SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Alimentary Canal and Reproductive Tract of *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae)

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Morfología General del Tracto Digestivo y Sistema Reproductivo de *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae)

RESUMEN - El estudio morfológico del tracto digestivo y el sistema reproductor de machos y hembras de *Hypothenemus hampei* Ferrari se describe a continuación. El tracto digestivo presenta un arreglo típico descrito para otros insectos. Ahora las diferencias morfológicas se encontraron en el buche del estomodeo, en donde se observaron estructuras espinosas y en el mesenteron se observo un número reducido de ciegos gástricos. Se evidenció por primera vez el comportamiento de la alimentación de las hembras de *H. hampei*, que requiere aparentemente de la ingesta del endosperma del café para poder producir huevos viables durante la oviposición. La presencia de burbujas de aire al interior del ventrículo anterior se cree que puede ser debido al hambre, más que a la respuesta al medio ambiente durante el vuelo como lo indicaban anteriores observaciones. El sistema reproductor de la hembra presenta dos ovarios y un arreglo estructural común a especímenes de la familia Curculionidae. El sistema reproductor masculino mostró una diferencia significativa con respecto a otros miembros de la familia Curculionidae e incluso a otra especie de la tribu Scolytinae presentando un aedeagus esclerotizado compuesto de varios orificios en la porción terminal, por donde el esperma se expulsa durante el apareamiento.

PALABRAS-CLAVE: Broca del café, morfología interna, comportamiento de alimentación, reproducción

ABSTRACT - The alimentary canal and the reproductive tract of males and females of *Hypothenemus hampei* Ferrari are described. The alimentary canal of *H. hampei* showed the crop with several spine-like structures and the midgut with few gastric caeca. We evidenced for the first time that adult females need to feed on coffee in order to produce viable eggs before and during oviposition period. The presence of air bubbles inside the anterior midgut may be due to starvation rather than the response of the environment during flying as previously reported. Two ovaries and the same structures and arrangements common to individuals of the Curculionidae beetles composed the female reproductive system. The male reproductive tract showed a significant difference with respect to other Curculionidae and even other Scolytinae species as it showed a sclerotized aedeagus with several pore-like structures in the terminal portion where sperm is released during mating.

KEY WORDS: Coffee Berry Borer, internal morphology, feeding behavior, reproduction

Coffee berry borer, *Hypothenemus hampei* Ferrari is one of the most severe insect pests of coffee in the world and feeds exclusively on coffee plants (Le Pelley 1968, Johanneson & Mansingh 1984). Adults of *H. hampei* damage coffee berries when reproducing inside the endosperm (Corbett 1933, Bustillo *et al*. 1998). Adult females of *H. hampei* bore into coffee berries, make an ovipositing chamber and start laying eggs in groups of 2-3 per day for 20 days (Bergamin 1956). Eggs hatching occur after eight days and the larvae feed inside the endosperm for 19 days. Adults emerge after eight days of pupal stage (Bustillo *et al.* 1998). *Hypothenemus hampei* reproduce by means of a sib-mating system. Adult females emerge and mate with their sibling males before abandoning the coffee bean in search of new coffee berries. Adult males are not able to flight and remain inside the coffee bean (Brun *et al.* 1995, Bustillo *et al.* 1998).

The alimentary canal in Curculionidae beetles can be divided in three regions: the anterior stomodaeum or foregut that is of ectodermal origin, the mesenteron or midgut that is of endodermal origin, and the posterior proctodaeum or hindgut that is also of ectodermal origin. All these regions are involved in ingestion, storage, digestion, absorption and of food and water balance (Borror *et al.* 1976, Calder 1989, Romoser & Stoffolano 1998). There gross morphology and histology of the Scolytinae beetles *Scolytus multistriatus* Marsham (Baker & Estrin 1974), *Dendroctonus* group (Díaz *et al.* 1998, 2003; Silva-Olivares *et al.* 2003), *Trypodendron lineatum* Oliv., *Gnathotrichus retusus* LeConte and *G. sulcatus* LeConte (Schneider & Rudinsky 1969), and *Ips pini* Say (Coleoptera: Curculionidae) (Hall *et al.* 2002) reported some features of alimentary canal which are useful for taxonomy of these beetles (Lopez-Buenfil *et al.* 2001).

The reproductive tract of the female produces and stores eggs, and receives and stores spermatozoa. This system is usually formed by the ovaries, which are comprised of a number of ovarioles, the lateral and the common oviducts, the vagina, the accessory glands and the spermathecae. The male reproductive tract produces, stores, and releases spermatozoa, and is formed by the testes, the *vas deferens*, the seminal vesicle, the ejaculatory duct, the penis or aedeagus, and the gonopore, as well as the accessory gland (Snodgrass 1935, Wigglesworth 1950, Blum 1985).

The internal morphology of beetles is now commonly used for taxonomic characterization of insect species in the same genus, or different insect genus in the same family (Russo 1926, Williams 1945, Robertson 1961).

Here, we describe for the first time the alimentary canal and the reproductive tract of males and females *H. hampei* in order contribute for the better understanding of this insect´s feeding habits and reproductive behavior.

Materials and Methods

This research was carried out at the National Center for Coffee Research – Cenicafé in Chinchiná, Colombia and the Universidad Nacional de Colombia in Manizales, Colombia. Single *H. hampei* infested coffee berries were collected in the field and then individualized in glass vials. These samples were stored at 23°C during the evaluation of the adult insects. Twelve insects were cold immobilized and dissected in presence of Ringer´s solution pH 7.2 and the alimentary and reproductive tract isolated.

Different techniques for the description of the internal structures of *H. hampei* were used. The morphology was analyzed on *H. hampei* females under a Zeiss Axiophot light microscope equipped with a measuring device in order to record size and length. The internal structures were previously stained with toluidine blue and eosin.

In order to reveal the histology of different organs of *H. hampei,* we fixed adults of *H. hampei* in a graded series of ethanol and then polymerized them with Spurr resin for further analysis at the light microscope following the procedure by Mercer & Birbek (1979)**.** Tissues sections 1-3 µm thick were obtained through cuts performed with the ultramicrotome LKB BROMMA Ultrotome-V 2088 with glass knife and then stained with Toluidine blue 2%. Another set of samples were fixed in 2% glutaraldehyde in PSB (phosphate saline buffer) and further dehydration in a graded ethanol series for analyses in a Philips XL 30 TMP Environmental Scanning Electron Microscope (ESEM).

Results

Morphology of the alimentary canal. The main structures at the alimentary canal of *H. hampei* are easily distinguishable (Fig.1). The length of the whole alimentary canal is three folds that of the female adult (Table 1). The foregut has occupies 16%, the midgut 44% and the hindgut 40% of the total length of the alimentary canal.

The foregut begins at the mouth (Fig. 2A) followed by the pharynx and the esophagus (Figs. 2B, C, D). This region is responsible for the transport of food to the crop (Fig. 2E) whose function is the temporal storage of food. The external surface of the crop shows several spine-like structures (Fig. 2E).. Followed by the crop, we find the proventriculus (Fig. 2F), which is the hardest and sclerotized section of the alimentary canal. The proventriculus is a spherical organ formed by eight sclerotized plates that are disposed in edge, and functions as gizzard and filter (Fig. 2G). Muscles that are responsible for the constriction of the proventriculus during the mortaring and filtering of food can be visualized (Fig. 2H). The cardiac valve is located at the end of the foregut (Fig. 2I). Circular muscles (Fig. 2J) that do not allow food to return from the midgut to the foregut surround this organ. The midgut comprises two distinguishable structures, the anterior and the posterior midguts (Fig. 3E). Both the anterior and posterior midguts are of the same length; however, the diameter of the posterior midgut is reduced by a third (Table 1). Closely to the midgut walls, we observed a great number of tracheae (Fig. 3A). The epithelium of the midgut is a single layer of cells surrounding the whole midgut (Fig. 3B, D). The food inside the midgut lumen is surrounded by the peritrophic envelope. This envelope is of Type II that is secreted around the cardiac valve and is projected along the midgut (Fig. 3B). The posterior midgut is characterized by the presence of two gastric caeca (Fig. 3C).

We observed that adult females do feed from the coffee beans during boring and ovipositing (Figs. 3E and 4A show digestion and excretion of food through the midgut and the hindgut). .We strengthen this observation after analyzing some *H. hampei* reared in coffee beans and observed this after 30 days when the adults were still feeding from them. We also observed the presence of air bubbles after three days of starvation of *H. hampei* adults. Despite the small size of the insect, the long hindgut allows clear observation of its structures (Fig. 4A). The hindgut starts with the pyloric valve that contains six Malpighian tubules (Fig. 4A). The Malpighian tubules are long and cover the entire midgut and hindgut. They are classified as Type II (Calder 1989) since they are clustered in two groups: one containing four tubules that end at the anterior part of the rectum, and the other containing only two narrower tubules that end at the base of the anus. The ileum is located right after the pyloric valve and it ends in a more constricted area (Fig. 4A). The

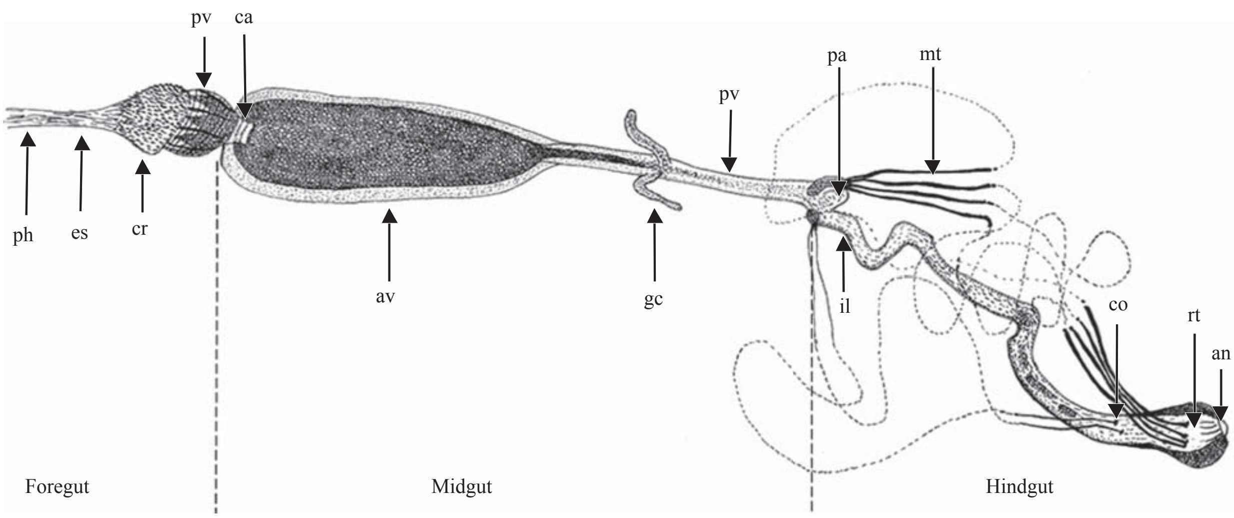

Fig. 1. Morphology of the alimentary canal of *Hypothenemus hampei*. (ph) pharynx, (es) esophagus, (cr) crop, (pv) proventriculus, (ca) cardiac valve, (av) anterior midgut, (pv) posterior midgut, (gc) gastric caeca, (pa) pyloric ampulla, (mt) malpighian tubule, (il) ileum, (co) colon; (rt) rectum, (an) anal sac.

Table 1. Average length of parts of the alimentary canal of *H. hampei.* (n = 12)

Region	Length (mm) x ± se
Alimentary canal	4.98 ± 0.115
Adult female	1.64 ± 0.031
Foregut	0.80 ± 0.007
Crop	0.25 ± 0.008
Proventriculus	0.29 ± 0.005
Midgut	2.20 ± 0.065
Anterior midgut	1.23 ± 0.038
Posterior midgut	0.95 ± 0.056
Gastric caeca	0.18 ± 0.007
Hindgut	1.98 ± 0.043

colon is easily visualized since it is enlarged and shows the insect's excrements (Fig 4A). The colon is surrounded by a group of muscles that aid in the peristaltic movement. The hindgut ends with the rectum, which is a more enlarged and sclerotized section, right before the opening of the alimentary canal or anus (Fig. 4C, D). Attached to the rectum we observed the female reproductive system, which is projected to the anterior part of the insect and is reposed on the ileum (Fig. 4B).

Morphology of the reproductive tract of the female *H. hampei*. The average length of the female *H. hampei* reproductive tract is about 76% that of the adult insects body (Table 2) and it is composed of two ovaries with two conical ovarioles each (Fig. 5A). Each ovary has a lateral oviduct that ends at the common oviduct (Fig. 5A). Even though there must be a separation between the common oviduct and the vagina, the later is not differentiated in *H. hampei*. At the dorsal side of the base of the vagina, we observed the accessory organs, which are the spermatheca and one accessory gland (Fig. 5A). The spermatheca, unlike the accessory gland, is strongly sclerotized (Fig. 5B). The vagina ends at the pygidium, which is the last abdominal segment that is exposed after the elytra (Fig. 5C). The ovarioles contain oogonia at the gemarium that eventually will become primary oocytes (Fig. 5A). The oocytes mature at the vitellarium, which is the basal part of the ovariole (Fig.5A, D). The four ovarioles present in *H. hampei* are asynchronous, since there are not mature oocytes at the same time. We found oocytes at different developmental stages, so when one ovariole was forming an oocyte, other was maturing another oocyte, other contained a matured one that was expelled to the lateral oviduct, and the other ovariole is empty (Fig. 5D). The eggs pass through the lateral oviduct to the common oviduct, then trough the vagina before being fertilized and expelled (Fig. 5E). The common oviduct and the vagina showed a muscle layer that contributes to the movement of the eggs to the exterior.

Morphology of the reproductive system of the male *H. hampei.* The male reproductive tract is almost as long as the adult male (Table 3, Fig. 6A). It begins with the aedeagus (Fig. 6B). The aedeagus is a prominent sclerotized tube that is about a fifth of the adult male length. This structure has not a true gonopore, but instead it is composed of small holes in which the sperm passes (Fig. 6C). There are two apodemes and one tegmen apodeme at the base of the aedeagus (Fig. 6B) that are jointed to muscles that serve as the site for contraction and expansion of the aedeagus. There is also one lateral apodeme that is attached at the VIII sternite and serves as support of the whole organ. The tegmen is an inverted Y-like structure that assists the

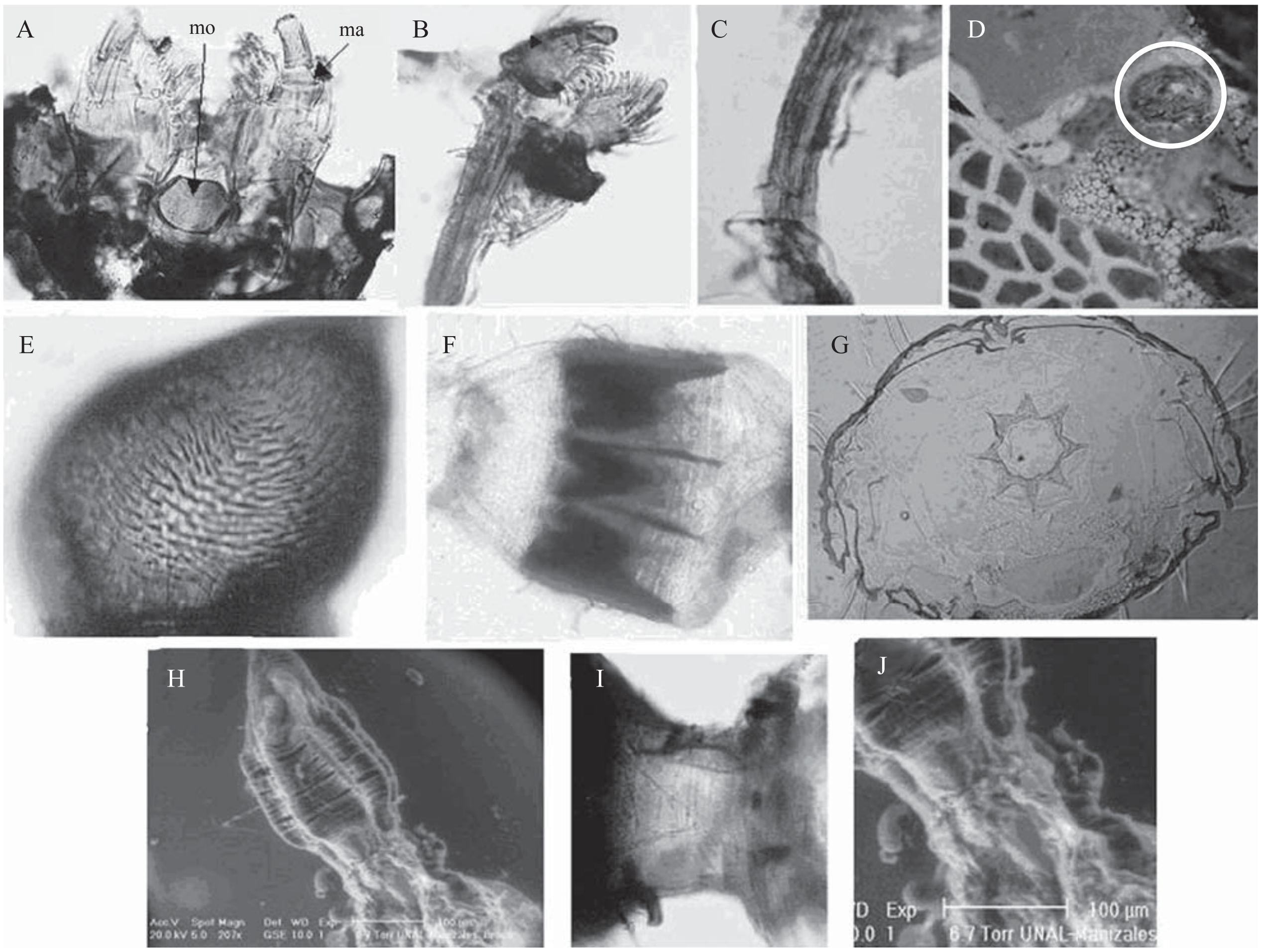

Fig. 2. Morphology of the foregut of *Hypothenemus hampei.* A) mouth parts showing the pre-oral cavity (mo) the mouth and the (ma) maxilla palpus; B) lateral view of the pharynx; C) Esophagus and pharynx; D) transversal cut of the esophagus showing the lumen; E) Exterior view of the crop; F) lateral view of the proventriculus; G) transversal cut of the proventriculus; H) ESEM circular muscles attached to the proventriculus; I) lateral view of the cardiac valve; J) ESEM. Muscles surrounding the cardiac valve.

movement of the aedeagus during mating. We found the ejaculatory duct after the aedeagus, which is a long thin structure that is connected to two seminal vesicles (Fig. 6D). There are two mesadenia (accessory glands) located between the seminal vesicles and the vas deferens. The *vasa deferentia* are between the seminal vesicles and the testes. The two testes are the biggest structures in the male reproductive system (Fig. 6A).

A brief hystological examination of the abdomen revealed the spatial organization of the different organs that composed the alimentary canal and the reproductive system in *H. hampei* (Fig. 7).

Discussion

This paper attempts to describe the morphology of the alimentary canal and the reproductive system of *H. hampei*, the most destructive insect pest on coffee crops. To our knowledge, this research gave the first description of these systems, and was aimed at providing biologists and scientists with basic knowledge of *H. hampei* internal structure. In general, the alimentary canal of *H. hampei* was similar to others Scolytinae beetles such as the *Dendroctonus* spp. (Díaz *et al.* 2000, 2003; Silva-Olivares 2003), *Trypodendron lineatum*, *Gnathotrichus retusus* and *Gnathotichus Sulcatus* (Schneider & Rudinsky 1969), *Ips pini* (Hall *et al.* 2002) and *Scolytus multistriatus* (Baker & Estrin 1974). The length proportion was also similar among Scolytinae. In *H. hampei* the foregut occupied around 16% of the alimentary canal, the midgut 44% and the hindgut 40%. These proportions were alike in species of *Dendroctonus,* in which the foregut occupied 15 to 25%, the midgut 42 to 52% and the hindgut 30 to 40% (Díaz *et al*. 1998).

The foregut begins at the mouth followed by the pharynx and the esophagus. This region is responsible for the transport

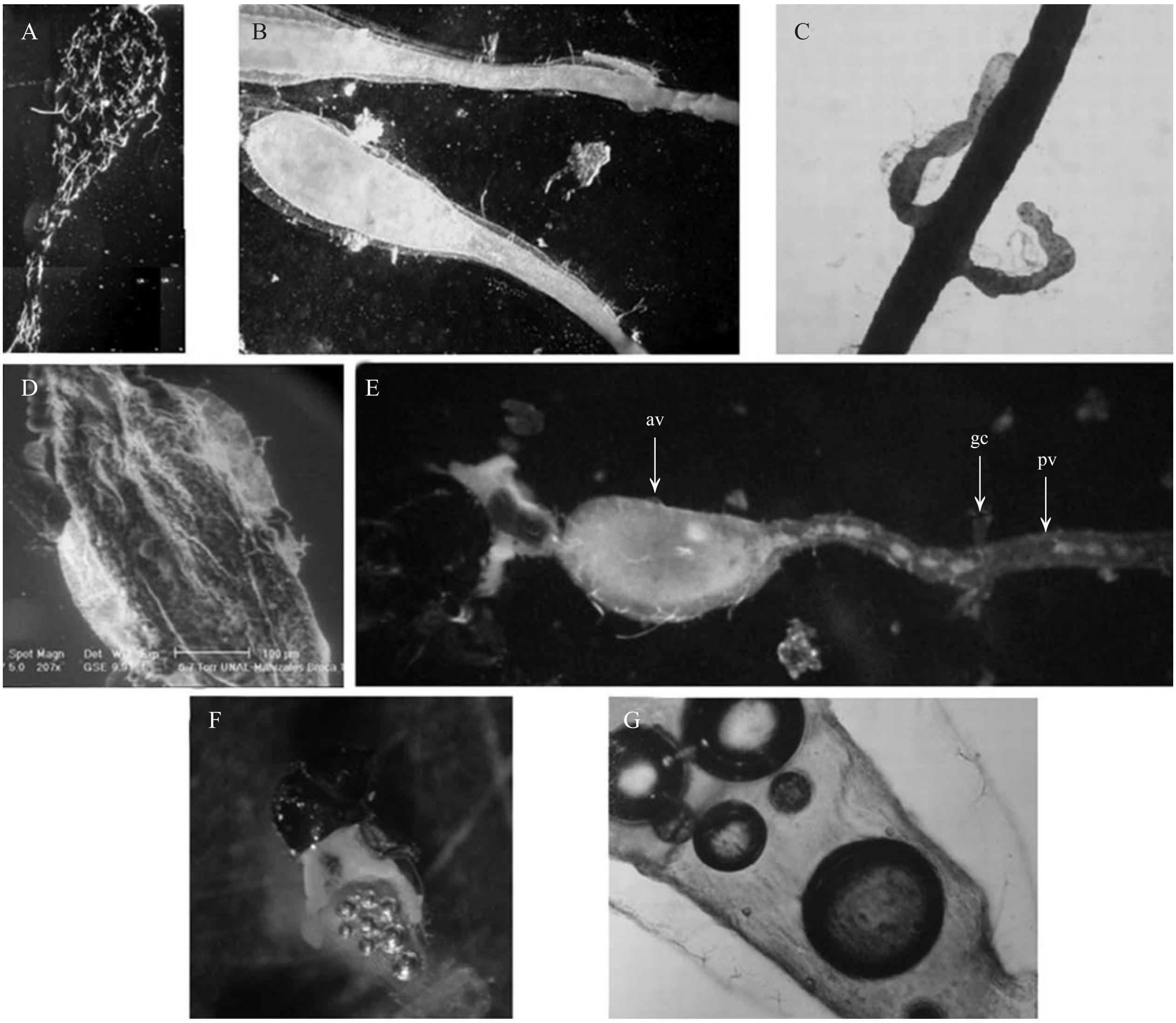

Fig. 3. Morphology of the midgut of *Hypothenemus hampei.* A) Visceral tracheal structures; B) dorsal view of the midgut showing the epithelium and the peritrophic envelope; C) posterior midgut with the gastric caeca; D) ESEM epithelium; E) Lateral view midgut; (av) anterior midgut, (pv) posterior midgut, (gc) gastric caeca. F) General view of the anterior midgut showing air bubbles inside. G) Lateral view the midgut showing the air bubbles.

of food to the crop whose function is the temporal storage of food. The spine-like structures in the crop surface may be involved in water absorption or may help in the contraction and expansion of the crop as a response during food storage. This can also be used as a characteristic in taxonomy to further resolve any given identification dispute within the genus *Hypothenemus*.

The midgut has an anterior and posterior region, which is common in all other described Curculionidae beetles (Wigglesworth 1950, Crowson 1981, Macgown & Sikorowski 1981). The gastric caeca can be used as a taxonomic tool since their position and number change according to the genus. Therefore, *H. hampei* had two gastric caeca in the middle portion of the posterior midgut while *Dendroctonus* has several gastric caeca at the terminal portion of the posterior midgut (Díaz *et al*. 2000). *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) have several gastric caeca in the anterior midgut (Sánchez *et al*. 2000), and *Metamasius hemipterus* L. and *M. hebetatus* Gyllenhal (Coleoptera: Curculionidae) that showed several gastric caeca in both anterior and posterior midgut (Rubio & Acuña 2006).

The fact that adults of *H. hampei* do feed from coffee beans do not offer any explanation about fecundity of this insect. However, Perez-Mendoza *et al*. (2004) indicated that there is oosorption in starved *Sitophilus oryzae* L. (Coleoptera: Curculionidae). This situation may indicate that *H. hampei* would require feeding from coffee beans to produce viable eggs.

The presence of bubbles inside the midgut after the *H. hampei* adults were maintained three days without

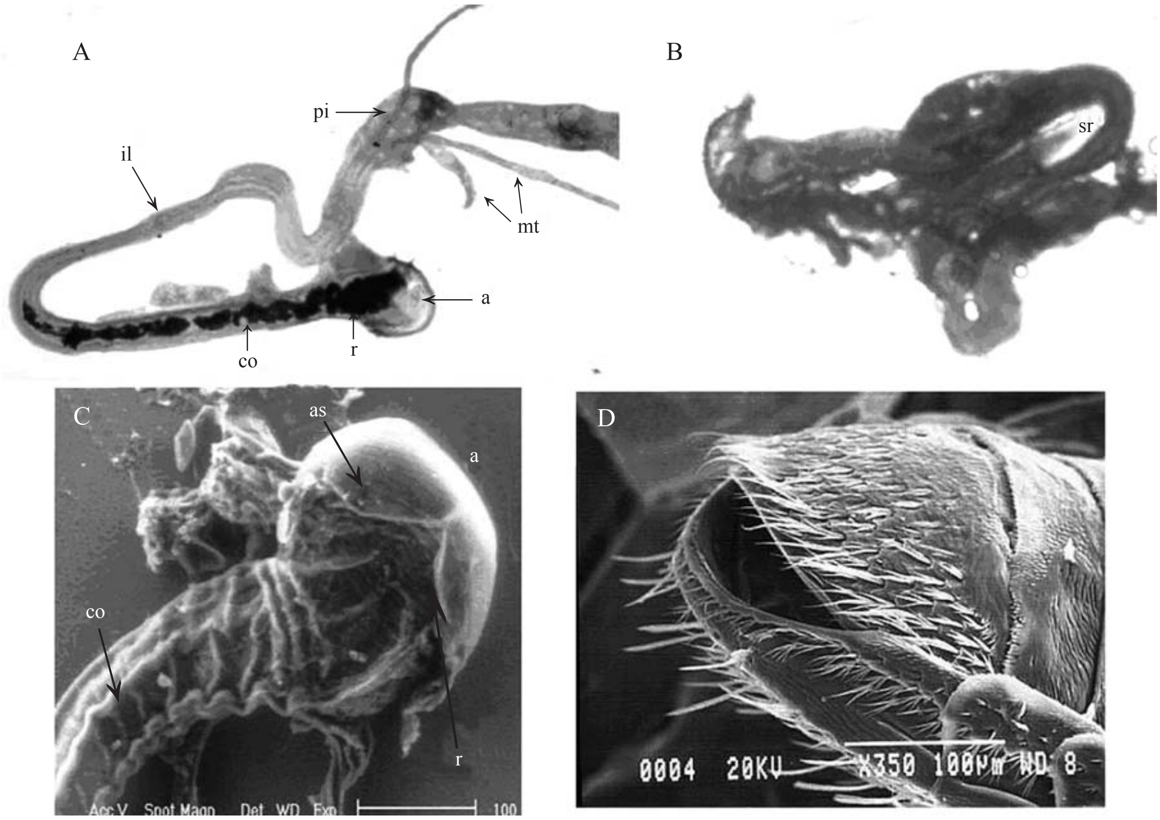

Fig. 4. Morphology of the hindgut of *Hypothenemus hampei.* A) view of the hindgut indicating the (pi) pyloric valve; (mt) three out of six Malpighian tubules; (il) ileum; (co) colon; (r) rectum and (a) anus B) lateral view of the hindgut and the *H. hampei* female reproductive tract; C) ESEM colon, rectum, (as) anal sac and anus; D) ESEM lateral view of the anal orifice.

Table 2. Average length of parts of the female reproductive system of *H. hampei.* (n = 12)

Region	Length (mm) x ± se
Adult female	1.64 ± 0.031
female reproductive system	1.24 ± 0.020
Ovariole	0.43 ± 0.015
Common oviduct	0.46 ± 0.007
Lateral Oviduct	0.04 ± 0.001
Spermatheca	0.13 ± 0.005
Accessory Gland	0.10 ± 0.003

Table 3. Average length of parts of the male reproductive system of *H. hampei.* (n = 12)

Region	Length (mm) x ± se
Adult male	1.26 ± 0.054
Male reproductive system	1.246 ± 0.0054
Testes	0.435 ± 0.026
Seminal vesicles	0.375 ± 0.004
Deferens vas	0.165 ± 0.0023
Accessory gland	0.21 ± 0.007
Ejaculatory duct	0.33 ± 0.0063
Aedeagus	0.254 ± 0.0103

any food, are believed to be a consequence of starvation, and not a response from the environment during flight as suggested by Díaz *et al*. (2000) in *Dendroctonus frontalis* Zimmermann.

The number of ovaries in the reproductive system of *H. hampei* females is similar to other Curculionidae, but there are differences among families within Coleoptera. In Buprestidae, *Buprestis aurulenta* L., there is only one ovary, but in Staphylinidae can be found six and almost two hundred ovaries in Meloidae (Crowson 1981). The oocyte maturation of *H. hampei* seems to be in agreement with the reported biology of this insect (Bergamin 1956). This author indicates that *H. hampei* lays 2-3 eggs daily during the first 20 days. The spermatheca, unlike the accessory gland, is strongly sclerotized since it is of ectodermal origin; this arrangement is common in all coleopterans (Crowson 1981).

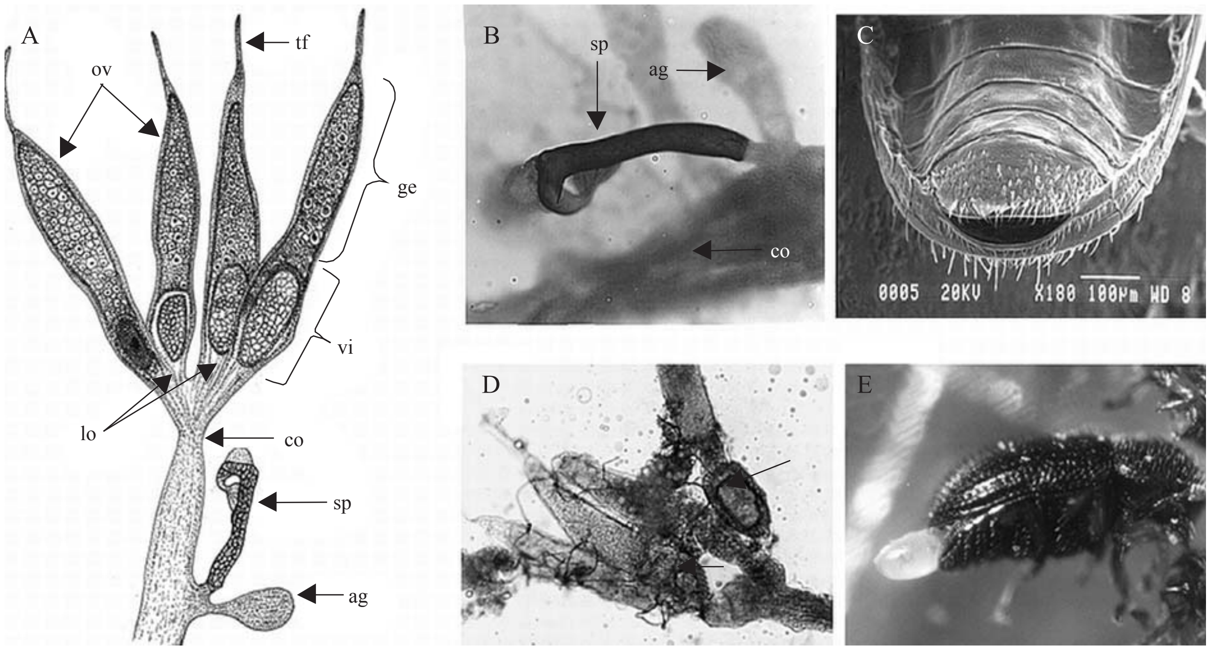

Fig. 5. Morphology of female reproductive tract of *Hypothenemus hampei.* A) General view of the reproductive tract, (ov) ovaries; (ge) gemarium; (vi) vitelarium; (lo) lateral oviduct; (co) common oviduct; (sp) spermatheca and (ac) accessory gland, (tf) terminal filament. B) Lateral view of the distal region indicating the spermatheca; accessory gland and common oviduct. C) ESEM General view of the genital opening at the last dorsal segment named pygidium. D) Lateral view of the maturation oocytes in the ovariole in different stages. E) Female *H. hampei* ovipositing.

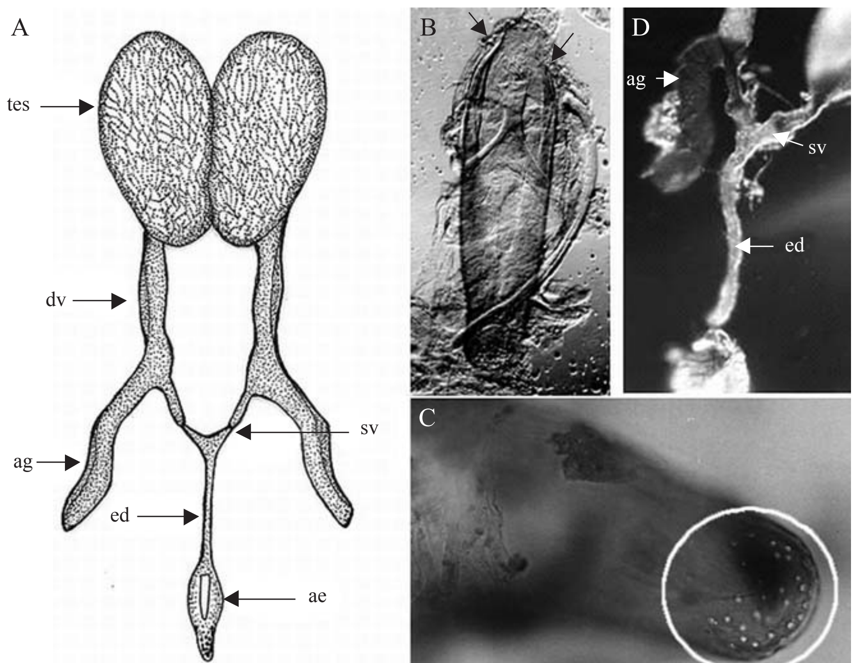

Fig. 6. Morphology of male reproductive tract of *Hypothenemus hampei.* A) General view of the reproductive tract of *H. hampei*, (ae) aedeagus, (ed) ejaculatory duct, (sv) seminal vesicles, (ag) accessory glands, (dv) deferens vas and (tes) testes. B) Lateral view of the aedeagus, where there are two apodemes (indicated with arrows). C) Amplification of the small pores in the distal region of the aedeagus; D) Lateral view of the middle portion of the male reproductive tract showing the ejaculatory duct, the seminal vesicles and the accessory glands.

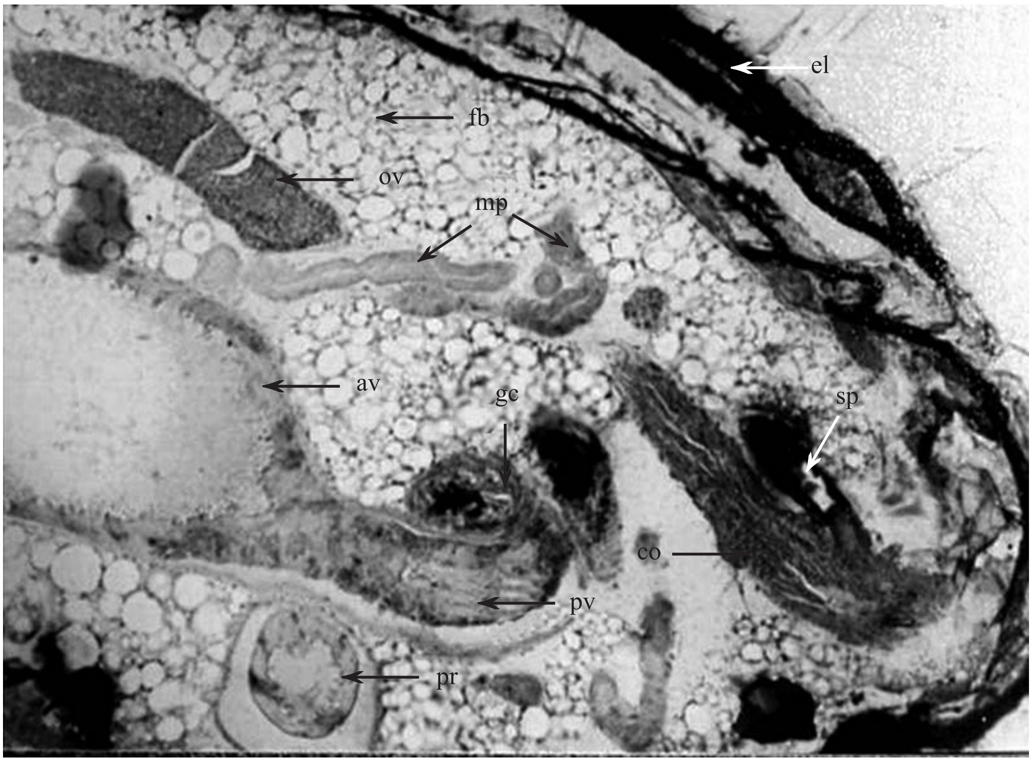

Fig. 7. Transverse section of the abdomen of *Hypothenemus hampei* female showing the position of the different organs that composed the alimentary canal and the reproductive tract. (el) elytra, (fb) fat body, (ov) ovariole, (mp) Malpighian tubules, (av) anterior midgut, (gc) gastric caeca, (sp) spermatheca, (co) common oviduct, (pv) posterior midgut and (pr) proctodeum.

The form of the aedeagus in the male reproductive system is very unique and has not been observed or documented in other coleopterans. This aedeagus does not have a single opening at the terminal portion, but several small pores in a sclerotized structure instead. These pores must serve as canals for the sperm ejaculation.

In summary, we described for the first time the morphology of the alimentary canal and the reproductive tract of *H. hampei*. The results showed typical structures and arrangements of these systems found in other beetles of the same family. However, we documented the feeding behavior of the adults of this insect-pest, the difference in number and position of the gastric caeca, the production of bubbles inside the alimentary canal as a consequence of starvation, and the unique structure of the aedeagus of *H. hampei* that shows several pore-like structures.

Acknowledgments

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