EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
E
MEDICAL ENTOMOLOGY & PARASITOLOGY

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Egypt. Acad. J. Biolog. Sci., 12 (2):49 – 59 (2020)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbse.journals.ekb.eg

Ultrastructural Morphology and Adaptive Behavior of *Leishmania infantum* in the Midgut of the Natural Vector, *Phlebotomus langeroni* (Diptera: Psychodidae)

Sanaa A. El Sattar[1*] and Bahira M. El Sawaf [2]
1-Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt.
2-Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt
E-mail: badersanaa@yahoo.com

ARTICLE INFO
Article History
Received:3/6/2020
Accepted:15/9/2020

Keywords:
Ultrastructure
Midgut
Leishmania infantum
Phlebotomus langeroni
Development

ABSTRACT

The development of *Leishamnia infantum*, the causative agent of visceral leishmaniasis, was studied by transmission electron microscopy in the midgut of the sand fly *Phlebotomus langeroni*, a natural vector. The suprapylarian *Leishmania* behavior was elucidated. The population of *L. infantum* promastigotes in the midgut was composed of several morphological forms that differ in cell shape, flagellum length, and attachment. Ingested promastigotes multiplied within the bloodmeal to small, oval, and slender promastigotes. The disintegration of the peritrophic matrix occurred simultaneously with the morphological transformation of parasites from procyclic forms to long nectomonads. These transformed into short nectomonads (leptomonads). The long nectomonads and short nectomonads attach to the abdominal and thoracic regions of the midgut in a highly specialized manner. The migration of parasites from the abdominal cardia occurred at the tenth-day-postinfection by different concurrently presented forms. These forms probably represent intermediate forms in the transformation sequence into infective metacyclics. Some morphological and physiological changes were observed in infected females compared to uninfected females. The pattern of variation was advantageous to the parasite, allowing development, establishment, and a continuous reservoir of dividing populations in the midgut.

INTRODUCTION

Leishmaniases are a group of diseases caused by the protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). The parasite is transmitted to the vertebrate hosts, including humans by the bite of the phlebotomine sand fly vectors (Diptera: Psychodidae: Phlebotominae). Females of two sandfly genera, *Phlebotomus* (Old World) and *Lutzomyia* (New World) are the only vectors of *Leishmania* species pathogenic for humans (Killick-Kendrick 1999). The symptoms of leishmaniases can range from mild self-healing cutaneous lesions to fatal visceral cases unless treated.

Visceral leishmaniasis caused by *Leishmania infantum* is a major neglected disease despite of its public health importance (WHO 2010; Maroli *et al.* 2013).

Complete life cycles of human pathogenic *Leishmania* species within the gut of sand fly hosts have been little studied. This has been restricted by the difficulty of laboratory colonization and mass rearing of the insect vectors. Particularly lacking are studies, at the ultrastructural level, of parasite adaptations to the gut in natural vector hosts. Ultrastructural aspects of proven natural host-parasite association have been explored in two instances: *leishmania donovani infantum* and the natural vector *Phlebotomus ariasi* (Killick-Kendrick *et al.* 1977), and *L. chagasi* the etiological agent of American visceral leishmaniasis in its vector *Lutzomyia longipalpis* (Walters *et al.* 1989; El naiem *et al.* 1992).

Early epidemiological field studies of visceral leishmaniasis in Egypt, led to the description and colonization of the hitherto unknown female of *Phlebotomus langeroni* (El Sawaf *et al.* 1985). This species acquires its highest epidemiological relevance in El Agamy, Alexandria, Egypt. *P. langeroni* is the natural vector species transmitting *Leishmania infantum* (MON-98) from the dog to humans (Youssef *et al.* 1989). The development of *L. infantum* in *P. langeroni* was described by light microscopy by El Sattar *et al.* (1991). The study showed the colonization of the parasite in the abdominal midgut, the forward migration to the foregut, and attachment to the armature region of the pharynx. This pattern of development of *L. infantum* in *P. langeroni* is typically suprapylarian.

Studies elucidating parasite/vector interactions have become the basis for new approaches to reduce transmission of several insect-borne diseases. Dostalova and Volf (2012) highlight the recent discoveries in this field: For example, the novel vector-based transmission-blocking vaccines (TBVs) (Coutinho-Abreu and Ramalho-Ortigao 2010). This approach aims at preventing transmission of pathogen by targeting molecules expressed on the surface of pathogen during their developmental phase within the insect vector. This strategy has been used successfully in identifying promising vaccine candidates for malaria control (Dinglasan and Jacobs-Lorena 2008) and has great potential in the research of leishmaniases. Despite the importance of visceral leishmaniasis as a disease and the extensive knowledge on the vector status of *P. langeroni*, no previous ultrastructural study of *L. infantum* development in the midgut of this sand fly vector has been reported. The present paper describes in detail the ultrastructural biology and adaptive behavior of the suprapylarian parasite *Leishmania infantum* in the midgut of its natural sand fly vector *Phlebotomus langeroni*. This study serves as a model for future investigations of the parasite in both specific and permissive sand fly species, which will contribute to the development of innovative control measures of epidemiological significance.

MATERIALS AND METHODS

Sandfly species*:*

Laboratory reared *Phlebotomus langeroni* was originally collected from El-Agamy, Alexandria, Egypt.

Sandfly rearing techniques followed the procedure described by Schmidt (1964).

Leishmania species*:*

The strain of *Leishmania infantum* used (MCAN/EG/87/RTC-3, LEM-1213 MON-98), was originally isolated from an infected dog in El-Agamy and grown in NNN medium.

Infection experiments*:*

Five-day-old *P. langeroni* females were experimentally infected using a membrane feeding technique with the pigeon-skin membrane at 26°C. Promastigotes from NNN medium cultures in log-phase growth were counted in a hemocytometer and mixed with defibrinated rabbit blood so that the final concentration was 2×10^6 parasites/ml. (El Sattar *et al.* 1991). Twenty to 50 females were used in the experiment. Engorged females were taken and held in cardboard cups with access to cotton wool soaked in sucrose solution and maintained at 24-26°C and 60-70 rh. Flies were inspected daily for up to ten days.

Preparation of guts for electron microscopy*:*

Females were dissected and examined for infection between 3 and 10 days postinfection. Suitable guts attached to the head were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4℃ overnight, then washed in the same buffer for 20h. Post fixation was in 1% osmium tetroxide in cacodylate buffer at 4℃ for 1.5 h. Guts were rinsed in distilled water and stained in 0.5% uranyl acetate at room temperature (22℃) for 30 min. The fixed guts were dehydrated in an ethanol/propylene oxide series, followed byembedment in fresh TAAB resin overnight at room temperature. Blocks were polymerized at 60℃ for 12-24 h. Ultrathin sections stained with 0.1% lead citrate were examined and photographed with JEOL Cx100 transmission electron microscope.The terminology for anatomical divisions of the sandfly gut and for the developmental stages of *Leishmania* follow those diagrammed by Walters *et al.* (1987) and Dostalova and Volf (2012).The transmission electron microscopy (TEM) was performed at the electron microscope unit. London School of Tropical Medicine and Hygiene, UK.

RESULTS

Parasite development and attachment in the midgut:

Electron micrographs of the midgut examined three days after the infectious bloodmeal showed a partial breakdown of the peritrophic matrix (PM) and some parasites invaded the ectoperitrophic space even before the bloodmeal digestion was completed (Fig. 1).

The sandfly guts examined four days postinfection showed that, the PM was completely disintegrated. The abdominal midgut was colonized by a population of small, oval and slender promastigotes, after being liberated from the bloodmeal into the ectoperitrophic space. These promastigotes were released in the lumen with an orientation toward the midgut epithelium (Fig. 2 A). Slender promastigotes were attached to the microvilli which line the entire midgut epithelium, bodily or via flagellum (Fig. 2 B, C) and occasionally into the cytoplasm (Fig. 2 D). Slender elongate nectomonad promastigotes were depicted six days postinfection. These forms were characterized by their elongate body. The kinetoplast and nucleus were widely separated (Fig. 3 A, B). Nectomonad promastigotes were attached to the midgut epithelium in an organized manner, with their flagella embedded deep between microvilli (Fig. 3 B). This form predominated in the abdominal midgut (Fig. 3 A, B). It is worth mentioning here that, a lipidic-like substance (inclusions) was present in the apical region of the midgut epithelial cells (Fig. 3 A). The enlarged promastigotes reproduced themselves and appeared to generate populations of short nectomonad promastigotes (leptomonads). This form was observed eight days postinfection and was characterized by short flagellum and decreased relative kinetoplast-nuclear distance. Short nectomonads were particularly prevalent in the cardia region of the thoracic midgut (Fig. 4 A, B). Ten days postinfection, some parasites appeared with pointed posterior ends while others had rounded posterior ends. Pear-shaped and oval promastigotes in different sizes were also observed (Fig. 4 C). These promastigotes probably represent intermediate forms in a transformation sequence from short nectomonads to infective promastigotes. Parasites in the cardia region appeared to have a distinct orientation towards the stomodeal valve of the foregut.

In this stage of parasite infection, the midgut cell nuclei were remarkably filled with chromatin (Fig. 4 C.). The microvilli area was enormous and clearly visible.

Generally, the midgut was continuously colonized by the promastigotes throughout the period of study.

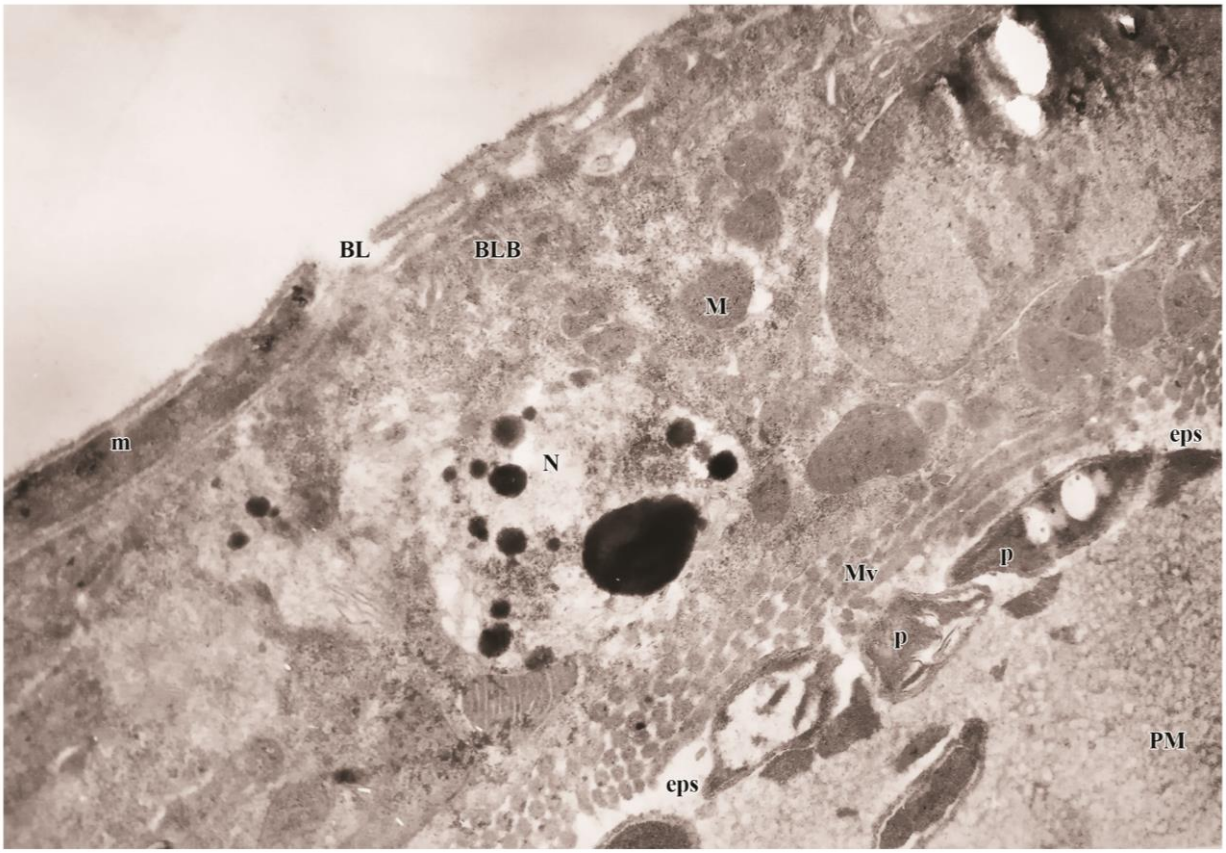

Fig. 1. Electron micrographs of cross sections of abdominal midgut of female *P. langeroni*, 3 days postinfection showing the peritrophic matrix (PM), ectoperitrophic space (eps), microvilli (Mv), nucleus (N), mitochondria (M), parasite (p), basal labyrinth (BLB), muscle (m), and basal lamina (BL). (x10,000).

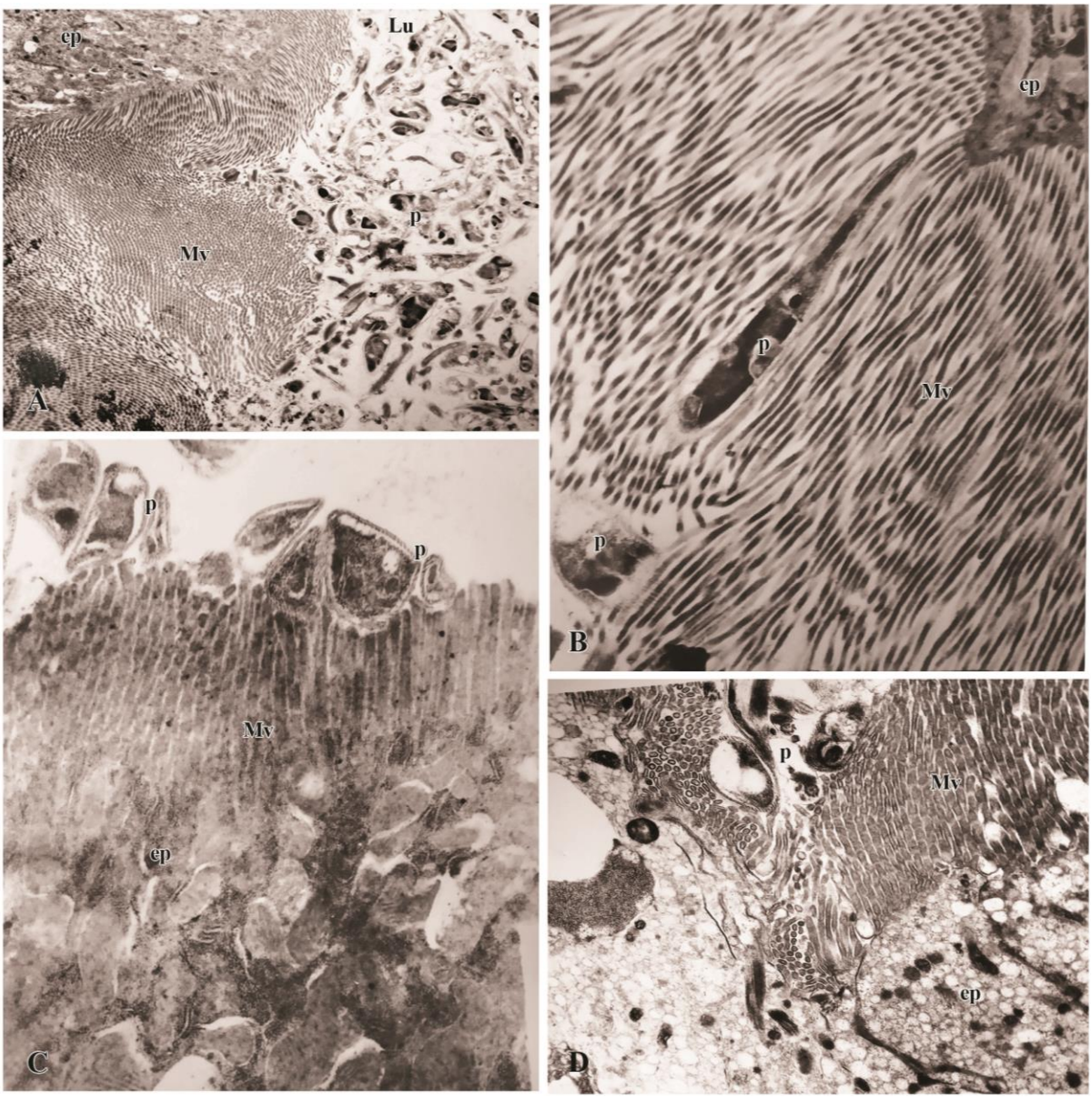

Fig. 2. Electron micrographs of cross section of abdominal midgut of female *P. langeroni*, 4 days postinfection showing, **A** small oval and slender promastigotes (p) in the lumen, (Lu) microvilli (Mv) and midgut epithelium (ep), **B** flagellar attachment to microvilli, **C** body attachment to microvilli and **D** flagellar attachment to cytoplasm.
A (x3,000), **B** (x10,000), **C** (x13,000) and **D** (x10,000).

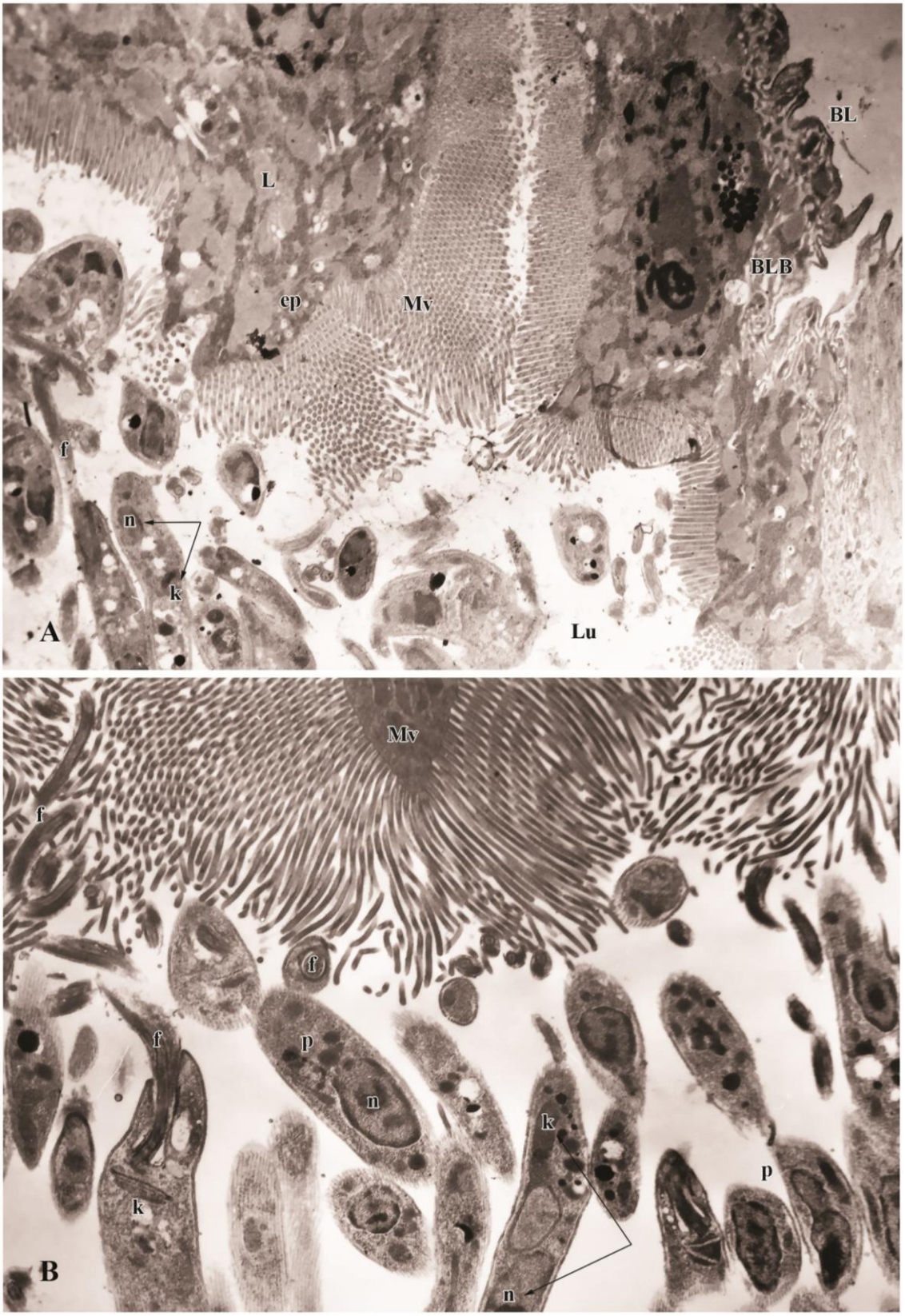

Fig. 3. Electron micrographs of cross sections of abdominal midgut of female *P. langeroni*, 6 days postinfection. **A** showing long nectomonad promastigotes (p), midgut epithelium (ep) and lipid inclusions (L). **B** long nectomonads promastigotes with kinetoplast (K), nucleus (n) and flagellum (f), inserted between microvilli (Mv).
A (x8,000) and **B** (x3,300).

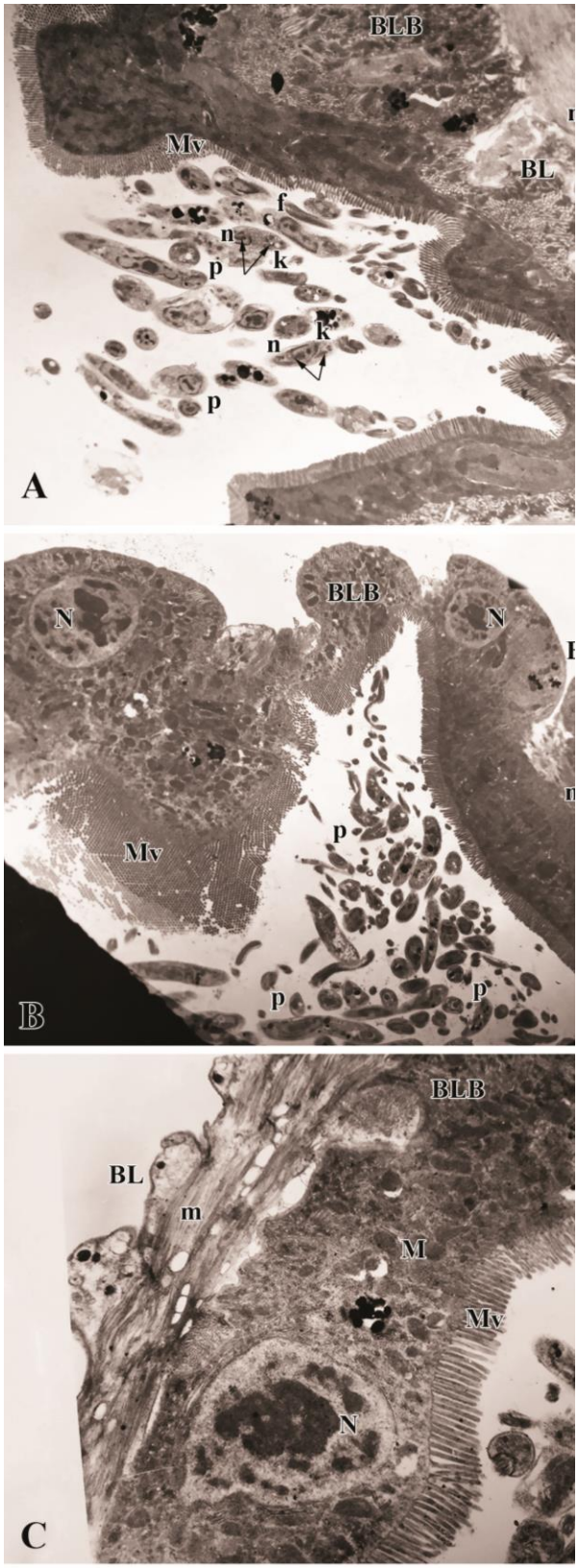

Fig. 4. Electron micrographs of cross sections of thoracic midgut of *P. langeroni* females (8 days postinfection **A**, **B**) and (10 days postinfection **C**) showing short nectomonads, **A** parasite attachment, **B** orientation to foregut and **C** oval, pear-shaped and dividing promastigotes in the cardia region.
A (x2,000) **B** (x1,600) and **C** (x5,000).

DISCUSSION

Leishmania-vector infections are complex and in many cases are species-specific. The life cycle of the parasite in the sand fly gut includes several morphological forms of the parasite promastigotes (nectomonads, haptomonad, and infective metacyclic) and paramastigotes. The distinct morphology of these forms was recognized after the use of electron microscopy (Killick-Kendrick *et al.*, 1974, Killick-Kendrick *et al.*, 1988, Walters *et al.*, 1987, 1989). Although visceral leishmaniasis (VL) is of major public health importance in the Old and New Worlds, the life cycle of human pathogenic *Leishmania* spp. within the gut of their sandfly host have been little studied. The life cycle and division sequence of promastigotes in the bloodmeal was reported only for *L. infantum* (The etiological agent of VL in France) in its natural host *P. ariasi* (Molyneux and Killick-Kendrick 1987) and *L. chagasi* (the etiological agent of American visceral leishmaniasis, in Brazil) in its vector *Lu. longipalpis* (Walters *et al.*, 1989; El naiem *et al.* 1992). In the current study, the cycle of development of the suprapylarian *L. infantum* in the midgut of the proven sand fly vector *P. langeroni* was studied for the first time by TEM.

During the period of study which started three days postinfection to the tenth day postinfection different forms of promastigotes were depicted. Promastigotes were present in the bloodmeal within the PM undergoing multiplication three days postinfection. The PM represents an important mechanical barrier to promastigotes and prevents their escape from the endoperitrophic space. The PM was disintegrated four days after infection by the sand fly chitinases (Ramlho-Ortigao and Traub-Sceko 2003; Ramalho-Ortigao *et al.* 2005) on its posterior end (Sadlova and Volf 2009). The kinetics of PM synthesis and disintegration differs among sand fly species (Walters *et al.* 1993; Pruzinova *et al.* 2015). No difference in timing of PM degradation was detected between females of *P. langeroni* infected with *L. infantum* promastigotes and those fed on uninfected bloodmeal (El Sattar and El Sawaf 2020). This finding was in agreement with that reported by Sadlova and Volf (2009) for *P. duboscqi* and *L. major* association. The authors proved based on histological and electron microscopy, that *L. major* chitinase does not have an important role in the disintegration of the PM in *P. duboscqi* and showed that the PM opens similarly in the uninfected females, Moreover, Coutinho-Abreu *et al.* (2010), based on a molecular study, reported that, targeting the midgut secreted chitinase PpChit 1, reduces *L. major* development in its natural vector *P. papatasi*. It can be concluded that the parasites taking advantage of sand fly chitinolytic activity within the midgut are the main mechanism for their escape. Studies applying molecular technologies have brought insights into several aspects of the parasite vector system and provide a promising target for transmission-blocking vaccines.

The attachment of *L. infantum* to the midgut microvilli of its vector *P. langeroni* was highly specialized. Deep insertion of *L. infantum* flagella between microvilli or bodily on the microvilli, was commonly observed. Flagella were sometimes seen embedded in the epithelial cytoplasm in the cardia region. This type of attachment suggests a significant adaptation of *L. infantum* for maintaining position in the midgut of *P. langeroni.* This behavior was previously observed by Walters *et al.* (1989) for *L. chagasi* and *Lu. longipalpis* association (a natural-parasite-vector association). The ultrastructure description of the midgut epithelium showed that the microvilli were found on the abdominal and thoracic midgut, providing an enormous surface area for the parasite attachment and establishment in the midgut. In addition to providing nutrients from the lumen (Gemetchu 1974). Long nectomonad promastigotes were the most dominant form in the abdominal midgut, moving towards

the thoracic midgut. This form is strongly motile according to Rogers *et al.* (2002). In the abdominal midgut, promastigotes attach by their flagella inserting them between microvilli (Bates 2008). The midgut binding is stage-dependent being limited to the forms found in the middle phase of development (nectomonad and leptomonad forms) but absent in procyclics and metacyclics (Wilson *et al.*, 2010). At this stage of infection (six-days postinfection), it was noticed that the apical region of the epithelium showed prominent lipidic inclusions. This was an unexpected result since it is generally accepted that for sand fly and other Diptera, the cell apparatus was reduced along with the digestive cycle. Lipidic inclusions and glycogen deposits disappeared from the posterior region (Rudin and Hecker 1982; Andrade-Coêlho *et al.*, 2001; El Sattar and El Sawaf 2020). The functional significance of this lipidic-like substance and how it relates to the parasite infection of the sandfly is unclear. However, it is possible that in infected females, synthesis and storage occurred, for utilization by the parasites in the relatively nutrient-deficient milieu, during their forward migration to the foregut. Walters *et al.* (1987) suggested that the switch in food source from the abdominal and thoracic midgut to foregut may be associated with the changes in the kinetoplast in both short promastigotes and paramastigotes. The long nectomonads reproduced themselves and appeared to generate populations of short nectomonad promastigotes also called leptomonads (Rogers *et al.*, 2002). Leptomonad forms accumulate in large numbers in the thoracic midgut and were particularly prevalent eight days postinfection. By day-10 postinfection, some promastigote forms appeared with pointed posterior end while others had rounder posterior ends or pear-shaped. These forms probably represent intermediate forms, of the haptomonads in the transformation sequence into the infective metacyclic stage. These attach as haptomonads in the stomodeal valve intima of the foregut (Sacks and Perkins 1985). Migration of *L. infantum* into the foregut was not described in this life cycle, however, infections of the pharynx, cibarium, and proboscis have been reported previously (El Sattar *et al.*, 1991).

The midgut was continuously colonized by promastigotes throughout the ten-day-period of study, maintaining a reservoir of infection flowing anteriorly into the cardia region of the thoracic midgut. The morphological and physiological changes observed in the midgut epithelium of infected females compared to uninfected females (El Sattar and El Sawaf 2020), illustrate a pattern of variation clearly advantageous to the parasites, allowing development, migration, establishment, and concurrently a reservoir of dividing populations in the midgut. This study underlines the complexity of the interaction of the insect vector with the parasite they harbor and transmit to the vertebrate host. Further research may reveal the functional significance of the processes involved in *Phlebotomus langeroni – Leishmania infantum* and other sand fly vector-parasite associations. This study serves as a model for future investigations of parasites in specific and permissive sand fly species and for comparison with ultrastructural development of other leishmanias, especially those causing visceral leishmaniasis.

ACKNOWLEDGMENT

We express our gratitude to Dr. Laurel L. Walters whose expertise was a great inspiration and an excellent guide for us in this scientific field. We also thank engineer Khaled Abu Almajd (freelance graphic designer), for the photo design of the electron micrographs.

REFERENCES

Andrade-Coêlho, C.A.; Santos-Mallet, J.; Souza, N.A. Lins, U.; Meirelles, M.N.L. and Rangel, E.F. (2001). Ultrastructural features of the midgut epithelium of females *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae). *Memórias do Instituto Oswaldo Cruz*, 96: 1141:1151.

Bates, P.A. (2008). *Leishmania* sand fly interaction: progress and challenges. *Current opinion in microbiology*, 11: 340-344.

Coutinho-Abreu, I.V. and Ramalho-Ortigao, M. (2010). Transmission blocking vaccines to control insect-borne diseases - a review. *Memórias do Instituto Oswaldo Cruz*. 105 (1).

Coutinho-Abreu, I.V.; Sharma, N.K.; Robles-Murguia, M. and Ramalho-Ortigao, M. (2010). Targeting the midgut secreted PpChit 1 reduces *Leishmania major* development in its natural vector, the sand fly *Phlebotomus papatasi*. *PloS Neglected Tropical Diseases*, 4(11), e901.

Dinglasan, R.R. and Jacobs-Lorena, M. (2008). Flipping the paradigm on malaria transmission-blocking vaccines. *Trends in Parasitology*, 24: 364-370.

Dostalova, A. and Volf, P. (2012). *Leishmania* development in sand flies: parasite vector interactions overview. *Dostalova and Volf parasites and vectors*, 5: 276.

El naiem, D.A.; Ward, R.D.; Young, P.E. (1992). An ultrastructural study on the early development of *Leishmania chagasi* (Kinetoplastida: Trypanosomatidae) in its vector *Luztomyia longipapis* (Diptera: Psychodidae). *Annales de Parasitologie Humaine et Comparee*, 67: 3-8.

El Sattar, S.; Shehata, M. and El Sawaf, B. (1991). The development of *Leishmania infantum* in *Phlebotomus langeroni* Nitzulescu (Diptera: Psychodidae). *Parasitologia*, 33 (Supp. 1).

El Sattar, S.A. and El Sawaf, B.M. (2020). The midgut ultrastructure of the sandfly vector, *Phlebotomus langeroni* (Diptera: Psychodidae). *Egyptian Academic Journal of Biological Sciences*, Vol.12(2): 19-25.

El Sawaf, B.M., Kassem, H.A., El Said, S. (1985). Description of the hitherto unknown female of *Phlebotomus langeroni* (Diptera: Psychodidae). *Journal of Medical Entomology*, 22(3): 312-314.

Gemetchu, T. (1974). The morphology and fine structure of the midgut and peritrophic membrane of the adult female, *Phlebotomus longipes* Parrot and Martin (Diptera: Psychodidae). *Annals of Tropical Medicine and Parasitology*, 68: 111-124.

Killick-Kendrick, R. (1999). The biology and control of phlebotomine sand flies. *Clinical Dermatology*, 17: 279-289.

Killick-Kendrick, R.; Molyneux, D.H. and Ashford, R.W. (1974). *Leishmania* in phlebotomid sand flies. Modifications of the flagellum associated with attachment to the midgut and oesophageal valve of the sand fly. *Proceedings of the Royal Society of London. Series B. Biological Sciences,* 187: 409-419.

Killick-Kendrick, R.; Molyneux, D.H.; Hommeel, M.; Leaney, J.A. and Robertson, E.S. (1977). *Leishmania* in phlebotomid sand flies. V. The nature and significance of infections of the pylorus and ileum of the sand fly by leishmaniae of the braziliensis complex. *Proceedings of the Royal Society of London. Series B. Biological Sciences,* 198: 191-199.

Killick-Kendrick, R.; Wallbanks, K.R.; Molyneux, D.H. and Larim, D.R. (1988). The ultrastructure of *leishmania major* in the forgut and proboscis of *Phlebotoms papatasi*. *Parasitology Research*, 74: 586-590.

Maroli, M.; Feliciangeli, M.; Bichaud, L.; Charrel, R.; Gradoni, L. (2013). Phlebotomine sand flies and the spreading of leishmaniases and other diseases of public health concern. *Medical and Veterinary Entomology*, 27: 123-147.

Molyneux, D.H. and Killick- Kendrick (1987). Morphology, ultrastructure and life cycles. Peters, W.; Killick-Kendrick, R., eds. The leishmaniases in biology

````
````

and medicine. *Academic Press, New York*: 121-176.

Pruzinova, K.; Sadlova, J.; Seblova, V.; Hamola, M.; Votypka, J. and Volf, P. (2015). Comparison of bloodmeal digestion and the peritrophic matrix in four sand fly species differing in susceptibility to *Leishmania denovani*. *PLoS ONE,* 10(6)e 0128203.

Ramalho-Ortigao, J.M. and Traub-Sceko (2003). Molecular characterization of Llchit 1, a midgut chitinase c DNA from the leishmaniasis vector *Lutzomyia longipalpis*. *Insect Biochemical Molecular Biology*, 33: 279-287.

Ramalho-Ortigao, J.M.; Kamhawi, S.; Joshi, M.B.; Reynoso, D.; Lawyer, P.G.; Dwyer, D.M.; Sacks, D.L. and Valenzuela, J.G. (2005). Characrerization of a blood activated chitinolytic system in the midgut of the sand fly vectors *Luztomyia longipalpis* and *Phlebotomus papatasi*. *Insect Molecular Biology*, 14: 703-712.

Rogers, M.E.; Chance, M.L. and Bates, P.A. (2002). The role of promastigotes secretory gel in the origin and transmission of the infective stage of *leishmania mexicana* by the sand fly *Lutzomyia longipalpis*. *Parasitology*, 124:495-507.

Rudin, W. and Hecker, H. (1982). Functional morphology of the midgut of a sand fly as compared to other hematophagous nematocera. *Tissue and Cell*, 14: 751-758.

Sacks, D.L., and Perkins, P.V. (1985). Development of infective *Leishmania* promastigotes within phlebotomine sand flies. *American Journal of Tropical Medicine and Hygiene*, 34: 456-459.

Sadlova, J. and Volf, P. (2009). Peritrophic matrix of *Phlebotomus duboscqi* and its kinetics during *Leishmania major* development. *Cell Tissue Research*, 337: 313-325.

Schmidt, M.L. (1964). A laboratory culture of two *Phlebotomus* species, *P. papatasi* and *P. orientalis. Bulletin of the World Health Organization*, 31: 577-578.

Walters, L.L.; Irons, K.P.; Chaplin, G. and Tesh, P. (1993). Life cycle of *Leishmania* (Kinetoplastida: Trypanosomatidae) in the neotropical sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae). *Journal of Medical Entomology*, 30(4): 699-718.

Walters, L.L.; Modi, G.B.; Chaplin, G.L. and Tesh, R.B. (1989). Ultrastructural development of *Leishmania chagasi* in its vector, *Lutzomyia longipalpis* (Diptera: Psychodidae). *American Journal of Tropical Medicine and Hygiene*, 41: 295-317.

Walters, L.L.; Modi, G.B.; Tesh, R.B. and Burrage, T. (1987). Host-parasite relationship of *Leishmania mexicana* and *Lutzomyia abonnenci* (Diptera: Psychodidae). *American Journal of Tropical Medicine and Hygiene*, 36(2): 294-314.

WHO. Control of the leishmaniases 2010. World Health Organization. *Technical Report Series*, 949: 186.

Wilson, R.; Bates, M.D.; Dostalova, A.; Jecna, L.; Dillon, R.; Volf, P. and Bates, P.A. (2010). Stage-specific adhesion of *Leishmania* promastigotes to sand fly midguts assessed using an improved comparative binding assay. *PloS Neglected Tropical Diseases*, 4 e816.

Youssef, M.; Shehata, M.G.; El Sawaf, B.M.; Boules, L.; Pratlong, F.; Amer, M. and Rioux, J.A. (1989). *Leishmania infantum* MON-98, zymodeme nouveau, isolé de leishmanioses viscerales humaines en Egypte (El Agamy). *Annales de parasitologies humaine et compare*, 64(2): 152-153.