

Functional principles of steerable multi-element probes in insects

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ABSTRACT

Hemipterans, mosquitoes, and parasitic wasps probe in a variety of substrates to find hosts for their larvae or food sources. Probes capable of sensing and precise steering enable insects to navigate through solid substrates without visual information and to reach targets that are hidden deep inside the substrate. The probes belong to non-related taxa and originate from abdominal structures (wasps) or mouthparts (hemipterans and mosquitoes), but nevertheless share several morphological characteristics. Although the transport function clearly differs (egg laying and acquisition of liquid food), the functional demands on the mechanical behaviour of the probe within the substrate tend to be similar. The probe needs to be thin to limit substrate deformation, and long, in order to attain substantial path lengths or depths. We linked the morphology across taxa to the different functional requirements, to provide insights into the biology of probing insects and the evolution of their probes.

Current knowledge of insect probes is spread over many taxa, which offers the possibility to derive general characteristics of insect probing. Buckling during initial puncturing is limited by external support mechanisms. The probe itself consist of multiple (3–6) parts capable of sliding along one another. This multi-part construction presumably enables advancement and precise three-dimensional steering of the probe through the substrate with very low net external pushing forces, preventing buckling during substrate penetration. From a mechanical viewpoint, a minimum of three elements is required for 3D steering and volumetric exploration, as realised in the ovipositors of wasps. More elements, such as in six-element probes of mosquitoes, may enhance friction in soft substrates. Alternatively, additional elements can have functions other than ‘drilling’, such as saliva injection in mosquitoes.

Despite the gross similarities, probes show differences in their cross sections, tip morphologies, relative lengths of their elements, and the shape of their interconnections. The hypothesis is that the probe morphology is influenced by the substrate properties, which are mostly unknown. Correlating the observed diversity to substrate-specific functional demands is therefore currently impossible.

We conclude that a multipart probe with sliding elements is highly effective for volumetric substrate probing. Shared functional demands have led to an evolutionary convergence of slender multi-element probes in disparate insect taxa. To fully understand 3D probing, it is necessary to study the sensory and material properties, as well as the detailed kinematics and dynamics of the various probes in relation to the nature of the selective pressure originating from the species-specific substrates. Such knowledge will deepen our understanding of probing mechanisms and may support the development of slender, bio-inspired probes.

Key words: spatial probing, multi-element probes, steering, parasitic wasps, hemipterans, mosquitoes, buckling avoidance.

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I. INTRODUCTION

Several insect taxa such as parasitic wasps (Hymenoptera), true bugs (Hemiptera), mosquitoes and flies (Diptera), and butterflies (Lepidoptera) include effective probers, which can find hosts for their larvae or food sources hidden in various substrates. The probes of these insects allow the delivery of eggs or fluids, such as venom, and/or the withdrawal of fluids, such as phloem sap and blood. Butterflies and flies that probe in solid substrates generally have rather short and stiff probes, with limited insertion depth (Krenn, 2010; Karolyi *et al.*, 2014). The presence of musculature inside the probe in butterflies (Krenn, 2010) presumably limits the length/width ratio (l/w), also called slenderness. This review focusses on slender probes that are generally present in the parasitic wasps, true bugs, and mosquitoes (Diptera: Culicidae). Parasitic wasps probe into a plethora of substrates, such as fruits (Ovruski *et al.*, 2000), galls (Ghara, Kundanati & Borges, 2011), and tree trunks (Heatwole, Davis & Wenner, 1962), in search for host larvae. Hemipterans target various plant tissues, including phloem and xylem vessels (Raven, 1983), and mosquitoes generally search for blood vessels in the dermis and hypodermis of vertebrates (Takken &

Verhulst, 2013). The highest ratios are found in species that need to probe deeply, such as the wood-probing wasp *Megarhyssa atrata* (Fabricius) ($l/w \approx 260$; Le Lannic & Nénon, 1999), and in xylem-feeding hemipterans, such as *Stomaphis graffi* (Cholodkovsky) ($l/w \approx 460$; Brožek *et al.*, 2015).

The probing behaviour of insects raises several questions. How can these animals insert and advance their slender probes into solid and tough substrates with no or minimal damage to the probe? How do they accurately steer their probes towards a target without visual cues? Answers to these questions may offer insight into the life-history traits or life cycles of pests and parasitoids, and could help to improve drilling tools for medical and engineering applications.

Insect probes usually consist of multiple slender elements that are connected along their length but can slide along each other. The egg-laying structures (ovipositors) of parasitic wasps generally consist of three such elements (Quicke *et al.*, 1994), the mouthparts of hemipterans consist of four elements (Labandeira, 1997), and those of mosquitoes contain six elements (Robinson, 1939). These probes are thought to have facilitated strong adaptive radiation of parasitic wasps and hemipterans, enabling the exploitation of a wide variety of niches (Goodchild, 1966; Sharkey, 2007). Hymenoptera

is one of the most numerous insect orders, with about 145000 recognised species (Huber, 2009; Vilhelmsen & Turrisi, 2011). For the order Hemiptera, about 82000 species have so far been described (Brambila & Hodges, 2008). The mosquito family Culicidae (Diptera), contains ‘only’ about 3200 recognised species (Foster & Walker, 2002). Although feeding on soft-bodied invertebrates occurs to some degree (George *et al.*, 2014), mosquitoes primarily obtain their blood meals from vertebrate skin, a substrate that is similar across species. This may be a reason why diversification within mosquitoes is limited.

We review the relationship between structure and function of multiple-element insect probes in terms of their mechanical and control properties. We discuss the capabilities of these probes in light of their functional demands for insertion and steering, and present an eco-morphological analysis based on basic probe characteristics. Similarities and differences across taxa in terms of functional morphology and mode of operation are critically evaluated.

II. FUNCTION AND THEORY OF PROBING

(1) Functional demands of probing insects

Probing into solid substrates is a complex process where several control, transport, and mechanical challenges need to be overcome.

First, sensors and effective motor control are needed to search, reach, and assess a target accurately inside the substrate (De Leon, 1935; Wang & Keller, 2002; Ruschioni *et al.*, 2015). Sensors are found, embedded in the cuticle, in almost all probes (Robinson, 1939; Leopold *et al.*, 2003; Kundanati & Gundiah, 2014; Zhang *et al.*, 2014). Because of their relatively small size, we do not expect that sensors have a large impact on the probe morphology and probing mechanics. Muscles for probe motor control are large and thus typically situated outside the probe. Both sensors and muscles are of great importance for the life history of the animals, but as we focus on the insertion and steering of the probe itself we will not elaborate on sensors and muscles herein.

A second challenge of probing is the transport of fluid or eggs (Gordon & Lumsden, 1939; Bronner, 1985; Tjallingii, 2006). Transport of (Newtonian) fluids through insect probes is often approximated using the Hagen–Poiseuille law (Daniel & Kingsolver, 1983; Loudon & McCulloh, 1999; Kikuchi & Mochizuki, 2008; Lee, Kim & Lee, 2009):

$$Q = \frac{\Delta P \pi R^4}{8\mu L}, \quad (1)$$

where Q is the volumetric flow rate through the probe, ΔP is the pressure difference over its length L , R is the radius of the probe, and μ is the dynamic viscosity of the transported fluid. Q is proportional to the fourth power of R , making R the most sensitive parameter. A 10-fold increase in R results

in a 10000-fold increase in the flow rate if the ΔP is kept constant.

The transport of eggs through the canal depends on a different set of factors, including the difference in the diameter of the egg and canal, the elasticity of the egg and of the canal, the normal stress of the egg on the wall, and the friction between the two. We are unaware of any measurements of the material properties, deformations of eggs in parasitic wasps, presence of friction-reducing lubricants or of any estimations of the energy needed for passing an egg through the ovipositor. We hypothesise that for canals with a diameter smaller than the size of the unloaded egg, the work required for transport increases with decreasing canal diameter, although to our knowledge there are currently no data to confirm this.

Third, insect probes are faced with several additional mechanical challenges when it comes to drilling and steering, including (i) puncturing the substrate without damage to the probe, (ii) advancing the probe into the substrate, (iii) reaching the target, and (iv) extracting the probe from the medium. We briefly introduce these challenges below, and discuss the underlying physical principles and solutions on how to overcome these in Section II.2. Other functional demands not directly related to probing and steering, such as the transport of fluids and eggs, will be addressed in Section IV.4.

Puncturing the substrate can be demanding because of substrate deformation and the presence of protective layers, such as stiff cell walls or viscoelastic structures in the substrate. Inserting a needle into a viscoelastic substrate (bovine liver) revealed that the force at the needle tip increases due to deformation of the substrate, until the substrate surface or internal tissue layers are ruptured, causing the force at the tip to decrease (Okamura, Simone & O’Leary, 2004; Elgezua, Kobayashi & Fujie, 2013).

During further insertion of the probe, the insect should overcome the friction along the length of the probe in addition to the cutting forces at the probe tip. Inserting a single-element probe into a solid substrate requires a pushing force that is large enough to overcome these forces and a sufficiently stiff probe to avoid buckling (Okamura *et al.*, 2004; Casanova, Carney & Sarntinoranont, 2014). A probe consisting of multiple reciprocally moving elements may be inserted more easily into a substrate than a single-element probe, because the former can reduce the net friction and pushing forces on the entire probe, as explained in Section II.2b (Vincent & King, 1995; Cerkenik *et al.*, 2017).

A host can be reached by steering the probe in the required direction. Steering requires a flexible probe. Flexibility depends on the stiffness of the probe materials and their geometry. Very little is currently known about the material properties of insect probes, except that they belong to the exoskeleton and consist of chitin and a variable protein matrix. The material stiffness of the cuticle, expressed as the Young’s modulus, varies between about 0.1 GPa in intersegmental membranes and 20 GPa in the elytra of beetles (Vincent & Wegst, 2004). We found only a few studies

that estimated the stiffness of insect probes; they reported a stiffness range of about 1–10 GPa, estimated based on either indentation force or material composition (Vincent & King, 1995; Kundanati & Gundiah, 2014). With respect to geometry, a slender probe is more flexible than a wide probe of the same material and length, because of its smaller second moment of area as explained below (Equation 2).

The final requirement in probing is retraction of the probe from the substrate. Most insects simply pull their probes out of the substrate (Pollard, 1973; Choumet *et al.*, 2012; Kundanati & Gundiah, 2014). This motion generates tensile forces within the probe and at its origin within the animal body. Slender probes can support much higher tensile than compressive stresses, and pulling on them is therefore not expected to induce damage to the probe. The probe–body connections are also expected to be capable of sustaining high tensile loads, considering that the probes are generally used multiple times throughout the lifetime of the animal. Because these tensile forces are expected not to damage the animal, we will not elaborate further on probe retraction.

The transport and insertion requirements, which have the greatest impact on the shape of insect probes, are contradictory. A large probe diameter may be advantageous for transport but makes the probe harder to insert and steer, due to reduced flexibility and increased friction. One disadvantage of inserting a probe with a large diameter is the reduced stress at the probe tip, which might hinder the puncturing and cutting of the substrate. This is less relevant for wasps, as all probes, as far as we know, have tapered tips (Hudson, 1970; Vincent & King, 1995; Ahmad *et al.*, 2012) that can concentrate the drilling forces onto a small area.

(2) Theoretical framework of the probing mechanics of multi-element probes

(a) Puncturing the substrate

Inserting a probe into a substrate requires an axial load, which, when not aligned along the probe axis, generates a bending moment. Even if the load is perfectly aligned with the probe axis, the probe still deforms (e.g. becomes thicker under compression). After structural buckling, the probe can return to its original shape when the load is removed, as long as the stress did not exceed the yield strength of the material. When the maximal load-bearing capabilities of the material (yield stress) are exceeded, plastic deformation occurs and the probe will not return to its original shape. The critical buckling load of an ideal (i.e. straight, homogeneous, and free from initial stress) beam as a function of its material and geometrical properties is given by the Euler equation (also see Fig. 1A):

$$P_{cr} = \frac{\pi^2 EI}{(KL)^2}, \quad (2)$$

where P_{cr} is the critical load at which buckling occurs, E is the elastic modulus of the material, I is the second moment

of area, which reflects the distribution of the cross-sectional area around the bending axis [for details on calculating I see, for example, Nash (1977)], L is the unsupported length of the beam, and K is the effective length factor of the beam accounting for its end conditions ('pivoted' or 'fixed'). Beam diameter has a very high impact on the value of P_{cr} , as I increases with approximately the fourth power of beam diameter.

(b) Advancing in the substrate

The second challenge of probing insects is to advance their probe through a solid substrate. To do so, the insect should overcome the friction forces along the length of the probe and the cutting forces at its tip.

Consider a cylindrical probe consisting of three or more slender elements that are interconnected longitudinally and can slide along each other (Fig. 1B). Vincent & King (1995) suggested that advancement of such a multiple-element probe through a solid substrate is achieved by pushing forward and pulling backward the individual elements in an alternating fashion. The motion of a single element depends on the external force by the animal's body (\mathbf{F}_{ext} ; either pushing: $\mathbf{F}_{ext} > 0$ or pulling: $\mathbf{F}_{ext} < 0$), the friction forces along the length of the element by its neighbouring elements (\mathbf{F}_{ngb}) and by the substrate (\mathbf{F}_{sub}), and the cutting force on the tip (\mathbf{F}_{tip}). These forces can be quantified using Newton's second law:

$$\mathbf{F}_{ext} + \mathbf{F}_{ngb} + \mathbf{F}_{sub} + \mathbf{F}_{tip} = m\mathbf{a}, \quad (3)$$

where m is the mass of the element and \mathbf{a} its acceleration. In a probe with n elements the sum of all forces on the probe is:

$$\sum_{i=1}^n (\mathbf{F}_{ext,i} + \mathbf{F}_{sub,i} + \mathbf{F}_{tip,i}) = \sum_{i=1}^n m_i \mathbf{a}_i. \quad (4)$$

In Equation 3, the friction forces between neighbouring elements $\mathbf{F}_{ngb,i}$ are not shown, because they cancel each other out, according to Newton's third law (action = –reaction). Advancement of the probe into the substrate is even possible with a zero net external force or even a net pulling force ($\sum_{i=1}^n \mathbf{F}_{ext,i} < 0$):

$$\sum_{i=1}^n \mathbf{F}_{ext,i} = - \sum_{i=1}^p (\mathbf{F}_{sub,i} + \mathbf{F}_{tip,i}) - \sum_{j=1}^r (\mathbf{F}_{sub,j} + \mathbf{F}_{tip,j}) + \sum_{i=1}^n m_i \mathbf{a}_i \leq 0, \quad (5)$$

with p being the number of protracting elements and r the number of retracting or stationary elements. In the case of a negligibly small contribution of the inertial term, this can be achieved by keeping the sum of the external friction and tip forces of the p protracting elements below the reverse

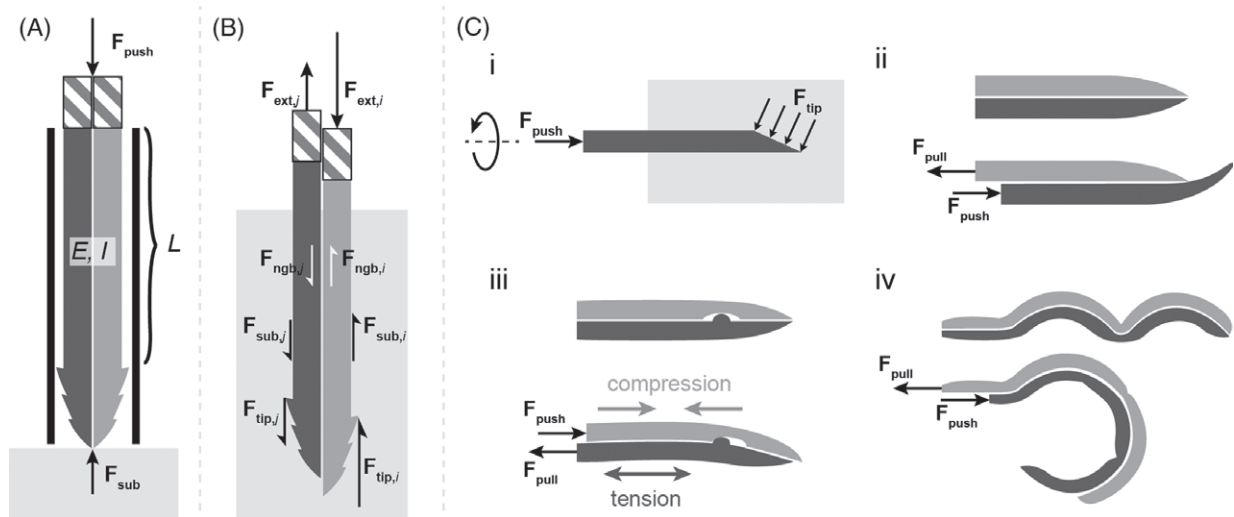


Fig. 1. General principles of puncturing, insertion, and bending of the probe in two dimensions. (A) Puncturing. The probe with Young's modulus E , second moment of area I , and length L (real probes are longer than depicted here) is positioned at a suitable location on the substrate and pressed against it (\mathbf{F}_{push} and surface reaction force \mathbf{F}_{sub}). The probe outside the substrate is stabilised along its length (black vertical lines). (B) Advancement within the substrate. Further insertion is achieved using a 'push-pull' mechanism (Vincent & King, 1995). \mathbf{F}_{ext} , pushing or pulling force on the valve; \mathbf{F}_{sub} , friction force of the substrate along the probe shaft; \mathbf{F}_{ngb} , inner inter-element friction force; \mathbf{F}_{tip} , force on the probe tip region. (C) Hypothesised bending mechanisms of insect probes. In all cases, the tip motion depends on the force generated between probe and substrate. The amount of bending (ii–iv) can be controlled by adjusting the amplitude of pro-/retraction of individual elements. Steering (ii–iv) can be achieved by the interplay of at least three elements (for clarity, only two are depicted). (i) An asymmetrical geometry of the probe tip region (bevel) leads to asymmetrical forces acting on it (\mathbf{F}_{tip}) during insertion, causing the tip to bend from a straight path. Steering can be achieved *via* rotation of the probe about the longitudinal axis (Webster *et al.*, 2006). (ii) 'Preloaded' elements (Pollard, 1969) curve towards each other when a low enough opposing force is present. This results in asymmetric forces as in (i). (iii) Longitudinally restricted movement of the elements by 'preapical stops' (Quicke, Fitton & Harris, 1995). The connections cause the build-up of tension and compression within the elements, thus generating bending moments within the ovipositor. (iv) Differential sclerotisation of elements causes the probe to bend when stiff (arches) and flexible regions (nodes) are aligned with each other (Quicke, 1991).

of the corresponding sum of the r retracting and stationary elements, that is:

$$-\sum_{i=1}^p (\mathbf{F}_{\text{sub},i} + \mathbf{F}_{\text{tip},i}) \leq \sum_{j=1}^r (\mathbf{F}_{\text{sub},j} + \mathbf{F}_{\text{tip},j}). \quad (6)$$

Equation 6 can be satisfied in various ways. First, the external surface area of the protracting element(s) can be smaller than that of the retracting and stationary element(s), which leads to lower friction on the protracting elements as compared to the friction on the retracting and stationary element(s). Second, by advancing only a minority of the elements (keeping r high and p low), the differences in the forces on the retracting and protracting elements are increased. Third, directional serrations on the elements 'anchor' them in the substrate during backwards pulling, whereas forward motion is possible with relatively low friction (Vincent & King, 1995). Furthermore, \mathbf{F}_{tip} may be reduced mechanically by using a sharp tip, or chemically by degrading the substrate (Le Lannic & Nénon, 1999).

(c) Steering the probe in multiple directions

Steering a single-element probe in 3D space (Fig. 1Ci) (Roesthuis, Abayazid & Misra, 2012) is theoretically possible

by combining forward translation, rotation along the longitudinal axis, and one-plane deflection (e.g. by means of a bevel that causes an off-axis reaction force on the tip). Rotation, however, requires a torsional moment on the probe shaft, which leads to a torsional stiffness-dependent angular lag between the tip and base of the probe or even structural failure of the probe. Probe rotation at the base can be avoided with alternative steering mechanisms available in multiple-element probes.

To simplify the discussion on possible steering mechanisms, consider a cylindrical probe consisting of two semi-cylindrical elements joined with little or no friction along the median plane of the probe (Fig. 1Cii–iv). One possible mechanism is based on the presence of a mechanical pre-stress in the distal region of the individual elements when the probe is straight (Fig. 1Cii) and is thus called the mechanism of preloaded elements. When such a distal region extends beyond the tip of the opposing element, the stress is reduced and the element bends inwards, forming a bevelled tip (Pollard, 1969). The distribution of the pre-stress, the extent of the protraction, and the mechanical interaction with the substrate determine the bevel shape as well as the resulting trajectory of the probe.

Another possible steering mechanism uses ‘preapical stops’ to build up tension and compression by respectively pulling and pushing at the base of the two elements (Fig. 1Ciii) (Quicke *et al.*, 1995). The generated tension difference at the opposing sides of the probe causes a bending moment distribution along the probe, which makes it curve. The resulting curvature of the probe depends on the exerted pulling and pushing forces, the second moment of area along the probe, and the interaction with the substrate.

Yet another mechanism relies on regional differences in bending stiffness and the ‘stress-free’ curvature of sclerotised regions along the two elements (Fig. 1Civ) (Quicke, 1991). The bending stiffness can be reduced by making the elements thinner (thereby decreasing the second moment of area) or by implementing a less-stiff material. Pulling and pushing forces on the two elements realign the mechanical regions of the elements, which results in a change in curvature.

All these proposed mechanisms are explained using two elements and, without rotation or twist along the longitudinal axis of the probe, can only result in bending in a single plane. When more elements are added, bending outside this single plane is possible. The multiple elements increase versatility in changing the shape of the probe tip (i.e. bevel angle) and consequently in adapting the probing direction. However, a larger number of elements also increases the complexity of the probe actuation, the required control mechanisms, and the transport through the probe, all of which will be addressed in Section IV.4. Probe element numbers vary in nature, which raises the question about the evolutionary pathways that led to this differentiation.

III. BASIC STRUCTURE OF MULTI-ELEMENT PROBES

In this section, we discuss the basic structure, the size, and the number of probe elements found in wasps, hemipterans, and mosquitoes. Differences in these characteristics may be affected by phylogeny, ontogeny, adaptation to substrate type, or other functional necessities, such as the transport of fluids or eggs.

(1) Parasitic wasps

The ovipositor of parasitic wasps evolved from two pairs of tubular appendages at the ventral side of the animal’s abdomen (Smith, 1970) and can reach several body lengths (Compton & Nefdt, 1988; Zhen *et al.*, 2005). The appendages have many synonyms in the literature, but we here call them valves (Quicke *et al.*, 1994). The dorsal pair of valves is commonly fused, resulting in a three-element probe, although the extent of the fusion varies among species (Quicke *et al.*, 1994). In the oldest extant hymenopteran superfamilies such as Xyeloidea and Tenthredinoidea (Sharkey, 2007; Peters *et al.*, 2017), the dorsal valves are partially fused, whereas wasps in the superfamily

Chalcidoidea have only a membranous connection between them (Quicke *et al.*, 1994).

In general, the fused dorsal valve contributes to about half of the ovipositor volume and half of its contact surface with the substrate. Each ventral valve contributes about a quarter to the ovipositor volume and likewise a quarter of the contact surface with the substrate, but exceptions occur. The egg canal is located between the three valves in the centre of the ovipositor (Quicke *et al.*, 1994) (Fig. 2A–C).

Internally, the valves are filled with haemolymph, tracheas, and sensory nerves, without musculature (Smith, 1969, 1972). Each ventral valve is linked lengthwise to the dorsal one with a rail-like connection which allows the valves to slide along one another (Smith, 1970, 1972; Quicke *et al.*, 1994; Nėnon *et al.*, 1997).

The ovipositors are often enveloped and protected by a pair of flexible sheaths, appendages that also originate at the ventral side of the animal’s abdomen (Smith, 1969; Vilhelmsen, 2003). These sheaths are not inserted into the substrate (Ahmed *et al.*, 2013; Kundanati & Gundiah, 2014; Cerkvėnik *et al.*, 2017).

(2) Hemipterans

The piercing-and-sucking mouthparts of hemipterans most likely evolved from a chewing mouthpart type that also allowed for simple piercing (Labandeira, 1997). Lengths of hemipteran mouthparts are rarely reported, but can reach up to at least one body length (Brožek *et al.*, 2015). The general sequence of insect mouthparts (from anterior to posterior) is: a labrum (upper ‘lip’), which acts as a cover structure, a hypopharynx, paired mandibles located lateral to the hypopharynx, paired maxillae, and a labium (lower ‘lip’) (Labandeira, 1997).

In hemipterans, these mouthparts have been transformed into the proboscis, a complex piercing apparatus in which the mandibles and maxillae are modified into four elongated, stiff cuticular appendages, termed stylets (Goodchild, 1966; Hamilton, 1981) (Fig. 2D). The maxillae taper from the base to their tip (Pollard, 1972; Leopold *et al.*, 2003) and generally interconnect with a rail-like mechanism similar to that observed in ovipositors of parasitic wasps. The maxillae form both the food and the salivary canals (Rosell, Lichty & Brown, 1995; Leopold *et al.*, 2003; Wang *et al.*, 2015) (Fig. 2E, F) and are located in the probe centre between the mandibles. The size of the food canal varies, depending on the animals’ size and their diet. Canals with the smallest diameters (<5 μm , but even reaching down to 0.5 μm) are found in hemipterans feeding on phloem sap (Auclair, 1963; Rosell *et al.*, 1995; Zhao *et al.*, 2010; Brožek *et al.*, 2015). Xylem-feeding hemipterans have food canals with diameters between 10 and 50 μm (Novotny & Wilson, 1997; Wang *et al.*, 2015; Malone, Watson & Pritchard, 2016). Predatory, haematophagous hemipterans are grouped at the higher end of this range (Cohen, 1990; Wenk, Lucic & Betz, 2010; Krenn & Aspöck, 2012). By contrast, the salivary canals, through which mainly anticoagulants, enzymes, immuno-regulatory, and anti-inflammatory substances are inserted (Fontaine

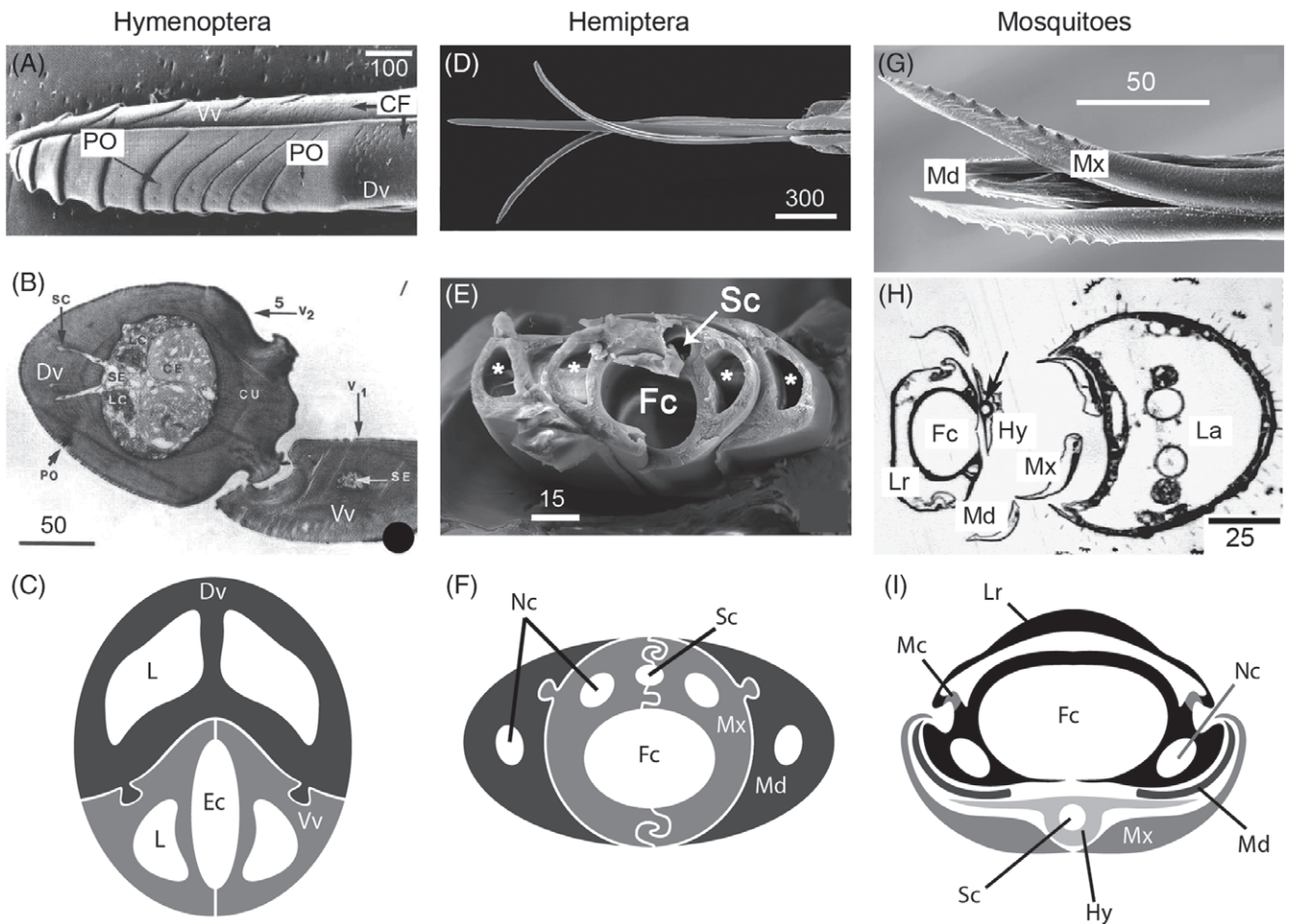


Fig. 2. Examples of insect probes. (A, B) Ovipositor of the parasitoid wasp *Megarhyssa atrata* (Ichneumonidae) Nénon, Kacem & Lannic (1997). (A) Scanning electron microscopy (SEM) image of the serrated dorsal (bottom, Dv) and ventral (top, Vv) valves. PO, pores; CF, cuticular formations. (B) Cross section through the distal end of the ovipositor. One of the ventral valves is missing. CE, cuticular epithelium; CU, cuticle; LC, longitudinal intravascular canal; SC, intracuticle canal; SE, secretion; v₁, ventral valve; v₂, dorsal valve. (D, E) Mouthparts of *Philagra albinotata* (Uhler) (Wang *et al.*, 2015). (D) SEM image of the mouthparts outside the protective labium, with flared out mandibles and maxillae (middle) that are usually kept together. (E) Cross section through the stylets fascicle (location not given in the original article). The mandibles envelop the maxillae that form the food canal (Fc) and the salivary canal (Sc). Asterisks denote the dendritic canals. (G, H) Mosquito mouthparts (proboscis) of *Anopheles stephensi* (Liston) (Krenn & Aspöck, 2012). Maxillae (Mx) and mandibles (Md) enveloping the labrum (Lr; not indicated in G) and hypopharynx (Hy; not distinguishable in G). La, labium; Fc, food canal. (C, F, and I) Generalised schematics of probe cross sections for Hymenoptera (C), Hemiptera (F) and mosquitoes (I). Ec, egg canal; L, lumen; Nc, neural canal; Mc, membranous connection. All scale bars in μm .

et al., 2011), have a constant width of less than 10 μm , irrespective of the diet.

The labrum is reduced to a cover plate at the base of the stylets, whereas the labium forms a sheath that houses the stylets and provides them with external support. Neither the labrum nor the labium penetrate the substrate (Pollard, 1973; Cobben, 1978).

(3) Mosquitoes

As in hemipterans, the probe of a female mosquito is called a proboscis, but contains six stylets. Mosquitoes insert their hypopharynx, labrum, mandibles, and maxillae into the substrate (Robinson, 1939; Clements, 1992) (Fig. 2G).

The stylet length depends on the diet and is largest in females of blood-feeding species (Wahid, Sunahara & Mogi, 2003). By contrast, stylets are reduced or are even absent in non-blood-feeding males and in species feeding exclusively on plant material (Wahid *et al.*, 2003); neither are addressed here.

The most dorsal stylet in the proboscis is the labrum, which forms the food canal with a diameter between 11 and 50 μm (Tawfik, 1968; Lee *et al.*, 2009). The labrum is shaped as a double-walled tube with a small opening on the ventral side. The two walls of the labrum form an inner lumen, are connected along their edges with a membrane, and are fused at their tips. The literature is not consistent on whether the hypopharynx (Dimmoc, 1881; Hudson, 1970) or the

mandibles (e.g. Robinson, 1939; Snodgrass, 1959) lie ventral to the labrum. Clements (1992) states that both arrangements are correct, with the hypopharynx lying ventral to the labrum at the base of the proboscis, and the mandibles at its tip. The maxillae are the most ventral of the stylets (Fig. 2G, H). The central lumen of the hypopharynx is the salivary canal, whereas the mandibles and the maxillae are solid slender rods (Waldbauer, 1962). Not all stylets are interconnected; only shallow connections between the maxillae and the labrum have been reported (Clements, 1992).

The labium terminates in two muscular labellae and houses the proboscis (Robinson, 1939; Snodgrass, 1959; Clements, 1992). Similar to the labium of hemipterans and sheaths of the wasps, the labium is not inserted into the substrate during probing (Robinson, 1939).

IV. MODE OF OPERATION

A probing session can be divided into a number of steps, each with specific functional demands (Section II). Execution of each step may vary among species. Similar considerations about the origin (phylogeny, ontology) and functional aspects (substrate adaptation, behaviour) as described in Section III apply here as well.

(1) Puncturing the substrate

As previously mentioned, puncturing a substrate with a slender probe poses a risk of buckling and damaging the probe. Across taxa, the most common way of preventing buckling during puncturing is by enveloping the probe in a structure, to provide lateral support and increase the effective diameter of the probe (thus increasing its second moment of area).

(a) Wasps

Sheaths protect the ovipositor in several wasp orders (Vilhelmsen, 2003). In most cases, sheaths support the ovipositor during puncturing and at the beginning of its insertion into the substrate. The sheaths are not inserted into the substrate, but gradually fold over their full length in an arc-like shape away from the ovipositor base with increased insertion (Compton *et al.*, 2009; Cerkvėnik *et al.*, 2017). Other buckling-prevention mechanisms include hardening the ovipositor tip (increasing E in Equation 2) with metal atoms, such as manganese and zinc (Quicke *et al.*, 1998), and shortening the probe's free length (L in Equation 2) by locking the ovipositor at the base (coxa) (Turrisi & Vilhelmsen, 2010), midway between their legs (Zhen *et al.*, 2005; Kundanati & Gundiah, 2014), or in specialised grooves on their abdomen (Heatwole *et al.*, 1962). If excessive buckling nevertheless does occur, the animals retract and straighten their ovipositor before resuming probing (Kundanati & Gundiah, 2014).

(b) Hemipterans

The segmented labium shaft that envelops the stylets is soft and moves away from the stylets by telescopic retraction into itself, the head, and sometimes the body (Weber, 1928; Pollard, 1969; Krenn & Aspöck, 2012). The labium tip is stiff and provides external support throughout the insertion process (decreasing K in Equation 2) (Dai *et al.*, 2014; Brožek *et al.*, 2015). Additional support can be provided by a flange of solidified saliva (Miles, 1968; Tjallingii & Esch, 1993; Leopold *et al.*, 2003; Tjallingii, 2006; Will, Furch & Zimmermann, 2013). The solidified saliva presumably stabilises the proboscis during probing (Miles, 1972), probably by fixing the end of the labium to the substrate (further decreasing K in Equation 2).

(c) Mosquitoes

Similar to hemipterans, the mosquito labium supports the proboscis during puncturing and further insertion. The labium folds away from the proboscis in a hairpin-like curve during probing (Ramasubramanian, Barham & Swaminathan, 2008). In contrast to hemipterans, the distal end of the mosquito labium is soft and longitudinally divided into two muscular labellae (Snodgrass, 1959). The labella presumably facilitate the puncturing of the skin by holding the stylets closely together (Snodgrass, 1959). Alternatively, the labellae might assist in puncturing by moving laterally upon being pressed onto the skin, thus stretching the skin and easing crack formation by the maxillae (Aoyagi, Izumi & Fukuda, 2008). In both instances, the stylets are more or less fixed, which decreases K in Equation 2.

(2) Advancing the probe into the substrate

Alternate movements of elements during probing have been reported for wasps (Vincent & King, 1995; Cerkvėnik *et al.*, 2017), hemipterans (Miles, 1958; Pollard, 1973), and mosquitoes (Robinson, 1939; Choumet *et al.*, 2012). However, the varying number of elements among taxa indicates that animals use different modes of operation.

(a) Wasps

In wasps, the valves can presumably be moved independently, as each valve has its own abdominal musculature (King, 1962; Smith, 1972; Fergusson, 1988; Vilhelmsen, 2000). Inter-valve friction is likely to be kept low, possibly with lubricants (Bender, 1943; Lyngnes, 1960; Copland, 1976).

The hypothesised reciprocal movement in wasps has been quantified solely in *Diachasmimorpha longicaudata* Ashmead (Braconidae) (Cerkvėnik *et al.*, 2017). Reciprocal movements were always observed when probing in relatively stiff substrates, but only occasionally in soft substrates (Cerkvėnik *et al.*, 2017). Wasps operate their valves at low speeds and accelerations (Cerkvėnik *et al.*, 2017) and satisfy Equation 6 by pushing one ventral valve forward at a time, while the

dorsal valve and the other ventral valve remain stationary. Furthermore, the 'stationary elements' were observed to be effectively pulled back rather than remaining still. This may, in combination with tip serration, increase friction with the substrate (Equation 6, Fig. 1B). The net pushing forces with which the valves are inserted by *D. longicaudata* were estimated to be very small (<200 pN) (Cerkvenik *et al.*, 2017). Advancing a single valve only a short distance beyond the tip of the stationary ones assures that the slenderness ratio of the protracted part is low, thereby preventing buckling. The maximal recorded amplitude of valve protraction in *D. longicaudata* is approximately 200 μm (Cerkvenik *et al.*, 2017), which is roughly 3.5% of the total ovipositor length (Leyva, Browning & Gilstrap, 1991).

(b) Hemipterans

In the four-element probe of hemipterans, the stylets are controlled independently by muscles located in the head (Weber, 1928; Miles, 1958; Cohen, 1990). The stylets are bathed in salivary secretions which presumably have a lubricating function (MacGil, 1946; Miles, 1968).

For a four-element probe, various patterns of stylet advancement in the substrate have been observed. In air or during insertion in gels, hemipterans either move all the stylets reciprocally or use both maxillae together as one (Weber, 1928; Miles, 1958; Pollard, 1970). We are aware of only one study that quantified the stylet motions and showed that in *Psylla mali* (Schmidberger) the mandibles are operated at half the frequency and twice the amplitude of the maxillae (i.e. 1.5 Hz and 5 μm compared to 3 Hz and 2.5 μm , respectively; Pollard, 1970). Hemipterans can probe by leading either with the mandibles or with the maxillae (Miles, 1958; Pollard, 1970, 1973; Cobben, 1978). Carnivorous hemipterans often use their mandibles only to anchor to their prey and then thrust their leading maxillae deep into the tissue (Cobben, 1978; Krenn & Aspöck, 2012).

Cutting forces at the probe tip during probing are reduced mechanically (e.g. using sharp stylet tips or disrupting fibrous material by sawing), chemically (e.g. dissolving/softening the material), or with a combination of both. The saliva of certain species contains enzymes (e.g. pectinases) that degrade the connection between cells and thus soften the substrate (Boyd, Cohen & Alverson, 2002). Species relying on pectinases generally take intercellular paths (Miles, 1968; Pollard, 1973; Oten, Cohen & Hain, 2014), whereas those mainly relying on mechanical work take predominantly intracellular paths to reach their target tissues (Miles, 1968). Flaring of the mouthparts deep in the target tissue has also been reported, which might occur to enlarge the opening of the food canal (Cohen, 1990).

(c) Mosquitoes

All stylets of the mosquito proboscis, with the exception of the hypopharynx, are controlled by individual muscles (Dimmoc, 1881; Snodgrass, 1959). As the stylets lack tight

inter-element connections (Fig. 2G, H, and I), we expect low friction between them.

We are not aware of any quantifications of stylet movements in mosquitoes. Observations of probing showed that mosquitoes rapidly move their maxillae and mandibles in alternate fashion along the labrum, which can be seen from the outside as vibrations of the stylet palps (e.g. Griffiths & Gordon, 1952; Kong & Wu, 2009). The labrum is tightly connected to the head and cannot move on its own. Instead, it is pushed inside the substrate by the vibration of the head (Aoyagi, Izumi & Fukuda, 2007). We hypothesise that the labrum muscles are predominantly used to bend this structure.

The stylets can become detached from each other and follow their own path in the substrate during insertion without apparent damage, despite bending at acute angles relative to each other (Choumet *et al.*, 2012). Whether this mechanical behaviour is intentional or not is hard to say. The proboscis tip flares out to a small degree during suction, which may help to open the entrance to the food canal (Choumet *et al.*, 2012).

To summarise, alternate movements of the probe elements are a widespread feature in insect probes. The pro- and retraction of individual elements presumably reduces the net insertion forces of the stylets and avoids buckling of the probe. Pushing forces can be reduced by sharp element tips or by softening the substrate with chemical secretions.

(3) Steering the probe in multiple directions

Several general steering mechanisms were described in Section II.2c. Below we discuss the different mechanisms in our taxa of interest.

(a) Wasps

At least some wasp species can direct their ovipositors in any direction with respect to their body orientation during probing (Fig. 3A–C) (Compton & Nefdt, 1988; Elias *et al.*, 2012; Cerkvenik *et al.*, 2017). Steering in the dorsoventral plane is hypothetically achieved by preapical stops (Quicke *et al.*, 1995), differential sclerotisation of the cuticle (Quicke, 1991), or preloaded valves (Pollard, 1969). Steering in a lateral direction could be achieved by rotating the ovipositor, predominantly using valves on one side of the ovipositor, or by bending the dorsal valve laterally. The latter may be facilitated by the membranous fusion of the dorsal valve (the so-called notal membrane) (Smith, 1970; Quicke *et al.*, 1994; Gerling, Quicke & Orion, 1998). We assume that the membrane allows for minute, independent movements of two halves of the dorsal valve. In chalcidoid wasps (superfamily Chalcidoidea), this membrane runs almost along the entire length of the dorsal valve (Quicke *et al.*, 1994) and we propose this to be the reason for the reported exceptional bending capabilities (Elias *et al.*, 2012).

The distal region of the ovipositor is usually geometrically asymmetric (e.g. Belshaw, Grafen & Quicke, 2003; Ghara *et al.*, 2011; Alves *et al.*, 2014), shaped as a bevelled tip.

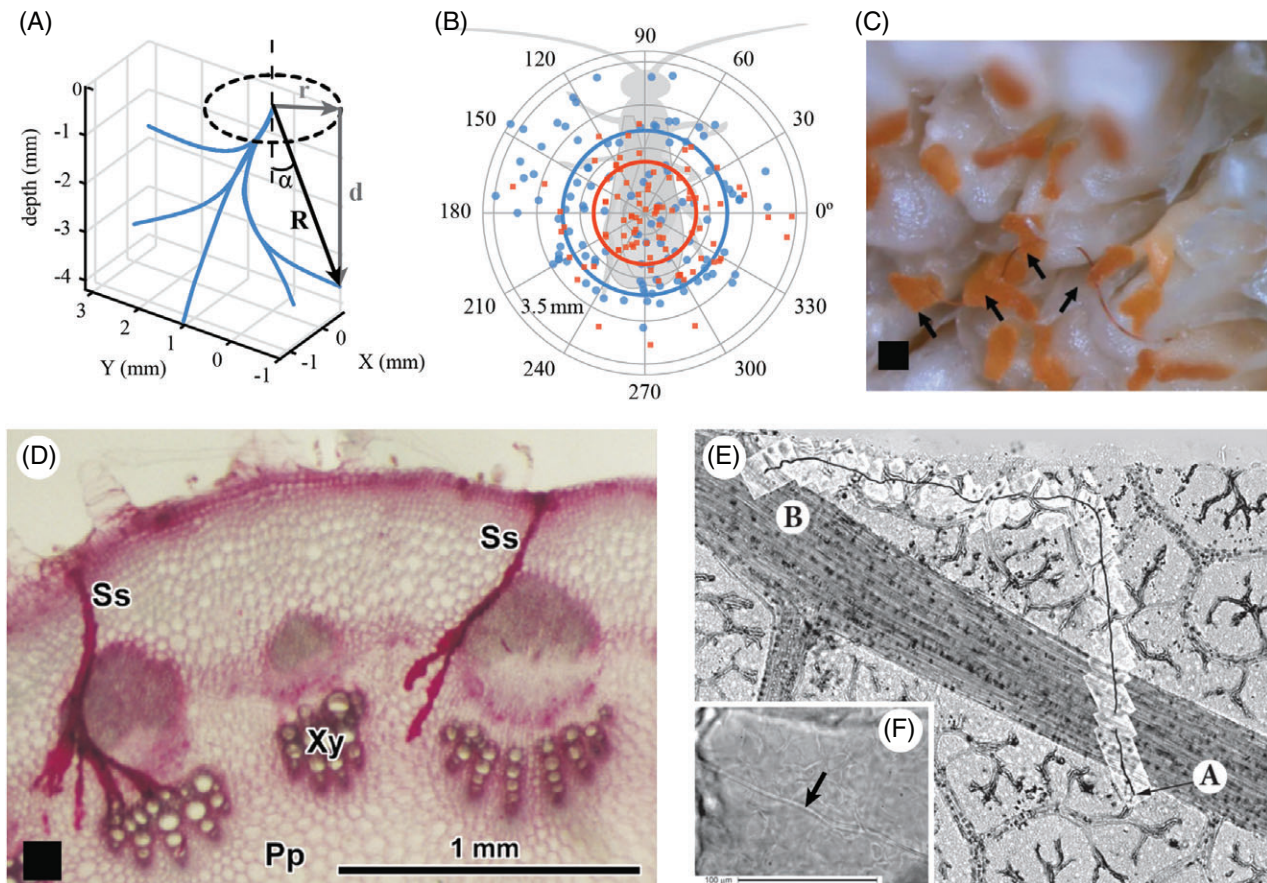


Fig. 3. Probing capabilities of hymenopterans and hemipterans. (A, B) Probing of parasitic wasp *Diachasmimorpha longicaudata* in artificial media (Cerkvenik *et al.*, 2017). (A) A three-dimensional example of a probing session during which the ovipositor was partially retracted and reinserted in a different direction (blue lines). The endpoint of an individual insertion can be described by its horizontal distance to the start point (r), and the depth in the substrate (d). From these parameters the insertion angle (α) and the position vector of the insertion trajectory endpoint (\mathbf{R}) can be calculated. (B) Top view of the insertion endpoints of many different wasps showing no directional preference and a range difference between the stiff (red) and soft (blue) substrates. (C) Parasitic wasp *Idarnes flavicollis* (Mayr) inserting its ovipositor into a fig fruit (Elias *et al.*, 2012). The ovipositor takes a sinuous path between inflorescences indicating active steering during insertion. Scale not given in original publication (D) Hemipteran *Homalodisca coagulata* (Say) stylet insertions into sunflower stem (light micrograph) (Leopold *et al.*, 2003). The salivary sheaths (Ss) show a clear branched pattern when probing for xylem vessels (Xy). Pp, pith parenchyma. (E, F) Hemipteran *Aulacaspis tubercularis* (Newstead) probing a mango leaf (Juárez-Hernández *et al.*, 2014). (E) Compilation of multiple high-resolution micrographs of stylet insertion (marked with a black line) through a mango leaf cleared with sodium hypochlorite. Scale not given in original publication. A, insertion site; B, last field showing evidence of the stylets. (F) A micrograph showing part of the stylet bundle (arrow).

The mechanics of bending of bevel-tip needles as the result of passive interactions with the substrate are well known (Fig. 1Ci) (Ko, Davies & Rodríguez y Baena, 2010), and this might also occur in ovipositors. Moreover, the valve movements may allow the wasps actively to change the shape and thus the bevel angle of the ovipositor tip. In *D. longicaudata*, the ventral valves appear to be preloaded, and their protraction strongly enhances the asymmetry of the distal region of the ovipositor. This is not true for the dorsal valve, and only drilling by leading with the ventral valves resulted in high curvature (Cerkvenik *et al.*, 2017). Theoretically, controlling the amplitude of valve movements also controls the size of the bending radius and curvature length.

(b) Hemipterans

Hemipterans are quite capable of fine control of the probing process, as observed from stylet paths within plant tissues that resemble those of ovipositor insertions (Fig. 3D–F) (Leopold *et al.*, 2003; Juárez-Hernández *et al.*, 2014). Due to the diversity in length and connections between stylets in hemipterans, various steering methods have been hypothesised (Pollard, 1969).

In Gerromorpha, an apical interlocking mechanism is created by interlocking lamellae (Cobben, 1978). A preloading mechanism has been observed in the mandibles of *Oncopeltus fasciatus* (Dallas) (Miles, 1958) and in the maxillae of *Eupteryx melissae* (Curtis) (Pollard, 1968, 1972), whereas a change of direction due to rotation of the inner

maxillary stylets along their longitudinal axis with respect to the mandibles has been observed in *Aphis gossypii* (Glover) (Pollard, 1969) and hypothesised for *E. melissae* (Pollard, 1968).

Solidifying saliva secreted by phytophagous hemipterans (Pollard, 1973; Leopold *et al.*, 2003) might help in steering by providing a low-friction environment for stylet rotation or may act as a fulcrum as mentioned in Section IV.4b.

(c) Mosquitoes

Flexibility is one of the most striking features of the mosquito probe: the proboscis can bend at least up to 90° with respect to the original direction during probing (Gordon & Lumsden, 1939; Choumet *et al.*, 2012). Steering appears to be mostly in the dorsoventral plane and is probably achieved by independent movement of the two lateral walls of the labrum (Gordon & Lumsden, 1939; Clements, 1992; Choumet *et al.*, 2012). Each wall is reported to have its own musculature which can move them in opposite directions along their longitudinal axes (Waldbauer, 1962; Clements, 1992). Because the labrum walls are fused at the distal end, their reciprocal movements induce bending due to generation of tensile and compressive forces within them. Contributions of other stylets of the proboscis cannot be assessed, due to the lack of quantification of mosquito probing kinematics. The maxillae and mandibles, albeit capable of back-and-forth movements, do not appear to contribute to steering (Choumet *et al.*, 2012). The hypopharynx does not have its own musculature (Dimmoc, 1881), so we assume that it is moved by head movements and is not involved in steering.

(d) Summary

A common solution for bending control in wasps, hemipterans, and mosquitoes is restricting the movements of the elements by interlocking or fusing them together. The presence of this steering mechanism is associated with probing in low-resistance substrates. It has been observed so far only in wasps parasitising hosts by exploiting existing tunnels in wood or nest entrances (Quicke *et al.*, 1995), in carnivorous hemipteran species feeding on soft tissues (Cobben, 1978), and in haematophagous mosquitoes (Clements, 1992). Steering can also be done by protracting individual probe elements, which probably creates or enhances the asymmetry of the probe's distal end, thereby promoting bending due to interactions with the substrate (Cerkvenik *et al.*, 2017). The influence of the substrate on steering mechanisms and capabilities, however, remains largely unknown.

(4) Additional considerations: transport of fluids and eggs

As mentioned above, an important function of insect probes is the transport of either solid matter such as eggs, or a combination of particles and liquids such as phloem and

blood. Apart from imposing a lower limit on probe width, the need to transport different substances is likely to have influenced the probe's structure and material composition. For example, to be effective, a probe should be able to withstand the inner pressure of the transported substance and should not leak. Here, we examine how the transported substances affect the shape and operational capabilities of insect probes.

(a) Wasps

Before laying an egg, some wasps paralyse or kill their host by injecting venom (Gauld, 1988). However, there is no connection between the ventral valves, which could potentially cause leakage. The ventral valves of many wasp species are equipped with thin cuticular flaps, located medially along the valves' common side, and projecting inwards into the egg canal (Fig. 4C) (Quicke *et al.*, 1994; Rahman, Fitton & Quicke, 1998; Dweck, Gadallah & Darwish, 2008). It is hypothesised that these soft flaps are forced out and thus overlap one another during injection of venom and egg laying. This effectively seals the crack between the ventral valves of the ovipositor along their entire length, creating a closed tube for fluid injection (Quicke *et al.*, 1994).

The second stage, egg laying, also poses a challenge, because the diameter of the (unloaded) egg of parasitic wasps is usually much larger than the diameter of their egg canal. The egg must thus be squeezed into the egg canal, which results in a considerable shape change of the egg (Fulton, 1932; Whiting, 1967; Austin & Browning, 1981; Austin, 1983; Bronner, 1985). Depending on the elasticity of the eggs, their passing along the egg canal could exert substantial mechanical stress on the inside walls of the ovipositor. The ovipositor expansion and its structural integrity also depends on the substrate surrounding the ovipositor. In relatively stiff media, the forces can be transferred to the substrate, which ensures that the ovipositor valves stay connected. In soft media, the ovipositor may expand, and the interlocking mechanisms must sustain the forces exerted by the egg. We could not find any empirical data on the material properties of the valve cuticle.

The passing of the egg through the egg canal is facilitated by small cuticular teeth/combs lining the inside of the egg canal (Austin & Browning, 1981; Rahman *et al.*, 1998). As these teeth/combs point towards the ovipositor apex, the friction between the inner wall and the egg is higher upon protraction of the valve than upon retraction. Thus, the animals slowly transport the egg through the egg canal by alternate valve movements (Austin & Browning, 1981). Most of the work of egg laying is probably done by abdominal muscles that move the individual valves.

(b) Hemipterans

Hemipterans take up food and inject saliva through two separate canals formed by their maxillae. The salivary canal is smaller than the food canal and located within one of

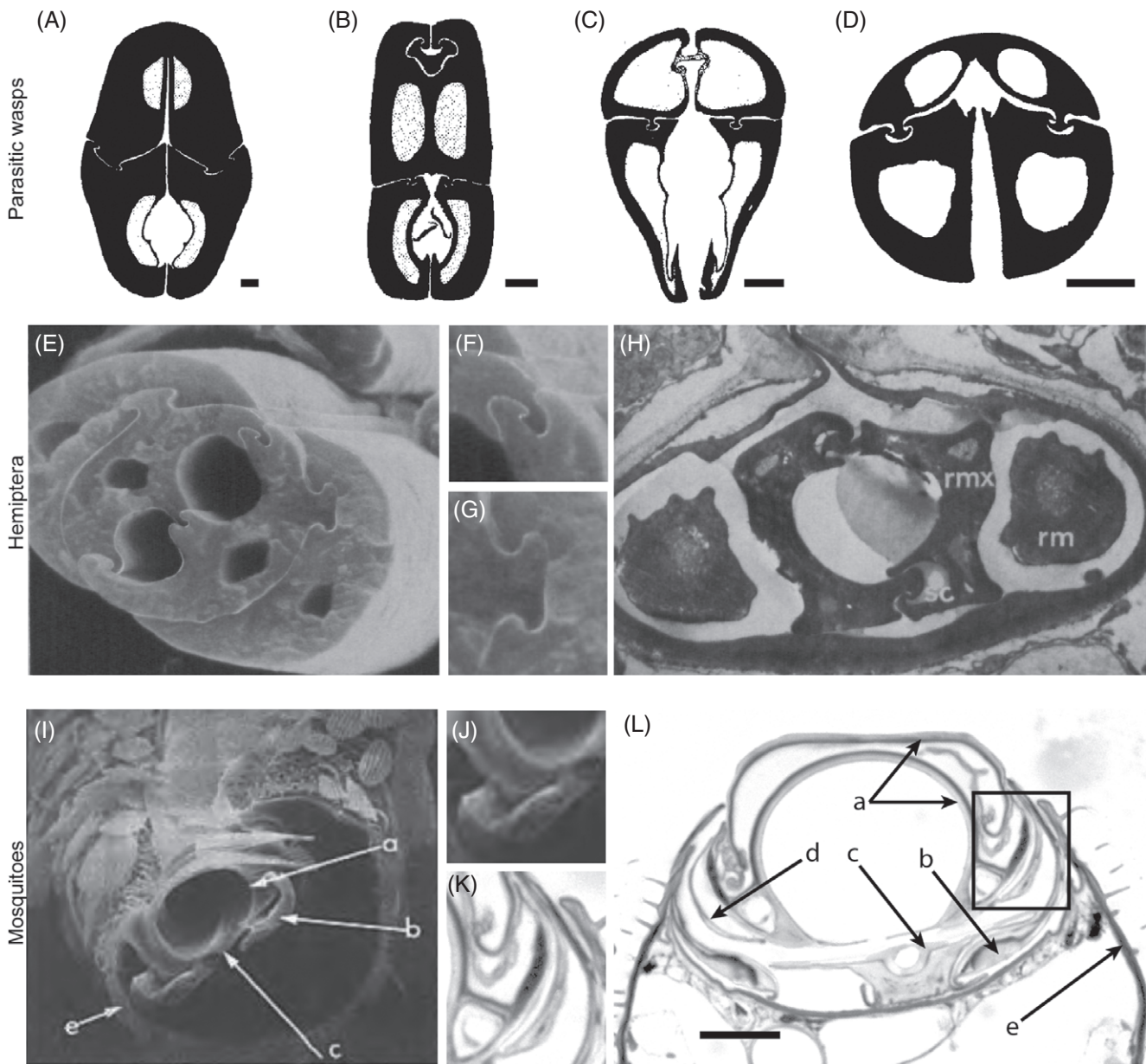


Fig. 4. Variation in element size and inter-element connections in probes of different taxa. (A–D) Cross sections through ovipositors of various parasitic wasps showing different kinds of inter-element connections. Note that a basic rail-like shape is present in all. The differences lie in the size of the connections, their orientation [diverging (A) or converging (D)], and the distance between them. A, B and C are from Quicke *et al.* (1994): 142, 103, and 93, respectively. Scale bars A–D: 10 μm . (E–H) Inter-element connections between mouthparts of hemipterans. Note the complex shape of the connection between the maxillae (E, F). The ‘rail type’ can also be present, for example, between the mandibles and maxillae (G). In H, the mandibles (rm) are not connected to the maxillae (rmx) that also contain the salivary canal (sc); Figures from Cobben (1978): 138C (indicated magnification 1400 \times) and 143C (indicated magnification 4680 \times). (I–L) Cross-section of the mosquito proboscis. The stylets are weakly connected and are held together by the labium on the outside of the substrate, but can flare out when inside the tissue: a, labrum; b, maxilla; c, hypopharynx; d, mandible; e, labium. Details of the maxilla–labrum connection are shown in J (detail of I) and K (detail of L). Indicated magnification of I: 495 \times . Scale bar in L: 10 μm . I and J are from Hudson (1970), fig. 1.1. Species: (A) *Coleocentrus* sp. (Acaenitinae); (B) *Lycorina* sp. (Lycorininae); (C) *Oedemopsis* sp. (Tryphoninae); (D) *Diachasmimorpha longicaudata* (Braconidae) (E–G) species of Thaumastocoridae; (H) *Hebrus ruficeps* Thomson (Hebridae), section at the base of the fourth labial segment; (I, J) *Aedes atropalpus* (Coquillett); (K, L) *Culex pipiens* (Linnaeus).

the maxillary interconnections. The muscular cibarial and salivary pumps power the suction of liquid food and the injection of saliva, respectively (Hamilton, 1981). Actual feeding dynamics of hemipterans are hard to measure, but it is proposed that pump pressures are correlated with the insect's food source, with higher pressures for more viscous fluids (up to 0.3 MPa for xylem feeders or haematophagous hemipterans (Bennet-Clark, 1963; Raven, 1983; Kim, 2013).

Phloem-feeding hemipterans do not seem to require active pumping of food, since the phloem itself is pressurised. This causes the sap to leak out on its own, although the net flow towards the animal can be aided by a pump (Raven, 1983). In these insects, the muscles of the cibarial pump may be used more as valves to reduce the flow of the phloem than to actuate pumping (Pollard, 1973). However, some phloem feeders also exploit xylem (Pompon *et al.*, 2011), in which case pumping is expected. We hypothesise that the relatively small food canal diameters in phloem feeders may help reducing the plant-pressurised phloem flow to a manageable rate.

Xylem-feeding hemipterans need to actively suck their food from the host, because the pressure inside the xylem is presumably negative, or in other words, under tension (Raven, 1983). Thus, to obtain a sufficient amount of food, the animals need to generate a relatively large negative pressure (Kim, 2013). How the insects achieve this is not completely understood. Xylem-feeding hemipterans have powerful muscular pumps (Raven, 1983; Wenk *et al.*, 2010) and relatively wide food canals that probably facilitate the uptake of liquids by lowering the required pressure differential during suction (Novotny & Wilson, 1997). In addition, they may feed primarily on sap that is under relatively low tension (Kim, 2013).

In haematophagous hemipterans (Bennet-Clark, 1963), the required negative pressure is expected to be higher than in phloem feeders, because the red blood cells in the blood make it a non-Newtonian fluid (Chien, 1970). Such fluids exhibit a higher viscosity with a decreasing flow velocity (Wells & Merrill, 1962), requiring a relatively high pressure differential to start the flow. Wide canals facilitate the passage of blood cells through the probe and help to prevent clogging the food canal. Similar to xylem feeders, haematophagous hemipterans possess powerful muscular pumps (Raven, 1983; Wenk *et al.*, 2010) and wide food canals (Wenk *et al.*, 2010; Krenn & Aspöck, 2012) that presumably facilitate the uptake of blood.

Saliva plays an important role in probing, but little is known about the injection dynamics of saliva in hemipterans. Phytophagous species are known to secrete two types of saliva, a gelling type and a watery type. The gelling saliva which forms the salivary sheath inside plant tissue may (i) reduce the friction between the stylets and the surrounding tissue (Miles, 1999), (ii) act as a fulcrum for the manipulation of the mouthparts (Cohen, 1990), and (iii) protect against leakage of pierced cells (Pollard, 1973). The watery saliva contains enzymes which can (i) soften the cell walls (e.g. Michael, 1989; Oten *et al.*, 2014), (ii) help with digesting

the food (Miles, 1972, 1999), and (iii) inhibit the immune response of the plant (Miles, 1968, 1999).

(c) *Mosquitoes*

Similar to hemipterans, the food canal of the mosquito is connected to the cibarial and pharyngeal pumps (Dimmoc, 1881; Kim *et al.*, 2012). Observations with micro-particle image velocimetry and real-time synchrotron micro-computed tomography (micro-CT) scans revealed that the two pumps act synergistically to take up large amounts of food rapidly (Kikuchi & Mochizuki, 2011; Kim *et al.*, 2012). Saliva is injected through the hypopharynx by the salivary pump (Snodgrass, 1959; Waldbauer, 1962). The mechanics of the saliva secretion is poorly understood, although it was suggested that female mosquitoes secrete it throughout the probing process (Griffiths & Gordon, 1952; Choumet *et al.*, 2012). The saliva may help to keep the stylets in close apposition (Robinson, 1939), but it also serves as an anticoagulant and anti-inflammatory substance (Ribeiro, Charlab & Valenzuela, 2001; Fontaine *et al.*, 2011).

V. VARIATIONS ON A THEME: COPING WITH SPECIFIC ENVIRONMENTS

There are important morphological intra- and inter-order variations in the multiple-element probes. Below, we explore and evaluate these variations in the light of functional demands imposed on the animal's life-history traits and lifestyles.

(1) Cross section of the insect probe

Although probes taper distally and are enlarged at the base, their cross section changes relatively little along most of their length (e.g. Dimmoc, 1881; Quicke *et al.*, 1994). The probes of the taxa we described are generally oval in cross section, with the largest observed variability occurring in wasp ovipositors (Fig. 4A–D) (Quicke *et al.*, 1994). This may be because parasitic wasps probe a great variety of substrates which pose different functional demands on the probing apparatus. Phytophagous wasps drilling in plant tissues, such as sawflies, have laterally compressed ovipositors, which is considered to be the ancestral state (Sharkey, 2007; Vilhelmsen & Turrisi, 2011). Similarly, the proboscis of many hemipterans and of mosquitoes is also compressed, albeit dorsoventrally (e.g. Cobben, 1978; Krenn & Aspöck, 2012) (Fig. 2E, H). By contrast, the ovipositors of wasp species (parasitoid or phytophagous) that penetrate hard substrates have oval or circular cross sections. (Quicke *et al.*, 1994; Vilhelmsen & Turrisi, 2011). The latter shape maximises the internal lumen through which the egg is passed for a given amount of substrate displacement (Quicke *et al.*, 1994; Vilhelmsen & Turrisi, 2011).

Individual elements differ in their cross sections within the probe, and their relative sizes and shapes show significant

differences among parasitic wasps and hemipterans (Cobben, 1978; Quicke *et al.*, 1994). Although the exact causation of this observed variation is unknown, it can be assumed that the morphology of individual elements is adapted to their specific function within the probe. The differences in cross sections of the ventral and dorsal ovipositor valves cannot easily be related to their functional roles, as all valves make extensive contact with the substrate during probing. Hemipterans and mosquitoes, however, have more-or-less nested probe elements (Krenn & Aspöck, 2012; Dai *et al.*, 2014), allowing us to make the following hypothesis: the elements that are considered to be the main stylets used in probing are largest (Gordon & Lumsden, 1939; Griffiths & Gordon, 1952; Choumet *et al.*, 2012), whereas the smaller elements normally have other functions, including clogging prevention (MacGregor, 1931) and injection of saliva (Dimmoc, 1881; Robinson, 1939).

In hemipterans, the cross-sectional shape of mandibular stylets seems to be associated with the evolutionary shift from a carnivorous to a phytophagous feeding style. Carnivorous (predatory and blood-sucking species) generally have oval or triangular (Wenk *et al.*, 2010) mandibular cross sections, whereas phytophagous species have either comma-shaped (Dai *et al.*, 2014) or rectangular mandibles (Brožek *et al.*, 2015).

We hypothesise that compressed probes have a preferred bending plane, whereas probes that are nearly circular are easier to steer in any direction and are thus suitable for animals aiming at moving targets, locations that cannot be reached in a straight line, or locations which are difficult to predict. Nevertheless, rectangular or even more complex cross sections are also present in wasps (Quicke *et al.*, 1994), probably in species that parasitise easily accessible hosts. This may indicate that the selection pressure for circular probes is most prominent in animals dealing with stiff substrates and those that need to steer in all directions.

(2) Alignment and interconnection of elements in insect probes

For the multi-element probe to function as described above, its elements used for drilling need to be aligned and kept closely together (Fig. 4), although in mosquitoes some elements may flare out (Choumet *et al.*, 2012). The inter-element connections also need to be strong enough to withstand force from the substrate or internal forces that could separate them, yet loose enough to enable the relative movement of the elements along one other.

In wasps and hemipterans, interconnections are ‘rail-like’. Wasps have a ridge with a mushroom-shaped cross section on the dorsal valve and a groove with an inverted mushroom shape in the ventral valves (Figs 2C, 4A–D). A similar, yet much more complex, ‘tongue-and-groove’ mechanism occurs between the elements of hemipteran mouthparts (Fig. 4E–H). The maxillae are usually interconnected by a twisted type of the ‘tongue-and-groove’ mechanism, where both maxillae contribute equally to the ‘tongue’ and the ‘groove’ part of the connection (Figs 2F, 4F) (Cobben, 1978;

Leopold *et al.*, 2003; Brožek & Herczek, 2004; Garzo *et al.*, 2012). When present, the maxillae–mandible connection can be of the shallow rim–groove type or of the mushroom type similar to that in wasp ovipositors (Fig. 4G) (Cobben, 1978; Brožek & Herczek, 2004).

We hypothesise that both the shape and the strength of the ‘tongue-and-groove’ connection depend on the stiffness and toughness of the substrate. A morphometric analysis of 113 ovipositor cross sections obtained from the literature indicated that the mushroom-shaped part is wider (thus possibly stronger) in wasps probing in materials classified as hard, such as wood, than in species probing in softer substrates or using exposed hosts (Gussekkloo, Heinen & Cerkvénik, 2016). The same considerations probably also hold for the proboscis of hemipterans, although we have found no studies that quantify this.

Additionally, in wasps, the length of the connection seems to depend on the species’ lifestyle. Endoparasitoid species, which lay eggs inside their hosts, possess ovipositors with ‘tongue-and-groove’ connections that extend all the way to the tip. This presumably facilitates the extrusion of eggs right at the tip of the ovipositor. In ectoparasitoids, which lay eggs on or adjacent to their hosts, the valve interconnections do not reach the ovipositor tip, allowing the eggs to be extruded earlier from the egg canal (Belshaw *et al.*, 2003).

Mosquitoes show little variance in the inter-stylet connections and possess shallow ‘ridge–groove’ connections between the labrum and the maxillae (Clements, 1992) and between the labrum and the mandibles (Snodgrass, 1959) (Figs 2I, 4I, J). The labrum–maxillae connections are thought to hold the stylets in a bundle during probing, perhaps aided by a viscous fluid (Robinson, 1939; Hudson, 1970). The strength of inter-element connections is relatively weak and does not prevent the flaring out of the stylets (Choumet *et al.*, 2012). Because mosquitoes probe in skin – a relatively soft viscoelastic substrate – we hypothesise that there is no selective pressure to develop strong inter-element connections in their proboscis. The flaring of stylets in the mosquito proboscis might even enhance probing capabilities by exposing more of the stylet surface to the substrate, thus increasing anchorage of the probe necessary for the push–pull mechanism.

The close apposition between probe elements may cause strong internal friction forces and may create a risk of clogging of the probe if, for example, dust/dirt particles become wedged between the elements. In addition, the introduction of liquid between the probe elements might hinder their movements (Quicke, 2015). How insects avoid such situations is not completely understood. The inner surfaces of ovipositors in many species bear small cuticular projections (ctenidia), which are thought to reduce friction between the valves by reducing their contact surface (Rahman *et al.*, 1998). Additionally, the inner surfaces of the ovipositors are also hypothesised to be hydrophobic (Quicke, 2015), although this is unlikely in sucking insects, because hydrophobicity may induce cavitation under negative pressure. However, the use of liquids as means of keeping the

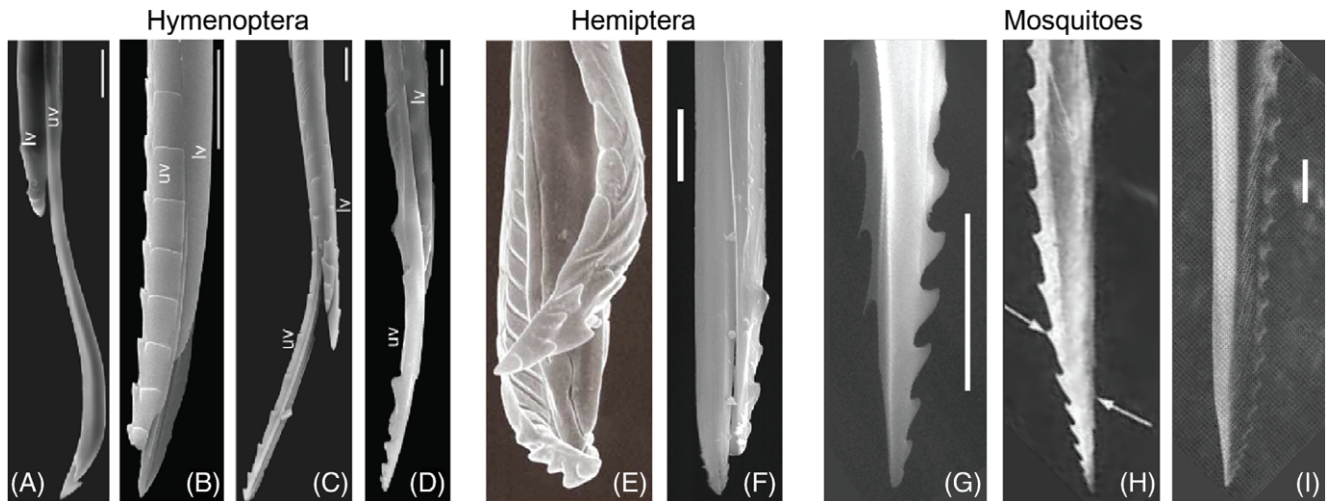


Fig. 5. Tip variations within insect orders. (A–D) Large variability is observed in the ovipositor tips of phytophagous and parasitoid wasps ranging from smooth valves, to small serrations, to strong serrations (Ghara *et al.*, 2011). The serrations can be present on either the upper valve (uv), the lower valves (lv), or all three valves. (A) *Ceratosolen fusciceps* (Mayr) (pollinating wasp), (B) *Apocryptophagus fusca* (Girault) (galler wasp), (C) *Apocryptophagus agragensis* (Joseph) (parasitoid), (D) *Apocrypta westwoodi* (Grandi) (parasitoid). (E, F) Different types of serrations on the outer wall of the mandibles of two species of hemipterans: (E) *Oncopeltus Fasciatus* (Dallas) (Angelini & Kaufman, 2004; contrast enhanced) and (F) *Sogatella furcifera* (Horváth) (Dai *et al.*, 2014) (G–I) Variation in shape of mosquito maxillae. Species differ in the number and size of stylet serrations. (G) *Aedes albopictus* (Skuse) (Kong & Wu, 2009), (H) *Aedes Atropalpus* (Coquillett) (Hudson, 1970), (I) *Anopheles farauti* (Laveran) (Lee & Craig, 1983). Scale bars: 10 μ m. Scale for E and H was not given in the original papers.

stylets together or even facilitating their sliding (lubricants) has also been proposed (Robinson, 1939; Pollard, 1973). Unfortunately, none of the cited authors elaborates on the physical mechanisms in favour of their hypothesis.

(3) Elements: shape and length

The greatest diversity in the morphology of the probes is found in their tips (Fig. 5). Attempts to correlate structural and material probe properties with substrate properties and life-history traits are only available for hymenopterans (Fergusson, 1988; Vilhelmsen, 2000; Belshaw *et al.*, 2003; Buffington, 2007; Polidori, García & Nieves-Aldrey, 2013; Elias *et al.*, 2018). For hemipterans and mosquitoes, we only found studies linking variation in tip shape and stylet lengths with feeding style (Cobben, 1978; Lee & Craig, 1983; Wahid *et al.*, 2003).

Highly sclerotised ovipositors occur in certain wasp species that probe in tough substrates (Ghara *et al.*, 2011). Strengthening of the tip by deposition of heavy metal atoms was also reported (Polidori *et al.*, 2013). The latter not only enables piercing of hard substrates, but also reduces wear of the probe (Quicke *et al.*, 1998).

The presence of serrations is also associated with substrate properties. An enlargement of the dorsal valve near its tip and large serrations on the ventral valves are generally found in wood-drilling ichneumon wasps, enabling stronger anchorage in the substrate during drilling (Belshaw *et al.*, 2003). Valves with shallow or no serrations and without enlargements are mostly associated with species operating on soft and nearly homogeneous substrates such as immature,

soft figs, whereas wasps probing in mature figs with heterogeneous tissues have not only more but also stronger and uneven serrations at the ovipositor tips (Ghara *et al.*, 2011; Elias *et al.*, 2018). Although large protrusions on the ovipositor tip are generally associated with hard substrates, an enlargement of the dorsal valve near its tip is also present in *D. longicaudata*, which parasitises hosts hidden in soft fruits (Cerkvenik *et al.*, 2017).

In species that probe for feeding, the situation is more complicated, presumably because mouthparts are used for multiple functions. In predatory hemipterans, the serrated maxillae are not only used for drilling but also for lacerating the prey tissue (Cobben, 1978). In blood-sucking representatives, the mandibles bear serrations, while serrations on the maxillae are reduced (Cobben, 1978; Wenk *et al.*, 2010). In this case, the mandibles presumably help in steering, and maxillary serrations are not needed, because the animals feed on liquid food. The mandibles of plant-feeding species are strongly serrated (Miles, 1958; Leopold *et al.*, 2003; Anderson *et al.*, 2006), indicating that, similar to wasps, hard substrates require large serrations. We expect that the mandibles aid in penetration, whereas maxillae nested between them have a role in steering. It would be worth investigating whether the mouthparts of phytophagous hemipterans show similar variation in their tip shape as observed in hymenopteran ovipositors. Mosquito species that feed on blood generally have more serrations on their maxillae than plant-feeding species that mostly feed on nectar or honeydew and do not penetrate plant tissues (Hudson, 1970; Foster, 1995).

In parasitic wasps, ovipositor length correlates with the required depth of probing (Ghara *et al.*, 2011). All valves are of equal lengths within individual ovipositors. Equal stylet lengths are also observed in bloodsucking mosquitoes and hemipterans (Cobben, 1978; Wahid *et al.*, 2003; Wenk *et al.*, 2010), whereas mosquitoes feeding only on plants have stylets of different lengths (Wahid *et al.*, 2003). The degree of stylet length reduction is species specific and presumably correlates with the diet of the animal (Wahid *et al.*, 2003). Surprisingly, predatory hemipterans also have stylets of different lengths with mandibles shorter than maxillae (Cobben, 1978). Mandibles are generally used for anchoring into their prey and maxillae for lacerating the tissue (Cobben, 1978). The available data are too limited to draw any conclusions regarding possible correlations between the length of mouthparts and their behaviour.

VI. DISCUSSION

Probes of parasitic wasps, hemipterans, and mosquitoes share several structural features despite originating in non-closely related taxa. The shared functional demands of buckling avoidance and reaching targets deep in solid substrates presumably led to convergent evolution resulting in structural and kinematic similarities. All investigated groups use external support structures for buckling avoidance and alternative movements of individual probe elements during probe advancement through the substrate. Nevertheless, probes differ in their detailed morphology such as the shape of their cross sections, the number and length of elements forming the probes, the strength of inter-element connections, and tip geometry. Presumably, at least part of the observed morphological diversity can be explained by additional functionality of the probes and adaptations to specific substrates. In man-made needles it has been shown that larger serrations on the outer surface increase anchoring in the substrate, which is important for proper functioning of the push–pull mechanism (Schneider *et al.*, 2009). Studies linking these parameters to the substrate properties are lacking, and it is currently unclear what characteristics are substrate-optimised.

To understand insect probing mechanisms fully, we need to analyse the variation in structural and material properties of the probes and their detailed kinematics inside substrates. Although the general characteristics of probes and their functioning seem obvious, few data are currently available. Only a small number of studies describe and quantify probing. Many proposed mechanisms of insertion and steering (Fig. 1C) were derived from inspection of dead animals (Waldbauer, 1962; Pollard, 1969, 1971; Quicke, 1991; Quicke & Fitton, 1995), with properties of *post-mortem* material unlikely to be representative of the natural condition of insect probes and their steering mechanisms. Better insight into the actual mechanisms of insertion and steering could be achieved by quantitatively analysing the movement

of probes inside the substrate, for example, by using high-speed videography and translucent media (Pollard, 1970; Cerkvėnik *et al.*, 2017).

Similarly, few studies focus on the structural and material properties of the probe. These can be obtained with high-resolution CT imaging or sequential transmission electron microscopy (TEM), using X-ray diffraction (Blackwell & Weih, 1980), small-scale three-point bending (Matsumura, Kovalev & Gorb, 2017), or nano-indentation experiments (Politi *et al.*, 2012). Due to the extremely small size of the probes, all these methods are either very expensive, difficult to execute, or both.

In addition, knowledge on forces involved in probing would complement our understanding of probe–substrate interactions. Quantification of forces *in vivo* is extremely difficult, but an estimation can be obtained from the size and arrangement of the muscles at the base of the probes (e.g. Pollard, 1968).

Understanding the probing mechanics and the relationship between the probe shape and the substrate also has important engineering implications. It could, for example, prove helpful in the development of novel, bioinspired, minimally invasive tools for medical purposes. Slender, steerable needles requiring small insertion forces can have several benefits, including minimal tissue damage, by-passing important structures such as blood vessels and nerves, and accessing hard-to-reach places within the body (Bano, Ko & Rodriguez y Baena, 2012; Leibinger, Oldfield & Rodriguez y Baena, 2016).

Multi-element steerable needles are already in use and more are under development (Scali *et al.*, 2017), but none are as slender and perform as well as insect probes. In most cases, steering is achieved by exploiting needle–tissue interactions arising from a bevelled tip (Fig. 1Ci; Section II.2b) (Elgezua *et al.*, 2013). In all cases, the needle is still pushed into the substrate with an external net pushing force. This requires stiff needles which hinders the minimisation of their diameters. Furthermore, the reported relative curvatures of needle insertions (Caborni *et al.*, 2012; Moreira *et al.*, 2014) are generally much lower than found in wasps (Cerkvėnik *et al.*, 2017), although some prototypes perform better when bending in one plane (Ko *et al.*, 2010). The restrictions in curving probably affect the accuracy and limit the versatility of these novel surgical tools. Needles based on insect probes may help to solve the challenges of minimisation, buckling, and steering. Development of multi-element needles capable of adjusting their tip asymmetry is already underway (Frasson *et al.*, 2010; Leibinger *et al.*, 2016; Scali *et al.*, 2017). Inserting such needles using reciprocal motion of elements decreases tissue strain and net insertion forces that might lead to tearing and damage (Leibinger *et al.*, 2016; Parittotokkaporn *et al.*, 2010). Insect-inspired probes might lead to the development of automated self-propelling probes, which will not only be useful for medical purposes, but may also be scaled up and employed anywhere where substrates need to be explored in depth, for example in construction or geology.

VII. CONCLUSIONS

(1) Initial puncturing of the substrate is always facilitated by external support mechanisms that prevent buckling and breaking of the probe.

(2) A multi-element design enables the probe to pull itself into the substrate by pro- and retracting individual elements and to steer by changing the tip shape resulting from the offset of the elements.

(3) Assuming no rotation or twist around the longitudinal axis of the probe, a minimum of three elements is required for 3D steering. Some probes possess more than three elements, which might be specialised for other functions such as injection of saliva and may have little influence on the steering of the probe.

(4) The probe tip is presumably adapted to a specific type of substrate, but information on the material properties of the substrate (and of probes themselves) is lacking.

(5) Understanding the probing mechanisms across various taxa will offer insights into their evolution, lifestyle, and ecological interactions, and may also aid the development of human-made probes.

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