



Insects locomotion, piercing, sucking and stinging mechanisms

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Abstract

This paper provides an overview of lessons from mosquitoes' locomotion and their painless piercing and from wasp stinging. Based on the understanding, conceptual schematics of bioinspired microneedle designs are presented for biomedical applications. In the first part, mosquitoes locomotory attributes, which are used by them to hunt a host, are described. Next, microanatomy, feeding process, and nanomechanical properties data of mosquitoes' labrum are presented. A hypothesis behind the painless piercing is presented followed by mosquito-inspired microneedle design guidelines. In the second part, wasp anatomy and stinging process are described. Their structure and nanomechanical properties data, modeling of the penetration process, and stinger-inspired microneedle design guidelines are presented.

1 Introduction

Some 10 million different species of animals and plants are known to exist on earth (Stork 2009; Mora et al. 2011; Barthlott et al. 2016). Of which, arthropods make up the largest category with roughly 5 million species (McGavin 1993; Chapman et al. 2013). The word, arthropods, is derived from two Greek words: *arthron* (joint) + *podos* (foot), meaning jointed-feet. These are spineless animals with segmented bodies, paired and jointed legs, exoskeletons, and bilateral symmetry. Arthropods, being one of the major class of animals, create medical problems (Service 1978; Durden and Mullen 2009; Goddard 2013). They attack other organisms, cause allergic reactions, release toxins and venoms, and may even cause death.

Figure 1 presents arthropods of medical-veterinary interest (Phylum, Arthropoda), which are classified on the basis of the well established ranking system (Parker 1997; Durden and Mullen 2009; Schmidt 2009; Stork 2009; Goddard 2013). Decreasing order of the ranking system is Phylum, Subphylum, Class, Order, and common names of the species. Starting from the top, Phylum *Arthropoda* is divided into four Subphyla *Hexapoda*, *Chelicerata*, *Myriapoda*, and *Crustacea*. The Subphyla are further divided

into six Classes and seventeen Orders. Each of the Order lists examples of the arthropods. The class *Insecta* includes all of bugs and are six-legged. This class is remarkably large and diverse.

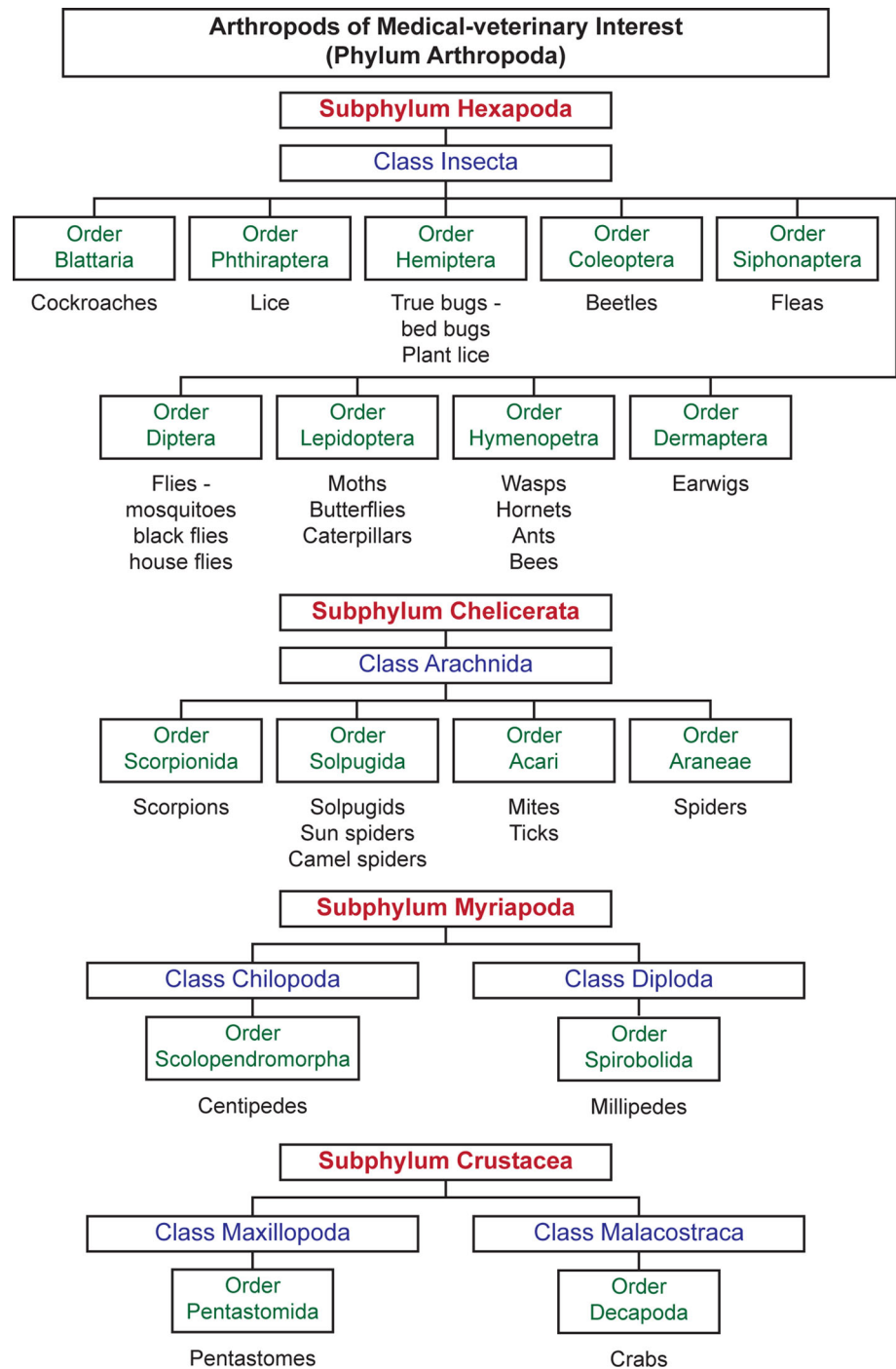
Some arthropods simply attack their prey with stingers and others feed on their host on plant nectar, blood, and other organisms. A few medically important arthropods are classified based on their attacking mechanism in Fig. 2 (Dickerson and Lavoipierre 1959; Durden and Mullen 2009; Chapman et al. 2013; Gurera et al. 2018). Arthropods are classified as biters, stingers, and piercers and suckers. The arthropods which bite with their mouthparts are classified as biters. Examples of the biters include caterpillars and spiders. Caterpillars use a pair of jaw-like-structures (mandibles) to bite onto their hosts. Spiders use a pair of sharp jaw-like-structures (spider fang) to bite onto their hosts, and later its serrated structure (chelicera teeth) to chew on them.

Next, the arthropods which sting into their prey with stingers located on their rear end and release a venom, are classified as stingers. Examples of the stingers include honeybees, wasps, and scorpions (Das et al. 2018). Among these wasps are known to cause most pain (Akre et al. 1981; Schmidt 2009). Honeybees sting using a pair of barbed stylets covered in a blunt sheath which moves back and forth alternatively while stinging the prey. The stylet is a medical name for slender, piercing part. To release venom, honeybees use openings found between the stylets. Wasps sting and release venom in a similar fashion to the honeybees. However, as compared to the honeybees, wasps have smoother barbed stylets (Akre et al. 1981). Scorpions

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Fig. 1 Arthropods of medical-veterinary interest (adapted from Parker 1997; Durden and Mullen 2009; Stork 2009; Goddard 2013)



use a sharp tail (aculeus) to sting their prey. The tail has an opening that releases venom to paralyze their prey.

Lastly, the arthropods that pierce and suck bodily fluids out of their hosts, with their mouthparts, are classified as piercers and suckers. Examples of the piercers and suckers include plant lice that suck plant sap, and bed bugs, flies, ticks and mosquitoes that suck blood. Blood sucking arthropods find a blood vessel and then suck the blood through an opening. Plant lice use two pairs of stylets (only

one pair is shown in Fig. 2) for piercing into their hosts, and use the opening formed between stylets to suck the plant sap. These stylets are covered in a sheath. Bed bugs pierce into their host using two pairs of stylets (only one pair is shown) and suck blood through the opening formed by one of the pair. Flies pierce the host using a pair of barbed stylets and suck the blood using the opening formed between the other mouthparts. Ticks use a pair of barbed stylets (chelicerae) by repeatedly flexing and relaxing the

Summary of selected medically important arthropods

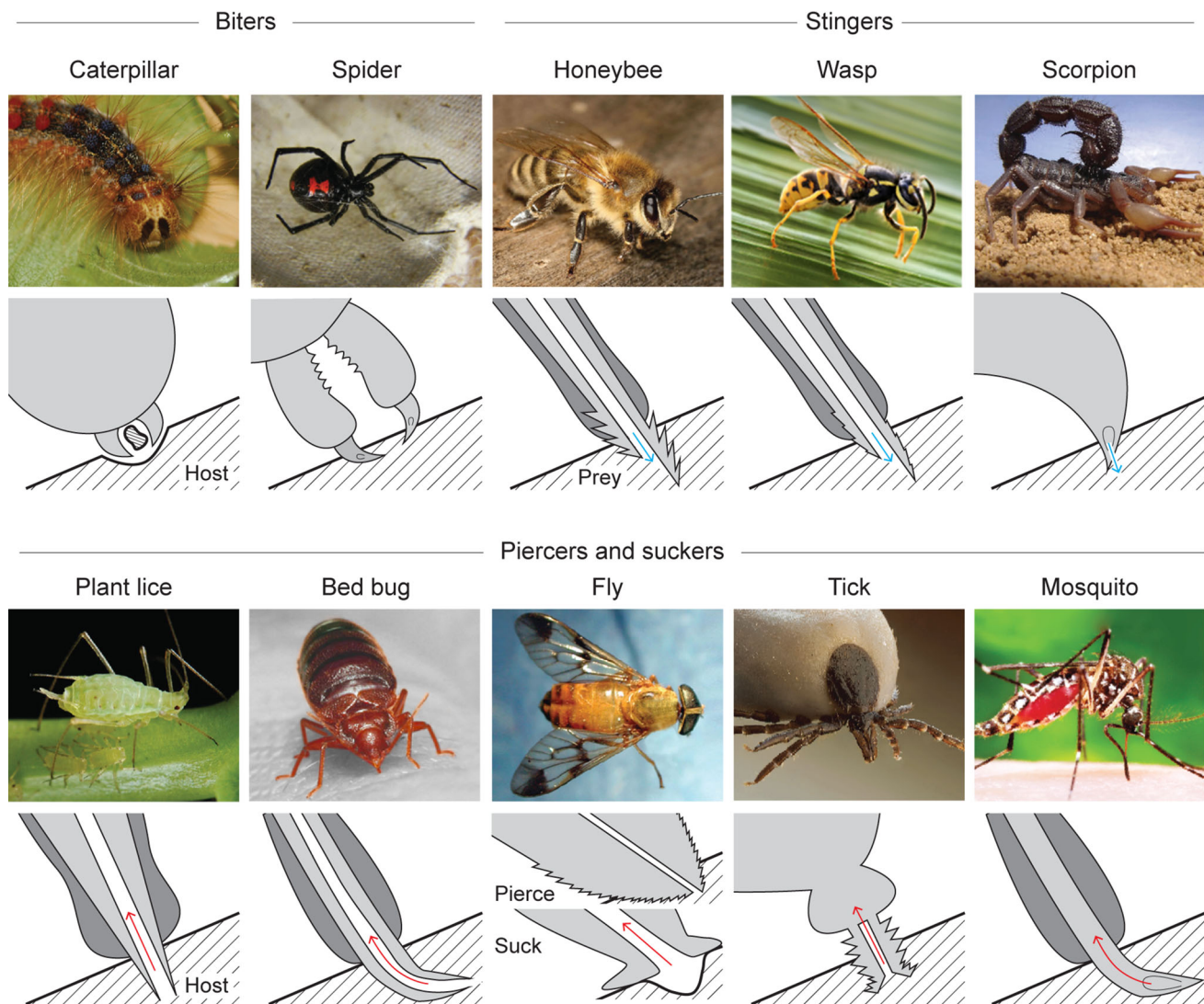


Fig. 2 Selected medically important arthropods classified based on their attacking mechanisms. The arthropods which bite their host with their mouthparts are classified as biters. The arthropods which sting with stingers located on their rear end and release a venom into their

prey are classified as stingers. The arthropods which pierce and suck bodily fluids out of their hosts are classified with their mouthparts as piercers and suckers (Gurera et al. 2018)

Table 1 Summary of number of deaths caused by various animals in 2015 (adapted from Gates III 2016)

Animal	Number of deaths
Mosquito	830,000 (~ 54%)
Human	580,000 (~ 38%)
Snake	60,000 (~ 4%)
Sandfly	24,200 (~ 1.5%)
Dog	17,400 (~ 1%)
Kissing bug	8000 (~ 0.5%)
Others (scorpion, lion, etc.)	15,370 (~ 1%)

tips of the stylets. After reaching a certain distance, the pair of stylets along with another mouthpart (hypostome, not shown) suck blood through the opening formed between the three. Mosquitoes use a vibrating bundle of six stylets (fascicle) to pierce into hosts. Then they use one of the stylets (labrium shown in the figure) to pierce into blood vessels and suck the blood (Gurera et al. 2018).

Biters only bite into their host with their teeth. A bite can introduce saliva as well as additional pathogens and diseases. The stingers penetrate the epidermis of the prey skin with a sharp tip with a hollow, narrow, canal through which the venom is injected for several minutes. It should be noted that all stings are not venomous. Piercers and

Morphological attributes of mosquitoes

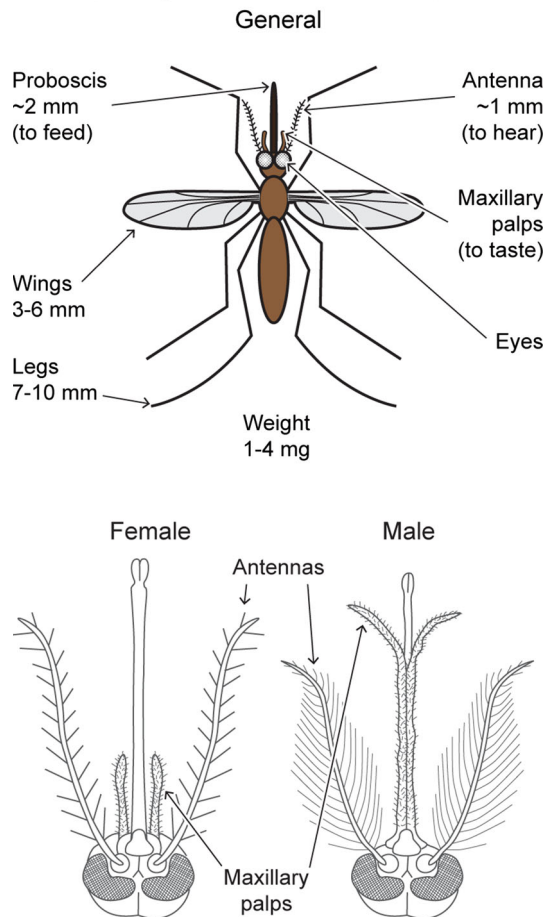


Fig. 3 (Top) A mosquito, generally, irrespective of its sex, can be morphologically described as shown. (Bottom) Moreover, there are visual differences between a female and a male mosquito. The male mosquitoes have longer maxillary palps and bushy antennae (Gurera et al. 2018)

suckers suck blood from the host and may infect with virus leading to malaria or other diseases. Stinging arthropods including honeybees, wasps and scorpions, sting other animals including humans mostly in self defense. They create problems ranging from minor pain including local swelling for a couple of hours, to a much severe pain, including cardiovascular compromise, anaphylaxis and even death due to respiratory block. Wasps and bees do not transfer human diseases or viruses from one human to another through stinging. Bees lose their stingers once they sting. Wasp stingers do not carry enough blood following a wasp sting to transmit a virus. However, since wasps and bees are scavengers, they are known to carry bacteria that can lead to infections during stinging. As a result, they are also known to spread diseases.

The mosquito's saliva can carry viruses and protozoa that it received from hosts that it had attacked previously. It is these viruses that pass on the diseases from host to host,

such as malaria, yellow fever, West Nile virus, dengue fever, and Zika virus and can cause death. It is considered to be the deadliest animal in the world. Mosquitoes, specifically by malaria alone, have been reported to cause deaths on the order of 1–3 million every year (Ito et al. 2002; WHO 2016). In 2015, mosquitoes piercing was reported to be responsible for about half of the total deaths caused by animals, as shown in Table 1 (Gates III 2016). The rest of the deaths, in decreasing order, were caused by humans, snakes, sandflies, dogs, kissing bugs, scorpions, lions, and so on.

Mosquitoes have unusual locomotory attributes. A study of their locomotion leading to bioinspiration is also of interest.

This paper provides an overview of lessons from mosquitoes' locomotion and their painless piercing and from wasp stinging (based on Bhushan 2018). Based on the understanding, conceptual schematics of bioinspired

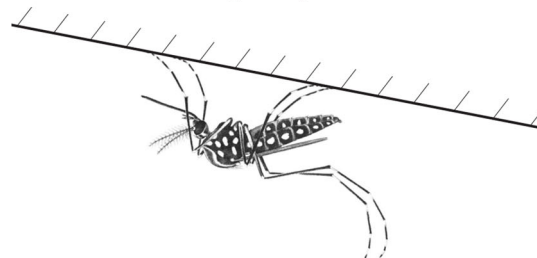
Locomotory attributes of mosquitoes

Standing on water



(Adapted from Kong et al., 2015)

Sticking to any surface



Flying in rain



(Adapted from Dickerson et al., 2012)

Fig. 4 Mosquitoes have three locomotory attributes, which are standing on water, sticking to any surface, and flying in rain

microneedle designs are presented for biomedical applications. In the first part, mosquitoes locomotory attributes, which are used by them to hunt a host, are described. Next, based on Gurera et al. (2018), microanatomy, feeding process, and nanomechanical properties data of mosquitoes' labrum are presented. A hypothesis behind the painless piercing is presented followed by mosquito-inspired microneedle design guidelines. In the second part, wasp

anatomy and stinging process are described. Then, based on Das et al. (2018), their structure and nanomechanical properties data, modeling of the penetration process, and stinger-inspired microneedle design guidelines are presented.

2 Mosquitoes' locomotion and painless piercing

A mosquito, irrespective of its sex, can be visually described as shown in Fig. 3 (top). It has a part to feed itself, three parts to sense, and two parts to move (Clements 1999; Chapman et al. 2013). The part to feed itself is known as the proboscis. The proboscis is used to pierce into a host and to suck fluids out of it. The proboscis has a length of about 2 mm and its diameter of about 80 μm . Next, three parts to sense are antennae, maxillary palps, and eyes, which helps mosquitoes to hear, taste, and see, respectively. The antennae in mosquitoes are about 1 mm long, and mosquitoes hear using them when vibrations are induced in the antennae by its surroundings (Johnston 1855). The maxillary palps taste various components of air, such as carbon dioxide (Kellogg 1970; Jones et al. 2007). As always, eyes are used by mosquitoes to see (Gao et al. 2007). The three sensing parts, antennae, maxillary palps, and eyes, exist in pairs. Next, two parts to move are wings and legs; however, the former is used more. Mosquitoes have a pair of wings and three pairs of legs. The wings are about 3–6 mm long and about 1 mm wide (Christophers 1960). The legs are about 7–10 mm long. Moreover, a mosquito weighs about 1–4 mg.

The features mentioned, however, do not appear the same for both sexes of mosquitoes, as shown in Fig. 3 (bottom) (Gordon and Lavoipierre 1962; Gurera et al. 2018). Visually, male mosquitoes are smaller in size, have longer maxillary palps, and have more bushy antennae (Foster and Walker 2009). There is also a difference in their hearing frequency. Best frequency of the female antennae is about 230 Hz and the male's is about 380 Hz. The males' best frequency happens to be the fundamental frequency of females flying, so that they may need it to find their mates (Clements 1999). Length of the female antenna is about 2 mm and male's mosquito is about 1.5 mm. Male and female mosquitoes also have different dietary preferences. Male mosquitoes feed on plant nectar instead of blood. Since the males do not have to pierce the skin, the piercing subparts of the proboscis are not used and they become nonfunctional.

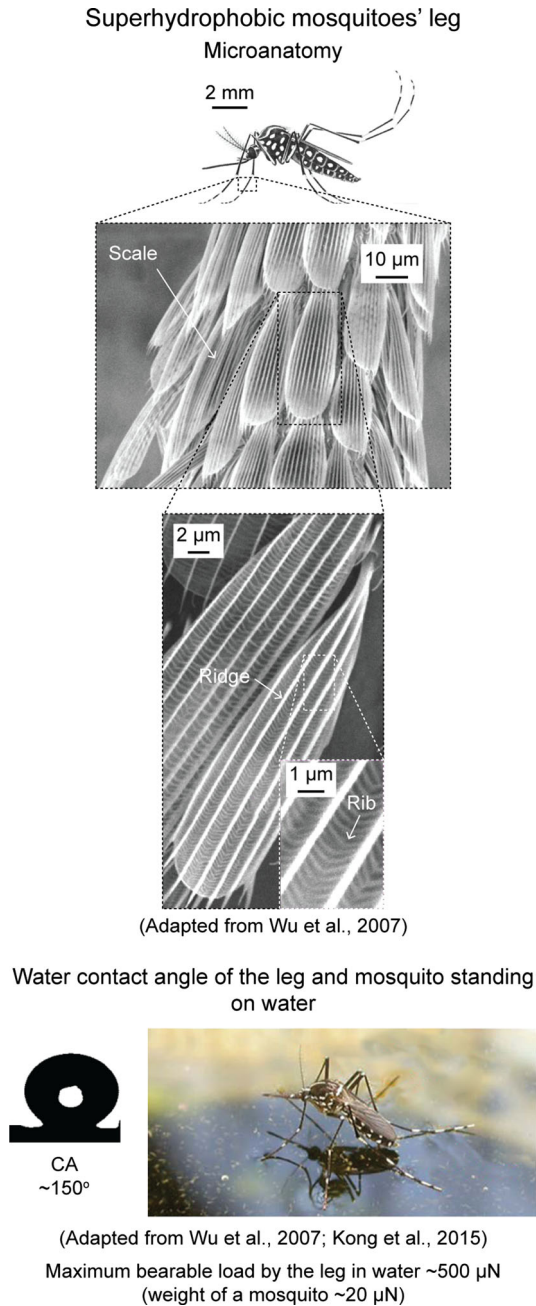


Fig. 5 Mosquitoes' legs are covered in micro- and nanostructures (top), which give them the ability to stand in water. (Bottom) A water contact angle measurement on the leg and a mosquito standing on water

Dry adhesion in mosquitoes' foot and its locomotion

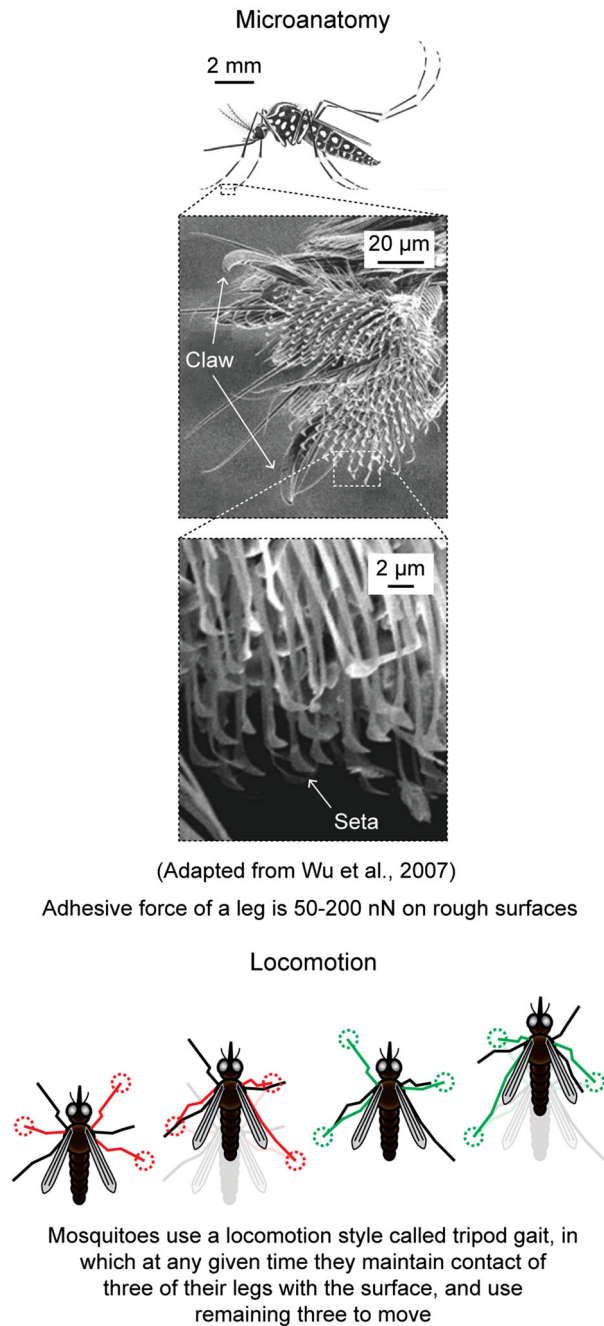


Fig. 6 Mosquitoes' feet have a combination of claws and setae (top). Claws help them attach to rough surfaces and setae help them attach to smooth surfaces. Mosquitoes use tripod gait to move (bottom). The dotted circles represent foot adhered to the surface

2.1 Locomotion

As shown in Fig. 4, mosquitoes have three unusual locomotory attributes: standing on water, sticking to any surface, and flying in rain (Dickerson et al. 2012; Kong et al.

2015; Gurera 2018a). These attributes give them an edge during hunting of a host.

2.1.1 Standing on water

As discussed before, mosquitoes have six legs, which are about 7–10 mm long, and its diameter ranges from about 200 μm at the base, to about 80 μm at the tip (Wu et al. 2007). The leg consists of three parts: femur, tibia, and tarsus. The femur is attached directly to the body and tarsus is the farthest part of the leg from its body. Tarsus is about 6 mm long whereas the other two parts are about 2.5 mm long. Being the thinnest part, the tarsus is more flexible. These parts, in turn, are attached together via joints.

As shown in Fig. 5 (top), the legs are covered with hierarchically microstructured scales (Wu et al. 2007; Gurera 2018a). The microstructures of the scales consist of longitudinally running ridges and cross running ribs. This, along with the orientation of the scales, along with the leg, provide superhydrophobicity with water contact angle $> 150^\circ$ on the surface, as shown in Fig. 5 (bottom). This

Mosquitoes' hydrophobic wings and antifogging eyes

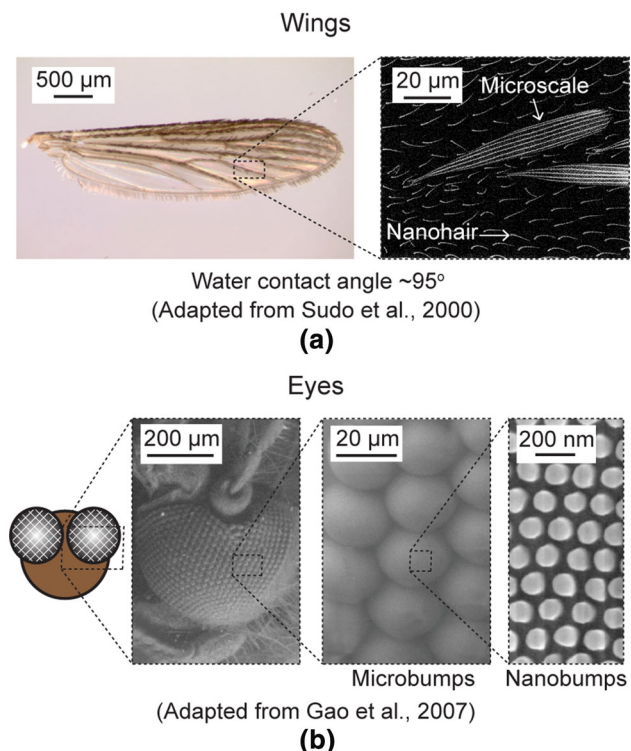


Fig. 7 **a** Mosquitoes wings have a combination of microscales and nanohairs, which makes the wing hydrophobic, and gives mosquitoes ability to fly in rain. **b** Mosquitoes' hemispherical eyes consist of hexagonally arranged micro- and nanobumps. The hierarchically structured eyes repel water and give mosquitoes ability to see in the fog

water repellency gives mosquitoes the ability to stand on water.

When standing on water, only the distal part of the leg, known as tarsus, is in contact with water (Kong et al. 2015). It has been reported that a tarsus can take load up to 20 times the weight of the mosquito, and the whole leg can take up to 23 times the weight of a mosquito. There are two key factors playing role in making tarsus such an efficient weight bearer: flexibility and angle of stepping in the water.

Since the tarsus is flexible, it means it can bend easily at a load. When a tarsus is pressed against water, it will continue to deform above the surface of the water. If it would not have been flexible, for the same load, the contact area of the tarsus and the water would have been lesser, and the tarsus would have pierced the water easily. However, in the case of actual tarsus, the contact area will keep on increasing and hence total pressure on water will decrease. Therefore, it will be difficult for tarsus to pierce water easily.

The tarsus is at an inclination when it comes in contact with water. The inclination angle also affects the standing abilities of mosquitoes. Similar theory, which was just discussed, can be applied here too—when the angles are low, they result in more contact area, hence lesser pressure is applied. This low pressure can account for the high-bearing loads. It has been reported that the largest forces appear in a range of 0°–20° inclination angle. At an inclination angle of about 70°, the bearing load appears to diminish, since the leg pierces water as soon as the contact begins (Kong et al. 2015). In practice, mosquitoes can change this angle by flexing or extending their legs accordingly.

2.1.2 Sticking to any surface

As shown in Fig. 6 (top), mosquitoes feet are a combination of claws and setae that help them attach (Wu et al. 2007; Chapman et al. 2013; Gurera 2018a). The distal part of the tarsus is referred to as the foot. The claws are used to attach to rough surfaces and the setae are used to attach to smoother surfaces. The setae at their end have a pad with a contact area of about $2\ \mu\text{m}^2$. These pads attach to a smoother surface using van der Waals force, in a manner similar to geckos. The mechanism can generate 50–200 nN force per leg (Pashazanusi et al. 2017). However, these forces are on the higher side if the surface is rough. The wettability of the surface, to which mosquitoes attach, also changes the mechanism of attaching. If the surface is hydrophobic, van der Waals forces are predominant. If the surface is hydrophilic, capillary forces also played a role to increase the adhesive force.

After attaching to a surface, it uses tripod gait, which is common in most six-legged arthropods, as shown in Fig. 6 (bottom). In the gait, at any given time it maintains contact of three of their legs with the surface, and use remaining three to move.

2.1.3 Flying in air and rain

Leading edge vortex is a commonly used mechanism for flying in nature (Dudley 2002). In the mechanism, flying species get an extra lift, by developing a vortex formed at the front edge of wings. The vortex decreases air pressure at the top of the wing, and gives them a lift. However, in the case of mosquitoes, the trailing edge vortex plays a crucial role in generating the lift, which is exclusive to mosquitoes (Bomphrey et al. 2017). During flapping, as the back edge of the wing flips around, it captures the swirling wave of the previous flap, allowing it to capture some of that energy. Another exclusive feature of mosquitoes is its high flapping frequency, of about 700 Hz, and low flapping amplitude of about 40°. The flapping amplitude is less than half of any other flying animal studied. The authors are unsure of any advantage of this mechanism.

When flying in rain, microscales and nanohairs on the wing, as shown in Fig. 7a, are beneficial (Sudo et al. 2000; Gurera 2018a). The structures help the wings remain hydrophobic, water contact angle on the wing is about 95° (Pal 1950). Only the veins of the wing are covered with microscales (Chapman et al. 2013), and the length of a scale is about 90 μm . The wing is also covered with hairs, which measure about 35 μm in length. These features are beneficial to mosquitoes during flying in rain.

Given the fact that the mass ratio of a raindrop to mosquito can be 2–50, a raindrop can produce a substantially larger force than the weight of a mosquito. Legs and wings are the most probable area of an impact since they are longest body parts of mosquitoes. The insect may be killed if it is flying too close to the ground. After getting an impact it takes the insect about 15–60 mm distance to recover (Dickerson et al. 2012). Also after recovering from an impact of a raindrop, especially, on its wing, it rotates up to an angle about 50°.

Flying in a variety of conditions, like fog, may adversely affect mosquito wing. In fog, as compared to rain, drops are smaller; a raindrop is on the order of 1000–5000 μm (NASA 2016), and a fog droplet is on the order of 1–50 μm in diameter (United Nations Environment Programme 1998). So, when a water drop is smaller than the distance between micro-asperities, it will follow the Wenzel regime, and stick to the surface. After an accumulation of drops, they all will coalesce to form a big drop that is attached to the wing. It has been reported that the big drop's water tension will be enough to overcome the bending stiffness of

Microanatomy of mosquitoes' proboscis

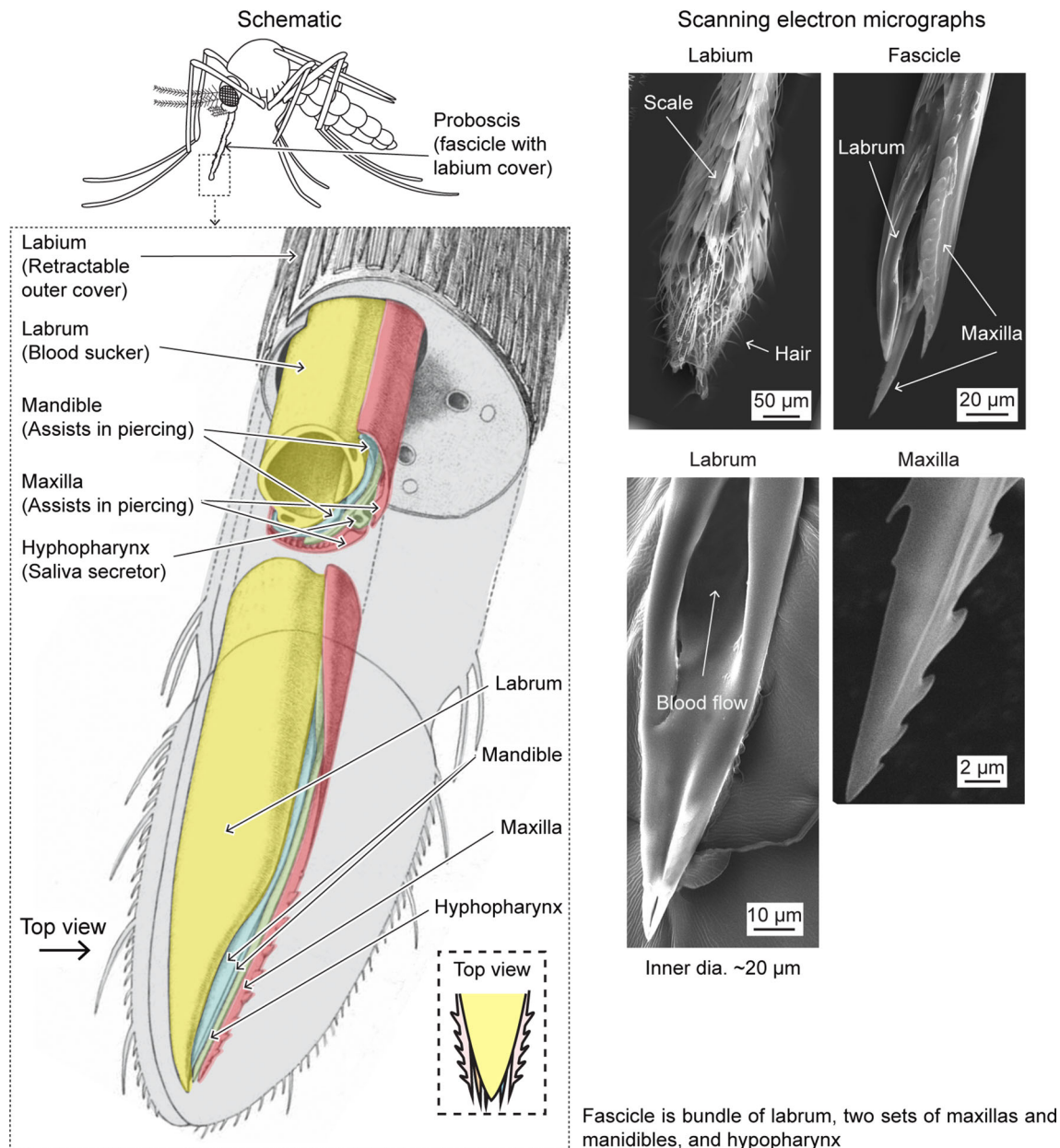


Fig. 8 The left column presents a schematic showing microanatomy of the mosquitoes' proboscis. The right column presents SEMs of the stylets of the proboscis (adapted from Gurera et al. 2018)

the wing (Dickerson et al. 2015). Most of the stiffness of wings are provided by its veins. However, they run in a longitudinal direction, hence the drop gets folded in the transverse direction. The authors of the study report that when a wing thickness is less than 50 µm, the wing folds; and thickness of mosquitoes' wing is on the order of 10 µm. In practice, mosquitoes attempt to remove the excess water by increasing flapping frequency, hard landing, and/or taking off at different angles from the ground.

Mosquitoes also have antifogging eyes, which help them to keep their vision clear (Gao et al. 2007). During fog, the wetting issue that existed on the wings does not exist in the case of the eyes. On the wing, the space between the asperities is more than the fog drop diameter. In the case of the eyes, it is not so. As shown in Fig. 7b, the eyes consist of hexagonally, closely packed microbumps with a diameter of about 25 µm, and loosely packed nanobumps with a diameter of about 100 nm and a spacing of about 50 nm (Gao et al. 2007). Since the spacing is less than the fog

Feeding process of mosquitoes

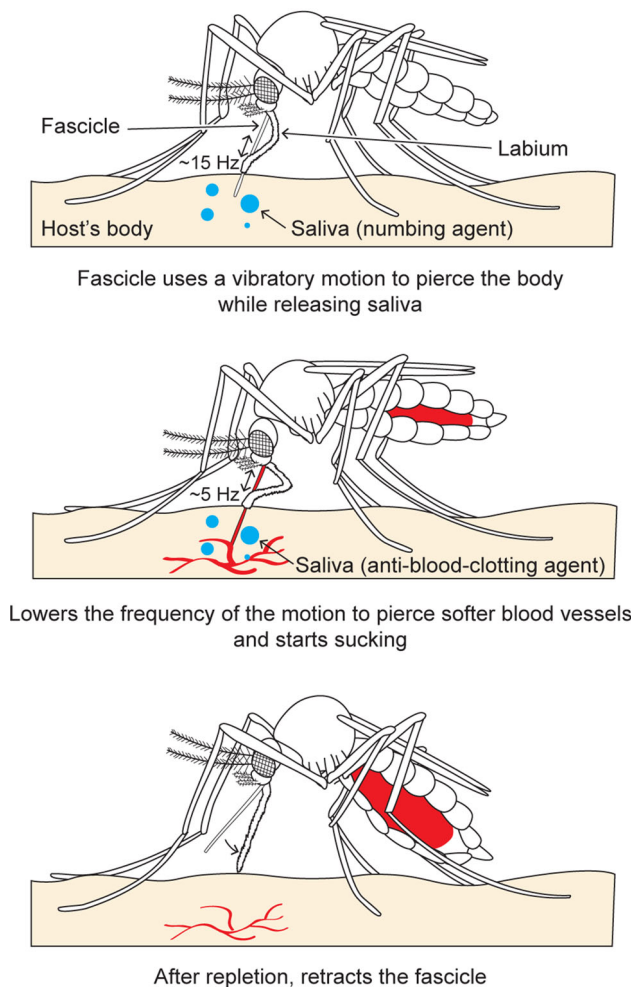


Fig. 9 Schematics of various steps in the feeding process used by mosquitoes (adapted from Gurera et al. 2018)

drop diameter, which is 10–50 μm in diameter, the drops follow Cassie–Baxter regime. The regime states that there is an air pocket formation between the drop and the surface; hence, the drop can roll-off easily. In addition, since the eyes, on a macroscale, are hemispherical, the rolling of drops may take place in real time.

2.1.4 Summary

Mosquitoes can stand on water, stick to any surface, and fly in rain. Mosquitoes are able to stand on water because of their hydrophobic legs, which are covered with hierarchically structured scales. They are able to stick to any surface due to dry adhesion in their feet, which is due to a combination of claws and setae. The foot can apply an adhesive force of one to two orders of nN on a rough surface. Lastly, mosquitoes are able to fly in rain because of their hydrophobic wings covered with microscale and

nanohairs and antifogging eyes covered with micro- and nanobumps.

2.2 Painless piercing

2.2.1 Microanatomy

Mosquitoes feed on a host using their mouthpart, known as the proboscis. A detailed schematic of the proboscis (left column) and its various scanning electron micrographs (SEM) (right column) are shown in Fig. 8 (Jones 1978; Gurera et al. 2018). The proboscis is a collective term used for mosquito's fascicle and its retractable, thick, outer cover, known as labium, as shown in the top of the figure. The labium being blunt does not pierce the host and retracts during the piercing. However, the fascicle, being sharper, pierces the host (Gurera et al. 2018).

A fascicle is a term for the bundle of stylets, shown in Fig. 8. The largest of them is a pointed and hollow stylet, the labrum, which sucks the blood. The others include a pair each of sharp mandibles and serrated-shaped maxilla, which sit on both sides of the labrum. These provide a major contribution to piercing since these are sharp and serrated. The maxilla, having serrated edges, also helps anchor the fascicle in place once inside the host. The final stylet of the fascicle is the hypopharynx which releases saliva during the initial piercing (Choumet et al. 2012; Gurera et al. 2018).

The mosquitoes' saliva serves several purposes including numbing pain during piercing and stopping the blood clotting during sucking. Mosquitoes' salivary glands contain 0.3 μg of protein with more than 1000 compositions which are responsible for various functions (Ribeiro et al. 2001). As an example for anopheline mosquitoes, anophensin family of protein, which is responsible for numbing, inhibits growth of a pain-inducing peptide known as bradykinin (Ribeiro et al. 2010). For the same species of mosquitoes, anophelin family of protein, which is responsible for anti-blood-clotting, inhibits processing of fibrinogen to fibrin protein that is known to clot the blood. Incidentally, the malaria parasite is also transferred into the host via this saliva (Gurera et al. 2018).

SEM micrographs of labium, fascicle, labrum, and maxilla are shown in the right column of Fig. 8. The labium is covered with scales all around it and has hairs at its tip. The hairs help in sensing a suitable piercing spot on the host (Foster and Walker 2009). The fascicle is shown with the labrum and a pair of maxilla bundled together. The labrum is shown which has an outer diameter of about 30 μm and the inner diameter of about 20 μm . The maxilla is shown with the serrated edges (Gurera et al. 2018).

2.2.2 Feeding

A detailed schematic of the feeding process of mosquitoes is shown in Fig. 9 (Jones 1978; Gurera et al. 2018). First, mosquitoes search for a suitable spot on the surface of the host, using the tip of its labium. After locating a spot, the fascicle is inserted inside the host at about 15 Hz with vibratory motion of about 40–90 μm (Kong and Wu 2010). The saliva, which acts as a numbing agent, is released under the skin by the hypopharynx (Gurera et al. 2018).

The insertion force used by mosquitoes is mostly in the range of 10–20 μN , and is about three times lower than the lowest reported insertion force reported by an artificial needle. When insertion forces are lowered, lesser tissue area will be deformed, which reduces nerve sensation. In other words, lesser pain or sensation will be felt by the host. Therefore, this vibratory motion is believed to help in reducing pain in the host. It is known that vibratory actuation in needles helps to reduce the insertion force. It should be noted that the labium is a retractable outer cover and it does not pierce but provides lateral support to the fascicle (Ramasubramanian et al. 2008). Without the labium, the fascicle will not be successful in piercing and will buckle instead (Gurera et al. 2018).

Next, once inside the host, the labrum constantly searches for a blood vessel to pierce in. The labrum being flexible, is able to bend at about 90° , and also can be maneuvered in any direction (Gordon and Lumsden 1939). It uses specialized sensors at the tip of the labrum which sense blood vessels (Hudson 1970; Foster and Walker 2009). It moves fascicle around in the skin tissue searching for an ideal place to tap for the blood source. After locating a blood vessel, a vibratory motion is used again to pierce in. However, this time only the labrum pierces and at a lowered frequency of about 5 Hz (Gurera et al. 2018). It uses a specialized pumping system for suction through its feeding tube, labrum. Mosquitoes use about 8 kPa pressure to suck blood out of their host (Chapman, et al. 2013). During the blood sucking, the injected saliva, in addition to acting as a numbing agent mentioned earlier, prevent blood clotting, prevent inflammation and keep the blood flowing.

There are reasons for the lowered frequency at end of the piercing process (Gurera et al. 2018). First, the material to be pierced, the blood vessel, is a softer material as compared to the skin. Therefore, the less vigorous motion will do the work. Second, there is an increase in friction between the labrum and the host's tissues. As the labrum keeps going inside the host, there will be an increase in contact area between labrum and the host. That may increase the frictional forces, hence mosquitoes are unable to keep up the same frequency.

Finally, after the mosquito is repleted, the fascicle is retracted in the labium cover.

Indentation locations on the labrums' tip

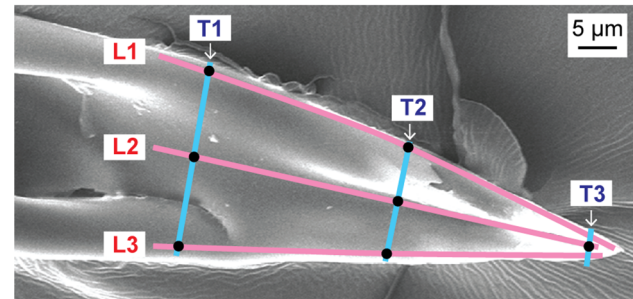


Fig. 10 The labrum's tip was characterized at presented seven locations. The locations were formed by the intersection of three longitudinal lines, L1–L3, with three transverse lines, T1–T3 (Gurera et al. 2018)

For completeness, it should be noted that finding a suitable location on the host can take on the order of 5–30 s depending on the age of a mosquito. Once inside the host, finding a blood vessel can take on the order of 150–400 s. The blood feeding can take on the order of 300 s. The average amount of sucked blood by mosquitoes is on the order of few milligrams, which is comparable to their bodyweight (Nayar and Sauerman 1975). Mosquito piercing generally cause a localized inflammatory response. The itchy bump one gets from mosquito bite is caused by dilating of blood vessels and host body's immune response to mosquito saliva, which is an allergen. Itching stops in several hours after piercing.

To sum up, in the painless piercing process, the tip of the labium is used to search for a suitable location on the host. Once the location is found, the fascicle pierces in and the saliva, which acts as a numbing agent, is released by the hypopharynx. A vibratory motion is used to pierce the sharp mandible and serrated maxilla inside the host. The serration in the maxilla also helps in keeping the parts anchored to the host's tissues. The wider labrum helps the entire fascicle pierce the skin. Once the labrum is in, mosquitoes lower their vibration frequency and start looking for a blood vessel using the sensor located at tip of the labrum. After locating a blood vessel, the labrum pierces the blood vessels and starts sucking in blood, until repletion. During the blood sucking, the injected saliva, in addition to acting as a numbing agent, prevent blood clotting, prevent inflammation and keep the blood flowing. Finally, the fascicle retracts and it goes back inside the labium cover (Gurera et al. 2018).

2.2.3 Nanomechanical property measurements of labium

To further understand the piercing process, material properties of mosquitoes' mouthparts are needed. During piercing, the thin mandible and maxilla initiate the

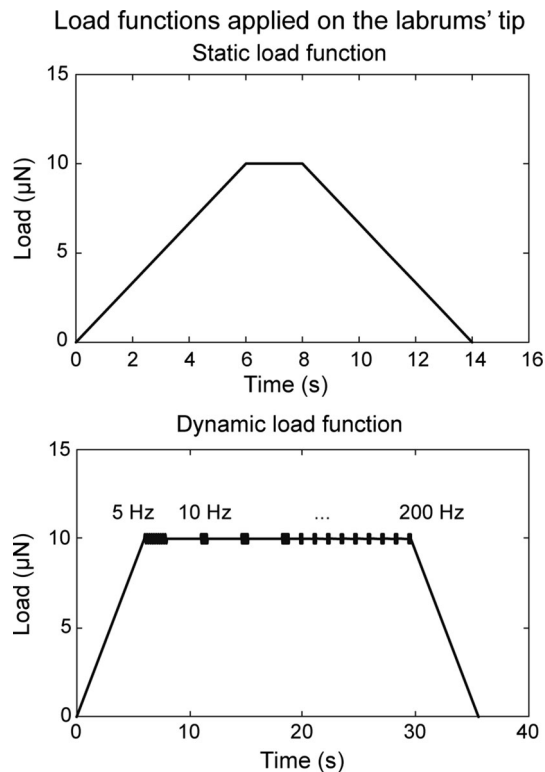


Fig. 11 Labrum's tip was characterized using two loading functions: static (top) and dynamic (bottom). The static load function has a peak load of 10 μN . The dynamic load function has a quasistatic load of 10 μN and dynamic load of 0.3 μN . The tested frequencies were 5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 Hz (Gurera et al. 2018)

piercing, but it is the labrum, which is about 35 times wider, which helps in the insertion of the entire fascicle into a host. Therefore, the labrum's tip has been chosen for nanomechanical characterization (Gurera et al. 2018).

2.2.3.1 Mosquito species selection The type of mosquito to be used in this study were selected based on its dietary preference and availability (Gurera et al. 2018). Females were selected because, as discussed earlier, only they feed on humans, whereas the males feed on plants. Among the females, a specie named *Aedes vexans* was chosen because they are aggressive piercers, high in population, and are found near residential areas (DeGroote et al. 2007). Moreover, it is one of the common species with an epidemiological significance (Lemine et al. 2017) as it was among the responsible species for spreading West Nile Virus across North America in 1999.

2.2.3.2 Nanomechanical characterization technique In order to measure local properties as well as gradients in mechanical properties over small regions of labrum with a thickness of about 10 μm , nanomechanical property measurements are needed with depth of penetration of few

hundred nm. A depth-sensing nanoindentation technique was used to measure elastic modulus and hardness under static and dynamic conditions (Bhushan et al. 1996; Bhushan 2017). A TI 950 TriboIndenter[®] (Hysitron, Inc., Minneapolis, Minnesota, USA), with a Berkovich tip (tip radius is on the order of 100 nm), calibrated with fused silica was used. In the technique, a ramp-up load is applied to the sample using a nanoprobe. As the load is being applied, the load and the probe's penetration depth are continuously measured. The load vs penetration depth data was used to calculate elastic modulus and nanohardness (Gurera et al. 2018).

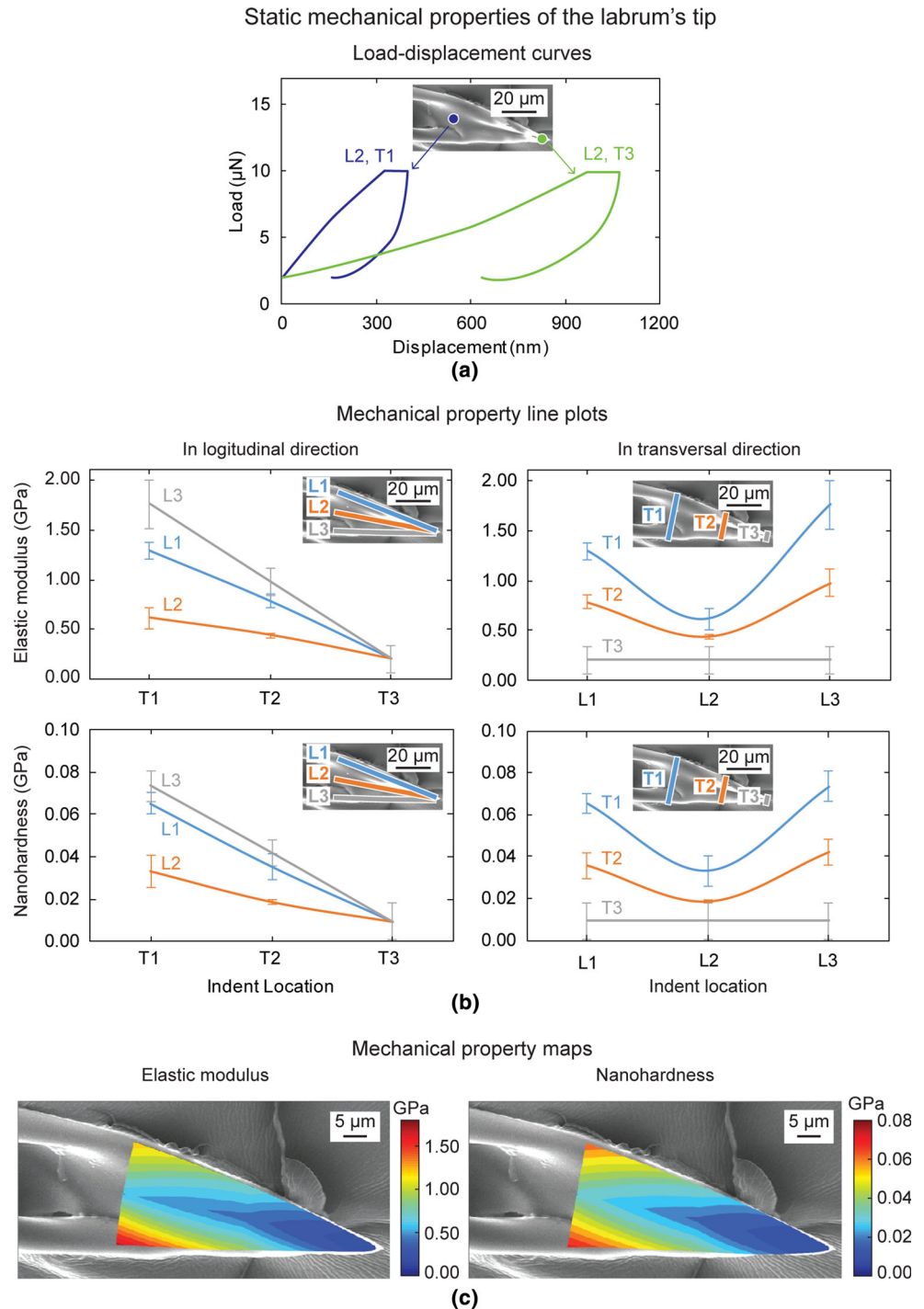
The labrum samples were prepared by dissecting the labrum from a mosquito and pasting it on a glass substrate using double sided tape. To characterize the labrum's tip, seven indentation locations distributed uniformly across the tip were chosen as shown in Fig. 10 (Gurera et al. 2018). The seven locations are defined by the intersection of three non-parallel longitudinal lines, L1–L3, with three parallel transverse lines, T1–T3. A nomenclature of the locations has also been chosen in the study. For example, the location formed by the intersection of L2 and T1 will be called as “L2, T1”.

Biological materials have viscoelastic material properties which are loading rate dependent. Since mosquitoes vibrate their labrum to pierce into their host, their properties were measured in dynamic conditions, in addition to static conditions. As mentioned before, mosquitoes use the insertion load of 10–20 μN while piercing a prey and the frequency of 5–15 Hz. Therefore, the measurements were made at a static load of 10 μN and at an oscillating load of the frequency ranging from 5 to 200 Hz, to measure static and dynamic effects, respectively (Gurera et al. 2018).

For static properties, a trapezoidal shaped load function was used in which the sample is loaded to 10 μN in 6 s, the load is held for 2 s, and then it is unloaded to zero-load in the last 6 s, shown in Fig. 11 (Gurera et al. 2018). The load cycle was based on the standard protocol used in nanoindentation studies (Hysitron 2018). For dynamic properties, a small dynamic load of 0.2 μN amplitude and a frequency sweeping from 5 to 200 Hz were superimposed on a static load of 10 μN , shown in Fig. 11. The frequencies used were 5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 Hz.

Measurements were made on five different labrum samples for each type of measurements, and their average values are reported. For each sample, all of the seven locations were tested right after the sample preparation. This was done because it is known that desiccation affects mechanical properties of a biological material. The penetration depths of all the presented data were measured to be less than 10% of the labrum's thickness—10 μm , except for the location—“L2, T3”, at end of the tip. This implies

Fig. 12 **a** Representative load–displacement curves at two locations—“L2, T1” and “L2, T3”. **b** Mechanical property line plots and **c** maps of elastic modulus and nanohardness. The tip has a gradient in mechanical properties, which decrease along L1–L3, and has a dip along T1–T3. The stiffness and hardness decrease along the tip and is lower in the middle across the tip (Gurera et al. 2018)



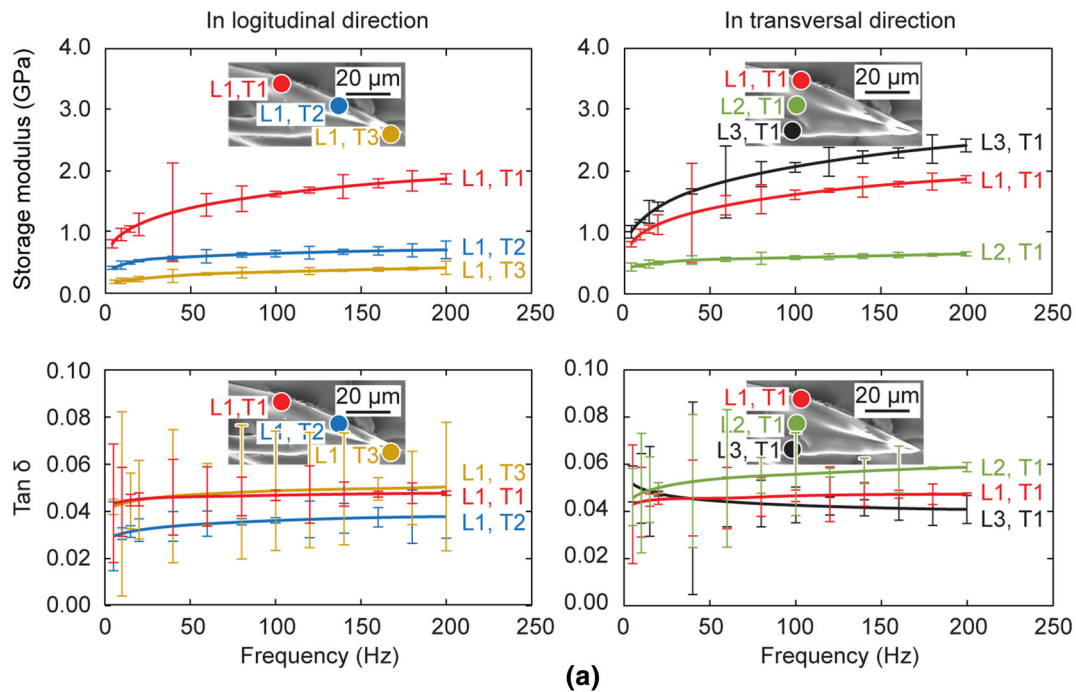
that there was no effect on the substrate on which the labrums were mounted. The data were taken in a temperature- and humidity-controlled environment, in which the temperature and relative humidity ranged from 21 to 23 °C and 40–50%, respectively (Gurera et al. 2018).

2.2.3.3 Experimental data Static properties data of the labrum's tip is presented in Fig. 12 (Gurera et al. 2018).

Figure 12a shows representative load–displacement data on two extreme locations. Note that the material creeps when the load is held for 2 s. Of the two locations, “L2, T1” and “L2, T3”, higher displacement was observed in the latter one.

Trends observed in the mechanical properties of the labrum's tip are shown as line plots in Fig. 12b and as maps in Fig. 12c. To understand the trends, discussion on

Dynamic mechanical properties of the labrum's tip Mechanical property line plots



Mechanical property maps

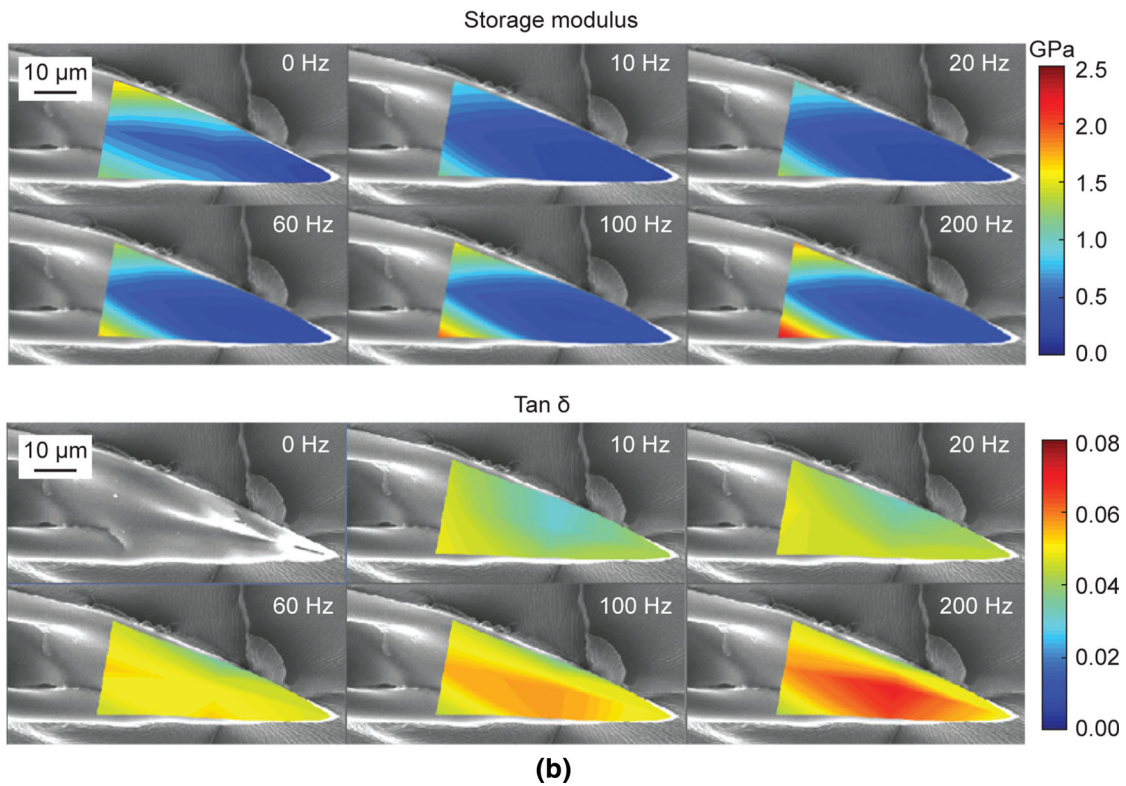


Fig. 13 **a** Line plots and **b** maps of storage modulus and $\tan \delta$ as a function of frequency. Storage modulus increases with the increase in the frequency, however the $\tan \delta$ does not change. Irrespective of the

frequency, the stiffness decreases along the tip and has lower stiffness in the middle across the tip (Gurera et al. 2018)

the plots follows. In Fig. 12b, the left and the right columns show the trends along L1–L3, and T1–T3, respectively. The solid lines shown are the average values and the error bars shown are one standard deviation from the average. It is observed that the elastic modulus decreases along L1–L3. Elastic modulus is a measure of elastic deformation and is also referred as stiffness. This implies that the end of the tip is compliant. It is also observed that the modulus along L2 is lower than along the adjacent L1 and L3. This implies that the tip is stiffer on sides as compared to its middle (Gurera et al. 2018).

Similar trends were observed in the case of nanohardness (Gurera et al. 2018). Hardness is a measure of resistance to plastic deformation. It is observed that the nanohardness along L2 is lower than along the adjacent L1 and L3. This implies that the tip is softer at end of the tip. It should be noted that the labrum tip is one order less hard than another piercing part, the human tooth (He and Swain 2011).

Dynamic properties data of the labrum's tip is shown in Fig. 13 (Gurera et al. 2018). Figure 13a shows trends in mechanical properties as a function of the frequency in a form of line plots and as maps in Fig. 13b. To facilitate the understanding of the trends, a discussion of the line plots follows. In Fig. 13a, the left and the right column shows the mechanical properties at all locations along L1, and at all locations along T1, respectively. The mechanical properties presented are storage modulus and $\tan \delta$. The solid-lines shown are curve fitted lines and the error bars are deviation of the average values from the fitted lines (Gurera et al. 2018).

Trends for storage modulus are presented, which are similar to those of elastic modulus. Storage modulus is a measure of elastic properties of the material. The storage modulus was found to be decreasing along L1–L3, however, only the trend along L1 is shown. As before, the modulus along L2 was also found to be lower, as compared to the sides L1 and L3. In addition, it was found that the magnitude of the storage modulus increases with the frequency, which implies that labrum's tip gets stiffer with increase in the frequency (Gurera et al. 2018).

Figure 13 also presents trends in $\tan \delta$ vs frequency (Gurera et al. 2018). $\tan \delta$ is a ratio of viscous properties to elastic properties. It is a measure of the amount of viscosity in a material as compared to its elasticity. It is the parameter used for comparing the dynamic nature of different materials. Given the scatter in the data, there appear to be no trends in the $\tan \delta$ values. However, the data is of the same order as that of dragonflies' wing (Sun and Bhushan 2012) and beetles' cuticle (Sun et al. 2016).

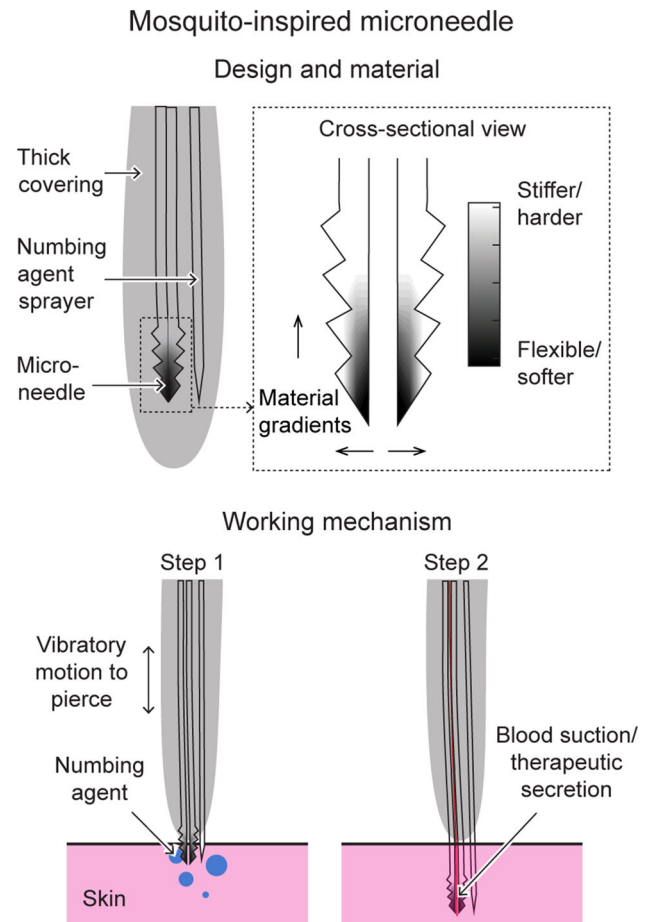


Fig. 14 Conceptual schematic of a painless mosquito-inspired microneedle, along with its material and design (top) and its working mechanism (bottom) (adapted from Gurera et al. 2018)

2.2.4 Relevance of nanomechanical properties in piercing mechanism

It is noted that elastic modulus and nanohardness decrease along the labrum, with end of the tip being most compliant and softest. Across the labrum, the properties are lowest in the middle. It is also noted that the labrum gets stiffer at higher frequencies. It is expected that the more compliant and softer end of the tip may cause lesser pain while piercing the host, because it will deform the skin less. The lesser deformation sensed by the nerve endings in the skin, will cause lesser pain. The end of the tip may also be compliant and soft because of the fact that it is used for sensing blood vessels inside the host. Furthermore, the stiffer and harder sides of the tip will also help the labrum to resist bending/buckling during piercing. Finally, increase in stiffness at the high frequency may maintain lower deformation at higher stresses (Gurera et al. 2018).

2.3 Lessons from mosquito piercing and conceptual schematic of a painless mosquito-inspired microneedle

A mosquito can insert a needle-like fascicle into host skin and draws blood for several minutes without host even noticing. The mechanism behind the painless piercing includes the combination of the frequency dependent graded mechanical properties, numbing agent secretion, serrated-design, and vibratory actuation (Gurera et al. 2018). As discussed earlier, the most compliant and softest end of the labrum's tip as compared to the rest of it, reduces the pain felt by the host. However, the compliant and soft end of the tip will increase the insertion force. To compensate for that increase, the serrated design and the vibratory actuation assist in easy insertion. The serrated design increases the number of high-stressed points on the skin, which means easy insertion, and vibratory actuation has also been known to lower the insertion force. In addition, the vibratory motion has also been known to increase stiffness of the labrum, which will limit any excessive deformation in the labrum's tip during the vibratory piercing. The pain will also be further reduced by the numbing agent secretion (Gurera et al. 2018).

There are conical-shaped microneedles commercially available that are made up of a medical grade polymer, of length on the order of 500–1500 μm (3M 2015; Brady and Donnelly 2016; NanoPass Technologies Ltd 2017). However, they induce pain when inserted. One can learn from the mosquito's piercing mechanism and this understanding could be used as a starting point to create a painless microneedle for biomedical purposes. A conceptual schematic of a painless, bioinspired microneedle has been proposed, shown in Fig. 14 (Gurera et al. 2018).

In Fig. 14 (top), the microneedle has a channel for fluid passage and serrated design, similar to the labrum and the maxilla, respectively (Gurera et al. 2018). The microneedle is also accompanied by a numbing agent secretor, which together are enclosed within a thick covering, similar to the hypopharynx and the labium, respectively. The tip of the microneedle shown in the cross-sectional detailed view is based on the combination of the mechanical properties discussed earlier. The microneedle is made up of a viscoelastic material having gradients in mechanical properties in both longitudinal and transverse directions similar to that of the labrum.

The microneedle also has a working mechanism similar to as of mosquitoes, as shown in Fig. 14 (bottom) (Gurera et al. 2018). The microneedle would be inserted using a vibratory actuation, which along with the serrated design to assist in piercing. At the start of insertion, a numbing agent would be secreted. The factors responsible for easy

insertion, along with the numbing agent secretion and the longitudinal gradient in mechanical properties are believed to reduce the pain. The lateral material gradient along with the increasing stiffness with the frequency will also help in a successful piercing, by keeping the microneedle away from bending/buckling and having higher deformations at higher stresses, respectively. In addition, the outer covering will prevent the entire microneedle from buckling. After successful insertion, either blood can be sucked or a therapeutic agent can be secreted, based on the need (Gurera et al. 2018).

2.4 Summary

Among various medically important arthropods which feed on living hosts, mosquitoes' piercing spreads viruses which causes the highest number of deaths annually. The primary cause of the deaths is malaria, which is spread by infected mosquitoes' piercing. Lessons from mosquito locomotion and painless piercing are presented. In order to survive, mosquitoes need to hunt for a host for blood. It is relatively easy for mosquitoes since they possess unusual locomotory attributes: standing on water, sticking to any surface and flying in rain, because of their superhydrophobic legs, dry adhesion in the foot, and hydrophobic wings and antifogging eyes, respectively. Mosquitoes pierce using their fascicle, which is a bundle of coherently functioning six stylets. Mosquitoes painlessly pierce using a combination of the numbing by releasing saliva to act as a numbing agent, the fascicle's serrated design, the vibratory actuation, and the graded and frequency-dependent mechanical properties of the labrum with a soft tip. Once they pierce the blood vessel, they suck the blood until repletion. After the repletion, the fascicle is retracted. Based on this understanding, conceptual schematic of a painless mosquito-inspired microneedle has been proposed (Gurera et al. 2018).

3 Wasp stinging

Female wasps sting in a manner similar to that of mosquitoes (Ross and Matthews 1991). The stingers provide an effective insertion into the victim's skin (Akre et al. 1981; Schmidt 2009). The proximal force required for wasp and honeybee stingers to pierce the skin are on the order of few mN. These stingers have tapered geometry, graded mechanical properties and refined insertion mechanics, which allow them to penetrate with very low puncturing force which prevents mechanical failure. The insight into stinger insertion mechanics can be translated into engineering design of a sophisticated microneedle system for biomedical applications.

Microanatomy of wasps' stinger

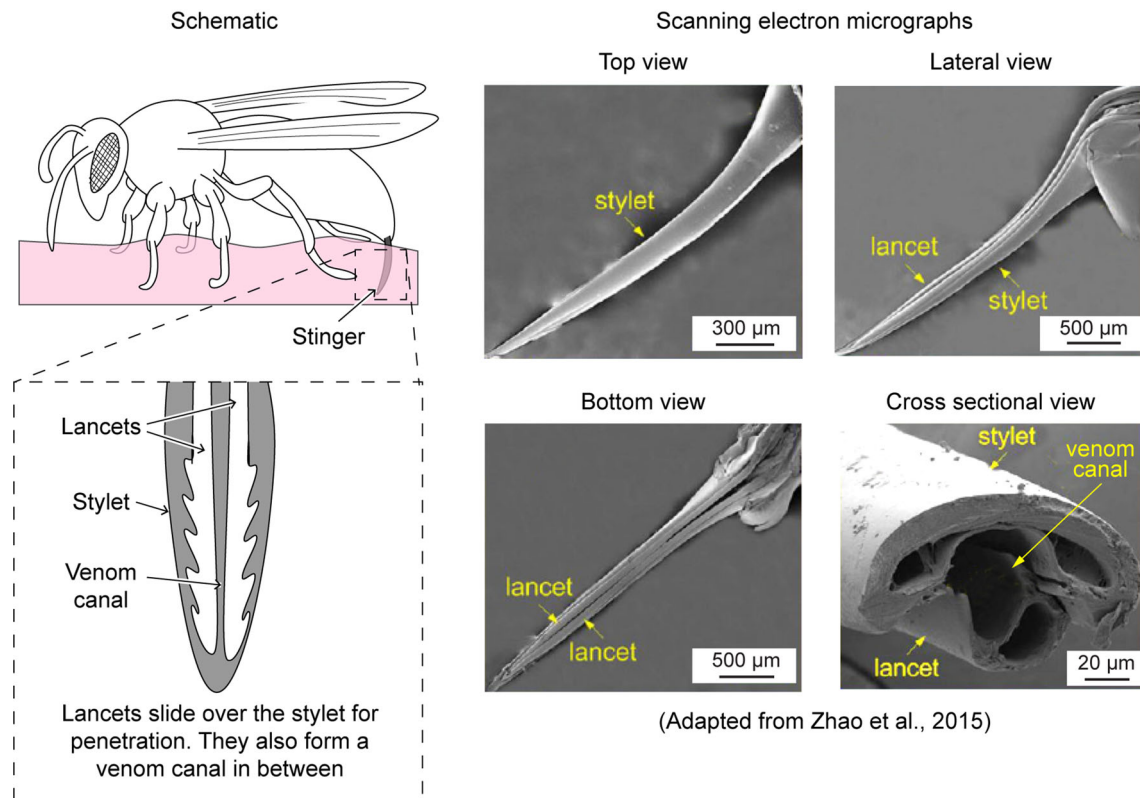


Fig. 15 The left column present a schematic showing microanatomy of the wasp stinger. The right column presents SEMs of the subparts of the stinger

Structure, geometry, nanomechanical properties, and insertion mechanics of stingers of various species have been studied (Politi et al. 2012; Wu et al. 2014; Zhao et al. 2015, 2018; Das et al. 2018).

In this section, an overview of wasp's microanatomy and stinging process is presented. Next, based on Das et al. (2018), an overview of structure, nanomechanical properties and modeling of the penetration process of wasp stingers are presented. Based on the understanding of the penetration mechanism, a design of a microneedle is presented.

3.1 Microanatomy and stinging process

The word, wasp, originated from a German word “webh” which means to weave. This was in reference to the nests they construct. Few of the examples of such wasps are tarantula hawk wasp and paper wasp. The body of a wasp is broadly divided into three parts—head, thorax and abdomen. The head contains a pair of sensory antennae, mouthparts for biting and licking, and two kidney-shaped clusters of compound eyes and simple eyes. The antennae are primarily the sensing structures, which help them hear, taste, and smell (Chapman et al. 2013). The biting mouth

part is a pair of mandible, which helps them in capturing, subduing, and processing prey (Schmidt 2009). The mandibles work as their jaws. They can chew wood fibers from dead trees and can cut through tough insect nets in a small amount of time. Their diet preference includes plants and other arthropods. The two different eyes serve different purposes. The compound eyes have multiple lens in it to gather views from the side. The simple eyes have one lens and helps to see in front of them. Moreover, inside the head, a tiny brain tends to the tasks at hand. Next, the thorax features six spindly legs and a pair of membranous wings. The legs and wings in many species provide enough strength for the wasp to fly away with a paralyzed victim in its grips. Lastly, the abdomen contains the majority of the wasps' organ systems and, in female wasps, a deadly stinger for self-defense and hunting. Females are bigger in size, since they produce eggs in their abdomen and they have the venom sac (Akre et al. 1981; Schmidt 2009; Gurera 2018b).

Although well known for their stinging ability, the wasps do not capture or subdue prey with the sting. Wasps capture their preys using their mandibles. The stinging is entirely used for defense, primarily against vertebrate predators. The Wasp sting hurts and produces pain,

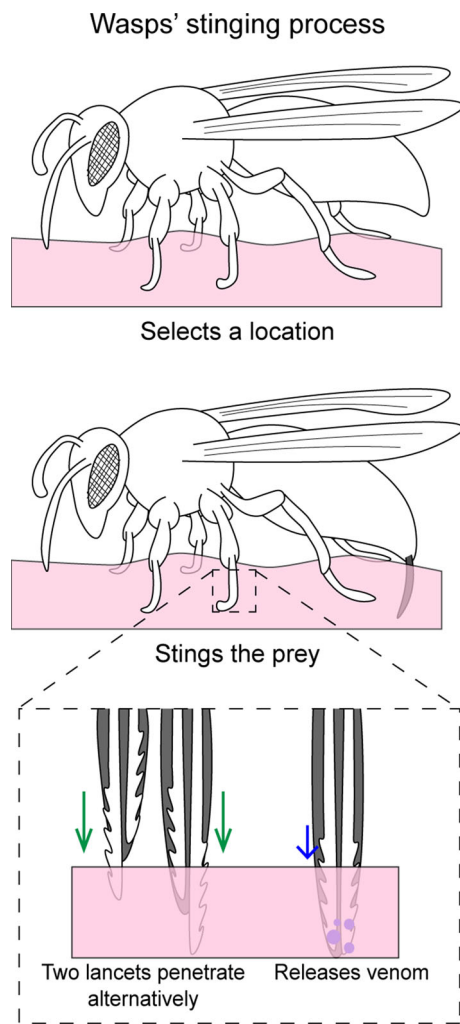


Fig. 16 After selecting a location, wasp stings the prey. The bottom schematic shows ventral views of stinger tip showing sting penetration by alternate forward strokes of the lancets, sliding against the shaft of the stylet. It then releases venom and retracts the stinger

swelling, redness, itching, and tenderness. The sting with its associated venom is a nearly perfect defensive system to counter attacks by large predators. The sting can penetrate the tough skin of the predator, and the venom is then delivered into richly innervated living tissue, where it causes immediate pain and tissue damage.

A schematic of the stinger and its various SEM micrographs (Zhao et al. 2015) are shown in Fig. 15. The stinger consists of two lancets and stylet (Akre et al. 1981). Lancets slide along the shaft of the stylet for penetration. They also form a venom canal in between. After selecting a location, the wasp first stings the prey and releases venom. Then it removes the stinger.

Schematics of various steps in the stinging process are shown in Fig. 16. Ventral views of stinger tip show stinging penetration by alternate forward strokes of the lancets, sliding along the shaft of the stylet, and lancets

pulled out of their track on the shaft. The tips of the lancets are equipped with tiny barbs to facilitate penetration. They use a sawing motion to cut through victim's flesh as each in turn is thrust forward and anchored in place by the barbs. Although the lancets lie side-by-side for a short distance after converging at the base of the shaft, their expanded bladelike ends overlap at the tip. The facing blade surfaces are each concave, creating a tubular venom channel between them (Akre et al. 1981). Venoms are highly complicated mixtures of pharmacologically and/or biologically active agents. They consist of biological amines, protein polypeptide toxins and enzymes. These agents cause the contraction of smooth muscle, stimulate glands of external secretion, increase capillary permeability, produce vasodilation with a resulting fall in blood pressure, destroy normal tissue barriers, and cause intense pain (Akre et al. 1981).

3.2 Structure, nanomechanical properties and modeling of the penetration process

The structure and nanomechanical properties of the wasp stingers have been measured and modeling of the penetration process has been carried out by Das et al. (2018).

3.2.1 Experimental details

The structure of the wasp stingers was analyzed using a 3D X-ray micro-computed tomography (μ -CT) technique. Nanomechanical property measurements were made using a depth sensing nanoindenter. The average elastic modulus (E) and hardness (H) on the surface along the longitudinal and transverse directions were measured. Next, stress and strain distributions in the stingers under compressive loading conditions were obtained by using their 3D computed tomography images and nanomechanical properties into a finite element model (Das et al. 2018).

3.2.1.1 Wasp species selection The female wasps (*Vespa vulgaris*) sting and these were collected from Ropar, Punjab, India (30.9664°N 76.5331°E). The samples were preserved in a deep freezer soaked in 50% ethanol solution between the time of collection and analysis. The stingers were extracted from the female wasps, located on the posterior end. The stingers were cleaned and dehydrated (stored in a sealed tube at room temperature for an hour and the solvent was allowed to evaporate) before the experimentation (Das et al. 2018). The specimen preparation procedure was found not to affect the physical properties of the stingers.

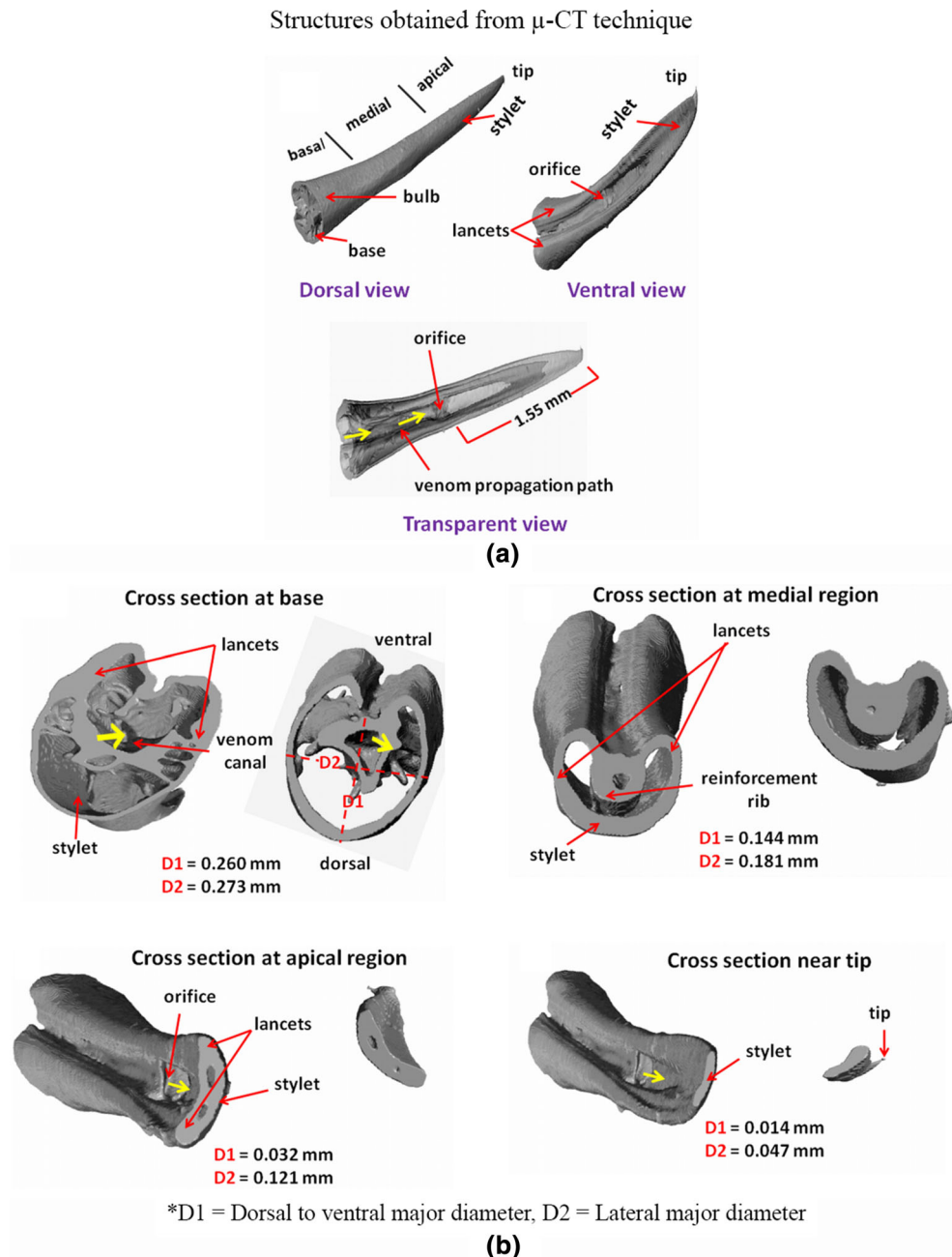
3.2.1.2 Structure analysis The 3D X-ray micro-computed tomography imaging of the stingers was performed using

GE Phoenix nanotom μ -CT system. It is a non-invasive imaging tool. The stingers were scanned at a resolution of 10 μ m at 280 μ A and 29 kV. The 3D scanned images were segmented based on grey value in Simpleware Scan IP software to build a 3-D model. In this segmentation process, the built-in median filter and the flood fill tool were used to reduce noise (Das et al. 2018).

3.2.1.3 Nanomechanical property measurements A depth-sensing nanoindentation technique, described earlier, was used for quasistatic nanomechanical property measurements of the stingers (Bhushan et al. 1996; Bhushan

2017). Five samples of wasp stingers were measured. The stinger has an intrinsic curvature and to avoid this curvature effect, the entire stinger was cut into three sections (basal, medial, and apical regions) and the ventral surfaces of each were put on the semi-solid epoxy block and stuck to it. After 2 h, the epoxy was cured with samples embedded in it. After that, on the flat region of the dorsal surface, indentation measurements were made. To obtain nanomechanical properties maps, a total of sixty indentations were performed along the longitudinal direction of the stinger, starting from the base (basal region) towards the tip (apical region) with an indent spacing of 40 μ m.

Fig. 17 Structures obtained using μ -CT imaging technique of **a** whole wasp stinger, and **b** cross section at the base, medial region, the apical region, and near the tip. It is observed that the stylet and lancets have hollow shaft and their radius decreases from the base to tip. The hollow structures diminish near the apical region and are transformed into solid structures. The cylindrical hollow venom propagation path is also visible (adapted from Das et al. 2018)



Indentation measurements in the transverse direction were made on three parallel longitudinal lines with spacing of 4 μm . Thus, the elastic modulus and hardness were measured at 3×60 points and elastic modulus and hardness matrices were obtained. The contour plots of the matrices were generated using MATLAB to get the 2-D mapping of elastic modulus and hardness of the surface of the stinger. The mapping images were superimposed onto the CT images of the stinger for a more clear visualization of the elastic modulus and the variations in hardness along the length of the stinger (Das et al. 2018).

To perform the quasistatic nanoindentation at the cross section of the stingers, the stingers were vertically embedded within a small semisolid epoxy block where the tip was placed inside the epoxy and the base was kept just outside the top surface of the epoxy block. Only the samples in which the cross-sectional surfaces became prominently focused were selected for nanoindentation from exocuticle to endocuticle with indent spacing of 2 μm . The elastic modulus and hardness map were generated (Das et al. 2018).

3.2.1.4 Modeling of the penetration process A numerical model was used to study the effect of penetration angle on the deformation of the 3D structures using measured nanomechanical properties. The segmented 3-D model of stingers obtained using μ -CT imaging was meshed in a finite element model of Simpleware Scan IP advanced meshing software with tetrahedral elements (C3D4). The number of elements in the model was 332,453. This meshed model was exported to ABAQUS-FEA (version 6.10) package for finite element simulation. For simulation, the average material properties obtained from nanoindentation were used. During simulation, the stingers were considered as linear elastic, isotropic and they were clamped at the base, with rotational and translational degrees of freedom constrained. The in-plane concentrated force of different amplitudes (10 mN, 30 mN, 50 mN, 80 mN and 100 mN) was applied along the central axis of the stinger at its tip and the resulting stress distribution, strain distribution and displacement, structural deformation/deformed shape were analyzed. The penetration angle of the applied force was varied from -90° to $+90^\circ$ and the corresponding stress and strain distribution were analyzed (Das et al. 2018).

In the second case, the gradient mechanical properties of the stingers were used. In Scan IP, the 3-D model of the stinger was divided into three sections and every section was defined by individual masks, followed by meshing with C3D4 elements. After exporting the model to Abaqus, the three different nanoindentation moduli were applied to these three sections. Then the simulation was performed

applying the same procedures as mentioned before (Das et al. 2018).

3.2.2 Results and discussion

3.2.2.1 Structure analysis of stingers The μ -CT images of the entire wasp stinger and different sections are presented in Fig. 17 (Das et al. 2018). Dorsal, ventral and transparent views of the structure of the entire wasp stinger are presented in Fig. 17a. The stingers have three major components; stylet, lancet and tip. There are paired lancets, one stylet and a tip. The stinger has an intrinsic curvature with a bulb type base, a slender medial region having constant diameter, and an apical region with decreasing diameter and tapered tip. It is observed that the two lancets converge in the apical region. In the ventral view of the stinger, the location of the orifice is called out and in the transparent view, the venom propagation cylindrical path is designated by an arrow. The venom is stored in the venom sack located at the lower abdomen of the wasp which is connected to the bulb of the stinger. The venom is transported through the hollow venom canal and transferred into the victim's body through the orifice (Das et al. 2018). Based on the dimensions measured in the μ -CT images, the wasp stinger has an average length of 2.67 ± 0.38 mm. The wasp pierces approximately 1.55 mm length section of its stylet into the victim's skin as shown in the transparent view of the stinger.

The wasp stinger was sectioned longitudinally into three regions: basal, medial and apical, and the μ -CT images are shown in Fig. 17b. In addition to the cross section of the base of the wasp stinger, the cross sectional view of the basal region from the inside of the wasp stinger was obtained through the post processing of the μ -CT image by Scan IP. Both of the cross sectional views of the base reveal the internal structure of the stylet, lancets and the venom canal. The stylet and lancets have hollow shafts and their radii decrease from the base towards the tip. The hollow shaft makes the stinger lightweight and the geometry of the decreasing cross section makes it strong. The cross section at the medial region are shown from two different directions, from inside to base and from inside to tip. A reinforcement rib is present in the ventral side of the medial region. This reinforcement improves the buckling resistance of the wasp stinger during its penetration into the victim's body. The cross section at the apical region and near the tip are also presented (Das et al. 2018).

The dorsal to ventral major diameter is denoted by D1 and the lateral major diameter is denoted by D2. The values of D1 and D2 in all regions are presented. The lateral diameter of the stinger in all regions is greater than the dorsal to ventral diameter. The diameters determined here are the average diameters of all five stinger samples.

Fig. 18 Nanoindentation data of the wasp stinger, **a** approximate sites of nanoindentation on stinger dorsal surface, **b** load versus penetration depth curve at different regions, **c** variation of the modulus and hardness along a length from base to tip, and **d** elastic modulus and hardness mapping. The mapping shows that the mechanical properties decrease along the length from base to tip of the stinger (adapted from Das et al. 2018)

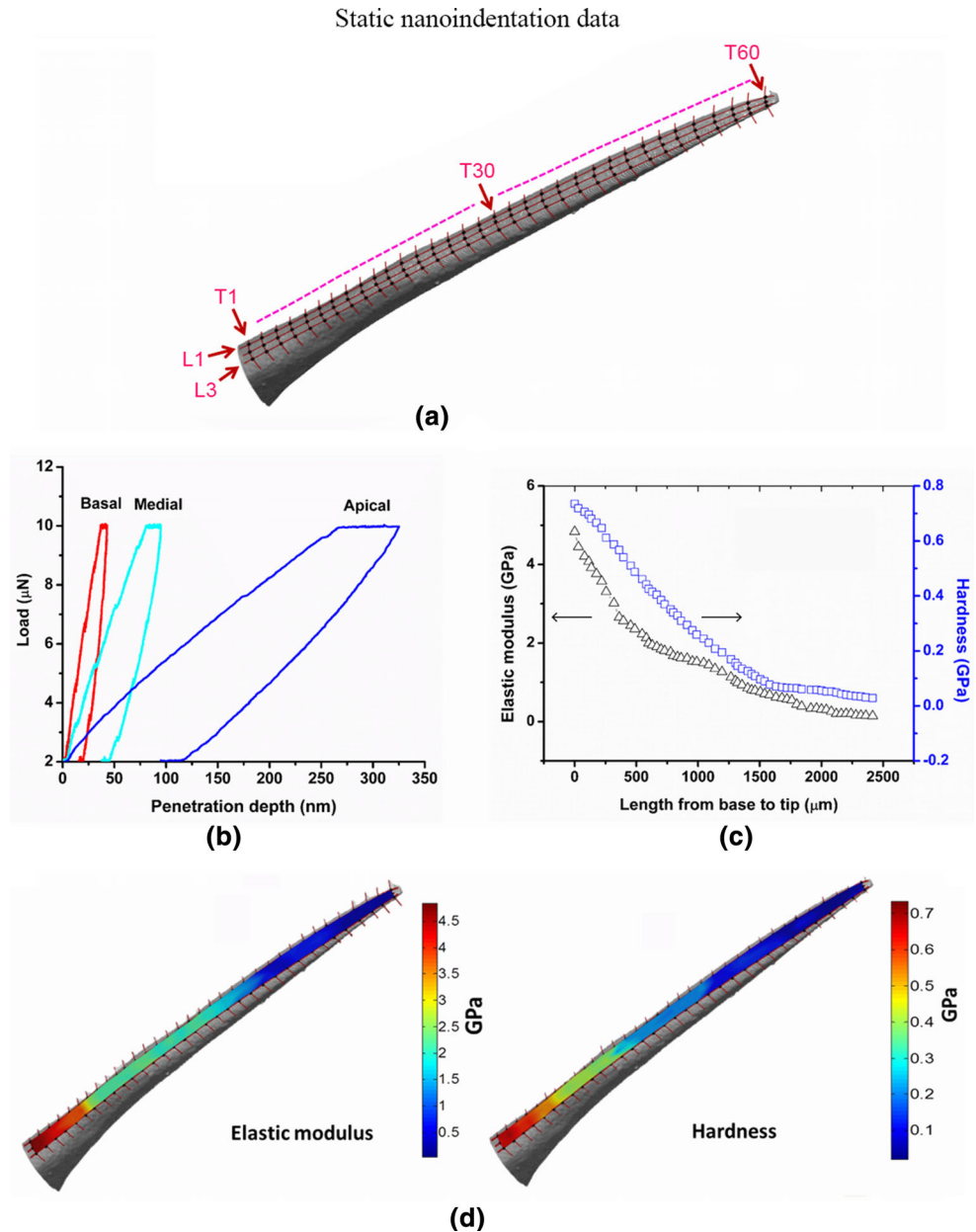


Table 2 A summary of quasistatic nanoindentation results of wasp stingers (adapted from Das et al. 2018)

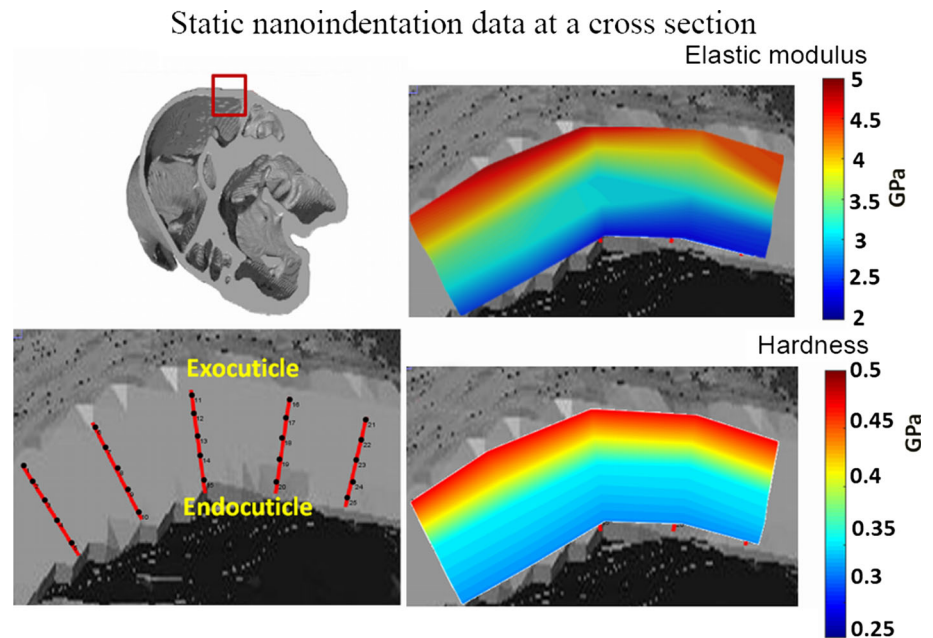
Region of interest	Elastic modulus (GPa)	Hardness (GPa)
Basal region	3.45 ± 1.36	0.56 ± 0.15
Median region	1.32 ± 0.44	0.31 ± 0.14
Apical region	0.47 ± 0.33	0.09 ± 0.03

To sum up, wasp stingers have hollow structural components with gradient geometry that makes them lightweight and mechanically strong. These stingers have an

intrinsic curvature and reinforcement rib at its medial region.

3.2.2.2 Nanomechanical properties of stingers The static nanoindentation data of the wasp stinger along the length are presented in Fig. 18 (Das et al. 2018). Figure 18a shows the approximate sites of nanoindentation denoted by the nodes of the mesh depicted on the dorsal surface of the wasp stinger, labeled as T1–T3 along the length and T1–T60 in the transverse direction. Representative load versus penetration depth curves at the different regions (apical, basal and medial) on the ventral surface are shown in Fig. 18b. The penetration depth at the basal region is minimum. It increases along the length towards the tip, and

Fig. 19 Nanoindentation data at a cross section of the wasp stinger, **a** the approximate region at basal cross section where nanoindentation performed and approximate nanoindentation sites, and **b** elastic modulus and hardness mapping. The cross section of the wasp stinger has hierarchical laminated structure and the mechanical properties decrease from exocuticle to endocuticle (adapted from Das et al. 2018)



at the apical region, it becomes maximum. The average values of elastic modulus and hardness determined from the load versus penetration depth curves at three regions are shown in Table 2 (Das et al. 2018).

The variation of elastic modulus and hardness as a function of the length from the base to tip, is shown in Fig. 18c. The elastic modulus and hardness at the basal region of the stinger is maximum and both decrease along the length from base to tip, at the region near the tip it becomes minimum. The elastic modulus mapping and hardness mapping of the stingers are shown in Fig. 18d. The images of the elastic modulus and hardness mapping were placed on the μ -CT image of the stinger. The mappings clearly exhibited the presence of the downtrend of elastic modulus and hardness along the longitudinal direction from base to tip of the stinger. Along the transverse direction, no measurable gradient of elastic modulus and hardness was observed.

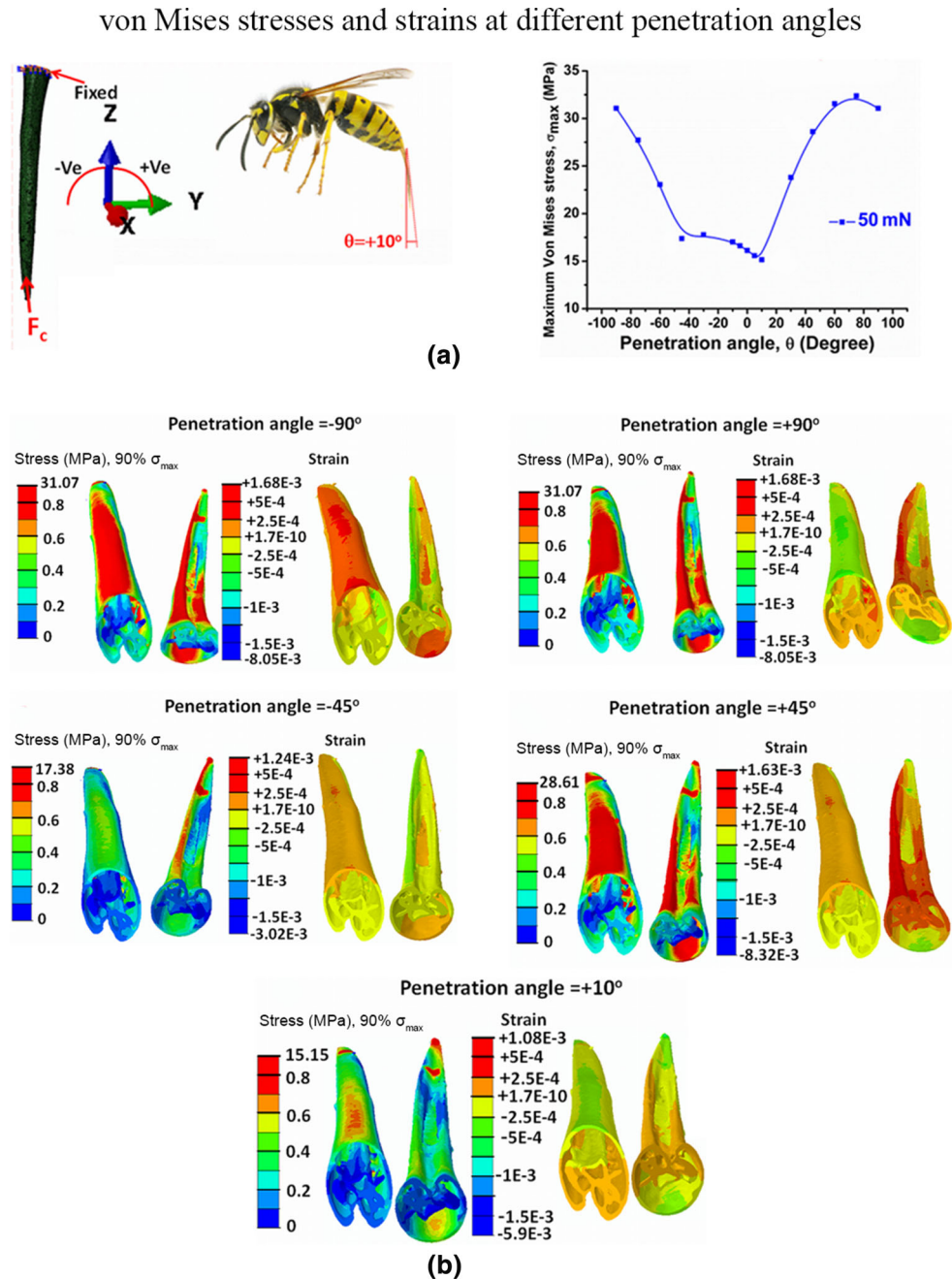
During penetration, the stingers undergo compression, bending and twisting. The basal region is subjected to the largest values of bending stresses and axial buckling during penetration, therefore this region is designed to have the highest elastic modulus and hardness. According to the structure analysis, the medial region of the stinger has the intrinsic curvature along with a reinforcement rib which minimizes the bending moments and stresses. Basal regions have the highest elastic modulus which decreases along the longitudinal direction towards tip. These characteristic material properties, in addition to the geometry, provide the necessary stiffness which prevents structural failure during penetration. The region near the tip has the minimum elastic modulus, however it is higher than that of

human skin by an order of magnitude. In addition, the tips of the stingers are sharp with required stiffness and have a refined insertion mechanism which enables them to puncture and penetrate easily through the human skin (Das et al. 2018).

Nanoindentation tests were performed at the cross section of the stinger at the stylet cross section. The nanoindentation sites are shown in Fig. 19a, and elastic modulus and hardness maps for the stinger are shown in Fig. 19b. From the elastic modulus and hardness mapping, it is clear that both the elastic modulus and hardness have a negative gradient along the length from exocuticle to endocuticle. These moduli and hardness distributions strongly favor the laminated structure of the cuticles which imply hierarchy in the structure of the stingers (Das et al. 2018).

The nanomechanical properties on the dorsal surface i.e. on the exocuticle were measured. The negative gradient of mechanical properties along the longitudinal direction from base to tip was observed along with the negative gradient in the cross section from exocuticle to endocuticle. The mechanical properties of the exocuticle of the stingers reveal that it is composed of chitosan, a hard substance. Chitosan is a biopolymer which is the deacetylated derivative of the chitin (Rinaudo 2006). Chitin is an important biopolymer which is the major structural component of spider fangs, insect cuticles, mollusk shells and exoskeletons of marine crustaceans like shrimp and crabs. The chitin contributes to the exceptional stiffness and fracture toughness of the cuticle. The orientation of chitin fiber differs in the different layers of the stinger cuticle (Das et al. 2018).

Fig. 20 von Mises stresses and strain distribution of wasp stinger, **a** variation of maximum von Mises stress ($\sigma_{\max}$) as a function of the angle of applied compressive force (F_c) under cantilevered condition of the wasp stinger, and **b** 90% of maximum von Mises stress and strain distribution maps for both the dorsal and ventral side of the stinger at a penetration angle of -90° , $+90^\circ$, -45° , $+45^\circ$, and 10° . The maximum von Mises stress and strain have a minimum value at the penetration angle of about $+10^\circ$. The wasp finally adopts this angle to insert its stinger into objects (adapted from Das et al. 2018)



In the cuticle, the isotropic protein resilin induces compliance on the stinger. The soft cuticle contains a higher amount of water, whereas the hard cuticle has a lesser amount of water. The resilin after hydration provides high toughness, high strain, long fatigue life and low stiffness into the cuticle. In addition, the hierarchy potentially improves the strength and toughness and specific physical properties of the structures (Das et al. 2018).

To sum up, the wasp stingers are mechanically and structurally graded based on the structural analyses. The geometry of stingers changes along the longitudinal

direction from base to tip. The nanomechanical properties decrease along the longitudinal direction of the stingers from the base to tip, and along the cross sectional layers from exocuticle to endocuticle (Das et al. 2018).

3.2.2.3 Modeling of the penetration process The base of the stinger was assumed to be planar in the X–Y plane, and the concentric force, F_c was applied at the tip of the stinger along the Z direction (longitudinal axis of the stinger), as shown in Fig. 20a (Das et al. 2018). The angle of applied force or penetration angle, θ was varied from -90° to $+$

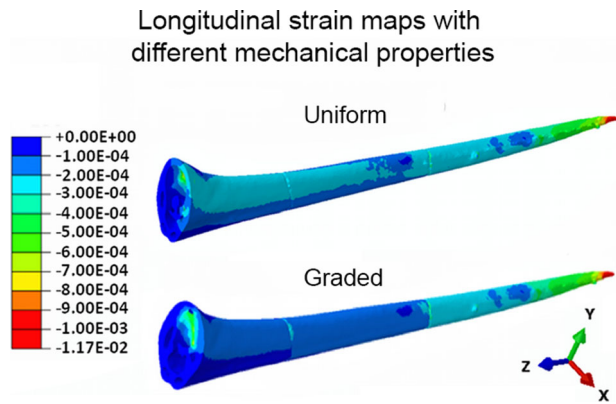


Fig. 21 Longitudinal strain distribution maps of the honeybee stinger in the axial direction with uniform and graded mechanical properties. For stinger, with uniform properties, relatively high compressive strain exists throughout the stinger, whereas for stinger with graded properties, it does not. This suggests more mechanical stability of the stinger with graded properties (adapted from Das et al. 2018)

90°. The maximum von Mises stress, $\sigma_{\max}$, as a function of penetration angle is plotted in Fig. 20a. The $\sigma_{\max}$ is very sensitive to the penetration angle. At a penetration angle of 10°, $\sigma_{\max}$ is minimum whereas at + 90° and − 90° penetration angle, it is maximum. It should be noted that the magnitude of the applied force was also varied, it was observed that the force amplitude did not affect the angle at which the stress becomes minimum (Das et al. 2018). The wasp gradually regulates its penetration angle from + 18° to + 9° during the penetration process. At the early stage of penetration, the wasp applies the force on the object surface by pressing the stinger tip and also rotating the stinger. Gradually, it increases the pressing force; in parallel, it adjusts the rotating angle. When the pressing force reaches an optimum level, the puncture occurs. At this stage, the rotating angle becomes almost fixed and the wasp deeply inserts its stinger. For this condition, the stinger can be treated as fixed at its base and its tip is subjected to a concentric compressive force. This fixed angle is believed to contribute to effective penetration of the stinger with minimum effort (Das et al. 2018).

The von Mises stress and strain distribution maps for both the dorsal and ventral side of the stinger at different specific penetration angles at an applied force of 50 mN are shown in Fig. 20b (Das et al. 2018). The maximum von Mises stress values are affected by the artefact of how the model is constrained and loaded. Therefore, 90% of maximum von Mises stress values were plotted. The stress distribution at the penetration angle of 10° shows that the small region near the tip and in the subapical region experience very high stresses. As the concentric force is applied at the tip, the subapical region always undergoes high stress at all penetration angles. The $\sigma_{\max}$ at 10° is about 15.15 MPa. The other sections of the stingers are

very low stressed regions having the stress range of 0–0.3 MPa. At other penetration angles, the stress distributions show that high stress regions develop all over the dorsal surface and ventral surface and also at their interface.

The strain distribution maps at different penetration angles are also presented in the Fig. 20b. At the penetration angle of + 10°, the dorsal surface at the basal region undergoes compressive strain of very small magnitude of about -2.5×10^{-4} to -5×10^{-4} . Whereas the dorsal surface at the apical region undergoes small tensile strain of about $+2.5 \times 10^{-4}$ under the effect of the applied compressive concentric force. The ventral surface also undergoes very small tensile strain, on the order of $+2.5 \times 10^{-10}$. At other penetration angles, the magnitude of either the tensile or the compressive strain becomes higher. At the penetration angle of + 10°, the $\sigma_{\max}$ is minimum and the overall strain is also minimum. Thus, the wasp adopts the penetration angle near the vicinity of + 10° which is used by nature.

To investigate the benefit of graded mechanical properties on the mechanical behavior of the stinger during penetration, the strain distribution maps of the stinger in the axial direction with uniform and graded mechanical properties, under the action of 50 mN concentric force at its tip, were obtained by Das et al. (2018). No difference in stress distribution was observed in either case for a constant applied force. Therefore, the stress distribution maps are not presented. The longitudinal strain maps are shown in Fig. 21. For a stinger with graded mechanical properties, the non-deformed region near the base is larger. Thus the overall stiffness of the structure increases with graded properties. It is also noted that the penetration angle with high stiffness is not influenced by the gradient in mechanical properties rather it is defined by its structural features (Das et al. 2018).

To sum up, the combination of structure, graded geometry, and gradient in mechanical properties enhances the mechanical stability of the biological structures which subsequently improves their survival in nature. The wasp adopts the penetration angle of about + 10° to insert its stinger into objects. This angle has significant relevance in maintaining the structural stability of the stingers. Additionally, the sliding mechanism of paired lancets plays a role in venom pumping mechanism.

3.3 Conceptual schematic of a painless, wasp-inspired microneedle

A conceptual schematic of a painless wasp-inspired microneedle is shown in Fig. 22. The microneedle should have decreasing diameter from base to the tip and should be made of materials that have gradient mechanical

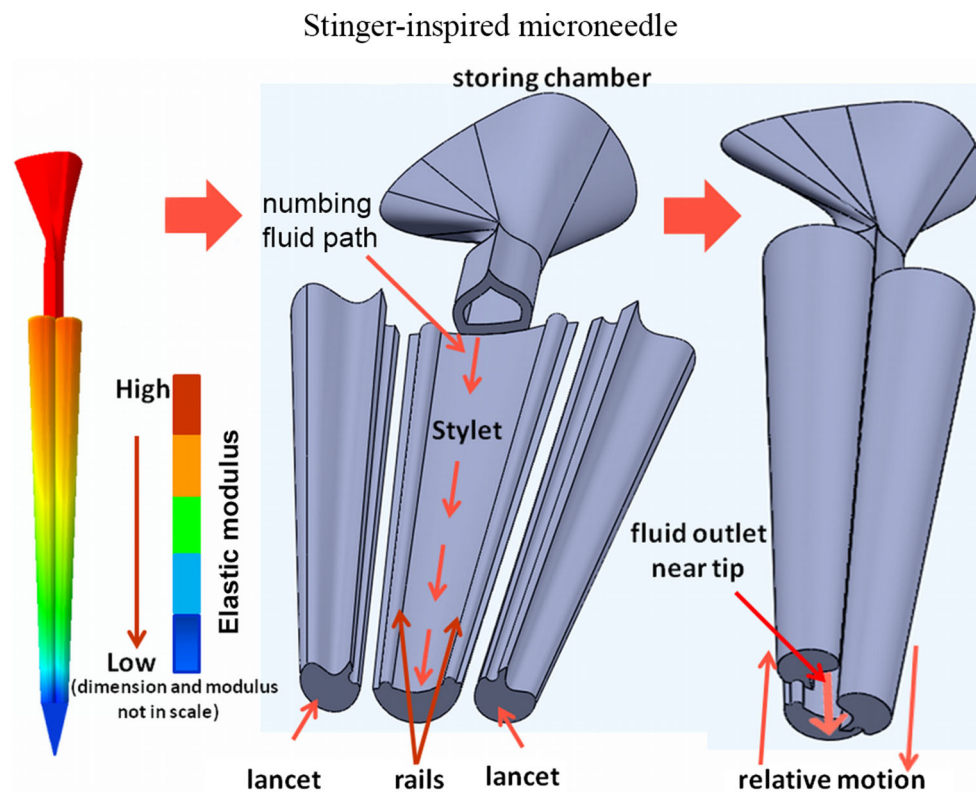


Fig. 22 The left image shows a conceptual schematic of a painless wasp-inspired microneedle mimicking the stingers with decreasing diameter from base to the tip and with graded mechanical properties. The color variation represents the functionally graded materials in the microneedle where the mechanical properties increase in longitudinal direction from tip to base. The middle image shows the magnified

view of the internal structure of the microneedle with all components (tip has been removed). Right hand image shows that a gap is generated near the tip due to sliding relative motion of lancets on the rails of the stylet. The numbing fluid stored in the storing chamber will be pumped through the one-way valve from the chamber to the targeted body (adapted from Das et al. 2018)

properties with a minimum at the tip and maximum at the base. The advantage of this structure with gradient in geometry and mechanical properties, has been already described. Because of the higher overall stiffness coupled with the less stiff tip, this microneedle is expected to be effective in obtaining less painful insertion with minimal dermal injuries, while maintaining mechanical durability (Das et al. 2018). To further improve the mechanical durability of the microneedle, it can be designed for use at a penetration angle ($\sim 10^\circ$ for wasp stingers) which produces minimum stress.

In addition, to supply a numbing fluid to minimize pain, sliding mechanism, as observed in stingers, may be implemented in the functioning of a microneedle system (Fig. 22). Because of this sliding motion, a gap for fluid transportation will be generated near the tip. The numbing fluid will be stored in the storing chamber of the microneedle, which will be transported through a one-way valve from the storing chamber to the targeted body. The force to purge the fluid into the targeted body can be generated by squeezing the storing chamber (Das et al. 2018).

3.4 Summary

In this study, the structure, nanomechanical properties and the mechanical performance during insertion of wasp stingers were investigated. The non-invasive imaging tool, micro-computed tomography was employed to reveal the structural features and graded geometry. The static nanoindentation measurements were carried out to measure the graded nanomechanical properties. Wasp stingers have graded mechanical properties, decreasing along their longitudinal direction starting from the base. Computed tomography images and the measured nanomaterial properties from nanoindentation were fed into a computational framework to determine the mechanical behavior of the stingers during penetration. The computation results predicted the penetration angle of about $+10^\circ$ for the wasp stinger which results in optimum stress and strain distribution (Das et al. 2018).

4 Closure

Arthropods are the largest group of the living organisms. They attack other organisms by biting, stinging, or piercing and sucking. In this chapter, the piercing and sucking mechanism in mosquitoes and the stinging mechanism in wasps are described. Based on lessons learned from mosquitoes and wasps, conceptual schematics of bioinspired painless microneedle designs are presented.

It is expected that this study will provide an impetus for fabrication of painless bioinspired microneedles requiring low insertion forces for biomedical applications such as micro-syringe needle system for drug delivery, hard tissue biopsy needle, and surgical suture.

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