Chapter 3
Anatomy and Physiology

Workers of *M. beecheii* collecting pollen from a water lily.

A great deal of variation exists on the external features of stingless bees; this is in strong contrast with the rather uniform body pattern found in the Apini, the other eusocial group of bees (Rasmussen and Cameron 2010). The stingless bees show notorious interspecific differences not only in size, but also in shape and color Ayala 1999). The size and number of mandibular teeth, the degree of body pilosity, the pattern of wing venation, and the size and shape of the corbiculae are
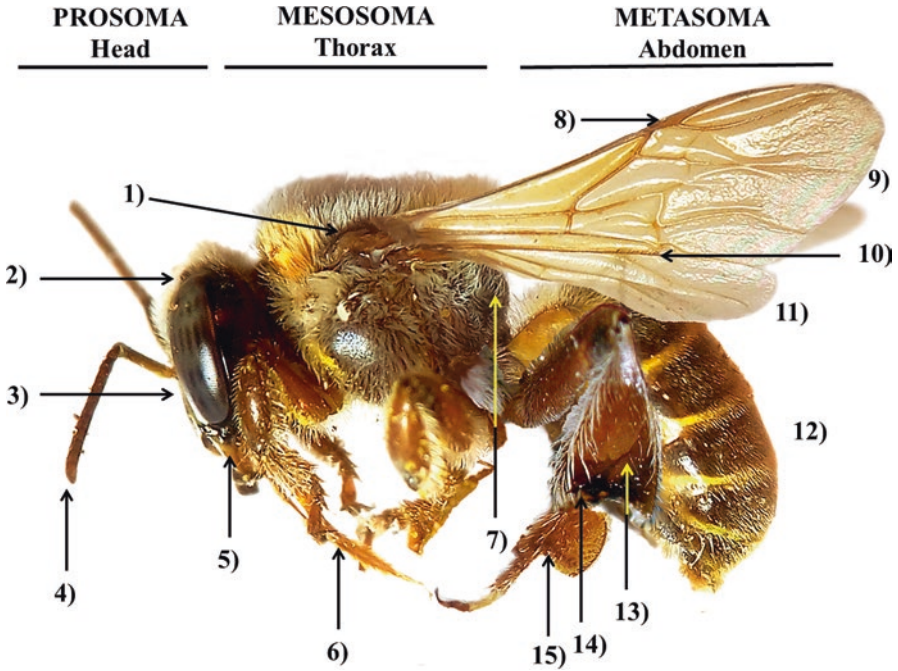
Fig. 3.1 External anatomy of a *M. beecheii* worker: (1) tegula; (2) ocellum; (3) compound eyes; (4) antenna; (5) mandibula; (6) proboscis; (7) propodeum; (8) pterostigma; (9) forewing; (10) hamuli on the hind wing; (11) hind wing; (12) abdominal segments A2 a A7; (13) corbicula; (14) penicillum; (15) basitarsum

notorious examples of such variability. Nonetheless, stingless bees exhibit a basic pattern of the body, which is similar to that of other Hymenoptera.

In this chapter a general description of the anatomy and physiology of stingless bees is presented. Descriptions proceed in accordance to the main segments conforming the Hymenopteran's body: the prosoma or head, the mesosoma or thorax, and the metasoma or abdomen (Fig. 3.1).

For some particular structures, differences are pointed between *Melipona* and other genera. Aspects related to the anatomy and physiology of the reproductive system are presented in Chap. 6, in the section referring to individual reproduction of gynes and males.

3.1   Prosoma

Structures found on the head or prosoma are important for the interaction of the insect with its environment, most acting as centers for feeding and the senses. The main organs here are the eyes, antennae, and proboscis.
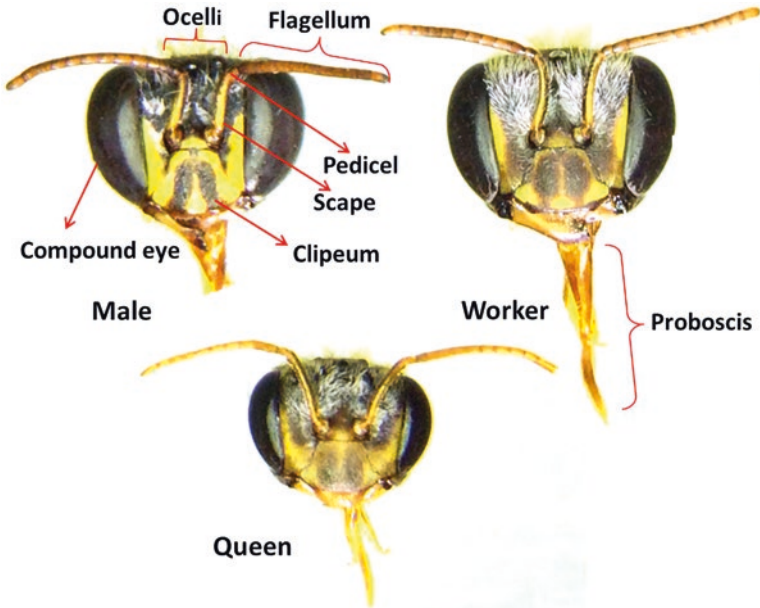
Fig. 3.2 Frontal image of the head of *M. beecheii* male, worker and queen. The ocelli and parts of the antennae are labelled on the male and the proboscis on the worker

Bees have two types of eye. One, is a set of three simple eyes called ocelli that are located at the frontal apex of the head (Fig. 3.2). The ocelli are not involved in the process of image production, but in the perception of light intensity. There is also a pair of large compound eyes located at both sides of the head. The large compound eyes are formed by small facets called ommatidia. Each ommatidium is a simple but complete optic unit with a corneal lens, a crystalline cone, and a light-sensitive pigment, the rhodopsin. It seems that the ommatidia act by capturing simple images in their visual area and that these are processed in the brain to obtain a more complex and wider image (Dade 1985).

At the front of the head there is a pair of antennae. Each antenna is formed by three segments; the larger one attaching the antenna to the head is the scape. After the scape, the following small spherical segment is the pedicel, which allows free movement of the distal section of the antennae called the flagellum (Fig. 3.2). The flagellum is divided into small segments called flagellomeres, of which females (workers and queens) have 10 and males 11. The surface of the flagellomeres is covered with chemical and mechanical receptors called sensorial plates or *sensilla*. There are different kinds of *sensilla,* each involved in the detection of the levels of CO_2, pressure, and temperature, as well as taste and smell, among others (Dade 1985). Inside the antennae, the Johnston's organ is involved in the reception of airborne sound. In *M. scutellaris* this organ seems capable of detecting the vibrations produced by forager bees through the movement of air particles at distances of up to 2 cm (Hrncir et al. 2008).

Underneath the head is the proboscis, an articulated structure used by the bee as a straw in the suction of nectar and liquids. When not in use, the parts of the proboscis

Fig. 3.3 Mandibles of a
queen, male, and worker of
M. beecheii. There is a
striking difference in the
size of the mandible and
sharpness of the teeth
between the worker and
both sexuals

(the maxillary palpi, labial palpi, and glossa) are folded behind the head. When in
use, the proboscis extends and the palpi form a tube around the glossa. The glossa is
longitudinally indented by the salivary canal. The saliva is produced in glands in the
head and thorax, and is used to dilute food. Muscles, underneath the head, create a
sucking pump on the proboscis and the liquids can be ingested (Dade 1985). Taste
seems to be mainly sensed at the tip of the glossa (the flabellum) and the palpi.

The mandibles are found in the lower part the head. These appendages are the
main tools for processing food (pollen) and building materials (wax, cerumen, and
resin), but also the most important weapons for defense (Fig. 3.3). Given that meli-
ponines lack a sting, the mandibles can be especially developed in some species.
Accordingly, the mandibles can vary in size, the number of teeth, and their sharp-
ness. Interestingly, highly defensive species have larger mandibles with more numer-
ous and sharper teeth (Shackleton et al. 2015). Sexuals generally make little use of
the mandibles, and these are significantly smaller (Fig. 3.3).

3.2   Mesosoma

The mesosoma or thorax is the second largest body segment. The organs involved
in the locomotion of the insect (walking and flight) are found here. The mesosoma
is formed by segments that have been reorganized in the transition from the larval to
the adult stage during metamorphosis. The first abdominal segment of the larva
(A1) moves into the mesosoma to form the last segment of the adult's thorax, and is
called the propodeum (Fig. 3.1). Between the propodeum and the first abdominal
segment of the adult (originally the larval A2), a narrowing of the body (wasp-waist)
called the petiole unites the thorax with the metasoma (abdomen). The petiole
allows great flexibility of the metasoma (Dade 1985).

The thorax carries two pairs of wings and three pairs of legs. Both types of
appendage are propelled by groups of muscles. The bee's legs are three pairs
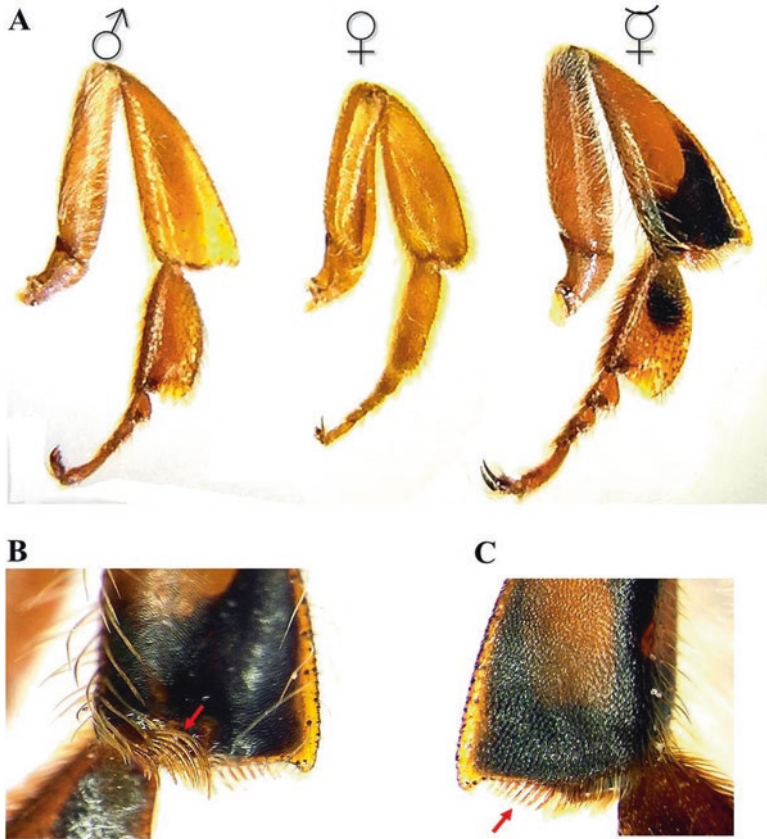
Fig. 3.4 (**a**) Hind leg of *M. beecheii* male, queen, and worker. The corbícula and details of the *penicillum* and *rastellum* (only found in the worker), are shown in images **b** and **c**, respectively

of segmented appendages. The segments of the legs, from the most proximal to the more distal are, the coxa (the first segment attaching the leg to the mesosoma), followed by the trochanter, femur, tibia, tarsus, and pretarsus. The tarsus is formed by five segments called the tarsomeres of which the first, the basitarsus, is wider and larger than the others. The basitarsus of the first pair of legs has a notch with hair that is used to clean the antennae (antenna cleaner). The third pair of legs is of particular importance in the workers of stingless bees (and other corbiculate Apinae), because the tibia forms the *corbicula* or pollen basket. The corbicula is a broad expansion of the tibia; it is slightly concave with curved hairs along the edge to help keeping the load in place (Fig. 3.4). The inner face of the basitarsus on the third leg is covered with thick short bristles (the pollen brush) that groom the pollen from the bee's body and transfer it to the corbiculae.

On the distal edge of the corbicula a group of short stiff bristles are known as the pollen rake or *rastellum*. The pollen rake is used during the transference of pollen

Fig. 3.5 Workers of *M. beecheii* visiting an achiote flower (*Bixa orellana*) showing the pollen loads (pellets) formed on the corbiculae. On the image to the right a worker with pollen loads and two workers with wax scales produced on the dorsal glands of the metasoma

from the legs to the corbiculae (Fig. 3.4). The *rastellum* is absent in the cleptobiotic genera (*Lestrimelitta*) and less developed in the Meliponini from Africa (Wille 1979). On the apical internal edge of the corbicula, a brushlike structure of stiff setae, exclusive of the stingless bees is the *penicillum* (Wille 1979). In the genera of cleptobiotic species the *penicillum* is greatly reduced; the males and gynes of all species lack it too (Fig. 3.4). It is suggested that the *penicillum* is also used to process the pollen loads. The pollen attached to the bee's body is brushed off by means of the first two legs. Then it is transferred from the second to the third leg of the same side by passing the second leg between the space formed by the *penicillum* and the basitarsus. Due to the curvature of the *penicillum* the pollen is pushed up into the corbicula to form pellets (Fig. 3.5). Stingless bees do not have a pollen press apparatus on the basitarsus like the honey bees (Wille 1979).

The wings of the bees are membranous structures, found in pairs on each side of the body. Each pair is formed by a large forewing and a smaller hind wing (Fig. 3.6). The wings have veins that act as mechanical support for their fine structure. The spaces among the different veins are called wing cells (Fig. 3.6). The pattern of forewing veins and cells is important in the taxonomic identification of species and lineages of stingless bees (Francoy et al. 2011).

Flight is produced by the action of two sets of muscles found in pairs inside the mesosoma, i.e., the longitudinal and dorsoventral (or vertical) muscles. These muscles act to generate the energy and movement required for flight. Thanks to the elastic cuticle between its segments, the mesosoma can expand and contract alternatively. The longitudinal muscles pull the mesosoma front to back and the vertical muscles pull it dorso-ventrally. The wing bases are inserted into a slit of the thorax (longitudinal slit) and by the alternative movements of the muscles, the wings are pushed up and down. The dorsoventral contraction elevates the wings, and the longitudinal contraction flips them down. Muscles contract and relax at a very high speed (200–250 beats per second), elevating and pushing the wings down in alternative movements, creating the necessary stroke for flight (Dade 1985).
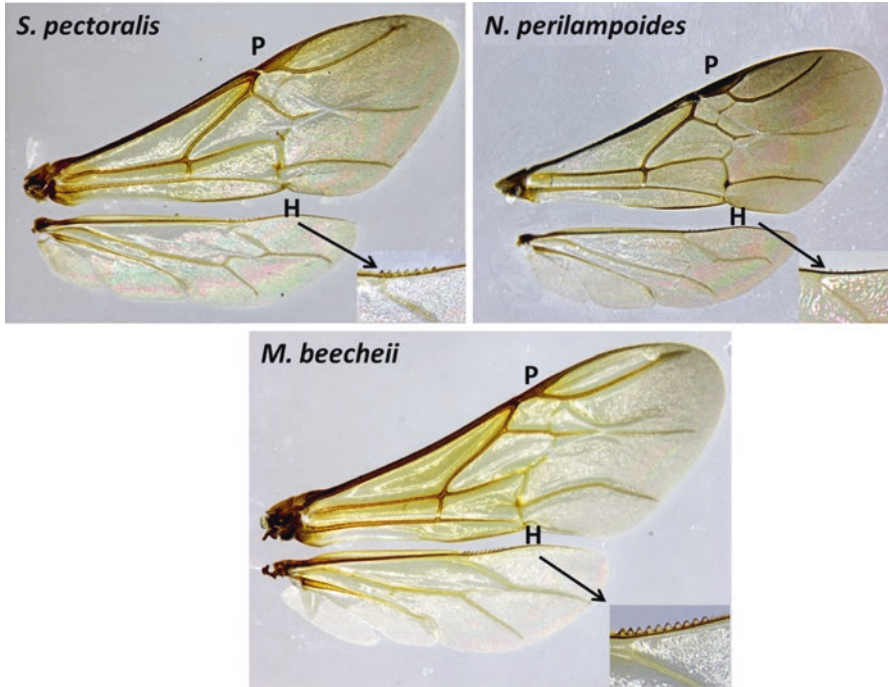
Fig. 3.6 Fore and hind wings of three Meliponini from Yucatan to compare the relative size of the pterostigma (P) and hamuli number (H), the latter in the amplified section. The species *Scp. pectoralis* and *N. perilampoides* have seven and five hamuli, respectively; *M. beecheii,* with a larger body mass, has ten

The mesosomal muscles produce a great amount of energy during contraction (Winston 1987). During flight the forewing and hind wing form a functional unit. This is achieved by means of a canal in the posterior margin of the forewing and a set of hooks (hamuli) on the frontal edge of the hind wing. When the bee expands its wings in preparation for flight, the hamuli on the hind wing clasp into the forewing canal, effectively becoming one structure.

Through this mechanism, the bee can increase the surface and strength of the wings, for a more powerful flight (Dade 1985). In the Meliponini, it has been found that the number of hamuli in the different species is correlated with body mass rather than wing length (Schwarz 1948). Non-*Melipona* stingless bees usually have between 4 and 10 hamuli while *Melipona* species have between 8 and 16 (Schwarz 1948). Queens have less hamuli compared with males and workers (Schwarz 1948). This is probably due to their low requirements for continuous and long flights, compared to the other individuals.
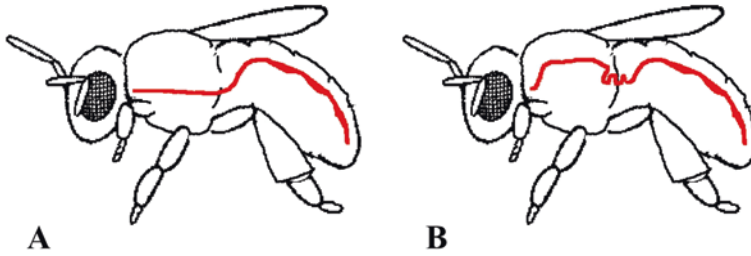
Fig. 3.7 Shape of the dorsal vessel (in red) in stingless bees: (**a**) type I aorta present in non-*Melipona* species and (**b**) type II aorta present in *Melipona* and *Meliponula*

Some fossil stingless are small, like the present *Plebeia.* Wille (1979) suggests that small size of their ancestors may explain some of the present anatomical features of the stingless bees, for instance, the reduced wing venation. Weak veins like those presently found in Meliponini, can be a hurdle for the strong flight needs of highly social bees. It seems that non-*Melipona* and *Melipona* stingless bees evolved different methods to compensate for the relative weakness of the veins. In the case of non-*Melipona* species, the forewing evolved to a comparatively larger size and the pterostigma is proportionally larger than in other corbiculate bees (Fig. 3.6). Large pterostigmas are characteristic of small Hymenoptera which provide additional support to the wings during flight (Michener 2001). In comparison, *Melipona* have comparatively shorter wings with a smaller pterostigma, but the number of wing hamuli has increased, probably resulting in a better union between fore and hind wings increasing flight capacity (Wille 1979; Fig. 3.6).

One portion of the circulatory system, the dorsal vessel, or aorta, is found in the thorax. This vessel shows differences between *Melipona* and non-*Melipona* species. In non-*Melipona* bees the thoracic portion of the aorta is normally straight, and it passes below the longitudinal thoracic muscles not between them; this type of dorsal vessel is known as type I (Fig. 3.7). In *Melipona* the thoracic portion of the aorta forms an arch between the longitudinal thoracic muscles and is known as type II (Fig. 3.7). It is thought that Type II aorta probably evolved as response for a better irrigation of the muscles or temperature regulation during flight in larger bees (Wille 1979; Winston 1987).

3.3 Metasoma

Most organs and systems of the bee are found in the metasoma or abdomen. The metasoma is formed by nine segments, each with a dorsal (tergite) and a ventral plate (sternite). The segments of the metasoma lack the pleurite or lateral sclerite between the terga and sterna found in the mesosomal segments. In contrast with the thorax, the bee's abdomen can expand notably due to the elastic membranes between the plates.
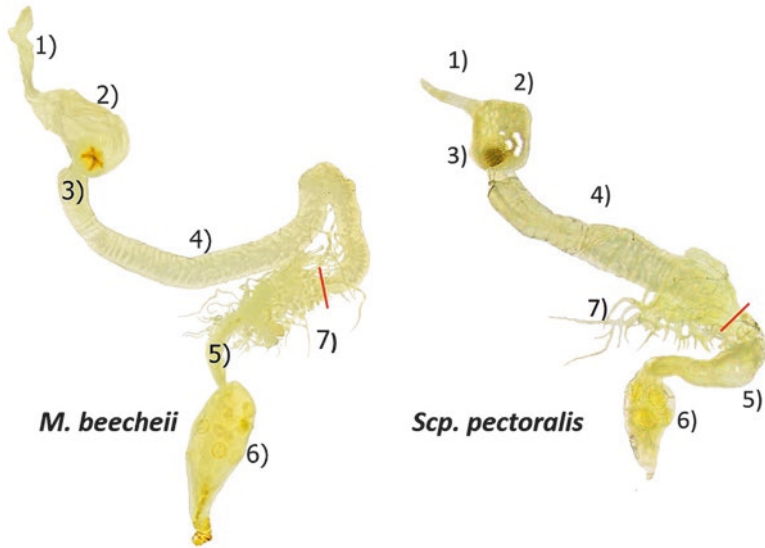
Fig. 3.8 Digestive tract of *M. beecheii* and *Scp. pectoralis* workers: (1) esophagus, (2) honey sac, (3) proventriculus, (4) ventriculus, (5) short intestine or ileum, (6) rectum, (7) Malpighian tubules. Elongation of the tract has occurred in *Melipona,* compared to other stingless bees, in particular of the ileum (5). The red lines indicate the start of the ileum

In the adult stingless bee, only segments A2–A7 are externally visible. Segment A1 has moved into the mesosoma to form the propodeum and segments A8 to A10 are located internally in the abdomen, some of them part of the vestigial sting.

The digestive tract extends from the proboscis in the head to the metasoma. In the abdomen, the digestive tract is continuation of the esophagus, a long and slender tube that extends from the head through the thorax. In the abdominal cavity, the esophagus expands to form a sac called the honey stomach or crop. When empty, the honey stomach is folded but its thin walls can expand notoriously to accommodate a large nectar volume (Fig. 3.8). The honey stomach does not have a digestive function; it acts like a reservoir for the nectar. The honey stomach is separated from the true stomach by a sphincter valve called the proventriculus which controls the passage of pollen and other solids from the honey sac. The true stomach or *ventriculus* is a thick muscular segmented tube where the digestion takes place (Fig. 3.8). A series of membranes inside the *ventriculus* (the peritrophic membranes), wrap around the pollen grains as they enter this part of the tract (Dade 1985). The peritrophic membranes may possibly act to protect the delicate lining of the *ventriculus* against the coarse or spinous exines of some types of pollen. In the *ventriculus* the pores of the pollen grains are broken by osmotic shock (Crailsheim 1990). In addition, the cells lining the *ventriculus* produce digestive juices and proteolytic enzymes penetrate the pores of the pollen. Inside the pollen, the enzymes digest the large molecules and a broth can exit the pores. Once in the *ventriculus* lumen, the nutrients

can absorbed into the hemolymph. Nutrients can be stored in the abdominal fat bodies as fat, glycogen, or protein (Dade 1985).

After the *ventriculus*, a thinner tube called the short intestine or *ileum* is found. In the insertion between the *ventriculus* with the *ileum* a series of tubules, called the *Malpighian tubules*, join the digestive tract. The *Malpighian tubules* are involved in the process of elimination of metabolic waste from the hemolymph. In the tubules, metabolic residues and liquid absorbed from the hemolymph form the primary urine that is poured into the ileum. Because bees feed on liquids (mostly nectar), it is suggested that the primary urine is hypo-osmotic. To keep an osmotic balance, the bees need to absorb ions actively from the urine while avoiding the absorption of excess water; a process performed in the *ileum* (Santos and Serrão 2006). After the *ileum,* the last section of the gut is the rectum. This is a large sac where the residues of the digestion and the hemolymph accumulate to produce the feces (Fig. 3.8). The rectum is involved in the absorption of water from the waste. Stingless bees in contrast to honey bees defecate inside the colony.

The length of the digestive tract shows differences between *Melipona* and other stingless bees. In non-*Melipona* species, the digestive tract is short (Fig. 3.8), compared with that of *Melipona*. A long digestive tract is considered a derived characteristic (Wille 1979). Notably, in the cleptobiotic *Lestrimelitta,* the ventriculus in addition to the ileum is also shortened (Wille 1979). This may be a result of a lifestyle involving the consumption of larval food and pollen, predigested by the hosts.

The bee's circulatory system is open; there are no vessels in the form of arteries or capillaries. Basically, there is one large vessel with two parts, the larger posterior part or heart, is found in the metasoma. The first part, the aorta, already described in Sect. 3.2, continues into the abdomen to form theheart. The heart is a muscular organ that pumps the hemolymph from the abdomen to the mesosoma and the head. The bee's hemolymph is a yellowish liquid composed of the plasma and cells called hemocytes. Hemocytes are involved in the immunity of the insect. The insect's hemolymph reaches all the organs and muscles carrying the nutrients and removing excretions. However, the hemolymph does not contain red cells and is not involved in the transport of oxygen. In the abdomen, the hemolymph enters the heart through a series of holes called the ostia. The walls of the heart pump the liquid to the aorta and to the head where the hemolymph is poured onto the brain from which it flows backwards to the abdomen (Dade 1985).

The bees, as other insects, breathe through the body cuticle by means of lateral orifices called *spiracles.* From these, the incoming air flows through ducts called *tracheae* to the different organs. The exchange of oxygen takes place in a series of microscopic tubes called *tracheoles* which are in close contact with the cells. The contractions of the abdomen pump the air in and out of the body (Dade 1985).

The bee's nervous system comprises the brain and a set of ventral nerve cumulus, called *ganglia*. The ganglia act as small satellite brains. It is important to note that in stingless bees the abdominal *ganglia* have migrated towards the front (cephalization) of the body (Wille 1979). This is more evident in *Melipona* (Fig. 3.9).

In some species, the abdominal *ganglion* three has migrated into the mesosoma. Thus, the thorax in these species carries two *ganglia* of mesosomal origin and one of metasomal origin (Wille 1961). It is believed that the cephalization of the abdom-

Fig. 3.9 Ventral nervous
chain of a *M. beecheii*
worker in which the four
abdominal *ganglia* are
observed

inal *ganglia* could have occurred as a result of the loss of a functional sting which would reduce the activity of the *ganglia* associated with this structure. In males, for instance, there is a trend in all bee taxa for a reduction in the number of abdominal ganglia (Wille 1979). Nonetheless, abdominal ganglion three also has a thoracic position in females of some bumble bees and orchid bees (Cruz-Landim et al. 1972), both having a functional sting.

The workers and males of some *Melipona,* such as *M. quadrifasciata* have five abdominal *ganglia*, while the queens have only four (Kerr and Nielsen 1966). In such species, the number of abdominal *ganglia* has been used to differentiate individuals with a queen genotype (Kerr 1950), but that due to nutritional factors are phenotypically workers (see Chap. 4). In *M. beecheii,* however, both queens and workers have four abdominal *ganglia* (Fig. 3.9; Darchen and Delage-Darchen 1975). In the adult bee, the last abdominal *ganglion* results from the fusion of the larval abdominal *ganglia* 7, 8, and 9 and is found in the fourth abdominal segment (Wille 1961).

The vestigial sting is found in the last portion of the abdomen. In the most primitive genera, it is still possible to find a small sac, probably homologue to the acid gland that exists in stinging bees. Interestingly, this sac is also found in the queens of Meliponini (Kerr and Lello 1962). Parts of the vestigial sting include the *valvulae* 1 and 2, which are the remains of the stylet of stinging bees (Packer 2003; Fig. 3.10). The *valvulae* 3 or *gonostylus* are the remains of the sheath. In stingless bees, valvulae 3 are robust, and straight, profusely covered with bristles, compared with the concave and smooth structures of stinging bees (Radović 1981; Fig. 3.10). In the stingless bees the bristles of the *gonostylus* may act as mechanical receptors. Interestingly, the contact of the *gonostylus* with non-sclerotized surfaces like the skin, triggers some defensive responses, like biting and abdominal movements of the workers that resemble the act of stinging (Radović 1981).
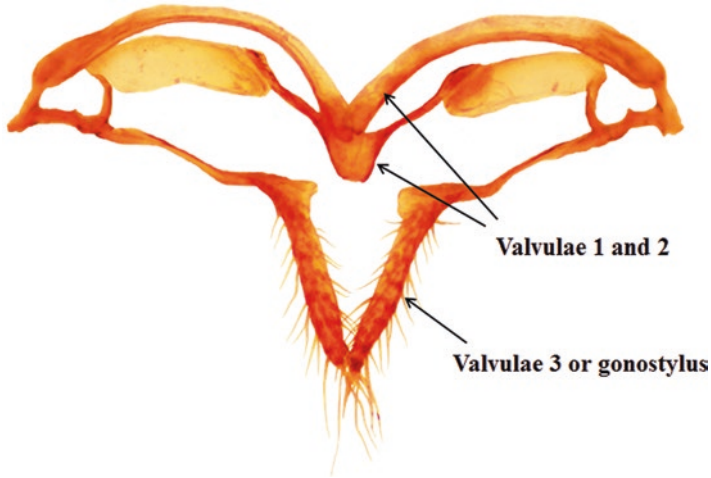
Fig. 3.10 Structure of the vestigial sting of a worker of *M. beecheii* in anterodorsal view. *Valvulae* 1 and 2 are the remains of the sting stylets and *valvulae* 3 or *gonostylus* are the remains of sting sheath. The *gonostylus* is covered with abundant bristles that may act as mechanoreceptors triggering defense responses

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