highly applicable to biological locomotory attachment systems, such as gecko feet or arthropod adhesive foot pads (Wohlfart et al., 2014). The previous models of the 'staple-pin' architecture (Pugno et al., 2013, Sahni et al., 2012) presume that initially the dragline is directly cemented to the substrate by means of the pyriform threads. However, our results show that the central part of the discs, where the dragline is attached, is rarely in contact with the substrate. This might be fundamental for the observed strong performance of the attachment disc: The separation of the dragline attachment and substrate attachment provides high flexibility of the disc structure and should support the force distribution within.

Further, we found that the architecture of an attachment disc follows a hierarchy from the dragline glued with dense bundles of pyriform fibers (conjunction) which spread and get separated towards the substrate, where they are separately cemented, distributed over a large area. This architecture might have several advantages: (1) A large contact area can be generated at low material cost, (2) forces may be distributed over a high number of branches and therefore the stress generated in each single structure is kept low, (3) propagating cracks may be arrested, because of the inhomogeneous material distribution, (4) peeling angles are kept low, which means that higher forces are necessary to detach the cementation (Pugno, 2011, Pugno et al., 2013).

Which part of the attachment disc fails under load highly depends on the adhesion energy between the cement and the substrate. Our results on glass show that the dragline is usually stronger than the pyriform silk network, in contrast to the observation by Sahni et al. (Sahni et al., 2012). This makes sense, because a failure within the disc is usually not accompanied by a total failure of spider attachment. For example, in the case of the conjunction failure, the dragline was further continuously pulled through the pyriform envelope, generating high friction. A bridge failure occurred stepwise in most cases. Both events significantly delay the total structural failure. Previous studies calculated the safety factor of draglines in spider fall scenarios, always assuming that failure occurs at the level of the dragline (Brandwood, 1985, Ortlepp and Gosline, 2008, Osaki, 1996, Osaki, 2003). This also holds for various models of web biomechanics, in which thread anchors were presumed as stable (Tarakanova and Buehler, 2012, Harmer et al., 2011, Ko and Jovicic, 2004, Lin et al., 1995, Cranford et al., 2012, Sensenig et al., 2011, Sensenig et al., 2012, Aoyanagi and Okumura, 2010). Although these studies show, that a high amount of energy can be absorbed by thread deformation, significant forces may reach the substrate anchors, especially in short threads. Our findings show that thread anchors can fail before the thread fails and should thus be considered in further estimations of the mechanical stability of spider webs. Additionally, non-uniform crack propagation due to non-continuous distribution of the pyriform glue leads to the further energy dissipation during detachment.

Our comparison of spider species with different ecology and phylogenetic background revealed that the high attachment strength is consistent with the relatively conserved structure of the discs. This indicates that web building and wandering spiders, as well as dwellers of the lower and the higher vegetation, might have similar demands on thread anchoring. However, in the crab spider *T. onustus*, the attachment strength is surprisingly less affected by the substrate.

Scaling effects on the attachment disc strength have very important implications for biology of species studied. Comparing large and small species indicates that safety factor of the disc attachment decreases with the spider weight, thus making both mechanical anchorage and its

function as a protection against falling from the height less efficient. This with spider size increasing loss of safety in climbing situations was previously hypothesized for the dragline (Ortlepp and Gosline, 2008) and leads to one of potential explanations of the extreme sexual size dimorphism in some spiders (Rodriguez-Girones et al., 2010). Males are more active in locomotion as they need to wander around, in order to find potential mating partners. In spiders that live on vegetation, males are often much smaller than females, which may be extreme in some orb web spiders and crab spiders, and which was previously interpreted by facilitation of the thread based locomotion (Rodriguez-Girones et al., 2010). Interestingly, the high sexual size dimorphism is absent in large wandering spiders (i.e. Ctenidae, Sparassidae), of which the arboreal representatives possess highly efficient adhesive foot pads (Homann, 1957, Wolff et al., 2013, Wohlfart et al., 2014, Lapinski and Tschapka, 2013). This is also the case for the large wandering spider *C. salei*, whose attachment discs exhibit only an insufficient safety factor. Future studies, testing the change in attachment disc performance during ontogeneny, may reveal scaling effects in the safety factor of dragline anchors.

For the spider the most uncontrollable part of the attachment disc performance is the adhesive strength between the pyriform glue and substrate. Our results show that cementation fails on surfaces with a low free surface energy like Teflon. This also counts, to some extent, for plant surfaces. The cuticle of plant leaves, stems and flowers is often covered with cuticular folds and crystalline waxes that create roughness on a micro- and nanoscale. Waxes may easily detach, if glued, which is reported to be an adaptation against herbivorous insects (Eigenbrode, 2001, Eigenbrode, 2004, Whitney and Federle, 2013). Spiders, although not being plant consumers, are faced with the same problem of adhesion reduction on these surfaces. Thus, they might have had the need to participate in the arms race of plants and herbivores, as they have to forage at the same sites. Whereas the basic architecture of the attachment disc seems to be relatively conserved (i.e. compare discs spun by Haplogynes and Entelegynes in (Schütt, 1996)), there might have been strong selective pressure on the wetting and adhesion properties of the pyriform glue. Convergently, insects that glue their eggs onto such waxy surfaces use proteinaceous cement (Voigt and Gorb, 2010), which might be functionally analogous to the pyriform glue. Crab spiders (Thomisidae), whose tested representative *T. onustus* showed no significant reduction of the attachment strength on the sycamore leaf surface and even on Teflon demonstrated remarkably high values, are often associated with herbal plants, where they capture prey items larger than themselves (Nentwig and Wissel, 1986). In contrast to many free hunting spiders, thomisids lack the hairy adhesive foot pads (Wolff et al., 2013), thus, they may largely rely on their exceptionally strong dragline attachment.

As the surface geometry and properties of natural surfaces may vary in a very wide range, the stability of web attachment points may be influenced by substrates. Comparing the arboreal *N. senegalensis* with *A. trifasciata* that occupies lower vegetation, no difference in attachment disc performance could be found, which speaks against adaptation of the pyriform glue to particular microhabitat. This, in turn, means that the evolution of the pyriform glue might have been driven towards high universality. This seems to be plausible, as surfaces may be highly inhomogeneous even within a particular microhabitat. On the other hand, there is certain sensibility of disc attachment to the substrate type. Possible ethological counter adaptations, like adjusting the number and/or size of anchors depending on the substrate or choosing proper attachment site, remain to be studied in the future.

Acknowledgements

We thank Prof. Dr. Jutta Schneider (University of Hamburg, Germany) and Dr. Clemens Schaber (University of Kiel, Germany) for providing experimental animals. Heike Scholz (Botanical Garden Kiel) and her colleagues helped collecting experimental animals in the greenhouses. Phillipp Hofmann and Mike Schindler (University of Applied Sciences, Bocholt, Germany) assisted in handling the living spiders during the experiments. Matthias Foellmer (Adelphi University, NY, USA) and an anonymous reviewer are acknowledged for their constructive criticism on the manuscript.

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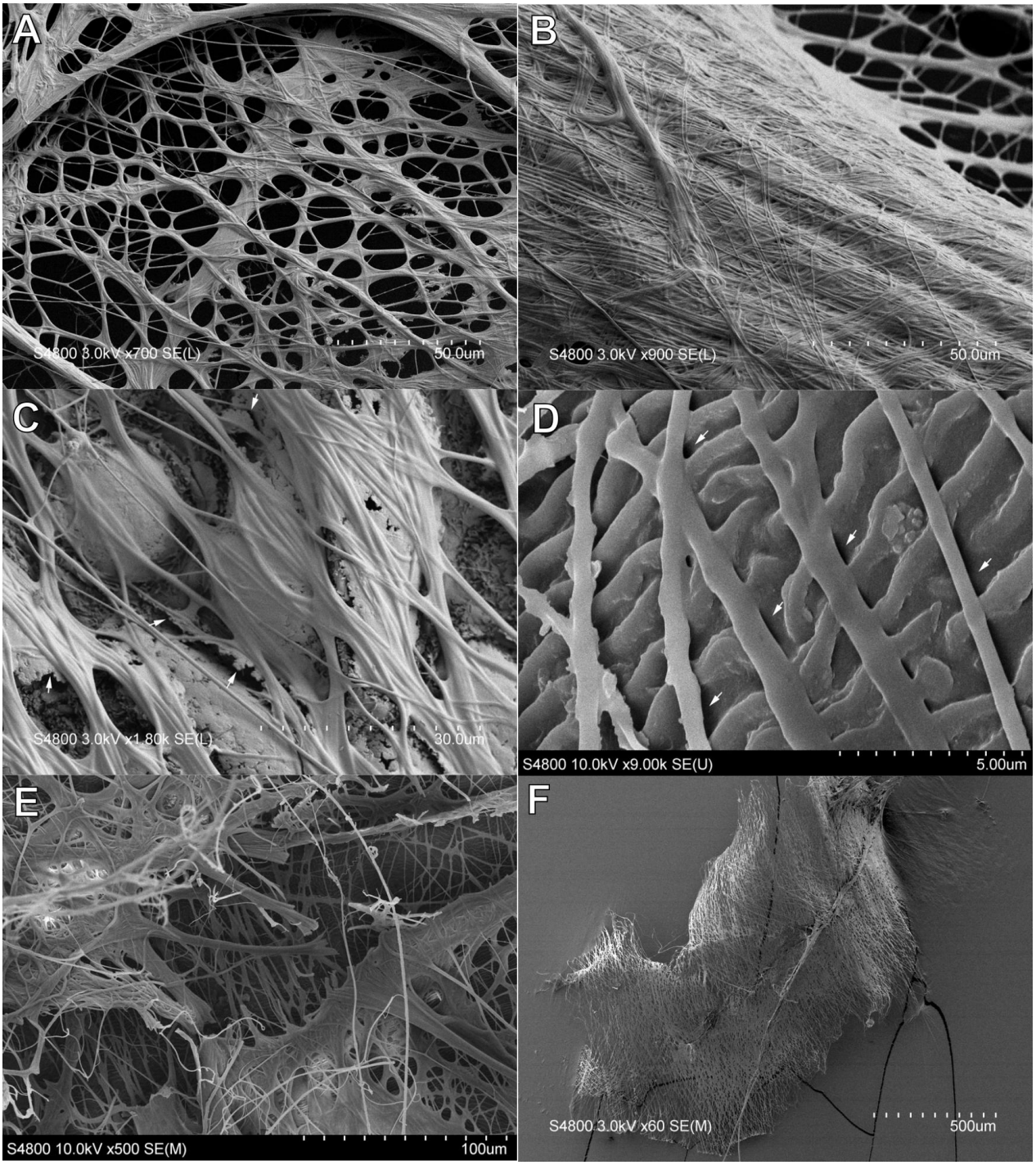
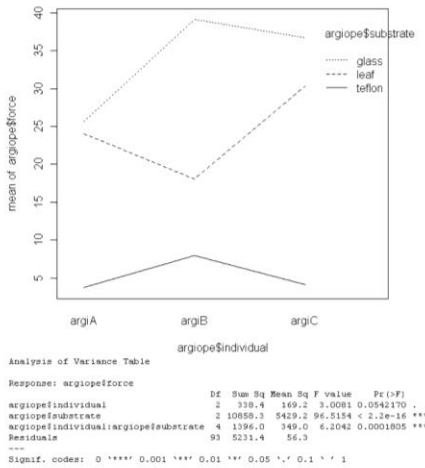
Supplementary Material



SM.8.1. (A) SEM image of the detail of the bridge region in an attachment disc spun by *Argiope trifascata*. (B) SEM image of the detail of the proximal baseplate region in a peeled off attachment disc spun by *Argiope trifascata* on the lower surface of an *Acer pseudoplatanus* leaf. (C) SEM image of the detail of the baseplate region in an attachment disc spun by *Cupiennius salei* on the lower surface of an *Acer pseudoplatanus* leaf. Arrows point to exemplary spots, were the pyriform glue is detached due to the contamination with plant waxes. (D) SEM image of the detail of the baseplate region in an attachment disc spun by *Argiope trifascata* on the upper surface of an *Acer pseudoplatanus* leaf. Arrows point to exemplary spots, where adhering of the pyriform glue is prevented by micro ripples of the leaf cuticle. (E) SEM image of the detail of the damaged bridge region in an attachment disc of *Argiope trifascata* after a tension test with a bridge failure event. (F) SEM image of a peeled off attachment disc spun by *Argiope trifascata* on the upper surface of an *Acer pseudoplatanus* leaf.

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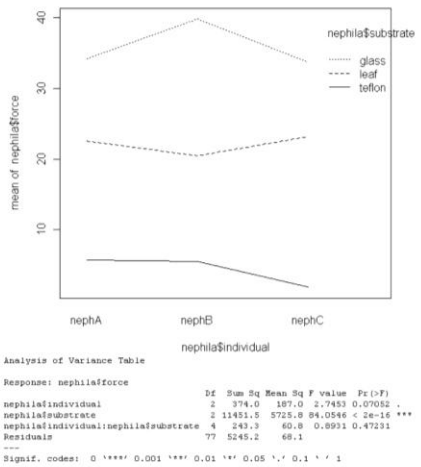
Analysis of Variance Table

Response: argiope$force
                                  Df  Sum Sq Mean Sq F value    Pr(>F)
argiope$individual                 2   338.4   169.2  3.0081 0.0542170 .
argiope$substrate                  2 10858.3  5429.2 96.5154 < 2.2e-16 ***
argiope$individual:argiope$substrate  4  1396.0   349.0  6.2042 0.0001805 ***
Residuals                         93  5231.4    56.3
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```




```
Analysis of Variance Table

Response: nephila$force
                                  Df  Sum Sq Mean Sq F value  Pr(>F)
nephila$individual                 2   374.0   187.0  2.7453 0.07052 .
nephila$substrate                  2 11451.5  5725.8 84.0546 < 2e-16 ***
nephila$individual:nephila$substrate  4   243.3    60.8  0.8931 0.47231
Residuals                         77  5245.2    68.1
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
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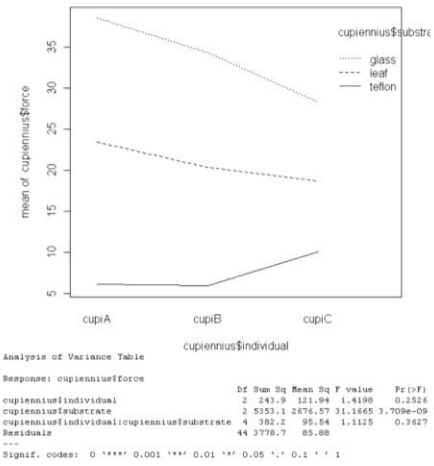


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Analysis of Variance Table

Response: cupiennius$force
                                  Df Sum Sq Mean Sq F value    Pr(>F)
cupiennius$individual              2  243.9  121.94  1.4198    0.2526
cupiennius$substrate               2 5353.1 2676.57 31.1665 3.709e-09 ***
cupiennius$individual:cupiennius$substrate  4  382.2   95.54  1.1125    0.3627
Residuals                         44 3778.7   85.88
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
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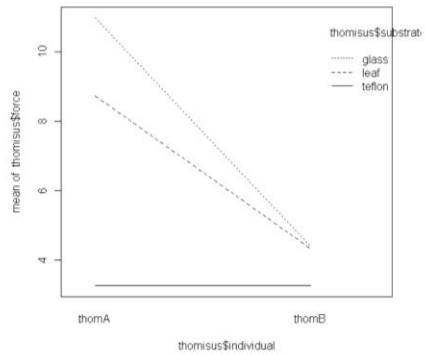


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Analysis of Variance Table

Response: thomisus$force
                                  Df Sum Sq Mean Sq F value    Pr(>F)
thomisus$individual                1 230.78 230.780  50.377 5.746e-10 ***
thomisus$substrate                 2 275.45 137.723  30.064 2.405e-10 ***
thomisus$individual:thomisus$substrate  2 121.46  60.729  13.257 1.152e-05 ***
Residuals                         76 348.16   4.581
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
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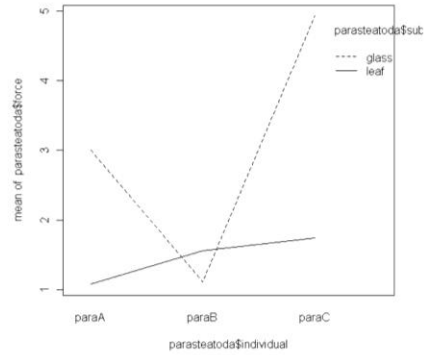


```
Analysis of Variance Table

Response: parasteatoda$force
                                  Df Sum Sq Mean Sq F value    Pr(>F)
parasteatoda$individual            2 27.615 13.8077  19.606 1.564e-06 ***
parasteatoda$substrate             1 12.119 12.1190  17.208  0.000188 ***
parasteatoda$individual:parasteatoda$substrate  2 17.789  8.8945  12.630 6.592e-05 ***
Residuals                         37 26.058  0.7043
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

SM.8.2. Test statistics for two-factor ANOVA with factors substrate and individual, for pull-off force data for each species tested.

9. Paper no.7*:

Spider's super-glue: Thread anchors are composite adhesives with synergistic hierarchical organization

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Abstract

Silk is a key innovation in spiders, fascinating both biologists and material scientists. However, to fulfil their biological function silken threads must be strongly fastened to substrates or other threads. The majority of modern spiders produce a unique and rather unexplored bio-adhesive: the two-compound pyriform secretion, which is spun into elaborate patterns (so called attachment discs) and used to anchor silken threads to substrates. Strong adhesion is achieved on a high variety of surfaces with a minimum of material consumption. Pyriform threads polymerize under ambient conditions, become functional within less than a second and can remain stable for years. They are biodegradable, biocompatible and highly versatile – the adhesion and the overall toughness of the attachment disc can be controlled by spinneret movements on a macroscopic level (Sahni et al., 2012). We found that the pyriform thread is a silk fibre that is coated with glue-like cement consisting of aligned nanofibrils, lipid enclosures and a dense, isotropic boundary layer. The threads are spun in a meshwork pattern that promotes stress distribution and crack arresting. Our results demonstrate, that hierarchical organization and fibre embedding may explain the high adhesive strength and flaw tolerance of a structure made by the same, rather simple type of silk glands.

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9.1. Introduction

Due to its high toughness, spider silk has caught the attention of material scientists and biotechnologists as a possible prototype for developing new biological materials for textile industry and medicine (Vollrath and Knight, 2001, Hinman et al., 2000, Schacht and Scheibel, 2014, Rising, 2014). Silk research concentrates mainly on the major ampullate silk (dragline silk) while other types of silk remain a rather unexplored source of innovation. By gene duplication the more advanced araneomorph spiders evolved up to eight different types of silks (Vollrath, 1992, Blackledge and Hayashi, 2006, Harmer et al., 2011), varying from ultra-tough nanofibres (aciniform silk) (Hayashi et al., 2004) to viscoelastic glycoprotein based glue droplets (aggregate secretion) (Sahni et al., 2010).

Pyriform silk is used to agglutinate silken threads or to affix them to substrates. It can produce strong adhesion, even on surfaces with a low free surface energy, such as Teflon (see ***chapter 8***). The pyriform glands are comparably small with a pear-shaped (name) lumen and a short duct opening into the nozzle-like spigot (Apstein, 1889, Kovoor and Zylberberg, 1982). These appear in large clusters on the anterior (first) of, typically, three spinneret pairs next to the major ampullate gland opening. This arrangement facilitates the fast attachment of the dragline (Eberhard, 2010). The pyriform gland exhibits two histochemically distinct parts each with a secretory cell type (Apstein, 1889, Kovoor and Zylberberg, 1980). The distal half of the gland produces the PySp spidroins (silk proteins), the proximal half secretes a colloidal fluid containing, as yet unidentified, acidic proteins and hydrocarbons (Kovoor and Zylberberg, 1980). The secretory products form two phases in both the gland lumen and the duct (Kovoor and Zylberberg, 1980, Kovoor and Zylberberg, 1982). Morphologically and histologically these glands are very similar to the single silk gland type found in ancient lineages of spiders whose threads adhere by means of an acidic protein coating (Palmer et al., 1982). Pyriform silk may thus have a very early origin and been highly optimized throughout evolution. With the evolution of insect flight, spiders began to spread out from their ground habitats into vegetation and to build aerial webs (Vollrath and Selden, 2007, Harmer et al., 2011). Plants often exhibit complex surfaces with anti-adhesive properties for staying clean and deterring herbivores (Whitney and Federle, 2013, Eigenbrode, 2004), among spiders there might have been a strong selective pressure for the best glue, which made the best foraging sites accessible (see ***chapter 8***).

The macroscopic structure of the attachment discs is the result of a highly conserved spinneret movement program creating numerous parallel loops of crossing silk fibres (Eberhard, 2010, Schütt, 1996). In the central part the dragline is glued, elevated and thus not in contact with the substrate attachment. This provides certain flexibility and a more homogenous stress distribution within the structure (see ***chapter 8***). Spiders may be able to spin attachment discs with different adhesion and overall toughness (Sahni et al., 2012): Through the coordinated action of the anterior spinnerets discs can be created that are attached to the substrate only at their margins and thus be easily detached by a small impact of the mobile prey. This kind of disc is used in unique traps called gumfoots (Sahni et al., 2012). Since attachment discs are frequently used by spiders they must have been selected for high economy in the course of evolution. The attachment disc of an adult (body mass 0.6-0.8 g) golden orb weaver (*Nephila senegalensis*) can hold 4-6 times its body weight when applied to a smooth glass surface (see ***chapter 8***), while containing only 2-10 µg (~0.001 per cent of spider weight) of material. Hence, the usage of pyriform silk by spiders may show means of applying glue in a way that minimises the use of material (Jain et al.,

2014). Furthermore, the intrinsic composite structure of the attachment disc can be a great source of inspiration for the engineering of nanocomposite and light weight materials. It is also noteworthy that pyriform silk may have a high potential as a natural glue or as possible component of synthetic biomaterials for medical applications and future green technologies (Hinman et al., 2014). The first progress made along this line of research is the recent identification, isolation and cloning of pyriform spidroins (Geurts et al., 2010, Hinman et al., 2014). However, a basic understanding of the mechanism of how the attachment disc functions is still lacking. Here we present the first comprehensive study on pyriform glue which (1) integrates ultra structural and micromechanical analysis, (2) reveals some synergistic effects in its hierarchical organization, and (3) gives insight into the structure-function relationships of the discs.

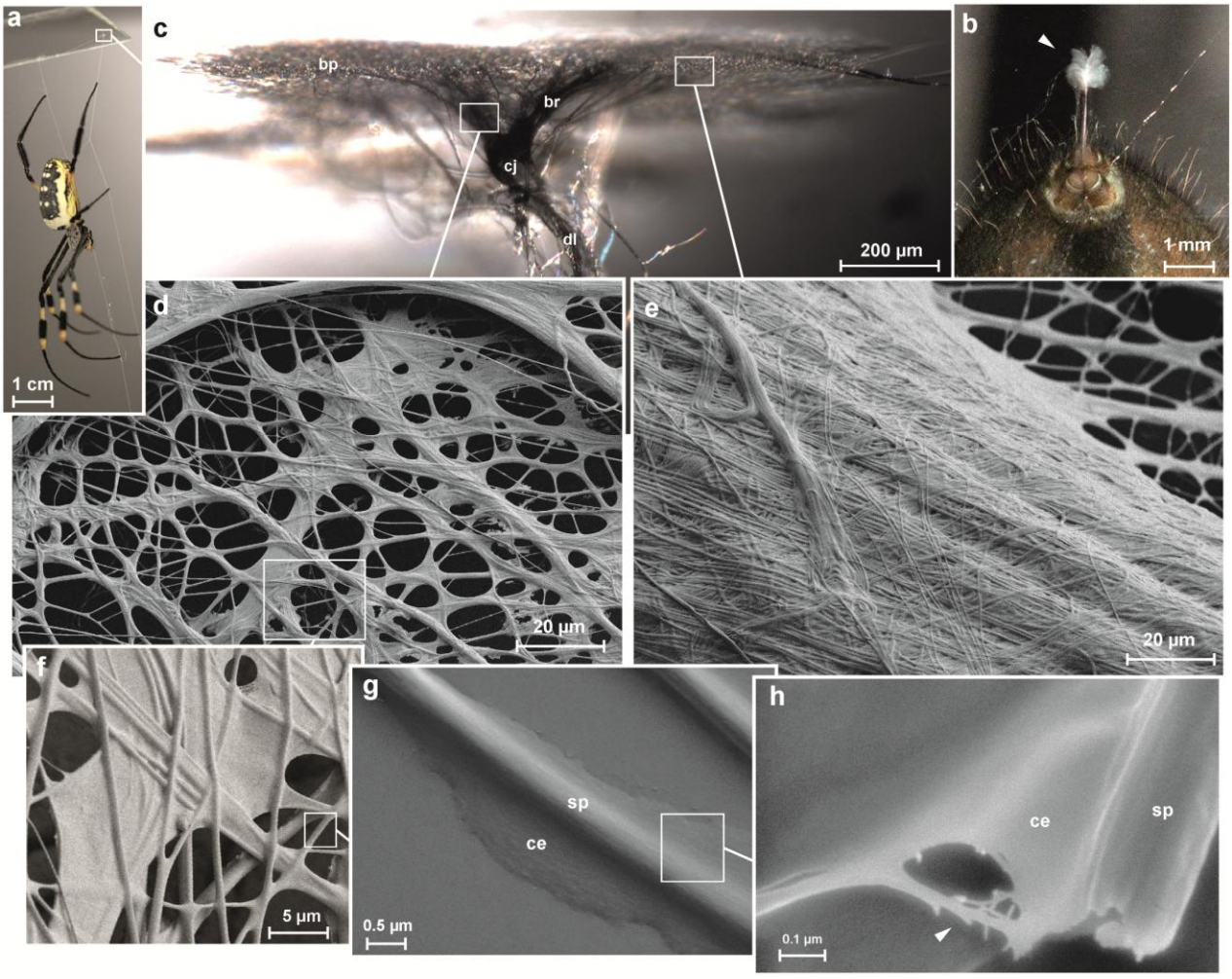

Fig.9.1. Hierarchical structure of spider silk anchors. Spiders, such as the golden orb weaver (*Nephila senegalensis*), can secure themselves on smooth glass by means of silk (a). The tough safety thread (*dragline*), made of major ampullate silk, is attached to the slide with pyriform silk (b). It is spun in an elaborate 3D-pattern called the attachment disc (c). It consists of an apical part where the dragline (dl) is glued (*conjunction*, cj), an intermediate network of pyriform threads (*bridge*, br) (d) and the basal substrate cementation (*baseplate*, bp) (e). The pyriform threads are applied in layers with shifted angles (f) and consist of two phases: a spidroin (silk protein) fibre (sp) and a glue coating (cement, ce) which is fluid-like just after extrusion and dries rapidly after application (g). The cement consists of aligned nano-fibrils, as seen at rupture sites (h).

9.2. Results

Spider attachment discs are divided into four functional parts (Fig.9.1.c): (1) the substrate cementation which can be regarded as a thin film (*baseplate*) (Fig.9.1.e); (2) a network of pyriform fibres between the baseplate and the cemented dragline (*bridge*) (Fig.9.1.d); (3) an envelope of pyriform fibres cementing the dragline (*conjunction*) and (4) the major ampullate silk threads anchored by the attachment disc (*dragline*).

The pyriform thread is a twofold compound material, a fibrous cement with an embedded fibre (Fig.9.1.g-h). By means of electron microscopy we found that the main fraction of the cement material is made up of nanofibrils (Figs.9.1.h; 9.2), consisting of 20-30 nm sized electron dense globular proteins connected by thin strains of a material with less electron density (Fig.9.2.e). This is very similar to the structure of the secretion before extrusion (Kovoor and Zylberberg, 1980). The cement nanofibrils are regularly arranged parallel to one another and to the spidroin fibre. They form stacked monolayers that get partially separated by strong shear forces

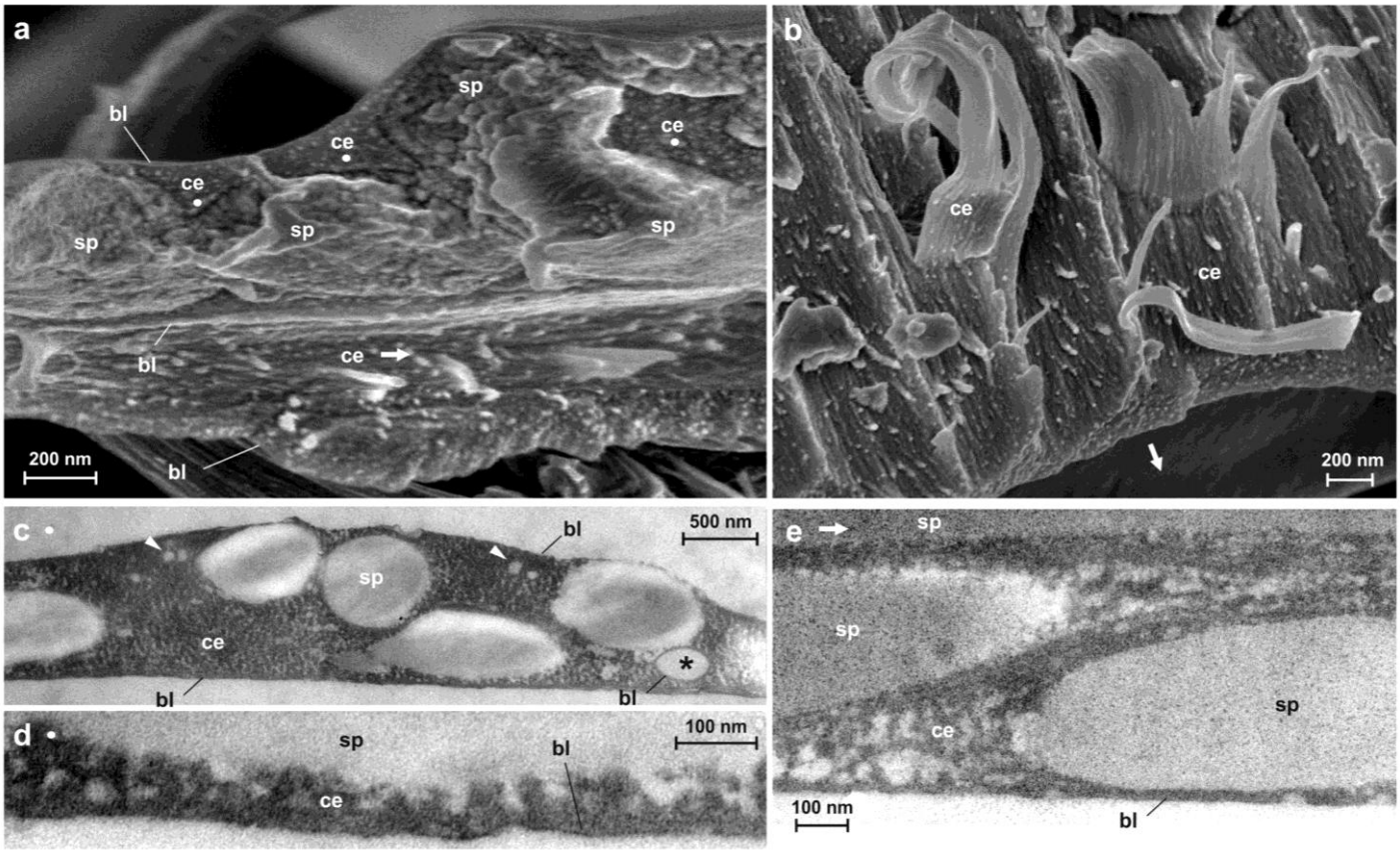

Fig.9.2. Ultrastructure of pyriform silk. (a-b) Cryo SEM micrographs of freeze fractured pyriform threads of *Nephila senegalensis*. (a) A transverse fracture of a thread crossing visualizing the different phases and its supramolecular organization. The pyriform cement (ce) consists of regularly arranged nano-fibrils aligned in the same direction as the embedded spidroin thread (sp) which consists of a less ordered material that, in contrast, does not show a smooth breaking edge. At the interface between the pyriform thread and the substrate the medium or earlier spun (already dried) threads show a thin boundary layer (bl), presumably a monolayer of regularly arranged macromolecules. Arrows and dots indicate orientation of polymer fibrils (dots indicate orientation perpendicular to the image plane). (b) A horizontal fracture, where spidroin fibres are ruptured, showing that the cement nanofibrils form layers. (c-e) TEM images show that cement fibrils are heteromeric polymer chains consisting of alternating electron dense and electron translucent parts. Spidroin fibres appear amorphous and less electron dense. The boundary layer has a high electron density. Occasionally droplets (presumably lipids, arrowheads) and air inclusions (asterisk) are found in the cement fraction.

during freeze fracturing (Fig.9.2.b). In transverse fractures the cement forms a rather smooth breaking edge with a regular arrangement of the globular proteins (Fig.9.2.a). In contrast, the embedded spidroin fibres exhibit a corrugated breaking edge indicating a more amorphous structure and higher toughness of the material (Fig.9.2.a). At the interface towards the substrate and the environmental medium (air) a thin (~10 nm) electron dense layer forms (Fig.9.2.a,c-e) which might consist of small, highly polar proteins that act as an intermediate agent between the substrate and the silk cement. In contrast to the cement and spidroin fibre, this part seems to be isotropic as it breaks in various directions. Surprisingly, when deep frozen at -140°C in liquid nitrogen for the purpose of freeze fracturing the attachment discs remained flexible and were hardly breakable, indicating a low content of unbound water.

We studied the spinning process using reflection interference contrast microscopic high speed videography (RICM-HSV) and Cryo scanning electron microscopy (Cryo-SEM). We found that the material is a low viscosity fluid, which is less organized when extruded (Fig.9.3.b). The embedded silk fibre is already differentiated against the glue phase and appears to be rather solid, but elastically deformable (Figs.9.3.f-l). The thread is extruded with a velocity of 5-9 mm/s. Within less than a second the cement is dried and no longer changes its shape when occasionally touched by a spider's cuticular structure. At high spinning activity, a condensation of minute droplets was observed on the glass substrate between the fibres (Figs.9.3.m-n), which we interpreted as water evaporated from the rapidly drying cement. If the threads are not directly applied onto the substrate, but with a short delay after extrusion, beads-on-a-string structures (BOAS) occasionally occurred (Fig.9.3.d). This is an indication, that the drying glue behaves like a viscoelastic fluid (Bhat et al., 2010). The spidroin thread is clearly embedded and does not directly adhere to the substrate, which is indicated by its occasional dislocation within the glue coating shortly after its contact to the substrate (Fig.9.3.f-l). Thus, the assumption of previous authors about strong interaction between the PySp thread and the substrate (Geurts et al., 2010, Hinman et al., 2014) is not supported by our data.

Once applied, attachment discs remain stable for a long period of time: We found that attachment disc samples of a *N. senegalensis* individual (on glass) stored for a year still retained, on average, three-fourths of the attachment force of freshly harvested ones (fresh: 39.8±8.9 mN, n=23; aged: 28.5±11.2 mN, n=13).

The adhesion of the pyriform glue outweighs the inner strength of the attachment disc on a smooth glass surface and the structure breaks internally (87% of 32 tests) or at the dragline (13%). When the glass is treated with APTES (3-Aminopropyl-triethoxysilane), which increases its hydrophobicity, the pyriform glue adheres less strongly. This is indicated by partial delaminating of the attachment disc during the tensile test (69% against 0% on untreated glass of each 32 tests) and reduced detachment forces (Fig.9.4.a). On glass treated with DMDCS (Dichlorodimethylsilane), which forms a strongly hydrophobic film on the surface, spiders are unable to attach their pyriform threads. This indicates the important role of hydrogen bonds in the adhesion of pyriform cement. Monolayers of water usually present on uncoated glass (and on most other surfaces (Hu et al., 1995, Miranda et al., 1998)) may act as a coupling agent and support adhesion.

On PTFE-foil (Teflon), which exhibits a similar water contact angle than that of the DMDCS treated glass, adhesion is significantly lower than on the APTES treated glass, but still sufficient to hold the body weight of the spider. The better attachment to Teflon than to DMDCS

treated glass might be explained by much stronger nano roughness of Teflon foil than of DMDCS treated glass (see Supplementary Material SM.9.1).

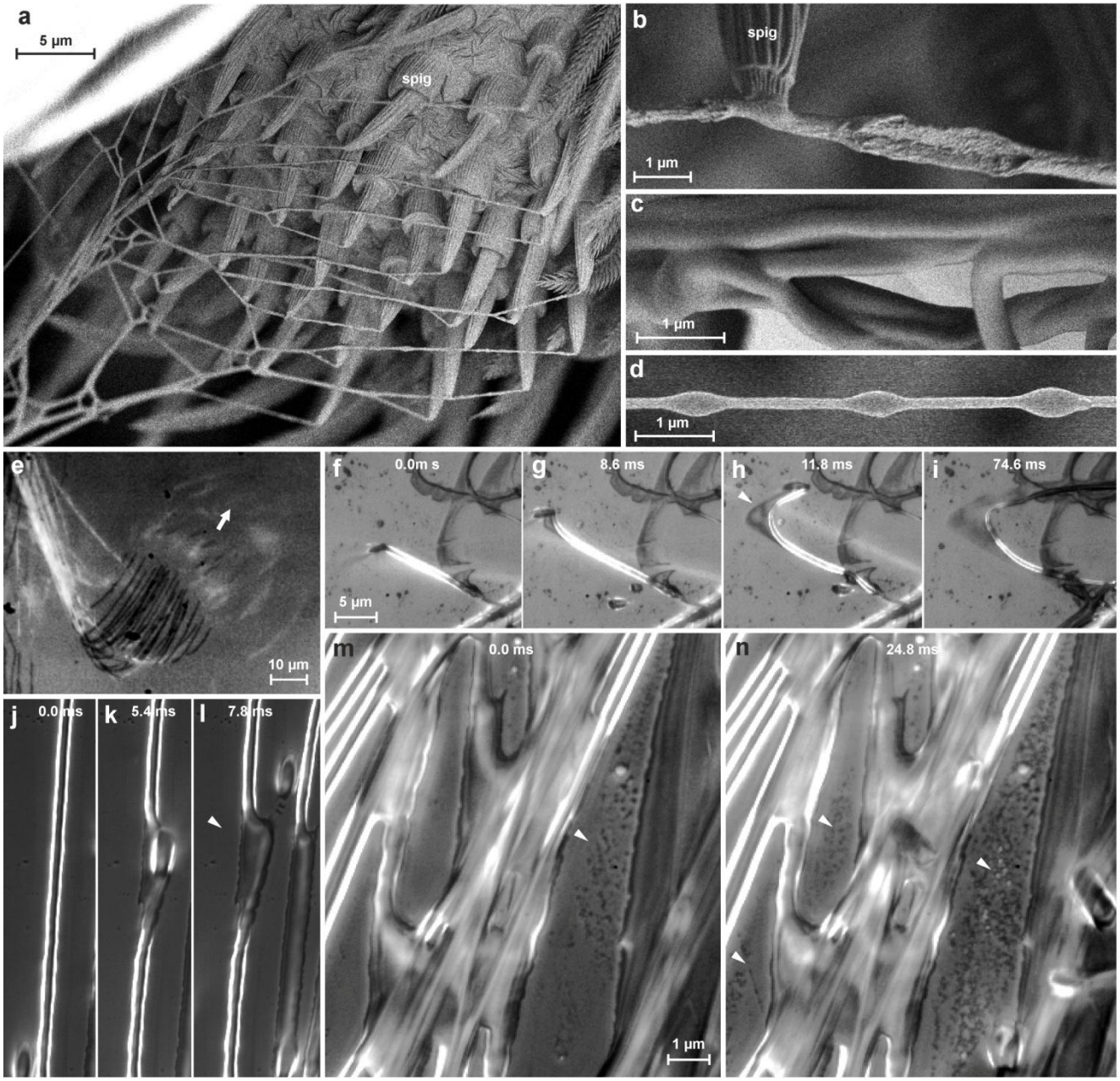

Fig.9.3. Spinning of pyriform silk. (a) During attachment disc spinning, pyriform silk is extruded by numerous spigots (spig) of the anterior lateral spinnerets, here in the cribellate orb weaver (*Uloborus plumipes*). The silk is liquid, when extruded, as indicated by the irregular surface of the thread (b). The same threads have a smooth surface at a greater distance from the spinneret, indicating post-spinning flow (c). Occasionally beads-on-a-string structures (BOAS) occurred, an indication that the drying glue behaves like a viscoelastic fluid (d). The application of the attachment disc onto a glass slide can be studied by means of RICM-HSV (view from below the glass slide), where direct contact appears darkened (a single spinneret of *U. plumipes* in action in (e), with an arrow indicating spinneret movement). Sequence (f-i) shows the application process in *Nephila senegalensis* at a turning point, showing that the internal spidroin thread is pulled over some distance at the distal point of the loop, smearing the cement phase over the substrate. This indicates that the thread is already solidified, while the cement is still in a fluid state. This is further supported by the observation that crossing (in this case: non-secreting) spigots take the thread with them over a short distance (j-l). When pyriform threads are applied at a high density, condensation of a liquid takes place on the glass in between (m-n), an observation which assumes water evaporation from the drying silk.

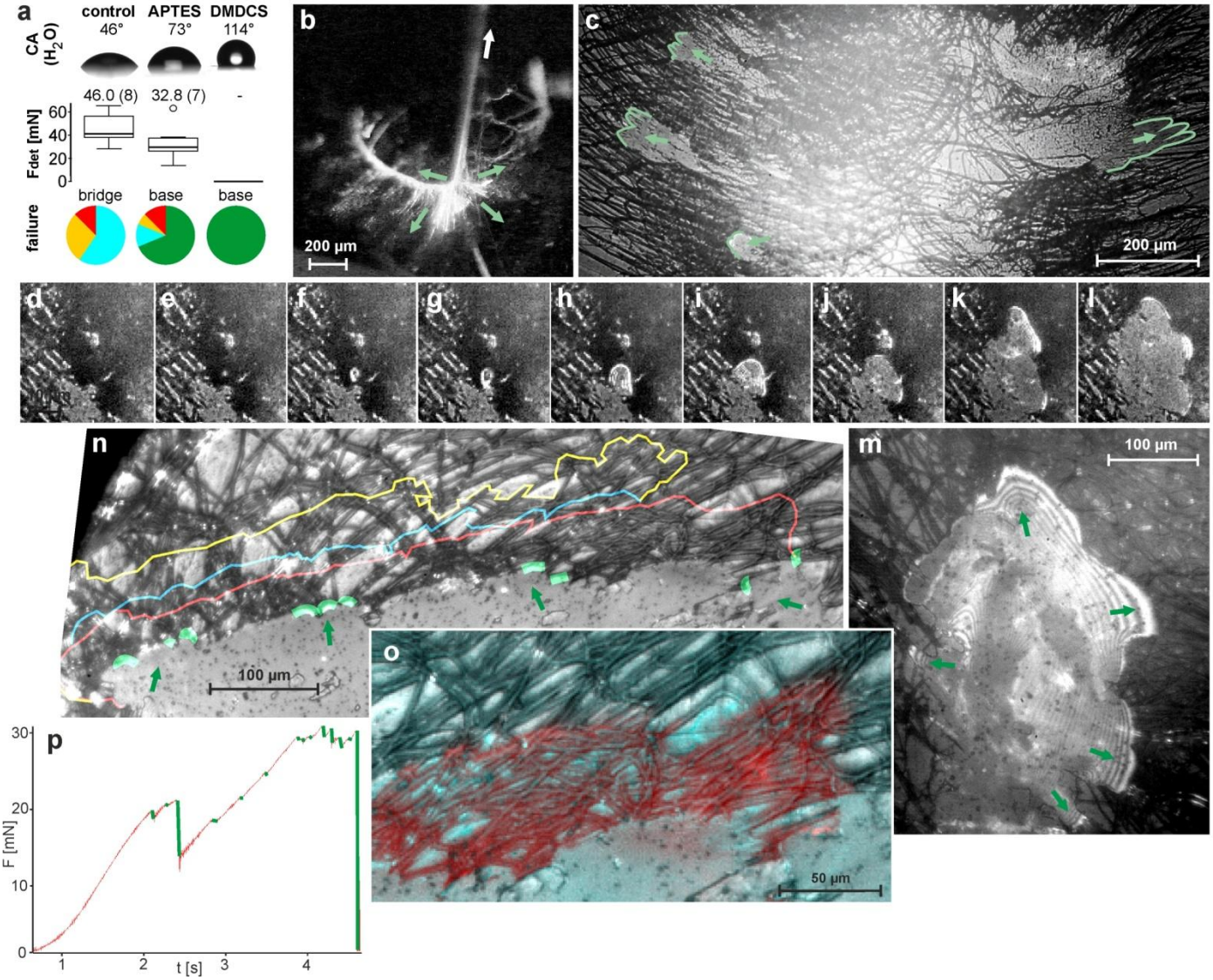

Fig.9.4. Fracture mechanics of pyriform silk discs. (a) Results of tensile tests of *N. senegalensis* attachment discs spun on untreated and silan-treated glass resulted in different detachment forces and failure modes. Above, the contact angle (CA) measurement of water droplets on the substrates demonstrates their differences in hydrophilicity. Box plots give the median and variance of detachment force (Fdet) data. Numbers above box plots give the mean values (in brackets number of individuals tested). Pie charts in the bottom indicate the proportion of failure modes, whereby red symbolizes dragline failure, yellow conjunction failure, blue bridge failure and green baseplate failure (total delamination) (for details see ***chapter 8***). These results indicate the importance of hydrogen bonds in pyriform silk adhesion. (b) When the dragline is pulled (arrow), various pyriform fibres of the bridge are put under tension (green arrows depict direction of acting tensile stress). (c) Due to their radial arrangement, stress is distributed to opposite sides of the symmetrical baseplate, leading to a simultaneous peel-off when spun on APTES-treated glass (green lines mark the peeling edges). (RICM-HSV frames, view from below the glass slide) (d-m) A stressed bridge fibre may induce a crack at the interface (arrowhead), which is initially circular and often propagates radial symmetrically first. The stress is distributed at the peeling edge, as indicated by interference stripes in the RICM image that occur when the separation between substrate and silken film is very low (m). (n) Crack arresting indicated by coloured lines. (o-p) Crack propagation is often stopped at crossing fibres. (o) A detail, with the next step failure marked in red. Please note that the crack propagation is stopped along transverse fibres. (p) The force-time curve during a tension test on APTES-treated glass shows that cracks (green marked force drops) are quickly arrested, followed by a further rise in the force. Shortly before the final rupture, the frequency of crack initiation increases because stress approaches the maximum that the attachment disc can withstand.

9.3. Discussion

The adhesion of silk threads is substantial for their function in protection, prey capture, locomotion or reproduction, as these always implicate an interaction with substrates or other previously spun threads. In insects and spiders silks have been shown to be coated with an acidic protein (sericin in *Bombyx mori* (Voigt, 1965)) or a glycoprotein (spider dragline (Augsten et al., 2006)). The thin draglines of small spiders can stick to smooth surfaces such as glass. However, the attachment is not sufficient to hold the body weight of the spider (Wolff et al., 2014a). Fastened with a pyriform anchor it can securely attach a spider even on Teflon (PTFE), which is well-known and applied for its extremely reduced surface interaction with other substances (see ***chapter 8***). Our results show an elaborate hierarchical structure of attachment discs and the combination of materials with different properties that may complement one another. In the following we discuss the role of different hierarchical levels from the molecular one to the macroscopical one and how these may interact synergistically.

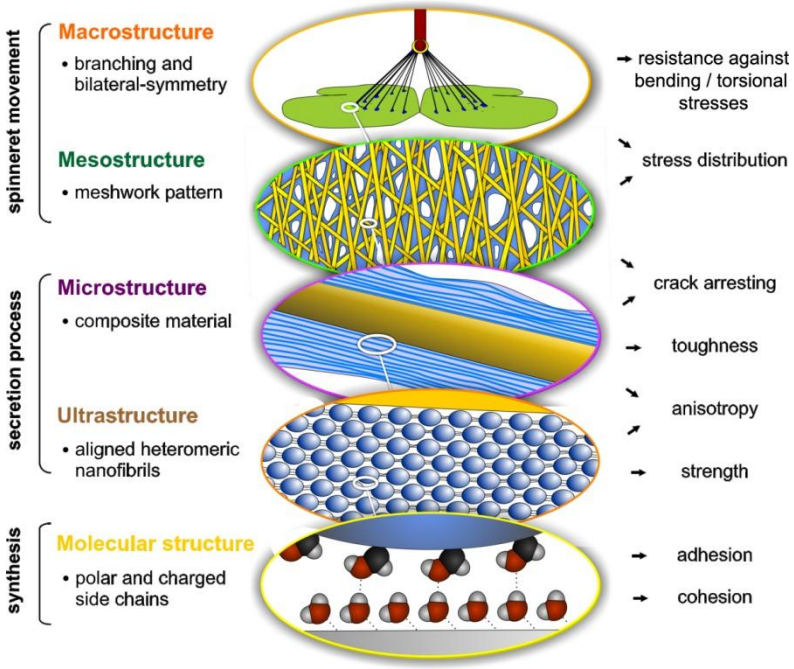

Fig.9.5. Hierarchical organization and functional correlates. Schematic illustration and description of the spider attachment disc structure summarizing the main hypotheses of this study. For further explanation, see text.

9.3.1. Molecular structure and Ultra structure

Previous chemical analysis of the pyriform proteinous fraction showed an extraordinarily high content of polar and charged amino acids (Kovoor and Zylberberg, 1980, Andersen, 1970, Geurts et al., 2010, Blasingame et al., 2009), which may result in strong interactions within the dried secretion film and with the substrate. The highly polar regions within pyriform spidroins also support self-assembly of the silk fibre (Geurts et al., 2010) and water-solubility (Blasingame et al., 2009), both being desirable properties for use in artificial spinning systems. Further, carbohydrate components were found in the cement fraction (Kovoor and Zylberberg, 1980), perhaps indicating the presence of glycoproteins. These play a universal role in adhesive cementation ranging from cells (Dranginis et al., 2007) to marine organisms (Hennebert et al., 2011). Our ultra structural analysis showed that there are inclusions of, presumably, lipids within

the cement. These could play a role as surfactants, improving the wettability of hydrophobic surfaces. We found that the cement consists of aligned heteromerous nano-fibrils of repetitive globular and fibrillar parts. Nano-fibrils with such geometry are hypothesized to provide a high resistance against bending, tensile and shear stresses, thanks to interlocking (Brown et al., 2012, Xu et al., 2014). This is of high relevance for the stability of the attachment under load.

The ultra structural analysis of freeze fractured pyriform fibres showed that both phases, the fibre and the cement, differ fundamentally in their fracture mechanics, indicating a difference in their mechanical properties. Previous amino acid sequence analysis of PySp showed that these spidroins are less organized but rather amorphic (Kovoor and Zylberberg, 1980, Blasingame et al., 2009, Geurts et al., 2010), which is confirmed by the results of our ultrastructural analysis. Such structure usually indicates high ductility. Material heterogeneity (although less pronounced) is also known from the major ampullate silk of spider draglines. These fibres consist of two different spidroins (MaSp1 and MaSp2). The MaSp1, located in the outer core of the fibre, exhibits a semi-crystalline structure, providing high strength (Brown et al., 2011). In the inner core, there is an additional protein, the MaSp2, which, in contrast, exhibits a disordered structure due to a high content of glycine, thereby providing high extensibility (Brown et al., 2011). Thus, due to the synergistic effect of both polymers the fibre exhibits high toughness. An analogy from artificial materials is the recent development of Engineered Cementitious Composites (ECC), which demonstrates that a strong, yet brittle material such as concrete can get tough and flaw tolerant through the inclusion of polymer fibres, because crack propagation is stopped at the interface between both materials (Li, 2003). The two fold compound structure of pyriform silk may reinforce the material in a similar way.

9.3.2. Micro- and Mesostructure

In a pyriform fibre the glue fraction usually constitutes less than half of the secretion. This might be substantial to quickly arrest the thread when applied to the surface. The glue coating must be thick enough to fill small cavities on a rough surface in order to generate a high contact area, crucial for adhesion (Johnson et al., 1971). On the other hand, it must be thin enough to prevent cohesion failure when drag forces are acting on the embedded fibre as long as the glue is in a fluid state (as indicated by partial displacement at turning points found during RICM-HSV recordings; Fig.9.3.f-i). It should further form a thin film on the substrate from which the solvent can quickly evaporate. A thin film exhibits high adhesive properties due to high elasticity (even if the material is relatively stiff) (Kendall, 1975). Depending on where the force is applied, the peeling line of a thin film (crack propagation zone) can be linear (uni-directional) or axisymmetric (multi-directional). The highest pull-off forces are achieved in the case of multidirectional peeling because the peeling line continuously increases, causing an increase in detachment resistance (Afferrante et al., 2013).

On smooth substrates with reduced adhesion (silanized glass) we often observed radial symmetrically-oriented crack propagation (Fig.9.4.d-m). Linear peel-offs only occurred in single threads at the margin of the attachment discs, where the pyriform threads are less dense and less interconnected. The propagation of cracks was significantly hindered by the material heterogeneity of the composite, leading to discontinuous peel-offs and high increases of detachment forces (Fig.9.4.p). Crack propagation is often stopped at threads arranged perpendicular to the crack propagation direction (Fig.9.4.n-o). Cracks are thus guided by the strong anisotropy (directionality) of the pyriform silk on both ultrastructural (cement nanofibrils)

and microstructural (embedded spidroin fibres) levels. Due to the crossover arrangement of pyriform fibres in the attachment disc basal plate (determined by spinneret movements during attachment disc spinning), cracks are trapped between thread interconnections and their propagation is stopped. Because the creation of a new crack demands significantly more energy than its propagation, the force necessary to detach the attachment disc rises. This principle is furthermore important for flaw tolerance. Most natural surfaces that are the target of attachment disc application are highly unpredictable and exhibit excessive heterogeneity in surface topography and/or surface chemistry. This means that flaws in the adhesive cannot be prevented. Cracks can easily be induced and propagate from such defects. By proper crack arresting the effect of adhesion defects is suppressed.

9.3.3. Macrostructure

The macrostructure of the attachment disc may substantially support energy dissipation, thus reducing stress concentration, because of the interconnections of pyriform threads in the *bridge* (and the *baseplate*). Because both the *dragline* and the *bridge* are not rigid, but rather ductile, energy might be dissipated by elastic and plastic deformation. The bi-axial symmetry of the attachment disc (determined by the arrangement of the paired spinnerets) leads to simultaneous peeling on opposite sites of the attachment disc, as observed in RICM-HSV peel off tests from APTES treated glass (Fig.9.4.b-c). Thus, the peeling angle is kept low, promoting stress distribution at the peeling edge and therefore higher pull-off forces (Pugno, 2011). Energy dissipation and stress distribution in both the macrostructure of the attachment disc and at the peeling edges (crack propagation zone) results in low stress actually acting on the substrate-cement interface. This might explain why the attachment discs achieve considerable attachment strength even if the substrate bonding is relatively weak, as on Teflon.

9.3.4. Conclusion

Spider attachment discs demonstrate the synergistic effect of structural parameters on different hierarchical levels from the molecular to the macroscopic, which altogether provide a high adhesive strength and toughness on unpredictable surfaces at an absolute minimum use of material (Fig.9.5). On the *molecular level* the high content of polar and charged side chains leads to strong interaction with substrates (adhesion) and within the material (cohesion). On the *ultrastructural level* aligned heteromeric nanofibrils lead to high anisotropy and strength. The composite structure of two phases (spidroin fibre and cement coating) creates toughness and anisotropy on the *microstructural level* and introduces a material heterogeneity, stopping crack propagation. The meshwork pattern, by overlays of pyriform threads applied in different angles, promotes stress distribution and flaw tolerance by crack arresting (*mesostructural level*). Finally, on the *macrostructural level*, the separated cementation of substrate and dragline, as well as bi-lateral symmetry, supports load energy dissipation and a reduction of sensitiveness towards bending and torsional stresses. These structure-function relationships are substantial for the basic understanding of attachment disc function and may significantly support the exploitation of this unique bioadhesive in biotechnological and biomimetic approaches, for both medical and technical applications. It further illustrates that an elaborate secreted structural material, such as silk, must be studied not only on the biochemical but also on the physiological and especially organismic (behavioural, phylogenetic and ecological) level. The ecological demands and

phylogenetic burden that determine and constrain the evolution of a biological material, must also be taken into account to understand and extract principles of a structure-function relationship.

9.4. Material and Methods

9.4.1. Animals and harvesting of attachment discs

Ten living individuals of the golden orb weaver *Nephila senegalensis* WALCKENAER 1842 (Nephilidae) were obtained from a laboratory stock of the Department of Ethology, University of Hamburg, Germany. Spiders were kept in reversed 500 ml plastic cups (with roughened walls and an apical hole closed with a piece of plastic mull) at 28-30°C and 60-70% relative humidity. They were sprinkled daily with water and fed weekly with juvenile locusts (*Locusta migratoria*) obtained from the local pet shop. For snap freezing experiments of spinning spiders, small cribellate orb weavers (*Uloborus plumipes* LUCAS 1846 (Uloboridae)) were collected in the greenhouses of the local botanical garden (this species was used for this particular experiment because of the restricted space in the set-up and its very frequent production of attachment discs). In high speed video recordings (HSV) of attachment disc production (see methodology below), we found no differences between representatives of *Nephila* and *Uloborus*, despite their different body sizes.

Attachment discs were harvested by holding the substrate sample with a long pair of forceps. The spider was induced to crawl onto the substrate by slightly touching the animal on the hind legs. When spiders were left sitting upside down on the substrate (usually the edges of the slides were grasped with the claws) they often secured themselves by means of an attachment disc. Spinning could also be induced by slightly shaking or blowing at the spider. The spider was given the freedom to move forward, without forcing it, until the dragline had a proper length. Then the dragline was cut, with a fine pair of scissors, at about 3 cm above the attachment disc. Substrate separated samples for ultrastructural analyses were obtained by letting the spider spin onto a piece of Teflon from which the attachment disc could be completely delaminated.

Samples of attachment discs delaminated from Teflon were weighed with using an ultra balance (UMX2, Mettler-Toledo Inc., Columbus, OH, USA) (dragline was cut off above the attachment disc prior to weighing).

9.4.2. Light microscopy (LM) and high speed videography (HSV)

Macro-morphology of attachment discs were studied using standard transmission light microscopy (phase contrast mode). Spinning of attachment discs was studied using reflection interference contrast microscopic high speed videography (RICM-HSV). This was conducted by means of an inverted microscope (AXIO Observer.A1, Carl Zeiss AG, Oberkochen, Germany), operated with coaxial light and a beam splitter reflecting the image onto the sensor of a high speed video camera (Fastcam SA 1.1, Photron Inc., San Diego, CA, USA). A 40x/0.65 lens and a 100x/1.4 oil immersion lens were used. Thin, cleaned (for cleaning protocol see below) cover slips were mounted on a Plexiglas slide having a median hole, fixed with double sided tape to reduce the bending of the slide under load possibly leading to unfocused recordings. Spiders were set onto the slide with the posterior end of the abdomen placed into the hole with the underlying cover slip and recordings were made with 5000 frames per second using continuous recording and post-triggering.

9.4.3. Electron Microscopy (EM)

Attachment disc samples on pieces of cover slips and delaminated samples were fixed on stubs with conductive double-sided carbon tape and sputter coated with 12 nm AuPd. Samples were imaged in a Hitachi S 4800 scanning electron microscope (SEM) (Hitachi Ltd., Tokio, Japan) at an acceleration voltage of 3.0 kV

Uloborus spiders were fixed on the tip of a bound piece of metal wire by means of a droplet of two compound dental wax (Polyvinylsiloxane, Coltène/Whaledent AG, Altstätten, Switzerland). The wire piece was glued onto a SEM specimen holder beside a small wooden block with an attached piece of tree leaf on an 80° sloped edge, serving as a spinning substrate. By bending the wire, the spider was brought into a position with the spinnerets pointing upwards and being close enough to the wood block to reach the leaf. When spiders started to spin an attachment disc, the entire specimen holder was immediately put into liquid nitrogen and the deep frozen sample was transferred into an S 4800 SEM equipped with a Gatan ALTO-2500 cryo system (Gatan Inc., Abingdon, UK), sputtered with 10 nm Au-Pd and viewed in the SEM with the stage cooled up to -120°C.

For freeze fracturing, *Nephila* attachment discs, initially peeled off the Teflon substrate, were vertically glued onto a specimen holder with Tissue-Tek® compound, shock frozen in liquid nitrogen and transferred to the SEM cryo system. Fracturing was executed by cutting and scraping the sample with a scalpel blade, mounted on a moveable metal stick within the super-cooled SEM prechamber. Then the samples were directly sputter coated with 8 nm AuPd and viewed at 3.0 and 10.0 kV.

For transmission electron microscopy (TEM) attachment disc samples were collected on ACLAR®-foil (Plano GmbH, Wetzlar, Germany), which is inert against the chemicals used in the TEM sample preparation process. Samples were fixed with 2.5% gluteraldehyde in PBS buffer and 1% osmium tetroxide, dehydrated in a series of ethanol solutions of increasing concentration and embedded in Epon resin. After Epon polymerization, the ACLAR®-foil was peeled off and a second layer of Epon was applied on the side where the foil was detached. 40 nm ultrathin sections were made with a Leica EM UC7 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany), mounted on copper grids and post stained with 1% uranyl acetate (20 min) and 2% lead citrate (7 min), rinsed in CO2 free aqua bi-dest, an observed in Tecnai G2 Spirit (FEI Corp., Hillsboro, USA).

9.4.4. Tensile tests

Glass slides (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) were cleaned by rinsing with acetone, ethanol and twice with distilled water and rubbed with KimWipe lab tissues. Some slides were bathed in 1% APTES ((3-aminopropyl)triethoxysilane) solution in a mixture of acetone and distilled water, rinsed three times with acetone and dried in an oven at 110°C for 1h. Other slides were exposed to vapours of DMDCS (Dichlorodimethylsilane) by placing them in sealed Petri dishes with droplets of concentrated DMDCS solution for one night and then rinsed with acetone several times until excessive, unbound DMDCS was removed. The contact angle of silan-treated and untreated glass slides was measured with DataPhysics OCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany) using 500 µl droplets of aqua bi-dest.

Freshly harvested *Nephila* attachment discs were tested by pulling on the upstream (last spun, previously directed towards the spider) dragline. Substrate slides were placed onto a lab boy and the dragline was fixed at a length of 10 mm onto the cantilever of a load cell force transducer

with 20 g force range (World Precision Instruments Inc., Sarasota, FL, USA) by means of a molten beeswax droplet. The force transducer was mounted on a micromanipulator (DC3001R with controller MS314, World Precision Instruments Inc., Sarasota, FL, USA), which provided constant (200µm/s) vertical movement. Force curves were recorded with AcqKnowledge 3.7.0 software (Biopac Systems Ltd, Goleta, CA, USA). Tension tests were simultaneously filmed using a Firefly pro GT 800 camera (Firefly Global, Belmont, USA) for the analysis of failure modes. The following failure modes were distinguished: (1) *dragline* failure (dragline brakes above or at the attachment disc), (2) *conjunction* failure (failure at the interface of the dragline-pyriform envelope), (3) *bridge* failure (breakage of the attachment disc above the substrate) and (4) *baseplate* failure (partial or total delamination of the attachment disc). Pull-off forces were taken as the highest force peaks measured during attachment disc pulling. In total, attachment discs of 8 individual spiders were tested with at least 3 attachment discs of each individual on each substrate. Data was analyzed using R software (R Core Development team, http://www.r-project.org/) whereby means of all attachment discs of the same individuals were taken as individual data points in comparative analysis.

Single tensile tests of attachment discs spun on APTES-treated glass slides were filmed with RICM-HSV (method described above) using 10x, 20x and 40x lenses and frame rates of 250, 2000 and 5000 fps.

Acknowledgements

We thank Prof. Jutta Schneider (University of Hamburg) for providing experimental animals. Theresa Gödel is acknowledged for characterizing the surface of substrates used in experiments by the means of atomic force microscopy. Thanks to Lars Heepe (University of Kiel) for constructive discussion and paper suggestions on adhesion physics. Victoria Kastner (Max Planck Institute for Developmental Biology, Tübingen) provided linguistic corrections of the manuscript. Two anonymous reviewers improved the manuscript by worthy comments and suggestions.

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Supplementary Material

SM.9.1. Comparison of the results of adhesion experiments on differently treated glass with those on Teflon. Characterization of test surfaces (water contact angle and mean roughness).

Material and Methods

Attachment disks of adult female Nephila senegalensis were harvested as described in the main manuscript. Tensile tests were performed as described in the main manuscript and Grawe et al., 2014.

Contact angle of silan-treated and untreated glass slides was measured with DataPhysics OCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany) using 500 µl droplets of aqua bi-dest.

Surface roughness was measured by means of Atomic force microscopy (AFM Typ NanoWizard, JPK Instruments AG, Berlin, Germany). The imaging and the force mapping was done with a Contact Cantilever (CONT, NanoWorld AG, Neuchâtel, Switzerland, resonance frequency 13 kHz, force constant 0.1862 N m-1). To characterize the scanning tip geometry the cantilever scanned a mica disk with colloidal gold particles (5.5, 9.3, 14.4 nm) (Pelco AFM Gold standard Kit Product No. 16205, TED Pella, Inc, Redding, CA). With the scanning probe image processing software (SPIP, Version 5.1.2, Image Metrology A/S, Hørsholm, Danemark) the radius (11 nm) was determied via blind tip reconstruction. Root mean squared surface roughness (RMS) was calculated with SPIP from 5x5 µm (glass slide) or 35x35 µm (PTFE foil) scans.

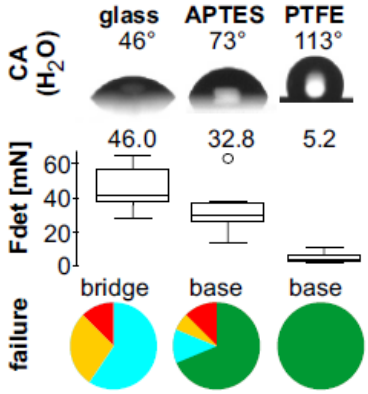
Results and Discussion

The PTFE foil used in previously published experiments (Grawe et al., 2014) exhibits a similar water contact angle like glass slides treated with DMDCS vapour.

The attachment forces of attachment discs spun on Teflon are significantly lower than on untreated and APTES treated glass, but still sufficient to hold the body weight of the spider.

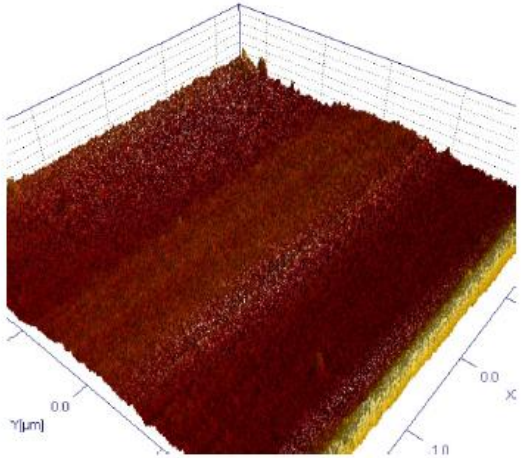
The dicrepancy with the results obtained on DMDCS treated glass (no application of attachment disc possible on this surface) might be explained by the difference in surface roughness.

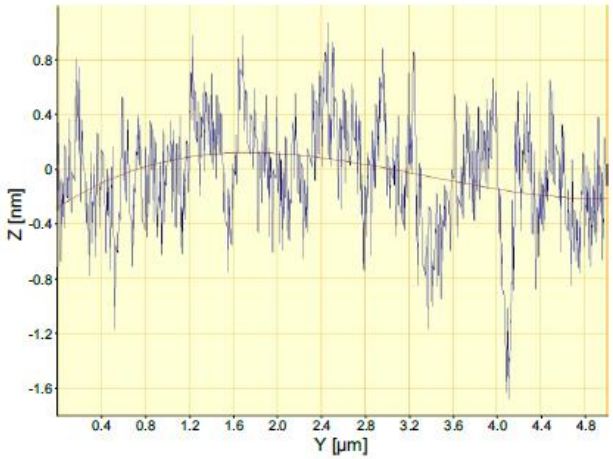
The PTFE foil exhibits a nano roughness, which may highly increase the contact area between the pyriform glue and the substrate. This is only possible if the pyriform glue has a low viscosity and surface tension in the moment of application.



Results of tensile tests of N. senegalensis attachment discs spun on untreated, silan-treated glass and PTFE (Teflon) foil resulted in different detachment forces and failure modes. Above, the contact angle (CA) measurement of water droplets on the substrates demonstrates their differences in hydrophilicity. Box plots give the median and variance of detachment force (Fdet) data. Numbers above box plots give the mean values (in brackets number of individuals tested). Pie charts in the bottom indicate the proportion of failure modes, whereby red symbolizes dragline failure, yellow conjunction failure, blue bridge failure and green baseplate failure (total delamination) (for details see Grawe et al., 2014)

Glass slide untreated

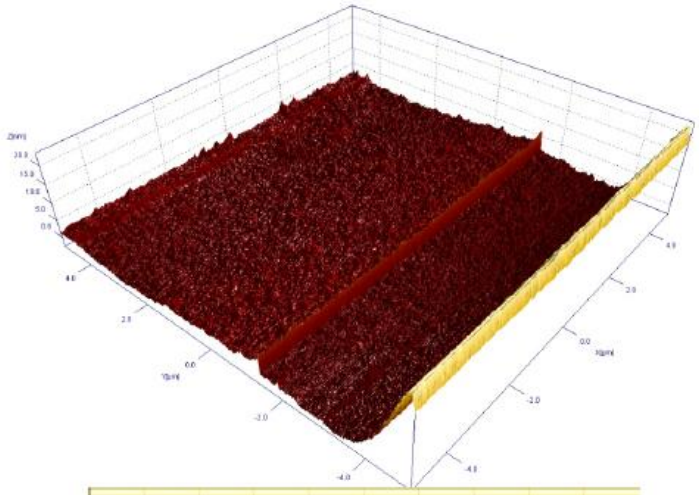


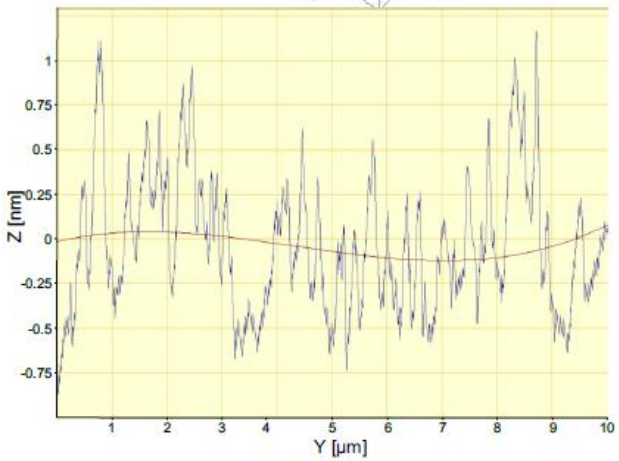


RMS 2.5 nm

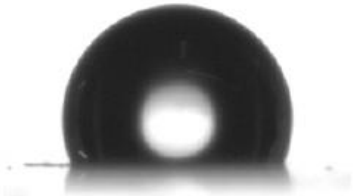
Mean CA 46°

Glass slide DMDCS treated



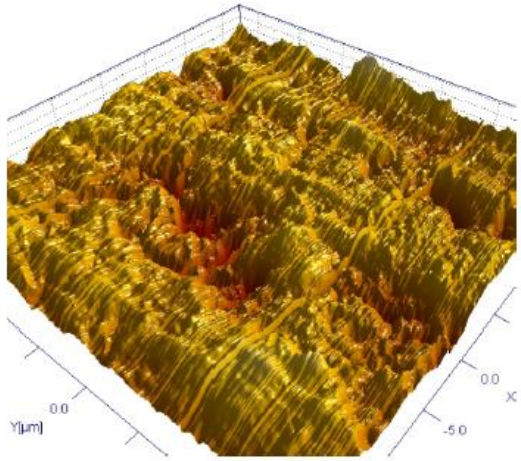


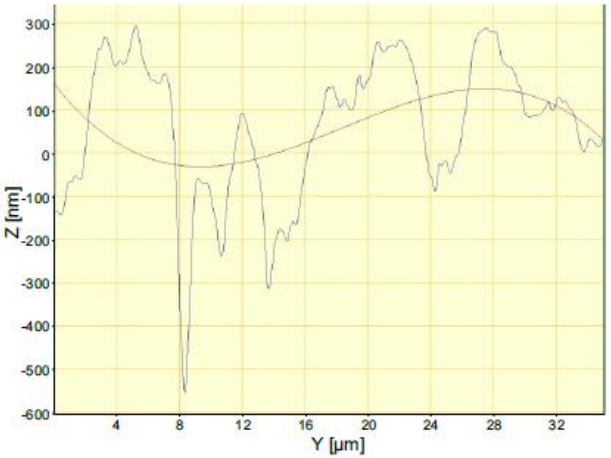
RMS 4.7 nm



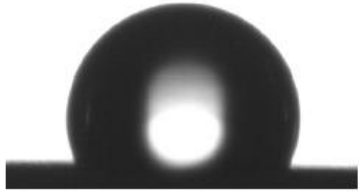
Mean CA 114 °

PTFE Foil





RMS 222.3 nm



Mean CA 113°

10. Discussion

10.1. Biological function and evolutionary trends of adhesive structures in arachnids

This survey elucidated the diversity of attachment devices and adhesive secretions in arachnids (see Tab.2.1). Some may be multi-functional, others serve very specific purposes. In the following I will discuss the factors that may have caused the evolution of attachment organs, and influenced their shaping. In order to understand the relationship between morphology and function, it should always be kept in mind that derived structures (apomorphies) can only evolve on the basis of precursor structures (plesiomorphies), which limits the possibilities of shaping (phylogenetic burden). Also the initial function of a structure may have been another than the actual one (functional shift). The comparison with analogies (homoplasies) in very distantly related taxa exhibiting a different body plan (like vertebrates), consisting of different biological materials, can help to understand the physical principles that favoured the evolution of a particular shape or property.

10.1.1. Ontogeny and the role of maternal care

In this study I found that in some arachnid orders showing maternal care attachment pads are present in the prenymphal instar (see ***chapter 3***). In these instances the attachment organs obviously specifically evolved to attach to the mother. Hence, this might be the primary evolutionary driving force for the occurrence of adhesive pads in these lineages. The structure of the prenymphal pads is rather simple. It is more or less a fluid filled bag with a non-sclerotized, softened cuticle. The softening of the membrane that contacts the substrate is achieved by a loosening of the cuticular material, presumably by the local lack of some matrix proteins. This seems not to demand lots of evolutionary steps, and may evolve rather quickly. Nonetheless the fibrillation seems to be a rather efficient principle, which is also frequently found in smooth adhesive foot pads of insects. At least, in the whip spiders the prenymphal pads can be regarded as the precursors of the locomotory pads of the subsequent free living instars. Hence, maternal care can start up the evolution of attachment structures.

10.1.2. Dispersal

Dispersal can be passive, by getting drifted by air or water currents. In these cases attachment is not demanded, except when reaching the new microhabitat site. Another strategy is phoresy, which means the transport with another, much larger animal. Athough this is a mode of passive dislocation it may demand some activity for a proper attachment. In many Acaridida and Gamasida there is a calyptostatic (pupa-like) deutonymph, the so-called hypopus, which attaches to phoretic hosts, in order to reach new habitats (Alberti and Coons, 1999). Various specific attachment organs have evolved for this purpose, like claspers, suckers or sticky secretions (Alberti and Coons, 1999). Phoresy is also known from pseudoscorpions, which attach to fly legs with means of their pedipalp chelae (Carl, 1994, Zeh and Zeh, 1992). In this case a multifunctional organ is used, which unlikely evolved in adaptation to phoresy. Depending on the size of the phoret the host may encounter significant costs due to an increased weight or drag, or even injury (Carl, 1994). Hence, counter-adaptations which reduce the attachment ability are expected if there is a frequent and/or specific phoretic relationship. This issue is highly overlooked in the study of

phoresy. The abundance of phoretic mites on honeybees is significantly reduced by grooming behaviour (Delfinado-Baker et al., 1992), but a structure-based reduction of the attachment ability is not known. In case of the pseudoscorpion-fly relationship, the fly tries to remove the phoret by rubbing its legs together, but it is unable to remove it by this means since the pseudoscorpion is then clamping even stronger (Carl, 1994).

10.1.3. Microhabitat choice and climbing demand

Adhesive foot pads are obviously used in locomotion, and associated with benefits and costs. Phylogenetic analyses with geckoes (Gamble et al., 2012), insects (Beutel and Gorb, 2001, Haas and Gorb, 2004) and spiders (Wolff et al., 2013) revealed that the evolution of adhesive foot pads, their gain and loss, is highly dynamic. Adhesive foot pads are usually intuitively thought to enhance the climbing ability of the animal, which however, is tested only in few exemplary studies in relation to the natural habitat and ecology of the animals. In some lizards (Elstrott and Irschick, 2004, Macrini et al., 2003, Zani, 2000), ants (Orivel et al., 2001), stick insects (Gottardo et al., 2015) and large ctenid spiders (Lapinski et al., 2015) a correlation between the inhabited stratum and the possession or size of adhesive pads was found. In lizards also a correlation between claw structure and perch height was found (Zani, 2000). Such observations are often explained with the climbing demand, which is assumed to be much higher in arboreal microhabitats. However, a plain relationship between arboreal living and the possession of adhesive pads or enlarged claws is no general rule. All examples above comprise relatively large animals, with the exception of ants. For most arachnids an adaptation of the attachment organs to the microhabitat is not as obvious. In hunting spiders, for instance, claw tufts are found both in arboreal species and ones that strictly live on the ground. Further, I found adhesive pads in species of whip spiders, pseudoscorpions and ricinuleids that have exceptionally been found at the ground. On the other hand there are numerous arboreal spiders that lack the adhesive foot pads, which is also true for most arboreal harvestmen and some climbing whip spiders. The analysis of hunting spider assemblages revealed that the relative abundance of pad-possessors increases with the height above the ground and the structuring of the micro-environment, which may indicate the differences in the ecological benefit of the pads (Wolff and Gorb, 2014). However, web building spiders extremely rarely have adhesive pads (Wolff et al., 2013), although being often dominant in above-ground habitats. This shows that the mode of living and the according amount and way of locomotion can play a much larger role in the evolution of locomotory attachment pads than the microhabitat niche. In order to understand the evolution of adhesive foot pads it would be necessary to study the functional role and efficacy of claws more in detail. Presumably, most natural surfaces provide enough possibilities for interlocking, but it may take time to find a sufficient foothold, and hence the risk of attachment failure might be higher when moving quickly. Therefore, it is possible that adhesive foot pads do not bring the benefit of a better general climbing ability (the ability to reach and dwell elevated sites), but the enhancement of surefootedness, hence permitting an increased climbing speed and manoeuvrability in structured microhabitats. This might be the more beneficial the higher the risk of being dislodged from the preferred site or caught by a predator, or, in the case of cursorial hunters, the faster the prey is.

Altogether, this shows that the evolution of locomotory attachment structures is ambiguous and may depend on different factors. Keeping this in mind, it seems to be even more difficult to estimate if the specific structure of the pads is adapted to the microhabitat surfaces. Natural surface structures are often very unpredictable and diverse in their properties, including

topography, surface energy and amount of adsorbed water, all of which highly influences adhesion. Hence, attachment structures should be rather universal and uniform than showing specifically adaptations. In spider hairy adhesive pads the structure and shape of the spatulate contact elements are very similar, despite of their independent evolution (Wolff et al., 2013). The tenent setae of acariform mites show very similar spatulae, which underlines their universality. Spatulae are the dominant contact structures in hairy adhesive pads, and hence believed to be the best solution for dynamic attachment (Varenberg et al., 2010, Gorb, 2001). However, their size and exact shape differs between the non-related insects, arachnids and lizards. This was explained with either the contact splitting theory (Arzt et al., 2003) or the phylogenetic burden (Peattie and Full, 2007), but not with specific adaptations to microhabitat surfaces. In spiders the spatulae are rather similar, but the size and shape of the tenent setae differs significantly between species, which is best explained by the phylogenetic history, neither the ecological niche nor body size (Wolff et al., 2013).

Nonetheless, at least in very small arthropods with narrow niches, the observation of direct adaptation of the pretarsal attachment structures to the micro-environment seems probable. Based on their observations on intertidal mites Pugh et al. (1987) argued that the pretarsal attachment structures are highly adaptive and that their structure reflects their ecological niche. In oribatids the attachment is primarily carried out with claws (Heethoff and Koerner, 2007), but species found in the canopy of rain forests exhibit arolia in the hind legs, which is presumed as an essential adaptation to settle on the tree leaves (Walter and Behan-Pelletier, 1993). In specialized herbivores and parasites, both of which in arachnids are only found among mites, adaptation of the attachment structures is most possible, as in these cases the substrates for attachment are specific and less variable. Since these substrates are of organisms, which can equally evolve and have a direct ecological relationship to the attaching animal, there might be an antagonistic co-evolution (see next sections).

10.1.4. The role of herbivore-plant interaction

Despite of some clades of mites arachnids are not herbivorous, but they are confronted with the evolutionary dynamics between herbivorous insects and plants either. Many plants exhibit anti-adhesive waxy surfaces that may prevent the attachment of herbivorous insects or their eggs (Eigenbrode, 2004, Whitney and Federle, 2013, Whitney et al., 2009, Gorb et al., 2008). Arachnids that settle on plants may have to enhance the efficacy of their adhesive devices to keep a sufficient safety factor. Spiders use attachment discs of pyriform silk to attach their safety lines and webs to substrates. In ***chapter 8*** we have shown that the adhesion of the attachment disc is reduced on leaf surfaces if compared with a smooth glass surface. The arising question is if spiders can sense the strength of their attachment discs and either choose the best attachment sites or accordingly adjust the size of the attachment discs. In *Eustala* spp. (Araneidae) it was found that dried dead plant stems were preferred as web attachment sites, which was explained with better camouflage (da Silva Souza et al., 2015). Dead or old leaves or stems often exhibit a reduced or damaged wax layer and an increased roughness by senescence (Neinhuis and Barthlott, 1998, Mechaber et al., 1996), which may facilitate gluing onto these surfaces. However, if the choice of foraging or web attachment sites is influenced by the adhesive capacity of the substrate surfaces has not been scope of a study yet. Nonetheless, it can be assumed that there is a high selective pressure on the glue efficacy. Studies on beetle eggs that are glued on the usually highly repellent waxy surface of *Asparagus officinalis* came to the conclusion that the ability of the glue

to wet the hydrophobic wax crystals is the key for the successful cementation of the eggs (Voigt and Gorb, 2010). In spider attachment discs we found that the wax crystal partly contaminated the pyriform glue leading to a reduction of efficacy, but the hierarchical structure of the attachment disc reduces the local stress on the cementation, which increases the overall attachment performance (see ***chapter 9***). This may be an adaptation to enhance the security of the thread anchors despite of the strong reduction of adhesion.

10.1.5. The role of predator-prey interaction

Most arachnids are predatory. Predation demands morphological, physiological and behavioural means of grasping and arresting prey. This survey demonstrates the frequent evolution of sticking devices or secretions specifically for prey capture. Raptorial appendages are present in various clades and obvious adaptations to catch prey. In spiders the evolution of hairy adhesive pads highly correlates with the loss of prey capture web building behaviour (Wolff et al., 2013). We have hypothesized that the locomotory pads, the claw tufts, are secondary developments, and hence the prey capture function was the initial selective benefit that lead to the evolution of the pads in spiders (Wolff et al., 2013). The pedipalpal smooth adhesive pads of solifuges are important means of prey capture, but also serve other functions, like climbing. However, it can be assumed that prey capture is the primary function of these organs as climbing is rarely performed in these soil dwelling organisms. In specific predator-prey relationships there is a great selective pressure on the prey, forcing the evolution of measures against being caught. Previous authors discussed the role of detachable setae or fluid coatings against the entrapment in spider webs (Opell and Schwend, 2007, Opell, 1994, Nentwig, 1982). As a counter-adaptation against prey loss some spiders evolved unique behaviours, like the release of elastic energy in a web that is hold pre-stressed (Uloboridae: *Hyptiotes*) (Marples and Marples, 1937), net-casting (Deinopidae: *Deinopis*) (Getty and Coyle, 1996) or the fishing with a single line (Araneidae: *Mastophora*) (Eberhard, 1980). In ***chapter 6*** and ***7*** we discussed the frequent use of glue in soil living predators for catching springtails, and the evolution of counter measures of the prey. Epigaeic springtails have evolved easily detachable scale-like setae, presumably as an anti-trapping device. Nemastomatid and sabaconid harvestmen have evolved hyper-bendable segments for clamping to compensate the loss of gluing efficacy (see ***chapter 7***). This shows that adhesion can play an important role in an ‘arms-race’ like evolution of prey and predator.

However, arachnids are also hunted themselves. Attachment and gluing can be effectively used to deter or immobilize predators (Betz and Kölsch, 2004), to fasten to the ground to avoid being picked up, or for camouflage. Some spiders (Theridiidae and Pholcidae) use sticky threads for defence (Vetter, 1980, Japyassú and Macagnan, 2004). However these probably did not specifically evolve for this purpose but for prey immobilization. However some structures or secretions specifically serve camouflage by catching soil particles, which has independently evolved in some spiders, harvestmen and mites.

10.1.6. The role of parasite host interaction

Parasites have an increased demand of attachment, since their life highly depends on their presence on a specific host. The surfaces of attachment may highly differ between host taxa, but may be uniform enough to evolve specific adaptations to attach to these surfaces. In astigmatid mites (Sarcoptiformes) the parasitic or phoretic deutonymphs show different types of attachment organs, which are related to the type of host: those that attach to arthropods have arolia, those that

attach to mammalian hair have reduced arolia but claw-like setae, and those that burrow into the skin or live in hair follicles have all attachment structures reduced (OConnor, 1982, Atyeo, 1979). The rules of parasite-host co-evolution would assume the evolution of counter-measures against parasite attachment in the hosts. However this might be limited as the parasites attach to multifunctional surfaces or structures, like cuticle, hair or skin, which may not be altered without the impairment of other functions. I could not find any published study results showing evidence for a parasite-host co-evolution of attachment and anti-attachment.

10.1.7. The role of sexual selection

Females may favour short copulations with multiple partners to enhance sperm competition (cryptic female choice) (Arnqvist and Nilsson, 2000, Parker, 1970, Thornhill and Alcock, 1983). In spiders and insects it was shown that females try to interrupt copulation, whereas males increase their reproductive success with the duration of copulation (Schneider et al., 2000, Elgar et al., 2000, Thornhill and Alcock, 1983). Hence, males may have a great benefit if they, or at least the copulatory structure, can strongly attach to the female. In insects also frequently mate guarding occurs as a strategy to secure paternity and avoid sperm concurrence (Alcock, 1994). In these cases the males often have claspers to stay attached to the females genitals (Gorb, 2001). In male leaf beetles the tarsal attachment pads exhibit specific setae that can generate higher adhesive forces on smooth surfaces, which is important to cling to the female's elytra (Pelletier and Smilowitz, 1987). In water beetles males attach to the female elytra via suction cups (Aiken and Khan, 1992). Suction cups are rather susceptible to irregularities of the target surface. Interestingly, the female elytra exhibits a fine surface roughness, which reduces the efficacy of suction cups (Miller, 2003, Green et al., 2013). Hence, it was assumed that this is a indication of the sexual conflict, which leads to a male-female co-evolution in an arms-race manner (Miller, 2003, Bergsten and Miller, 2007). Interestingly, likewise in acaridid mites males attach to the female by suction cups, but, in contrast, the female seems to *improve* the attachability of the male in this case. The females often exhibit conspicuously smooth surfaces at the attachment site, and in some mites even the immature female possesses tubercles that perfectly fit into the modified tube-like suckers of the males (Witaliński et al., 1992). This contradicts the sexual conflict hypothesis, although also in females of acaridid mites multiple copulations frequently occur and are obviously advantageous (Witaliński et al., 1992). The reason for this phenomenon may lie in the parasitic habit of these species, which means low densities of potential mating partners (Witaliński et al., 1992). If the period of receptivity of the female is short and the chance of male encounter is low it is beneficial to bind *any* found partner already before maturity, to ensure its presence when the eggs have to be fertilized (Witaliński et al., 1992). In general, specific attachment structures of males to attach to females, like clamping legs, are strikingly frequent among parasitic mites, and the attachment often already takes place before the maturity of the female. The abundance of mating partners may thus play an important role in the evolution of mating related attachment devices.

In the case of lock-and-key systems, like in entelegyne spiders, there might be an antagonistic co-evolution of the attachment ability. It is an exclusive mechanical interaction as the copulatory structures usually do not bear sensory structures ('numb genitalia') (Eberhard and Huber, 2010). It is barely possible to separate entelegyne spiders in copula without injuring the male (pers. observation on Gnaphosidae), which underlines the strength of the attachment. However, in females selection may favour a reduction of the attachment ability and hence an

alteration of the structures the male structures interlock with in order to be able to interrupt and control the mating duration. This would increase the selective pressure on the interlocking ability of male pedipalpal structures. This hypothesis to explain the often very high complexity of pedipalps and vulvas is highly neglected and should be studied more intensely.

Spermatophores also often exhibit a complex capsule with protuberances. These structures are highly variable and species specific, which leads to the presumption of a lock-and-key mechanism (Weygoldt, 2000). However, the external genitals of the females are often rather simple and non-sclerotized (Peretti, 2004). Hence, the structure may be shaped by mate choice in the way that females use the tactical cues given by the spermatophore structure to 'assess' the quality of the male (Peretti, 2004). In a broad comparative approach including both insects and arachnids it was found that spermatophore complexity generally cannot be explained with a lock-and-key mechanism, but might rather be shaped by female choice and the demand of protection of the sperm against environmental impacts (Proctor et al., 1995). Though, some of the protuberances often play a role in interlocking with the female genital tract, in order to increase the duration of sperm transfer (Jacob et al., 2004, Weygoldt, 2000). This part of the interaction may be under the influence of sexual conflict and thus shaped by sexual selection.

Another strategy of males in securing paternity is the production of mating plugs, which seal the female genital opening (Uhl et al., 2010, Kuntner et al., 2009, Parker, 1970). In contrast to insects, this is much more common in arachnids than mate guarding. It was shown that the efficacy of these plugs varies (Herberstein et al., 2012), which may be due to an antagonistic co-evolution of females and males. However, structures or surface coatings that reduce the attachment ability of the plugs, which should consequently evolve then, have not been found yet. This may be explained by the frequent research focus on the male genitals alone (Ah-King et al., 2014). In some cases the plugs are produced by the females, even during copulation (Aisenberg and Barrantes, 2011). The underlying biological function of this is not entirely clear, but it is assumed that the female by this means ensures the paternity of her offspring by the partner of her choice. This implicates that a rigorous mate choice is made before the copulation.

These examples illustrate, that attachment structures and glue are frequent means of mate choice and securing paternity and can have specifically evolved under the pressure of sexual selection and sexual conflict.

10.2. Analogies and comparative mechanics

10.2.1. Claw and spines

Claws are the most ubiquitous attachment devices, and equally found in arthropods, vertebrates and some annelids. The shape and material of the claws may vary, but the basic structure is strikingly similar. The base is broad and flexibly hinged to absorb stress and provide certain resistance of the attachment during movement. The shaft is curved ventrally, which enhances interlocking capabilities by exposing the tip to cavities on corrugated surfaces. The tip is highly pointed to cover a broad range of interlocking possibilities.

Spines to interlock with surface asperities or crevices are very widespread among arthropods and important supports of the claw increasing the possibilities of interlocking with small scale surface asperities. In arachnids the spines are also of high importance for prey capture. In spiders they can highly change their angle permitting an activation-deactivation mechanism of the capture basket. As the long spines could hinder the locomotion in a structured terrain this might be very beneficial. The mechanism is based on the hydraulic locomotion principle of arachnids. Capture baskets build by the spinated walking legs are also known from other insects, like dragonflies (Gorb, 1999), but in these the spines are always erected. Specialized raptorial pedipalps or legs independently evolved in the Pedipalpi (Uropygi+Amblypygi), Laniatores (Opiliones) and some mites. Similar raptorial legs also evolved in some insects, like mantids or mantis flies (Ass, 1973, Frantsevich and Wang, 2009). A strikingly similar feature is the distally directed tilting of the spines. At first glance this seems counter intuitive, because a proximal tilting would represent a barbed leg, whose grip can hardly be escaped. Presumably, the release of a struggling prey item from this grip would be more difficult, which would be critical if the prey turns out to be harmful. Hence, for a predator it is important to control the attachment to the prey to reduce the risk of getting injured. This may also explain, why the scopula setae of spiders are orientated in a manner that they produce the highest friction when sheared towards the body, which puzzled previous authors (Niederegger and Gorb, 2006).

10.2.2. Adhesive foot pads

Lots of natural surfaces, especially those of plants, exhibit a regular surface roughness in very small length scales, too small to provide sufficient hold for claws or spines. Consequently, adhesive pads that can generate strong friction and adhesion on smooth and micro-rough surfaces have evolved for multiple times among terrestrial animals, often with striking morphological and functional similarity (Federle, 2006, Arzt et al., 2003, Gorb and Beutel, 2001). The mechanics of the foot pads may differ between insects and spiders due to the differences in pretarsal anatomy and muscular control. Further, the number of walking legs differs between insects and arachnids, but also between different arachnid orders. Nonetheless, very similar structures have evolved assuming a similar mechanical and physical function.

Hairy adhesive pads are equipped with spatulate structures, like in insects and geckoes. The tenent setae of spiders and trombidiform mites are branched and equipped with small spatulae, very similar to geckoes. In insects branching of the tenent setae does not occur and the spatular dimensions are much larger. A fluid secretion into the contact is essential to generate a significant contact with the substrate, because the large spatulae are not compliant enough to generate such a close contact with the substrate that van der Waals forces get effective.

Comparable tenent setae are present in some laniatorid harvestmen and ricinuleids, and the similarity suggests, that the setae in these arachnids are also fluid mediated.

The smooth adhesive pads of most arachnids obtain their compliancy by a fibrillation of the underlying cuticle, just like in many insects. In the analogue pads similarly the fibrils do not run perpendicular to the epicuticle, but are tilted distally. This was assumed to be important for an anisotropy of friction generation (Gorb, 2007, Bullock et al., 2008), but may also simply be a result of the cuticular formation, as the fibrils originate from horizontally stacked cuticular layers.

In many highly specialized ectoparasitic mites the arolia are in the shape of large discs with the ambulacral stalk medially attaching, e.g. in *Chirodiscoides caviae* (Alberti and Coons, 1999). Such mushroom-like contact elements also occur in male leaf beetles, were they serve the attachment to the smooth elytrae of females (Pelletier and Smilowitz, 1987). It was shown, that mushroom-like shaped contact elements may produce significantly higher adhesive forces than spatulae (Spolenak et al., 2005), because they permit a uniform stress distribution and hence high resistance, when pulled perpendicular (Carbone et al., 2011, Heepe and Gorb, 2014, Gorb et al., 2007). This might be beneficial for a durable, but not a fast dynamic attachment. This is supported by the finding that in arachnids these contact elements are only found in parasites and some secreted attachment structures (see ***10.2.4.***).

Insects that exhibit both tarsal and pretarsal adhesive pads may bring some of the pads in contact only if additional safety is required, e.g. during wind gusts or when walking on anti-adhesive surfaces, a movement which can be performed by rapid preflexes (Eberhard et al., 2009, Endlein and Federle, 2013). In the case of the anystid mites, which exhibit both hairy and smooth pads in different leg portions, I could not find a similar behaviour. Both pads were equally used when walking horizontally, vertically or upside down. Hence, the function of this unusual configuration remains unclear.

10.2.3. Dry or wet adhesion?

It was previously presumed that a thin homogeneous fluid film between the contact element and the substrate is essential for good adhesion in all kinds of smooth adhesive foot pads (Drechsler and Federle, 2006, Barnes, 2007) and in hairy adhesive foot pads consisting of un-branched setae with spatulae too large to sufficiently adapt to the micro- and nano-roughness of a substrate (Gorb et al., 2010, Dirks et al., 2009). Occasionally larger amounts of secretion emergence have been observed, which is attributed to the removal of contaminating dirt particles (Clemente et al., 2010). In hairy pads with a high density of very small spatulae, such as in geckoes and spiders, it was assumed that fluids barely play a role and the adhesion is primarily attributed to van-der-Waals forces (dry adhesion) (Autumn et al., 2002, Kesel et al., 2003). This means that the structure alone is responsible for the good adhesion, which renders them most interesting for biomimetic applications (Autumn and Gravish, 2008). However, this issue is still controversially discussed, as traces of secretions have been found on the spatulae of geckoes using very sensitive methods (Hsu et al., 2011). It was thus assumed that structural adhesive pads always imply some kind of secretion, but in very small spatulae the amounts are usually too small to be seen. Though, at nanoscale adsorbed water molecules, which are usually present on natural surfaces, even in a dry environment, have a considerable effect on adhesion (Jang et al., 2004, Asay and Kim, 2006). Experiments with geckoes and spiders show an effect of humidity on pad adhesion, which may be attributed to the amount of adsorbed water and hence a capillary effect (Niewiarowski et al., 2008, Huber et al., 2005, Wolff and Gorb, 2012b), as proposed by previous

authors (Homann, 1957, Roscoe and Walker, 1991). Water may also be absorbed by the contact element softening its material and leading to higher compliancy (Prowse et al., 2011).

For the first time Peattie et al. (2011) comparatively studied the adhesion of different arachnids to glass surfaces, revealing the occasional presence of fluid secretions. It remained unknown, where those secretions came from and if they are functionally important for adhesion. The data presented for spiders show an extreme wetting of the setae, such that the spatulae are submerged. Adhesion models show that in a hairy pads a secretion only enhances adhesion if it only wets the space between the spatula and the substrate (Huber et al., 2005). If the fluid film is thicker than the spatula, slipping occurs (Wolff and Gorb, 2012b). Hence the observed secretion could rather serve detachment or the cleaning of the setae. Further, lipidoid secretions are even more present in web-building spiders, such as orb-web spiders (Araneidae) (Kropf et al., 2012), cob-web spiders (Theridiidae) and cellar spiders (Pholcidae) (own observation), all of which do neither have scopulae nor claw tufts. In those the secretion prevents getting stuck in the own web (Briceño and Eberhard, 2012, Kropf et al., 2012). This shows that the foot secretions are not necessarily correlated with the presence of an adhesive pad.

In the smooth arolia of pseudoscorpions, scorpion and whip scorpion nymphs, ticks and anystid mites small amounts of lipoid fluids were often left behind, which underlines the importance of fluids for the function of smooth adhesive pads. In comparison with water or many water-based solutions lipids have the advantage of much lower evaporation rates and a good wetting behavior of hydrophobic surfaces, which are widespread among plants and animals. In the case of the prenymphal arolia the substrate is highly specific; hence the fluid properties might be adapted to the surface chemistry of the mother's cuticle.

In amblypygids, which possess spatulae we found that the pad is functional in both a wet and a dry mode (see ***chapter 4***). This is the first known case of an arolium working with dry adhesion, which is attributed to the unique microstructure. We could not directly relate a variation of the attachment strength to the amount of the secretion, so it remains unclear if the secretion enhances or reduces adhesion.

10.2.4. Glue

Strong, quickly drying glues conglutinating natural surfaces are widespread among animals and base on diverse substances. Studies on biological glue focus on some model organisms, like barnacles, hence the mechanisms to create strong bonds with biological substances are only exemplarily known. A key role might be the switch from an initial low cohesion and good, universal wetting behaviour to the eventual high cohesion and toughness. This may be achieved by very different ways like polymerization, evaporation of a solvent or non-Newtonian fluid properties.

The anal pedicels of Uropodina mites are remarkably analogous to the byssus threads of mussels used for an extremely robust anchorage on substrates, however, with the difference that mussels use a radial network of such threads (Qin and Buehler, 2013, Smeathers and Vincent, 1979). A combination of long, tough fibrils and a compliant homogeneous cement material may be the best solution to stay attached despite of high stress impacts. The mushroom shaped structure of the adhesive disc is very beneficial for a durable attachment, as I discussed above. Other secretory products with a comparable structure and mechanical function are the spermatophores, found in various arachnids, and equally in insects (Davey, 1960). The difference to the pedicels and byssus threads is that the stalk must be stiffer to provide an upright position. A

joint-like constriction underneath the sperm carrying apical section reduces the stress working on the adhesive disc. These analogies underline the importance of mushroom-like structures for the creation of a durable attachment.

The use of viscoelastic substances for prey capture seems to be widespread. The prey capture glue of nemastomatid harvestmen is a viscoelastic fluid (see ***chapter 6***) and the viscid glue of spider capture threads recruits its adhesion from glycoproteins behaving like viscoelastic solids (Sahni et al., 2010). The benefit of viscoelastic substances is their shear thickening behaviour, which means initial low viscosity and fast spreading on the prey surface, but the generation of high cohesive forces, when the prey is struggling wildly. Hence, it is not surprising that prey capture secretions of some insects (Betz and Kölsch, 2004) and carnivorous plants (Erni et al., 2011, Gaume and Forterre, 2007) exhibit similar properties.

10.3. Noteworthy unique features and their potential for biomimetics

10.3.1. Transverse-oval arolium

A transverse strip-like contact area of the arolium seems to be a special feature of arachnids. Such geometry of smooth adhesive pads is not common in insects. In arachnids it independently evolved three times: in whip spiders, pseudoscorpions, and anystid mites. This shape may permit a high anisotropy of friction and adhesion and enable a rapid switch between stress distribution when forces act perpendicularly and stress concentration by bi-lateral peeling. This principle could be used for the design of adhesive grippers, e.g. for fabrication or robotics. In order to transport large panes or sheets usually suction cups are used, which, however, may deform the material due to the generated local pressure difference. Soft, fluid mediated pads may produce much lower stress on the material. Narrow broad pads could carry components with smooth surfaces by generating high friction, and release them by a transverse peeling mechanism.

10.3.2. Micro-patterned arolium

The development of micro patterned dry adhesive tapes is the primary aim of biomimetic studies on the hairy adhesive pads of geckoes, insects and spiders. The main problems that limit the direct transfer of found structural principles on technical solutions are different application demands, the upscaling to human relevant sizes and the restrictions of fabrication processes. The most successful innovation is an adhesive tape based on mushroom shaped microstructures inspired by the modified tenent setae of male leaf beetles (Gorb et al., 2007). This material can produce high adhesive forces, when the force acts perpendicular, but is sensitive to substrate roughness (Kasem and Varenberg, 2013) and pull-off angles below 45° (Heepe et al., 2014). Because the microstructures bend away from the surface during sliding (Varenberg and Gorb, 2007) it is also not very useful for friction generation. Spatulate structures are assumed to be more effective at low pull-off angles and can generate anisotropic high friction forces when sheared over smooth surfaces. Further, they can better adapt to rough surfaces. The main problem is the production of flexible spatulate pillars structures, which is hard to achieve at microscale. The spatulate hexagonal structures found in amblypygids are unique among attachment devices in animals studied so far. We found, that they can produce high adhesive forces and may also have a drainage effect, which enhances the efficacy on wet surfaces (see ***chapter 4***). Similar microstructures may be easier to produce with polymers than seta-like ones and could be used in applications that demand high friction generation on smooth and wet surfaces.

10.3.3. Prehensile feet of harvestmen

The tarsal flexor system of the long-legged harvestmen already fascinated scientists more than hundred years ago, because the control and mechanism is simple, but the efficacy is high. In insects, and some other arachnids, like solifuges or scorpions, the tarsi are also subsegmented, but never in such an excessive extend that they can be used as prehensile organs. Further, in contrast to harvestmen in insects the tarsi bend upwards, which can be an important mechanism to peel off the adhesive pads (Bullock et al., 2008). Hence, the prehensile tarsi of harvestmen represent a unique system, which can effectively attach without the necessity of adhesive structures. This principle might be interesting for the design of grippers for long objects, like cables, poles or pillars. It might also be used for tree climbing equipment.

10.3.4. Attachment discs

The spider pyriform silk, investigated here in detail (***chapter 6*** and ***7***) show a remarkable hierarchical structure, which is not known in that extend from other biological glues. In artificial materials the embedding of flexible fibers was found as an effective means to increase the toughness of brittle materials, like concrete (Li, 2003). For adhesives this principle is to the best of my knowledge not applied yet. The hierarchical structure of attachment discs may further teach lessons how to apply a limited amount of glue best to achieve the highest bonding strength, even on hardly wettable surfaces. Future research on the exact composition of the pyriform glue and its material alteration during the quick drying process may lead to novel sustainable and strong adhesives.

Further, the investigation of spider spigots could lead to innovations that enhance the efficacy of glue nozzles. The pyriform spigots are remarkably clean and adhering remains of dried secretion, which could plug the gland opening, are seldom found. The study of the structural, chemical and physical principles that act to prevent glue contamination is thus very promising.

10.3.5. Harvestmen glue and droplet bearing setae

The fast spreading and good adhesion of nemastomatid glue to the Lotus-like surfaces of springtails are quite fascinating. The fluid switches between low and high viscosity extremely quickly. Studying the macro-molecular properties of the fluid may provide insights into mechanisms that can enhance the wettability of glues or paints on microstructured and repellent surfaces.

The anchorage of a fluid glue droplet onto the secretory seta with microtrichia independently evolved in Palpatores harvestmen and mesostigmatid mites. This principle could be interesting for droplet catching devices, e.g. for gluing with minute amounts in microfabrication, or for printing technologies. Maybe this principle is also an opportunity to influence the contact angle of fluids without adding surfactants, because the embedded microtrichia can locally deform the droplet.

10.3.6. Minute suction cups

Among reversible adhesive structures, suckers can generate much higher attachment forces than smooth and hairy pads, but this is only true at macro scale (Spolenak et al., 2005). Previous theoretical models indicated that the function and thus evolution of suction cups with a diameter smaller than 10 µm is unlikely (Spolenak et al., 2005). Further, suckers are much more effective in a fluid or even viscous medium than in air, and are quite sensitive to roughness of the target surface. Keeping this in mind, the occurrence of minute suction cups in terrestrial acaridid mites is quite astonishing. One would assume that smooth or hairy adhesive pads that, in contrast, *increase* their efficacy at small scale would be a much better solution. The acaridid suckers are close to the estimated limit, hence, the exact physics of these structures are ambiguous. Studies on the material properties and biomechanics of these minute suction cups could bring new insights in potential means of efficacy enhancement of suction cups at small scale, which might be of high interest for micro-fabrication and micro-robotics. The great advantage of such micro-suckers in contrast to stickers is the controllability of the adhesive force by the elevation or depression of the sucker plate.

11. Conclusion

Arachnids show a high diversity of attachment structures and sticky secretions. Some of them are strikingly analogue to structures of other animals, like claws, spatulate setae, smooth adhesive pads with internal fibrillation, or viscoelastic prey capture fluids. This reveals the evolutionary optimal solutions for similar demands like surface attachment. On the other hand this study revealed some very unique and rather unknown structures that may be regarded as key innovations, leading to great ecological success and diversification, like the pyriform glue of spiders or the pedipalpal secretory setae in harvestman. I found unique configurations of smooth adhesive foot pads, like the transverse-oval arolium, which independently evolved three times among arachnids, as well as the first arolium that can produce a high adhesive strength even in the absence of mediating fluids due to spatulate hexagonal microstructures, and the perhaps most simple adhesive pad in scorpion prenymphs that can be controlled by an antagonistic work of a muscle and internal pressure. These discoveries may be of high relevance for our understanding of the function and evolution of attachment devices, as well as for arachnological and entomological research. The findings may also be a source of inspiration for biomimetic approaches and demonstrate that the high neglecting of arachnids in biomechanic and biomimetic research is unjustified.

12. References

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Eidesstattliche Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation abgesehen von der Beratung durch den Betreuer nach Inhalt und Form meine eigene Arbeit ist und alle benutzen Quellen angegeben sind.
Diese Arbeit wurde weder ganz noch zum Teil im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt. Die wesentlichen Teile der Arbeit sind in Fachzeitschriften veröffentlicht.
Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden.

Kiel, _____

Jonas Wolff