Investigation of the Hemidesmosome Protein Components and their roles in the attachment mechanism of *Leishmania*

by

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PhD Thesis

In Biological Sciences

Lancaster University

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June 2014

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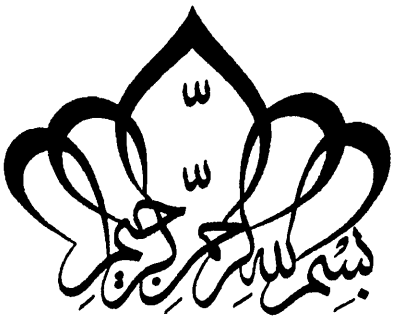

﴿إِقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الإِنسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الإِنسَانَ مَا لَمْ يَعْلَمْ ﴾

القران الكريم –سورة العلق, اية (1-5)

﴿Read in the name of your Lord , who has created (1) Has created the man from a colt (2) Read and your Lord is the Most Generous (3) Who has taught by the pen (4) Has taught the man that which he did not know﴾

Holy Quran- Soraht Al-Alaq (1-5)

A journey of a thousand miles begins with a single step

Lao-tzu, The Way of Lao-tzu
Chinese philosopher (604 BC - 531 BC)

Dedication

Lovingly I dedicate this thesis to my inspiration and heroes; my father and my mother, who planted in my soul the confidence that I can make my dreams come true. Who taught me that, the best kind of knowledge to have is that which is learned for its own sake and even the largest task can be accomplished if it is done one step at a time.

****

بكل حب أهدي رسالة الدكتوراة ....

إلى والدي العزيز.. علي

من كلله الله بالهيبة والوقار.. إلى من علمني العطاء بدون إنتظار .. إلى من أحمل أسمه بكل إفتخار..

إلى ملاكي في الحياة أمي الحبيبة.. زينب

معنى الحب و الحنان والتفاني .. إلى بسمة الحياة وسر الوجود.. إلى من كان دعائها سر نجاحي وحنانها

بلسم جراحي ...

لكما يا ملهمي و قدوتي .. يا من زرعتم في روحي الثقة بقدرتي على جعل أحلامي حقيقة ... يا من علمتوموني أن تحصيل العلم لذاته شرف ما بعده شرف.. و أن المهمة مهما كانت صعبه تتحقق بالإصرار و المثابرة و السعي لها خطوة بخطوة ..

أسال الله أن يمد في عمركما لتشاهدا ثماراً قد حان قطافها بعد طول إنتظار... وستبقى كلماتكما نجوم أهتدي بها اليوم وفي الغد وإلى الأبد..

أبنتكم الممتنه لكم دائما و أبدا

رويدا

Acknowledgments

In Name of Allah, The most Gracious, The most Merciful, who guidance and inspire me, who is taking my hand through this life. Sincere thanks and gratitude to Umm-Al-Qura University for funding my study and for the opportunity that being given to me by them to complete my higher education.

I would like to extend my thanks to my supervisor Dr. Paul Bate, who is the kindest person I ever knew. Thank you for your believing in me and honored me to work under your supervision. Thank you for your support, patience and understanding during all the time. Thank you for teaching me variable lessons in research field and in my future path.

I would like to thanks Mrs. Michelle Bates and Dr. Manal Jamjom for being supportive and for their helps and kindness.

Big and deep thanks to my sister Rania, my brothers; Mohsin, Hasan and Majed, my little siblings, my auntie Amnah, my family, my cousins and my best friends for their sympathy and their believing in me. I love you and I would not reach what I reached without you.

Yours Sincerely ...

Rowaida

Declaration

This thesis has not been submitted in support of an application for another degree at this or any other university. It is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated.

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List of Abbreviations

VL	Visceral leishmaniasis	PM	Peritrophic matrix
CL	Cutaneous leishmaniasis	PSG	Promastigotes secretory gel
PKDL	Post-kala-azar leishmaniasis	IF	Intermediate filament
MCL	Mucocutaneous leishmaniasis	α-ß	Alpha –Beta
HIV	Human Immunodeficiency virus	IFAB	Intermediate filament associated proteins
DCs	Dendritic cells	HBSS	Hanks balance salt solution
PMNs	Polymorphonuclear neutrophils	FBS	Fetal bovine serum
LPG	Lipophosphoglycan	kDa	Kilo Dalton
PPG	Protephosphoglycans	WHO	World helath orgnazation
IgG	Immunoglobulin G	EVA	Ethylene vinyl acetae co-polymer
IL	Interleukin	PES	Polyethersulfone
IFN-γ	Interferon gamma	PGCs	Polycistronic gene clusters
L.	*Leishmania*	SL-RNA	Spliced leader RNA
NO	Nitric oxide	LSGs	*Leishmania* specific genes
iNOS	Nitric oxide synthase	*L.*	*Lutzomyia*
DAT	Direct agglutination test	DMSO	Dimethyl sulfoxide
IFAT	Immuno fluorescence test	DIG	Digoxygenin
ELISA	Immunosorbent assay		
rK39	Immunochromatographic strip test		
P.	*Phlebotomus*		
DEPC	Diethylpyrocarbonate		
SDS.PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis		

Abstract

During their life cycles trypanosomatids differentiate into distinct forms within their hosts. The parasites use their flagella to attach and anchor themselves to the surface of the gut wall in their sand fly vectors. On cuticular surfaces the flagellar tip becomes expanded and forms hemidesmosome-like structures, which enables the parasites to attach strongly to the gut wall. This attachment process occurs in all trypanosomatids and is believed to protect the parasites from elimination by the action of digestive system of the gut so they can survive and multiply, and is also proposed to be an important element of the transmission mechanism. The identity of *Leishmania* hemidesmosomal protein molecules and their function in the attachment mechanism is not known.

In order to investigate this, *L. mexicana*, *L. major* and *L. tarentolae* promastigotes were cultured in the presence of various materials in attempts to replicate this attachment phenomenon in vitro. Most of the materials were discontinued due to their poor attachment performance when cultured with the parasites. However, *L. mexicana* promastigotes were able attach well to Ethylene-vinyl acetate copolymer (EVA) forming expanded flagella as confirmed by scanning electron microscopy. The EVA material generated high quality and quantity of parasites to use for protein investigations.

Protein extraction followed by SDS-PAGE gel staining of selected materials that showed good attachment, revealed a wide range of proteins bands (24.4 kDa – 88 kDa) in each material, except EVA showed only three bands(24.4 kDa, 31 kDa and 36.5 kDa). However, these may have been false positive results and most of bands were probably from media supplements, and therefore molecular approaches were applied for their sensitivity and specificity advantages.

Total RNA was extracted from attached promastigotes of *L. Mexicana* that adhered to EVA surface. A cDNA library was conducted and screened by RT-PCR, cDNA probes and Northern blotting. Screening results showed several genes identified as hypothetical proteins and expressed by trypanosomatids or *Leishmania* species only.

To find specific genes highly or only expressed by attached forms, levels of these genes expression were compared between *in vitro* attached (haptomonad) promastigotes, metacyclic and log-phase promastigotes. Results showed that the three stages expressed some genes at a similar level (LmxM.17.0870, LmxM.09.1505, LmxM.17.0810 and LmxM.05.0450), while other genes were more highly expressed by log-phase promastigotes (LmxM.01.0620, LmxM.36.3780, LmxM.31.2500, LmxM.30.2270, LmxM.08.0410, LmxM.31.1090, LmxM.36.3620, LmxM.36.5850 and LmxM.05.0450). Metacyclic promastigotes also showed different expression levels of several genes (LmxM.11.0930, LmxM.31.0180, LmxM.36.5060 and LmxM.36.2450).Three putative haptomonad-upregulated genes were identified (LmxM.18.1620, LmxM.32.0940 and LmxM.29.3025) although due to time restrictions it was not possible to determine if these were associated with hemidesmosomes. Further investigations are recommended by using new technical approaches.

Chapter 1

Introduction

1.1 General Background

The history of leishmaniasis (cutaneous/oriental sore) begins early in human records ,as described in tablets from the 7th century BC found in the library of King Ashurbanipal, some of their contents derived from 1500 to 2500 BC (Manson-Bahr, 1996). An Arabic physician and scientist named Avicenna in the 10th century also described in his books a skin lesion known as Balkh sore found in northern Afghanistan, Jericho and Baghdad (Manson-Bahr, 1996). Visceral leishmaniasis (VL) was probably first described in 1824 in Jessor, India, where a patient had fevers and his condition diagnosed as malaria, but did not response to malaria treatment (quinine). Later in 1862 the disease outbreak became an epidemic in Burdwan (Elliott, 1863), and cause of the disease remained unclear until Leishman and Donovan described *Leishmania donovani* in 1900 (Hoare, 1938), and the disease was named after them.

Since that discovery *Leishmania* has been identified as a genus of flagellated blood and tissue protozoa (haemoflagellates), which belong to the family Trypanosomatidae of the order Kinetoplastida. *Leishmania* are responsible for various different types of diseases known as leishmaniasis (Beck and Davies, 1981). According to the WHO (2013) leishmaniasis is distributed over both the Old World and New World (Fig. 1) affecting 88 countries with a prevalence around 12 million cases of leishmaniasis. Of the 88 endemic countries, 22 are in the New World and 66 in the Old World with an

estimated incidence of ~1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of VL per year. More than 90% of the CL cases occur in Iran, Afghanistan, Syria, Saudi Arabia, Brazil, and Peru, while more than 90% of the VL cases are reported from India, Nepal, Bangladesh, southern Sudan and north-east Brazil. The annual mortality of leishmaniasis worldwide based on hospitals reports deaths is about 20,000 to 40,000 (Alvar *et al.*, 2012) and the at risk population estimated as 350 million people (WHO 2013). The parasites are transmitted through the bites of small insect vectors named phlebotomine sand flies (Fig. 2) (Service, 2008). The main clinical forms of leishmaniasis are classified as cutaneous, mucocutaneous, diffuse cutaneous and visceral leishmaniasis with different clinical features and geographical distributions (Markell and John, 1999).

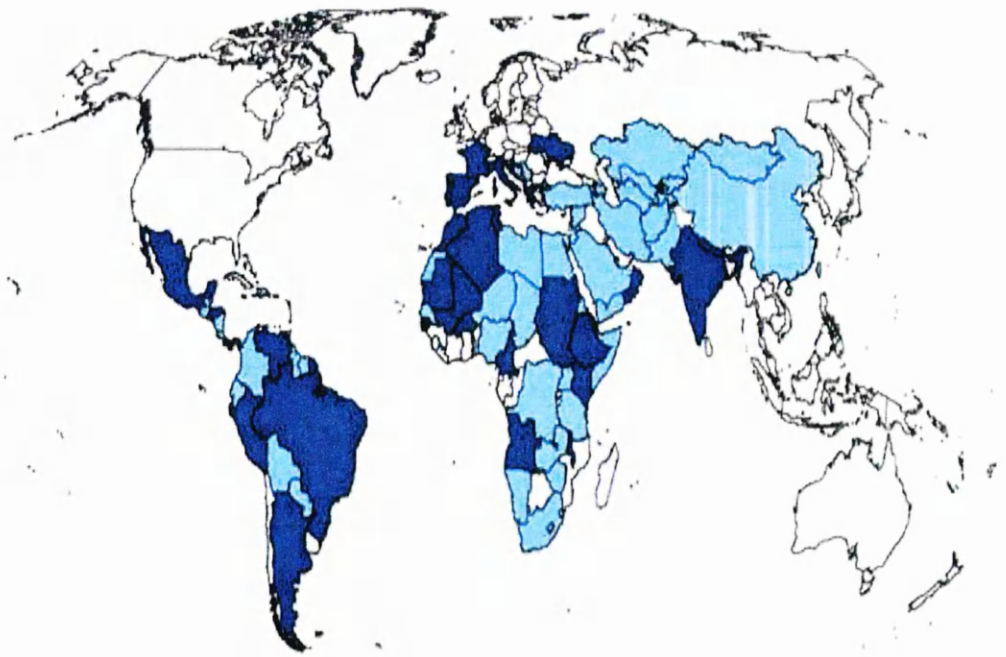


Figure 1. World-wide distribution of leishmaniasis. *Leishmania*-endemic countries and those reporting *Leishmania*/HIV co-infection in 2001. (Taken from Desjeux and Alvar, 2003).

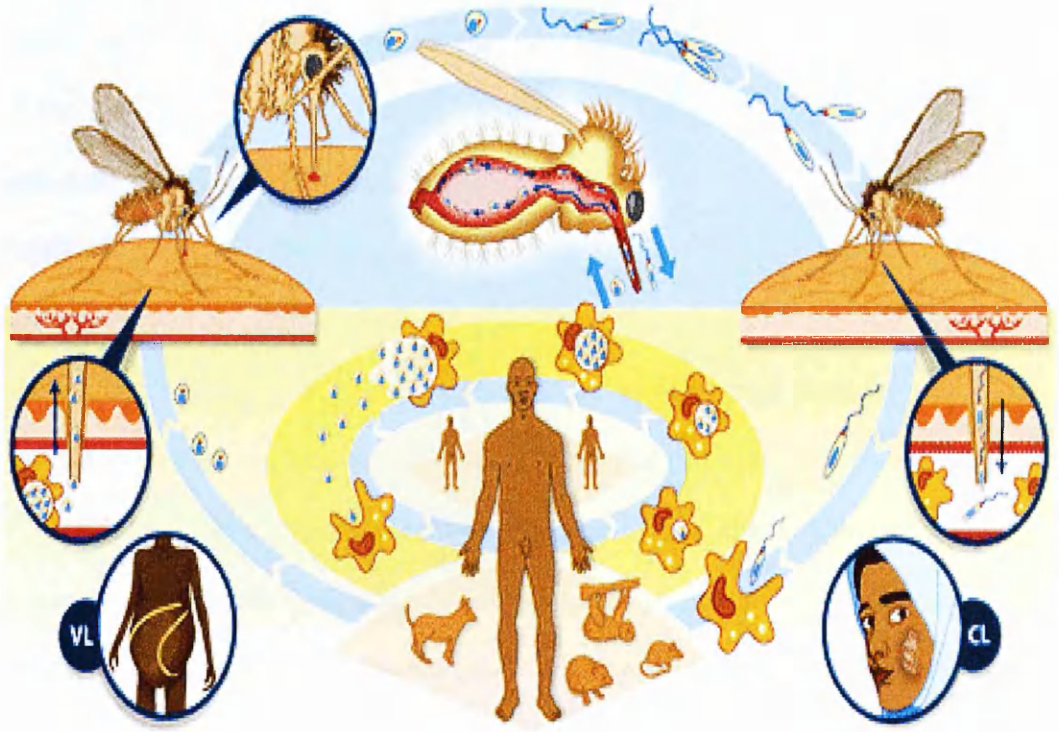


Figure 2. *Leishmania* life cycle. *Leishmania* has a two host life cycle, inside the sand fly vector and inside the mammalian host. (Taken from TDR/Wellcome Trust).

1.2 *Leishmania* Classification

Leishmania species belong to the family Trypanosomatidae (Markell and John, 1999). Other genera in the family Trypanosomatidae include *Trypanosoma, Endotrypanum,* and *Crithidia*. The basic classification of *Leishmania* according to Lainson and Shaw (1987) is summarized as follows:

Kingdom - Protozoa

Sub-Kingdom - Protozoa

Phylum - Sarcomastigophora

Sub-phylum - Mastigophora

Class - Zoomastigophorea

Order - Kinetoplastida

Sub-order - Trypanosomatina

Family - Trypanosomatidae

Genus - *Leishmania*

The various *Leishmania* species have similar morphological features which are difficult to distinguish (Lainson and Shaw, 1987). For that reason, their classification is based on a combination of their extrinsic characters (e.g. disease clinical symptoms, and geographic distribution) and intrinsic characters (e.g. immunological, biomedical and molecular aspects) (Lumsden, 1974). In addition to these, several techniques have been developed to identify *Leishmania* species such as the isoenzyme electrophoresis method, considered as the reference technique for identification of *Leishmania* species (Lanotte *et al.*, 1981), monoclonal antibodies (serodeme) methods (Grimaldi *et al.*, 1987) and molecular (DNA-based) techniques (Marfurt *et al.,* 2003). The human-infective members of the genus *Leishmania* have been divided into two subgenera, *Leishmania (Leishmania)* and *Leishmania (Viannia),* based on their developmental position in the midgut of the sand fly. The subgenus *Leishmania* (*Leishmania*) refers to those parasites whose life cycle occurs in the midgut and foregut of the alimentary tract in the sand fly. The subgenus *Leishmania* (*Viannia)* includes parasites that have a prolific and prolonged phase during their development in the sand fly, in which they are attached to the hindgut wall by their flagella, and later migrate to the other regions (midgut and foregut) (Chance and Evans, 1999). The subgenus *Leishmania (Leishmania)* is distributed over both Old World and New World. On the other hand, *Leishmania* (*Viannia)* is only localized in the New World (Lainson and Shaw, 1987).

Under these two subgenera different species were classified, (Table 1) (Ross, 1903; Lainson and Shaw, 1987).

Clinical Disease	*Leishmania* Species	Geographic Location
Cutaneous leishmaniasis	*L. tropica* complex	Old World
	L. tropica	
	L. aethiopica	
	L. major	
	L. mexicana complex	New World
	L. mexicana	
	L. pifanoi	
	L. amazonensis	
	L. garnhami	
	L. venezuelensis	
	L. braziliensis complex*	New World
	L. peruviana	
	L. guyanensis	
	L. panamensis	
	L. lainsoni	
	L. colombiensis	
	L. infantum	Old World
	L. chagasi	New World
Mucocutaneous leishmaniasis	*L. braziliensis* complex*	New World
	L. braziliensis	
	L. guyanensis	
	L. panamensis	
	L. mexicana	New World
	L. tropica	Old World
	L. major	Old World
Visceral leishmaniasis	*L. donovani* complex	
	L. donovani	Old World
	L. infantum	Old World
	L. chagasi	New World
	L. tropica	Old World
	L. amazonensis	New World

Table 1. Classification of *Leishmania* species based on their disease type and geographical distributions (Markell and John, 1999). The *L. braziliensis* complex (*) are members of subgenus *Viannia*, the other species are in subgenus *Leishmania*.

1.3 Epidemiology and Clinical features

Leishmaniasis can be subdivided by geographical distribution and different clinical spectrums. The clinical manifestations of leishmaniasis range from localised self-healing infections to disseminated infection of the entire reticuloendothelial system, and inner organ infection that may become fatal.

1.3.1Visceral Leishmaniasis (VL)

Visceral leishmaniasis, which is also known as kala-azar (black disease), is the most progressive form of disease with a mortality rate approaching 100% if untreated. The disease is caused by the *L. donovani* complex. In Old World countries such as India, Bangladesh, Nepal, Ethiopia, Kenya, Southern Sudan, and Somalia, visceral leishmaniasis results from *L. donovani* infection (Fig.3). In Europe, North Africa and China, *L. infantum* is reported as the cause of VL. In Mexico, Central America, Venezuela, Colombia, Brazil, Argentina, and Paraguay (New World) *L. infantum* is also responsible for VL, described as *L. chagasi* in some of the older literature but now recognised as a junior synonym of *L. infantum*. The main reservoir of *L. infantum* is the domestic dog, while *L. donovani* is mainly an anthroponotic infection. Recently, due to migrations of large populations from rural into urban areas and large cities with poor living conditions, the disease has been seen in areas where it used to be rare. Vulnerable malnourished people with poor immunity are more severely affected. The disease is clinically characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver(Fig. 4) and anaemia (which may be serious). If the disease is not treated, the fatality rate in developing countries can be as high as 100% within 2 years(WHO

2013, Ashford and Bates, 1999; Markell and John, 1999).

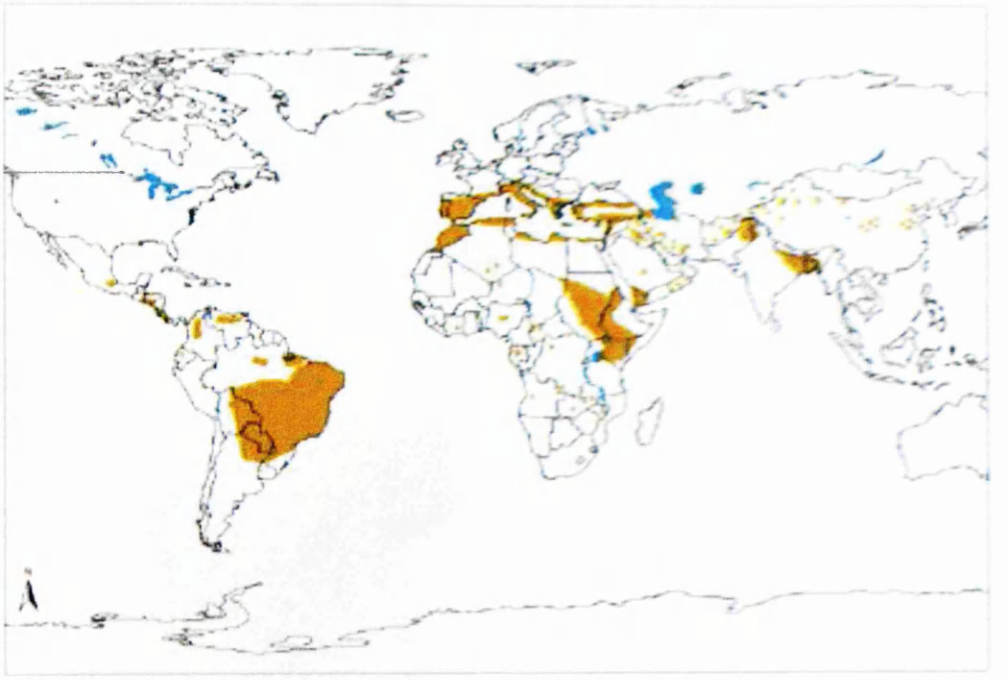


Figure 3. Distribution of VL (WHO, 2013).

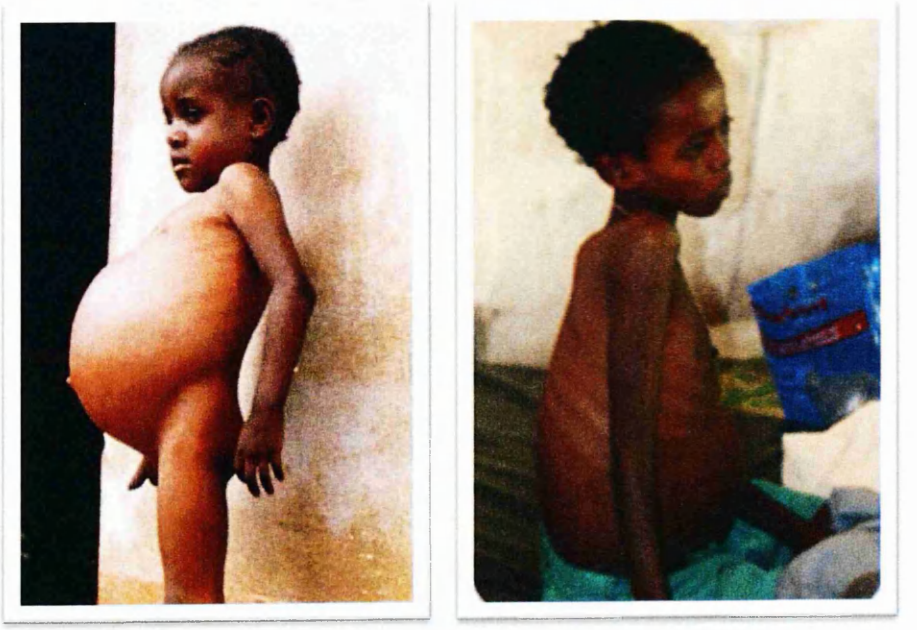


Figure 4. Hepatosplenomegaly due to VL (WHO/TDR/Desjeux/CDC).

1.3.2 Post-kala-azarDermal Leishmaniasis (PKDL)

PKDL is a condition that sometimes appears in patients who have recovered from visceral leishmaniasis. It characterized by a nodular rash, hypopigmented macular lesions, or micropapularmeasles-like lesions that appear on individuals who have recovered from VL after a period of 6 months to years (WHO, 2013) (Fig.5)

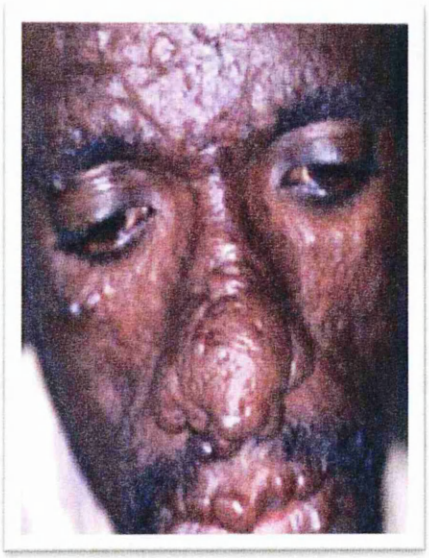


Figure 5. Post kala-azar dermal leishmaniasis (WHO/TDR)

1.3.3 Cutaneous Leishmaniasis (CL)

Cutaneous leishmaniasis caused by the *L. tropica* complex, *L. mexicana* complex and *L. braziliensis* complex. In the Old World it is widely distributed, found in Asia (Armenia, Turkmenistan, Azerbaijan, Uzbekistan, India), the Middle East (Afghanistan, Yemen, Saudi Arabia, Iran, Syria, Jordan) and Africa (Sudan, Egypt, Algeria, Tunisia, Chad, Niger and Kenya) (Fig. 6, 7). In the New World CL is found in areas including south central

Texas, the Yucatan peninsula of Mexico, Guatemala, Belize, Venezuela, and the Amazonia region of Brazil (Fig. 8).

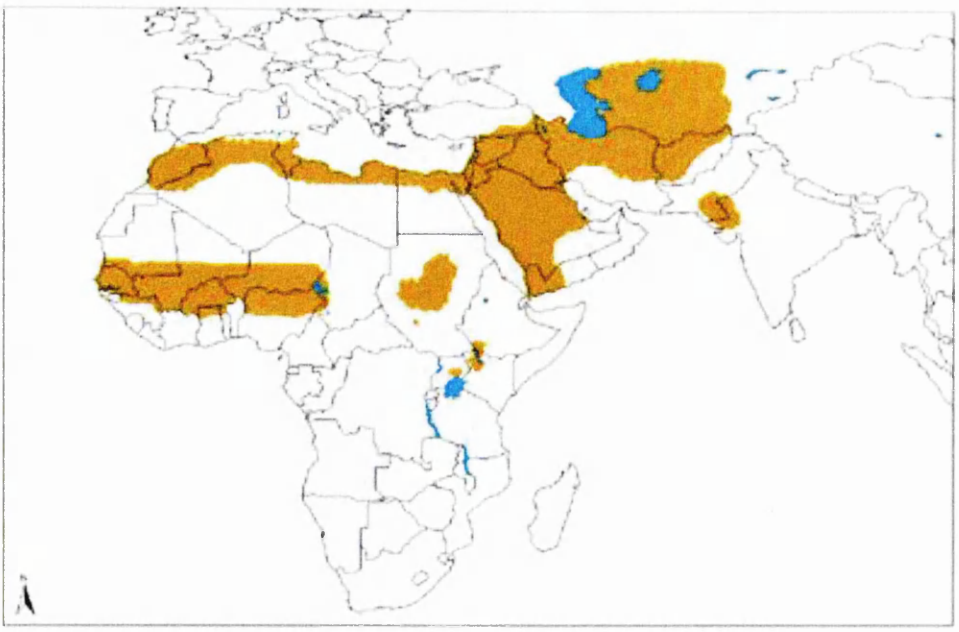


Figure 6. Distribution of CL from *L. major* in the Old World (WHO, 2013)

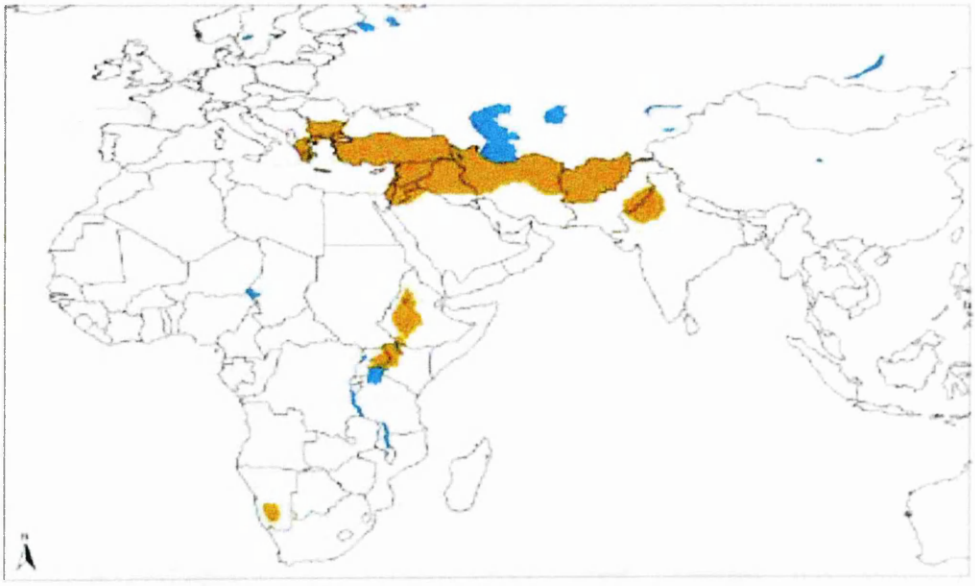


Figure 7. Distribution of CL from *L. tropica* in the Old World (WHO, 2013)

Figure 8. Distribution of cutaneous and mucocutaneous leishmaniasis caused by *L. braziliensis* in the New World (WHO, 2013).

Primarily CL is considered as a rural disease and rodents are the most important reservoirs of infection. CL is also known as Oriental sore, where the lesion is confined to the skin and is characterized in the beginning of the infection by appearance of a small red papule that grows in diameter and ulcerates within a few months time. Usually CL is a self-healing disease but it leaves unpleasant scars on infected areas that may negatively affect patient psychology (Fig.9) (Ashford and Bates, 1999, Markell and John, 1999).

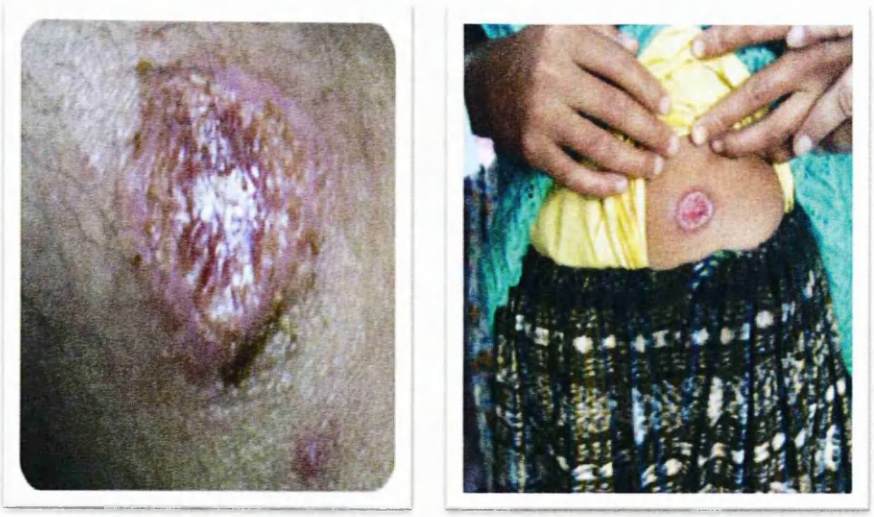


Figure 9. Cutaneous leishmaniasis on the arm and body (WHO/CDC).

1.3.4 Mucocutaneous Leishmaniasis (MCL)

Mucocutaneous leishmaniasis is mainly caused by *L. braziliensis* in Latin America (Brazil, Colombia, Ecuador, Paraguay, eastern Peru, Bolivia and Venezuela) (Fig. 8). The disease is mostly like cutaneous leishmaniasis in the beginning, but then it spreads to the oral and nasal mucosa and if untreated results in very disfiguring tissue destruction (Fig. 10) (Ashford and Bates, 1999, Markell and John, 1999).

1.3.5 *Leishmania*-HIV co-infection

Leishmania parasites also cause severe forms of leishmaniasis in AIDS patients, where the risk of VL infection increases by 100 up to 2320 times in endemic regions. Many countries have reported *Leishmania*-HIV co-infection, and in Europe about 70% of the cases in adults are associated with

HIV (Fig. 1). The clinical presentation depends on a combination of factors including the host immune response and the parasite species, invasiveness, tropism, and pathogenicity (Person *et al.*, 1996; Rees and Kager, 1987; Ashford and Bates, 1999; WHO, 2013)

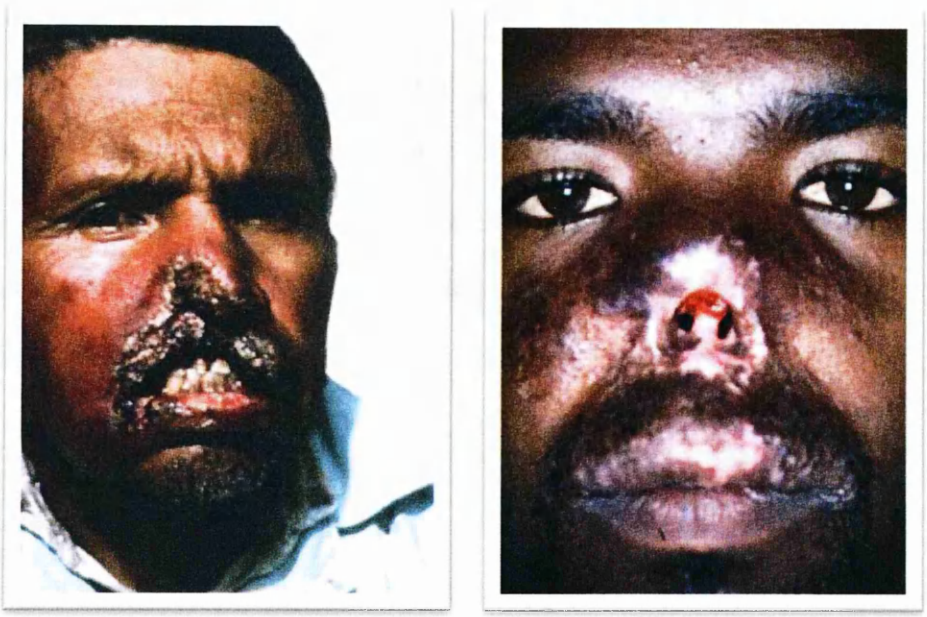


Figure 10. Mucocutaneous leishmaniasis (WHO/TDR/Crump).

1.4 Immunology and Proliferation

Leishmana parasites are transmitted to humans or animals through the bite of an infected female sand fly. When an infected fly feeds on a mammal it releases metacyclic promastigotes into the skin. The immune system responds to the infection with infiltration of macrophages, neutrophils and dendritic cells (DCs) into the bites site, as they are the first line of defence against such infections. Promastigotes are taken up by phagocytosis into macrophages and occupy a parasite vacuole that is part of the phagolysosomal system. After the internalization into the phagosome

promastigotes differentiate into non-motile forms, amastigotes. Macrophage lysosomes fuses with the phagosome and release their contents, but the parasites are resistant to the hydrolytic enzymes and not affected by them. Amastigotes divide several times and finally the macrophage is ruptured releasing parasites into the tissue spaces where they can invade other macrophages (Franklin and Harold, 1994).

The parasites have an amazing ability to manipulate immune cell activity and behaviour so they can survive and proliferate inside the host. The interactions between immune cells and the parasites impact on the final outcome of the disease and understanding of these interactions has an important role in drug and treatment mechanisms (Dong and Jude, 2012).

At the initial infection site neutrophils and macrophages migrate to the site of the bite. Recent studies have proposed that many parasites takenup by neutrophils, which are present in infection sites in large numbers. As neutrophils have a short life span in the blood stream and tissues it is proposed that the parasites use them as an intermediate host prior to uptake by macrophages silently without leading to cell activation (Peters *et al.,* 2008; Peters and Sacks, 2009; Laufs *et al.,* 2002; van Zandbergen *et al.*, 2004). In a study by van Zandbergen *et al.*, (2004) they found the infection of polymorphonuclear neutrophils (PMNs) by *L. major* causes a delay in programmed cell death and secretion of significant levels of MIP-1ß, which has a role in attraction of macrophages to the infection site.

Interaction between macrophages and parasites generally occurs by receptor-mediated processes resulting in phagocytosis. Promastigote-macrophage interaction is promoted by surface molecules on both. On macrophages, surface complement (CR) 1, fibronectin, CR3 (Mac-1), and mannose-fucose receptors have roles in attachment and

binding between promastigotes and macrophages (Kane and Mosser, 2000). On promastigotes, surface lipophosphoglycan (LPG), GP63 and protephosphoglycans (PPG) play critical functions in stimulation of phagocytosis by macrophages (Yao *et al.,* 2003; Naderer and McConville, 2008). In amastigotes, the interaction with macrophages has been explained by the binding between host IgG-coated parasites and Fc receptors (FcyR) on macrophages that facilitate amastigote phagocytosis (Miles *et al.,* 2005).

Generally activation of macrophages is classified into two pathways; classical and alternative. Classical pathway macrophages are activated by products of Th1 and NK cells. Cell cytokines IL-12, IFN-γ IL-1, and TNF-α stimulate the production of inducible nitric oxide synthase (iNOS) by macrophages, which generates the toxic nitric oxide (NO) that kills intracellular parasites (Mosmann and Coffman 1989; Liew *et al.,* 1990; Bronte and Zanovello, 2005). Alternative activation contrasts with the classical pathway. It is activated by production of $CD4^{+}$T-cells that support Th2 cell development and cytokine secretion, IL-4 and IL-13. IL-4 induces polyamine biosynthesis through upregulation of the arginase activity, and this helps parasites to survive and proliferate intracellularly. Other T cells and infected macrophages also secrete immunoregulatory cytokines such as IL-10 and TGF-ß that deactivate the infected cells and prevent parasite elimination by the immune system (Gordon, 2003; Kropf *et al.,* 2005).

1.5 Diagnosis

Diagnostic techniques have been poorly developed in the *Leishmania* field because of the lack of funding for a disease that mainly occurs in poor countries, where people also have poor knowledge of disease aetiology and limited facilities for diagnosis. However, *Leishmania* diagnosis requires that clinical features of the disease are supported by laboratory parasitological diagnosis, as none of the clinical features are unambiguously diagnostic. Correct diagnosis is the key for the right treatment and also early diagnosis will help to reduce the treatment period. Leishmaniasis can be diagnosed by using various methods including detection and identification of the parasites in the tissues and immunodiagnostic techniques (Myler and Fasel, 2008).

The earliest method to diagnose leishmaniasis is the detection of parasites in patient's tissue samples. Biopsy samples can be stained with Leishman's or Giemsa's stain, or used to inoculate cultures that can be directly examined for the presence of promastigotes. Microscopic examination of stained samples looks for intra macrophage amastigotes (Leishman-Donovan bodies). In VL samples are collected from liver, spleen, lymph nodesor bone marrow, while in CL samples are taken from skin lesions. The sensitivity of lymph node aspiration has been reported as 52%-58% (Sidding *et al.*, 1988; Zijlstra *et al.*, 1992), while bone marrow aspiration is better at 52%- 85% (Sidding *et al.*, 1988; Zijlstra *et al.*, 1992; Chowdhury *et al.,* 1993; Bryceson *et al.*, 1996). Aspiration from the spleen is the most sensitive technique for VL at 90%-97% (Sidding *el al.*, 1988; Zijlstra *et al.*, 1992), but should only be performed by an experienced clinician as it can cause fatal haemorrhage.

Serological methods based on antibody-antigen reaction with high sensitivity include the direct agglutination test (DAT), immunofluorescence test (IFAT), enzyme-linked

immunosorbent assay (ELISA), and immunochromatographic strip tests (rK39), and are used in diagnosis of leishmaniasis (Santarem *et al.,* 2005, Sundar *et al.,* 1998, 2002).

1.6 Treatment and Vaccination

Chemotherapeutic treatment for leishmaniasis is usually administered by intravenous or intramuscular routes. Drug doses and duration of therapy vary depending on whether the patient has cutaneous, mucocutaneous or visceral leishmaniasis (Ashford and Bates, 1999; Markell and John, 1999). The standard and first line treatment for all leishmaniasis chemotherapy for many years was (and in many locations remains) the pentavalent antimonial drugs (Pentostam, Glucantime). Treatment with pentavalent antimonials has been practised since 1947, but *Leishmania* parasites are now showing resistance to antimonial drugs in about 10-25% of cases (Myler and Fasel, 2008). Parasite drug resistance has been reported in Southern Europe, Iran, South America, and North East India (Faraut-Gambarelli *et al.*, 1997; Hadighi *et al.*, 2006; Jackson *et al.*, 1990; Rojas *et al.*, 2006; Sundar *et al.*, 2000). Amphotericin B is often used in areas where parasites have been found to be resistant to pentavalent antimonials. Amphotericin B has not seen widespread use because of the level of toxicity in its original formulation, however, liposomal formulations (Ambisome) are much less toxic, although more costly (Mukherjee *et al.,* 2007 ; Sundar *et al.,* 2004).

The first oral treatment for visceral leishmaniasis is miltefosine, introduced in 2002 in India, where about 95% of cases were cured. Clinical trials in Colombia have suggested that miltefosine could also be used for cutaneous leishmaniasis. The drug was originally developed to treat types of cancer, and when tested on *Leishmania* parasites miltefosine was shown to interfere with phospholipids and sterols in

Leishmania (Myler and Fasel, 2008; Lux *et al.,* 2000; Rakotomanga *et al.,* 2007). However, parasites have been shown to easily develop resistance to miltefosine. There remains an unmet need for new antileishmanial drugs, as those available are either too expensive, require long courses of treatment, or are associated with parasite drug resistance.

There are many ongoing efforts to develop safe, affordable, *Leishmania* vaccination. Candidate vaccines include recombinant proteins, DNA-based vaccines, *Leishmania* antigens expressed in BCG and *Salmonella* (Connell *et al.,* 1993; Ghosh *et al.,* 2001a; Yang *et al.,* 1990), and immunization with sand fly components (Morris *et al.,* 2001; Valenzuela *et al.,* 2001). However, to date no vaccine is available for human use, although vaccines for use in dogs are now available (Dantas-Torres, 2006).The difficulty in achieving a successful vaccine is due to different factors. One is that most of the candidates have been tested against *L. major* infection, and it is not sure if they can be efficient against other *Leishmania* species as they have different antigens and mechanisms of pathogenesis. Secondly, experiments are conducted using animal models, and the vaccines may not have similar effects on human leishmaniasis. Also there is the possibility that resistance to vaccines might be developed by parasites (Myler and Fasel, 2008). Therefore, to date no vaccine has been successfully developed for use and more studies are required (Mutiso *et al.*, 2013).

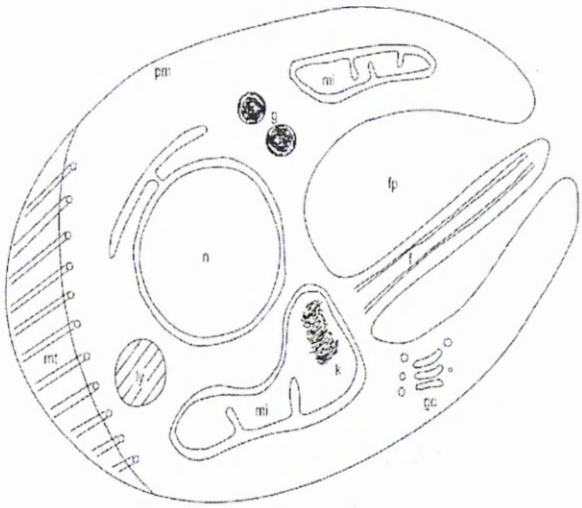
1.7 Parasite Morphological Features

During the parasites life cycle, *Leishmania* organisms exist in two distinct morphological forms known as amastigotes and promastigotes (Beck and Davies, 1981). Amastigotes are small rounded to ovoid bodies 2 - 5μm wide, containing a nucleus and kinetoplast, and a non-motile flagellum that does not extend out of the

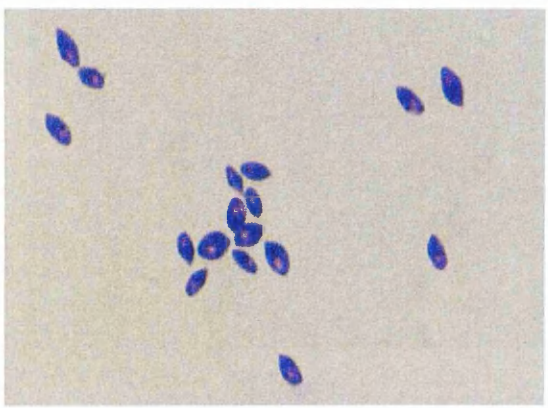
flagellar pocket (Fig. 11). They are intracellular organisms found in phagocytic immune cells including macrophages and monocytes. Promastigotes differ from amastigotes in their elongated slender bodies with size about 10-20 μm. Promastigotes are motile forms by action of their long free flagella at the anterior end of the body near the kinetoplast (Walters *et al.*, 1993, Chiodini, 2001) (Fig. 12), and they are extracellular parasites found in the alimentary tract of the sand fly (Beck and Davies, 1981).

In smears of the gut of sand flies heavily infected with some *Leishmania* species, large rosettes of parasites are sometimes observed attached to each other by their flagella, which are oriented towards the centre of the mass (Molyneux and Killick-Kendrick, 1987). A wide variety of promastigotes are seen *in vitro* cultures. However, in long-term cultures, it is not possible to relate such forms to a particular sequence of development as in the sand fly. Likewise, it is incorrect to assume that promastigotes cultivated in vitro are representative of all the stages found in the sand fly (Ashford and Bates, 1999).

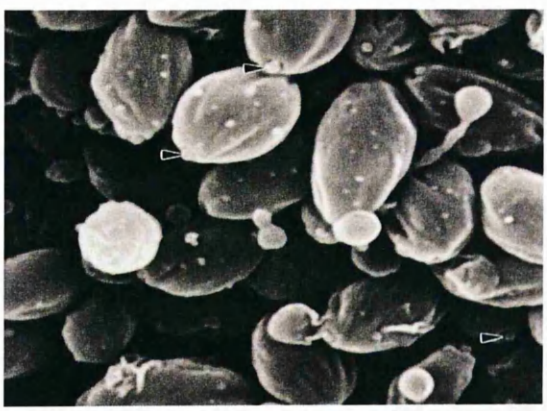
Figure 11. Morphology of Amastigotes



(A) Ultrastructure of a *Leishmania* amastigote. Amastigotes possess a central nucleus (n) and adjacent kinetoplast (k) within a single branching mitochondrion (mi). The flagellum (f) arises from a flagellar pocket (fp) but does not extend beyond the cell body. Lysosomes (ly), glycosomes (gl) and Golgi complex (gc) are found in the cytoplasm. Rows of microtubules (mt) run just below the plasma membrane (pm). (Taken from Ashford and Bates, 1999).

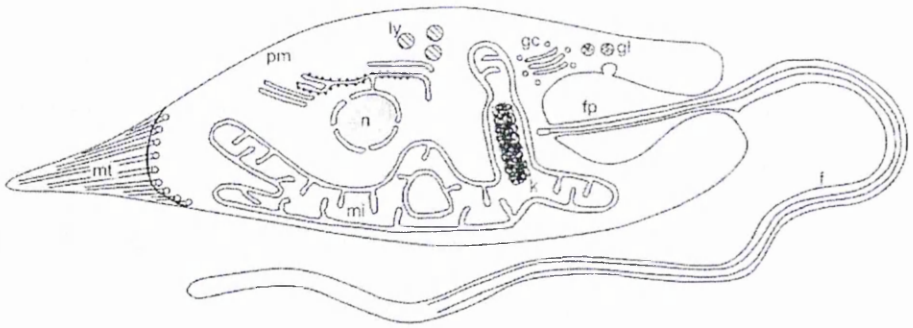


(B) Light micrograph of Giemsa-stained amastigotes from Grace's insect culture medium.

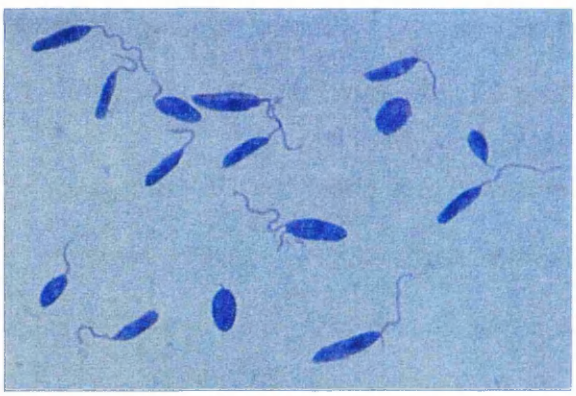


(C) Scanning electron micrograph of amastigotes showing flagella slightly extended beyond the cell body. Arrowhead denotes individual flagellum. (Taken from Wakid and Bates, 2004).

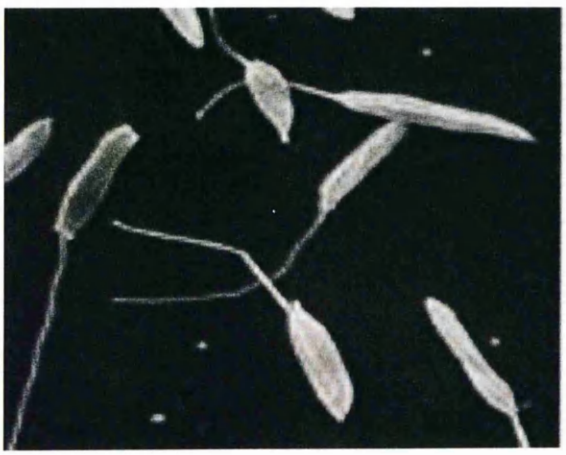
Figure 12. Morphology of Promastigotes



(A) Ultrastructure of a *Leishmania* promastigote. Many of the features found in the amastigote stage are also found in the promastigotes. Some differences are that cell body is elongated, the cell body and the kintoplast has a more anterior location relative to the nucleus. (Taken from Ashford and Bates, 1999).



(B) Light micrograph of Giemsa-stained promastigotes from M199 culture medium.



(C) High power scanning electron micrograph showing several promastigotes. (Taken from Wiese, USS/link).

1.8 Molecular Biology

Genomic databases of trypanosomatids (Tritryps: *L. major*, *Trypanosoma brucei*, *T. cruzi*) have revealed a conserved core proteome of about 6200 genes that are assumed to mainly fulfil housekeeping functions (EL-Sayed *et al.,* 2005b). In *Leishmania* there are an additional ~1000 genes are specific to *Leishmania* (LSGs), which are randomly distributed among the genome. Some of these LSGs have known functions, such as involvement in parasite metabolism, but the functions of the majority are still unknown (Ivens *et al.,* 2005). Further efforts in recent years have resulted in gene sequencing of three *Leishmania* species (*L. infantum, L. braziliensis* and *L. major*). The sequenced genomes exhibit more than 8300 protein-coding and about 900 RNA genes. There are about ~200 genes that are different in their contents between three *Leishmania* genomes sequenced (species-specific differences), which may be related to the differences in their pathogenesis.

The arrangement of *Leishmania* species genomes are similar, consisting of 34 to 36 chromosomes, mainly in a diploid state, with sizes ranging from ~250 Kb to ~4 Mb (Myler and Fasel, 2008). *Leishmania major* and *L. donovani* complexes have 36 chromosomes, while the *L. mexicana* complex (New World) have 34 and the *L. braziliensis* complex have 35 chromosomes, due to the previous fusion of chromosome 8 + 29 and 20 + 36, and the fusion of chromosome 20 + 34, respectively (Wincker *et al.,* 1996; Britto *et al.,* 1998). The protein coding genes are organized on the same DNA strand in long polycistronic gene clusters (PGCs) of tens to hundreds of genes. These are arranged either head-to-head (divergent) or tail-to-tail (convergent), or interrupted by RNA genes causing splitting of the PGCs into two or

more head-to-tail arrays (sequential) (Myler *et al.,* 1999; Worthey *et al.,* 2003; Myler and Fasel, 2008).

Leishmania telomeres are relatively small and consist of a few different types of repeat sequence (Myler and Fasel, 2008). In the *L. major* Friedlin sequenced genome the telomere has a repeated structure (ACCCTA) and studies showed that recombination between different groups of telomeres can occur, causing sharing of telomere proximal protein coding genes and sub-telomeric sequences (Chiurillo *et al.,* 2000; Sunkin *et al.,* 2000; Myler and Fasel, 2008).

Leishmania has unusual characteristics regarding the regulation of gene expression. *Leishmania* genes have no introns in their transcription units, the protein coding regions do not get interrupted (Mair *et al.,* 2000 a). The groups of genes in PGCs are co-transcribed to yield polycistronic pre-mRNAs (Myler *et al.,* 1999; Worthey *et al.,* 2003; Ivens *et al.,* 2005). Posttranscriptional processing involves a *trans*-splicing mechanism in which a small capped RNA of 39 nucleotides is added, the spliced leader RNA (SL RNA), to the 5' terminus of the mRNA, and a 3' cleavage and polyadenylation (Ullu *et al.,* 1996). Various studies have proposed that the trans-splicing and polyadenylation processes in *Leishmania* are linked and have common regulatory signals (LeBowtiz *et al.,* 1993; Matthews *et al.,* 1994).Gene expression regulation is exclusively a post-transcription process where control of mRNA stability and translation is exerted. These processes are determined, at least partly, by sequences within 3' untranslated region of the relevant gene (3' UTR) (Fig. 13). Such control of gene expression has been shown to be involved in regulation of *Leishmania* differentiation within macrophages (Clayton, 2002; Boucher *et al.,* 2002; Folgueira *et al.,* 2005; McNicoll *et al.,* 2005).

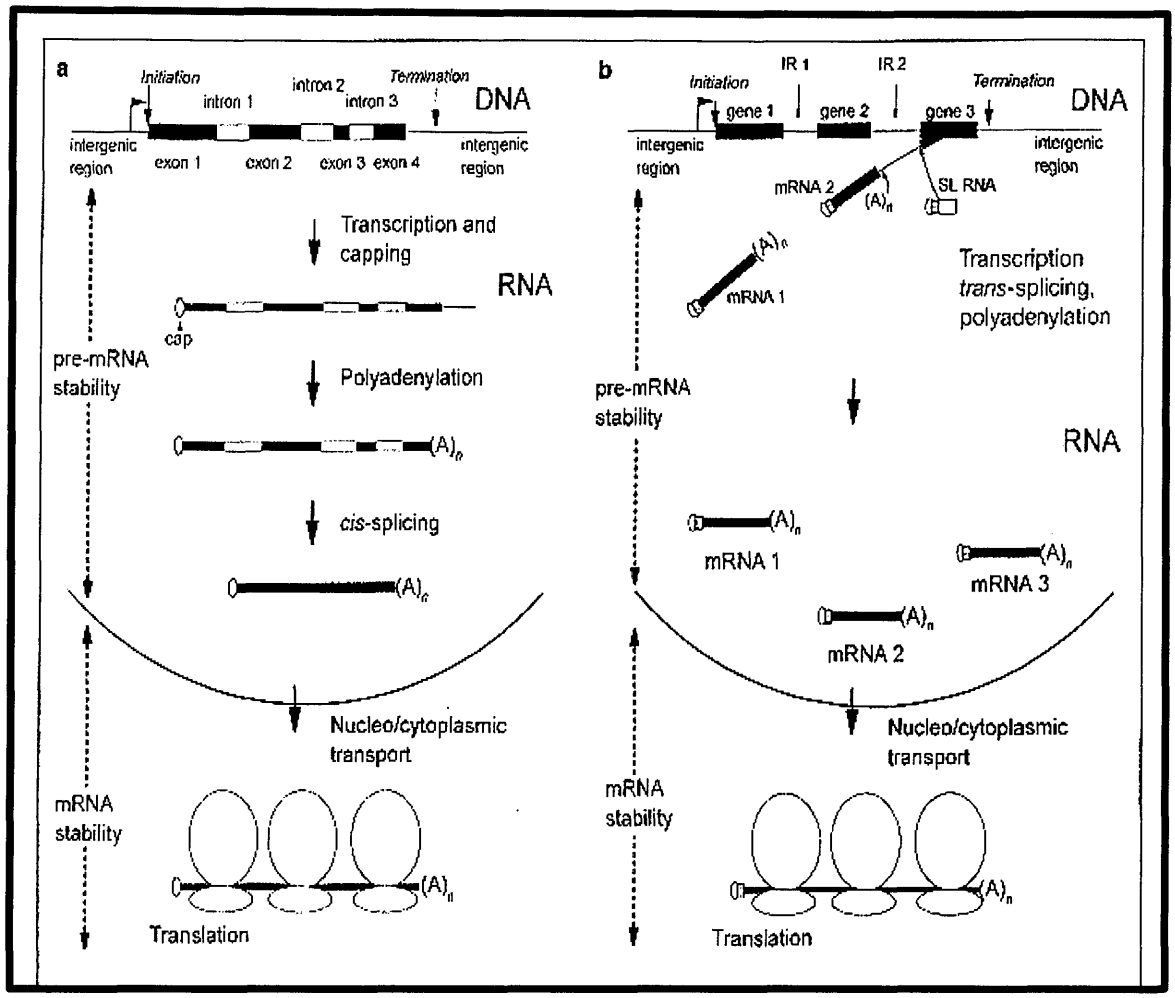


Figure 13. Comparison of mRNA synthesis in higher eukaryotes (a) and trypanosomatids (b). *Leishmania* genes have no introns their transcription units and gene expression regulation is exclusively a post-transcription process. The Posttranscriptional process involves a *trans*-splicing mechanism where a small capped of the spliced leader RNA (SL RNA) is added to the 5' terminus of the mRNA, and a 3' cleavage and polyadenylation (Stiles *et al.*, 1999).

Chapter 2

Literature Review

2.1 Cuticle structure of the sand fly gut

During the life cycle of *Leishmania* inside sand fly, some stages (ex, nectomonads and haptomonads) attached to the cuticle surface of vector gut. Many studies have been conducted using light and electron microscope to understand and describe the structure and composition of insect gut. Both methods were agreed that the cuticle line is actually secreted by a single layer of epidermal cells that covers the entire surface of the insect including fore-gut and hind-gut, while the mid-gut is lined with microvilli. The cuticle is composed of several layers, starting from the outside: cement and wax, then epicuticle, then exo- and endocuticle (Fig. 14, Fig. 15) (Wigglesworth, 1948, Vincent and Wegest, 2004, Bruck and Stocken, 1972 (A) and (B), Filshie, 1970). The cuticulin has a thickness of 12 – 18 nm and it is noticed that the cuticulin line have sublayers too that been seen during their development process (Locke, 1966). Beneath the cuticulin the protein epicuticle layer is deposited which characterized by different thickness forms (Locke, 1974, 1976). Through the protein layer wax canals were shown penetrated and connected to the pore canals in the procuticle (region of the cuticle located between the epicuticle and epidermal cells, and it constitutes the main part of the total cuticlele (Andersen, 1979)) and terminate in the cuticulin or on its surface (Bruck and Stocken, 1972 (A), Locke, 1961). Based on cuticle examination; some studied proposed that the cuticle is covered by lipids in a wax form which secreted from wax canals and they related that to the formation and transportation the

lipids within the cuticle, where in some insect the wax layer showed covered by a cement layer. Both may play a role in cuticle protective and permeability barrier properties (Andersen, 1979, Locke, 1961). The chitin is another composition of the cuticle line of sand-fly gut. It is consisting of arrangements of highly crystalline chitin nanofibres embedded in a matrix of protein, polyphenols and water, with small amounts of lipid (Vincent and Wegest, 2004) Chitin is an acetylated polysaccharide akin to cellulose in its structure. The monosaccharide units are linked together through β-1,4 links that make the molecule very straight and ribbon-like. The chitin chains are arranged anti-parallel form (the α form) and combine into a highly crystalline structure within which the sugar residues are heavily H-bonded imparting stiffness and chemical stability. Within the body of the cuticle the chitin is organized into nanofibres with about 3 nm in diameter and about 0.3 μm long, each containing 19 molecular chains (Atkins, 1985).

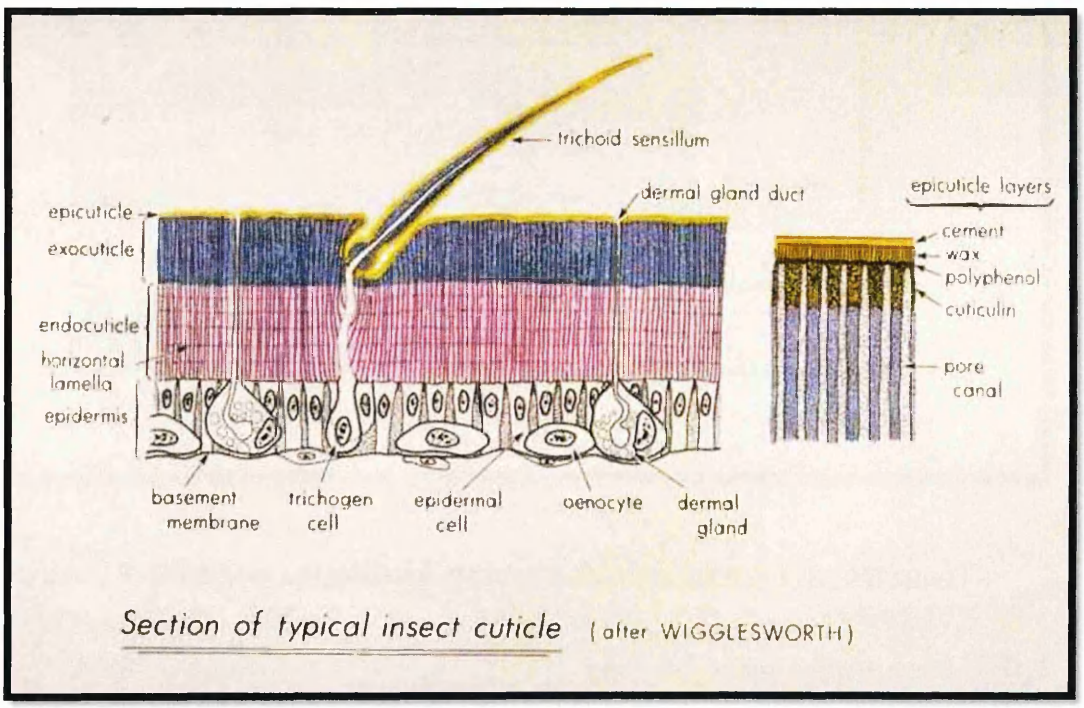


Figure. 14. Section of typical insect cuticle. (Wigglesworth, 1948)

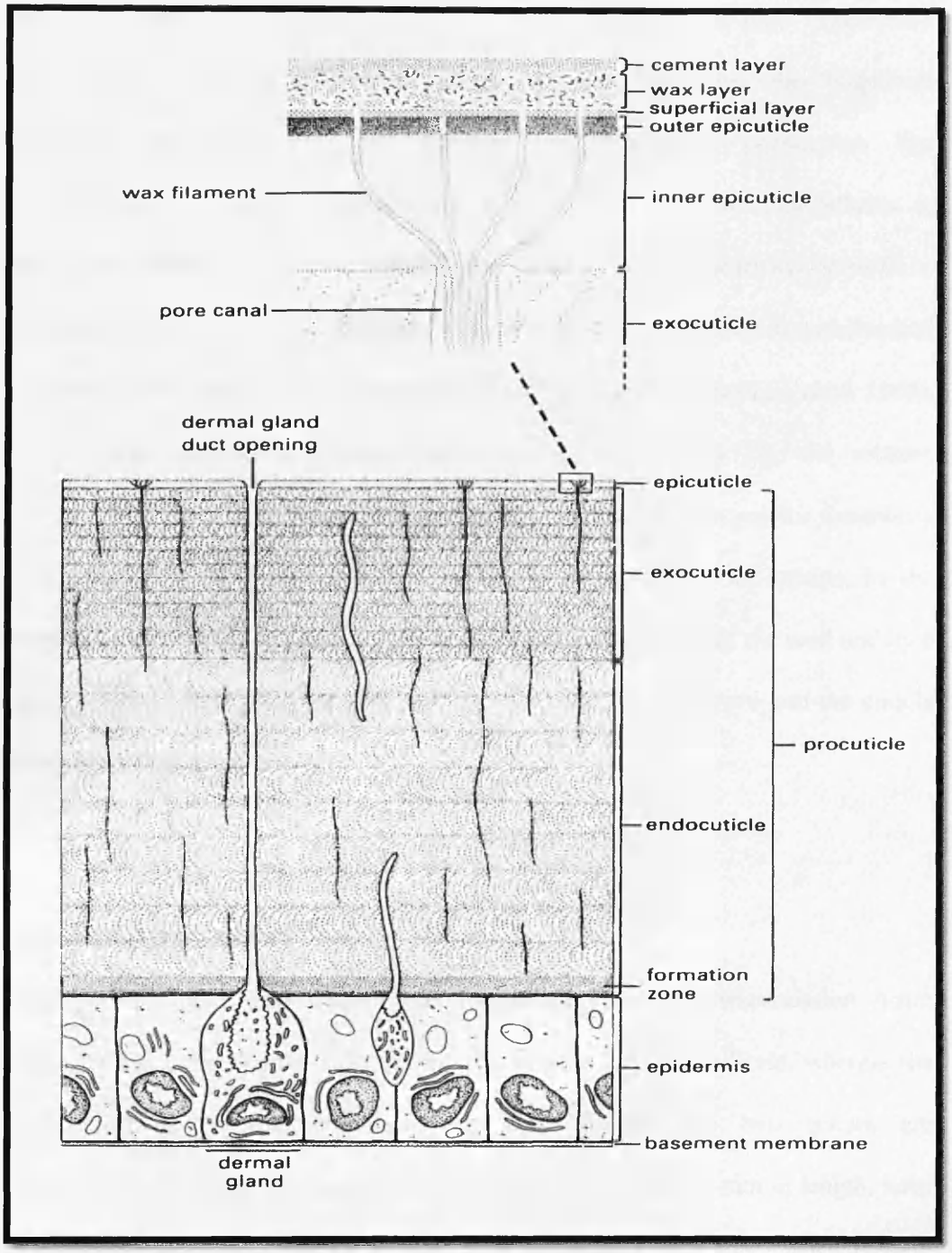


Figure. 15. Structure of insect cuticle layers. (entomologyryk.blogspot.co.uk)

The vectors body has being a harbour for several organisms not only *Leishmania*. Many studies introduced different organisms, bacteria, fungi and other flagellates organisms, discovered live and transmit by attachment phenomena like Trypanosomads. *Serratia entomophila* is a pathogenic bacterium that adheres to the foregut cuticle of *Costelytra zealandica* by fimbriae to the epicuticular superficial layer (Binnington, 1993). The flagellate *Crithidia fasciculata* parasite found attached in clusters to the lining of the gut of different mosquito species (Scolaro *et al*, 2005). Parasites also attached to different parts of the vector body like the rectum; *Trypanosoma lewisi* showed attached to the rectal wall of the *Nosopsyllus fasciatus*is by penetration of the flagella between the highly folded wall of the rectum, by the wedging of the expanded flagella in these folds and irregularities of the wall and by a zonula adherens functional complex between the flagellar membrane and the cuticle of the rectum (Molyneux, 1969).

2.2 *Leishmania* life cycle

Female phlebotomine sand flies are responsible for leishmaniasis transmission. Adult females of the genus *Phlebotomus* transmit the disease in the Old World, whereas the genus *Lutzomyia* is responsible in the New World. The two genera are morphologically similar; the adults are very small in size, 1.5-3.5 mm in length, have large black eyes, a hairy body and hold their wings in a "V-shape" when resting. Females feed on plant juices and suck blood from a variety of vertebrates such as dogs, livestock, lizards and rodents. Many females of *Phlebotomus* and *Lutzomyia* bite mammals, including humans (Fig. 2). People can be bitten by female sand flies at night, but biting also can occur during the day in dark rooms or in cloudy forests (Service, 2008).

Most human leishmaniasis is zoonotic, transmitted from an animal reservoir, and the degree of human involvement in the transmission process is different from area to area (Service, 2008). For example, in some areas sand flies transmit the disease between animals with minimal or without human involvement, whereas in other areas animals play an important part as reservoir hosts of human infection. In India, the disease can be transmitted from human to human by sand flies with no role for animals in its transmission (Bates, 1994; Silva *et al.*, 2002; Service, 2008).

The *Leishmania* life cycle is complicated. Female sand flies become infected when they feed on the blood of an infected animal or human reservoir (Fig. 2). Sand flies are pool feeders that create a small wound in the skin and then take up blood, so rather than acquiring parasites direct from the blood they originate from macrophages in the skin. They ingest macrophages that are filled with amastigotes within that blood meal. The amastigotes are released in the midgut and transform into procyclic promastigotes (Fig. 16, 17). In vitro amastigotes transform into promastigotes within 24-48 hours in suitable culture media and temperature (about 26℃), and amastigote transformation to promastigotes and cell division occur together (Bates, 1994). The procyclic promastigotes are the multiplicative forms which responsible for initial expansion of the parasites in the sand fly (Lawyer *et al.*, 1990; Rogers *et al.,* 2002; Gossage *et al.*, 2003; Lawyer *et al.*, 1987; Walter *et al.*, 1987; Walter *et al.*, 1989b; Nieves and Pimenta 2000). The procyclic promastigotes are found in the midgut or hindgut (*Viannia*) and multiply rapidly, 2-3 days after the blood meal, into elongate nectomonad promastigotes (Service, 2008; Gullan and Cranston, 1998; Bates and Rogers, 2004). Nectomonads measure over 12μm in length, these are initially free in the lumen. They have a typical kinetoplast about 2μm from the anterior end and a flagellum emerging anteriorly. The free flagellum is approximately the same length as

the body but may be up to 20μm in length. The overall shape is that of a spindle with the posterior end gradually tapering to a point. Nectomonads are electron dense due to a high ribosomal content (Molyneux and Killick-Kendrick, 1987).

Nectomonad promastigotes in sand fly midgut try to escape from the peritrophic matrix (PM), which is a structure surrounding the blood meal (Fig. 16). This matrix facilitates the digestive processes of the sand fly and works as a defence line against microorganisms and parasites (Gullan and Cranston, 1998; Tellam, 1999). Nectomonads secrete chitinase and protease enzymes in the anterior region of the peritophic matrix, which may accelerate the matrix breaking down (Rogers *et al.*, 2002; Tellam, 1999; Marquardt, 2000). When they manage to escape from the PM they move forward in the alimentary tract. Nectomonads attach to the sand fly midgut epithelium by inserting their flagella between the microvilli, and is mediated by interaction between their flagella and lipophosphoglycan (LPG) (Sacks *et al.*, 2000; Pimenta *et al.*, 1994; Butcher *et al.*, 1996). Evidence from a study conducted to determine the function of LPG proposed that the LPG works as a ligand that enables parasite attachment to the sand fly midgut (Jacobson, 1995).

The second function of the nectomonads is as a strongly motile form, which is spread the infection further from the abdominal midgut in the sand fly. This motile form is responsible for migration of the parasite population to the sand fly thoracic midgut (Rogers *et al.*, 2002) (Fig. 16). The third function of the nectomonad is development into leptomonad forms, which occurs 3-7 days after the blood meal. Leptomonad promastigotes are about 6-8μm in length and accumulate in the lumen midgut. The transformation to leptomonads occurs when the infection has spread to the anterior midgut and the cuticular surface of the stomodeal valve at the junction with the

foregut (Bates and Rogers, 2004). The leptomonad term is a new and recent addition to the *Leishmania* life cycle description (Rogers *et al.*, 2002; Gossage *et al.*, 2003). The use of a new term for these promastigotes was based on their functions, which differ from nectomonad or metcyclic promastigotes. The main function of leptomonads is the production of a gel-like substance named promastigote secretory gel (PSG) (Rogers *et al.*, 2002; Stierhof *et al.*, 1999). The PSG plays an important role in the infection by helping in create a blocked fly (Bates and Rogers, 2004).

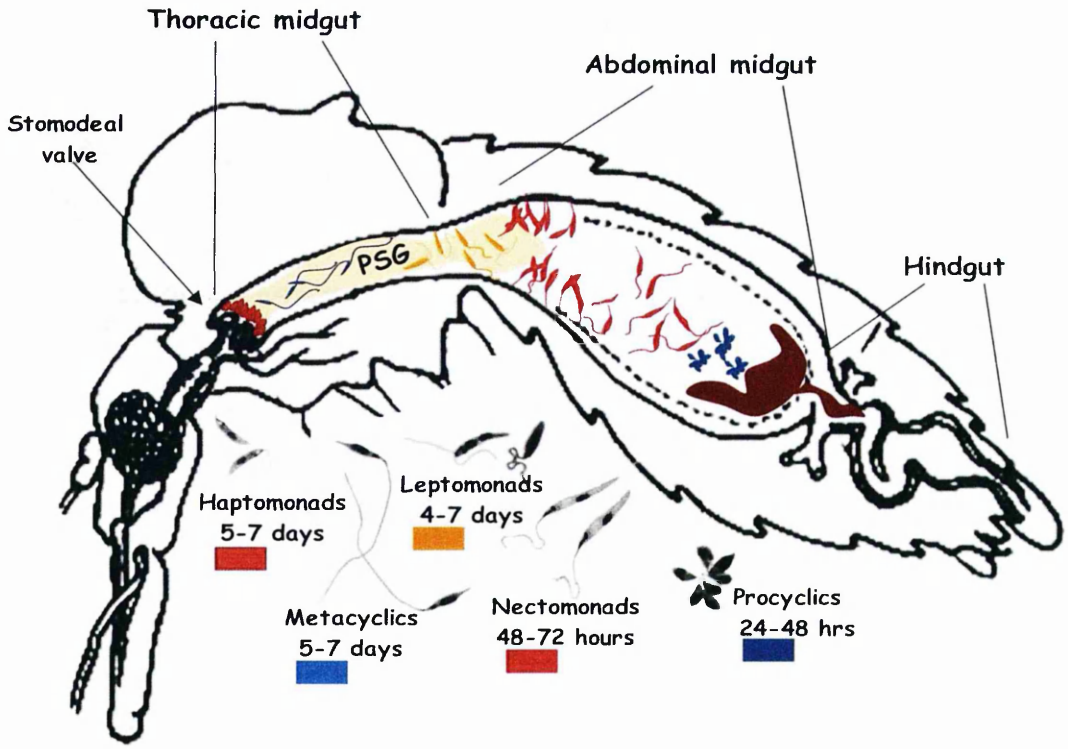


Figure 16. Diagram of the sand fly alimentary tract containing an infective blood meal. The figure depicts a partially digested blood meal with dividing promastigotes in the abdominal mid-gut, a partially degenerated peritrophic membrane, and escaped promastigotes with some attached to the microvilli via their flagella. The sequential morphological development of promastigotes and regions of the gut in which these forms are typically found are also shown. (Taken from Kamhawi, 2006).

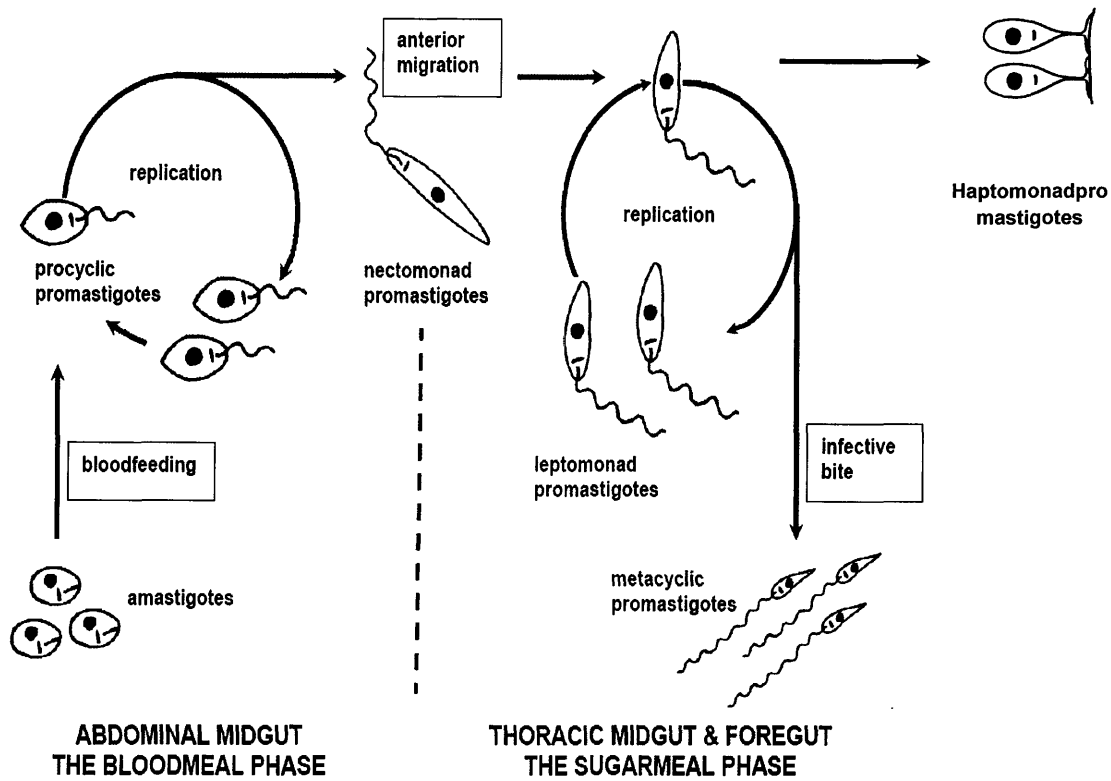


Figure 17. Development of *Leishmania* inside the vector sand fly. Amastigotes within the infective blood meal transform to procyclic promastigotes. Procyclic promstigotes divide and differentiate into distinctive nectomonad promastigotes, which accumulate in the anterior part of PM. Some of the nectomonads attach to the midgut epithelium inserting their flagella to the microvilli, while other nectomonads remain motile and spread the infection in the sand fly midgut. The development of leptomonad forms is either from the motile or attached nectomonads. The haptomonad form is found attached to the cuticular surface of the stomodeal valve, their origin is uncertain either coming from the leptomonad or the nectomonad forms. The population of leptomonads multiplies and differentiates into metacyclic promastigotes the mammal-infective forms. (Taken from Bates, 2007).

The haptomonad promastigotes are a population found in the anterior midgut of the sand fly, and they are different to leptomonads. Haptomonads are short, fat, and usually less than 12μm in length and sometimes as short as 5-8μm. The kinetoplast is anterior in position and the free flagellum measures up to 15μm in length. They are electron lucent promastigotes, which attach to the cuticular lining of the stomodeal valve by expansion of the tip of their flagella and formation of hemidesmosome-like structures (Killick-Kendrick *et al.*, 1974; Molyneux and Killick-Kendrick, 1987; Walters *et al.*, 1989). The origin of haptomonads is uncertain, whether it is from nectomonad promastigotes or from leptomonad promastigotes (Fig. 17) (Bates and Rogers, 2004).

The other major population that originates from leptomonads found in the sand fly midgut is the metacyclic promastigotes (Rogers *et al.*, 2002; Gossage *et al.*, 2003). They are about 5-8μm, non-dividing, slender highly motile forms with long flagella. The metacyclics are found in the anterior midgut and mouthparts of the sand fly after four to twelve days of taking the infective blood meal. In fact the previous feeding on sugars from plant juices is necessary for parasite development in sand fly midgut. Metacyclics are the infective form for mammals, when the sand fly feeds on a new host metacyclics are inoculated into that host and initiate infection (Walters *et al.*, 1989; Service, 2008). (Fig. 16, 17).

In the mammalian host metacyclic promastigotes quickly invade host cells and transform into intracellular amastigote forms. They multiply by longitudinal fission and cause host cell damage (Beck and Davies, 1981). Amastigotes invade a wide range of cells from the reticuloendothelial system, such as monocytes, macrophages and polymorphonuclear neutrophils of the blood, lymph, and bone marrow. The extent

of the invasion depends on the species and host immunity. The amastigotes from macrophages are then taken up by a sand fly when it feeds on infected host and the parasites continue their life cycle (Beck and Davies, 1981, Bray and Alexander, 1987) (Fig. 2).

2.3 Parasite-vector interaction

During the life cycle of the parasites inside the vector midgut, they pass through potentially hostile environmental conditions and encounter physical barriers that need to be overcome and require the parasites to have special mechanisms and special ways of interaction to survive (Sacks and Kamhawi, 2001).

During the bloodmeal the vector midgut starts to secrete various digestive enzymes such as trypsin, carboxy-peptidase, chymotrypsin and aminopeptidase. Secretion of these proteolytic enzymes creates a harsh environment for parasites to survive (Borovsky and Schlein, 1987). It was found that the concentration of these enzymes reached the peak within 24 to 48 hours after feeding and nearly 50% of the parasites were killed during that time (Shatova *et al*, 1984; Pimenta *et al*, 1997; Schlein and Jacobson, 1998), which is a big challenge facing the parasite to overcome to complete the life cycle. Adler (1938) investigated the action of midgut digestive enzymes on parasites. He studied the early survival of *L. tropica* inside the midgut of *P. papatasi* flies, which is not a natural vector. He found that by decreasing the percentage of rabbit serum that was fed to the infected fly, the infection rate was enhanced. He explained this finding that as a result of lowering the protein serum in the bloodmeal the production of protease enzymes was

decreased too, and that protected the parasites from being killed or destroyed by the action of the enzymes. Other studies conducted by Borovsky and Schlein (1987) supported Adler's finding; they studied the survival of *L. donovani* in *P. papatasi* by adding soybean trypsin inhibitor to the vector bloodmeal and found that this addition boosted the survival of parasites in the midgut. A Study observed that amastigotes of *L. major* infection caused a significant suppression of alkaline protease, trypsin and aminopeptidase activity during the first 30 hours after ingestion of bloodmeal into *P. papatasi*, the natural vector of *L .major* in comparing with the effect of amastigotes of *L. major* in *P. langeroni* , non-natural vector, was unclear (Dillon and Lane, 1993)

Based on those results it is obvious that the ability of the parasites to live inside unfavorable vectors is enhanced by decreasing levels of digestive enzymes. From that it could be postulated that the survival of *Leishmania* species inside their favorable vectors is promoted by the ability of the parasites to resist the digestive enzymes actions or it may be due to their capacity to reduce or modulate the level of the secreted enzymes (Sacks and Kamhawi, 2001), as had been observed by Schlein *et al.*, (1986; 1987) where *L. major* inhibited production of proteolytic enzymes within the *P. papatasi* (normal vector) midgut. The effect of the parasites on the production of proteolytic enzymes was also reported when *P. duboscqi* flies were fed on blood containing galactosamine. The *L. major* parasites inhibited the lectin action which led to enhancement of the gut infection (Volf, 1998).

Another challenge facing the parasites during their presence in the midgut is the peritrophic matrix (PM). The PM is secreted by the epithelium midgut and is composed of a chitin network consisting of proteins and proteoglycans. The PM has multiple functions; within the first 4 hours of the bloodmeal the PM makes a cylindrical sheet that encloses the meal in the abdominal midgut, which functions as semi-permeable barrier for the digestive enzymes (Blackburn *et al.,* 1988; Gemetchu, 1974; Terra, 1990; Walters *et al.,* 1993). The PM also works as a protective shield against microbes, which in this case the parasites need to escape from in order to survive (Feng, 1951; Walters *et al.,* 1992). Observations on the behaviour of *Leishmania* within unnatural vectors with *L. donovani* infection in *P. mongolensis* (Feng, 1951) and *L. panamensis* in *P. papatasi* (Walters, 1992), revealed that the loss of *Leishmania* infection in those vectors was due to the inability of the parasites to escape the PM. In contrast observations from monitoring *L. major* within *P. papatasi* found that the parasites which successfully transformed were those who managed to escape rapidly from the peritrophic matrix (Cihakova and Volf, 1997). Their way to break-through the PM is by secretion of chitinase enzyme, which leads to breakdown the matrix and facilitates their escape (Schlein *et al.,* 1991). To confirm the action of chitinase on the intact of PM, studies were conducted involving chitinase inhibitor (allosamidin) treated flies. The PM structure in experiments where allosamidin was added to the bloodmeal of *P. papatasi* appeared more intact and thick for up to 7 days. The effect of allosamidin was to delay *L. major* from escape, and when they finally escaped the infection was lost (Pimenta *et al.,* 1997). Study of the protein code of the chitinase enzyme found it is antigenically and

enzymatically different among *Leishmania* species, which may contribute to differences in species vector interactions (Shakarian and Dwyer, 1998, 2000; Sacks and Kamhawi, 2001).

2.4 Mechanisms of parasite attachment

After the parasites pass through the peritrophic matrix, they continue their life cycle and ultimately differentiate into metacyclic promastigotes. Promastigotes are seen attached to the gut wall, which prevents the parasites being eliminated by the action of the gut (Sacks and Kamhawi, 2001). The first observation of the attached parasites was by Adler and Theodor (1927) using light microscopy. Later more studies were pursued devoted to investigate the attachment process and subsequent ultrastructural images by electron microscopy and showed the parasites attached to the epithelial lining of the midgut by their flagella (Killick-Kendrick *et al.*, 1974; Warburg *et al.*, 1986; Walters *et al.*, 1989).

The attachment to the gut occurs in several different ways. The flagellar binding to the midgut is most likely to be a receptor–ligand interaction system as there is no obvious ultrastructural modification of flagella during their binding to the midgut (Bates, 2008). In agreement with this, midgut attachment of promastigotes can be meditated by the surface molecule lipophsphoglycan (LPG), a ligand that plays an important role in the attachment mechanism as described in many studies (Jacobson, 1995; Kamhawi, 2006; Silva *et al.*, 2009). The first described receptor that was reported to interact with LPG was a 65kDa protein from *P. papatasi*, which bound to LPG of *L. major* species (Dillon and Lane, 1999). The best characterised receptor from the sand fly that binds to LPG in the midgut is a galectin molecule (Kamhawi *et al.*, 2004). PpGalec is a specific receptor expressed mainly by *P. papatasi* and

P. duboscqi that only binds to poly-Gal (β1-3) side chains on the LPG ligand of *L. major* (Kamhawi *et al.*, 2004). In vitro, anti-PpGalec antibodies were used and the binding of the PpGalec receptor to *L. major* LPG was inhibited, and when *P. papatasi* flies were fed with bloodmeal containing anti-PpGalec antibodies, the survival and development of *L. major* parasites was reduced, indicating that the binding of *L. major* LPG receptors to PpGalec receptors in *P. papatasi*is essential for *L. major* infection in *P. papatasi* (Kamhawi *et al.*, 2004; Myler and Fasel, 2008). Many studies have investigated the structure of LPG structure and the attachment sites among different *Leishmania* and vectors, and have found there are significant variations in LPG structure and LPG-mediated receptors between different species that may contribute to the specificity of the parasites-vector interaction (McConville *et al.*,1990; 1995).

Another way that promastigotes attach to the midgut is via a LPG-independent mechanism that may occur in certain sand flies and with a limited range of *Leishmania* species (Myskova *et al.*, 2007; Volf and Myskova, 2007). In fact, this LPG-independent mechanism was first observed when LPG1-/-mutants of *L. mexicana* were shown to be capable of maturing and produced an infection in *Lu. longipalpis* (Rogers *et al.*, 2004).Although this mechanism is still not fully understood and needs to be investigated further and the details clarified, it will add to the variety of attachment mechanisms in *Leishmania* parasites (Bates, 2008).

The subject of this project concerns a third mechanism of attachment. Trypanosomatids share a common mechanism of attachment with each other, in which they expand their flagellar tip and form hemidesmosomal-like structures. This hemidesmosome-like structure has been known for a long time (since the 1970s),

where trypanosomatids had been seen to adhere themselves to chitinous surfaces of the vector midgut (Molyneux, 1977; Molyneux *et al.*, 1987). Various studies have simulated the attachment mechanism in vitro by using different materials. Investigation of *Crithidia fasciculata* and *Trypanosoma congolense* attachment *in vitro* found the attachment of those parasites was similar to the mechanism that occurs within their vectors (Brooker, 1971; Gray *et al.*, 1981).In the *Leishmania* life cycle the stage that is characterised by forming hemidesmosomal-like structures is haptomonad promastigotes. They use this structure to anchor themselves to cuticle-lined parts of the gut (foregut, stomodeal valve and hindgut) (Wakid and Bates, 2004). Despite the fact that the ultrastructure of the hemidesmosome has been described for a long time, it is still undescribed biochemically (Bates, 2008).

Since that time, several studies have been conducted to investigate the hemidesmosomal mechanism of attachment in trypanosomatids. The results showed that attachment of trypanosomatids in their vectors takes place on chitin-lined surfaces. Many studies proposed that chitin has a role in that attachment (Wallbanks *et al.*, 1989; Stiles *et al.,* 1990). Other studies conducted have shown that the mechanism of attachment is based on hydrophobic interaction (Schmidt *et al.*, 1998; Kleffmann *et al.*, 1998). Later, the attachment mechanism of *Leishmania* promastigotes was investigated using a new and quantifiable in vitro assay system, and indicated that flagellar attachment is mediated by a non-specific hydrophobic interaction in *Leishmania* species (Wakid and Bates, 2004). According to observations made during some in vitro attachment studies, it is believed that the attachment process in trypanosomatids is necessary for metacyclic parasites production but not for parasite proliferation (Fish *et al.,* 1987; Hendry and Vickerman, 1988; Bonaldo *et al.,* 1988; Vickerman and Tetley, 1990; Thomas and Dean, 1990).

2.5 *Leishmania* Flagella Structure and Biology

Flagella in all parasites play an important role in parasites movement and attachment to their host. They are similar in their function and structure to cilia with differences in length and beating action (Gibbons, 1981). Both of them are composed of a mass of microtubules known as the axoneme (Warner, 1974). All kinetoplastid protozoa have a similar flagellum structure. Studies of ultrastructural cross-sections of *Leishmania* flagella have shown no difference between them and flagella of other kinetoplastid parasites. However, the length of the flagellum changes from stage to stage during the *Leishmania* life cycle. The flagella of the amastigotes are small and short in length. They arise from the flagellar pocket but do not extend beyond that (Ashford and Bates, 1999) (Fig. 11).

Leishmania promastigote flagella have an axoneme structure that consists of nine pairs of outer doublets and two central single microtubules. This axoneme is connected to the basal body, where the flagella originate. Beside the axoneme, another structure emerges from the flagellar pocket known as the paraflagellar rod. The paraflagellar rod is present all along doublets 4-7 and located in the plane bisecting the axoneme through its two central microtubules (Molyneux and Killick-Kendrick, 1987; Vickerman and Tetley, 1990). In attached promastigotes the flagella condense and become shorter than in non-attached forms. Flagellar tips become extended and form foot-like structures that are known as hemidesmosomes (Walters *et al.*, 1989) (Fig.18).

2.6 Structure of Microtubules

Microtubules are fundamental components of the cytoskeleton, playing important roles in cell motility and cell morphogenesis (Hyams and Lloyd, 1993, Amos, 2000,

Downing, 2000). They are highly dynamic components (Mitchison and Kirschner, 1984; Desai and Mitchison, 1997; Nogales, 2000; Howard and Hyman, 2003). *In vivo*, the stability of microtubules is controlled by microtubule-associated proteins (Hirokawa, 1994; Mandelkow and Mandelkow, 1995). Microtubule basic structure consists of globular subunits of heterodimers of αβ-tubulin. Both proteins have a molecular mass of about 50 kDa (Desai and Mitchison, 1997) and consist of a similar sequence of 450 amino acids; about 40% of this sequence is identical (Luduena, 1998).

In addition to the αβ-tubulin proteins, another type, γ-tubulin, was discovered. It is located in the centrosome and it is the starting point of the microtubule assembly (Shiebel, 2000). The axoneme contains nine outer pairs of doublet microtubules and two central single microtubules that form the 9 + 2 array structure (Fig. 19). The outer doublets of the 9 array consist of A and B subfibres. The A subfibre is made of a complete microtubule with 13 protofilaments, but in case of the B subfibre the microtubule is incomplete with 10 protofilaments. Both A and B subfibres are connected to each other by filaments of protein tektins, localized in basal bodies and occurring in 1:17 weight ratio to tubulin (Stephens and Lemieux, 1998).Both inner and outer arms of dynein are connected to every A subfibre at regular intervals and that is what creates the motility in cilia and flagella (Goodenough and Heuser, 1985a, 1985b, 1984). The nine outer doublet microtubule structures are seen attached to the two central single microtubules at each A subfibre by radial spokes and spokeheads (Gibbons, 1981). The central pair of the single microtubules is surrounded by an inner sheath. A bridge connects this central pair, while the outer doublets are bridged together by highly elastic inter-doublet linkers. These linkers, nexin proteins, are

normally about 20 nm, but the diameter can stretched to reach up to 200nm (Bozkurt and Woolley, 1993).

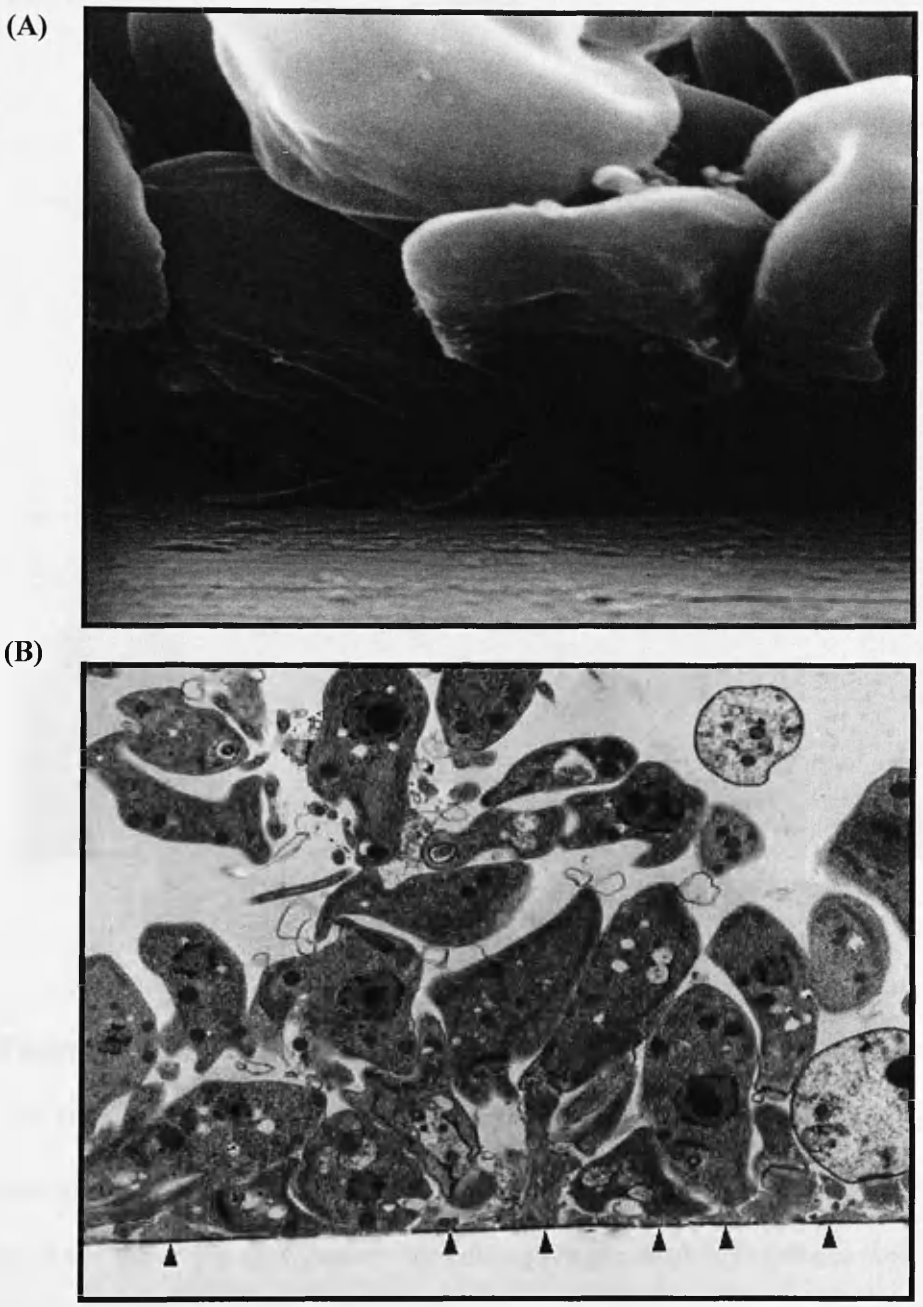


Figure 18. Electron micrographs of *Leishmania* flagella. (A) High power scanning electron micrograph from underneath showing promastigotes attached to the surface with flagella and expanded flagellar tips. **(B)** High power transmission electron

micrograph showing expanded flagella tips of in vitro attached promastigotes with formation of hemidesmosome plaques (arrowheads). (Taken from Wakid and Bates, 2004).

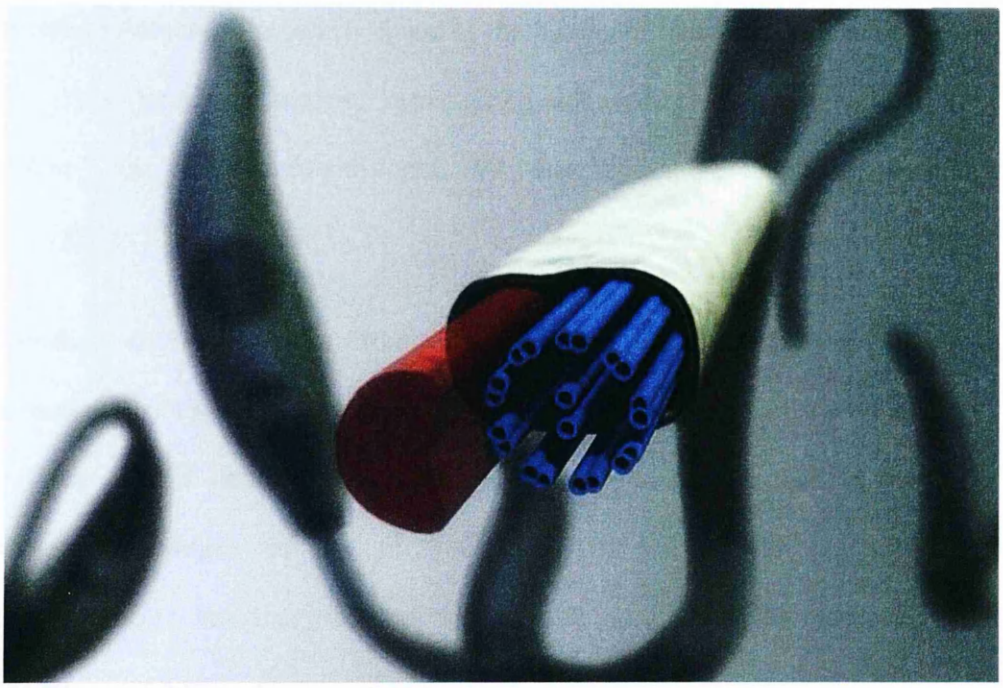


Figure 19. 3D simulated picture of flagellum axoneme. The illustration shows the 9+2 array structure that consists of nine outer pairs of doublet microtubules and two central single microtubules (blue), the paracrystalline paraflagellar rod structure is shown in red and in grey/green is the cell membrane bilayer. The image was created in Blender based on scanning and transmission electron micrographs by Wheeler R. (2013).

2.7 The Desmosome and Hemidesmosome

The desmosome and hemidesmosome are terms used to describe structures in kinetoplastids that are similar to structures found in other species in vertebrate epithelial cell layers (Brooker, 1971a; 1971b). Desmosomes are cell surface attachment sites for intermediate filaments at cell to cell contact points, while hemidesmosomes are primarily found at the basement membrane zones of epithelia. These two structures mediate cytoskeleton adhesion to the extracellular matrix (Fontao *et al.*, 1999; Green and Jones, 1996; Borradori and Sonnenberg, 1996; Hieda *et al.*, 1992).

Hemidesmosome molecules from epithelial cells are classified into membrane molecules, plaque molecules and matrix molecules. Characterization of hemidesmosomal proteins showed that membrane molecules include the bullous pemphigoid antigen protein of 180 kDa (BP 180), and heterodimers of hemidesmosome integrins (α6β4 integrin). Plaque molecule identification showed the bullous pemphigoid antigen of 230 kDa (BP 230), intermediate filament (IF) associated proteins of 300 kDa (IFAP 300), and plectin, which is an IF-associated protein related to IFAP 300. Laminin-5, which is also referred to as GB3 antigen, epiligrin and kalinin are the matrix molecules and they serve as ligands for α6β4 integrin (Hieda *et al.*, 1992; Jones *et al.*, 1994, Green & Jones 1996, Borradori and Sonnenberg, 1999).

The ultrastructure of trypanosomatid hemidesmosomes has been known for many years but knowledge of the hemidesmosome function and the biochemical modification of parasites flagella in the attachment mechanism remains very limited (Borradori and Sonnenberg, 1999; Bates, 2008).

2.8 Flagellar proteins and their roles in trypanosomatid life cycles

Several studies have been performed *in vitro* to try and identify flagellar plaque molecules, and investigate their architecture to understand the process of attachment and detachment. Identification and understanding of these molecules could provide valuable information to help to understand the interaction between the parasites and their vector hosts. One study showed an abundant protein in attached *Trypanosoma congolense* epimastigotes with a molecular mass of about 70 kDa, and the authors proposed that this protein is likely to be a major constituent in the hemidesmosome structure (Beattie and Gull, 1997). However, this study has not been followed up and it is likely that this protein was in fact contaminating serum albumin (Ginger, personal communication). Studies on proteins associated with the flagellar attachment zone in *Trypanosoma cruzi* trypomastigote and epimastigote cytoskeletons, using monoclonal antibodies, resulted in identification of protein bands of molecular masses higher than 750 kDa up to 2500 kDa. A giant protein of 2500-3000 kDa was found in the anterior end of the cell body microtubules near the desmosomal junction of promastigote forms of the flagellate *Phytomonas serpens* (infects plants) (Ruiz-Moreno *et al.*, 1995; Baqui *et al.*, 1996).

Results of another study demonstrated the existence of a novel class of mega dalton phosphoproteins in promastigote forms of trypanosomatids, which appeared to be genus specific with distinct cytoskeletal functions. Promastigote forms of *Leishmania tarentolae* and the trypanosomatids *Leptomonas samueli* and *Phytomonas serpens* express cytoskeletal giant proteins with apparent molecular masses of 1,200 kDa (Lt 1200), 2,500 kDa (Ls 2500), and 3,500 kDa (Ps 3500), respectively (Baqui *et al.*, 2000). Polypeptide similarity between Lt 1200 and Ps 3500 was recognized using polyclonal antibodies. The anti-Ls 2500 serum also cross reacted with Ps 3500, and

with a 500-kDa polypeptide of *Leishmania tarentolae*. Their results also indicated that Ps 3500, Ls 2500, and Lt 1200 are phosphorylated *in vivo* at serine and threonine residues, whereas, *in vitro* examination of cytoskeletal fractions revealed that only Ps 3500 and Ls 2500 are phosphorylated. Heat treatment (100°C) of high salt cytoskeletal extracts demonstrated that Ps 3500 and Ls 2500 remain stable in solution, whereas Lt 1200 is denatured. In addition, there is also evidence that Ps 3500 and Ls 2500, in contrast to Lt 1200, seem to be autophosphorylating serine and threonine protein kinases, suggesting that they might play regulatory roles in the cytoskeletal organization (Baqui *et al.*, 2000).

Another study reported the occurrence and subcellular distribution of actin in trypanosomatid parasites. The results showed that about 10^6 copies per cell of actin (42.05 kDa) were present in the *Leishmania* promastigote (Sahasrabuddhe, 2004). Analysis of the primary structure of this protein showed that the unusual characteristics of the protein may be related to the presence of highly diverged amino acids in the DNase I-binding loop (amino acids 40-50) and the hydrophobic plug (amino acids 262-272) regions of *Leishmania* actin. The subcellular distribution of actin was studied by employing immuneelectron and immunofluorescence microscopy. The protein was present not only in the flagellum, but also found in the flagellar pocket, nucleus and the kinetoplast, and also localized on the nuclear, vacuolar and cytoplasmic face of the plasma membranes. The results clearly indicate that *Leishmania* contains a novel form of actin which may structurally and functionally differ from other eukaryotic actins (Sahasrabuddhe, 2004).

A recent study was conducted on the life cycle proteome of *Leishmania donovani* promastigotes (Harder *et al.*, 2010). They found that the correct formation of the

flagellum has a strong impact on the characteristics of *Leishmania* parasites. Deletion of the gene for one component of the outer dynein arm docking complex resulted in promastigotes assuming an amastigote-like morphology, as well as loss of motility. Other studies have found that the paraflagellar rod proteins are important to maintain the form and normal function of flagella and any gene deletions lead to losing the motility of promastigotes (Tull *et al.*, 2010; Lahiri and Bhattacharya 2006). Myosin XXI, a novel isoform, was found to be expressed more predominantly in the promastigote stage of *Leishmania* as an abundant protein (Katta *et al.*, 2009).

Comparison of the proteomes of procyclic and metacyclic promastigotes identified 25 protein spots that were differentially expressed during metacyclogenesis (Mojtahedi *et al.*, 2008). Proteins involved in protein synthesis were less abundant in metacyclic promastigotes, while proteins involved in motility, including paraflagellar rod protein 1D, α-tubulin and β-tubulin were more abundant. Also, two mitochondrial enzymes (succinyl-CoA synthetase β subunit and cytochrome *c* oxidase subunit IV) were expressed as different isoforms in the life cycle stages. Down-regulation of proteins related to synthetic pathway in metacyclic promastigotes is consistent with the arrested growth in this life cycle stage, while up-regulation of proteins related to motility in metacyclic promastigotes is in agreement with the high motility observed in the metacyclic promastigote stage (Mojtahedi *et al.*, 2008).

2.9 Project Hypothesis

During the *Leishmania* life cycle, the parasites attach to cuticular surfaces of the gut wall (foregut, stomodeal valve and in some species hindgut) and transform into haptomonad forms. These form structures that connect the tip of the haptomonad flagellum with the surface of the sand fly gut called hemidesmosomes. Our hypothesis

is that the hemidesmosome proteins play an important or essential role in the parasite's life cycle. The proteins in the hemidesmosome are proposed to control and regulate the attachment mechanism. Therefore, if those proteins were identified and their genes were knocked out or knocked down, it may result in the formation of structurally abnormal hemidesmosomes. In that case, the parasites may be unable to attach, or they may achieve a weak attachment such that they readily detach, so they lose their ability to complete the life cycle because the hemidesmosome fails to function properly.

Determining the identities of *Leishmania* hemidesmosome proteins provides a good starting point to study hemidesmosome function. Until now the knowledge about hemidesmosome flagellar protein molecules identity and their function are not sufficient for a full understanding of the hemidesmosome mechanism formed by *Leishmania* haptomonad promastigotes.

Therefore the overall aim of this study is to establish the molecular identity of *Leishmania mexicana* hemidesmosomal proteins.

Chapter 3

Materials and Methods

3.1 Parasite cultures

Experimental work was performed with *Leishmania mexicana* (MNYC/BZ/62/M379), *Leishmania major* (MHOM/IL/80/Friedlin) and *Leishmania tarentla* (LV 108). They were cultured as axenic amastigotes and promastigotes.

3.1.1 Culturing of amastigotes

Grace's insect cell culture medium (GIBCO 11300-043) with L-glutamine and without sodium bicarbonate was used as the base medium for culturing amastigotes. Grace's medium was prepared by dissolving the powder (the whole bottle), plus 0.35g sodium bicarbonate $NaHCO_3$ (Sigma S-8875) in 1L of distilled water. The pH was adjusted to 6.2 by adding 1M NaOH. The medium was filtered using a Corning Incorporated (431096) 250ml filter system, 0.22μm PES (Polyethersulfone) with vacuum pump to sterilize the medium and then stored at 4°C.

80 ml of prepared Grace's insect medium was supplemented with 20 ml of heat inactivated fetal bovine serum (FBS) (Hyclone, research grade, Perbio CH30160.03), 1 ml of BME vitamin 100X stock (Sigma B6891), which had been frozen in 5 ml aliquots at -20℃ before use, and 0.25 ml gentamycin sulphate (Sigma G1272, 10 mg/ml stock) diluted to 25μg/ml final concentration. The pH was adjusted to 5.5 by adding 1M HCl. The medium was then filtered as above to reduce the risk of any contamination and sterilize the medium before use. The complete medium was stored at 4°C.

Amastigotes were cultured using 25 cm^2 cell culture flasks in 10 ml of complete medium or 75 cm^2 flasks in 50 ml of medium at 32℃. Amastigote cultures were monitored, maintained and passaged at 5 x 10^6/ml starting density until sufficient numbers were generated to be harvested for protein extraction (~10^9 per extraction). Parasite growth was monitored by haemocytometer counting. A 50µl volume of parasite culture was mixed with 50µl of 4% (v/v) formalin. The formalin kills the parasites and in the case of promastigotes (below) stops movement, which makes the counting much easier. A small drop of the mixture was placed on the haemocytometer slide and examined using a 40x objective under the light microscope. 5 squares were counted, the 4 corners and one square in the centre. The density of the parasites in the culture was estimated using the formula: density = number of parasites counted in 5 squares x 5 x 2 (for dilution) x 10^4/ml.

3.1.2 Culturing of promastigotes

Medium M199 containing Hank's salts, L-glutamine, 25mM HEPES and L-amino acids (GIBCO 22350) was used as the base medium for culture of promastigotes. A 500 ml bottle of M199 (supplied as liquid) was supplemented with 5 ml of BME vitamin 100X stock, 1.25 ml gentamycin sulphate, 2% sterile urine and 55 ml of FBS (10% final concentration). The complete medium was filtered using a Corning Incorporated (431096) 250ml filter system, 0.22µm PES (Polyethersulfone) with vacuum pump to sterilize the medium, the pH was adjusted to 6.9, and then stored at 4°C until required.

Promastigotes were cultured using 25 cm^2 cell culture flasks in 10 ml of complete medium at 25°C. Promastigote cultures were initiated at 5 x 10^5/ml, then monitored, maintained and passaged until they reached log phase or stationary phase (depending

on experimental requirements) (stationary phase typically 2-4 x 10^7/ml) with sufficient numbers to establish cultures as techniques required.

Parasite growth was monitored by haemocytometer counting. A 50 µl volume of parasite culture was mixed with 50 µl of 4% (v/v) formalin to immobilise the parasites. A small drop of the mixture was placed on the haemocytometer slide and examined using a 40x objective under the light microscope. Five squares were counted, the four corners and one square in the centre. The density of the parasites in the culture was estimated using the formula: density = number of parasites counted in 5 squares x 5 x 2 (for dilution) x 10^4/ml.

3.2 Examination the ability of *Leishmania mexicana* to attach in vitro to different materials

In order to find a material that can provide a good quantity and quality of attached promastigotes several materials were tested.

3.2.1 Melinex plastic sheet

Smooth Melinex plastic sheets (Agar Scientific L4103) were cut into small square pieces 15mm x 15mm. Melinex squares were washed with distilled water and 70% ethanol, then left to dry in a sterile environment in a tissue culture cabinet before use. They were then cultured with 2 ml volumes of promastigote culture in sterile 12 well tissue culture plates (Costar, 12 well cell culture cluster 3513). The plates were sealed with ParaFilm to protect the cultures from contamination and incubated at 26°C.

Cultures were monitored using an inverted microscope and fed regularly every 2-4 days by removing ~1ml and replacing with fresh medium in order to keep the parasites alive and active. The Melinex squares were removed at different time points, checked in two ways, by using an inverted microscope to look at the attachment in

live parasites and by staining. In the latter case squares were fixed with absolute methanol, then left to dry, and later they were stained with 10% (v/v) Giemsa's stain (improved R66 solution Gurr, VWR 350864X) in 10mM phosphate buffer for 10 minutes. Melinex squares were rinsed with tap water and left to dry, and then examined using oil under a light microscope (100X) by putting a drop of water between a glass slide and the Melinex square to hold it firmly in place. To count the parasites on each Melinex square 10 random fields were examined and the average number of attached promastigotes per field calculated. The number of attached promastigotes per Melinex square was estimated by multiplying the attachment density per field x 9000 (Area of one field = $25x10^3$ μm^2; area of one cover slip = $225mm^2 = 225 \times 10^6$ μm^2) (Wakid and Bates, 2004).

Melinex squares were used as a control for attached parasites on different materials.

3.2.2 Polycarbonate membrane filters

Polycarbonate membrane filters (Steritech Corporation, PVP-Free 1.0μm x 47 mm) were used. Two types of polycarbonate membrane were cultured with promastigotes (P/N: PCTF1047100; hydrophobic surface and P/N; PCT1047100; hydrophilic surface). Filters were removed from their packs with sterilized forceps and incubated with 5 ml promastigotes culture in disposable polystyrene round Petri dishes (100mm x 15mm) with lids, sealed with ParaFilm, and then incubated at 26°C. The cultures were checked every day under an inverted microscope. Filters were collected on different days using sterilized forceps and washed twice by shaking them gently in round Petri dishes with 10 ml Hank's balanced salt solution (HBSS) to remove un-attached promastigotes. Then they were transferred to a 50 ml universal tube with 5 ml HBSS and vortexed for 5 min to remove un-attached promastigotes.

3.2.3 Chitin

Chitin powder (Sigma C-7170) was used to culture with *Leishmania* in vitro. The chitin powder was used directly following sterilization with 70% ethanol, then prepared in 1M NaCl solution (Shibata *et al.*, 1997), 1 g of chitin in 100 ml NaCl. This chitin was used either without further treatment (un-sonicated) or following sonication at different powers, times and volumes (Table 3.1).

Type	Power Voltage	Volume/ml	Time	Type of sterilization
Un-sonicated	--------	20	----------	70% ethanol
Un-sonicated in NaCl solution	--------	20	----------	Autoclave
Sonicated in NaCl solution	24	10	5	Autoclave
Sonicated in NaCl solution	7.5	20	10	Autoclave
Sonicated in NaCl solution	12	15	15	Autoclave

Table 3.1 Different sonication conditions of chitin solution.

1x 10^{8} /ml of parasites were cultured with the various chitin preparations in 25 cm^2 culture flasks and incubated at 25°C.

3.2.4 Agarose

1% Agarose for routine use was used (Sigma A9539), 1 g agarose was prepared in 100 ml Medium 199 and autoclaved. After cooling but before becoming solid (~45°C) 10 ml of Agarose/M199 medium was taken and supplemented with 25 µl gentamicin sulphate, 1.1 ml FBS and 100 µl BME vitamins. 1 ml volumes of supplemented Agarose/M199 were used to cover the base of a 25 cm^2 culture flask and 3 ml to cover a round Petri dish. They were sealed with ParaFilm and incubated at 25°C.

Based on monitoring cultures and after determining the best time to collect, they were harvested. The flasks or Petri dishes were washed with M199 several times to remove

the unattached parasites. The agarose was then cut into small pieces and collected in a 50 ml universal tube with 5 ml M199. The tube was vortexed for 1 min then centrifuged for 10 min at 3000g. The supernatant medium was discarded, keeping the medium in the bottom of the tube containing the parasites. The medium in the bottom of the universal tube was transferred to a microfuge tube and centrifuged for 5 min at 10,000g. Slides were prepared and stained with Giemsa's stain to examine the morphology of the promastigotes from the agarose culture.

3.2.5 n-Octacosane

Supplied as a waxy powder, n-Octacosane (Sigma O-2126) was sterilized with 70% ethanol and left to dry in the culture cabinet hood. About 1g of sterilized n-Octacosane used in culture with promastigotes in a 25 cm^2 culture flask. n-Octacosane also melted and as coated surface of Melinex plastic sheets.

3.2.6 ParaFilm

ParaFilm, the plastic packaging tape (Pechiney W154952) was cut into squares, washed with 70% ethanol, and left to dry in a sterile environment. Then they were cultured with the parasites in round Petri dishes, sealed with ParaFilm and incubated at 25°C.

3.2.7 Paraffin wax (Candle wax)

Shavings of candle wax were prepared from household candles using a scalpel blade. The shavings were sterilized with 70% ethanol and left to dry in the culture cabinet hood. When they were dried, the shavings were cultured with the parasites in a 25 cm^2 culture flask and incubated at 25°C.

3.2.8 Polystyrene, Sepharose and Sephadex

Micro particles based on Polystyrene (Fluka 74491), CM Sepharose^{TM}Fast Flow (Amersham Pharmacia Biotech AB 17-0719-10) and Sephadex (Sigma G-25-150), which are similar sized spherical particles, were used to test parasite attachment behaviour. 0.1 ml of each suspension was sterilized with 70% ethanol, left to dry in under sterile conditions. Then they were cultured with promastigotes in 25 cm^2 culture flasks and left in the incubator at 25°C.

3.2.9 Ethylene-vinyl acetate co-polymer (EVA)

Hot Glue Sticks (Bostik Findley Limited) were used to generate EVA to culture with promastigotes. The glue sticks (small cylinders ~0.75 cm diameter x 5 cm length) were melted at high temperature (> 80°C) using a Bostik glue gun. When the stick is melted it was extruded to different forms based on the techniques and the purpose of use, and then cooled with distilled water. EVA once cooled became a solid flexible structure, which was then washed with distilled water, sterilized with 70% ethanol and left to dry in a culture cabinet hood. They were then cultured with promastigotes and incubated at 26°C.

All cultures with the different materials were checked and monitored every day for attached parasites using inverted microscope.

3.3 Preparation of parasite cultures for protein extraction

All cells from different materials were collected from cultures depending on the material type (below).Then they were washed by centrifugation and resuspension with HBSS twice at 3000g for 10min, then lysed for 30 minutes by using ice cold MME buffer (0.2 % (v/v) Triton X-100, 10mM MOPS, 2mM EGTA, 1mM $MgSO_4$, 1:1000

protease inhibitor cocktail (Sigma P8215)). Protein extraction was performed as described later.

3.3.1 Polycarbonate membrane filters

The filters were collected from cultures on different days using sterilized forceps. They were washed twice by shaking them gently in round Petri dishes with 10 ml Hank's balanced salt solution (HBSS) to remove un-attached promastigotes. Then they were transferred to a 50 ml universal tube with 5 ml HBSS and vortexed for 5 min to remove un-attached promastigotes and those that had attached loosely. Then they placed in a Petri dish and cut into small pieces with sterilized scissors. The filter pieces were gently re-suspended in 5 ml ice cold MME buffer. The samples were incubated for 30 min resulting in cell lysis.

3.3.2 Chitin, candle wax and ethylene-vinyl acetate co-polymer

Chitin powders, candle flakes and pieces of EVA, which were cut into small sizes 1mm x 1mm, were collected from cultures after they were examined with an inverted microscope and showed parasites attached to them. They were transferred into 15 ml universal tubes and washed by centrifugation and resuspension with HBSS twice at 3000g for 10min. They were then collected and incubated with 1 ml of cold MME buffer for 30 min.

Protein extraction was carried out for both free promastigote flagella and attached forms, comparing and looking for different proteins based on protein banding appearance on SDS-PAGE gels.

3.4 Isolation of *Leishmania* flagellar proteins

3.4.1 Isolation of *Leishmania* flagellar proteins from free, normal promastigotes

The protocol used was based on Schneider *et al.* (1988). Parasite cultures were harvested by centrifugation at 1500g for 10 min, the supernatant medium discarded and the cell pellet re-suspended in 10 ml of ice cold M199. The medium was used without any supplements i.e. no serum. The suspension was centrifuged at 1500g for 10 min, the supernatant removed and the cell pellet re-suspended again; this step was performed twice providing three washes in total for the cell pellet. After the final wash, the pellet was gently re-suspended in 1 ml of ice cold MME buffer, resulting in cell lysis, and the lysate incubated on ice for 30 min. During the incubation the lysate was vortexed 6 times for 15 sec each time, at 5 min intervals. Following incubation a 40 µl volume of the suspension was collected in small labeled microfuge tube as total proteins.

The remaining lysate was centrifuged at 3000g for 10 min in a refrigerated microfuge at 4°C. A 100 µl volume was retained from the supernatant in a microfuge tube and kept as soluble proteins. Then the remaining supernatant was discarded and the pellet re-suspended in 1 ml MME buffer. The mixture was centrifuged at 3000g at 4°C for 10 min. The supernatant was discarded and the pellet re-suspended in 0.5 ml MME buffer. From this suspension 40 µl was collected in one small microfuge tube as insoluble proteins. To the remaining volume 115 µl of 5M NaCl (final concentration = 1M NaCl) was added. Then, the mixture was vortexed ~10 times, each time 15 sec, over a10 min period and kept on ice in between vortexing. Later, the mixture was centrifuged at 12,500g in a refrigerated microfuge 4°C for 30 min. Avoiding the pellet 100 µl was removed from the suspension and kept in a tube labeled as cytoskeleton.

The remaining supernatant was discarded and the pellet re-suspended in 1 ml SMME (MME buffer, 1M NaCl, +1:1000 protease inhibitor) buffer, followed by centrifugation at 12,500g, 4°C in a refrigerated microfuge for 30 min. Finally, the supernatant was removed and the pellet re-suspended in 100 µl MME buffer and the tube labeled as flagella. A 10 µl volume of insoluble, cytoskeleton and flagella protein extractions was taken for preparation of smears on microscope slides to confirm the extraction. Flagella appeared free (not intact to the membrane) thread like structure on Giemsa stained slides.

3.4.2 Isolation of *Leishmania* flagellar proteins from promastigotes attached to materials

The protocol used was based on Schneider *et al.* (1988), and modified to provide good quality and sufficient quantity of proteins, as the expected number of the promastigotes was not high. This procedure was the same basic method for protein extraction of attached parasites with some different steps based on the specific materials, which are described within the results. When the materials were ready to collect, they were transferred from their culture into a universal tube with 10 ml HBSS or M199 (no supplements), vortexed for 5 min and then centrifuged twice at 3000g for 10 min to remove unattached parasites. Then the materials were transferred to another universal tube and incubated with 1 ml of cold MME buffer for 30 min on ice. The medium that was used to wash the materials was re-centrifuged for 10 min at 3000g and the pellet was collected. The pellet was also incubated with 1 ml cold MME buffer for the same duration. During the incubation the lysate was vortexed 6 times for 15 sec each time, at 5 min intervals, followed by centrifugation at 3000g, 4°C in a refrigerated microfuge for 30 min. Finally, the supernatant was removed and the pellet

re-suspended in 100 µl MME buffer. The fractions were removed into separate tubes and both stored at -80°C until used for analysis.

3.5 Protein Assay

The concentration of the protein in samples was estimated by using a BCA™ protein assay kit (Thermo Scientific 23227). Serial dilutions of standard proteins were prepared ranging from 0 to 2000 µg/ml (0, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000, 2000). To make these, tubes containing 150 µl of diluent buffer (MME buffer) were prepared. Then 150 µl of 2mg/ml bovine serum albumin (BSA) was added into tube one and mixed, then 150 µl removed and added to tube two, repeating the process from high concentration to low concentration. 50µl of these standard dilutions were transferred to a second duplicate set of tubes. Microfuge tubes were prepared and labeled with protein samples, and 108µl of diluent buffer added, then 12µl of protein samples added, final dilution was (1:10). 50µl of the diluted test samples were then transferred into two duplicate sets of tubes.

Working reagent was prepared according to the manufacturer's instructions by adding 50 parts of reagent A to one part of reagent B (50:1). 1 ml of working reagent was added to each 50 µl volume of standards and samples, and mixed well. The tubes were incubated at 37 °C for 30 min, then cooled to room temperature and the absorbance (Optical density, OD) read at 570 nm. The mean absorbances of blanks were subtracted from all other standard and protein samples. OD versus protein concentration was plotted in µg/ml, then the calibration curve used to estimate the concentration of proteins in test samples using Microsoft Excel. An example of an equation of the linear standard curve used to read protein concentrations in samples is:

Linear Standard curve equation; $y = 0.0008x + 0.00343$

$R^2 = 0.9977$

(y) = standard optical density reading, (x) = standard concentration

Total protein concentration in samples (x) was calculated by inserting the (y) value in the standard equation:

$$x = \frac{y - 0.0343}{0}.0008$$

(y) = sample optical density reading, (x) = unknown protein concentration

Amounts of total proteins were derived in µg/µl.

BSA(µg/ml)	OD1	OD2	Mean	OD mean subtract blank
2000	1.77	1.75	1.76	1.68
1000	0.93	0.96	0.94	0.86
500	0.55	0.59	0.57	0.49
250	0.35	0.37	0.36	0.28
125	0.23	0.24	0.24	0.16
62.5	0.16	0.17	0.17	0.09
31.25	0.13	0.13	0.13	0.05
15.62	0.11	0.11	0.11	0.03
7.81	0.10	0.10	0.10	0.02
0	0.08	0.08	0.08	0.00

Table 3.2 Example of standard readings for BCA protein assay.

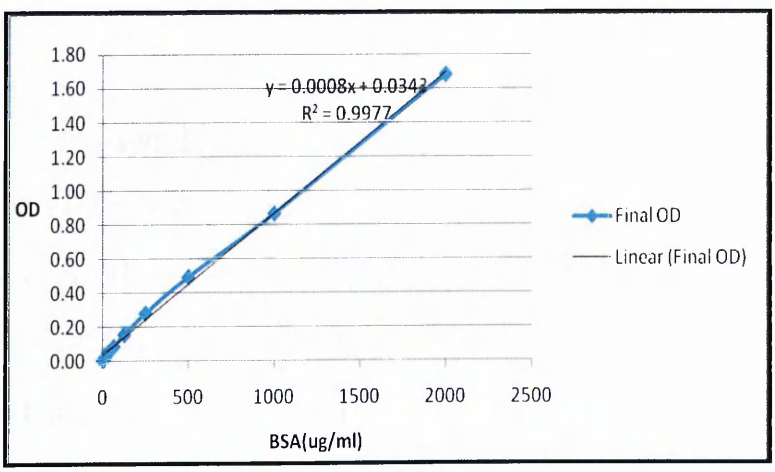


Figure 3.1 Example of standard curve for BCA protein assay.

Unknown protein	OD1	OD2	Mean	OD mean subtract blank	µg/ml	µg/µl
A	0.90	0.89	0.90	0.82	975.88	0.98
B	0.46	0.48	0.47	0.39	443.38	0.44
C	0.99	1.06	1.02	0.94	1135.88	1.14
D	0.42	0.44	0.43	0.35	397.13	0.40
E	1.59	1.57	1.58	1.50	1832.75	1.83

Table 3.3 Example of estimations of protein concentration for various extractions. Note, standard equation was different in each extraction.

3.6 SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) analysis

3.6.1 Sample preparation

The required volumes of the samples were calculated to give either 5µg or 10µg per lane. 5X concentrated sample buffer (Pierce 39000) was added to 4 volumes of protein sample and mixed. Then, a volume of 1X sample buffer (Pierce 39000) was added to adjust the total loading volume to 15µl in each lane of the gel. Also about 15ml of the sample buffer was added directly on the materials tested for attachment. The loading samples and the tested materials were heated in boiled water for 5 min to extract the proteins and denature the sample. The tubes were then centrifuged at 3000g for 2 min to precipitate any insoluble material prior to gel loading.

3.6.2 Gel Electrophoresis

SDS-PAGE was performed using 8-16% Pierce pre-cast protein gels with size of 1 mm x 12 wells (Thermo scientific 25223). Gels were electrophoresed in running buffer (Tris base, Hepes, and SDS dissolved in 500 ml distilled water, pH 7) and the lanes were flushed using a plastic pipette. A 5µl volume of Pierce®-3 colour protein molecular mass markers (Thermo Scientific 26691) was loaded into a marker lane.

Then the prepared protein samples were loaded into the gel, the lid placed on and attached to the power supply and ran at adjusted voltage ~ 90-100 for 30 min to 1 hour. When the bands reached near the end of the gel the power was turned off and plates separated gently using a spatula and the gel gently slid into a small plastic box with distilled water.

3.6.3 Coomassie blue staining

Gels were washed 3 times in distilled water for 5 minutes to remove SDS. Then each gel was stained for 30 min – 1 hour with gentle shaking on a rocker with 0.05% Coomassie brilliant blue R-250 (Sigma B0149), (0.16g Coomassie blue dissolved in 50 ml methanol, 50 ml distilled water and 10 ml acetic acid, and filtered). Gels were de-stained in two solutions; one hour in the first solution (50 ml methanol, 50 ml distilled water, acetic acid 10 ml) and then left to de-stain overnight in the second solution (7.5% acetic acid in 100 ml distilled water).

3.6.4 Silver staining

Silver staining (Pierce 24612) was also performed to stain gels. The gel was washed for 5 min twice in ultrapure water. Then it was fixed in 30% ethanol, 10% acetic acid solution for 15 min twice, followed by washing for 5 min twice in 10% ethanol, and then in ultrapure water for 5 min also twice. The gel was then sensitized for 1 min in Sensitizer Working Solution (50µl sensitizer with 25ml water) and then washed for 1 min twice with water. After the sensitization step, the gel was stained for 30 min in Stain Working Solution (0.5ml Enhancer with 25ml Stain) and then washed twice each time for 20 sec with ultrapure water. Then it was developed by immersing in the Developer Working Solution for 2-3 min or until bands appeared. When the desired band intensity was reached, the developer solution was replaced with prepared stop

solution (5% acetic acid) with sufficient volume to cover the gel and incubated for 10 min.

3.6.5 Protein molecular mass identification

Molecular masses of proteins were estimated from the stained gel by measuring distances of calibration proteins bands in millimetres then plotting these values versus calibration molecular masses (15.2, 24.4, 31.5, 47.7, 77.6, 105, 207 kDa) to draw a standard curve. Migration distances of unknown proteins were plotted on the standard curve and molecular masses determined. Calibration proteins were run with each gel and a standard curve was drawn for each preparation (Hames and Rickwood, 1990).

3.7 Preparation of nucleic acids

3.7.1 Culture preparation of EVA for RNA extraction

Promastigotes were cultured as described in 3.1.2 using 75 cm^2 cell culture flasks in 60 ml of complete medium at 25°C. Promastigote cultures were initiated at 5 x 10^5/ml, then monitored and maintained until they reached stationary phase (4-6 x 10^7/ml). 12 or 24 well plates (Thermo Scientific 150628) were used to culture EVA with promastigotes. 3ml volumes of promastigote cultures were added to each well with 3-4 pieces of EVA. Plates were sealed with Para Film and incubated at 25 °C. Cultures were monitored using an inverted microscope until the parasites attached to the EVA. When they were ready the pieces were removed from wells into a 50ml universal centrifuge tube with 10 ml Hank's balanced salt solution (HBSS) for washing. The EVA pieces were vortexed vigourously to remove unattached parasites then washed three times in Petri dishes containing fresh HBSS, after which attached parasites were ready for extraction.

3.7.2 RNA extraction

For each EVA RNA extraction about 10 to 20 plates were processed. For each plate EVA pieces were transferred into 15ml centrifuge tubes with 2ml of standard Trizol (Invitrogen 10296-010) and incubated at room temperature for 5 min. 400µl of chloroform (Sigma 107K3528) was added, mixed and incubated at room temperature for 10 min. Tubes were then centrifuged at 12,000 x g for 15 min at 4°C. The top aqueous phase was then transferred into a fresh tube. 1ml of isopropanol (Sigma 49296APV) was added and the mixture vortexed for 5-10 sec, incubated at room temperature for 10 min, then centrifuged for 8 min at 12,000 x g at 4°C, and the supernatant discarded leaving behind the RNA pellet. 2ml of 70% ethanol was added to wash the tubes and discarded. Another 2ml of 70% ethanol added to resuspend the pellets, and centrifuged for 5 min at 7500 x g. The ethanol was removed and the tube left to dry on ice. RNA dissolved in 30µl-70µl of molecular grade (RNAse-free) distilled water. Absorbance of A260/A280, and A260/A230 were read usinga Nanodrop spectrophotometer to evaluate quantity and quality of the RNA. 1-1.5% agarose gel electrophoresis was also used to evaluate RNA quality (integrity of ribosomal bands). The RNA was stored at -80°C until use. RNA was prepared from free parasites in cultures usingthe same method, Trizol being added directly to cell pellets.

3.7.3 DNA extraction

25 cm^2 cell culture flasks of *L. mexicana* promastigotes were harvested and washed three times with 10 ml cold HBSS at 300 x g, each time for 10 min. A Qiagen DNeasy Blood and Tissue kit (69504) was used to purify total DNA. Cell pellets were

resuspended in 200 µl PBS. 20 µl of proteinase K and 200 µl of buffer AL were added to the suspension pellet and mixed by vortexing, then incubated at 56°C for 10 min. 200 µl of ethanol (96-100%) was added and mixed by vortexing. The mixture was carefully pipette onto a DNeasy mini spin column placed in a 2 ml collection tube. The tube was centrifuged at 6000 xg for 1 min. The flow through was discarded, the DNeasy column transferred into a new 2 ml collection tube, 500 µl of AW1 buffer was added, and centrifuged at 6000 xg for 1 min. The flow through was discarded again, the DNeasy column transferred into a new 2 ml collection tube, 500 µl of AW2 buffer was added, and centrifuged at 20,000 x g for 3 min to dry the DNeasy membrane. The flow through was discarded and the DNeasy column placed in 1.5 ml microcentrifuge tube. AE buffer (50 µl- 200 µl) was pipetted into the column and incubated at room temperature for 1 min. The column centrifuged for 1 min at 6000 x g and the elutate was collected. The absorbance at A260/A280 was read to evaluate the quantity and quality of the DNA. 1-1.5% agarose gel electrophoresis was also performed to evaluate DNA quality (high molecular weight, no degradation), then DNA stored at -20°C until use.

3.8 cDNA library construction

A custom uncut cDNA library was constructed by Invitrogen using total RNA isolated from attached parasites. The library was supplied in the pENTR™ 222 vector (Fig.3.2) and maintained in the *Escherichia coli* DH10B™ T1 Phage Resistant strain. It contained about >3 x10^6 of primary colonies, was supplied in 80% S.O.C. medium, 20% glycerol, stored at -80°C.The genotype of DH10B™ -T1^R is: F' *mcr*A Δ(*mrr hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara*, *leu*)7697

*gal*U *gal*K λ⁻ *rps*L *nup*G *ton*A. The *att*L1 and *att*L2 sites permit site-specific recombination of the entry clone with a Gateway® destination vector. A kanamycin resistance gene is used for selection in *E. coli* and the pUC origin for high-copy replication and maintenance of the plasmid.

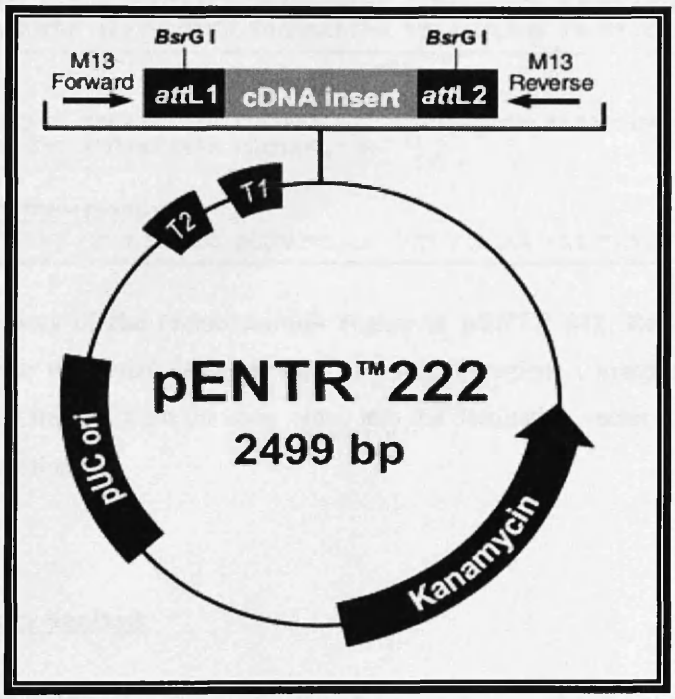


Figure 3.2 The pENTR™ 222 vector map.

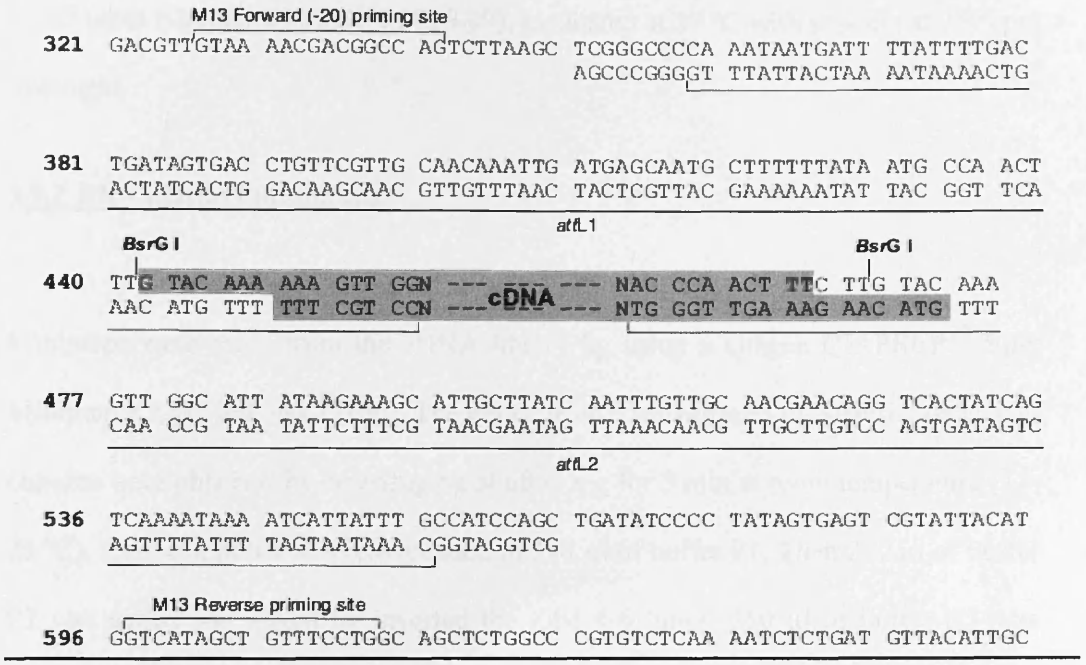


Figure 3.3 Features of the recombination region in pENTR 222. Restriction sites are labeled to indicate the actual cleavage site. The shaded regions correspond to the DNA sequence that will transfer from the entry clone into the destination vector following the LR recombination reaction.

3.9 cDNA library analysis

3.9.1 Colony picking

LB broth was prepared by dissolving 12.5 g of LB broth powder (Sigma L3152) in 500 ml dH_2O, then7.5 g of bacteriological agar (OXOID 33441765) was added to the broth and swirled to mix. The liquid was then autoclaved for 40 min to sterilize, left to cool to 50°C and 50µg/ml of antibiotic kanamycin sulphate (Invitrogen, GIBCO 854038) was added. LB agar was poured into the plates and left to solidify under sterile conditions. Aliquots of 100 µl from a 1:10 dilution of the cDNA library were spread on each plate, and then incubated at 37°C for 24 hrs. Individual colonies from plates were picked and inoculated in 5 ml LB broth containing 50 µg/ml kanamycin in

12 ml tubes (Greiner E11080K0 2016-09), incubated at 37 °C with shaking at 250 rpm overnight.

3.9.2 DNA plasmid minipreps

Minipreps were made from the cDNA library by using a Qiagen QIAPREP ® Spin Miniprep Kit (27104 and 27106). The pelletsfrom1.5mlvolumes of bacterial overnight cultures were obtained by centrifuging at 6800 x g for 3 min at room temperature (15-25 °C). Each cell pellet was resuspended in 250 µl of buffer P1. Then 250 µl of buffer P2 was added and mixed by inverted the tube 4-6 times. 350 µl of buffer N3 was added and mixed, and the tube centrifuged for 10 min at ~ 17,900 x g. The supernatant was transferred onto a QIAprep spin column, and centrifuged for 1 min. The flow through was discarded and the spin column washed with 750 µl of buffer PE, then centrifuged for 1 min and the flow through discarded again. The spin column was transferred into a new 1.5 ml microcentrifuge tube and 50 µl of EB buffer (10mM TrisCl, pH 8.5) was added, left at room temperature for 1 min then centrifuged for 1 min and eluate was collected. Product absorbance was read atA260/A280 to evaluate quality and quantity of the plasmid DNA, then stored at -20°C until use.

3.9.3 Analytical restriction digests

Analytical small scale digests for screening cDNA constructs were carried out in a total volume of 20µl using 2µl of 10x enzyme buffer, 2µl of 10x BSA buffer, 0.5µl of *Bsr*GI enzyme (BioLabs 0081108) and made up to 20µl with molecular grade water to digest approximately 300ng/µl of DNA (typically 2.5-3µl of miniprep DNA). Then digests were incubated for 2 hours at 37°C. After incubation, the whole reaction was

mixed with 4 µl DNA loading buffer and loaded on a 0.5-2% agarose gel depending on the size of the DNA fragments to be resolved (usually 1%).

3.9.4 Dye terminator cycle sequencing

Plasmid DNA samples from the cDNA library were sequenced using a GenomeLab^{TM}Dye Terminator Cycle Sequencing (DTSC) Quick Start Kit (Beckman Coulter PN 608120) and M13 Forward and Reverse primers. Sequencing reactions were carried in 96 well micro-plates and with 300ng of DNA samples. DNA and an appropriate volume of molecular grade water were added to the plates wells, mixed well and denatured at 95 °C for 3 min. After cooling down on ice, 8 µl of DTSC master mix was added to each well and plates incubated in a PCR thermo-cycler for 30 cycles(90°C for 20 sec, 50°C for 20 sec and 60°C for 4 min) for 3 hrs. Reactions were stopped by added to each well 5µl of Stop Solution/Glycogen mixture (2µl of 3M sodium acetate pH 5.2, 2µl of 100mM Na_2-EDTA pH 8.0, and 1µl of 20 mg/ml of glycogen). 60µl of cold 95% (v/v) ethanol/dH_2O from a -20°C freezer was added to each well and mixed thoroughly. Immediately the plate was centrifuged at 12,000 x g at 4°C for15 min. Carefully, the supernatant was removed with a micropipette, and the pellets rinsed 2 times with 200 µl cold 70% (v/v) ethanol/dH2Ofrom a -20°C freezer. After each rinse, the plate was centrifuged immediately at 12,000 x g at 4°C for 3 min. After the second centrifugation the supernatant was carefully removed with a micropipette, and each pellet resuspended in 40 µl of the Sample Loading Solution. Then, the plate was left to dry for 10 minutes. Each sample plate was prepared for loading into the sequencing instrument by adding one drop of light mineral oil per well (provided in the kit or Sigma M 5904).Sample plates were loaded into the

instrument, a Beckman Coulter CEQ ™ 8000 Genetic Analysis System, and the desired method was started.

3.10 DNA sequences data analysis and primers

Data that obtained from sequencing cDNA clones were analyzed by using the *Chromas Lite 2.01* programme to assess sequence quality, and genes identified by BLAST analysis on the *TritrypDB* website. Specific primers for PCR and RT-PCR of selected genes were designed through *Primer 3 (v. 0.4.0)*. These were:

Control Gene: 60S ribosomal protein L10a, putative

Forward Primer	5' GTCCTGAAGGTGGACAAGGA 3'
Reverse primer	5' AGCGACACGAGGAAGTTGAT 3'

Trypanosomatid-specific genes

LmxM.18.0620

Forward Primer	GTCCTGAAGGTGGACAAGGA
Reverse Primer	AGCGACACGAGGAAGTTGAT

LmxM.01.0620

Forward Primer	CCTGCGGCTGTATAAGAAGG
Reverse Primer	GGTACGGCTGTTCGTGTTCT

LmxM.03.0640

Forward Primer	GAAGGACAACGCGAAGAGTC
Reverse Primer	CAACCCCTTCAAAACCAGAA

LmxM.05.0450

Forward Primer	TGGATATTCGACTGGTGCTG
Reverse Primer	GTTCAGAGGCGAGCAAAGAC

LmxM.08.0410

Forward Primer	AGTTTTTACGCGTGGGACAG
Reverse Primer	TGTGTCCCACTTCTGCTGAC

LmxM.09.1505

Forward Primer	CGAAAGAGCATTCGTGAGC
Reverse Primer	CCGGATAGCCTGCAGAATC

LmxM.17.0810

Forward Primer	CTCTGCAAGCTCGGCTAGAT
Reverse Primer	GTACATCCCGTTCAGCACCT

LmxM.17.0870

Forward Primer	TTGTTAGCCAGAGCGACCTT
Reverse Primer	ACCCTGAAGCCATTCACATC

LmxM.27.0650

Forward Primer	TGAAGGTCGAGCTGACAATG
Reverse Primer	CGGAAATAGCGAAGACGAAG

LmxM.30.2270

Forward Primer	GGCACAGACTGCTACTCACG
Reverse Primer	TGCCGACTTTTGGACTCTCT

LmxM.31.0180

Forward Primer	GCCCAAGAAGAATCCGTACC
Reverse Primer	GACGGTAGCCCTTCAGCTC

LmxM.31.1090

Forward Primer	CGGCTTTTGAGTTTTTCTCG
Reverse Primer	TGTGCCATAGCGAATCCATA

LmxM.31.2500

Forward Primer	TACTCGCACAGCAACTACGG
Reverse Primer	CAAGCAGCGACTGCAGATAC

LmxM.36.2450

Forward Primer	AGGACGAACATGACCACCTC
Reverse Primer	AATCCGCTGCTGATACTGCT

LmxM.36.3620

Forward Primer	ATGCAGCAGAGCCTCATCC
Reverse Primer	GCACGCGAACTCTTCGTAGT

LmxM.36.3780

Forward Primer	TTATGACGACGCAGAAGTGG
Reverse Primer	GAATGTCACCGGCTCGTAGT

LmxM.36.5060

Forward Primer	TGTCGAGATCAAGGATCGTG
Reverse Primer	CTTCTCCCCTTGGTCATTCA

LmxM.36.5850

Forward Primer	TATCAGGGGACTGGATCTGC
Reverse Primer	ACAGACTCGTTCGCCTTGTT

LmxM.25.0920

Forward primer	GTGCTTCACTGAGTTTGCGA
Reverse primer	AGACCGATACAATGACCGCA

***Leishmania*–specific genes**

LmxM.23.1020

Forward primer	ACCAGCAGTGCGATAAGGAC
Reverse primer	AACTCGTTCTTGGGGTCCAC

LmxM.34.3180

Forward primer	CAGGGAGCGCTTCTCAAAGA
Reverse primer	CTCGGTGCATGAACAGGTCT

LmxM.29.3025

Forward primer	GCGGTGCGAGAGCAAAAATA
Reverse primer	CATCTAAGGGGACGGCGAAG

LmxM.27.1350

Forward primer	ACTGGGTGAGCGACATCAAA
Reverse primer	TGAGCTTCGTGTAGAACGCA

LmxM.12.0905

Forward primer	GGGGATGTGCTGCGGTAG
Reverse primer	GGAGAGGGCAAGCAGAGAG

LmxM.28.1165

Forward primer	ACGTGCGCTGTACACTGAAG
Reverse primer	AGAGAAGAGCGCTCAACAGC

3.10.1 PCR amplification

PCR was used to analyse *Leishmania* DNA and plasmids DNA with a HotStar®Taq *Plus* Master Mix Kit (Qiagen 203643). Using 0.2ml 8 Strip PCR tubes (Starlab 14022900), a final volume of 20µl was used (10µl of 2X HotStar-Taq Master Mix, 1µl of 10µM Forward primer, 1µl of 10µM Reverse primer, 2µl DNA template at 300ng/µl and made up with molecular grade water to 20µl)and mixed by vortexing. Samples were run using the following thermocycler conditions: initial denaturation at 95^0C for 5 min, 30 cycles of denaturing at 94^0C for 30 secs, annealing at 60^0C for

30 sec and extension at 72^0C for 1min per kb target, final extension at 72^0C for 10mins, then hold on 4 °C.

3.10.2 RT-PCR amplification

Leishmania mexicana RNA from attached forms, metacyclic promastigotes, and log-phase promastigotes were tested with various specific gene primers. SuperScript® III One-Step RT-PCR System with Platinum ® *Taq* high Fidelity (Invitrogen 12574-030) was used in 15µl total volumes (7.5µl of 2X Reaction Mix, 0.6µl of 10µM/µl Forward Sense primer, 0.6µl of 10µM/µl Reverse Anti-Sense primer, 1µl of 10ng/µl RNA Template, 0.3µl of SuperScript® III RT/ Platinum ® *Taq* high Fidelity Enzyme Mix, and up to 15µl with autoclaved molecular grade water). The mixtures were incubated using the following thermal cycling program: 45°C for 30 min, 94°C for 2 min, 28 cycles of 94 °C for 15 sec, 60°C for 30 sec, and 68°C for 1 min, final extension at 68° C for 5 min, then hold on 4°C.

1% - 1.5% agarose gels were prepared; about 1-1.5 g of agarose (Sigma 100M9432V) was dissolved in 100 – 150 ml of 1X TAE buffer and autoclaved for 3 min. 3µl of GelRed (Gentaur, BioTium 41000) was added. Products were analysed by mixing 4µl of 6X DNA sample buffer with the products, and 10µl volumes were loaded onto gels. 5µl of a DNA ladder was loaded into another lane and gels run at 120V for 20 min. Then the gel was incubated in GelRed stain for 15 min, washed twice in water, and photographed.

3.10.3 RT-PCR result analysis

Gene bands intensities were measured by using the Image J programme. The measurements of the genes of interest and the control gene bands were subtracted

from the measurement of their own background (comparable area having been chosen which is lower than the gene measurement). The results from the subtraction were used to find the ratio between the gene and the control. Table 3.3 gives an example of how the calculation was done.

Bands	Area	Mean	Min	Max
Haptomonads	3432	73.237	9	255
Metacyclic	3432	57.856	13	197
Promastigotes	3432	103.067	14	255
*Haptomonads	3969	97.874	13	255
*Metacyclic	3969	99.739	17	255
*Promastigotes	3969	100.735	14	255

Background Bands	area	mean	min	max
Haptomonads	3432	10.683	7	19
Metacyclic	3432	13.182	8	38
Promastigotes	3432	14.065	9	62
*Haptomonads	3969	12.735	6	23
*Metacyclic	3969	14.851	9	29
*Promastigotes	3969	12.376	6	22

L.mex life stage	Bands	Background	Subtract
Haptomonads	73.237	10.683	62.554
Metacyclic	57.856	13.182	44.674
Promastigotes	103.067	14.065	89.002
*Haptomonads	97.874	12.735	85.139
*Metacyclic	99.739	14.851	84.888
*Promastigotes	100.735	12.376	88.359
	Gene	Control	Ratio
H 365850	62.554	84.989	0.736
M 365850	44.674	57.962	0.771
P365850	89.002	140.303	0.634

Table 3.4 Example of methodology for gene and control measurements for the RT-PCR results. Analysis shows data from *Lmx.M*.365850 gene. * Control bands.

3.11 cDNA library screening using cDNA probes

3.11.1 Reverse transcription of cDNA probes

Reverse transcription of RNA to produce cDNA from attached parasites and log-phase promastigotes with a QuantiTect Reverse Transcription Kit (Qiagen 205310) was used to prepare probes to screen the cDNA library. 1µg of template RNA of attached and log-phase promastigotes was processed. RNAase was deactivated by incubated for 2 min at 42°C. Reverse transcription reactions were performed following the manufacturer's instructions.

The DNA products from reverse transcription were cleaned by using a QIAquick PCR Purification Kit (Qiagen 28104). The cDNA samples were mixed with PB buffer, then placed in a QIAquick spin column in a 2 ml collection tube, and centrifuged for 30–60 sec and the flow through discarded. Then the column was washed with PE buffer, and centrifuged again for 30–60 sec, and the cDNA eluate collected by adding water (pH 7.0–8.5) to the centre of the QIAquick membrane and centrifuging the column for 1min.

3.11.2 cDNA DIG labelling

The cDNA Library was screened by labelling cDNA probes with digoxygenin (DIG) using a DIG High Prime DNA Labeling and Detection Starter Kit I(Roche 11745832910, version 13). The final product of cDNA template synthesis from reverse transcriptase was denatured by heating in a boiling water bath for 10 min, and then quickly chilled in an ice. 4 µl of DIG-High Prime was added to the denatured DNA, mixed and centrifuged briefly for 1 min. The mixture was incubated for 20 h at 37^0C and the reaction stopped by adding 2 µl 0.2M EDTA, pH 8.0.

The efficiency of the labelling step was tested by performing serial dilutions of each labelled probe prepared from different RNA samples and compared to positive DNA control. Table 3.4illustrates the serial dilutions prepared for the samples, according to the manufacturer's recommendations.

Tube	DNA (µl)	From tube #	DNA Dilution buffer (vial 3) (µl)	Dilution	Final concentration
1		Diluted original			1 ng/ µl
2	2	1	198	1:100	10 pg/ µl
3	15	2	35	1:33	3 pg/ µl
4	5	2	45	1:10	1 pg/ µl
5	5	3	45	1:10	0.3 pg/ µl
6	5	4	45	1:10	0.1 pg/ µl
7	5	5	45	1:10	0.03 pg/ µl
8	5	6	45	1:10	0.01 pg/ µl
9	0	-	50	-	0

Table 3.5 Dilutions prepared for testing probe labelling efficiency.

1µl volumes of tubes 2-9 from labelled probes samples and the labelled control were spotted on to a nitrocellulose membrane. The nucleic acids were fixed to the membrane by cross linking with baking for 30 min at 80^0C - 85^0C. The membrane was then transferred into a plastic container with 20 ml Maleic acid buffer and incubated with shaking for 20 min at room temperature. After that the membrane was incubated for 30 min in 10 ml freshly prepared Blocking solution, followed by a 30 min incubation in 10 ml of antibody solution (contains anti-DIG antibody conjugated to alkaline phosphatase). The membranes were washed with 10 ml washing buffer, twice for 15 min, then equilibrated for 2-5 min in 10 ml of detection buffer. The membrane was then incubated in 2 ml of freshly prepared colour substrate solution in an appropriate container in the dark without shaking. Colour started to form within a

few minutes and the reaction stopped when desired spots intensities were achieved by washing the membrane for 5 min with 50 ml of TE-buffer. The intensity of the spots from the labelling reactions were compared to controls to enable calculation of the amount of DIG-labelled DNA. If the 0.1 pg dilution spots of probe and control are visible then the labelled probe has reached the expected labelling efficiency. If this was not achieved fresh probes were prepared. Probes were kept and re-used providing they showed good signal strength.

3.11.3 DNA transfer and fixation

In order to screen the library gridded nitrocellulose membranes were used (Amersham cat. no. RPN.1737c). About 5µl - 8µl of the cDNA library was plated out on a LB plate with 50µg/ml kanamycin sulphate (Invitrogen, GIBCO 854038) and incubated overnight at 37°C. Then 100 individual colonies were picked up used a sterile yellow tip, and each colony was resuspended in 50 µl sterile saline. 3 µl volumes of the colony suspensions were spotted in duplicate on nitrocellulose membranes and allowed to dry. DNA was released and fixed by placing the membranes on 3MM filter paper soaked in 0.5 M NAOH for 5min, then on filter paper that was soaked in 1M Tris-HCl pH 7.5 for 5 min, and finally on filter paper that was soaked in 0.5M Tris-HCl pH 7.5 and 1.25M NaCl for 5min. The membranes were then left to dry on 3MM filter paper and baked in a vacuum oven at $120C^0$for 30 min. They were stored dry at 4^0C in sealed bag ready until use.

3.11.4 Hybridization and immunological detection

For hybridization an appropriate volume of DIG Easy Hyb buffer (10ml/ 100cm^{2}filter) was preheated to the hybridization temperature of 50°C. The membrane was pre-hybridized for 30 min with gentle agitation in an appropriate container. DIG-labeled DNA probes were denatured (about 90 ng/ml) by boiling for 5 min and rapidly cooling in ice/water. The denatured DIG–labeled DNA probe was then added to the preheated DIG Easy hyb buffer (3.5 ml/10cm^2 membrane), and mixed well but gently without forming bubbles. The pre-hybridization solution was then replaced with probe/hybridization mixture and the membrane incubated overnight at 50°Cwith gentle agitation in a sealed container.

The membrane was then washed twice for 5 min each in 2x SSC, 0.1% SDS at 15 to 25^{0}C under constant agitation. This was followed then by washing twice for 15 min each in 0.5x SSC, 0.1% SDS, pre-warmed and maintained at the wash temperature of 65 to 68^{0}C under constant agitation (high stringency wash). After the hybridization and post-hybridization washes, the membrane was rinsed briefly for 1-5 min in washing buffer. Then it was incubated for 30 min in 100 ml blocking solution, followed by incubation for 30 min in 10 ml of antibody solution. After that, the membrane was washed 2 times each for 15 min in 100 ml washing buffer. The membrane was then equilibrated for 2-5 min in 20ml detection buffer, followed by incubation in 10 ml of freshly prepared colour substrate solution in the dark without shaking during colour development. The reaction was stopped when the desired spots appeared by washing the membrane for 5 min in 50 ml of PCR grade water or with TE buffer. The membrane was then photographed using a ChemiDoc image system (BioRad).

3.12 Northern blots

3.12.1 PCR for probe synthesis

Primers for candidate genes were designed using *Primer 3* and synthesised by Life Technologies (Invitrogen).

LmxM.20.1290

Forward primer	GAGATGGATGAGGAGGACGA
Reverse primer	TCACTCCACCTTCGCTTTCT

Annealing temperature 60 ^{0}C

LmxM.31.0020

Forward primer	TGCTCGACGCTCATTCAGTA
Reverse primer	TTTGCTGATGCTTCTGTTGC

Annealing temperature 60 .7 ^{0}C

LmxM.33.3790

Forward primer	ATCACTGCTGTGTGGCTTTG
Reverse primer	TGTCAATCAGCGAGACTTGG

Annealing temperature 59.9 ^{0}C

LmxM.18.1620

Forward primer	TTACCGGCCTTGGTATGTTC
Reverse primer	AAACACCAACGGAGAACCAC

Annealing temperature 59.8 ^{0}C

LmxM.32.0940

Forward primer	CCACTTTGGTAGCCCACTGT
Reverse primer	TTCATCAGGGATGGGAACTC

Annealing temperature 60 ^{0}C

Control primers 60S ribosomal protein L10a, putative

Forward primer	GTCCTGAAGGTGGACAAGGA
Reverse primer	AGCGACACGAGGAAGTTGAT

Annealing temperature 60 ^{0}C

PCR reactions in a total of 20µl were prepared by mixing 10 µl of 2X HotStarTaq Master Mix (Qiagen203643), 1 µl of 10µM forward and reverse primers, 100-200 ng/µl of DNA template and molecular grade water. Reactions were run using the following PCR cycling parameters; initial denaturation was at 94^{0}C for 5 minutes, followed by 30 cycles with a denaturation step at 94 ^{0}C for 30 seconds; annealing at 60^{0}C for 30 seconds, and extension for 1 minute at 72 ^{0}C. The final extension was for 10 minutes at 72 ^{0}C.

The PCR products were then run on 1% agarose gels to confirm that the correct size of the product had been synthesized. Then the PCR products were incubated overnight with 4 µl DIG-High Prime at 37 ^{0}C for DIG labelling, followed the manufacturer's protocol (DIG-High Prime DNA Labelling and Detection Starter Kit, cat No. 11 745 832 910, Roche) to generate labelled DNA (for details see 3.11.2).

3.12.2 RNA separation by gel electrophoresis

All the equipment for Northern blots was sprayed with anti-RNase spray, then washed with DEPC-water (diethylpyrocarbonate) 1ml/liter distilled water, then autoclaved for sterilization and RNase deactivation. All tips and tubes were RNase-free. Water and buffers were treated with DEPC, and then autoclaved to destroy the DEPC.

RNA samples of attached forms and free promastigotes were prepared by mixing 3 µl of 6M glyoxal,12 µl dimethyl sulfoxide (DMSO), 3 µl of 0.1M phosphate buffer (0.1M NaH_2PO_4, 0.1M Na_2HPO_4, pH= 7.0), then RNA samples added and brought to the final volume of 30 µl with DEPC-water. The samples were incubated at 50^0C for 50 minutes and then chilled on ice for 10 minutes. RNA samples and RNA size ladder were mixed with 2.5 µl RNA loading buffer (50% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol, 0.1M phosphate buffer, 6M glyoxal) and then loaded on to 1% agarose gels prepared in 10mM phosphate buffer pH 7.0. The gels were run at 60 V in phosphate buffer in presence of magnetic stirrers to re-circulate the buffer during the electrophoresis and prevent the accumulation of H^+ and maintain the neutral pH as the glyoxal will dissociate with RNA at high pH (above 7), then the gel stained with GelRed for 30 min and photographed.

Alternatively a formaldehyde-free RNA Gel Kit (Amresco, N726) was used, which is a non-mutagenic, safer alternative to formaldehyde-containing agarose gels that can be performed on the bench without using the fume hood. Further incubations and buffer recirculation during electrophoresis are unnecessary because the absence of glyoxal. Gels were prepared according to the manufacturer's instructions. Gels were run at 80 V in 10X running buffer without presence of magnetic stirrers or a post-stain step, and photographs taken.

3.12.3 RNA transfer to nylon membranes

Gels were equilibrated in 100 ml of 20X SCC (3M NaCl, 0.3M $Na_3C_6H_5O_7$, pH 7) at room temperature for 15 min, twice. Then the RNA samples transferred to the nylon membrane (Boehringer Mannheim) by the capillary transfer method based on

Sambrook and Russell (2001). The transfer vessel was filled with 20X SSC to just below the level of the gel. 3 Whatman grade filter papers were pre-soaked for 5 minutes in 20X SSC and placed on the vessel before placing the gel, so the filters were beneath the inverted gel. Then a pre-soaked nylon membrane in 20X SSC was placed onto the inverted gel (no air bubbles). Two pieces of Whatman grade filter papers, pre-soaked in 2X SSC for 5 minutes, were placed on top of the nylon membrane to facilitate the SSC gradient and encourage capillary transfer of the RNA. Layers of papers towels were placed on top and a glass plate was placed on the top of the towels, whole system weighted by adding 1Kg on the top. The RNA was left to transfer overnight at room temperature. Once the transfer was completed the nylon membrane was washed briefly in 2X SSC, then baked at 120^0C for 30 minutes to cross-link the RNA samples.

3.12.4 Hybridization and immuno-detection using the DIG system

The DIG High Prime DNA Labeling and Detection Starter Kit I (Roche, cat. no. 11745832910, version 13) was used to label DNA probes for hybridization and subsequent colour detection by enzyme immunoassay. The DNA probes were generated with DIG-High Prime according to the random primed labelling technique in special developed reaction mixture containing digoxigenin-dUTP, alkali-labile, and all reagents were premixed in an optimized 5X concentrated reaction buffer. The blotted RNA on the nylon membrane was hybridizing by using alkali-labile form of DIG-11-dUTP. The immunodetection was performed by using anti-digoxigenin-AP Fab fragment and then visualized with colorimetric substrates NBT/BCIP.

After the completion of transfer and the cross-linking of the RNA samples to the nylon membrane, the membrane was then hybridized with an appropriate volume of DIG Easy Hyb buffer (10ml/ 100cm^2 filters) preheated to the hybridization temperature of 50°C. The membrane pre-hybridized for 30 minutes with gentle agitation in an appropriate sealed container. DIG-labeled DNA probe was denatured (about 90 ng/ml) by boiling for 5 minutes and rapidly cooling on ice. Denatured DIG –labeled DNA probe was added to the preheated DIG Easy Hyb buffer (3.5 ml/10cm^2 membrane), and mixed well gently without forming bubbles. Pre-hybridization solution was replaced with probe/hybridization mixture and the membrane incubated at 50^{0}C overnight with gentle agitation in a sealed container.

When the incubation was finished the probe/hybridization DIG-buffer was poured off and the membrane washed twice for 5 minutes each in 2x SSC (0.1% SDS at 15 to 25^{0}C under constant agitation. This was followed by washing twice for 15 minutes each in 0.5x SSC (0.1% SDS, pre-warmed to wash temperature) at 65 - 68 ^{0}C under constant agitation.

After the post-hybridization washes, the membrane was rinsed briefly for 1 - 5 minutes at 20^{0}Cin washing buffer (0.1 M Maleic acid, 0.15 M NaCl, pH 7.5, 0.3% (v/v) Tween 20). Then the membrane was incubated for 3 hours in 100 ml blocking solution (1:10 in Maleic acid buffer). Then it was incubated for 30 minutes in 10 ml of antibody solution (Anti-Digoxigenin-AP 1:5000 (150mU/ml) in blocking solution). The membrane was then washed 2 times each for 15 minutes in 100 ml washing buffer, equilibrated for 2-5 minutes in 20 ml of detection buffer (0.1M Tris- HCl, 0.1 M NaCl, pH 9.5 (20^{0}C), followed by Incubation in 10 ml freshly prepared colour

substrate solution (40µl of NBT/BCIP stock solution to 2 ml of Detection buffer), stored away from the light in the dark without shaking during colour development. The reaction was stopped when the desired bands appeared by washing the membrane for 5 minutes in 50 ml of TE buffer(10mM Tris-HCl, 1 mM EDTA, pH 8.0). The washed membrane was photographed for the bands.

3.13 Electron Microscopy

3.13.1 Sample preparation for scanning electron microscopy

Leishmania mexicana stationary phase parasites were cultured with pieces of Ethyl Vinyl Acetate (EVA) for 2 days. The EVA was checked for attachment of parasites with an inverted microscope. The EVA pieces were processed by the Electron Microscopy Unit at Glasgow University.

3.13.2 Sample preparation for transmission electron microscopy

L. mexicana stationary attached parasites were prepared as above. The EVA was vortexed in 10 ml of HBSS and washed 3 times to remove unattached promastigotes. The EVA samples were fixed in 4% paraformaldehyde, 2% glutaraldehyde overnight. Then they were washed with Medium 199 and once more with Fetal Bovine Serum (FBS). Several chemicals (acetone, acetic acid, hydrochloric acid, 100% absolute ethanol, 100% absolute methanol and xylene) were used to separate the attached parasites form EVA surface. Experiment continued by using Xylene. EVA were incubated in 500 µl FBS and 1ml xylene for 5min. The liquid was collected and centrifuged for 5min at 10,000g to separate xylene from serum. The parasite pellet was washed in 1% paraformaldehyde and resuspended in 200 µl 1% paraformaldehyde. Smears were prepared form collected pellet, then fixed in methanol

for 10 min and stained with Giemsa's satin and florescent dye to confirm the separation. Then the sample was processed by the Electron Microscopy Unit at Liverpool University.

3.14 Confocal Microscopy

L. mexicana attached parasites were prepared as previous. The EVA was vortexed in 10 ml of HBSS and washed 3 times to remove unattached promastigotes. EVA was fixed in methanol overnight then sample was processed by the Confocal Microscopy Unit at Lancaster University/School of health and medicine.

Chapter 4

Results

4.1 Attachment to different materials

Different species of *Leishmania* were tested for their ability to attach various materials in various media, using Melinex as a control substrate (Wakid and Bates, 2004). The overall aim was devise a system suitable for large scale preparation of attached promastigotes amenable to biochemical and molecular analysis. Comparison of the growth of parasites in Medium 199 and Grace's insect medium demonstrated better growth in the former, so experiments were carried out using Medium 199. *Leishmania major*, *L. mexicana* and *L. tarentolae* were used to test the best materials that provide good attachment as described in this chapter. Normal promastigotes and mutant promastigotes of *L. mexicana* were cultured with some materials. Mutant *L. mexicana* promastigotes CAEP 63 (CRK3::Hyg/CRK3::Ble [pTEXcrk3his], CRK3 null mutant with a his-tagged version expressed from the pTEX vector), were cultured with Melinex and Filter paper membrane in Grace's insect medium but during monitoring they did not show clear signs of attachment. Experiments using mutant promastigotes were discontinued. All experiments were using low subpassages of normal promastigotes cultured in 25 cm^2 culture flasks and Medium 199 with 10% FBS and 2% urine, experiments performed investigating attachment to various materials.

4.1.1 Melinex Plastic Sheet

Melinex was cut into 15mm x15mm squares, sterilized with 70% ethanol and left to dry in a sterile tissue culture cabinet. They were then cultured with promastigotes in sterile 12 well culture plates sealed with ParaFilm and incubated at 26°C. Different

starting densities and different sub-passages were used. The culture medium was replenished once within one week. The Melinex squares were removed after 7 days, washed with HBSS, fixed with methanol and stained with Giemsa's stain.

Testing of different starting volumes showed that a higher starting volume gave a higher attachment rate (Table 4.1).

Promastigote culture	Starting volume	Attachment 7 days
Low starting volume	0.25 x10^8/0.5 ml	154800 / 225μm^2x 10^6
High starting volume	1 x 10^8/2ml	605700 / 225μm^2x 10^6

Table 4.1 Comparison of *L. mexicana* attachment in cultures initiated with different volumes. Cultures had the same starting densities but different volumes were used and attachment measured after 7 days of incubation.

To investigate any difference in attachment ability between different sub-passages of promastigotes (since transformation from amastigotes), Melinex squares were cultured with 1 x 10^8/ml of 2 different sub-passages and the number of parasites counted after 7 days of culturing (Table 4.2).

Promastigote Sub-passage	Attachment 7 days
SP3	918000 / 225μm^2x 10^6
SP10	811800 / 225μm^2x 10^6

Table 4.2 Comparison of attachment rate between different sub-passages of *L. mexicana* promastigotes. Subpassage 3 (SP3) and subpassage 10 (SP10) cultures were tested for their ability to attached to Melinex squares.

This experiment indicated that attachment rate decreased with increase in passage, although attachment was still quite good after 10 subpassages (~12% decrease). These experiments indicated that parasite attachment was occurring as previously reported (Wakid and Bates, 2004).

The parasites attached to the smooth surface of the Melinex and their attachment increased with time. Microscopic examination of the stained Melinex showed them attached in rosette shaped clusters. Cultures after few sub-passages showed many more clustered parasites in suspension, which is in agreement with the higher numbers of attached parasites than shown by cultures that have been through many sub-passages. The attachment behaviour of promastigotes was more active in cultures with few sub-passages and with time the rate of attachment of promastigotes decreased (Wakid and Bates, 2004).

Promastigotes attached to Melinex were examined by scanning electron microscopy (Fig. 4.1). Foot-like extensions previously shown to include hemidesmosome-like structures were observed (Wakid and Bates, 2004).

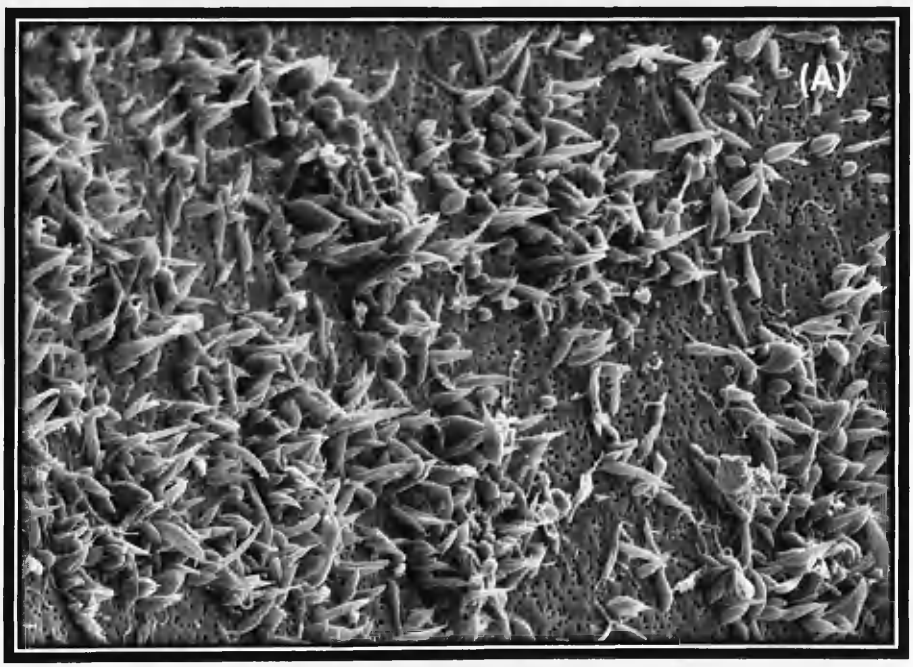
Figure 4.1 High magnifications of scan electronic micrographic pictures of parasites attached to the Melinex plastic sheet by forming hemidesomosoe foot like structure (arrowheads)

(A) HV= 10KV, Mag=4000X, Bar= 5μm

(B) HV= 10KV, Mag=14000X, Bar= 2μm

4.1.2 Polycarbonate membrane filter papers

Polycarbonate filter papers were cultured with *L. mexicana* and *L. major* promastigotes. Two types of filter surface were used: hydrophilic and hydrophobic (1.0μm pore size x 47 mm). The cultures were initiated at several different densities and the best starting density was found to be $1x10^8$/ml with about 5 ml of the promastigote culture. Based on light microscopic examination the parasites started to attach to the filters within a few hours of culturing time. Although quantitation was not possible in this system, it could be seen that the parasites attached at a higher rate to the hydrophilic type than to the hydrophobic filters. Both filters have two sides; a shiny side and a dull side. Examination by scanning electron microscopy showed the parasites attached to both sides similarly (Fig. 4.2). From the electron micrographs it is hard to confirm whether the parasites are attached to the filters by forming hemidesmosomes or just penetrate their flagella inside the pores, as the ends of the flagella cannot be seen.



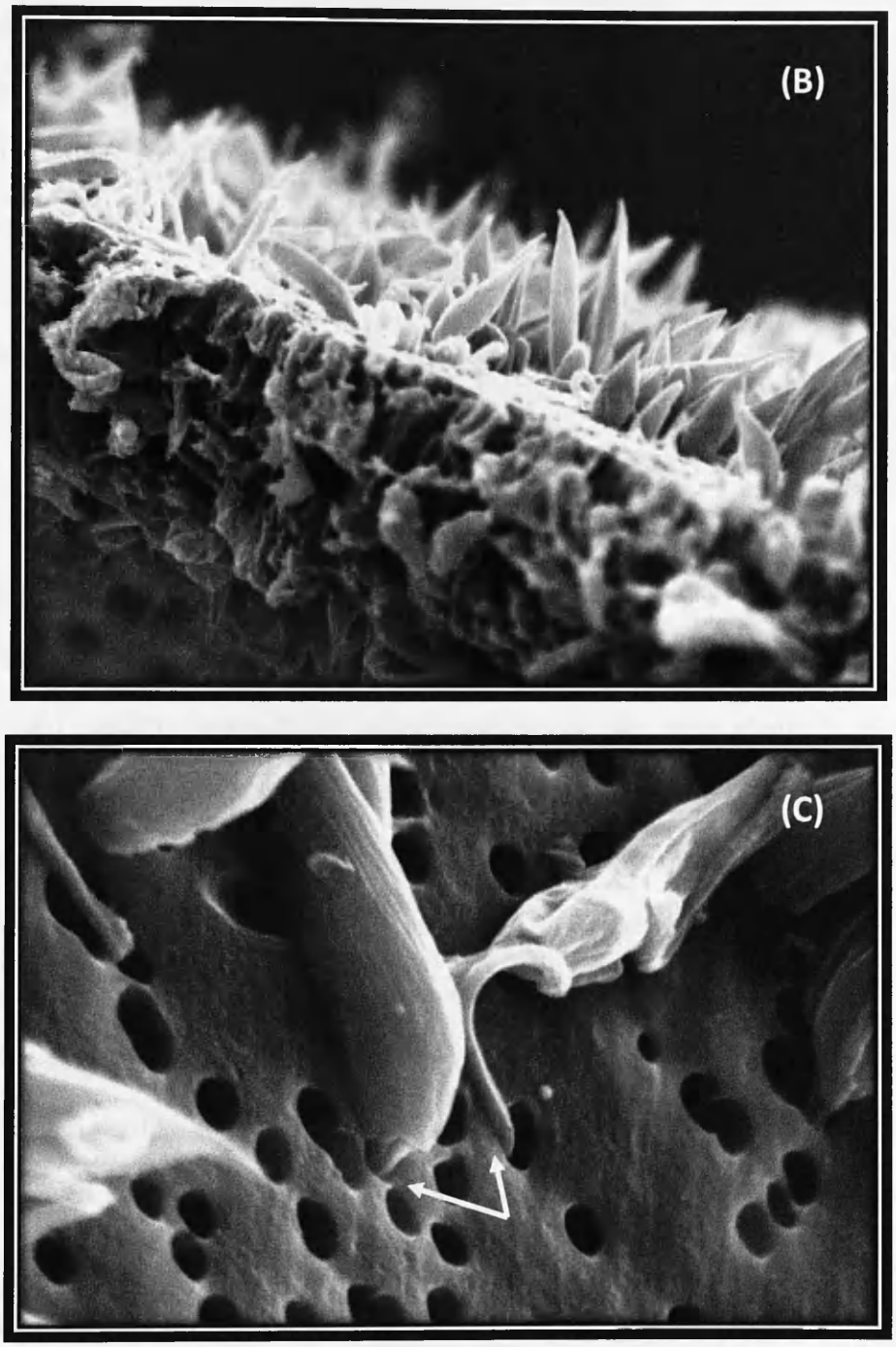


Figure 4.2 High power of scanning electron microscopy showing how the parasites insert their flagella inside the pores (arrowheads) of hydrophilic and hydrophobic filter papers. (A) HV = 10KV, Mag = 1000X, Bar = 20μm, (B) HV= 10KV, Mag = 2500X, Bar = 10μm, (C) HV = 10KV, Mag = 1000X, Bar = 2μm.

4.1.3 Chitin

Different chitin flake preparations containing various sizes of particles were made and used in culture with *L. mexicana* promastigotes. The cultures were started at the same density (1 x 10^8/ml), and inverted microscopic examination showed that the parasites attached to the chitin in the all preparations, but in higher numbers to the large particles. However, the density of their attachment was not very high. The parasites attached to chitin based on microscopic examination of the cultures for 7 days then rate of the attachment decreased.

4.1.4 Agarose-coated Petri dishes and flasks

Different initial densities were used to start the cultures. When the cultures were examined using an inverted microscope attached parasites were observed, most of these were clustered around the edge of the agarose-coated area. That was maybe because the edges were rough and the promastigotes prefer to attach to such areas rather than a smooth surface (Wakid and Bates, 2004). Parasites isolated from the agarose and examined by light microscopy on Giemsa-stained microscope slides showed varying structure, some were large stumpy forms with short flagella. Others had a small dot-like structure at the end of the flagellum and yet others had unusual round-ended flagella with a balloon-like structure that was unlike the normal end of the flagellum in free promastigotes. It was postulated that this might represent a hemidesmosomal expansion. About 13.9% of the attached parasites from agarose cultures had such flagella. However, during checking of the cultures under the inverted microscope, promastigotes with the unusual flagella collected from the agarose were also found swimming freely in the medium, indicating that these may not be the haptomonad forms. It was difficult to get clear judgment on the attachment based on microscopic examination.

4.1.5 n-Octacosane wax and ParaFilm

The n-octacosane wax and ParaFilm were both used in cultures with 1.3ml of *L. mexicana* and 2.5ml of *L. tarentolae* promastigotes. The density used to start the cultures was $0.2x10^8$/ml.

Two forms of n-octacosane were used, melted (and allowed to re-solidify) and non-melted (used direct from the bottle and sterilized). Both showed attachment of the promastigotes of both species within 3 days but the attachment density was low in comparison with other materials. Similarly, the parasites started to attach to ParaFilm within 3 days and were in low number. Most of the parasites were gathered around the ParaFilm edges. In the following days the numbers of attached parasites did not rise and remained low. Based on microscopic examination the non-melted form of n-octacosane was better than the melted one. The non-melted gave a good view and the parasites actually attached to it more than the melted one.

4.1.6 Paraffin wax (candle wax)

L. mexicana promastigotes were used to test the parasites attachment in vitro to candle wax. The parasites were cultured at different densities and the optimum starting density that was subsequently used was $1x10^8$/ml. About 3 ml of culture for each species was incubated with candle wax flakes. The cultures were monitored and examined by inverted microscope. The microscopic observations showed the parasites became attached to the wax within 2 days and they also could attach within one day if the starting density was higher than $1x10^8$/ml.

4.1.7 Polystyrene, Sepharose and Sephadex

Polystyrene, Sepharose and Sephadexare are inert chemical materials available as small beads that were tested as possible attachment substrates for promastigotes. Polystyrene is characterized by its clarity and glass-like appearance. It is manufactured by free-radical polymerization of phenylethene (styrene). It can be used as an electrical and thermal insulator (Daintith, 2008).The gel-filtration medium Sepharose, which is based on cross-linked agarose is a widely used affinity matrix that is activated with epichlorohydrin allowing various ligands to be coupled (Cammack *et al.*, 2008). Sephadex is a gel-filtration medium based on cross-linked dextran, which is also used for electrophoresis (Cammack *et al.*, 2008). These three materials have quite similar microscopic structure; they are spherical in shape with a smooth surface. The concept of using the beads was that the promastigotes may attach to the round surfaces in an organized way and provide a good picture of the attachment mechanism. Then they could be examined by electron microscopy to examine the flagella/molecules surface attachment site that was formed by the promastigotes. Another idea was that since the surface area is high, they can be used in suspension cultures and that may provide large quantities of attached promastigotes in a simple manner, which are easy to handle.

0.1 ml of beads were cultured with 3ml of promastigotes at an initial density of $1x10^8$/ml and then incubated at 25°C. The following day the cultures were examined under the microscope. The parasites did not attach to the spherical beads. They were swimming freely around the beads with no signs of attachment.

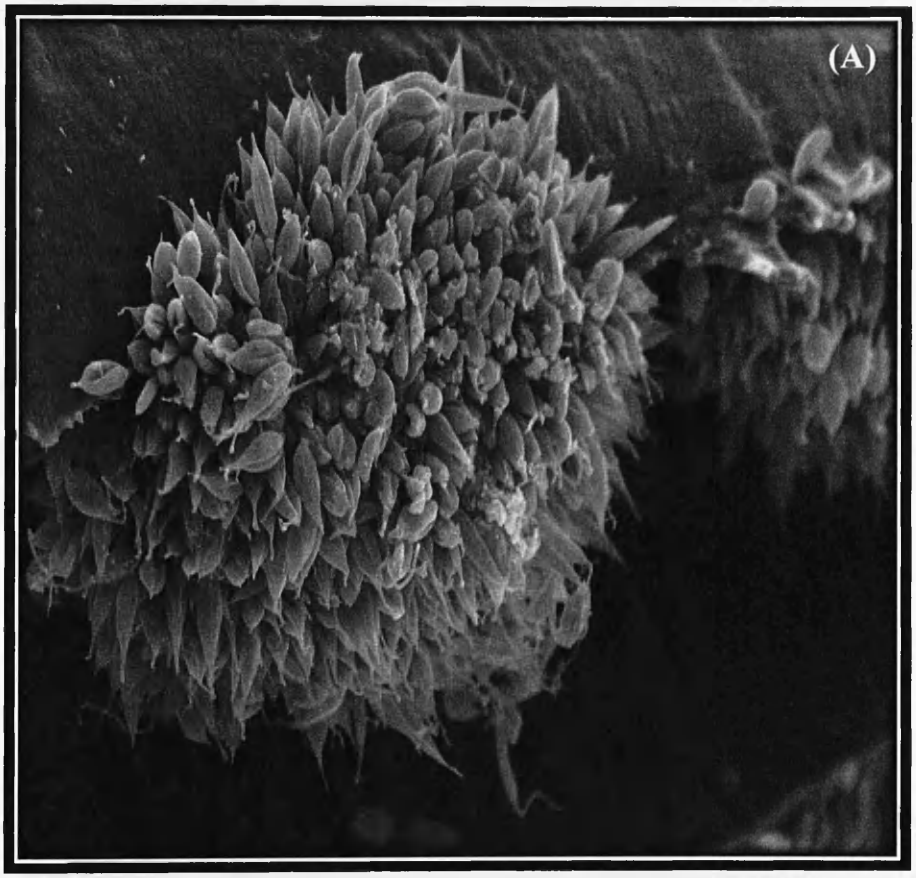
4.1.8 Ethylene-vinyl acetate copolymer (EVA)

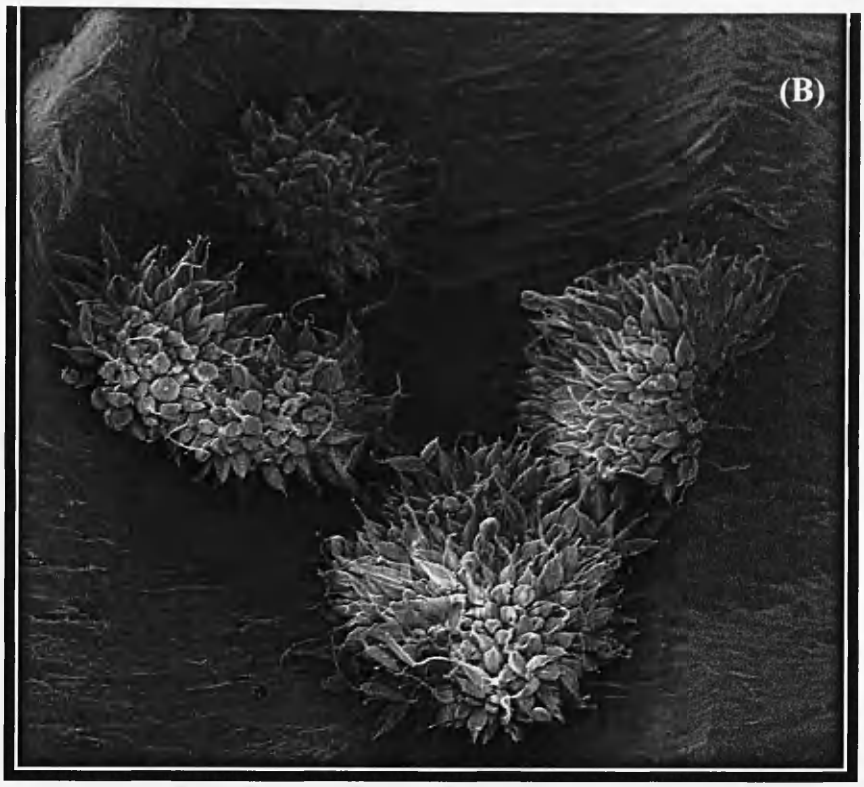
Initially EVA was used by forming long thin lines of EVA and letting them solidify. Then they were cut into very small pieces about 2mm x 1mm, washed with distilled water then sterilized with 70% ethanol and left to dry in sterile cabinet hood. The pieces were incubated with *L. mexicana* parasites in a 10 ml culture from an early sub-passage (SP3), starting density of 0.2 x 10^8 /ml and incubated at 26°C. The promastigotes attached to the EVA pieces in higher numbers than to any other of the materials that have been used in this project. The rate of the attachment was high within a few hours and increased on the following days, which was clear from examining the EVA under the inverted microscope at different times (Judgment based on microscopic examination because counting was difficult). The promastigotes attached permanently to the EVA pieces, remaining attached after vortexing the pieces vigorously for 10 sec. Through looking at them regularly by inverted microscope it was found that the populations attached to the pieces remained attached to the same site until they died. The rate of attachment was higher with a freshly transformed culture (0 sub-passage) and started to decrease at 8 sub-passages. Similar results were previously reported with Melinex (Wakid and Bates, 2000).

EVA pieces with attached promastigotes were examined by electron microscopy. The pictures showed that the parasites attached to the plastic and to each other and grouped in clusters (Fig.4.3).Attached parasites have round bodies and short flagella. High magnification pictures showed the parasites attached to the EVA by expanded tips from their flagella indicating the presence of hemidesmosomes (Fig. 4.3)

Transmission electron micrographs were prepared to examine the flagellum plaque that forms when the parasites attach to a surface, but unfortunately satisfactory

pictures could not be taken of parasites attached to EVA despite several attempts and modifications of the protocol. The composition of the EVA did not appear to be compatible with fixation and embedding process in resin required for transmission electron-microscopy (for more pictures see the Appendix 6.1)




(B)


(C)

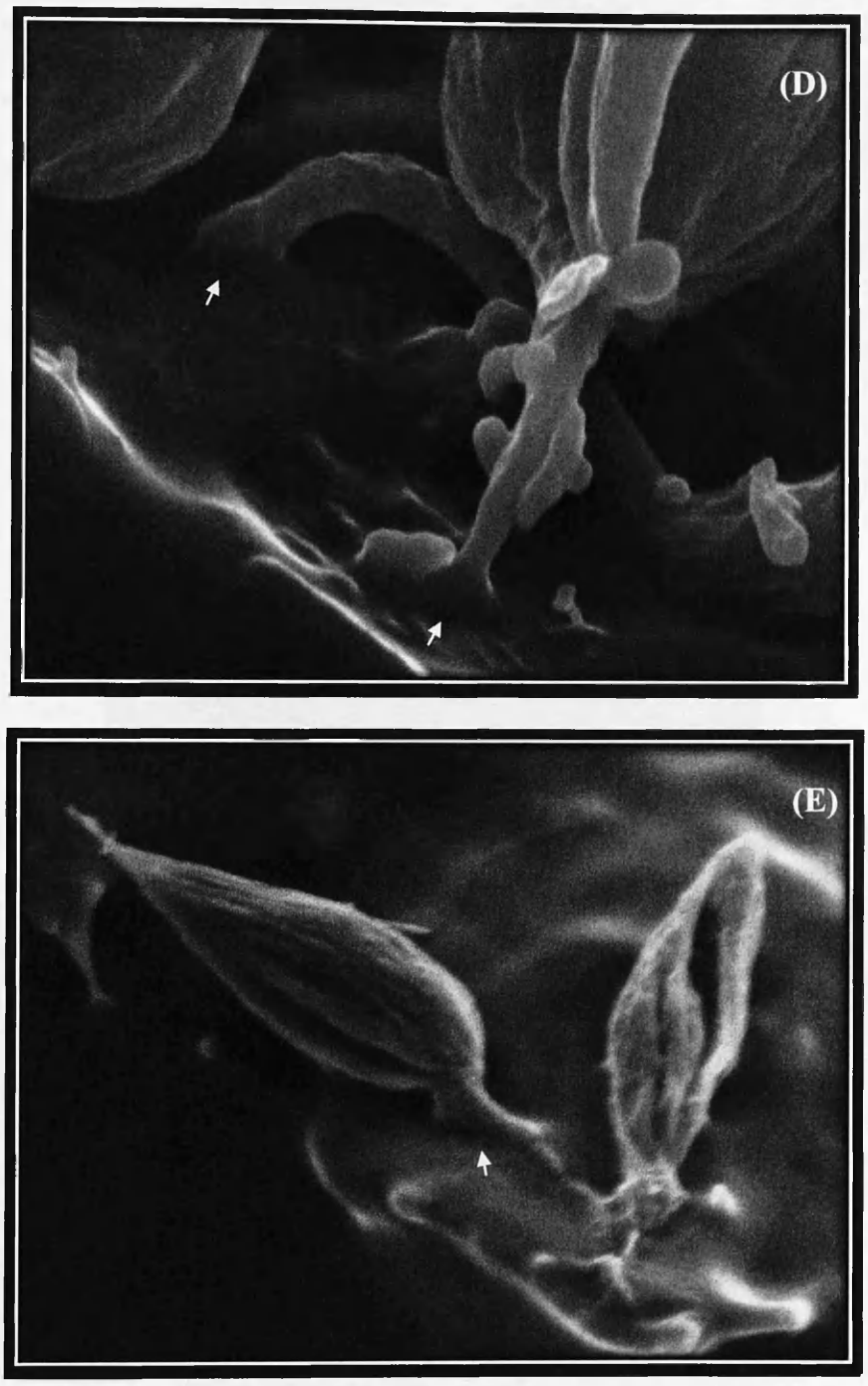


Figure 4.3 Scanning electron microscopy photos are showing attached in cultures (**A** and **B**) Parasites appeared with rounded bodies and short flagella attach to the EVA surface by forming hemidesmosome foot like structure (**C**, **D** and **E**) **(A)** HV=10KV, Mag=250X, Bar= 100µm **(B)** HV=3KV, Mag=500X, Bar= 50µm, **(C)** HV=10KV, Mag=4500X, Bar= 5µm, **(D)** HV=10KV, Mag=20000X, Bar= 1µm, **(E)** HV=6KV, Mag=5000X, Bar= 5µm

EVA pieces with attached promastigotes were also examined by confocal microscopy. The pictures showed that the parasites attached to the EVA surface grouped in clusters (Fig. 4.4).

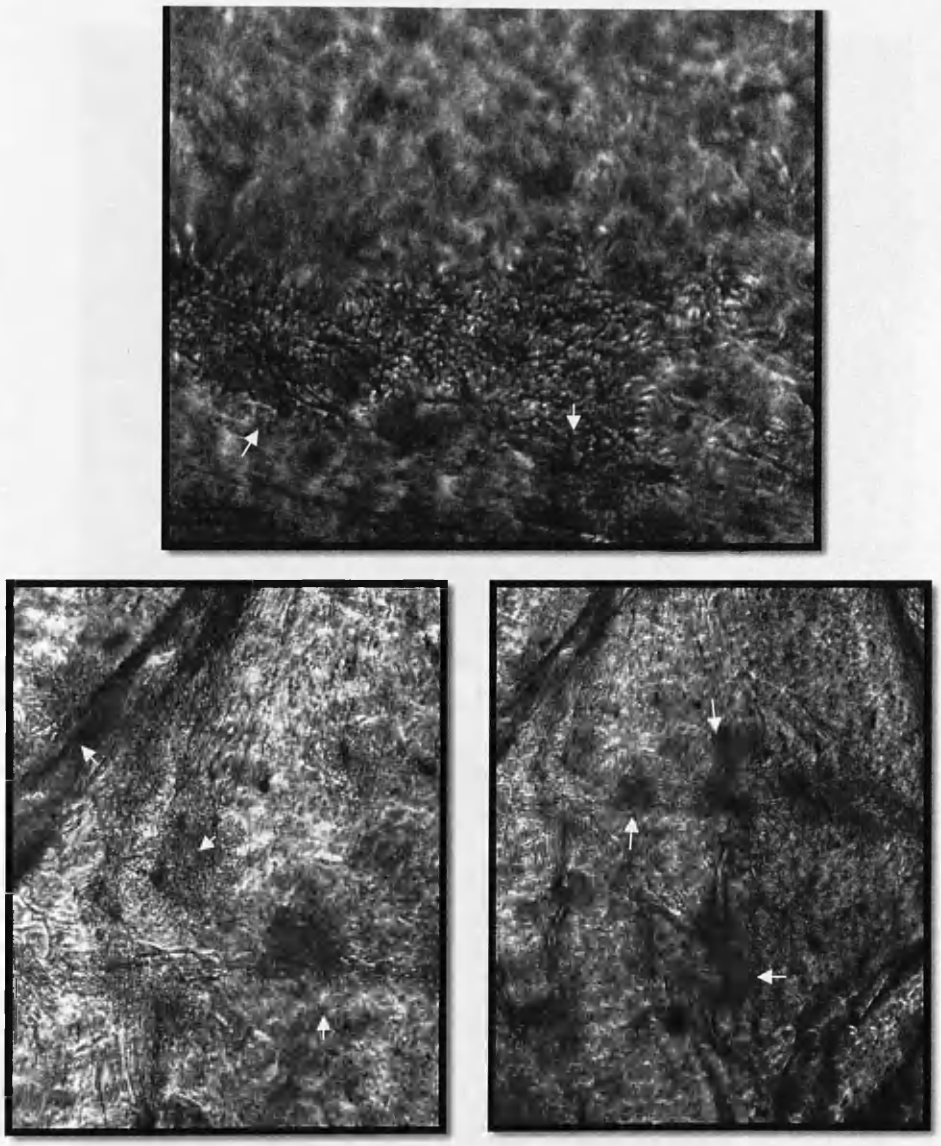


Figure 4.4 Confocal microscopic pictures showing *L. mexicana* promastigotes attached to EVA surface in clustered forms (arrowheads).

Attached parasites of *L. mexicana* to EVA were separated by xylene. Pellet was collected and smears prepared, fixed and stained by Giemsa'a stain (Fig. 4.5) showed parasites with rounded bodies and short flagella

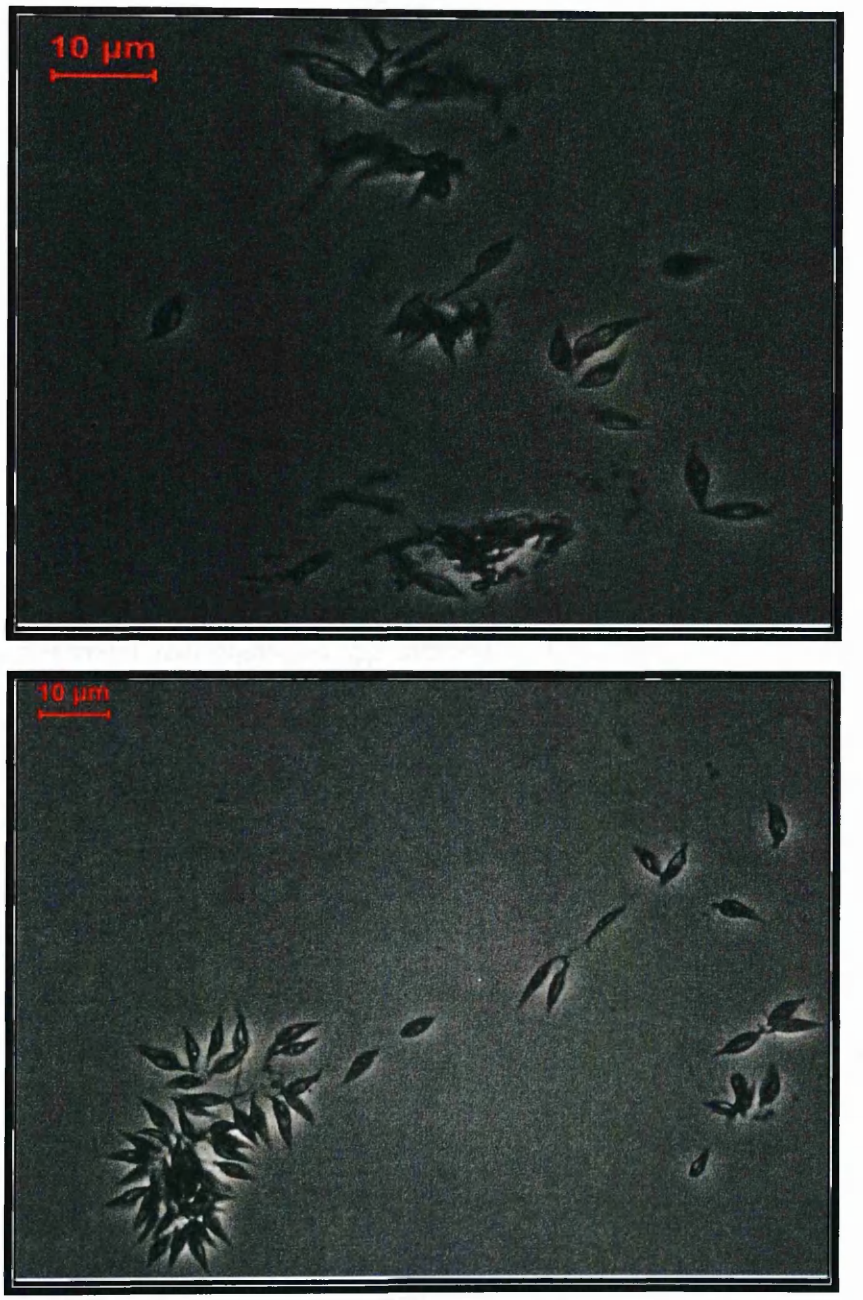


Figure 4.5. Gaima's satin of *L. mexicana* promastigotes after separated from EVA surface by xylene showing rounded bodies with sort flagella.

4.2 Biochemical characterization

Promastigotes attached to various materials and free promastigotes were extracted in MME buffer and SDS-PAGE buffer to search for proteins associated with attached forms.

4.2.1 Polycarbonate membrane filter papers

Polycarbonate filter papers were cultured with *L. mexicana* and *L. major* promastigotes. Two types of the filters surface were used: hydrophilic and hydrophobic (1.0μm x 47 mm). The cultures were started at several different densities and the best starting density was found to be $1x10^8$/ml with about 5 ml of the promastigote culture. The experiment was repeated 5 times. Filters stained with sliver stain (see below) and with coomassie blue stain (see Appendix)

As the attachment was higher on the hydrophilic filters further experiments were carried out using this type. The results showed *L. mexicana* parasites had an optimal attachment rate on the third day from initiation of cultures, and after three days attachment was reduced. Filters were extracted with MME buffer to remove soluble proteins, then the filters were extracted with SDS-PAGE sample buffer, this fraction should contain cytoskeletal and hemidesmosomal components. Protein bands of various masses were detected by SDS-PAGE, estimated as 24.4 kDa, 37-38 kDa, 44.5 kDa 47.7 kDa and 49kDa (Fig. 4.6, Table 4.3). The masses of *L. major* proteins masses identified using the same protocol are shown in Figure 4.7 and Table 4.4.

The filter papers provided a quick method to generate attached promastigotes of *Leishmania* in vitro considering the short time that the parasites took to attach to the filters. Their reliability and ease of handling make them good materials. However, the

results obtained from filters may potentially be false positive. The filters may have adsorbed culture media components from the serum. Besides, there is uncertainty about the parasites being attached to the filter by the desired mechanism, as this was not clear in the electron microscopy photos.

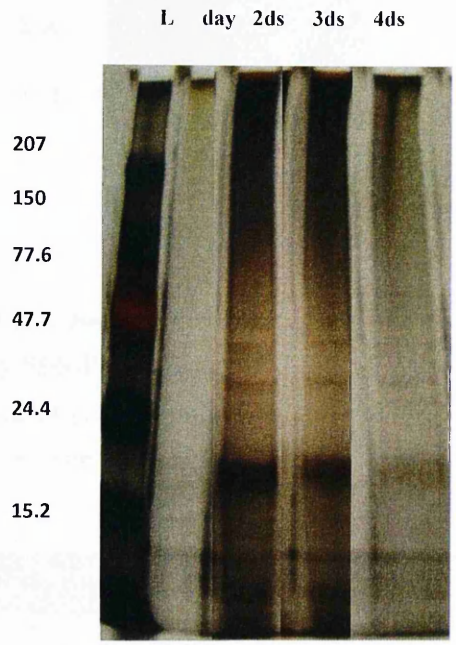


Figure 4.6 Silver staining of *L. mexicana* promastigote proteins collected at different days from hydrophilic filter papers separated by SDS-PAGE. The loaded volume of sample was 10µg per lane. **L** is the ladder of molecular mass standards. (ds = days).

2 days	3 days	4 days
24.4	24.4	24.4
37~38	37~38	37~38
44.5	44.5	44.5
49	47.7	47.7

Table 4.3 The molecular masses of *L. mexicana* promastigote proteins collected at different days from hydrophilic filter papers.

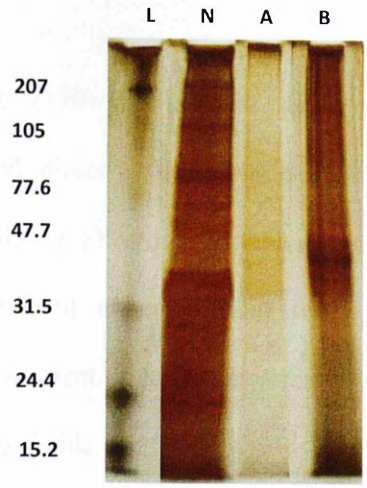


Figure 4.7 Silver staining of *L. major* promastigotes proteins extracted from hydrophilic filter papers separated by SDS-PAGE. Loaded volume of sample was 10μg per lane. **L** is the ladder. **(N)** Normal *L. major* promastigotes. **(A)** Extraction with MME. **(B)** Extraction by direct addition of SDS-PAGE sample buffer to the filters.

Normal Promastigotes	Proteins extracted by added directly sample buffer	Proteins extracted by MEE
18.5	23~24	27.8
26.8	24.4	34.5
28.8	26.4	34.8
30.5	27.5	39.9
33~34	30.9	40.9
35~36	33~34	75~76
36.9	34.2	100.5
40.1	36.5	-
43~44	39.9	-
46.9	41~42	-
59.9	42.1	-
77.6	44.5	-
88.9	72.1	-
110	75~76	-
165~166	92.1	-
200	137~138	-
207	155~156	-

Table 4.4 Molecular masses of *L. major* proteins from hydrophilic filter papers.

4.2.2 Chitin

Chitin powder (Sigma C-7170) was used in culture with *Leishmania* in *vitro*. The chitin powder was used directly following sterilization with 70% ethanol then prepared in 1M NaCl solution (Yoshimi Shibata *et al.*, 1997), 1 g of chitin in 100 ml NaCl. This chitin was used either without further treatment (un-sonicated) or following sonication at different powers, times and volumes (as described in Chapter 3; Materials and Methods, Table 3.1).

1x 10^8 /ml of *L. major* parasites were cultured with the various chitin preparations in 25 cm^2 culture flasks and incubated at 25°C for a week. The chitin particles were subjected to sequential extraction in MME buffer and SDS-PAGE sample buffer to extract the proteins and run the gels. From previous work MME buffer containing the non-ionic detergent Triton X-100 is predicted to extract soluble proteins from attached promastigotes leaving cytoskeletons and hemidesmosomes still attached, which would then be extracted into sample buffer by the ionic detergent SDS. In the MME preparations a large smear of proteins was revealed with a few discrete proteins bands appearing mostly in the chitin preparation that was sonicated for 10 min at 25% of full power (Fig. 4.8). The molecular masses of those bands were estimated as 27-28 kDa, 33-34 kDa and 42.2 kDa (Table 4.5). The smear presumably contains various promastigote proteins and probably also components of serum from the culture medium adsorbed onto the surface. In the other MME preparations the bands were faint with only one band of 33-34 kDa or no bands (Figure 4.8, Table 4.5).

The sample buffer treated chitin preparations were loaded on the SDS-PAGE gel and the proteins bands appeared more clearly and intensely than those extracted with MME buffer.

The protein molecular masses of these were estimated and evaluated. The proteins of the un-sonicated chitin preparations showed more intense bands than the other preparations. The molecular masses of these bands are summarised in Table 4.5. The 5 min and the 15 min sonicated chitin particles showed bands similar to the unsonicated chitin particles but lower in their intensity. The chitin solution that was sonicated for 5min had the largest number of proteins of molecular masses 27-28 kDa, 28.9 kDa, 31.5 kDa, 36.9 kDa, 45-46 kDa, 92.1kDa and 143-144 kDa. The 15 min sonicated chitin preparation showed several bands (Table 4.5) whereas the 10 min sonicated preparation showed only two weak bands of 92.1 kDa and 143-144 kDa. *L. major* promastigotes have a range of proteins with molecular masses from 18 to 207 kDa (Table 4.5). In comparison with the bands from the promastigotes attached to the chitin some were of similar mass: 28 kDa, 33-34 kDa, 30 kDa, 36.9 kDa, 43-44 kDa and 46.9 kDa. However, experiment with chitin was preformed only once and without further analysis we cannot be sure if these are the same proteins or this is simply a coincidence.

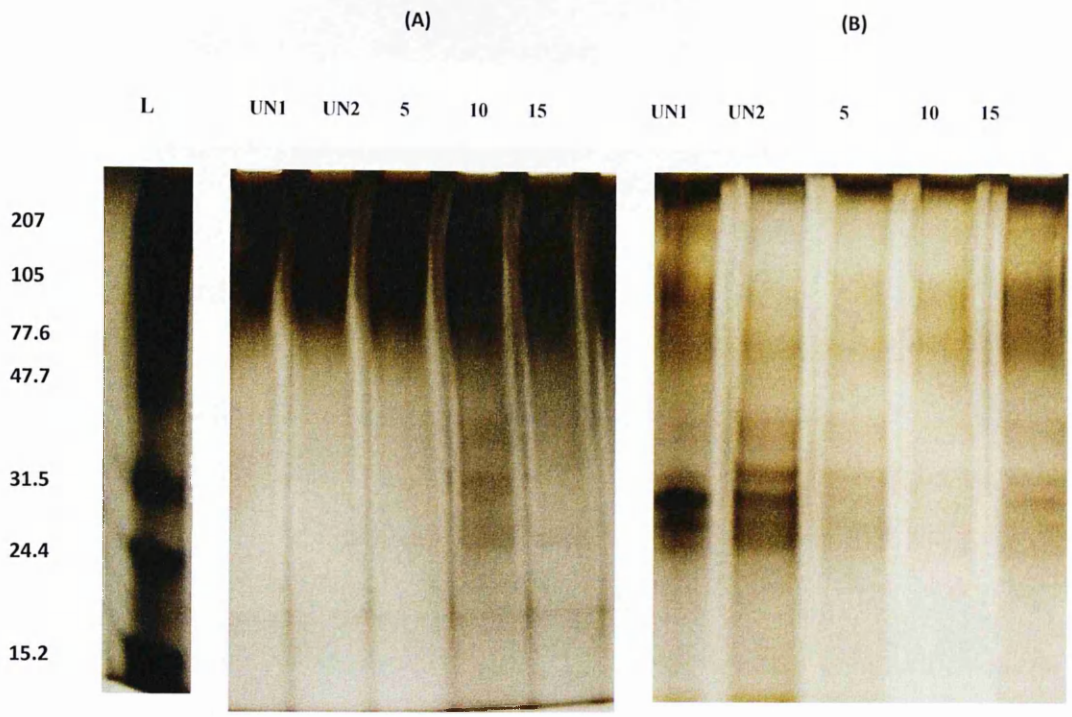


Figure 4.8 Silver staining of *L. major* promastigote proteins from chitin separated by SDS-PAGE. Loaded volume of sample was 10µg per lane. **(UN1)** proteins from un-sonicated chitin, lane **(UN2)** proteins from un-sonicated chitin, lane **(5)** insoluble proteins from 5 min sonicated chitin, lane **(10)** proteins from 10min-sonicated chitin and lane **(15)** proteins from 15 min-sonicated chitin. L is the ladder. **(A)** indicates the proteins extracted in MME buffer. **(B)** indicates the proteins extracted by subsequent addition of SDS-PAGE sample buffer.

(A)

Unsonicated chitin 1	Unsonicated chitin 2	sonicated chitin/5 min	sonicated chitin/10min	sonicated chitin/15min
			27~28	33~34
			33~34	-
			42.2	-
Nil	Nil	Nil		

(B)

Unsonicated chitin 1	Unsonicated chitin 2	sonicated chitin/ 5 min	sonicated chitin/10min	sonicated chitin/15min
26.9	27~28	27~28	27~28	26.1
28.5	28.9	28.9	91.2	30
30.9	30.9	31.5	143~144	32.9
36.1	36.5	36.9		37~38
90.2	44.2	45~46		46.5
134~144	87~88	92.1		92.1
	143~144	143~144		

Table 4.5. The molecular masses of proteins from chitin extractions. (A) Treated with MME, **(B)** treated with sample buffer.

4.2.3. Paraffin wax (candle wax)

Shavings of candle wax were prepared from household candles using a scalpel blade. The shavings were sterilized with 70% ethanol and left to dry in the culture cabinet hood. When they were dried, the shavings were cultured with 1 x 10^8/ml of *L. mexicana* and *L. tarentolae* parasites in a 25 cm^2 culture flask and incubated at 25°C for 2 weeks with monitoring for attachment. The experiment was performed once for each species. After collecting the wax from the cultures and in order to prepare it for the protein extraction process and loading on the gel, the wax was treated in two different ways. The first method was to incubate the wax with MME buffer for 30

min, then separating the wax from the buffer, adding the sample buffer to it, boiling for 5 min and loading onto the gel. The second method was to take the separated wax from the MEE buffer and incubate it with sample buffer for 1 hour at 37 °C, then collect the sample buffer without the wax and boil for 5 min before loading onto the gel. The proteins bands seen on the SDS-PAGE gel from the wax directly treated with sample buffer appeared more intense in both species (Fig.4.9). Molecular masses of protein bands from *L. mexicana* preparations were: 18.5 kDa, 22.2 kDa, 24.4 kDa, 30.9 kDa and 32.1 kDa (Table 4.6). From *L. tarentolae* the protein bands were estimated as:17-18 kDa, 21-22 kDa, 24.4 kDa, 32.1 kDa, 33-34 kDA, 36.5 kDa, 43.1 kDa, 52.2 kDa, 54.5 kDa, 61-62 kDa and 87-88 kDa (Table 4.7).

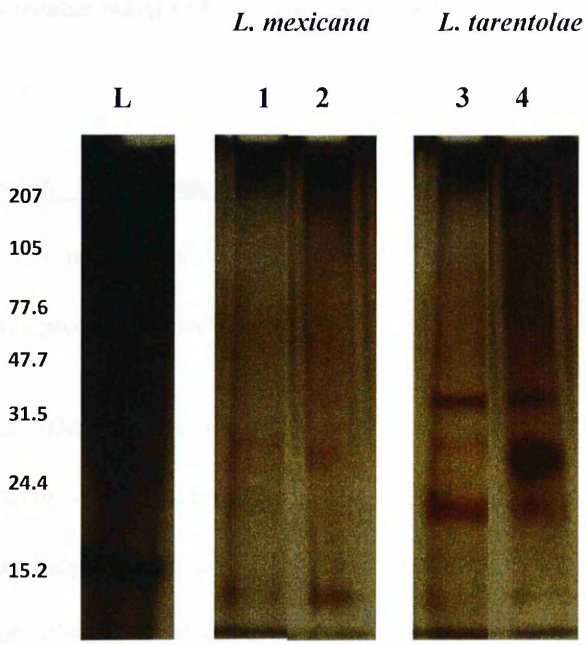


Figure 4.9 Silver staining of promastigote proteins from candle wax separated by SDS-PAGE. Loaded volume of sample was 10µg per lane. **(1)** Proteins of *L. mexicana* from wax treated by adding sample buffer at 37C°, lane **(2)** proteins of *L. mexicana* from wax treated by MME, lane **(3)** proteins of *L. tarentolae* from wax treated with sample buffer at 37C°, lane **(4)** proteins of *L. tarentolae* from wax treated with MME. **L** is the ladder.

Proteins extracted by direct addition of sample buffer	Proteins extracted by direct add of sample buffer at 37°C	Proteins extracted by MME
30.9	24.4	22.2
-	18.5	32.1

Table 4.6 The proteins molecular masses of *L. mexicana* from candle wax.

Proteins extracted by direct addition of sample buffer	Proteins extracted by direct addition of sample buffer at 37°C	Proteins extracted by MME
33~34	24.4	17~18
52.2	32.1	21~22
-	43.1	32.1
-	61~62	36.5
-	87~88	54.5

Table 4.7 The proteins molecular masses of *L. tarentolae* from candle wax.

4.2.4. Ethylene-vinyl acetate co-polymer (EVA)

Pieces of 2mm x2mm EVA were cultured in 25^{0}C flasks with a starting density of 1 X 10 ^{8}/ml of *L. mexicana* promastigotes for one week.

Pieces were treated with MME buffer, or boiled with sample buffer for 5min, or incubated with sample buffer for 1 hour at 37 °C then boiled in sample buffer for 5 min. The samples were then loaded on SDS-PAGE gels. The experiment was performed 6 times. The proteins bands from all preparations appearing on the gel were few and their molecular masses estimated as 24.4 kDa, 31 kDa and 36.5 kDa. The bands from EVA directly treated with sample buffer were more intense than those treated with MME buffer (Figure 4.10, Table 4.8). The results obtained were from a few pieces per preparation and we assume that by increasing the numbers of EVA pieces we will get more biological materials.

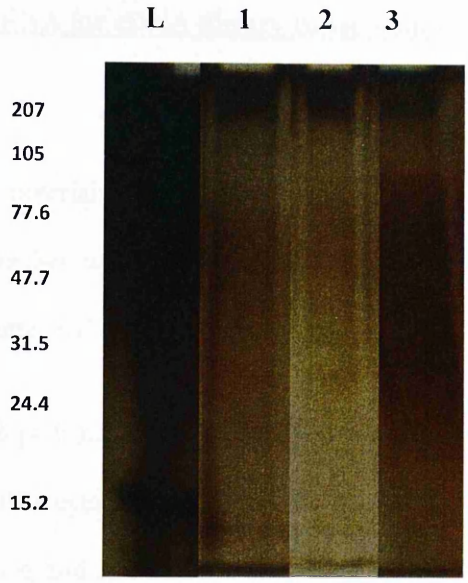


Figure 4.10 Silver staining of *L. mexicana* promastigote proteins extracted from EVA pieces separated by SDS-PAGE. Loaded quantity of sample was 10µg per lane. **L** is the ladder. **(1)** Proteins bands from pieces treated by adding sample buffer at 37C°. **(2)** Proteins bands from pieces treated with MME. **(3)** Proteins bands from pieces treated by adding sample buffer.

Proteins extracted by direct sample buffer extraction at 37 ℃	Proteins extracted by direct addition of sample buffer	Proteins extracted from MEE
24.4	31	24
-	36.5	31.5

Table 4.8 Protein molecular masses of *L. mexicana* from EVA pieces.

4.3 Molecular Approaches

4.3.1 Generating total RNA for cDNA library construction of attached promastigotes

From testing different materials the best one that provides highly pure and good quantity of attached parasites was Ethylene-Vinyl Acetate Co-polymer (EVA). This was chosen to generate attached promastigotes to use in molecular approaches.

Use of EVA was developed according to experimental requirements. When testing materials just a few small pieces were used, about 1mm x 1mm. Preparation of small EVA was time consuming and not easy to deal with, but it was acceptable in these experiments because they were only testing the parasites ability to attach and only a few pieces were required. Later the size of pieces used in biochemical analysis and SDS-PAGE were enlarged to 2mm x 2mm and more pieces with a scratched surface were produced to enhance the attachment, which consumed more time and required a high amount of handling. For generating total RNA to use in molecular experiments we maximized the size to be between 5mm x 5mm and 1cmx 1cm. This size was the best and easiest to deal with during culturing, washing and lysis of the cells.

L. mexicana promastigote cultures were initiated at 5 x 10^5/ml and cultured in 75 cm^2 cell culture flasks in 60 ml of complete Medium 199 at 25°C. When the cultures reached stationary phase (4-6 x 10^7/ml),3ml of parasite culture was incubated per well in 12 or 24 well plates with EVA then lysed using Trizol reagent (section 3.7).

4.3.2 Total RNA extraction from EVA plates

Total RNA was extracted as described in 3.7.2 from four batches of EVA pieces in 37 plates (12 wells) producing RNA at a concentration of 1.024µg/µl (Table 4.9). The RNA was loaded on to a 1% agarose gel electrophoresis to check RNA integrity, which was in good condition (Fig.4.11). The experiment was repeated and the Trizol extract sent to the Life Technologies (Invitrogen) company to construct the cDNA library from attached parasites of *L. mexicana*.

Extractions	No. of Plates	Conc. µg/µl	A260/A280
1	10	0.191	1.9
2	11	0.171	2
3	10	0.491	1.9
4	6	0.171	1.9

Table 4.9 The yield of RNA from each batch extracted from *L. mexicana* parasites attached to EVA.

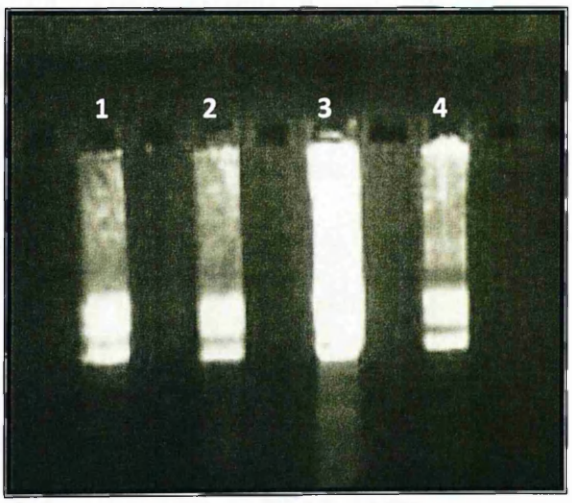


Figure 4.11 1% Agarosegel showing intact RNA samples extracted from 37 plates of attached *L. mexicana* promastigotes to EVA. Numbers represent the different extractions. 2ug of total RNA was loaded per lane.

4.3.3 cDNA library construction

The library was made by the company and supplied in 80% S.O.C. medium, 20% glycerol in the pENTR™ 222 vector and maintained in the *E. coli* DH10B™ T1 Phage Resistant strain. It contains 3.6 x10^7 cfu of primary colonies and average insert size of 1.6 kb. About 87% of the library contains inserts.

4.3.4 cDNA library screening

4.3.4.1 RT-PCR analysis of randomly picked clones

The library was tested by sequencing 48 randomly selected colonies from the cDNA library plated on agar plates. Plasmid DNA of the 48 colonies was sequenced in our lab using a Genome Lab™Dye Terminator Cycle Sequencing (DTSC) Quick Start Kit. Sequence data was analysed using the *Chromas Lite 2.01*programme and *TritrypDB* version 6.0, where information about the genes was provided (see Appendix 6.4 for more details).

Genes results were based on the NCBI database and matched genes from *L. mexicana* MHOM/GT/2001/U1103. The *L. mexicana* U1103 strain was isolated from the ear lesion of 30-year old male patient in Guatemala (Rogers *et al.*, 2011). 24 of these genes according to the database information from both NCBI and Tritryp DB are characterized proteins and found to be expressed by different species of trypanosomatids and *Leishmania* as well as by other organisms as documented in OrthoMCL database version 5.0 (Table 4.10).

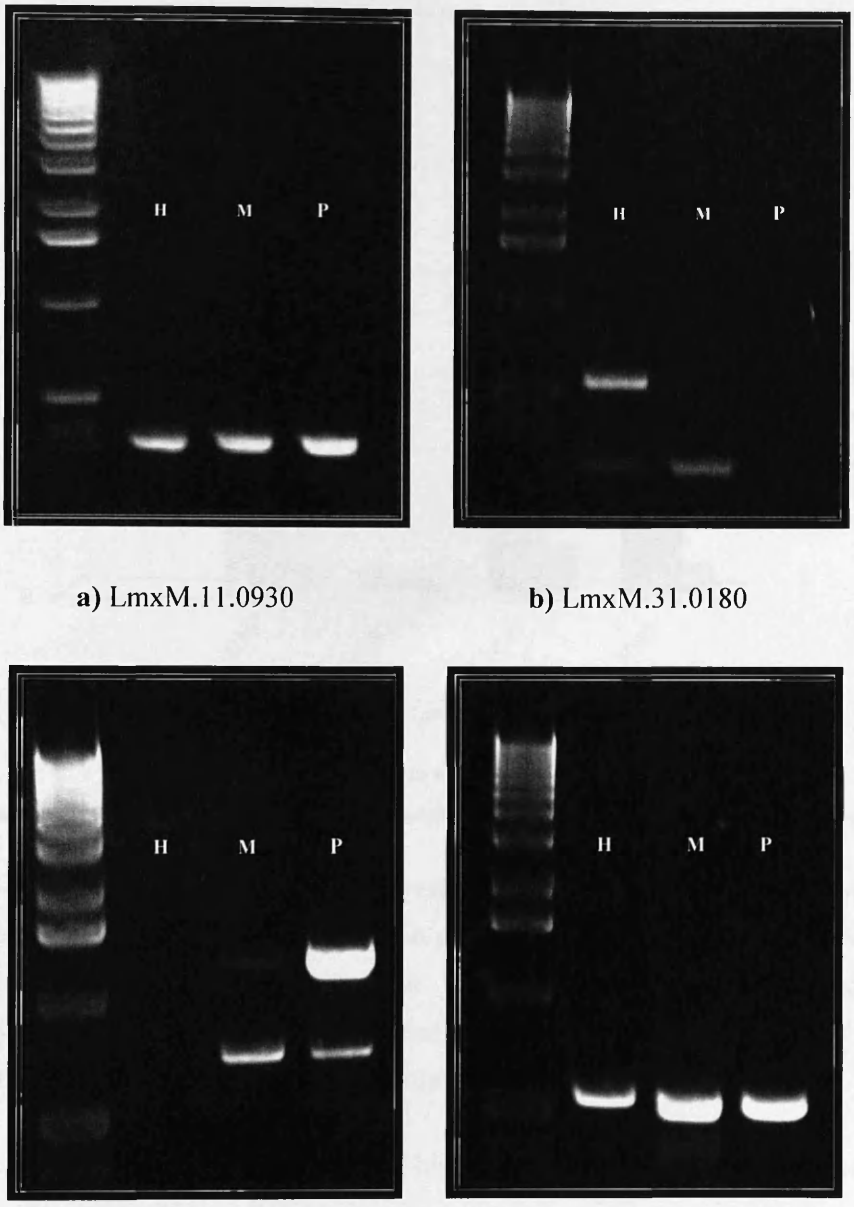
Gene ID	Gene Description	Feature	Gene copy
LmxM.30.0450	amastin- putative	*Leishmania only*	Single
LmxM.36.3990	hs1vu complex proteolytic subunit-like,hs1vu complex proteolytic subunit-like, threonine peptidase, Clan T(1), family T1B	*Trypanosomatids only*	Single
LmxM.29.3440	DNA ligase I, putative	*Trypanosomatids only*	Single
LmxM.25.0910	cyclophilin a	*Trypanosomatids only*	Single
LmxM.32.0792	beta tubulin	*Trypanosomatids only*	Single
LmxM.22.0420	40S ribosomal protein S15, putative	*Trypanosomatids only*	Single
LmxM.03.0440	60S acidic ribosomal protein P2, putative	*Trypanosomatids only*	Single
LmxM.13.0280	alpha tubulin	*Trypanosomatids only*	Single
LmxM.34.3780	60S ribosomal protein L27A/L29, putative	*Trypanosomatids only*	Single
LmxM.13.0560	60S ribosomal protein L18, putative	*Trypanosomatids only*	Single
LmxM.33.2900	ribosomal protein L3, putative	*Trypanosomatids only*	Single
LmxM.14.1270	ubiquitin/ribosomal protein S27a, putative	*Trypanosomatids only*	Single
LmxM.03.0570	Quinone oxidoreductase, putative	*Trypanosomatids only*	Single
LmxM.28.2560	40S ribosomal protein S17, putative	*Leishmania only*	Single
LmxM.34.3780	60S ribosomal protein L23, putative	*Trypanosomatids only*	Single
LmxM.34.0600	60S ribosomal protein L18a, putative	*Trypanosomatids only*	Single
LmxM.13.0390	alpha tubulin	*Trypanosomatids only*	Single
LmxM.33.2870	ribosomal protein L3, putative	*Trypanosomatids only*	Single
LmxM.26.0170	60S ribosomal protein L7, putative	*Trypanosomatids only*	Single
LmxM.28.2740a	activated protein kinase C reseptor	*Trypanosomatids only*	Single
LmxM.08_29.1740	histone H2A, putative	*Trypanosomatids only*	Single
LmxM.36.1430	translation elongation factor 1-beta, putative	*Trypanosomatids only*	Single
LmxM.09.1220	AAA family ATPase	*Trypanosomatids only*	Single

Table 4.10 Summary of identified genes from the random colonies selected from cDNA library.

In addition 24 hypothetical proteins of unknown functions were revealed by sequencing and database searching, some of these were *Leishmania*-specific other found in other trypanosomatids as showed in following table (Table 4.11).

Gene ID	Description	Feature	Gene Copy
LmxM.11.0930	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.23.1020	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.36.3620	hypothetical protein, unknown function	*Trypanosomatids only*	Single
LmxM.30.2270	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.36.1430	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.05.0450	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.31.2500	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.36.5060	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.08.0410	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.34.3180	hypothetical protein, conserved	*Leishmania only*	Single
LmxM.36.2450	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.29.3025	hypothetical protein, conserved	*Leishmania only*	Single
LmxM.01.0620	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.36.5850	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.17.0810	Hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.09.1505	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.17.0870	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.27.0650	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.36.3780	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.03.0640	hypothetical protein, conserved	*Trypanosomatids only*	Single
LmxM.27.1340	hypothetical protein, unknown function	*Trypanosomatids only*	Single
LmxM.09.1210	hypothetical protein, unknown function	*Trypanosomatids only*	Single
LmxM.31.0180	hypothetical protein, conserved	*Leishmania only*	Single
LmxM.31.1090	hypothetical protein, conserved	*Leishmania only*	Single

None of the identified genes seemed likely to be components of hemidesmosomes, having different established functions within the cell. However, it was considered possible that one or more of the hypothetical proteins might be hemidesmosomal candidates. Although these are only randomly selected clones, this possibility was based on the assumption that such genes should be highly expressed (since the hemidesmosome is a structural component) and also should be conserved in other trypanosomatids (since all make hemidesmosomes). Therefore the expression of these hypothetical proteins was investigated further by comparing three different stages of *Leishmania mexicana*; attached promastigotes; log-phase promastigotes; and metacyclic promastigotes, using the semi-quantitative RT-PCR technique. Primers were designed specifically for each gene and after RT-PCR, samples were loaded on 1% agarose gels and photos were taken. Band intensities were measured (see 3.10.3) to determine their relative expression normalized to a control gene. The RT-PCR results showed variety of gene expression patterns; some genes showed similar expression by the three stages; other genes were expressed mainly or highly by one of the three stages examined. Data analysis showed four genes that were highly expressed by metacyclic promastigotes comparing with the two other stages. These hypothetical proteins were LmxM.11.0930, LmxM.31.0180, LmxM.36.5060 and LmxM.36.2450 (Fig. 4.12, 4.11).



a) LmxM.11.0930 **b)** LmxM.31.0180

c) LmxM.36.5060 **d)** LmxM.36.2450

Figure 4.12 RT-PCR analysis of metacyclic upregulated genes.10 µl/lane of RT-PCR products from **(a)** LmxM.11.0930, **(b)** LmxM.31.0180, **(c)** LmxM.36.5060c and **(d)** LmxM.36.2450 were loaded onto 1% agarose gel, showing their expression by haptomonads **(H),** metacyclics **(M)** and log-phase promastigotes **(P).**

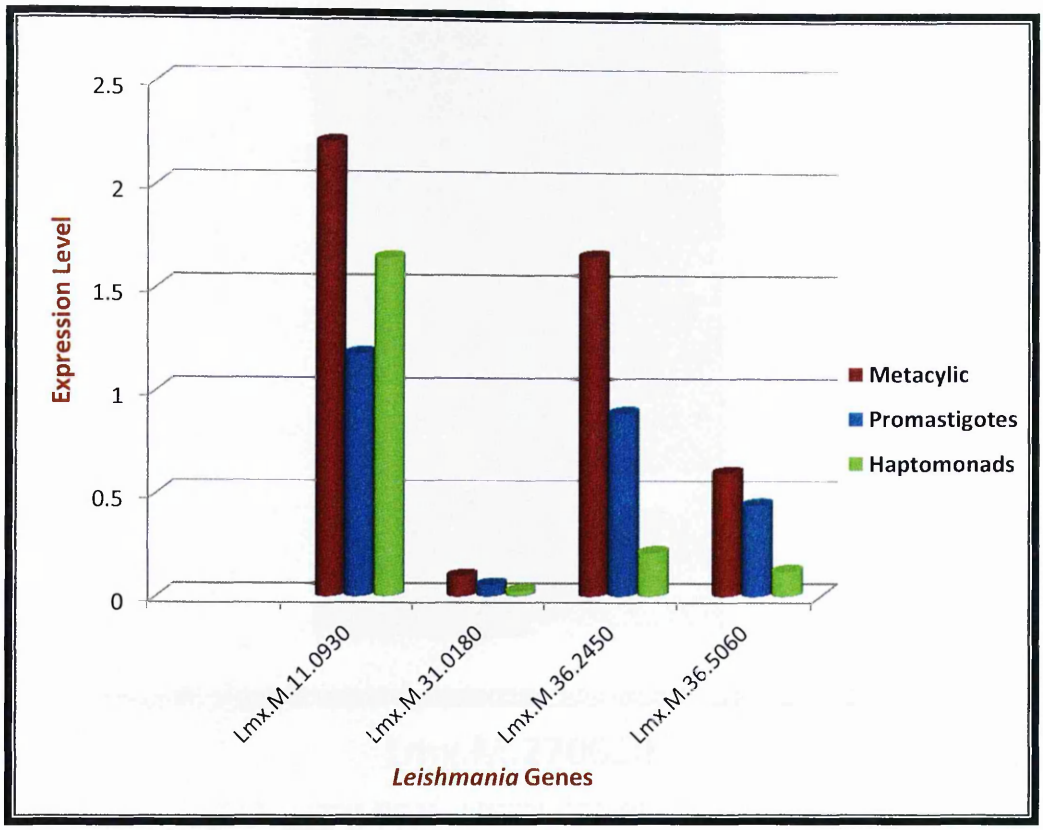
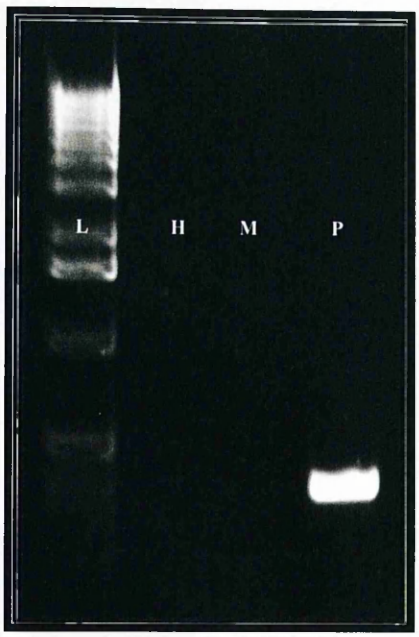


Figure 4.13 Quantitation of RT-PCR expressions of metacyclic upregulated genes. The graph represents levels of expression for the genes indicated comparing metacyclics with attached and log-phase promastigotes. The level of expression in each sample was individually normalized to that of the control gene ribosomal protein L10a to allow for between sample variation in RNA quantity or quality.

Several genes (11) were expressed at higher levels by log-phase promastigotes compared to the two other stages examined. LmxM.27.0650 gene was found to be expressed only by log-phase promastigotes (Fig. 4.14), while LmxM.01.0620, LmxM.36.3780, LmxM.31.2500, LmxM.30.2270, LmxM.08.0410, LmxM.31.1090, LmxM.36.3620, LmxM.36.5850 and LmxM.05.0450 were expressed by all stages but more highly in promastigotes (Fig. 4.15, 4.16). For LmxM.25.0920 only RNA samples for attached forms and log-phase promastigotes were available (Fig. 4.17, 4.18) but showed higher expression in log-phase promastigotes.



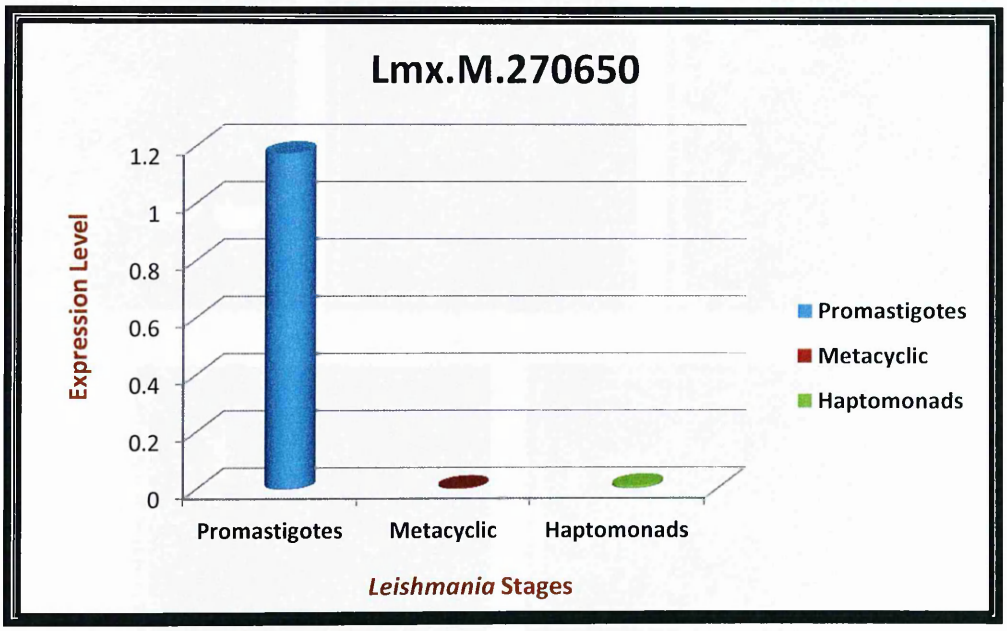


Figure 4.14 RT-PCR analysis of a putative log-phase promastigote specific gene. The gel and graph show that Lmx.M.270650 is only expressed by promastigotes and was absent in haptomonads and metacyclics. (H) haptomonads, (M) metacyclics, (P) log-phase promastigotes.

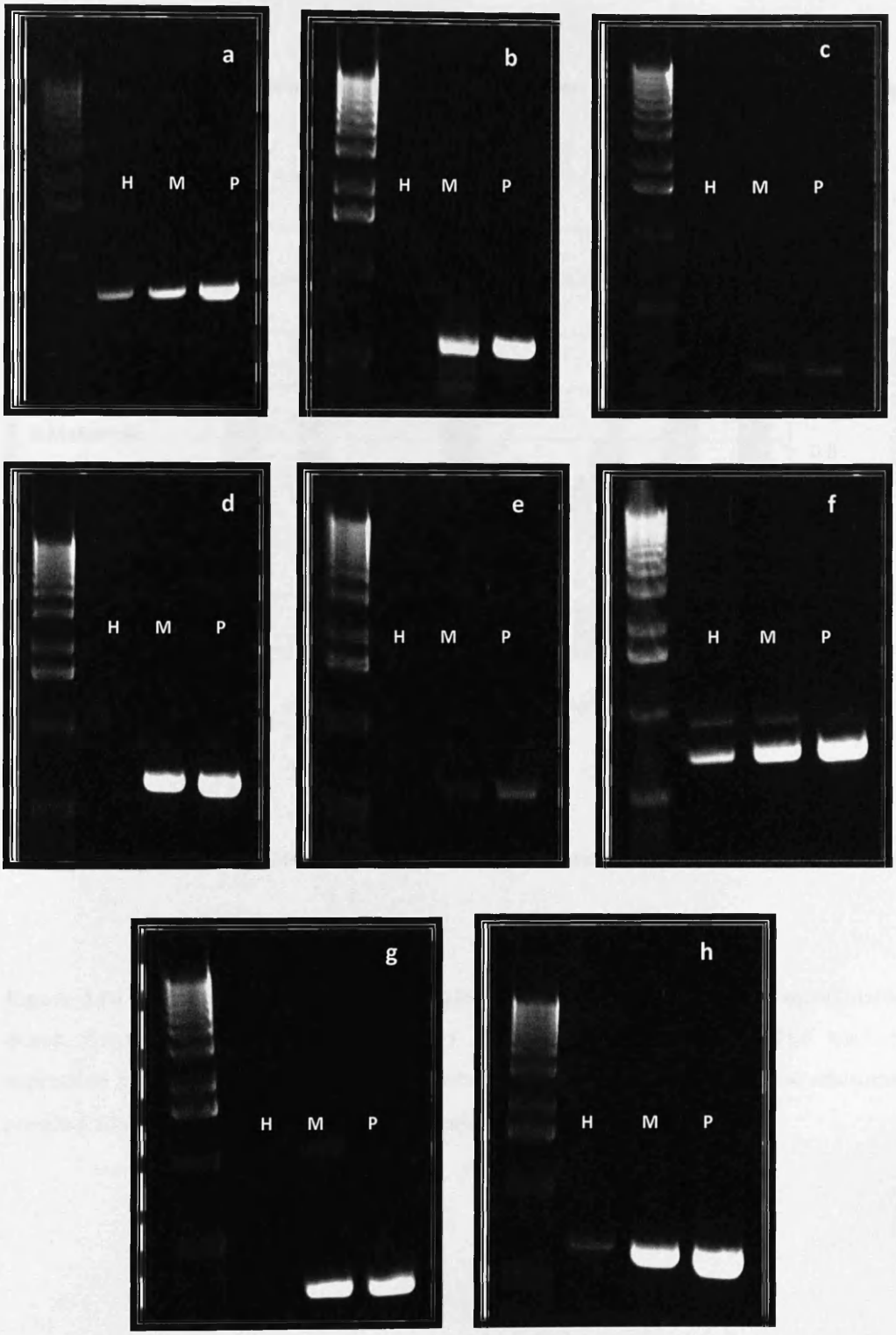


Figure 4.15 RT-PCR analysis of log-phase promastigote upregulated genes. Agarose gels showing RT-PCR bands of: a) LmxM.01.0620; b) LmxM.36.3780; c) LmxM.31.2500; d) LmxM.30.2270; e) LmxM.08.0410; f) LmxM.31.1090; g) LmxM.36.3620; and h) LmxM.36.5850. (H) haptomonads, (M) metacyclics, (P) log-phase promastigotes.

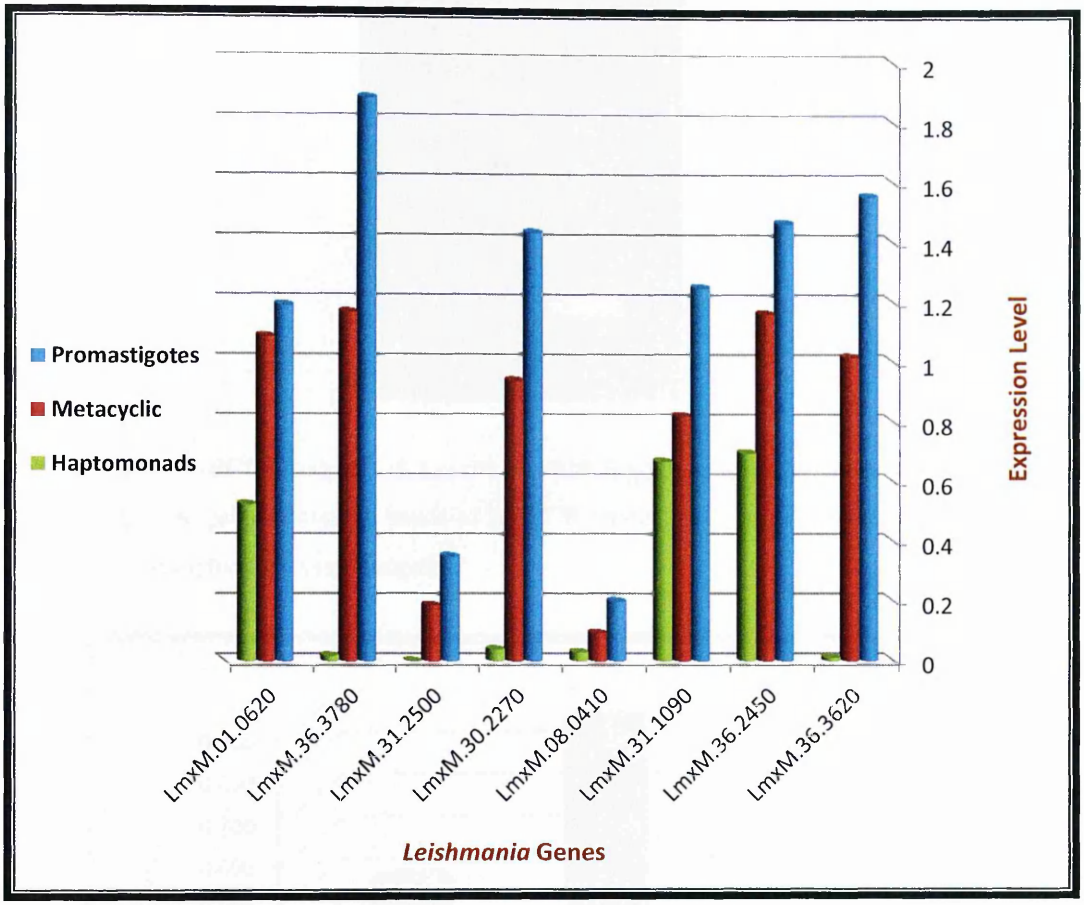


Figure 4.16 Quantitation of RT-PCR expressions of log-phase promastigote upregulated genes. Graph is showing bands that highly expressed by promastigotes. The level of expression in each sample was individually normalized to that of the control gene ribosomal protein L10a to allow for between sample variation in RNA quantity or quality.

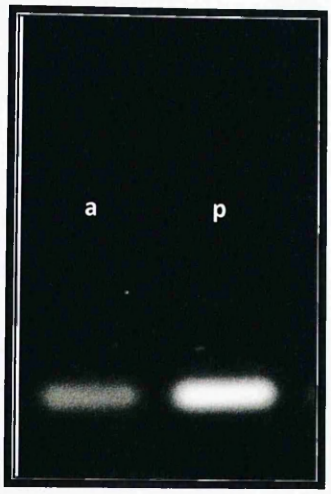


Figure 4.17 RT-PCR analysis of LmxM.25.0920 log-phase promastigote upregulated gene. 1% agarose gel showing the bands of RT-PCR products of comparing between attached form (a) and log-phase (p) promastigotes.

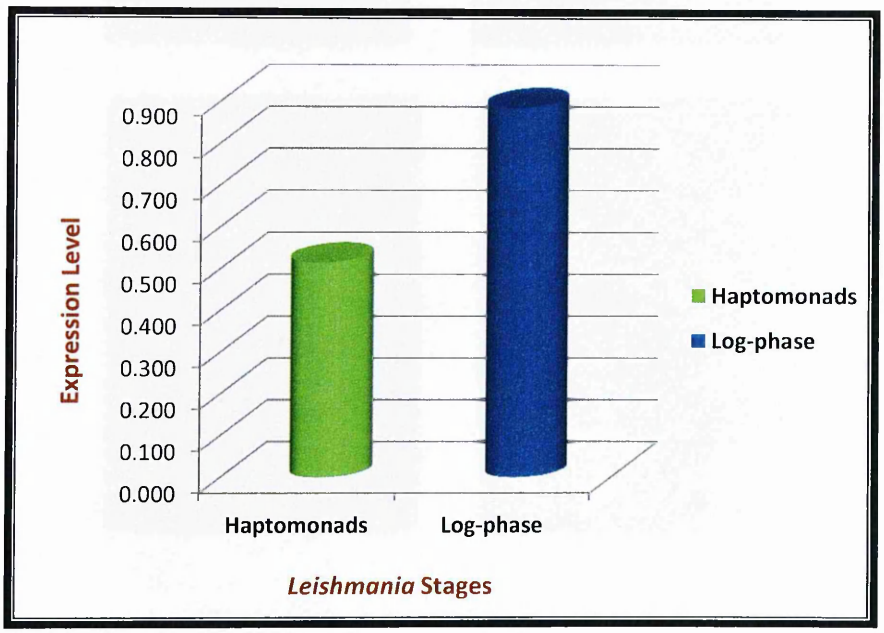


Figure 4.18 Quantitation of LmxM.25.0920 log-phase promastigote upregulated gene expression. The graph is showing the expression level of LmxM.25.0920 gene comparing haptomonads and log-phase promastigotes.

Four genes were found to be expressed at similar levels in attached forms, metacyclic promastigotes and log-phase promastigotes: LmxM.17.0870, LmxM.09.1505, LmxM.17.0810 and LmxM.05.0450 (Fig. 4.19, 4.20). The level of expression varied between the different stages (Fig. 4.20).

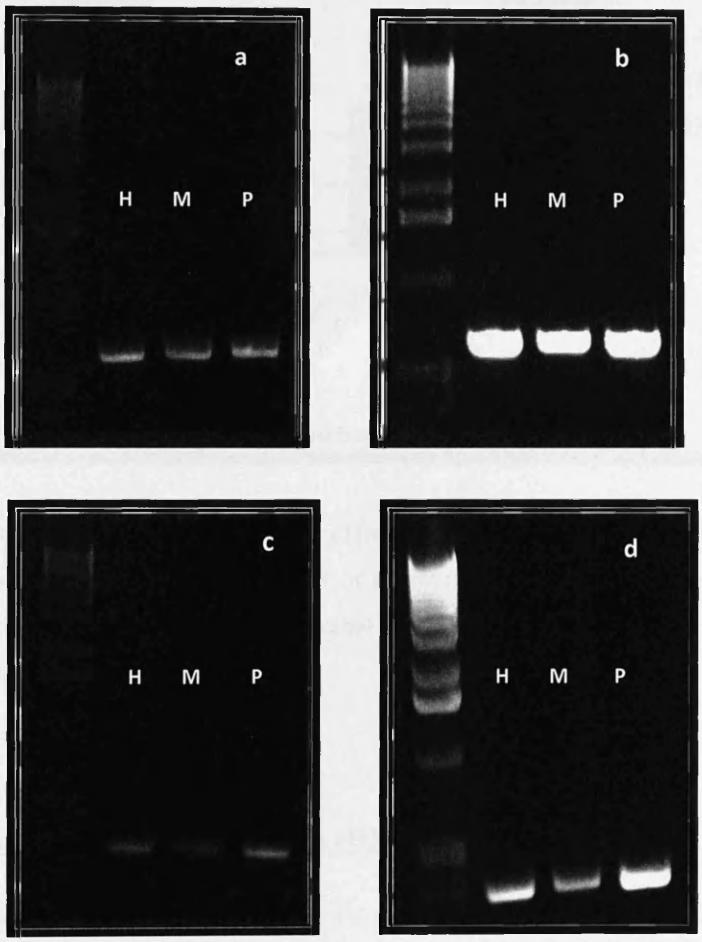


Figure 4.19 RT-PCR analysis of constitutively expressed genes. Agarose gels showing RT-PCR bands of: **a)** LmxM.17.0810; **b)** LmxM.17.0870; **c)** LmxM.09.1505; and **d)** LmxM.05.0450 **(H)** haptomonads, **(M)** metacyclics, **(P)** promastigotes.

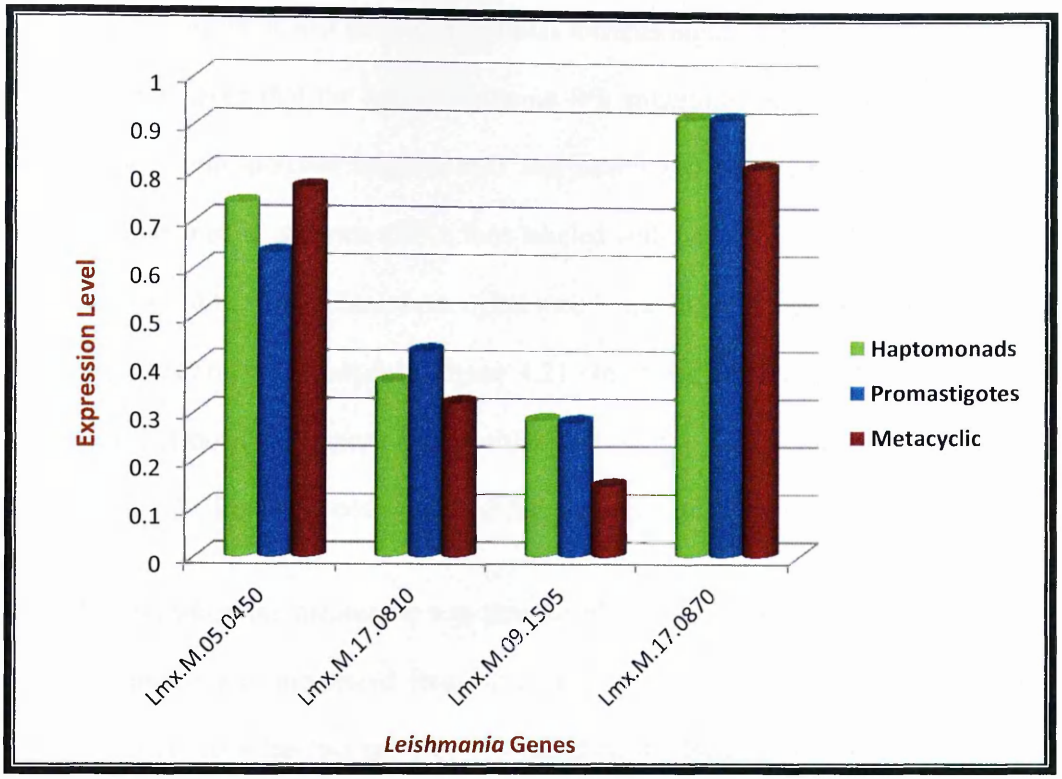


Figure 4.20 Quantitation of constitutively expressed genes. Graph showing expression of indicated genes in different stages. The level of expression in each sample was individually normalized to that of the control gene ribosomal protein L10a to allow for between sample variation in RNA quantity or quality.

4.3.4.2. Screening of cDNA library with cDNA probes

Analysis of randomly picked clones from the cDNA library by RT-PCR indicated that the library was high quality and revealed clones with varying patterns of expression, including some putative metacyclic-specific genes. However, none of those analysed showed haptomonad-specific expression that would be consistent with a hemidesmosomal protein gene. Therefore the cDNA library was screened further by spotting colony resuspensions on nylon membranes and using a DIG High Prime DNA Labeling and Detection Kit to generate and screen with cDNA probes. This technique enables the screening of hundreds of genes at the same time, is straight

forward and reliable. It will have a natural bias towards highly expressed genes, which was desirable given that the hemidesmosome is a structural component. Total RNA was isolated from attached haptomonads and unattached culture promastigotes and reverse transcribed to generate cDNA then labeled with DIG. A total of 1654 colonies were screened, 64 to 100 colonies per nylon membrane sheet. An example of a typical screening experiment is shown in Figure 4.21 (for more details see Appendix 6.6). Colony suspensions were spotted on positively charged nylon membrane and probed with total cDNA from attached forms and log-phase promastigotes.

After hybridization the membrane was developed and the intensity of the individual spotted samples was monitored frequently; 45 minute, 1 hour and overnight, and comparison between the two samples was based on the intensity of pairs of matched pairs of spots. As expected there was variation in spot intensity within a membrane, this reflecting the expected variation in mRNA levels from highly expressed genes (intense spots) to weakly expressed genes (weak or no signal), the latter being genes that only require small amounts of protein. A number of differentially expressed genes were identified, the spot being distinctly stronger in intensity with one probe or the other. 25 genes were genes identified as being more highly expressed in attached haptomonad promastigotes and 20 as being more highly expressed in unattached culture promastigotes. The corresponding colonies for each of these spots were grown, plasmid DNA isolated and sequenced to determine the identity of the corresponding cDNA clones and their genes. The results of the sequencing are summarized in Tables 4.12 and 4.13.

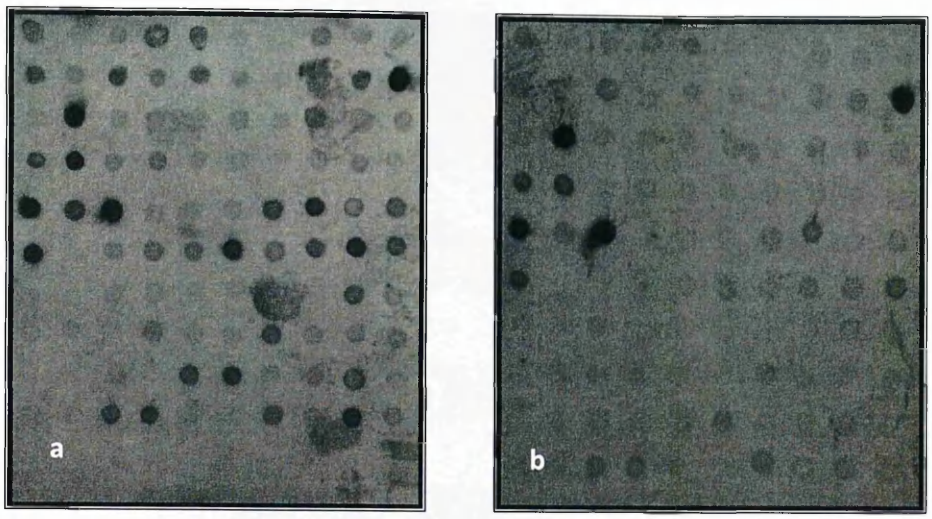


Figure 4.21 Screening result showing duplicate arrays of resuspended colonies. The two nylon membranes were probed with cDNA from **(a)** log-phase promastigotes and **(b)** attached promastigotes.

Gene ID	Description	Features	Gene copy	Identity %
LmxM.10.0405	GP63, leishmanolysin	In other organisms	multiple	99
LmxM.34.3870	nucleoside diphosphate kinase, putative	In other organisms	multiple	99
LmxM.36.1610	universal minicircle binding protein, putative	In other organisms	multiple	99
LmxM.04.0750	60S ribosomal protein L10, putative	In other organisms	multiple	99
LmxM.14.0650	fatty acid elongase, putative	In other organisms	multiple	99
LmxM.22.0030	60S ribosomal protein L11 (L5, L16)	In other organisms	multiple	100
LmxM.12.0990	surface antigen protein, putative	*Leishmania* only	multiple	77
LmxM.31.0020 *	hypothetical protein, conserved	*Trypanosomatids* only	single	99
LmxM.33.3790 *	hypothetical protein, conserved	*Trypanosomatids* only	single	100
LmxM.36.0370	phosphatidylinositol-4-phosphate 5-kinase-like protein	In other organisms	multiple	99
LmxM.25.0910	cyclophilin a	In other organisms	multiple	100
LmxM.25.0910	cyclophilin a	In other organisms	multiple	100
LmxM.29.0860	surface protein amastin, putative	*Trypanosomatids* only	multiple	95

Gene ID	Description	Feature	Gene Copy	Identity %
LmxM.31.0920	vacuolar proton-ATPase-like protein, putative	In other organisms	multiple	99
LmxM.32.0940 *	hypothetical protein, conserved	*Trypanosomatids* only	multiple	99
LmxM.34.1890	60S ribosomal protein L5, putative	In other organisms	multiple	92
LmxM.36.3590	cysteine synthesis		multiple	99
LmxM.13.0390	alpha tubulin	In other organisms	multiple	78
LmxM.20.1290 *	hypothetical protein, conserved	*Trypanosomatids* only	single	97
LmxM.32.0720	60S ribosomal protein L6, putative	In other organisms	multiple	99
LmxM.18.1620 *	hypothetical protein, conserved	*Trypanosomatids* only	multiple	100
LmxM.32.0940	hypothetical protein, conserved	*Trypanosomatids* only	multiple	99
LmxM.32.0720	60S ribosomal protein L6, putative	In other organisms	multiple	99
LmxM.18.1620	hypothetical protein, conserved	*Trypanosomatids* only	multiple	100
LmxM.32.0940 *	hypothetical protein, conserved	*Trypanosomatids* only	multiple	99

Table 4.12 Genes identified as showing higher expression in attached promastigotes. Those indicated with an asterisk (*) were subsequently investigated by Northern blotting.

Gene ID	Gene name	Features	Gene copy	Identity %
LmxM.36.1040	hypothetical protein, conserved	*Trypanosomatids* only	single	99
LmxM.23.0890	hypothetical protein, conserved	*Trypanosomatids* only	single	99
LmxM.34.3700	Gim5A protein, putative,glycosomal membrane protein	In other organisms	multiple	92
LmxM.09.1340	Histone H2B	In other organisms	multiple	98
LmxM.13.0280	alpha tubulin	In other organisms	multiple	100
LmxM.34.1890	60S ribosomal protein L5, putative	*Trypanosomatids* only	multiple	98
LmxM.25.0910	cyclophilin a	In other organisms	multiple	100
LmxM.17.0860	hypothetical protein, conserved	*Leishmania* only	single	88
LmxM.06.0410	60S ribosomal protein L19, putative	In other organisms	multiple	95
LmxM.19.1640	hypothetical protein, conserved	No information!	multiple	77
LmxM.10.1225	hypothetical protein, conserved	*Trypanosomatids* only	single	99
LmxM.36.5120	40S ribosomal protein SA, putative	In other organisms	multiple	99
LmxM.27.1130	intraflagellar transport protein IFT88, putative	*Trypanosomatids* only	single	63
LmxM.34.3670	hypothetical protein	*Trypanosomatids* only	single	100
LmxM.36.1635	poly-zinc finger protein 2, putative	*Trypanosomatids* only	multiple	99
LmxM.36.1640	universal minicircle sequence binding protein (UMSBP), putative	*Trypanosomatids* only	single	99
LmxM.17.0083	elongation factor 1-alpha		multiple	99
LmxM.29.1100	DREV methyltransferase, putative	In other organisms	multiple	70
LmxM.13.0570	40S ribosomal protein S12, putative	In other organisms	multiple	99
LmxM.29.0180	2-hydroxy-3-oxopropionate reductase, putative	In other organisms	single	100

Table 4.13 Genes identified as showing higher expression in log-phase promastigotes.

Of the genes identified as being expressed more highly by haptomonads compare to log-phase promastigotes several were not considered likely candidates for hemidesmosomal proteins due to their known function and/or their occurrence in other

organisms beyond trypanosomatids, for example, LmxM.14.0650 fatty acid elongase. Others appeared to be *Leishmania*-specific and were not considered or that reason. However there were 5 genes with appropriate properties: proteins of unknown function conserved amongst trypanosomatids but not in other organisms (and showing higher expression in haptomonads according to the screening results): LmxM.31.0020, LmxM.33.3790, LmxM.20.1290, LmxM.18.1620 and LmxM.32.0940. These selected genes were further investigated by Northern blotting to determine their level of gene expression. For each blot, 3µg of total RNA from attached promastigotes and unattached promastigotes were ran on a 1% agarose gel, blotted on to nylon membranes and probed with a probe specific for one of the five selected genes. Photographs were taken to ensure the quality of RNA prior to blotting experiments (Fig. 4.22). RNA was transferred overnight to positively charged nylon membranes, then hybridized and developed. Figure 4.23 shows the results of representative blots together with the control gene (LmxM.18.0620, 60S ribosomal protein L10a, putative).

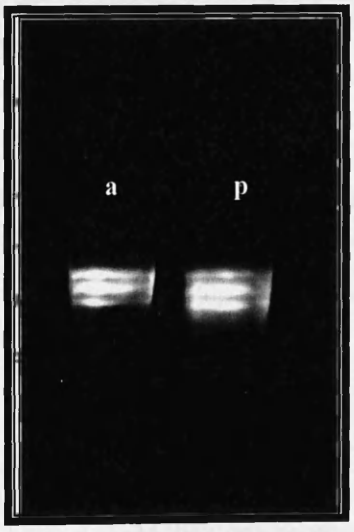


Figure 4.22 Total RNA from *Leishmania* promastigotes. 3µg of total RNA of **(a)** attached and **(p)** log-phase promastigotes on a 1% agarose formaldehyde-free gel.

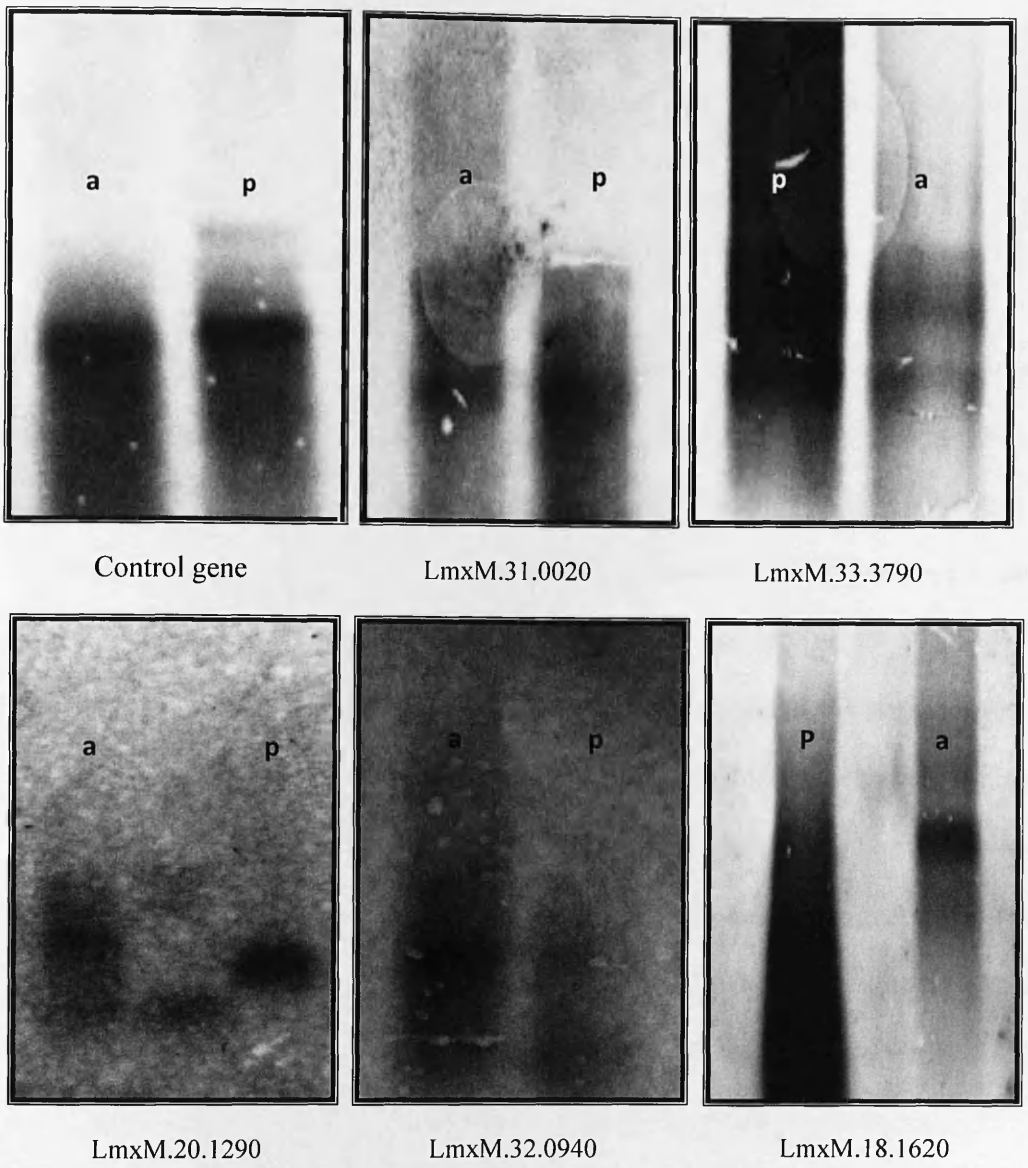


Figure 4.23 Northern blots of five selected genes from screening the cDNA library. The control gene used was the 60S ribosomal protein L10a, putative. Lanes contained RNA from either **(a)** attached forms promastigotes, **(p)** free log-phase promastigotes.

The results of the Northern blots indicated that for three of the selected genes LmxM.31.0020, LmxM.33.3790, LmxM.20.1290 expression was equal or higher in the normal promastigotes, which differed to the colony screening results. However,

two of the genes, LmxM.18.1620 and LmxM.32.0940, showed stronger signals with attached promastigotes. Band intensity for the control gene was similar in both RNA samples.

4.3.4.3 Analysis of *Leishmania*-specific genes

Although hemidesmosomal genes would be predicted as conserved amongst trypanosomatids, this depends on the quality of the underlying genomic databases, which are known to contain some errors in sequence assembly and/or annotation. Therefore, the expression pattern of some of the *Leishmania* only genes found in the library was investigated (Table 4.14). These genes were analyzed by RT-PCR, three times, to estimate the gene expression level in attached and log-phase promastigotes. PCR products were loaded on 1% agarose gels, then stained for 30 minute to 1 hour with GelRed and photographed. The gels showed the intensity of the bands from the two RNA samples (Fig. 4.24) and the graph shows the quantitation of the expression level (Fig. 4.25). In most cases expression was slightly higher in log-phase promastigotes than in attached promastigotes. The exception was for LmxM.29.3025, which is expressed by attached parasites more than by log-phase promastigotes.

Gene ID	Description	Gene Copy	Identity (%)
LmxM.23.1020	Hypothetical protein, conserved	Single	97
LmxM.34.3180	Hypothetical protein, conserved	Single	99
LmxM.29.3025	Hypothetical protein, conserved	Single	91
LmxM.27.1350	hypothetical protein, unknown function	Single	98
LmxM.12.0905	Hypothetical protein, putative	Single	73
LmxM.28.1165	Hypothetical protein, conserved	Single	100

Table 4.14 Genes identified as *Leishmania*-specific investigated by RT-PCR.

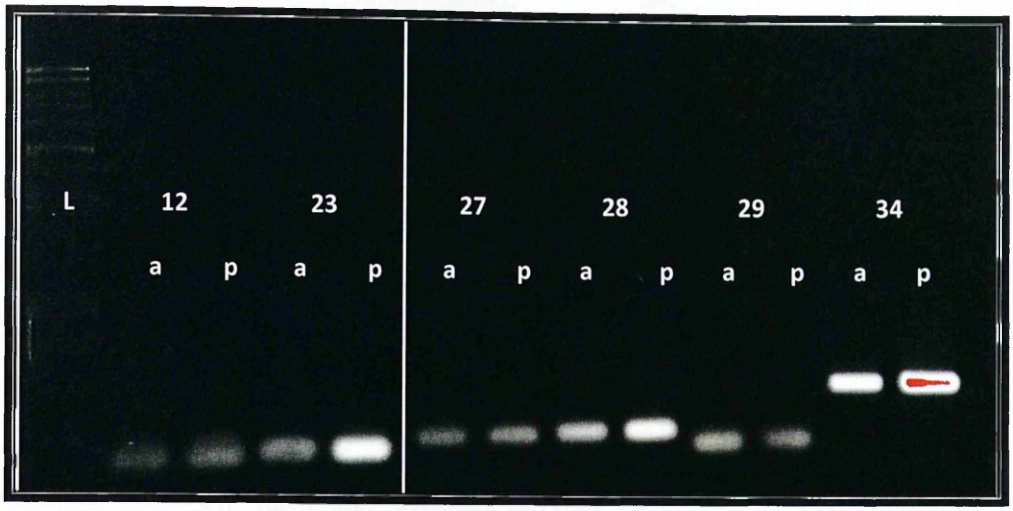


Figure 4.24 RT-PCR products of *Leishmania*-specific genes. The gel shows band intensity for 6 different genes in RT-PCR with RNA from attached forms **(a)** and log-phase promastigotes **(p).** The genes are LmxM.12.0950 **(12)**, LmxM.23.1020 **(23),** LmxM.27.1350 **(27),** LmxM.28.1165 **(28),** LmxM.29.3025 **(29)** and LmxM.34.3180 **(34).**

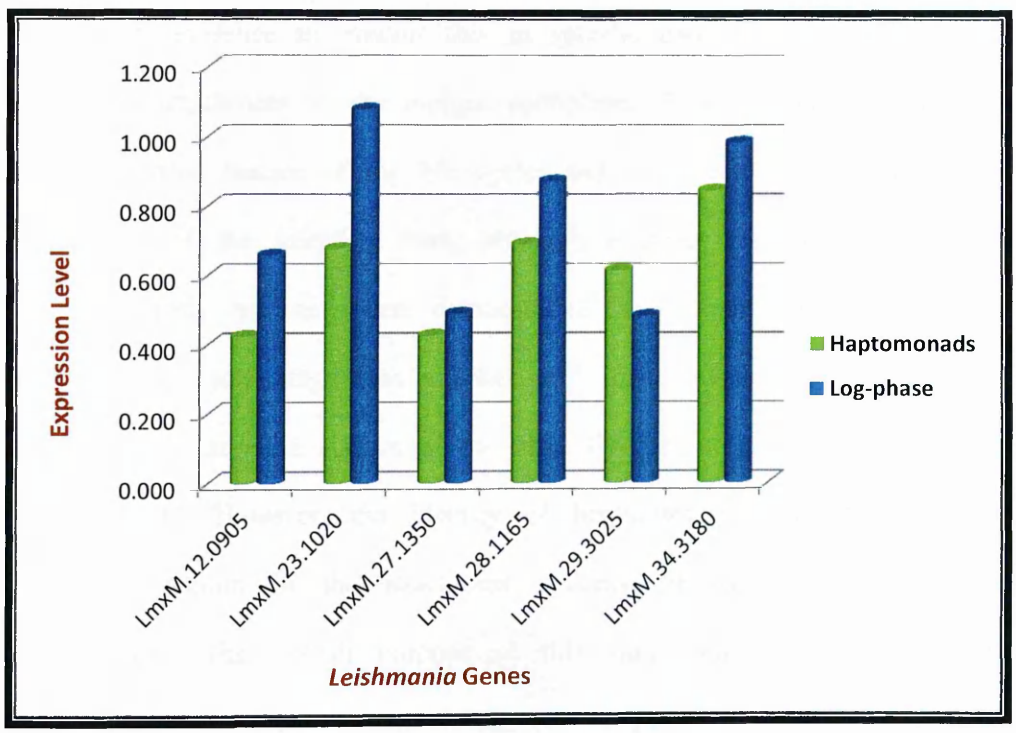


Figure 4.25 The chart par is showing the variations of *Leishmania* genes expression by attached (haptomonads) and log-phase promastigotes.

Chapter 5

Discussion

5.1 General discussion

Previous studies of the life cycle of *Leishmania* have shown that the parasites change both their form and function as they differentiate from one stage to another, including some life cycle stages are that capable of attaching to their host by their flagellum (Bates and Rogers, 2004, Wakid and Bates, 2004). These attachment processes are believed to be important for the parasites in order to complete their development in the sand fly vector, and there is strong experimental evidence to support this in specific cases, for example the role of LPG in attachment to the midgut epithelium. This attachment is regarded as an important feature of the life cycle, and may even be essential for the development of the infective form, although a direct role for attachment in metacyclogenesis has not been demonstrated. Haptomonad promastigotes are a unique life cycle stage that attaches and forms hemidesmosomes between the flagella tip and the surface of the sand fly foregut or hindgut (Bates and Rogers, 2004). However, the identity of hemidesmosome protein molecules and their function in the attachment process are still poorly understood (Bates, 2008). The overall purpose of this study was to investigate these proteins in order to identify them and improve our understanding of *Leishmania* hemidesmosomes.

The first objective of this study was to develop an in vitro attachment system capable of generating haptomonad forms in sufficient quantity to enable biochemical and molecular investigations to be undertaken. Although attachment had been previously studied in *Leishmania* and other trypanosomatids, existing methods were only small scale. Therefore, various different materials were tested whose properties suggested they might be useful substrates for attachment, yielding an amount of attached parasites suitable for the purposes of the project. To make a fair comparison, at the beginning it was important to standardize the in vitro cultivation method by using a standard starting density and sub-passage. To achieve this growth and attachment by forming hemidesmosomes were tested by culturing with a material previously used in other projects, plastic Melinex sheets (Wakid and Bates, 2004), to be a control measure with different starting densities of *L. mexicana* promastigotes cultures.

The monitoring and microscopic examination of the cultures with Melinex proved that starting with high density yielded a high rate of attachment as in previous studies (Berens *et al*, 1976; Beattie and Gull 1997; Ghosh *et al*, 1999; Wakid and Bates, 2004; Harder *et al*., 2010). Subsequently a starting density of 1 x 10^8 /ml was used in all experiments. Cultures only a few sub-passages after initiation and transformation from lesion amastigotes showed many clustered parasites in suspension in rosettes, linked by their flagella, which suggested that the numbers of attached parasites would be higher than with cultures that have been through many sub-passages. This was clear from the attachment behavior of the promastigotes, which was much more active in cultures with few sub-passages, because with time the rate of attachment of promastigotes decreased. Examination of Melinex by scanning electron microscopy at

high power confirmed the forming of hemidesmosomal-like attachment and was used as a reference for the attachment to other materials, and as had been previously shown (Wakid and Bates, 2004).

Various combinations of three different species, *L. major*, *L. tarentolae* and *L. mexicana*, were cultured with different materials; polycarbonate membrane filter papers; chitin powder, agarose gel, n-Octacosane wax, candle wax, Parafilm, polystyrene, Sepharose, Sephadex and Ethylene-Vinyl Acetate Copolymer (EVA). Based on microscopic examination, generally the attachment of *L. major* to the materials was low in comparison to the other species, although whether this was a species-specific effect or just relates to the specific strain used in this study is not known. A similar result was obtained by another member of the laboratory also working on *L. major* (M. Bates, personal communication). In contrast, the lizard parasite *L. tarentolae* attached at a good scale similar to *L. mexicana,* but amongst all of them *L. mexicana* gave the best rate of attachment and also was an easy species to deal with during the culturing process. No statistical analysis of promastigotes attachment to different materials is presented as due to their varied shape, size and physical properties counting and estimating their number on the tested materials in a comparable way by microscopic methods was either difficult or impossible. These conclusions are therefore based on careful observations.

Most studies of trypanosomatid attachment have concluded that the mechanism is dependent on some form of hydrophobic interaction (Wakid and Bates, 2004). Plastic materials such as polycarbonate Ethylene-vinyl acetate copolymer, Melinex plastic

sheet and polystyrene Petri dishes, together with the materials paraffin, n-octacosane, chitin and agarose were used because of their hydrophobic properties. A previous study described a weak attachment of *T. cruzi* to chitin (Kleffmann *et al.,* 1998). In this study the parasites attached to chitin based on the microscopic examination of the cultures for 7 days. However, the rate of the attachment was low and did not increase with time, and that may because the parasites in the insect midgut and in vitro releasing a chitinase enzyme that lysis the chitin framework to help the parasites to migrate and escape the PM (Schlein *et al.*, 1991; Rogers *et al.*, 2008). So their attachment may be temporary or it is only happening in the vector sand fly.

The attachment behavior of the parasites to the plastic materials and hydrophobic substances has been observed in vitro in this study. Here *Leishmania* promastigotes attached to different materials and showed a high rate of attachment to the materials made from plastic. The promastigotes were also seen attached to the wall of the culture flask and the surface of the Petri dishes. Several previous studies have used plastic materials to study and investigate the attachment system in trypanosomatids. All showed the reliability of plastic and hydrophobic surfaces to create a good adhesion surface for the parasites (Beattie and Gull, 1997; Schmidt*et al.*, 1998; Kleffmann *et al.,* 1998; Wakid and Bates, 2004), and for this reason most of the materials were chosen with these properties in mind.

Kleffmann *et al.* (1998) and Schmidt *et al.* (1998) stated that the *Trypanosoma cruzi* epimastigote attachment mechanism requires a hydrophobic surface and the parasites are not able to attach to hydrophilic surfaces, as the superficial layer of the rectum of *Triatoma infestans*, the vector of *T. cruzi*, was shown to be hydrophobic. In this study the *Leishmania* promastigotes attached to hydrophobic as well as to hydrophilic

substances as shown on polycarbonate filter papers. However, examination by inverted microscope did not clarify whether the parasites were attached by forming hemidesmosome or other mechanisms. So photographs of these membranes were taken by scanning electron microscopy. Parasites seemed anchored via their flagella inside the filter pores so it was difficult to confirm the attachment mechanism. However, parasites were definitely attached because observations showed that the parasites remained on the membrane during extensive washing and vortexing. As the *Leishmania* promastigotes attached to the hydrophobic and hydrophilic materials this may indicate that the hydrophilic surfaces became coated with hydrophobic molecules, for example lipids from the serum, assuming the mechanism is the same in both cases.

Agarose-coated Petri dishes did not deliver a good attachment of promastigotes. Giemsa-stained slides showed that most of the parasites seemed clustered around the edge of the agarose-coated area, and there were morphologically similar parasite forms within the cultures, which indicated the majority of the population were not attached parasites. Kleffmann *et al.*, (1998) found only 3% of *Trypanosoma cruzi* epimastigotes attached to the hydrophilic surface of agarose, which they considered an insignificant result. Here, it was difficult to confirm whether the parasites were actually attached to the agarose surface, were just lying on the surface or sticking there because of the natural consistency of the agarose, when looking at them with the inverted microscope. For these reasons the agarose experiments were discontinued and other materials examined.

The attachment to the n-octacosane, paraffin wax and ParaFilm also was not significant; the numbers attached were low and did not achieve the required level for

this study. The n-octacosanewas used before to study the attachment ability of *Leishmania* promastigotes in vitro (Wakid and Bates, 2004). The n-octacosane was melted and used to coat the Melinex surface to enhance the parasitic attachment mechanism. These authors found that Melinex coated with n-octacosane had a positive effect on the attachment process. It enhanced attachment in comparison with the other methods that were used in the study. The substances polystyrene, Sepharose and Sephadex gave negative results. The parasites did not attach to them at any point. However, polystyrene was used before to culture *T. cuzi* epimastigotes and they attached at a significant rate. These parasites attached to polystyrene Petri dishes (Kleffmann *et al,* 1998), while in this study microparticles based on polystyrene were used. The concept of using these was that the promastigotes may attach to the round molecules in an organized way and provide good pictures of the attachment mechanism but the approach was not successful. The reasons why the parasites did not attach to the beads are unknown. Presumably, it may due to the difference in the chemical components between the two substrates of polystyrene or the beads are not compatible with the needs of the parasite. It could also be that the pH of the culture media or enzymes released by the promastigotes made the surface unsuitable for attachment.

Ethylene-Vinyl Acetate Copolymer (EVA) was a new material to try, chosen based on its thermoplastic properties. Trypanosomatids attach to materials made plastic or have plastic as a major component in their composition. Also EVA physically is characterized by its softness, flexibility and clarity of appearance which make formation to the desired shape and handling during the whole experiment easy. The chemical and physical properties of EVA made it a good candidate to use in our study. It was the best substrate that generated attached promastigotes with high quantity and

good quality. About ~ 90 % of the population were attached forms. Attachment of *L. major* comparing to *L. mexicana* was obviously different. *L. major* poorly attached to EVA while *L. mexicana* attached at high numbers. This may be because of selective attachment or the specific attachment behavior of the parasites when they attach to the sand fly midgut during their life cycle (Pimenta *et al.*, 1994). The easy preparation, culturing, handling and washing of the EVA minimized any contamination with media, serum or any other supplements. Also the flexibility of EVA made it applicable to use for any purpose; it was used as small pieces for electron microscopy or as large pieces in any shape (round flat with rough surface) for protein and RNA extraction. The only drawback was that, according to both Glasgow and Liverpool University electron microscope units, it was hard to get transmission electron microscope pictures due to incompatibility between the resin that is used in the fixation process and the nature of EVA. They did not bind together, which is an important step for the sectioning step in the transmission electron microscope procedure, which resulted in unsuitable images. The purpose of transmission electron micrographs was to look at the hemidesmosomal plaque in cross section that is formed by attached parasites (Wakid and Bates, 2004). However, based on scanning electron micrographs it was very convincing that attachment was occurring by hemidesmosomes, their appearance being essentially identical to the previous study (Wakid and Bates, 2004).

Materials that showed good parasite attachment were used in experiments aimed at biochemical characterization of proteins extracted from attached promastigotes. Polycarbonate membrane filter papers, chitin powder, n-Octacosane, paraffin wax and Ethylene-Vinyl Acetate Copolymer (EVA) were used in these experiments. The filter papers provided a quick method to generate attached promastigotes of *Leishmania* in

vitro considering the short time that the parasites took to attach to the filters. Their reliability and ease of handling made them good materials. However, the results obtained from filters may potentially be false positive. The filters may have adsorbed culture media components from the serum. Also there is a possibility that some of the flagellar components of free promastigotes attached to the filter and after they were treated with MME buffer were re-absorbed by the filter. That may have resulted in the appearance of proteins bands of whole free flagella of promastigotes mixed with proteins from attached forms. Besides this there was uncertain confirmation of the parasites being properly attached to the filter as this was not clear in the electron microscopy photos. MME-buffer treated chitin samples were loaded on gels and resulted in a range of bands with different molecular weights. However, the experiment with chitin was performed only once and without further analysis we cannot be sure if these are the same proteins or this is simply a coincidence.

Paraffin wax as an enhancement factor for *Leishmania* promastigotes in vitro has also been shown previously. It can raise the rate of parasite attachment in vitro when it is melted and used to coat the surface of materials (Wakid and Bates, 2004). In this experiment, paraffin candle wax was used in a different form, as a non-melted form by shaving flakes from candles, after melting and solidification as flat sheets, and as big and medium sized round shaped wax particles. The best form was the wax flakes; the parasites attached to them more than other forms. It also provided a clear view of parasites attachment through the microscope. However, with the other forms of wax the outcome was not clear and it was difficult to judge if there was attachment. The advantages of using candle wax includes it cheapness, providing a good quantity of materials to culture with the promastigotes and it gives a clear view of parasite attachment under the microscope. It is also easy to prepare and culture. The

disadvantages were as follows: they were hard to collect from the cultures as most of them adhere to the wall of the flask and the tube during the washing steps, which resulted in losing valuable parasitic biological materials. Also their potential contamination with the culture media during their collection, that may give false positive results on SDS-PAGE. If the wax flakes were left over a week in the incubator they turned into one piece. They also melted and became solid material when boiled for loading onto the gel and for those reasons it was disqualified from further use in this study.

EVA was used to generate attached promastigotes and then used to extract proteins. The numbers of bands from EVA were few in comparison with the other materials that were used for biochemical identification and that confirmed previous suspicions about the contamination of these with media components. The results obtained were from a few pieces per preparation and it assumed that by increasing the number of EVA pieces more biological material will be obtained. The molecular masses from protein extraction and SDS-PAGE gels were estimated manually and may include a percentage of inaccuracy. Also the proteins band results could include those from other stages of *Leishmania* promastigotes and culture supplements. Due to these uncertainties and unclear results and also that identification of these proteins by SDS-PAGE required a large amount of biological samples, alternative approaches were sought to circumvent these problems. Investigation based on sensitive and specific molecular approaches which required less biological materials was therefore pursued.

Sufficient quantity and quality of RNA was generated to construct a cDNA library of *L. mexicana* EVA attached promastigotes for genetic investigations. A cDNA library is made from mRNA which therefore reflects proteins synthesis in the cells from

which it was extracted. Based on this premise, a cDNA library from attached parasites was constructed to search for hemidesmosome protein genes. The library was then screened by RT-PCR, colony hybridization and Northern blotting. RT-PCR is a sensitive method that allows detection of small amounts of mRNA and any variations in expressions among genes, and provides rapid results (Morrison *et al.,* 1998). Semi-quantitative RT-PCR is performed, as in this study, by measuring the relative expression of a target gene versus a housekeeping gene (ribosomal protein L10) and this measurement used to investigate the physiological changes in gene expression (Pfaffl, 2001), applied here to detect the gene expression level among the three *Leishmania* stages.

The results obtained from RT-PCR showed that the majority of the genes were highly or only (at detectable levels) expressed by log-phase promastigotes, despite the library being constructed from haptomomad mRNA. These are possibly proteins that are involved in division and active multiplication processes. Also they could be proteins for promastigote growth and structural components, for example, cytoskeletal proteins, which are required high levels. Lu *et al.,* (2007) and Garcia-Martinez *et al.,* (2007) observed that about 50 and 70% of genes expressed in log-phase of bacteria and yeast have good correlation between mRNA and protein levels. The variation in gene expression between *Leishmania* stages (log-phase, metacyclic and attached promstigotes) is reflected in the differences in individual gene functions, as each one of those stages has a special distinctive role during the parasites life cycle. Previous work reported that proteins which are involved in metabolic and signaling pathways are characterized by a strong correlation between mRNA and proteins levels, while those in large complexes have a weaker correlation (Schmidt, 2007). Screening the library by RT-PCR was performed for limited numbers of colonies, the technique

applied to estimate the level of gene expression. Despite the advantages of RT-PCR, it is used as a semi-quantitative method that provides indirect evidence about gene expression levels and also there is always a minor percentage of variability. Therefore, screening the cDNA library was continued by using hybridization with cDNA probes.

Library screening enables the expression of thousands of genes to be assessed in parallel at the same time. The measurement of expression levels is based on observing the difference of intensity of colony hybridization between the different probes used on the membranes. Screening of colonies was repeated many times and in each run there were few differences from previous ones. Screening by hybridization is a critical technique has many factors that could cause biases such as the hybridization handling process, incubation temperatures and scanner settings at membrane photographing. In general experimental conditions may cause differences in samples in replicate experiments that make distinctions between differentially and constantly expressed genes difficult (Yang *et al.*, 2002), so during the application efforts were made to minimize the variations and optimize the technique accuracy.

1654 colonies were collected from agar plates of the cDNA library; spotted, hybridized with probes of log-phase and attached phase promastigotes, washed and finally stained with the colorimetric substrate NBT/BCIP. The results demonstrated variation in colony intensity between the two stages. Both promastigote forms have same intensity for some colonies, but similar to the results from RT-PCR the majority of the colonies were more intense on the log-phase promastigotes membrane. However, five genes from among the large number of screened colonies were more intense in attached forms and characterized as hypothetical proteins conserved amongst trypanosomatids only. They were ideal candidates for this study and to

confirm their expression level Northern blot analysis was performed, which the most accurate technique for measuring gene expression because it deals with the RNA samples directly without potential interference from various factors like the amplification step in RT-PCR and variability in membrane hybridization methods. However, this technique does require large amounts of high quality RNA, in comparison to RT-PCR and hybridization where requirements are modest. Nevertheless, Northern blotting was feasible using the EVA method developed in this study. The results indicated that three of the five genes were more highly expressed in log-phase promastigotes compared to attached forms, in contrast to what was expected from the screening. The other two genes were more encouraging, although the results cannot be considered conclusive from these data alone. LmxM.18.1620 and LmxM.32.0940 appeared to be more highly expressed in attached promastigotes, and are worth further investigation.

Hypothetical assumptions about the proteins of hemidesmosomes are summarized as follows. Proteins that are involved in creating hemidesmosomes are expressed only by attached promastigotes in high level or they may be found in other stages but at much lower level, as the hemidesmosome is a unique structure of the attached forms and not seen in other stages, and those proteins should be produced in abundant quantity to regulate and form the structure. These should be proteins only expressed by attached forms or proteins that already exist in other stages but when promastigotes differentiate to different stages those proteins decrease or increase according to the parasites need, in the case of hemidesmosome formation they should increase. Screening the library showed that the attached parasites shared with the log-phase and metacyclic promastigotes manyproteins being expressed at a similar level and only a few that were higher in attached forms. Results that were obtained from identification

of 1702 cDNA colonies by RT-PCR, screening by cDNA probes, and Northern blotting showed very few potential proteins specifically expressed or being more highly expressed by attached parasites. The quality of the cDNA library was good but by screening large numbers of library genes the relative lack of success may have many explanations. Perhaps there are no specific proteins for hemidesmosome-like structures and they are composed of proteins that are also expressed by other stages. That could be confirmed by localization techniques for genes to determine the location of their protein products in promastigote morphology. Or it might be there are proteins which are only expressed to form hemidesmosome structures but they are few in number and scattered among the library which needs more screening. Another possibility is that the mRNA sequence length of hemidesmosomal genes is too long to be included in the constructed cDNA library as the library if they are particularly large proteins and that may the reason why they have not been found despite the large number of colonies screened.

Six genes that are found only in *Leishmania* species were found by the cDNA library screening process. The genes were analyzed by RT-PCR to determine their expression level in comparison with log-phase promastigotes as they may be specific genes for attached parasites but not hemidesmosomes. The results showed no significant higher expression among attached promastigotes, five were higher in log-phase promastigotes, one gene being expressed more by attached forms but only slightly more than the log-phase promastigotes. Due to the restricted time of the project localization could not performed to determine the location of the proteins from genes that were expressed by attached promastigotes, which may those proteins located in the hemidesmosome structure.

Identified proteins included those with known function. For example, tubulin (α and ß) proteins were repeatedly detected among the screening process and expressed by all stages. That is expected as tubulin is a fundamental component of the *Leishmania* cytoskeleton structure that is also responsible for cell shape and is involved in biological activity such as cell division, ciliary and flagellar motility and intracellular transport (César *et al.*, 2013). The alpha tubulin was found on chromosome 13 similar to *L. braziliensis* (César *et al.*, 2013), while beta tubulin was found on chromosome 32. Other proteins frequently observed were ribosomal proteins. In eukaryotes, ribosomal proteins are part of its translational apparatus and function in cell growth and apoptosis regulation mechanisms (Naora, 1999).

In conclusion, unfortunately this study did not successfully identify the genes that are involved in hemidesmosome structure. However, a much improved attachment system was developed and two genes were identified that appear to be upregulated in haptomonads, although whether they are truly stage-specific or associated with hemidesmosomes remains to be determined. Most of the genes identified were expressed by other stages and none of them being expressed at sufficient level to be considered as attachment-specific proteins or proteins involved information and regulation of hemidesmosomes. Results from the approaches that were applied were appropriate to the study purpose, but they were also time consuming and needed lots of effort to optimize.

5.2 Project difficulties

Most of the obstacles faced during this study were derived from the culturing of the promastigotes. After several sub-passages the promastigote attachment rate decreased and they gradually lose their ability to attach. In addition *L. mexicana* promastigotes

have a tendency of reverting to the amastigote stage in stationary phase, the non-motile forms (no flagella). Also the time of culturing was different from one material to another. Some of them provided results in a short time while some needed a much longer time. The decision of at what time the parasites should be collected was also critical. There were times where the parasites were left for a long time in attempts to provide large quantities of attached promastigotes but this ended in losing the parasites as they then died resulting in lost time. There was also difficulty finding a robust system to figure out the numbers of the attached promastigotes in the cultures. That was not easy as the counting on most of the materials that were used in this project was hard. Choosing the materials to test with the parasites in fact was a time consuming process, because many substances were tried based on previous studies as well as new materials in order to try and generate significant results. The handling of some of the materials such as chitin and wax particles was difficult as they are in powder form and the particles are small. It was also hard to collect them from the cultures. Many were lost during washing steps and the extraction process. Also they were mixed with the culture media M199 that contains supplements and some of the proteins bands detected on the gels may refer to some component of the Medium 199 and not to the haptomonads.

Library screening techniques were critical, choosing suitable methods and performing them was time consuming. Each technique consumed lots of time to optimize and then experimental replications and results analysis were not easy. Sample preparation for transmission electron microscopy was difficult, as it turned out EVA was not suitable for fixation process, and it was hard to devise an alternative way to separate the attached parasites from the EVA surface and collect a pellet to process for electron microscopy. After several attempts a pellet was finally collected but the process was

too harsh on the parasites and resulted in destruction of the parasites structure and unsuitable have transmission electron micrographs. Screening the cDNA library by hybridization was the most time consuming technique due to it needing large quantities of RNA from attached parasites, and it took a long time to culture and extract the RNA form the huge numbers of cultures. Also the application involved many factors that affected the outcome, and consequently results were variable and confusing. In some cases an experiment with the same samples required repetition over 3 times to get confident results and took a long time to be completed.

5.3 Future work

The hypotheses within this study may be tested by further modifying the study plan and by applying new approaches. The hind gut of a model insect such as Manduca sexta, a very large caterpillar, could be used as an alternative attachment surface for *Leishmania*. This approach may also use to get clear and efficient transmission electron microscopy images. The lizard *Leishmania tarantolae* is known to attach as a haptomond form to the hindgut cuticle of *Lutzmomyia longipalpis* and this also might be used for TEM studies. Screening could be conducted by searching among completed sequenced data base of *L. major, L. infantum and L. braziliensis* by using a bioinformatics approach. Further investigation also could be applied to identify genes by generating antibodies or transfecting parasites with GFP-tagged genes so the subcellular localization of potential hemidesmosomal gene products could be investigated. Finally, gene knock down or gene knock out for hemidesmosome specific gene could be performed to inhibit the adhesion process and that could provide more information about the proteins function and their role in hemidesmosome structure.

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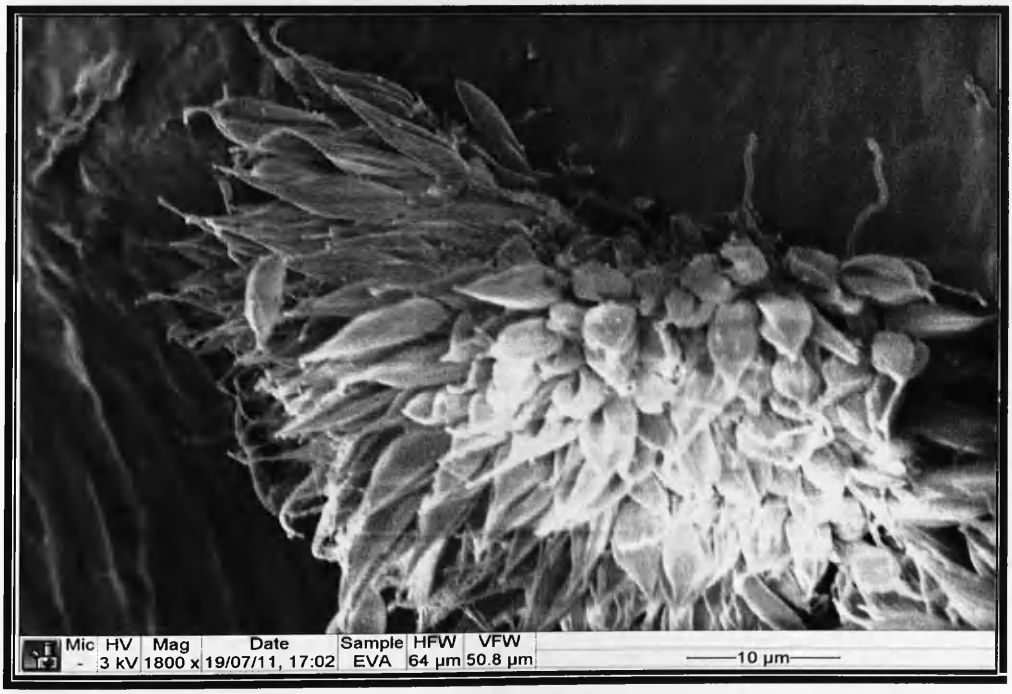
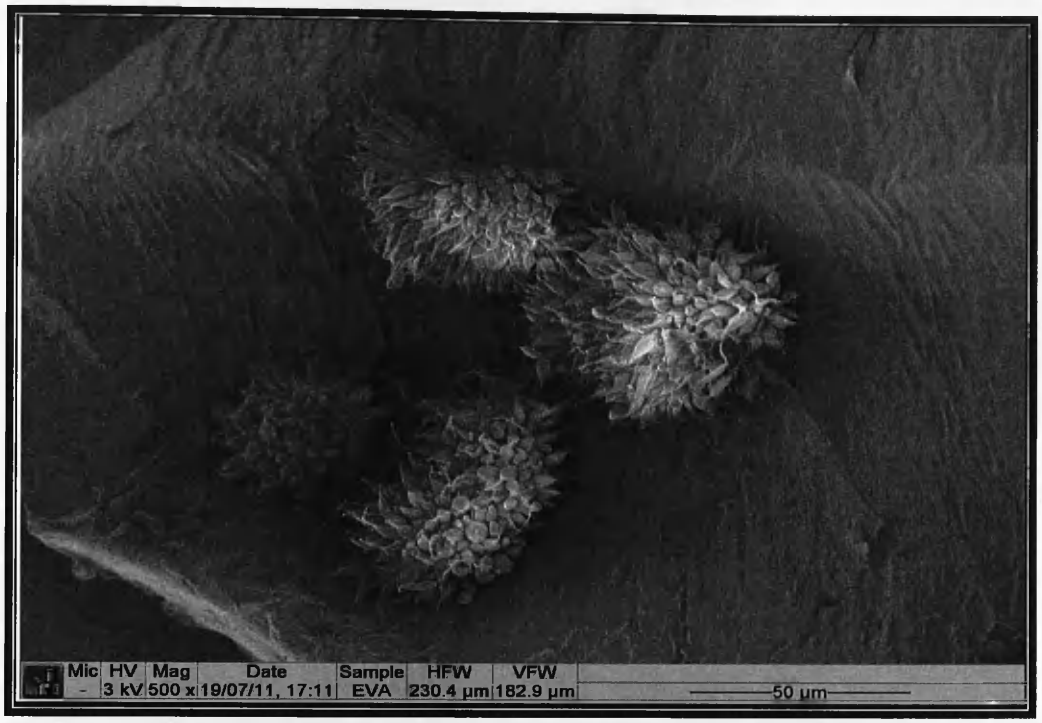
Chapter 6

Appendix

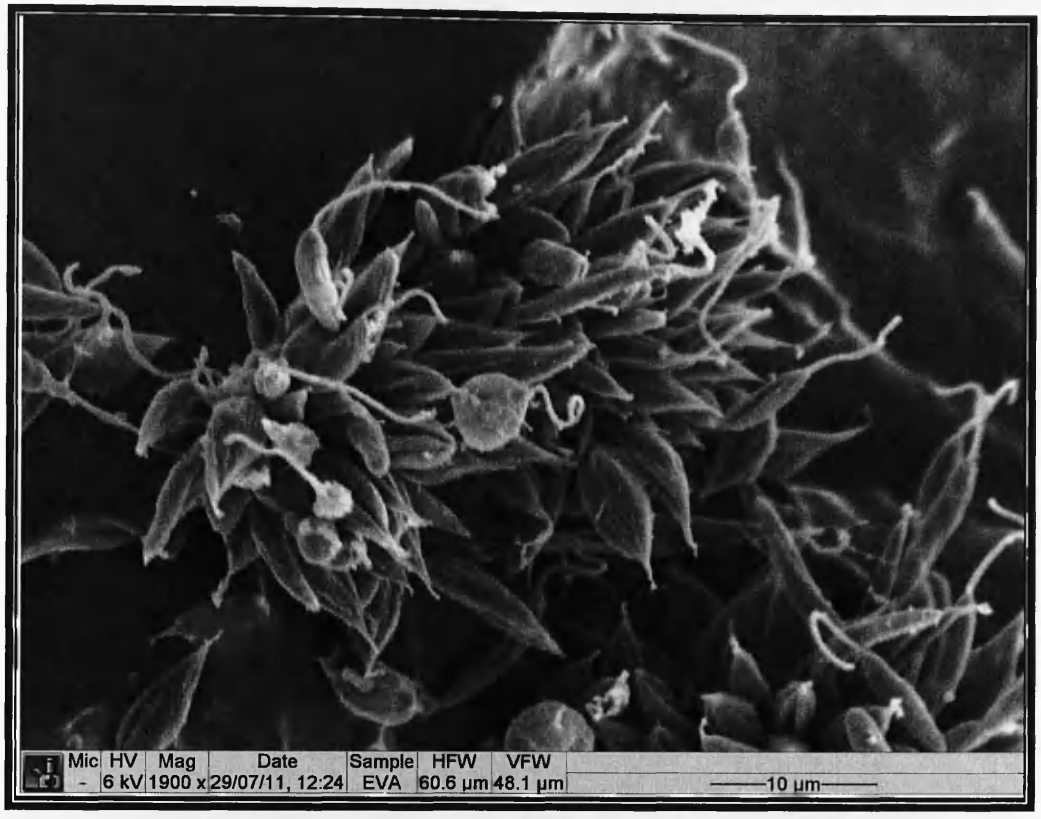
6.1 Appendix

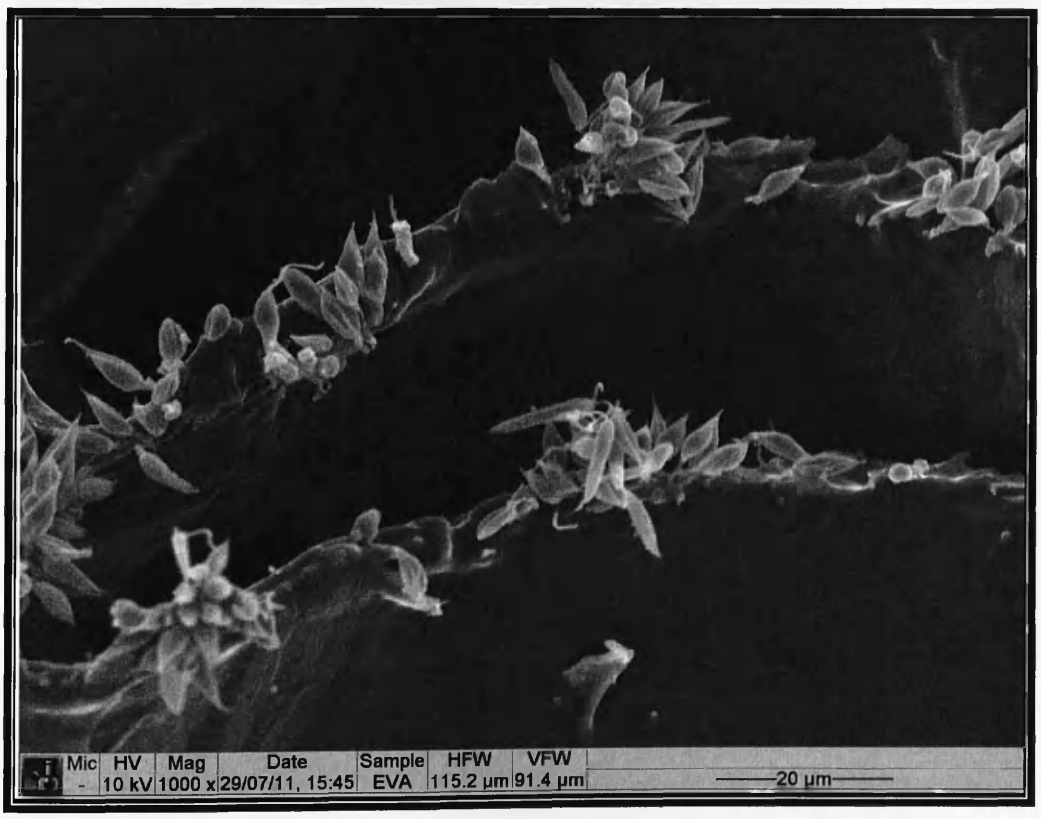
a. Complete Pictures of scanning electron microscopy of *L .mexicana* attached to Ethyl Vinyl Acetate Co-polymer (EVA) surface in clustered form.



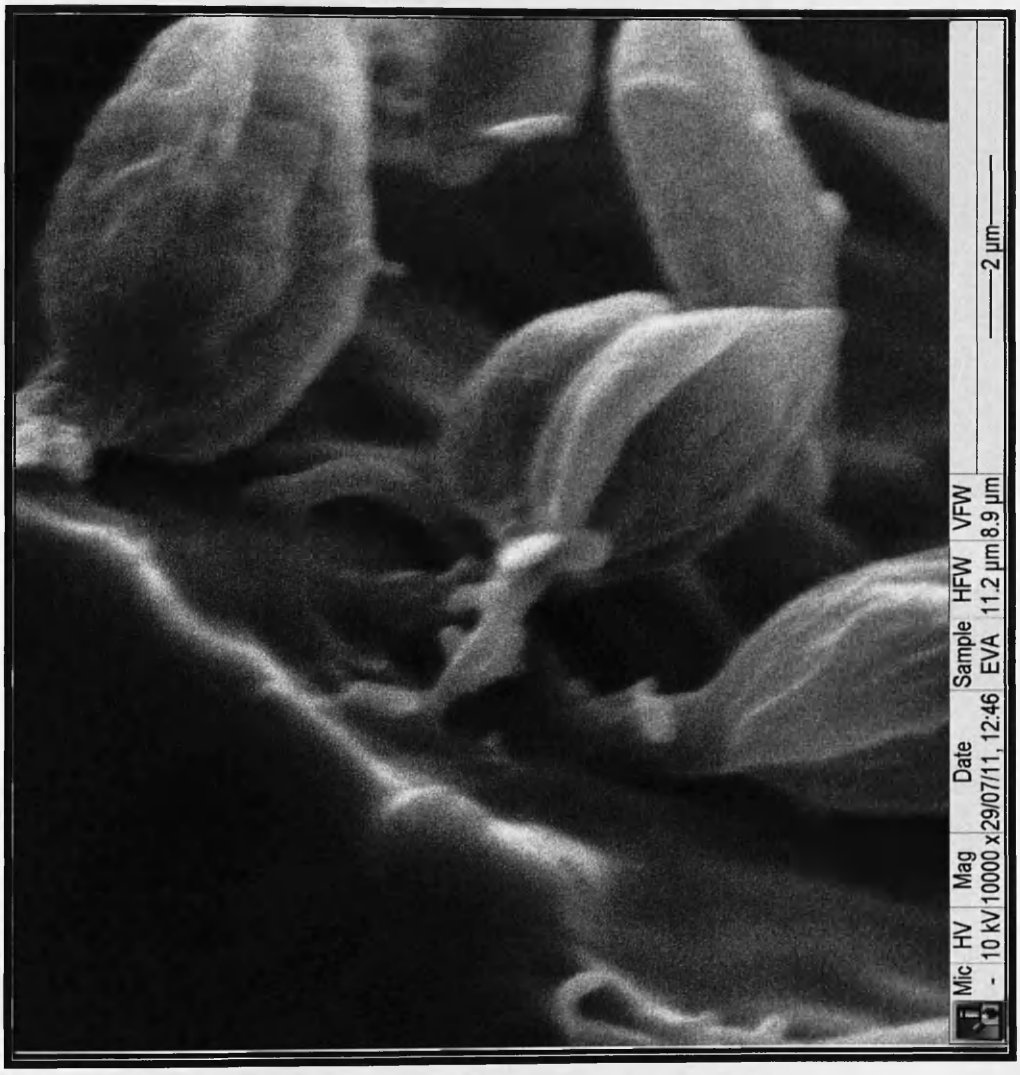

Mic HV Mag Date Sample HFW VFW
- 3 kV 500 x 19/07/11, 17:11 EVA 230.4 µm 182.9 µm
50 µm

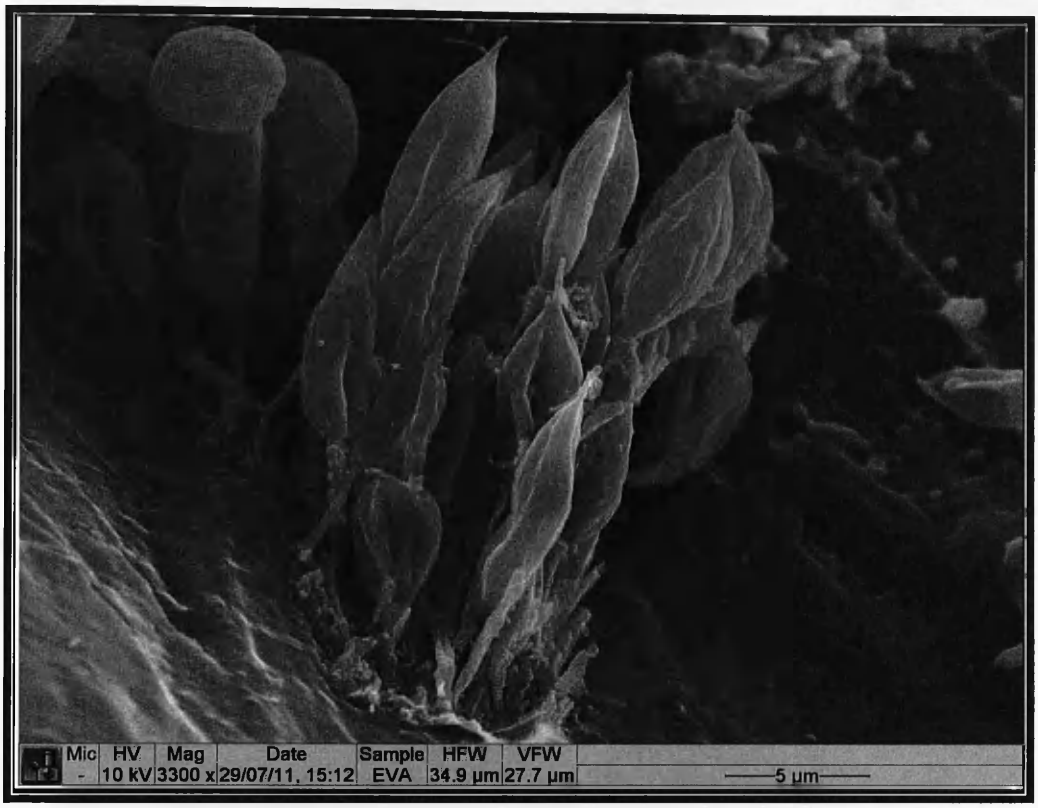

Mic HV Mag Date Sample HFW VFW
- 10 kV 250 x 29/07/11, 15:36 EVA 460.7 µm 365.8 µm
100 µm

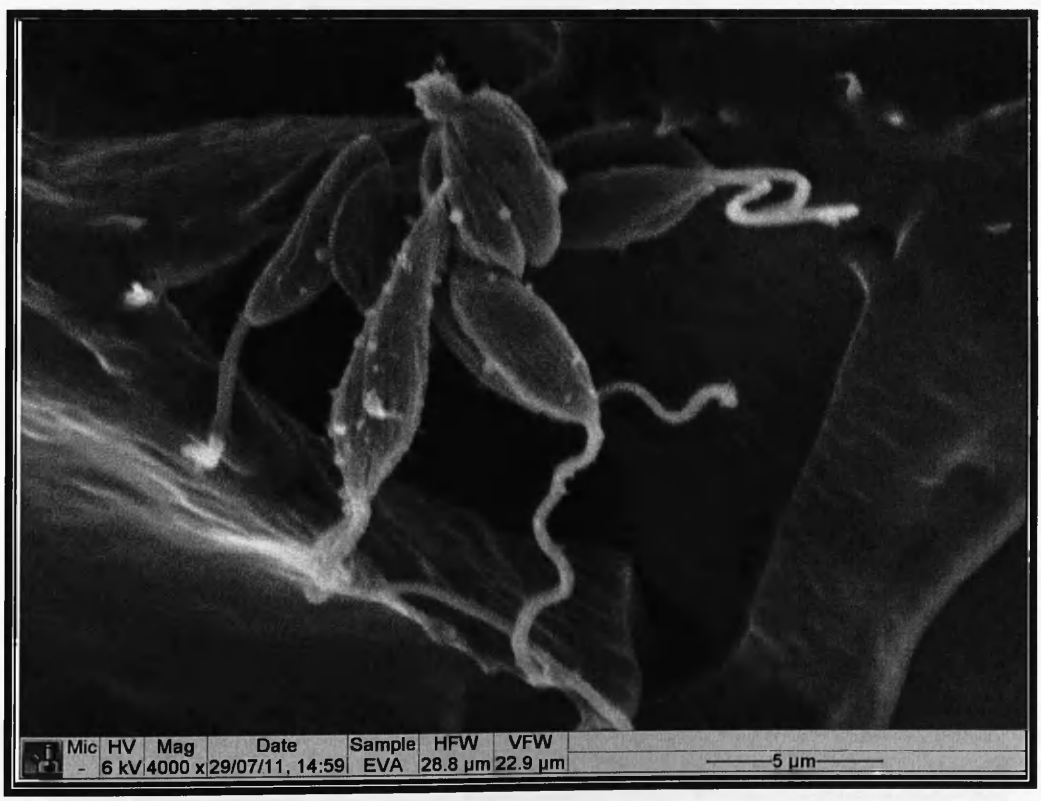

Mic HV Mag Date Sample HFW VFW
- 6 kV 1900 x 29/07/11, 12:24 EVA 60.6 µm 48.1 µm
10 µm

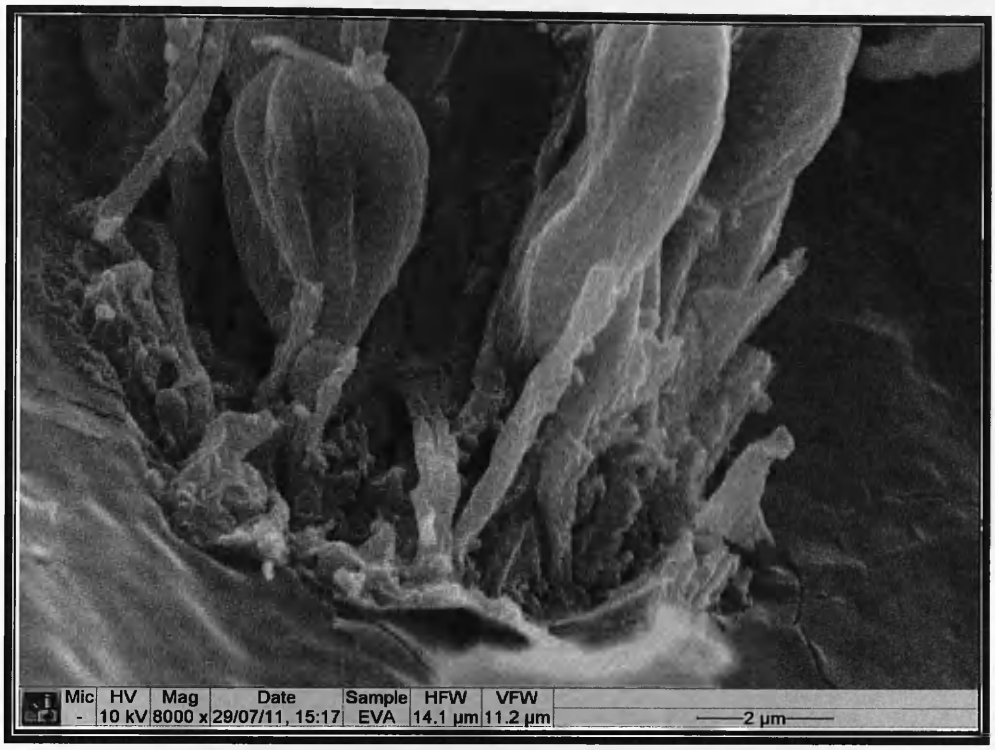

Mic HV Mag Date Sample HFW VFW
- 10 kV 1000 x 29/07/11, 15:45 EVA 115.2 µm 91.4 µm
20 µm

b. Complete Pictures of scanning electron microscopy of *L .mexicana* attached to Ethyl Vinyl Acetate Co-polymer (EVA) surface by forming hemidesmosome-like structure.

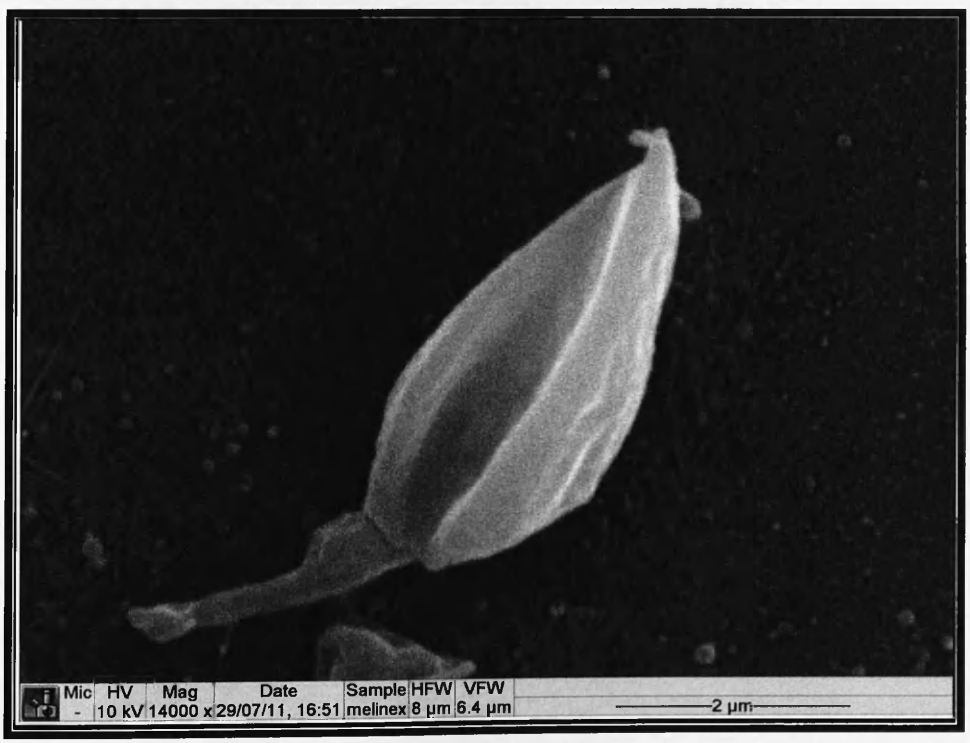



Mic HV Mag Date Sample HFW VFW
- 10 kV 3300 x 29/07/11, 15:12 EVA 34.9 µm 27.7 µm
5 µm


Mic HV Mag Date Sample HFW VFW
- 6 kV 4000 x 29/07/11, 14:59 EVA 28.8 µm 22.9 µm
5 µm

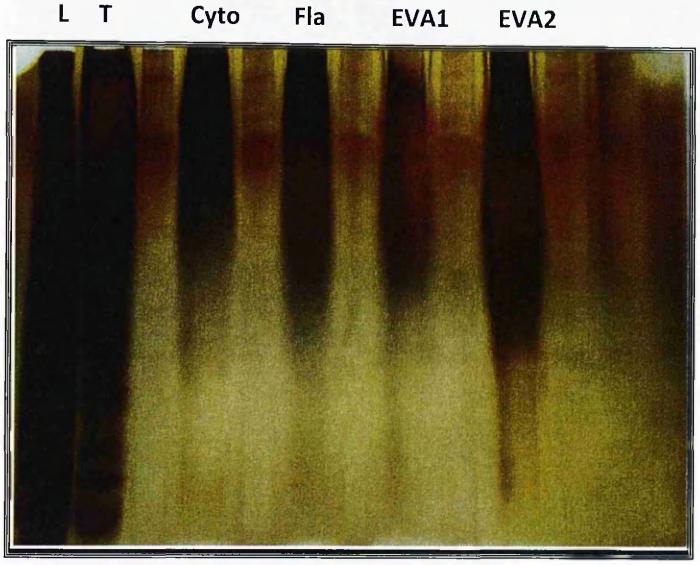


c. L.mex attached to Melinex plastic sheet by hemidesmosome



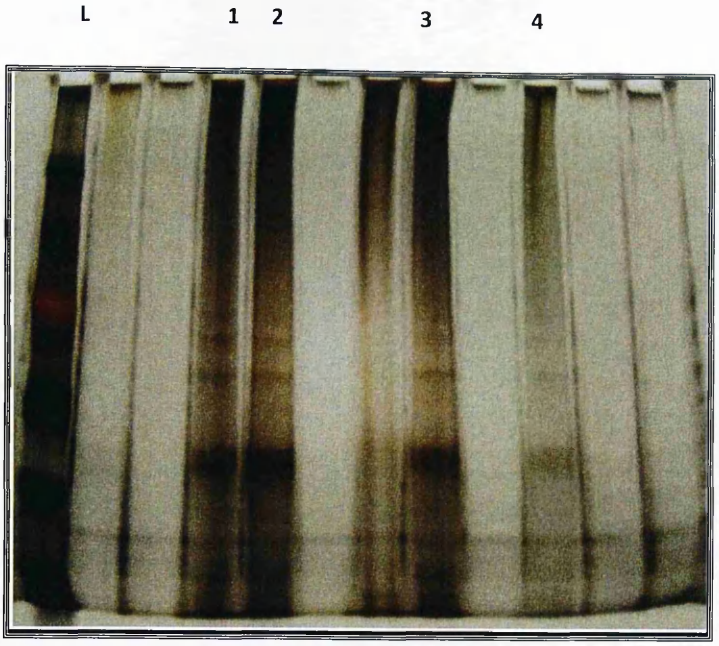
6.2 Appendix

a. Complete data of SDS-PAGE gels of *Leishmania* species extracted proteins from different materials.

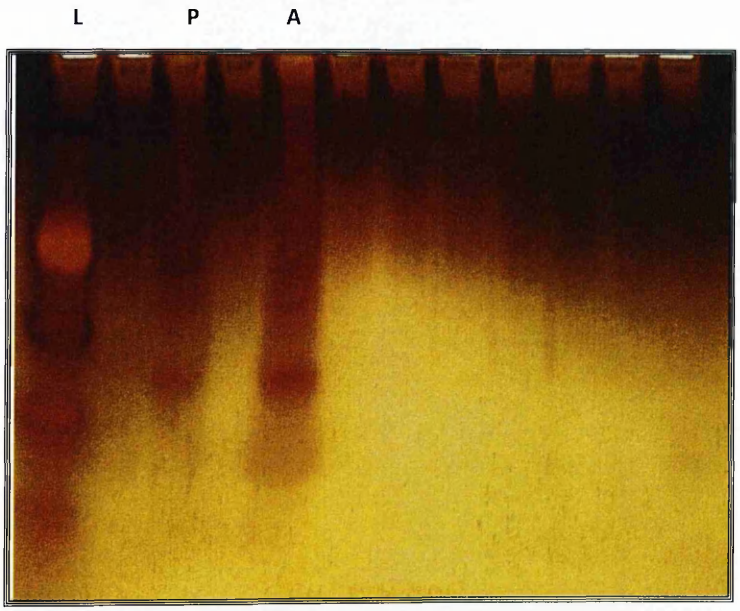


Proteins from attached *L.mex* to EVA comparing with free log-phase promastigtes proteins, silver stain.

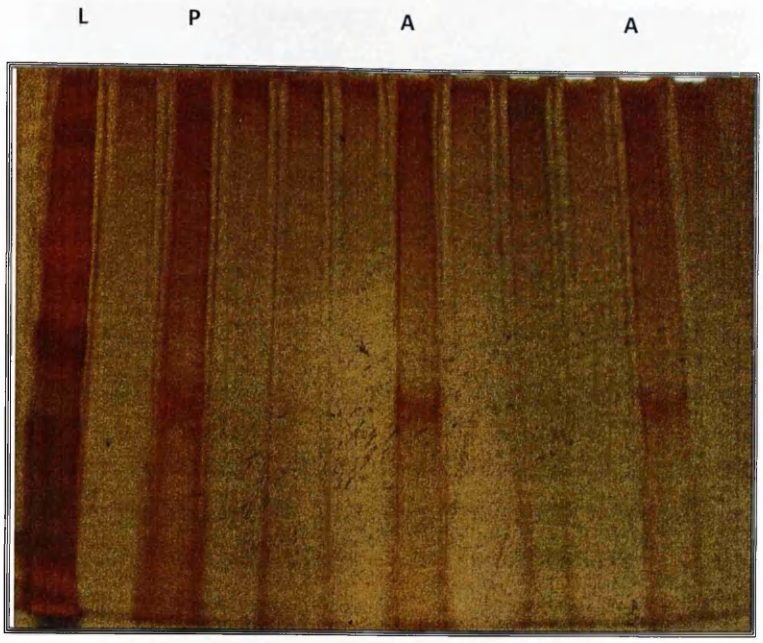
EVA proteins extracted by MME and SMME buffers stained with sliver stain.



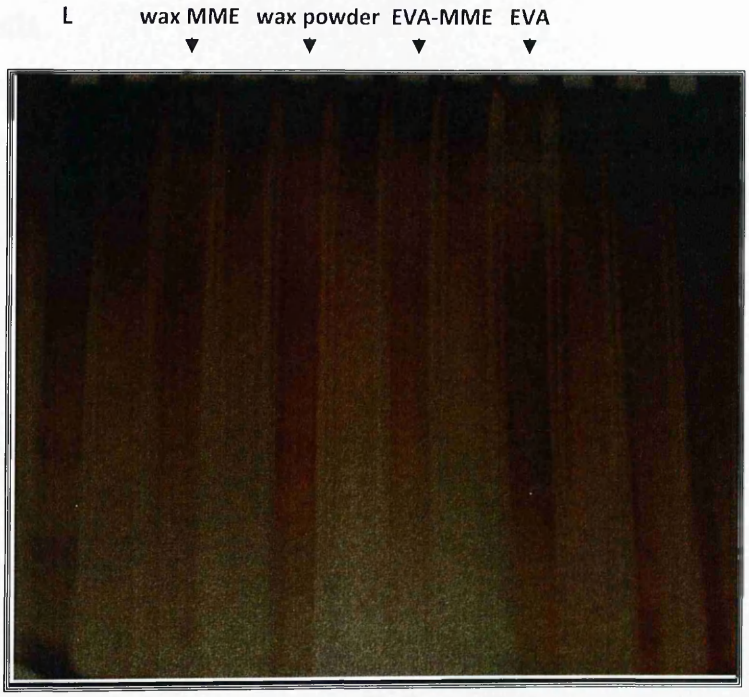
Proteins extractions from hydrophilic filter membrane of attached promastigotes on different days. Numbers are referring to days. Silver stain.



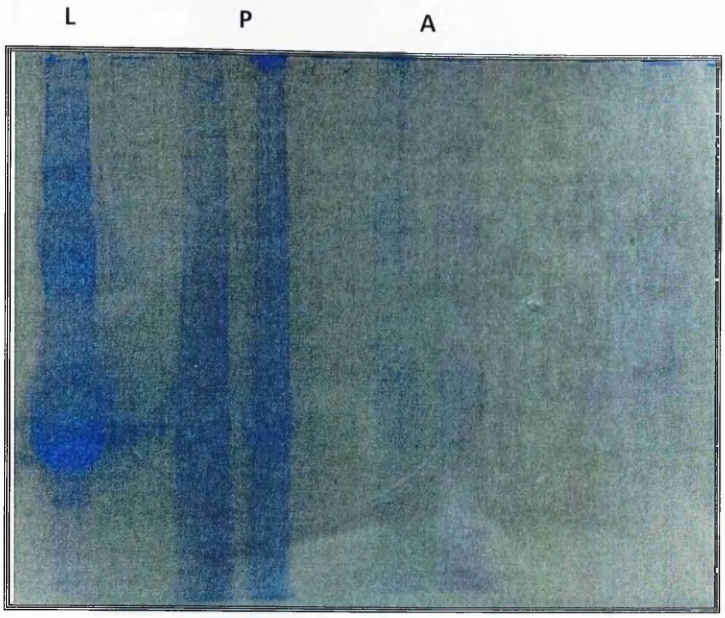
Two days cultures of filter membrane. (A) attached promastigotes, (P) free log-phase promastigotes. Sliver stain



One day cultures of filter membrane. (A) attached promastigotes, (P) free log-phase promastigotes. Sliver stain.



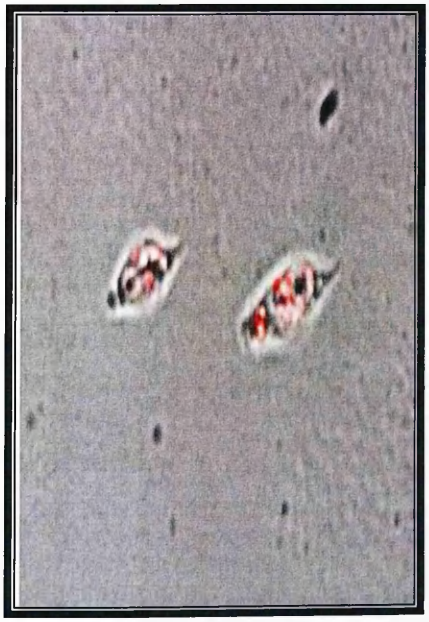
Proteins extractions from attached promastigotes with MME treated- and non-treaded wax and EVA. Silver stain



Proteins extarction from (A) attached promastigotes, (P) free log-phase promastigotes from filter papers, stained with coomassie blue stain.

6.3 Appendix

Fixed L.mex attached on Melinex plastic sheet stained with fluorescent dye



6.4 Appendix

Complete data of sequenced genes identification by *Chromas Lite 2.01* programme and *Tritryp DB* version 6.0

Parasites	L.mex
Gene ID	LmxM.11.0930
Gene name	Hypothetical proteins, conserved
Feature	Trypanosomads only

Sequencing length	377 aa
Molecular weight (MW)	42633 Da
Isoelectric Point (ISP)	10.30
Signal peptide	no
Gene copy	Single

DNA sequence

ATAATCGATCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGA
TAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGT
ACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCGCAAACCAGGAGAAAA
GAGCAGTTTCCGCAGATGAGACACCGCACCACAGACTACATTTACTCATTATTCGTTTAA
TCGTGTTTTAATAGAGCAAAGCGCTGCAGCGCCGATAATAGACAGACATCTTTTTTTTTT
CGCACAACGAGAATCAAAACGTAGAGATAACGCCTAACCCCGCATGTGAAAAAAAAGTGG
ATAATAAAGAACTATGAGAAGTGCCACCACCATTCAGAAGAGAGCATTCATCCCTGGCGC
AGTCATCCCCCCTCCACACTGCCTATTGACTGGATGTCGACAAAACATTNGGGTATATT

Protein Sequence

MVFDVNGDFVENEQQFVEAARKSINLTIKLQNTSGMSELIARVLEDDDRRASGTISTSAL
DFDELLRTTPRFNFLSGDHPLFRRYNKRLSQQRQAAALIAQRTAEAELQRQVEIKERLRR
ALEEEAALQKKKEQEKAERQRMRAAQEPARFVPEEPFLKIIKDESAFGQVEREVKRSEPP
AEPKVESKTEVATSEAITNTATGTSTTLSNDVAATISAEELLVLVGVPTEAMETTTPALE
DLAEAIALPLPENTATYVALPAEEYTLCSGERVVSIIKKRSGPIPAPPPGKPPVINKAAA
SRLNEPVKRAKPVEKMVRQRTPSNEQPYKSRHRSRSPRSRRRSERDHSPKTRSRHHRRDD
PSSHRYHKNSRDSKHRR

Parasites	L.mex
Gene ID	LmxM.30.0450
Gene name	amastin, putative
Feature	*Leishmania* only

Sequence length	195 aa
Molecular weight (MW)	21752 Da
Isoelectric Point (ISP)	8.13
Signal peptide	No/3

DNA sequence

ACTATAGGGGATATCAGACTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCT
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AGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTTGTTTTTTTTTGCCTCCCCTCTCAC
TATGGCAATTAGTGTCCGTTACAGGCATTTATGCCACATCACGGTTCTCCTCATGTCCGC
TGGCCGCTGAACCGCACAGCAGTACCACTAGCCGTGAAAAACGCGTGAAAGCTGCGTCTA
TGCCGTCACGAAGCACCGCATAGCCGGCCATACCGTAGACACAAACAATTTTCGAGAAGC
AGAGAACCGAAAGCGTGTTCACGTACCAGTCACCGGCACACTCGGCACACAGTCCCGAGA
GTCGGACACGCCCCGTCCCGCTCACTGCGCTGCCGCACCTTCACGGTTGGCGGGGCCATG

TCCCCNCCGCAGTACCGTGATGCCGGCTTGCTCGGTTGCCCGCTTCGCGCGCGGGGCTCC
CCATCCGCCCAATGCCGACGGCCGCCTGGTGCGCCGTCGCGTGCGCGCTGCGGCACCCGG
GCACCGCTCGTCCCGAAGCACCCCCGGCGCTCCCGCACAGCGCGGGCCACCNTGGGGTCA
CCCCGCGCAGGGCGCCCCCGCCCGGACGGCAGCCGCCCCGCTTTCCGGAGCCGCCCCAGT
CCCCCGGCCGCCTTCGCCCCCGGCCCGNCCTCGCCCCTGTCGTCGCGCCCCCCCCCGCCG
GTTCTTGTCGGTGCCGTCGCTCGGGCCGCCTCTGCCCGCCTCCCCCGCGCCCCCTCCTTC
CGCTGAGGCGCCGGCGCCCTGNGACATGGCGCTGTTGCG

Protein Sequence

MAVKLGVIIYVVLQLIAFVFVMIGTGVDMFYIKPEFIVVHKFCVTLWGGKSNCRKPQITL
PLNVGWGDCPRIRDNFRAAEAFAIISIFVYGAALLFGCLLLYCCACFRWLCLVLNIVGAV
TAGFVWALMVVTYRIKEPNCQPLSLAYNFGTGFSLFVFAWALDIFNIIFLMLPWQLGQSG
KEAEEHAAVTKAAEQ

Parasites	L.mex
Gene ID	LmxM.36.3990
Gene name	hs1vu complex proteolytic subunit-like,hs1vu complex proteolytic subunit-like, threonine peptidase, Clan T(1), family T1B
Feature	Trypanosomads only

Sequence length	226 aa
Molecular weight (MW)	24694 Da
Isoelectric Point (ISP)	6.12
Signal peptide	No/0

DNA sequence

ATCGATTCACTATAGGGGATATCAGACTGGATGGCAAATAATTATTTTATTTTGACTGAT
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CAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTACAACAACGACAGAATCA
CCGGCAGCGCCCAAACAGAAGAAAAACACAAAGAACAAGAAAAAGATGGTGCGCATAAAA
ATGCGATTTTCACGAAAGCGAGATACACGAGGAAGCGGAGGTCGTGCACTGGCTCGCGCT
ACAATACTGAAAAGCTGATGGTGCTGCAAAGAAGGGACGACGCTGCATGCCAACCGCGGA
GATCAGTCACATGCTTACACCTTCCCCTATTCCTTGGCCTGCGCCTCATTCTCTGCCTTC
TCTGCCTTTTCCGCCTTCTCCGCCTTCTCCGCCTCCTCCTTCTTTACCGCTCCTTCCTCA
CGCGTCAAGATCTCCACATCCCAGTTGCTGTTGCTGAAGACATCGATGTCGGTCGCAATT
CTCATCGCCTTGCGCGCGATGCGCTCTGCATCATAGCCGTCAACGTCAATAAGCGCACGA
GCCGCCGCCTTGGCGTACGTGCCACCCGAGCCAATGGCGATGATGCCGTCGGCCTCAGGG
GTGATTACGTTGCCCTGCCCGTCAATCTCCAACGTCTCCTCTTTGCTGCACACAATGAGG
GACGCCTCGAGGCGGCGGAGGGCACGGTCCGTCCGCCAGTTCTTTGCCAGCTCACCGCGG
CGCGAGACGCCTGTCCCGGAAGTTCGTTCAGCTTGTTTTCCAGTTTTTCCATCAACGCAC
AAGCATCGCCGTGCTGCGGCCAAGCAATTCAACGTGTCGTGAGCTGCCAGCTGCAGCCTA
CTTTCCCCATACCCTCNAGTCCCTAGTCAAGGG

Protein Sequence

MFRRLATRSTSFVTGAAVQARHTTILSVRKGNKVILIGDRQVTLGERIVAKSSACKLRKL
NDNVVIGFAGSTADAFALMEKLENKLNDFPEQLSRAAVELAKDWRTDRALRRLEASLIVC
SKEETLEIDGQGNVITPEADGIIAIGSGGTYAKAAARALIDVDGYDAERIARKAMRIATD
IDVFSNSNWDVEILTREEGAVKKEEAEKAEKAEKAEKAENEAQAKE

Parasites	L.mex
Gene ID	LmxM.29.3440
Gene name	DNA ligase I, putative
Feature	Trypanosomads only

Sequence length	767 aa
Molecular weight (MW)	85023 Da
Isoelectric Point (ISP)	6.08
Signal peptide	no

DNA sequence

TNATAATCGATCTCCTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGAC
TGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTT
TGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGTGTGTGTGTGTGT
ATGCGTAAAAGAGAGGGTGAGAAGGTGCAGTGATGAACTTATTCACCGCGGCCCTCTCTA
GGGTGCCTCCAACTTGACGCCCACGCGCTACATGTGCGCTGCAAGAAAAGTAAAGAAGAA
AAAAGGTAAATGTGGCCAAGAACGCCGCTTGCAACGAGCGAGAAACGTTAGCGGCAACGA
GCGGTCGCGAGATGTGCCAGCGCGTTTCGTTTAGCGGGACAGCTCACTCGGCGTCCCCGT
TCGCGTCGGGCTGCGCAGCCGAGGACTGCGCCTTGTACATGTCCGCCACCTGCTGTGCAC
TTGTGGCGTCTGCGGGTTTCTTGTCCTCGCGCTGGCGCAGGTAGCGAGGAAAGCGCAGTG
CGATACCTTTGTTGGGGTCCACGAGCCCCACGGCTGCCTGATGAACTGGCGACACTGACA
AGTCCGCCGCCTTCACCTCCCACACCTGCGCCTCGGTGAGCCAAACATCCGGCTCCTCTC
CCCGGCACGGTAGTAGCGCGGCTTGTCGTCCACCACGAACGACTTCAGGGACTGCGTGAG
CTCCTCCAGCNCTCCGTCCTGAAAGCGGGTGCCGATTTTGCAGATGCTCTGGTACTCGTC
CGCCTCCGGGTCGTACCACGCAACCAGG

Protein Sequence

MHQTTFERFVGRKAPAGGPSGPDAPREESKRAATEANREDRRRPREEEKVAGPYVSSTPA
SSSDSRLRTSWNEPTNAYYAQHVAEYKALVTDVPPPTATSMAKLLQESSFDPVTTFEAVW
LPPRVPATAPTAGASEPVPFAAVVDVLADISATGSRLECLKQLTFLLLAVIERCPEDLVP
VMYLVINKHAPQHEGVELGIGDAVLVKAVAECCGMTEARAKEEYRQSGDLAEIAQMHKQK
QSTLMKPKPLSAQSVFKTYKEIAMMSGRDVMRRRSDLIKGLLRDAQGPEVNLIVRGLQQK
MRIGLAEPSALAAVGYAFALHFLGDAQMHQMDVVQLQTLLNTGADSVTRIFYEVPSLDVV
LSAVLANGFMTLVPGSSIAKRYAKDLSIRPGLPVKPQLAYPTSSITVILDRLQGKKFTSE
YKYDGERAQIHYDKEKGFYIFSRNSETHTGKYPDVISMLPKVFDPAEVDSFILDSEVVAV
HPETGVLQAFQVLQHRGRKNIAEEDVIIPVCVFVFDILYFNGEPQLNKTLQQRRELLWRC
IHPLPAKLSFATYLDSDKVEDVQTFLERSIADGCEGLMVKTLEEEANYTPAKRSHYWLKL
KKDYMDGVTDTLDLVPIAAFYGKGKRTGVFGGFLLACYDPKADEYQSICKIGTGFQDEEL
EKLTQSLKSFVVDDKPRYYRAGGEEPDVWLTEAQVWEVKAADLSVSPVHQAAVGLVDPNK
GIALRFPRYLRQREDKKPADATSAQQVADMYKAQSSAAQPDANGDAE

Parasites	L.mex
Gene ID	LmxM.25.0910
Gene name	cyclophilin a
Feature	Trypanosomads only

Sequence length	177 aa
Molecular weight (MW)	18794 Da
Isoelectric Point (ISP)	7.94
Signal peptide	no

DNA sequence

GTNTAATCGTACTCACTATAGGGGATATACAGACTGGATGGCAAATATATGATTTTATTT
TGACTGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCA
ACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTTCTTATTTCGGGCAAAGAGAAAAA
AAACAAAAAAATGCGGCCCTTTTTGCCGTTGATTCTCCCGGAACCCCCCCCTTATCCCCC
CCCCCCCCCCCCTAGAACCCTCTCCCTCCCCCCCCCACCCCCTGGGCAAAACCCTCGAC
ATGCCCCCCTTGCCCCCACCTTGTTCGTTTTTTGCTTGGCGTAATTCCGGAAAAAAAATA
TTGTGTGCTTTTGGTGGTGCGCTTGTGTCTTTTTCTTGTCTCCCTTTTCTTTTGTTAATC
TTTTATTTTTTTCCGATTTTTTTCGTCTTCTTTTTAAGAGAGCTGTGGTGGGTGGTGGTT
GGTTACTTTTTTTTTCTGGGAGTCCTTGAAGGCTAAAAAGCAAAGGGGGGCCGGGATGGG
GGAGGACTTTCCCCCCGATTTCCCCCTGTGGAAATTTTTGAAACGCGTCCCGCCTTTTCC
CCCTCCCCTCCTGCTTTTTTTTTTTTCCGCCCTTACCCCCGTTGCCCCGGCGGCTCGGTC
CCCNGCGTTTGAACCCCGGTTTTGGCGGAAAGGCCGGTCCCCGTTATTGTTCTGGGTGCC
TTTTTCCTTTTTTTCTACTCCCCCCTGTTGCGAGGGTTTCCCCCCTTTTTCTTGTCGCCG
TGGGGTTCCTCCCCCCTCCGGGTTTTCCCTTCTTTGGTGTGGAGCGCCTCCCTCCCCTTG
TTGTGTTTTTTTGTCTTTCCTCTTGT

Protein Sequence

MPYKPYYPVVESNPKVWMDIEIGGKSAGRVTMELFADAVPQTAENFRVLCTGEKGFGYSN
SPFHRVIPDFMCQGGDFTAGNGTGGKSIYGSKFADESFVGKAGKHFGPGTLSMANAGPNT
NGSQFFLCTAPTSWLDGKHVVFGQVLEGYDVVKAMEAVGSRSGVTSKPVRVSACGQL

Parasites	L.mex
Gene ID	LmxM.23.1020
Gene name	hypothetical protein, conserved
Feature	*Leishmania* only

Sequence length	117 aa
Molecular weight (MW)	12507 Da
Isoelectric Point (ISP)	3.8
Signal peptide	No

DNA sequence

CTATAGGGGATATCAGACTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTCGCGCTCATTTACGCAGCCAAGACGCCG
CCGTCTATTAGGAAGCACTGCCCGTCCGCTCCTCTGCACACACTTGGCCCTCGTTGTTGT
TTGCGATTTCTGGTCTTGACATTTTCCGTAGGCTCAGGCTCGACGGTGACGCATTTCGCT
TGTCTTGCATGTCGCTCACTTCCCGCTAGACATCATGTCCCGTCAGCGCCCCTCCCCCCT
CTCTCCTCCTCTGAGCCGAGTCCTCCGGGTCTCTTTTGTTTTCGCCCGCGACGACCTCCG
GAAGTGCATACAGGCATGTCATTCTTCAGACCTCCTCATGACAAAGGCGCGTGTCGGTCA
TCATGCGCGACAGGAAGCAAAAGCTCGTCAACTGAGTGGCTGAAAAAAGTGTGTAGGCGC
AGACGTGGTGAGCTTTTTCTTTTCCTCTCCGTGTCCGTCCACTGTGCCGTAAAGCATGAG
GTGATACTTCCCACTCGACCTTTCGGGCTGTCCCAATCCCCCCTCCCCTCNTCGCGTGGT
GCGAAGTGGCGGTACGCCCGCGCTACGGCAGTGCGCCGACTCCGCATATGCGAGCCCGGG
CACTGCCTCATACTCGCCCCCCTGCCCTCCCGGGTCGCCTCCCCGTCGCTGTCTTCGTGC
CGGGTGCCCCCCCCTGGAGCTTTCCTCCGAATGCCCCGGCTCCCCGCCCCCGGAATGCAAC
GCCGGCTTGGATTGCTCGGTCGCGCACGCTCCCCTGCTTGTGGTCGACCCGGCCGTCCCG
GTCCCGAGCCCCCCGTCGGGACAAACG

Protein Sequence

MGSFCAKPDAVPKDNAHEGEKNYEAGGEKSFNDANVEAKSPHQQCDKDDAAKDVEETDDA
AKDVEAADDAAKDVEAADGEAKCAEAVDMEEKQEAFEVDPKNELNDVEGQAGETQDE

Parasites	L.mex
Gene ID	LmxM.32.0792
Gene name	beta tubulin
Feature	Trypanosomads only

Sequencing length	443 aa
Molecular weight (MW)	49725 Da
Isoelectric Point (ISP)	4.45
Signal peptide	No

DNA sequence

TTATCGATCTCCTATAGGGGATATCAGCTGGATGGCAATAATGATTTTATTTTGACTGAT
AGTGACCTGTTCGTTGCACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTAC
AAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTCGCTTCGCCAGGAGACATC
GAGAACAAATTTGACATGGTAGAAAAGAATAGAAAGGCGCCGGCGTGTTGTGCCGGAGGG
GGATGCTCACTCGCTCCGACACGCCCCACGTGACGCCCGCCTCCCTCCATCCCCCGCCTC
TCCAGACTCGCCCAGTGACTCCACTCTTCGGCAAACAGGTGTGCTGCACGCGCGGCGCGA
CACCTAGGCGAACGCACAAAGGGGGTGTGGAGCAAGTGGTGCGAGGAGGTCACAGGGGAG
GCGGAGGAGGGAGAAGACGAAAGCGCGCGCGCAAGCGAGTGTCGCTGCACCGGGGCACTG
TGCCAGCCTGCCCAACTTTTTGTACAAAGTTGGCATTATAAAAAAGCATNGCTCATCAAT
TTGTTGCACGAACAGGTCACTATCAGTCAAATAAATCATATTTGGGGCCAACCGATATAN
GCTCNGATNATNTGGACTGGCAGTT

Protein Sequence

MREIVSCQAGQCGNQIGSKFWEVIADEHGVDPTGSYQGDSDLQLERINVYFDESAGGRYV
PRAVLMDLEPGTMDSVRAGPYGQLFRPDNFIFGQSGAGNNWAKGHYTEGAELIDSVLDVC
RKEAESCDCLQGFQLSHSLGGGTGSGMGTLLISKLREEYPDRIMMTFSVIPSPRVSDTVV
EPYNTTLSVHQLVENSDESMCIDNEALYDICFRTLKLTTPTFGDLNHLVAAVMSGVTCCL
RFPGQLNSDLRKLAVNLVPFPRLHFFMMGFAPLTSRGSQQYRGLSVAELTQQMFDAKNMM
QAADPRHGRYLTASALFRGRMSTKEVDEQMLNVQNKNSSYFIEWIPNNIKSSICDIPPKG
LKMSVTFIGNNTCIQEMFRRVGEQFTGMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLVS
EYQQYQDATVEEEGEFDEEEEAY

Parasites	L.mex
Gene ID	LmxM.36.3620
Gene name	hypothetical protein, unknown function
Feature	Trypanosomads only

Sequencing length	103 aa
Molecular weight (MW)	11807 Da
Isoelectric Point (ISP)	4.37
Signal peptide	No
Gene copy	Single

DNA sequence

TAATCGACTACTATAGGGGATATACGACTGGATGGCAATAATGATTTTATTTTGACTGAT
AGTGACACTGTTCGTTGCAACAAATTGATAAGACAATGCTTTCTTATAATGCCAACTTTG
TACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCAATCTCGCGCCGATA
CGGTACCTTCACCCACTCTACCCACTCTTTCGCCCTCGACCCACACAAAACGACATACAC
GCATACATAGACTTATAGAGCCGTGCGGCTGTGTGTGTGTGTGCGTGTTTGTGTCGCC
GTTTCCAGGCGAGACACATACACGGTGAAAAGGAAAGAGAGAAGAAAGGCGGAGAAGACA
CAAGCATCGCACAAGGAGAAAAATAAACAAGCACACGCGCATGCATACACACACAGACAC
ACACAGACAGACGCAAGACACACACACGCAACGAAAAAAAGAAAAGAAAAAGGGAAGGAA
AACAAGACAAAAGTACGCTATCACATCGTACGTGTATATATACNGATGATAGAAGCGAAA
AAAGACGGATCGGTGATGCGCTTTGCGTATGTGTGTGTGTGTGTGTGTCTCGCTCTTC
GGGGGGAGAAAAGCGTGTGATCTGGTGGCCGCGGGTGTGGGTAGGTGCGTGTTTGGGTAA
CTGGGCGTGCGAACCCGAACGTTTCCCCTCCTGGCGTTTCCCTCTTTTCCTCCCTGCCCC
CTTTGGAAGCCGGAAGTTGGGGGGGGGAACCGGGGGTTTTGGGGGGGAAGGGGGGGAAAG
GGGGGG

Protein Sequence

MQQSLIPGHNNNRHLTYEEVENNIGGLPVTDNRIQDLFDSLDVDHSGAVSVEVVKRFYMG
LEHYGVDLSDKELDEMVRKHAATHEESLNYEEFACFVLSLAQW

Parasites	L.mex
Gene ID	LmxM.22.0420
Gene name	40S ribosomal protein S15, putative
Feature	Trypanosomads only

Sequencing length	152 aa
Molecular weight (MW)	17436 Da
Isoelectric Point (ISP)	10.8
Signal peptide	No

DNA sequence

TNATAATCGATCTCCTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGAC
TGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTT
TGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCGTTCTGTTTTCGT
AGTCGTCGGAGTTTTGCGCGCGAGGGGGTGGGTGAATGCCCCTCCCCCCTCCCAACACGC
AAGAAAATCAGAAAAGAAAAAGAAAAAGGAAACGGCCAGACCGAGAAGGGGGGAGGGCAG
GAGCATCTGAAGAGAGACTCGTGCCATCACGCAGGTCGACTGTTGCCTCTCGCCTGCGTG
CCCGCGACTCCCTCACCCCGCACGGAAGATGAAGACGACGAGCGCAAGAGGCGGCGGCGA
GGGCAAGGCAGAAAGAGATGAAGGCAGAGTTCCCCGACAAAGAGAGAGAGCGACGCTTTC
CTGCGTGCACACCGTTCTCACCCTCACGCGACCATTCAAAAGCTTCATCCTCGCCGCCCT
CCCCTTCATAACCCGTTCACGTACACAGAGAGCGCCTACTTGATCGGGATGAAGCGGGAG
GAGTGCGTGGCACCCACACCAGGGCGACCGTGCAGCACCGGGCGGTAGCTCATCGAGAAC
TCGCCAAGGTAGTGGCCGATCATCCTCACCCCTTGAATCTCCACAGCCGTTTGAAACTGG
GGTGGCCCGTTGGTTAGAATCGCCCACCCACGGGGACCCCCCACCCATTCTTCCCGGGCG
TTGAATCACCAACGGTTCGCCGCCANGGTGCGTTTTTGAACAGCCCTTCGGGCTTTTCGC
CAAACATCAACATACATAAAACAAAAAAGCAAGCCCTAAGGAACACGGGCGGCGGGCGGT
CCCTTGGCGGTATGTTGCCGGCCCCC

Protein Sequence

MASNITAERYEQLKKERTFHKFTYRGLEIDPLLALSEEEFKALVHARARRNMNRHADRRP
PVLLKRLREAKKHVKVGEKPKAVKTHLRDVVITPEMVGSVVAIYNGHQFNAVEIKGEMIG

HYLGEFSMSYRPVLHGRPGVGATHSSRFIPIK

Parasites	L.mex
Gene ID	LmxM.03.0440
Gene name	60S acidic ribosomal protein P2, putative
Feature	Trypanosomads only

Sequencing length	110 aa
Molecular weight (MW)	11034 Da
Isoelectric Point (ISP)	4
Signal peptide	No/3

DNA sequence

TTATCATCTCCTATAGGGGATTCAGCTGGATGGCAATATGATTTATTTTGACTGATAGTG
ACCTGTTCGTTGCACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGA
AAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGGGGCGCGCAGAGGAAGGGGAAA
CGCGCGCGTAAATAAAATCGAAAAAGAAAAGAGAGTAACGACACACGCACACACACACAC
ACACACACACACGCATGCAGGCAGGCAGCGCTCGCACGCATGCCCGGGTGTCGCAGAGCA
TGCGGCGGACAACCATGTGCTCGCCTATGTCAGTCATCACGCGTCGATCGACTTAGTCGA
ACAGACCGAAGCCCATGTCGTCGTCGCCCTCCTCCTCGGGCTCGTCCTTCTTGTCCTCCA
CCTTGCCGCCGGCCGCTGCAGCGGAGGCAGCGCCGGCCGCCGGAGACGGGGCGGACGTGG
CCGTCGGGGCCTGTGCGGCGGCGGCCGCCATCAGAGTCTCCACAGACTTCTTCTCCAGGA
AGCGGGCAAAGATAATGGGCAGGGTGGGGCGCATCTCCACGCCGGCGGCCTTCACCGCCG
CGGCGATGTTCTCGGCCGAGTGGGCAGCCGGCGTCGCTCAGCATGAGCCGCGTACTGCAC
CCAGGTTCAGCGACTGTGATGATGACAGTGCCTAAGAGCACTTTGTACAGTGCATATAAA
ACTGTCTATGTCACACGTCATCATAATATTTGGCACA

Protein Sequence

MSAETLACTYAALMLSDAGLPTSAENIAAAVKAAGVEMRPTLPIIFARFLEKKSVETLMA
AAAAQAPTATSAPSPAAGAASAAAAGGKVEDKKDEPEEEGDDDMGFGLFD

Parasites	L.mex
Gene ID	LmxM.30.2270
Gene name	Hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	462 aa
Molecular weight (MW)	48784 Da
Isoelectric Point (ISP)	4.94
Signal peptide	No/1
Gene copy	Single

DNA sequence

CTATAGGGGATATCAGACTGGATGGACAAATAATGATATTTATATTTGAACTGATAGTGA
CCTGTAAACGTAAGACAACAAATATGATAAGACAATGCTTTCTTATAATGACCAACTTTG
TACAAGAAAGTTAGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTCGTGCATGGTATGCT
TCCACATGACAAGTGGTGGTGGTGGTGATCACCTTTGAGACGAGAGCAAACACGAGTTGC
TTGAATGAAAACTATTAAGACGTCGCTGGCCTGCTTCCCGTTTCCGTTTCGCGTCCCCAC

GTCACCATTACAACAGCGAGCTCGTCGACAGATGTGCACGCTATACAACACACAACCAAC
AAGCGCAACGAGGATGACCATCCCGATAAGACAACACNCGGGGCGGGGCAACCCCTGGAA
GCCCCCCTCCTAAGGGGAACGAAAGCCCCGAACCTGGCCCCCTACGGAAACACGGGGGGG
CCTTTGCCGTTCGACACGGGGCTCTTCTTGCCCTCCCTCGCTCCTCCCTCGCTTGCGTGC
TGCGTCTCCCGCGAATAGGCGGGCCTGGGNGCACTGCACTTCTGGATCCCAAAGCGGGCC
GCGTCCAGGGTCCTGGTCGAGGGCGGAGGCACCGGTGTCCGCCGTGCGGCTAGTGGAGGG
GGTGGCCTAGGCACTTGCCGCTTGGGCTCGGGGAATGAGTTGGGCCGCCGCAGGCCCGGA
CCATCTGGAGACCCTGTGCTCCGCCTGCGTGTGCCTTGCCGTATTTCGGTGTGGAGGCCC
TCCCCTTGGGTGCCCGGGGGAATCGATGCTGCCTTTCGGGGCCCCCTCCCCTGCTGCGGC
TTCTGTGCCCCGCCGTCCCCGGGTTGGTGCGGGGGTGCGCNGCCCGCGCCCTGCCGGGGG
TTCTGTTGGTTTGTTTCTCGGCCTTGTTCGGTGNGGCCCTGCCCCGTCCGGCCCCCGGC

Protein Sequence

MSSDSGVNAALLNLCAQLQGGDARPVANEGPSAPNHAEVSGETAEQASPSAAACATALAA
TPAAAAQGPARPASDYEWLRNALVSVESPEKRVKQLLVHMENQTTEGKPGPLVQEERLEA
LAELADMVEDVNWAAEFALMQGPKRLLDVMRRERAAHPLLATGSRDASASVEAADTTGTG
DREAQTATHDGISSSAVPLFTELAMIVAHSAQLNAPVQAAYEAAHWEDIILPFMGDCIAA
VQHLLHLGSDGQVGSAHGDSTEAATVAAGAASLMRLLGALLHACSCLCRDCSPNTVSFFQ
ASGLAVIVEALRLTRALPESIVVGTAEDGRTPAVVTSIANDAASDQEVDDIYAPLLGTTH
KVTARALFFVAYLASTSVSSEEIIQLTCRHAESRNSGERVQKSAARALTALVEKSPKAIK
EAVHTLMPHRLHEWRTQVRRAVGDRETQDERLHFLDALDRIS

Parasites	L.mex
Gene ID	LmxM.36.1430
Gene name	translation elongation factor 1-beta, putative
Feature	Trypanosmads only

Sequencing length	208 aa
Molecular weight (MW)	22954 Da
Isoelectric Point (ISP)	4.49
Signal peptide	No

DNA sequence

CTATAGGGGATTCGACGGATGGCAATAATGATTTTATTTTGACTGATAGTGAACCTGTTC
GTTGCACAATTGATAAGCATGCTTTCTTAATAATGCCACTTTGTACAGAAAGTTGGGTGA
ATTCTTTTTTTTTTTTTTTTTTTTTTTCGCCCTGCTGAGTCTTGAGCGGCTTCTCTGCCTT
TTTCTTTCCTTTGAGGTGAGGGTCCGGAGAGCTCTCGACCGTGCGGAGGACGACGGGAAA
GGAAGCTTTATGCCCGGTGTATATCCGCCGAACGTGCTAGCCCAGCTTGGGCCGTCGGCT
GCGGGACGAACCCGCGGGGCCGACCAGAGCGACGTGCGGGTGCTTAGAGTGGANGATCNT
TTGGGCGANGGCACTTCGTCCGTTCTCTTCGTGAGACGCGCTGTCTGTCGCTGTGCCCCC
TGCCGCGACGACCCACNCNCNCGGCNCTTCCTGGCTCCTCGGTTGTGCCCCGTCGCCCTT
GTGCGCCCTCGAGTGTTGCCGGNGGCGCCCTCTGAGACGACGCCGCTCTGGCGTGCCGAC
NCGCTGGTGGGGCNCCTGCGCTCCCTCGCTGCCTCCCCTTCCGTTTTCGGGCTTCCCTTG
TGGTTTCTCCCACCTTTTTGGTCCATGGTTGGCTTNTTGCAAGCTTGCCTCATTGTGCCC
GNCGTCCTTCGTCTTNTCTTGGGCCCGGCTGCTGCGTGCTTCCCTTGCGGAATCTCTCTC
CGGTGNGCGCTTCCAGCTGGTGCTTTGTGGCCCTGTGCTA

Protein Sequence

MSTLKEVNGRLNAQPFVAGFSPSSEDARIFSEMFGNHPNVIQWVARMASYYQAERDEMLN
AGSEKKATEPAKATAPAPAAAAEDDDDIDLFGETTEEEKAALEAKKAKDAEKKKAKKEVI
AKSSILFDVKAWDDTIDLGALAKKLHAIQRDGLIWGDHKLVPVAFGVKKLQQLIVIEDDK
VSGDDLEEMIMGFEEEVQSMDIVAWNKI

Parasites	L.mex
Gene ID	LmxM.05.0450
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	196 aa
Molecular weight (MW)	22358 Da
Isoelectric Point (ISP)	4.58
Signal peptide	No
Gene copy	Single

DNA sequence

GTNATATCGTACTCCTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGA
CCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGT
GAATTCTTTTTTTTTTTTTTTTTTTTTTGTCTTCACGCTGTCAATGAGCTTTCGAGACAAGATAGAGGA
CTTCTTGCCACTTTACCGGTGAGCATATACAGAAAAACCTTTAGTGATCGCCTTTTCCAATGCCGCGCA
TAACGCCACATTCTATATGTATATTATATATATATATAATTTTTTCTCTTCGATCATGCACATGTCGAC
ACACTAGCACAACAAGAGAGCTTTTTTGACACCGATCCCTTCACAAATTTCTCACTTCTTCGTCGTGGC
TTTCACAGCATCATCAAACCACGGGTAATCCTTCGACACCTCCGCCAAATCCTCGCTCGTAATCTCCGT
CTCCTTGTCGAGGCCCATGACCTCGTGGATGATGCCGTTGTCGTCCTTGCCGCGGAGCTTGCAGCCGAG
CCCGATACACATAAAGTCGAGCAGTCCCTGTATGCCAAACTGCTCAGCTGCCGTCATTGTCAGGAGGTA
ATGCTTCTGGTTCAGAGGCGAGCAAAGACGCTGGTTGAAGAAGTCATTATTCCACTTGTCTGTCACAAC
GTAAGAGAAATCACGGTAATGCATGGGCGGGTCAGCTGGCTAGCAGCATGGCATCTACACCGTAGTTGG
ACGCCTAGCTAATAGAATGCTCAGGACTGCGGCTGGGTATCCAACCTTGGGATCCTCCCCCACCTTCCC
ATGTACGGGCCTGTTGTGTTATTTTGACCCCAATTACTCCTCTCCATGCCAATTTATGTCCGCTATTCA
CACATAGATCAACTCCATCTATTTCATAACCCCCCCCCGTGAGAATTGNTAAAAGATACCCACTCCTCT
CCG

Protein Sequence

MADEGSMDIRLVLKGPNGEFKVDRDLYIIVHCDDGKYIEVSKNYTKQCPFIEEVEGEIPE
FGYPAAVLEHLIRWAVHYGVDGHAASQLTRPCIYRDFSYVVTDKWDNDFFNQRLCSPLNQ
KHYLLTMTAAEQFGMQGLLDFMCIGLGCKLRGKDDNGIIHEVMGLDKETEITSEDLAEVS
KDYPWFDDAVKATTKK

Parasites	L.mex
Gene ID	LmxM.31.2500
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	162 aa
Molecular weight (MW)	19266 Da
Isoelectric Point (ISP)	9.43
Signal peptide	No
Gene copy	Single

DNA sequence

TNTATCTACTCCTATAGGGGATATCAGCTGGATGGCAATATATGATTTTATTTTGACTGATAGTGACCT
GTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAA
TTCTTTTTTTTTTTTTTTTTTTTTTGTCTCTGTTGCCCACATAAAACGAATGCGCATTCAGGCAAACCA
GCCACATACTTGTGTTTGTCGGTTGCGGTCCCCAGTCTCAGCCCCGCGGCGGATTGTGTGTAAGTCCAG
AGTCTCTCACCGCAGCCCAGCCCTCGTGCCGTGGGACAAGGAAAAGCAGCTAAGCAGACCCCCAGCCCA

CCCGTGCGTGCGTGCGTGCGTGCGAGAGAGTGACTGAGTGGGACAGAGAAAGACGAGTCAAAAAA
AAGGCACGACAAAGACGCGCAGAAGACACCGGCGCAGCCAACCAGCGTAAGCGAGCTCGCTATGCCTTC
GCAGCACAAACACGGTGTGTCTCACGACAGCAATGCGAAAACTTGGCAGGCACGCCCCCGAAACACGAG
CGTGAACCTCCCAAAGCCGCAGCCGCACCTCGACTGAGAAGAGCCGTTGGCGAGATGCAGCGAGGCAGC
CGCTCCTTCTCCCCTCTCCGCGGTGTTGATGATCCGTGCTCCCGCTCTGAGTTGCGTTCCCTGCGGGCC
TAGCGCCGGCGCCCTCCTCGAAGTAGTTGTTGTTGTACTATCGGCGNGCTGGTCAGGCGAGGCCCCCTC
CGCCAACTTTTTGGTTCCAGTTGGCTTTTTTAAAAAGCTTTGCTCTTTATTTGGTTGCCACGAACGGGT
TTTTTTTTTTTCATTTAATTTTTTTTTGGGGGGCCAGCTTTAAAATGCGGGTTTTTTACTTTTTGTGTT
GGGAATTTTGTTTTGCTTTTCCCGAAAATGTGCAGAATGTAGCTT

Protein Sequence

MAGAPPVSFVHTVTRTLQHDIRTFTHWDPAVNKLQCAIRDAAYLVIDLPRFFMYSHSNYG
AMRRYIRQSRINKGKINPEDFKDVDTNVLRESLIKYAQAKGPLQKIDFRWFYWMYAVMII
YVGWQGMYLQSLLDARVDRMGASLEQRRDMYDDDYLEEVRPR

Parasites	L.mex
Gene ID	LmxM.36.5060
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	440 aa
Molecular weight (MW)	48261 Da
Isoelectric Point (ISP)	6.52
Signal peptide	No
Gene copy	Single

DNA sequence

GACCAGCTCTTAAAGACTCGGACCCCAAATAATGATTTTAATTTTGACTGATAGTGACCT
GTTACGTTAGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAA
AGTTGGCTGTACTTTATTGTGGGCGTCTCTCACACAGCTCGACAATCGAAAGAAAGAAGA
GCAAGTCTCTTTGCGTCCTATTCAATTTTGTTTTCCGAGTTCGCGTTTTCGCGTTTACGT
GTATGTGTGTGTGTGCAGCCGGCAGCCTCTTTCTTCCGGATCCGCACGCGCCAAGTCGCT
TCGCGTATCACTCTTTCTTACAACACCACTTGCATTGCAAGAGAGGCCCTCATGTCGTGG
GCAGCACTCGTGAAGAGTATAGCAGCCGGAGGAGGCGACCAACTTCACGGCGAAGCCACG
AGTCACCGTGTCCGCCGTCGACCCATCGCAGAATGATGCGGAAGAGGCAGCTGCAGAACC
CGCAGCACGAGACGGCAGAGGTGAGGCTGCCGTCGTCCTCCGGAGGTCTTCTCATCTTAG
ATGCCTTCGTTCGTCGTCGGGGGCTTGGAACTTCTTTGTCTCCACCGCCGATGCGCTGGT
GACGACGCCGCTTGTGATTGTCCAGATCGATGATCGTGCATCGCGGGAACTGTGGAAGCG
GCTACCGCTCCTAGTGACGGTGCTTGACCCCCCCCCCGGGAGCCGTCGCGTCGTNGTTGCT
GTGGCGAAGCCCACGGTACTTTGTCTTGTCCCGCCCGTCTTCGCCTTGCGCCCCCCGCTG
GCTGTNTGGGGTGGTTGGTCCCCGCCAACCGATCGACTCGCTCCTCCGGNTGGACCGGGG
ACCCCGCGCCGGGTCCGGTTATGCCGGGGGGAA

Protein Sequence

MSWAALVKSIKPEEATNFTAKPRVTVFAVDPSQNDAERQLQNPQHETAEVRLPSFSKGLL
ILDANAIIKGMDNFVSTADALVTTPQVIVEIKDRASRELLERLPHKVTVLDPTPEAVAAV
VACAERTGDFGAMSRTDIRLCALALDCCKVGGFLGEPIEPRPPQVNPGNADKVQVMTEEM
GEDDSDDEREKSEPAQAQSASSGSMPGWGDWSNEAKGTDGEGGAGDADEDDGEGEWITPE
NIQDVQSGTRRTGRAFEAGMACVTSDYAMQNTLMHLGVPIVGTNGIHIRELRLWMMRCTA
CFTLVGDTTRQFCPECGSGDTLRRVNYVVNDQGEKKLYINFRKRISTRGTIYNLPKPRGG
MRGTNRNLVLREDQLAHVIRGTTSSKVKAHQVMQNDDGALATFGEAPKLKKKNLADPRAY
SSYHKYNVNEKKKVRAAHRK

Parasites	L.mex
Gene ID	LmxM.08.0410
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	1401 aa
Molecular weight (MW)	148526 Da
Isoelectric Point (ISP)	7.35
Signal peptide	No
Gene copy	Single

DNA sequence

TAATCGTACTCACTATAGGGGATATCAGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCT
GTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAA
TTCTTTTTTTTTTTTTTTTTTTTTTGGTCAGCTGTGGTTGCTTCAGACGACTCACTTCACGTGTGCTCG
CCGGCCCCATCCCTGAGACTCTTCTCGGTGCGGTGCAGAGGTGAGAGGGACGGGGTGGGTGCGAGGAGG
CCTTGAAAGGCGCGGAGAGAGCAAGAATAGGTCTGCGTTGAAGGTGGTGGGTGGTTGGACAGAAGAACA
TACAACGGAGACGGAGCGAGACAGCACCCTATTCACCCCCATCACCCAAATGATCAACTCTATGGTCAG
AGACATCCTCATCAACACACACACACAGTCACACACAGAGGCAACACACCATAACAAGAAACTGAAAGG
CACAACGGTGAGCACAGGGAGGGATGAGCCACTATACACATACCCGCGTCCGCACGTGTGCATACACCC
ATGACCATATATATATATATATCCATATACATGGGTATCGCTATCTATGTATGTATGCGCATACACTCA
TCTCTCTCTCTATATATACCCTAGTGGCATGTAGAGCTACATCAGGAAGAGCACACACCCAGTCCCACC
CACCCNCACNCCCCCCCCCCCCCCACAGAGCANGCGTGCAGACACAGCACACGAAAAGATGTCCGCACC
GCGCATCGCCCCCGACGAAGGATGATACAATCCCAAAGACTCGTAAGGTACAGTCACCTTTTGGTCCAA
GTGGCTTTATAAAACCTTGCCTCTCTTTTGTGCACGAACGGGTTCTTTGTTAAATAATTTTTGGGGCCG
GCTCCATACTGCATCAATA

Protein Sequence

MALSPTAYISPGCSAQCQQCVSAKPDSFAFASAQSISLYRVSTANVTIPTSTSNTPAQAQ
QQQQQSLETIAITNYPLVTLFGHGANAAIGAFSYNDDYMACLTPQNKQILLWRLKDAETL
TAKKITSTALSDTFKREGNPSTMCLAGRHHVLCGTNTGRLISLNTNLDNAVPHSVAIPPS
QQRGEARQSRSPLSPRNAYSVPAAAAPLSSLNPSGTSNAAAAAVESVECIVAATARPEVV
ACGTSDGTLCLLTLNASTGLLVTASLCPFPAKEKSDTVLDVNPLPVTSLAFEPTSAQYLV
VGSQDGALALCDMNKNSIVQTFEVNKLPEKHISSIAWIPGEAGAFYTASTDSPVLRKWTV
SSKSVVGSVSIMMMHPTPQQRCSGACGAADASNSADAEHSSSRTGIRSVACIDQTRVVVG
LTNGAVKVYDVAQQRLECDIVTGHTDATLSCKLSKHDRDQAATGGVDGMIRVWNLRTLSQ
QYSIPVGPVMVHSVDWSPNGKHLIAALGSGEVVMYSTSTNRESWRTPVFSELVYRVCWAA
GDSSLIAATSRSGVAVLSSKDGKVVRRYPATRGAFYGVDIEPTKSKMIAAGSHDHRIYVY
NLSSSSDRPVHVLAGHTDAVCDVAYNPTALNYLLSGSYDGTLRVWDLSSNDTHTISVSSR
ALKGHADRVRSVAWCSLAPYLVISGSADASIRLWDIRNGVAITTVRGHNADVVAISSHVD
RPLTFLSAARDSTLVAWNVALLRQVYLDAALGTLESCIVADPSSLMGVAASNVTVSQVAG
AAVQRLAKELAECASRPAERLQKLVSFFEFPNGAAEVAEMALCAVDPAAYQVAVAEGKTA
ATGLVVPARSLAEAARARATYTNERAHGKSVNAAGPSYKKQRLLEAADEFLRVGQLEAYC
DLLVEAEEWDRAIAASPAISRAYWRSVCQKAAEAMEATGDARAVAYYIIGEHAHKAAQLL
TRLSERHYDAATVVCQTCPQVAEDPQQQQANEPPHNTTVDVNSVTAATQQLQRQRAAVLK
RYANPQLFAAVLLAYGHHDEAVNVLQHCGDVVLAHLLVHTVPLREQASIDTAFRLSMLQS
ARQQKWDTALTCATRQSNPYDALATVLALFQTAQGKQLVGKTTAPSLTPGNLSTLQGVGE
RLKTFYEQVRGECGKLQLPLDAAAIQQRHAHDGLASQNQLAAMVLVADPSSGPMTDGAIL
QSLSGFMESLLAVALKEIDGATTPFYLRQAYNVSAYVSLPFETPSKAGGNLSVSLSASAV
ISTMTPEHKRFLALAFIVAALMAVKVYRFPKLLNSAFTKARELAAASGSASLSTILTNTQ
GALGTYSPHSKEVDCSSVGCTVPALSSEGRQIVSVLTGDPVCGAVYVLEDGSSFISKSEA
LAWMLCSHFSPLGSGARLTAL

Parasites	L.mex
Gene ID	LmxM.13.0280
Gene name	alpha tubulin
Feature	Trypanosomads only

Sequencing length	5.48 aa
Molecular weight (MW)	60185 Da
Isoelectric Point (ISP)	5.55
Signal peptide	No/3

DNA sequence

GATAAGAATAGATAAAACGAACGGCCAGTCTTAAGACTCGGGCCCCAAATAATGATTTTA
TTTTGACTGATAGTGAACCTGTTCGTTGCAACAAATTGATGAGCAATGCTTTTTTAATAA
TGCCAACTTTGTACAAGAAAGTTCGGAGCGCGCCGTGTGCATGATTGCCAACTCGAACCG
CGATCGCTGAGGTGTTTGCCCGCATCGAACCACTAGTTCGATCTGATGTACAGCAAGCGC
GCGTTCGTGCACTGGTACGTGGGTGAGGGCATGGAGGAGGGCGAGTTCTCCGAGGCGCGC
GAGGATCTCGCTGCGCTGGAGAAGGACTACGAGGAGGTTGGCGCCGAATCCGCCGACGAC
GTGGGTGAGGAGGACGTCGAGGAGTACTAAGGTGCCCTCGTGCCCCTCGGTGATGATGTC
GGTGCACGCGTGCGTGTGCTGCGGAGCCGCTGCCNCCGCGACTGTGTGTGTGTGCGC
GTGACGACCGGCTCGAGGGAGGAGGAGCGCGCGTGTGCGTGCGATGCGCCCCTCTCT
CTTTTTTTTTTTCGCTCTCGTCGGCGTTTTTTTTTTTTGGGGGGGCGGCGGGGCGGGGTG
GCGGGGGCGGCCACCCCTCTCCGGCGTNTTTCCGTTTCCGCAGATAAACTNCCTTACCTC
GTGCCGTGCGTTNTGTCCTGCNCNTGCCAACCCTCCNCCCCTTCCCCAATGGAAGCTGTG
T

Protein Sequence

MGLMRTDTHVHAPPSSRSAALLLLLLPCAGRLPRVSSPHLALSLLSSPLPSPPLLADTQH
ARCTHAHAHRNPRSSSLSFFEQTPLNRLLTPLPSFSAMREAICIHIGQAGCQVGNACWEL
FCLEHGIQPDGSMPSDKCIGVEDDAFNTFFSETGAGKHVPRCIFLDLEPTVVDEVRTGTY
RQLFNPEQLVSGKEDAANNYARGHYTIGKEIVDLALDRIRKLADNCTGLQGFMVFHAVGG
GTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTAVVEPYNCVLSTHSLLEHTDVATM
LDNEAIYDLTRRSLDIERPSYTNVNRLIGQVVSSLTASLRFDGALNVDLTEFQTNLVPYP
RIHFVLTSYAPVVSAEKAYHEQLSVADITNSVFEPAGMLTKCDPRHGKYMSCCLMYRGDV
VPKDVNAAIATIKTKRTIQFVDWCPTGFKCGINYQPPTVVPGGDLAKVQRAVCMIANSTA
IAEVFARIDHKFDLMYSKRAFVHWYVGEGMEEGEFSEAREDLAALEKDYEEVGAESADDM
GEEDVEEY

Parasites	L.mex
Gene ID	LmxM.34.3780
Gene name	60S ribosomal protein L27A/L29, putative
Feature	Trypanosomads only

Sequencing length	145 aa
Molecular weight (MW)	16070 Da
Isoelectric Point (ISP)	11.18
Signal peptide	No

DNA sequence

TCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACC
TGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAA
GTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCCGTGCGTGCTGTTTTGGTTGTCTG
TTGCAAAAACCACCGCCTCTCTCAGTGAGCACCTCACGCGCTAACTTTCACGCACTCCCT
TCTTTTTCGCGTGCCCCCTCCCTGCCTCAGTCCCTCCTACCCCTTACCCGCACACTCATT
GTGGCACAAGACCGTCACTGCGAGAGGGCGTGCTGGCGCGACAAAAAGGAATGGGATGTG
CCCCGGTTCTCCGTCACCGTCTCCACGGAAGACCTTGCGGGGTTTTCTTTGGTGAATCTA
CCTCAGGCATTCACACGCCGAAAAGAGAGGGACAGACGGCAGAGGAAGAGAGAACACACG
AGTAAGCAGCCCCAGAGAGGGGGTGAGGACGCAACCAAGCAAAAGTCAGAGGAAGCAAAG
AAAGAAAAGAGAAACAACGCTCCTCCCCCTCGCACTCACGCCTGGAGCACCACAGCGCCG
CCGGCCTGGCGTATCTTCTTGTCGGCCAGCTTTGCTCACGTAGCGCGCCCTTCACGATGC
ACGGNACCTGGATGTGGGCCGTTGCCCAGCAGCTTTCGCGTACCCCGCTCGACTGCAGGG
TTCACCACCAGGGCAAAGTTTCCCGCCCCCTTCGTTCGCCCTTTCGCCCCGNCCGCCCTC
CCGCCAGCCAAATTCAGGGGCCCCGA

Protein Sequence

MPTRFKKCRHQRGSTFCGYGRVGKHRKHESGRGNAGGMHHHRINFDKYHPGYFGKLGMDH
YHRKKNPMWKPTINLNNLSRLIAAEAAAKATKGGTLPVVDLQSSGYAKLLGNGHIQVPCI
VKARYVSKLADKKIRKAGGAVVLQA

Parasites	L.mex
Gene ID	LmxM.34.3180
Gene name	hypothetical protein, conserved
Feature	Leishmania only

Sequence length	192 aa
Molecular weight (MW)	21452 Da
Isoelectric Point (ISP)	4.02
Signal peptide	No/1

DNA sequence

TGATAATCGATCTCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGA
CTGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACT
TTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTT GCTCGCCATGAGT
GGCCGTGTAAAATTAAGAGAGAAGCACAGCGGACCTGCGCAAGAAAGCGCGCATGTGCAA
CACAGCGCCACCACTATCATACATGTGCAGCTCCCTGACACGTGTGCACACACGCACATG
CATGCTCCTGCAGCACAGCCGCACGCGAAAACGTATCAGCAAGGCGGATCACACAGATAT
CGATCCCTCTCACGATTATCCTCTCCGCCATGGGGAACAAGAAACGAGAGGCCTCCACAG
CAGACTCCACGGGATCCAGCTCTTGTTGGTGGTACGACAAACGCAGACCTGTGCTAGCAG
CAGTAATACGCACATTACCAACTCTACTGCGCTGATCATCCGGAGGGGGAGGAGTGAGGA
GGGGGGGGGGGGGG

Protein Sequence

MRTPVLGAGVAPLEPVPVEASSASGEEGGEPGAGDKDTDRERFSKMWMYCLQQMQGHEVG
MLMDMMPPDIQELYSDKLSDEYLRDERDSMDKGVHALRAAQKMVEELTPEQLAHIEEFLV
ETDALNPEANRCHGKAHHPRGDDDNREEEEDDGDDDGEDLFMHRDEGMGSDEEEWLLEQM
IAAGDKTSATAK

Parasites	L.mex
Gene ID	LmxM.36.2450
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	451 aa
Molecular weight (MW)	49811 Da
Isoelectric Point (ISP)	7.97
Signal peptide	No/4
Gene copy	Single

DNA sequence

GTTAGAATGATAAAACACGACAGTGCTTAAGACTCGGACCCCAAATAATGATTTTATTTT
GACTGATAGTGACCTGTTCGTTAGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCA
ACTTTGTACAAAAAAGTCGGCCGCCATCGAACTTCAGCTCTTCGTACGCGTGCGACATTT
TGCGGCATGGCACCCACCCCTGTCATTCACCCATGCGACACGCATCACATCACGACCAGC
ACCCCCGTCTCCATCACGGCAGCCTCGTACGTGTTGCTGAACGCCAACGGCAGTGGCACG
GAGCTTGACGCGATGAGCACAGCGAGGATTGAAGGGGCGCGGCTCCGGTGGGACCGACGA
CAGAGCCTTGTGGTGTCATTTGTGTCGGGGGTGTTGTCGGGGTACCAGTGCATCATTGAC
CCCATCACAGAGGAGGCGACCCTCTCCACAGAGTCTGCCGCGCGCGCCACGATCGTCGGC
GGTGTGGAGTCCATCGTGTGTCGGCGTGCCGACGCCCTATACTACGCCGTCGAGCGCGTG
AACTCGACGCGGTGGCTGCCATGTGACGAGGCCTTCTACCTCCCGGCTGGGCGGTGGTGT
GTGCGGGCCCGCGCCACACTGTACTGCGACCACNGCCGCTTCGCGATCGCGATTGTCTTC
GCGGTGAGTATGATCTGACCCGTGCTTAGGATTTGGATGGCGGGAGGAGGGGGAGCTGCC
TGGGATCCTGCCCGCCGCCTCCTGATGCTCCTGCGTTCTTTCCTCTTTCTTGACCCTGGC
GTTGTGCTTCCTCGGTGGGTGTCCACTGTCTTGCCCGCTGCCTCCTGTTCTGCTCTTGTG
CCGGACGACTGCTTNTGGTGTTCTTGATGGCGGGGGGGACCCCCCCTCCCGGGTTGNTTT

Protein Sequence

MQDWQIAVLVIVIVVFVGLATPLIYLIYRFGSRRRRYSSKRTNMTTSRYADSSCCAKYGI
LDAGDAFAQLFRGDPRIHSSTHLLYFVDFQGELHWINFNKKPAAKIDTESAIQQYIFETF
HPIVEEDNRVVPLPPEMLALSYINYHQTLVVLDLNRLLARDAEFGARMRHTAERPLLLSR
LVADGADRAVLTALSRYGHTAGDAFVPPVTQTSGQCPALVTASPLQQYQQRIEVDLSTSR
ANPMSTTPAVSTSLPNTHAPPPTVQPPVAPPLLPPSNFSSSYACDIFGMAPTPVIHPCDT
HHITTSTPVSITAASYVLLNANGSGTELDAMSTARIEGARLRWDRRQSLVVSFVSGVLSG
YQCIIDPITEEATLSTESAARATNVNGVESIVCRRADALYYTVERVNSTQWLPFDKAFYL
PAGRWCVRARATLYCDNSSKTIAKVFTVSMI

Parasites	L.mex
Gene ID	LmxM.29.3025
Gene name	hypothetical protein, conserved
Feature	Leishmania only

Sequencing length	95 aa
Molecular weight (MW)	10369 Da
Isoelectric Point (ISP)	4.59
Signal peptide	No

DNA sequence

GTTAGATGTAAAACACGACCGTATTAAAAACACGGACCCCAAATAATGATTTTATATTGA
CTGATAAGTGACCTGTTACGTTGCACAAATTGATGAGCATGCTTTTTAATAATGCCACTT
TGTAACAAAAGAGGTTGGGTTTCTGTACTTTATTGTCGCGAGTGAAACTAGACTCCGTCG
TGAACGGACCGCCATCGTCGGAGAGACTCTAGACGTGCAGTGTCAATACCACATTATACC
CACACCGACTCTCATATACTTATATATATATACACGCAGAACGACGGAACGCCTCCGT
GCACACGTGCGCAGAGAGTGTTGCTCTGCTTCTTTTCACATACTCCACGCACACGCACAC
CCGAACGAGGAGGGATGGATCCGACTTAGCTGTTTCAGGACTTTGCCTGCTCTGTGCGGT
GCGAGAGCATGATGCGGTGGTGTTTGTCGCCTGTCCGGAGGGCGGCCGCTACGGCGATGT
GCGCAGGACCTTGGCCGGTTTGCTGCAGCGTCCCTTCGGCTCGGTGCACGTGGCCCCGCC
GGTCGGCGACTTCGCCGTCCCCTTAGATGATGATCGGTAGTCGAGTCGGCTGAGAATGAG
TGATCGTCCCTGCCCGCATGTTAGACGGCGACGGAGGGGGGCGGGTGGGAGTTTGAGTGC
CCGCGCTCTCCGAGTGGAGCCCCCCGCGCGAGCGAGAGANGCCCGANTCCCCTCCCTCCC
GNTCGTCCCGCTGCCGTGCCTTTGAGCTCTTGTCGCNCTCGGAGGGGAGGCTAGCTTCCT
GTNTGCGTCTACTGTTGTCTCTTGAGTGCCGTTCTTCCTTGCGCTGTCCCCCCCTTTCCT
TCCACCGTGCTCGTCTCTTCGTCCCCTTTGTCGTCTCATTGTCT

Protein Sequence

MDQTKLFQDFACSVRCESKNTVVFVACPKGGRYGDVRKTLAGLLQRPFNSVHVAPPVGDF
AVPLDDDQVVESAENEVIVPARILKATEGGGWEFE

Parasites	L.mex
Gene ID	LmxM.13.0560
Gene name	60S ribosomal protein L18, putative
Feature	Trypanosomads only

Sequencing length	198 aa
Molecular weight (MW)	22107 Da
Isoelectric Point (ISP)	12.16
Signal peptide	no

DNA sequence

TNATAATCGATTCTCCTATAGGGGATATCAAGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTG
ACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGG
TGAATTCTTTTTTTTTTTTTTTTTTTTTTTGTTGGGCCGATGCGTGAACGAAGGGGCGAAAAACAAAACA
AAACAAAAGTGAAAAGAGAAGAGAAACGTCACAGGGGAAAGACATCACGGAGAGCGGCACGCACCTCCC
ACGGAAGGGACGCGAGCGCGTGGAGGCTGGCGCGCACGCCGTCGCACAGCCACGCCCACAATCTCGAAT
CACCGCCATCTTGCTTAGACGTGGCGGAAGGCCTTGCGCTTGTACGAGCGACCCGTGCGGCGGCCGCGC
TTCGTCTCCTTGCCCCGGTTGGTCGCGTAAGGCTTCGAGTGGCTGCCGGGCACGCCAGAGGCGCCGAAG
TGGCGCACCGACTCGCGGCCAGACTTGCGACCACGCAGCAGGTACGTGTTCTTGCCAGTCGGAGCAATC
ATGGCGAGCTGGTCGAAGGTCAGGCACTCACCGCCGGCGGCGACGATGCTCTGGCGCGCGCTCTTGGAG
AAGCGCAGGGCGCAGACGCGCATCGCGGGGATGCGGGCCATGCGCACATCATCCAGAACGTCACCGACC
ACGACGGCGATCGGGGCGTCCTGTTCTTCGCGGTGAAGACAGCCTGCCTTATGACGACGGCATGCGCTA
CCGAGATCGGGCCCGTGCTGCGCTTGATCGGCCTGTCCGACTGTTACCAACCCTCCCTCAAGACTTTCC
TGACNACTAATTGGGTCNAATCTTTGGAATCCAACTTGATCNACGT

Protein Sequence

MGVDLTGISKKSRVIRHHTYSTNPYIKLLIKLYKFLAKRTSSGFNKVVYQRLIKSRSNRA
PISLSRIAVVMKRKAVFTAKNRTAPIAVVVGDVLDDVRMARIPAMRVCALRFSKSARQSI
VAAGGECLTFDQLAMIAPTGKNTYLLRGRKSGRESVRHFGASGVPGSHSKPYATNRGKET
KRGRRTGRSYKRKAFRHV

Parasites	L.mex
Gene ID	LmxM.33.2900
Gene name	ribosomal protein L3, putative
Feature	Trypanosomads only

Sequencing length	419 aa
Molecular weight (MW)	47544 Da
Isoelectric Point (ISP)	11.69
Signal peptide	No

DNA sequence

AACTNTAATCTATCTCCTATAGGGGATATCAGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTG
ACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGG
TGAATTCTATTATTTTTTTTTTTTTTTTTTTTTTTTTGTGTATGTGACAAATGACATTACGGTTATGA
AAAACAAAAACAAAATAAGATGCATTCATGCAGTGCAGCTTCAGCGCGAAAAAAAAAGAAAAGGTAGTC
AAGTGGCTCCGCCTCGCAGGAAGAGGCGGACGCGCCTGCGACCCTGGCAGACACGAAAAGGTCACGCAG
AGAGGCAGACGCCCACAACAAGACGTGTCTTTTGCGCTGGCATGCTTACTTCTTCCTCCCTTCCAACTT
TTTTGTACAAAGTTGGCATTATAAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTTACTATT
AGTCAAAATAAAATCATTATTTGGGGCCCGACCGGTTATACTGGCTCTACTTGTGTTTGTCATTGTATG
TGACTGGGAGACTCCGTAGACTNCGTTACCNAGAGTGGGAACTGCCGGCTCATNCAAGGCTATCGAGAT
GGGCCNTNTNTGTGTGT

Protein Sequence

MSHCKFEHPRHGHLGFLPRKRSRQIRGRARAFPKDDATQKPHLTSFMVFKAGMTHIVRDV
DRPGSKVNKKEVVEPVTILEAPPMVIVGIVGYRQTPVGLKTIGTVWAHHTSVEFRRRYYK
NWKQSAQLAFSRQKQFANTKEGRIAEARTLNAFAKKASVIRVIAHTQLRKLRNHRVGVKK
AHVQEIQINGGNVAAKIALAKSLLEKEVRVDSVFQQSEACDVCSVTKGHGTEGVVKRWGV
ACLPRKTHRGLRKVACIGAWHPARVMYTVARAGQHGYHHRTQLNKKIYQIGRSVAVEPNQ
ATTTYDLTAKTITPMGGFVGYGTVRNDYVMLKGSVSGPRRRVMTLRRPMAPQTSRQLKEK
IVLKFIDTSSKIGHGRFQTKKEKSQWFGPLKKDRIRREERLRKERAARAVERKAKAAKK

Parasites	L.mex
Gene ID	LmxM.01.0620
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	566 aa
Molecular weight (MW)	59566 Da
Isoelectric Point (ISP)	9.52
Signal peptide	No/0
Gene copy	Single

DNA sequence

GTNATAATCTTACTCCTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTG
ACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGG
TGAATTCTTTTTTTTTTTTTTTTTTTTTTTGTTGCAAGGAGGTAATCATCGGGAAAATAGAGATGGGAAA
CAGCTTCTACGTGTACATCGGCATCCTCGCCTTTCCGTCGTTTTTCGGACTCCATGTCATCATCAGTGC
GACCCCAGGCCTCCCCCTCCACCCACTCCCACTCCACAGCCATGGAAGCTGCGCCAGTGGCGAACCCAG
CCACATATACGCAACGGCATGCAGACAGACGCAAGGGGGTGCTTCCTGGAAGGGCTGGGGTGATGGCGA
CCCGATGACCATCTTCCATCATATTGCTGCGGACACGCACGCACGCACTCCTCCCGCCCCACGCCTCTG

TCTCTCTGTGTGTTGTGTGTGTGTGTGTGCGTGTGGGTGTCCGTGTACCTCTCCCGTTGCGCACGCT
CATCGGCAACCGACGCGTTCCCCCGCAGGAGGGGAGGAGGGCGGGGACGTGGCGTGAGACCCCATCCCC
CCCCCGCCTTGCCCGTTGCTGATCTTTCCCTAATGGCTGTACATCGAGGGGGGTCCGAGGAGGATACTT
CATCTCCCGACTCAGCACCGAGCGCCGAGCCGCCGGTGCTCTCGTGTTCGATCCGACCCCTTTTCTACC
GTGACTCCGCGATGAAAAACTGCCCTTTCCGACATCCCCGCGGC

Protein Sequence

MTSPDALDCLSGAAATAASHTTANSVGFVPKMANPPAHDTANNCGLNDAAYSPTVSPSYN
TGAIPQEQSKMLAGMLAKLPELDLGEMSRVKSCFSAVLGEGRENIVNALELRVVLGELGL
YPSEAELNLVLRAYRDRVNLVTLTRYLRLYKKEFWINHAAAAAGGAGAVRSAPLSAAPRS
HQAFHVSHPMVASRAGAFGVGGGMDEDTLKAFIALGGGEDGSGEILASTLRDAIRGFGLT
IDIDSMIRTVDVHHSGVLGYVDFCALWLQSTSTSFEPGEAGGAGVFMGEALQRESADNAG
PDTYRRRSSHGSTMSDTRQRLMSLLATTPRLTSLSGGVLRRRSMTQAPEQASTPSHARHC
SPGGTHFFQNTNSRTVTPAYSPQAGGAGNGHGFDGAMACNSILTPPPTPITEEEHMLLVE
MYLFPEKYKTVRRAPRFAADLGGGGGGGSPVYGAAGREAALHSSGSLHQKRLSRPSASG
SGSRGRGRSQARGLGNTSGGADGGGAVADFFPPRSSNVYRPPSPMILSLRNSTAYRNRLK
RLEEQKRERKGWKPPAGAATTQQQQQ

Parasites	L.mex
Gene ID	LmxM.14.1270
Gene name	ubiquitin/ribosomal protein S27a, putative
Feature	Trypanosomads only

Sequencing length	152 aa
Molecular weight (MW)	16970 Da
Isoelectric Point (ISP)	10.24
Signal peptide	no

DNA sequence

TTATCGATCGCACTATAGGGGATATCAGACTGGATGGCAATAATGATTTTATTTTGACTG
ATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTG
TACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTCGGGTCGTTTTTCTCA
TTTTCGAAGAGAAATAAAACAAAACAAAACGAAAAAAAGAAACGGAGAAAATGAGCCCAC
GCACACACAGAGAGAGAGAGACGCAGACAGGGGAAGTGGGGGGAGGGTACAGACCGCAAC
GCGGCGACACACACACACACGCTGCGGCAGCGCACTTCTCAGGCAGGTGTGCGTCCGCTC
ACCTGTTTGTTTGTTTGTTTTTGTATGCGGCCGTCCTCCGTTTTGCCGCGTTGCCTTCTG
TTATCCTTGTCTTCGTCCCTCGCGTGTGGTTGCCCCGCACACTCTACTTGCTCTCCGCCT
TGTAGGTGAGGTGGCACTTGCCGCAGTACTGACGGTCCTTGTGCTGGGCCATGTACACGC
CGGCGCCGCACTGAGGGTGCGGGCAGTCCTGGCGCGTACGCTCCACCTTGTAGGAGCCGT
CGTCGTTCTCTGTCACCTTGAAGTACTTGAGTGCGCGCATCTTCTCCAGCTTGTGGCGGT
GCGTCGGCTTCTTCGGCTTCGTGAAGATGCGCTTCTTCTTCTTCTTGCCCTTACCACCCT
CCACCGGGATGACCACGTCGACGGTGGACTCCTTGGACCGACCGTAAGCGGCGAGCGTCG
CCTCNCGGCGAGGCACATGCCCGCGAAGAAGAGGTGCCCTGCGTCCGTGGGCTGAGCCTG
AGGAGGCNCGTGTCTCGCGACCGCCCGCCACGACGGCGCGGTTCGACAAATGCGCGTAAG
CATAGTCGCNTTTGTCGTGGCGAATGTACG

Protein Sequence

MQIFVKNAAGRSVAVRVSAEDTVAFLKAQANVTQGNLFFAGMCLAEEATLAAYGLSKEST
VDVVIPVEGGKGKKKKKRIFTKPKKPTHRHKLEKMRALKYFKVTENDDGSYKVERTRQDC
PHPQCGAGVYMAQHKDRQYCGKCHLTYKAESK

Parasites	L.mex
Gene ID	LmxM.03.0570
Gene name	Quinone oxidoreductase, putative
Feature	*Leishmania* only

Sequence length	332 aa
Molecular weight (MW)	34494 Da
Isoelectric Point (ISP)	6.66
Signal peptide	no

DNA sequence

ATAATCGATCTCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGAC
CTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTG
AATTCTTTTTTTTTTTTTTTTTTTTTTGCACAGACACACACACACACACATACACACAGGCACACCTAG
CACACTCGCCTCTTCTCAGTCCATGCACGTCAAGAGCAGCTTGCCCGTTGTCTTGCGCGACTGCAGGTC
CGTGAGCGCCTGTGGCGCTTCATGCAACGGGTACTCACGACCGATCGTCAGCTGCACCTGCTTCGACGC
CACCCACCCAAACACCTCGGAGGTGCGGCGCTGCGCTTCCTCGGGGGTGCGCATGAAGTCGCCCAACGT
CGGGCGCTGCAGGTACACGCTGCCCGCGCGCGACAGCTCCAATGGCGAAACTGGCGGCACCGCGCCGCT
CGCGTTGCCGAATGTCATCATCAGGTCCTCGCGGGTAAACAGCACACCGCGGTCCCACCCAACTTTTTT
GTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCACTATCAGTCA
AAAATAAAATCATTATTTGGGGCCCCGAGCTTAATACTGGCCGTCGTTTNTCATCGTNTTGACTGGGGA
AGACTCCGTGCTAACGTTACCGCGAGAGTAGGAACTGCCCGGCCTTAATTTCCCCGANGG

Protein Sequence

MEVIATAHGGPEVLAVRPSSHTPDATQLEEGQVLVRNAYAGVNFIDTYFLCGLYKKPAMP
YVVGEEGAGAVVKVGAGVPESMLGKRVAYFGGAGCTGSYAAFTVAPASALREVPDGVTDA
EAAAVMCQGLTAHYLVDSSYPCGPGSTVVVHAAAGGTGLLVCQMAKLRGARVIGICGGAE
KATLARSVGRADVVIDYVATPDWAPLVRAAAPQGVDAVYDGVGQATFAGSLSVLRPRGYM
ITFGNASGAVPPVSPLELSRAGSVYLQRPTLGDFMRTPEEAQRRTSEVFGWVASKQVQLT
IGREYPLHEAPQALTDLQSRKTTGKLLLTCMD

Parasites	L.mex
Gene ID	LmxM.28.2560
Gene name	40S ribosomal protein S17, putative
Feature	Trypanosomads only

Sequence length	143 aa
Molecular weight (MW)	16409 Da
Isoelectric Point (ISP)	11.49
Signal peptide	no

DNA sequence

TATCGACTCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGTGAATT
CTTTTTTTTTTTTTTTTTTTTTTGCGGTCAGATCAACGAAACAATGAAAAGGAAATAAAATTTAAAAAG
GAAAGGCAGCTACGGCGTAGAACGCCGGGGCGCGGAGGAGCAGAGGGGCGTCGGTACAAACACGCGCGT
ATCGCCTGTGCACCTACTTGGAGCTCTTCTGCTGGCGGCGGCGCGGAGCCGCCTTCACCTTCGGCGCGG
CGGCATCGGCACGCTTCACGCGGCGCGGCACACCGATCTCCAGGCGCTGCAGCATCTGCATCGTCTTCT
TGTCCCACGCTCACGCCAGACTGGATCGCCTTGTCCACGTCGGACGTCGCGGGGGCGTGGTCCATGCGG
CGCTCGCGCTCCTCCTCCTGCAGCTTCAGCGAGATGCCGCGCACGGGGCCGCGCGCCAGGCGCTTCATG

ATGTGAGTCGCGTACCCAGCGATCTTGTTCTTCAGCCTTCTTAGACTCCGCTATCCTCACGTCCATGAC
GACGCGCTTGTTCTGGTAGAAGTCAAAGTTCCGCTNCGAAGTGTACTTCTCCCCCACCTGGCCGGCCAA
CTTTTTTGTTCNAAGTTGGGCNTTTTAGAAAGGCCTTTGCCTCNTTTTTTTGTTTGCAACGAAC

Protein sequence

MGKIRTKTVKRASRQVVEKYYSKLNFDFYQNKRVVMDVTIAESKKLKNKIAGYATHIMKR
LARGPVRGISLKLQEEERERRMDHAPATSDVDKAIQSGVSVDKKTMQMLQRLEIGVPRRV
KRADAAAPKVKAAPRRRQQKSSK

Parasites	L.mex
Gene ID	LmxM.34.3780
Gene name	60S ribosomal protein L23, putative
Feature	Trypanosomads only

Sequence length	145 aa
Molecular weight (MW)	16070 Da
Isoelectric Point (ISP)	11.18
Signal peptide	no

DNA sequence

TTCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTT
GCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTT
TTTTTTTTTTTTTTTTTTCTTTTTTGACTTGAAGAGAAACAAAAAGAAAGTTTGTGTTGCCCTGCGCCG
CGTGATGCACTGGAACCGAGTACCTCATACGCAGCGAGCTACGGCACGCGGATTAGACGATGGCGGGGG
CGTGCGTGGAGATCTTGGGCCACAGGTCCGCAGACTCCTTTGCCACCGGGCCGGCAATGCCGGAACCCT
TCATTTCACCCTTGGGGTTCACGATCACGCCAGCGTTGTCCTCGAAGTAGATCACCGTGCCATCCTTGC
GGCGCCAGCTCTTGCGCTGACGGATGATCACAGCGTTCAGCACCTTCTTGCGCAGCTCCGGCTTGCCCT
TCTTCACAGAGCACATCACCATATCGCCCAGGGCAGCAGACGGCAGACGGTTAAGACGGCCGTGGTAGC
CCTTCACGGAGATGACGTACAGGTTCTTGGCACCGGTGTTGTCCGCGCAGTTTACCACCGCGCCGACGG
GCCCAACTTTTTTTGTACAAAGTGGGCATAATAAAAAAAGCATTGCCTCATCAATTTGGTTGGCAACGA
AACAGG

Protein Sequence

MPTRFKKCRHQRGSTFCGYGRVGKHRKHESGRGNAGGMHHHRINFDKYHPGYFGKLGMDH
YHRKKNPMWKPTINLNNLSRLIAAEAAAKATKGGTLPVVDLQSSGYAKLLGNGHIQVPCI
VKARYVSKLADKKIRKAGGAVVLQA

Parasites	L.mex
Gene ID	LmxM.36.5850
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequence length	520aa
Molecular weight (MW)	57286 Da
Isoelectric Point (ISP)	10.6
Signal peptide	No
Gene copy	Single

DNA sequence

ACTACCCCATAACTCATACAATCCACTCAGTGGGTATNACACACATGTNCNAGNGCNTACTGAGNTCAC
ACAAAAATGAATACTTATACTTTGCATCNGCATCAGTAGCACCNGCATAACTGAAAAGTCAACAAAAGT
CACATAGNCAAAGATCAATACAATAAATAAGCTCCAAACAAAAGACAATCAAANAAAAAAGCCCGTGGC
CGCTAAAACTCCAATTTTCCTCCCTCTTTTTTTTTTTTTCTTTTTTTCCCCCCCCCCCCCCCAAAAAAAT
GCTCCCCCCAATCAAAGATAAAACCAAAACCCCCAAAAAAAAAAAAAGCCAAAAAAGGCACAAAAAAAAA
AAACCCCCCGGCGCCCCCCCCCCCCACAACCCCCGGGTTCCACACCCGCCCCCCCCACCCCCCAAAATG
CTCTCCCCCCGGGCGGTTTGGTGTGTTTTCNTCCATCACCCACCCCCCCCCCCCCCCCCCCCCACCCCC
CCCCCAGAGAACCCCCCCCCACCCACTCACCACCTCTGCCAGCGCCG

Protein Sequence

MTQETTPQQNGEPAGLSSRQRARKNRSGENAARVIRGLDLLSDSGERRPNTGRLHCPPPH
NNFSSPREFFAGPSHPPRLFTGIRRYPNGNRDSVGAMLTDEYFMTNYDDSTITGRNGGAL
RRGSSRSPMRGGSGAYGSVAKRGEGALRQKKAHRRANSINGATAQFRCRDGLTNNCIVTA
PEAPWRCGVKITHPQGGMDAPYFEDNDPRPPRNVPPVTGKRHVPPPQKDAKMFGQVPPPK
EGEDVFQSSLPPCHKTSNKANESVDVLNLHYYTADELNEKPHPVKQLGPRKCESQELPPR
KPPVIKPINTPAKQEHDVLGTGRWGFPEKEHPRGLARGLCRPPHDTANLFYGGMLLADDN
AGSARHQESVGSKPGSLSARTDQRHPRAMDSRRTRSPSGSVSARTDSRYPAQGSRVAAHP
SRQGEAHHGSSRSSSPKKRADPVFDDNYRPHRMVFKKNAGDPNILHYYDPNIDSMPAAPP
RRPIPRSNMESHLDDLNTPHTIGRARGEFCKNSRSTIALV

Parasites	L.mex
Gene ID	LmxM.17.0810
Gene name	Hypothetical protein, conserved
Feature	Trypanosomands only

Sequence length	379 aa
Molecular weight (MW)	39392 Da
Isoelectric Point (ISP)	7
Signal peptide	No
Gene copy	Single

DNA sequence

ACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTCGTTCTTTCGGGCGTGTCAAGTCACCGCCGCATTTAATGGGTGCATGTCACAGC
GTTCTCTGGGAGAGACACGAGACAGACACAGAGAGAGAAAGGCTGCGGCAGACATCGAACACCATACAC
ACCGTATGCCGCATCAAACATGCACACCACTGTGGTTAGCAAGAAGCATAAGGTAACCGCACAAGCCGT
TCAGTTGATCGGCCGACCGAGGTGGACTTGACTATCGTGTCGCGCCACCAGGCCCACGCACGGTTGCCT
CTGCCTCAGCGCCAGAGGGGGACGGTGAGGGGAAAAGGGATGCTTAGAACTCTCCTCTCCTACTTTGTG
AGGTGTTGCGAGGCTGTGGCAGCTTCGTCTTCCCACGCCTTCAGCGCTGCCAGCACATCCGCCGACACC
ACCGGCTCAACGACCAGTGGCAGAGGTGTTTCATGTAACGTCGGACGCAGGTACATCCCGTTCAGCACC
TGCAGGGCCCGCACCGCGGCGGCGGCTGAGGTGAACACCACCTCAAGCGTCTTCGTGTTTGGGCGGATT
GCAGCCACTGTGTCACGCGAGAAGATCACCAGCGCGCGGCGTTGCGAGCAAGACGGGAAAGGCTGAGCG
CTCTGAATACAG

Protein Sequence

MQQELLLLTRRVAQLESTADGLHGKLNEVHRSIGEVVLGQRNTDMLVQQMERRHLAGPAG
THAATGSGTGEGVAQSTTAPLSDACASPSASHDGGASLAAATHAATTVAPGAFPSSAQLA
EQVATLQARLDQLTTNIFAADRLGAIVDRMAASGIDTEKVVASVLPAETPSSRSSLLQQL
QRRYALGTFTDAAGVTRLSSQVVRVHNVPLNMGAMEVRELCVQHVCKGKNADELVSCMVH
RRANSIPAVAAVAGHACHPVPEVVDSPTDAAPPRYLSRKAASKPSSPSLASADAAALGDL

PRGDTVAAIRPNTKTLEVVFTSAAAAVRALQVLNGMYLRPTLHETPLPLVVEPVVSADVL
AALKAWEDEAATASQHLTK

Parasites	L.mex
Gene ID	LmxM.34.0600
Gene name	60S ribosomal protein L18a, putative
Feature	Trypanosomands only

Sequence length	179 aa
Molecular weight (MW)	20779 Da
Isoelectric Point (ISP)	10.88
Signal peptide	no

DNA sequence

CTATAGGGGATATCAGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAAC
AAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTT
TTTTTTTTTTTTTGGTTTGGCGTGTAAAATGACGGAAACAAGCACAAAAGAAAAAAGGAAACAAAGGAA
CGAAACACGCGAACAATGACGTCACGCCAAAGTTCGAGAACCTTCACGGAGCACACGTCAAGCAGCGCG
GCACGCCGTCCCCTTTATCTGCCGTTGTCCGCCGCTTTTCCTTTTCTCGCTTTCCCGATTCCCTTCCTT
CACCGCTTGTCGCCGGCGGCCAGTGCCGATGTTCCCCCGCCCGGCAACAGCAACACATGCGCGTATGTG
TGTCGGCGGAGGTGGGGGCAACGCCGCTGGATCACCACCGCGAACCAACGCAAAGACGTAAGCCAGCAG
GATCCAGAGGGGGGAGGGAGGGGGGCGAGAAAAACGAAAGGCGAGAGAACATCGGCAGTTGTGCACCCA
CGCCACCCGCAAAGAACGAGACAGCCGCCACAATCGTACTTGGCAAGCACCTGCCTGCACCGACGACGA
CACCGACACACAAACACGCACACACGAGATCTGCACCCCCTACCAGCAGCCAGGAGACAAGGGAAGAGG
GCAGAGGGAATGGGCGTGCCCACCTACACCCACAAATCACATACGGATACAATCCCATATATACGCATG
TGTGTGCCCGTACACTCCCGTTCTCCCCCTCCTCCACTGTTCTGCCTCACTAAATTCTCCCTCCGCCAC
CCCGCGCGCTTCGTGTTCTTCTGACAAAGATGCCG

Protein Sequence

MVKPHLRHYQVVGRESPSEKNPEPTVYKFEVFAPNFVVAKSRFWRMMRVKNKVKSTHGDV
LSCNVVKDAKLVARNYLVDIAYYSQRCGYTRMVKEFRDVSKTGAVSQAYHDLASRHRARY
HNIEVLNVKSIPDHEVKHLSIAQYHAPNLSFPLLQRRTKAARKDRAIFVKKNTKRAVVA

Parasites	L.mex
Gene ID	LmxM.13.0390
Gene name	alpha tubulin
Feature	Trypanosomads only

Sequencing length	548 aa
Molecular weight (MW)	60185 Da
Isoelectric Point (ISP)	5.55
Signal peptide	No /3

DNA sequence

TATATACTACTACACTATAGGGGATATCAGACTGGATGGCAATAATGATTTAATTTTGAC
TGATAGTGACCTGTTACGTTGACAACAAATTGATAAGACAATGCTTTACTTATAATGCCA
ACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCATCTATCAA
ACGACGGGGAGGGAGAGGCAAGGCCCTCCTCCACAGATACACAGCGCTACGGCTACGACG

CGCGTGTGCATGCGCGGCAGACGCACGCACGAGGAAGGAGAGGACGGCGAACGGAAGAGA
ACGCCGGAAGAAGCGTGGCCGCTCCGCCATCCGCCCCGCCGTCCTCTCCGANACNAACGC
CGACGAGAGCGAATTTTTNCNTAGAGAAAGGGGCGCATCGCCCGCNCGCGCGCGCTCCTC
CTCCTCCCTCGGGCCGGTCGTCCGCGCCCCCCCCCNCGTCGCGGTGGCGCGGCTCCCGCT
GCGCCCCGCCCGCGTGCCCCTACTCTCCCCGTGGGGCCGAGTGCCCCTTGGTCCTCCTCG
CCGTCCTCCTCCCCCCTGTCGTCGGCGGATGCGGCGCCATCCTCTCGTAGTCCTTCTCCG
CGCCGCGGATCCTCCGCGCCTCGGGACTGCCCTCCTCCTGCCCCCCCNCGTNCCGTGCCG
AACCCCCTGCTTTCTTGGTCCNCTGTGTCGTCGGCCACCCTCGGATCCGTCGGTGCATTT
CCCCGCCCGCCTTCGGGTCCCCGCCCAGTGGGTGTTTGTCCACTACCCTGG

Protein Sequence

MGLMRTDTHVHAPPSSRSAALLLLLLPCAGRLPRVSSPHLALSLLSSPLPSPPLLADTQH
ARCTHAHAHRNPRSSSLSFFEQTPLNRLLTPLPSFSAMREAICIHIGQAGCQVGNACWEL
FCLEHGIQPDGSMPSDKCIGVEDDAFNTFFSETGAGKHVPRCIFLDLEPTVVDEVRTGTY
RQLFNPEQLVSGKEDAANNYARGHYTIGKEIVDLALDRIRKLADNCTGLQGFMVFHAVGG
GTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTAVVEPYNCVLSTHSLLEHTDVATM
LDNEAIYDLTRRSLDIERPSYTNVNRLIGQVVSSLTASLRFDGALNVDLTEFQTNLVPYP
RIHFVLTSYAPVVSAEKAYHEQLSVADITNSVFEPAGMLTKCDPRHGKYMSCCLMYRGDV
VPKDVNAAIATIKTKRTIQFVDWCPTGFKCGINYQPPTVVPGGDLAKVQRAVCMIANSTA
IAEVFARIDHKFDLMYSKRAFVHWYVGEGMEEGEFSEAREDLAALEKDYEEVGAESADDM
GEEDVEEY

Parasites	L.mex
Gene ID	LmxM.09.1505
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	121 aa
Molecular weight (MW)	14051 Da
Isoelectric Point (ISP)	11.53
Signal peptide	No
Gene copy	Single

DNA sequence

TATACTACTACCTATAGGGGATATACAGACTGGATGGCAATATATGATTTTATTTTGACT
GATAGTGACCTGTTACGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTT
TGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTAAATGTTGTCTTTC
GTCAAGTCTCCCTGAGCCAACTAAGAGAAAGAGGGAGAGTTTGTAACTCCCTGACACACC
TCTGAAGATTTCTCCAAGAGGTGCCCACCAACGAACCTGTCACCACACTCATGATTAATG
CTTGTTACCTGTTGCGGGCCCGGATAGCCTGCAGAATCGTCCGGAACCCACAAAACCCAC
GCAGTGGGTCGTGGCACGACTCCTCCGCAGATCCTCCTTGTACCCTTGGTACGGGAGCGG
CTTGGGTATACCTGCCTCTTCGTGAAGCCGGCACTCCACTGCGTNCNGACCAGGATTCAT
GCGGCCATTTAGTACATCCTTCGGTACAGCTCNCTAATTCCCGAAGCCCCGGCGCTTCGC
GTCGCGCTCCTTTTTCTGCTTCCGGAAGACGCGCCTCTCCACCTTCGACCAATTCGAGTT
CAGGTCTTTATGCCCGCCTTCTTTCGACGCTCCGAATGCTCTTTCGGGCCGCCGCNCAAG
CTTCATGGCTACNCGTTGGCGACCCCGCGATGATTGGAACAGCCGAGGGTGGACCGACCC
GTCCCCGTGATGCGACTTGGAGCCGGGTGGGGTGCCGTTCTGTGCGCGAGGGTCGTGCNT
TGGTCGAACCCTTTTGTCCGGTGCTTTTAGGCTGCCTCTTGTGCCGACGGTCTTCGTCTT
TNCTTTTGGGCGCTGNTCGTGTTNGCTCGCAGGACCTCTCTCCGTCCGGGTGGGCCGCTT
TTTGGGCCGGGGAGCCTTTTGTGTGTCCCTCGGCTCGCG

Protein Sequence

MKLVRRARKSIRERRMKACINDLNSNLSKVEMRVFRKQKKERDAKRRGSGISELVPKDVL
NGRMNPGLYAVECRLHEEAGLPKPLPYQGYKEDLLRSRATTHCVGFVGFRTILQAIRARN

Parasites	L.mex
Gene ID	LmxM.33.2870
Gene name	ribosomal protein L3, putative
Feature	Trypanosomads only

Sequencing length	419 aa
Molecular weight (MW)	47544 Da
Isoelectric Point (ISP)	11.69
Signal peptide	no

DNA sequence

ATACACTCACCTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTG
TTACGTTGCACAAATTGATAAGACATGCTTTCTTATAATGACCAAACTTTGTAACAAGAAAGTGGGTGA
ATTACTTTTTTTTTTTTTTTTTTTTTGTGGCTGGGAAAGAAAATCAAATAACGTTAAAGAAATTCAGGA
AATGAGACTTCAACATTAGACACCCCGAAGGGCCCCAACTAGTTTCAAGGGGAACGAGACAAATGGACC
CGCCCTAATTTCGGAAAAGGACAAGAAGTTGGTTTACACGGTTCCATTTTCAACTAAGANAGGCCCCAT
TTCCCACCCAATNAAC

Protein Sequence

MSHCKFEHPRHGHLGFLPRKRSRQIRGRARAFPKDDATQKPHLTSFMVFKAGMTHIVRDV
DRPGSKVNKKEVVEPVTILEAPPMVIVGIVGYRQTPVGLKTIGTVWAHHTSVEFRRRYYK
NWKQSAQLAFSRQKQFANTKEGRIAEARTLNAFAKKASVIRVIAHTQLRKLRNHRVGVKK
AHVQEIQINGGNVAAKIALAKSLLEKEVRVDSVFQQSEACDVCSVTKGHGTEGVVKRWGV
ACLPRKTHRGLRKVACIGAWHPARVMYTVARAGQHGYHHRTQLNKKIYQIGRSVAVEPNQ
ATTTYDLTAKTITPMGGFVGYGTVRNDYVMLKGSVSGPRRRVMTLRRPMAPQTSRQLKEK
IVLKFIDTSSKIGHGRFQTKKEKSQWFGPLKKDRIRREERLRKERAARAVERKAKAAKK

Parasites	L.mex
Gene ID	LmxM.17.0870
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	444 aa
Molecular weight (MW)	48291 Da
Isoelectric Point (ISP)	4.99
Signal peptide	No
Gene copy	Single

DNA sequence

ATTCACTATAGGGGATATCGTCGGATGGCAATAATGATTATTTTGACTGATATGACCTGT
TCGTTGCACAAATTGATAAGCAATGCTTCTTATAATGCCAACTTTGTACAAGAAAGTGGG
TGAATTCTTTTTTTTTTTTTTTTTTTTTCACGAAAGCCACTGAAAGGAAATAGCATTCAC
AGAAAACATTCTGCAGGAGAAGATTTCCTTCGCAGCACTATATCATGGCAGGGTGAGTTG
TTTGCTAAGCCAATGTCCTAACGTATCATACTCTTACGCCTTTTTGGAGGTAGAAGG

Protein Sequence

MTTADDVCGRYTLSHCDGKVTPAKATLTIHRCGETLTAHATVANDLRGTVQYENCHIVGS
LHSTGNEASPAQESVEQALSKGFADGFNVVVEINQVLLKNANSSFVFERSWKLSDLNGEH

AIIAINDQSPNQEMTISFTPDGNGGSFVTANIANSLRGNCQIDAGLLRGDLATTQGKADE
SSMQVEKVISEGFQQGFHVCTNDSGILLQSSEANIQLCRIVSQSDLEGEYVLKSFNGAAV
PTRNQPSIVFKPVNTNEVEISIVVANRIRGTAALNQNVLSSEEPLMSTRMVGTEEESQLE
GAFNVGFQYGLETISHGNELTLKNQDCKFVLVKAVAPAAQHGGPTYKGTHCNKCFKAEGN
GLLFRIVNEHEKKWAFYNDTEDLRIRVHATFGARSKIEALDNAKMYKNDDGRYAVEVTVD
PQATEMFIQGDVNGFRVLYDAQPI

Parasites	L.mex
Gene ID	LmxM.27.0650
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	167 aa
Molecular weight (MW)	19658 Da
Isoelectric Point (ISP)	10.29
Signal peptide	No
Gene copy	Single

DNA sequence

TTACTCACTATAGGGGATATCAGACTGGATGGCAATAATGATTTTATTTTGACTGATAGT
GACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAA
GAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGTTTTTTTTTTTGTTTTCATA
GTTATTCTTGTTTTTTCCCCTTCGCCGCTTAAAGGAGGTGTGGGGGATGGTGTCTGGCAG
AATAAAAACAGACGTCCGTGAACAAACTCGTGCTTATAAATGTATTCATGTTCCGTTGCG
CAACTGCTCAGGCGCGGTGGGCGGAAGCCGCGGGAGGGGAGGAGCACGCGCGTCATCATC
CAGCTTACCTGCCAAACTACTACTACGCTATAAGAGGGAAGAAAAGAGAACAACGGGGAG
GGGAGCGACATCTTCCAACAACAGAACACACACATACTCGGCAACAGTGCGGACTCTGGA
AAAGGAGTGGGAATGGCGCACATTCGGTGCTCGAAATATTAGCGCGCGCGCATGTGAGCA
TGTGCGCGCAACGGGAACTGCGATACCTTTTGAAGCGGCGGTCTGTGTGTGTGTGTGTGT
GCGCGCGTGGGTGGGTGGGTAGGTGAGGGAACAAAAATGCTGAAGGCNTGCCCCGACGGG
TATGCCCCGGTGGAGGGGAGAGGCTGTTCACCCGCCTTGGCGGGCCCCCGCGCCCCGTGA
TGCGCCGAGACGCCCTGATGATTCCACCCCTGGAGTCCNTGTGCGGTGCTGATCGGCCCC
TTTTTCGCTTCCCCCGGGGTGGCTTCCCCTT

Protein Sequence

MKVELTMQYLDEWMLRWRKFQTESDWRIEKNRQWWRQANIVTAGAVMGSLVMYTAGTATI
RRQFGAPHFFDVGVDAKIKEAICDTITSRWRYTPQGYGRLMVVGLPTFFVFAISEHIQER
RRLRAYVRQNTVFGEQARRLVQNGKIEEYLAVDIKASLPQNQTQLYA

Parasites	L.mex
Gene ID	LmxM.26.0180
Gene name	60S ribosomal protein L7, putative
Feature	Trypanosomads only

Sequencing length	290 aa
Molecular weight (MW)	33115 Da
Isoelectric Point (ISP)	11.2
Signal peptide	No/2

DNA sequence

GATCACTATAGGGGATATCGTCGGATGGCAATAATGATTTATTTTGACTGATATGACCTG
TTACGTTGCACAAATTGATAAGCAATGCTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGTGAAAGAACAACAAACGAACCAAGCAAG
CGGGGCGACCACCCCGGCATGCGAGAAAAGAAAAGGAAACGGGTGTTTCACGGAACGGCA
GCAGAGGCCCACCCACCGNCAAAAAAAGNCCGAACGGGGGCAACACTTGNAAAGACCCCC
CCAAAGGGGNACTTCCCAAACCATTTTGGAACCAAAAACCCCCCAATTTCCCCCCCAACC
CGGG

Protein Sequence

MRFMFLFPLALSSRLTLDSPPLSGNATPHTAFKKKHTAMPTHSVYGNASDMPAVPAPESA
IKRAAFKQQQTESFKKAVVARKAAKAALKKTAYLRARKYSREYRGAEKKLVTLRRQAASH
GNYYLEAKPKVVVVTRIRGIAKVNPKQRKILQLLRLRQIFNTVFVKMNKPMENMLRAVEP
YIAYGYPSLATVRAMVYKRGYLKINGQRVKITDNQMIKDKYNNADIVCAEDMVNQIYTCG
KHFRTVMHGMWPFKLAPPAGGMRQKRRHFVEGGDYGNRDTLINRFLARMI

Parasites	L.mex
Gene ID	LmxM.36.3780
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	316 aa
Molecular weight (MW)	36038 Da
Isoelectric Point (ISP)	6.25
Signal peptide	No
Gene copy	Single

DNA sequence

ACTCACTATATGGGATATCAGCTGGATGGCAATAATGATTTATTTTGACTGATAGTGACC
TGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCACTTTGTACAAGAAAG
TTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGTATGCGGACCCCACTGTTCTCTCCC
TTTTACGCCCTATTGAAGAGATGCTTCTGGGCGCCGTAAAAAATCGATAGGAACACCCAT
TCTGCAACAATACTCGCCTCTCTCTCTGACAGCCTTCTTGGTTCCGTTTAAGTTTTCCCA
AACAANCAAAAACAACCTTNGGGGGTGACAACCTTGGCCAAACCAAACAAGGNGTCCCGC
ACTCGGAATTTTTTAAA

Protein Sequence

MDAIKTYTYKSGAVYEGTFDGNMRSGRGHWTHPQGERYEGEYKDNKQNGLGVYIFSETGK
KYLGNWEAGQMNGEGLYFFNLDCTAYYFGNYTKDKKDGDGHYMYETGVMTTQKWNMGALL
KEEETPPSEIVECAVKIKELMDAVRAVAPKELGDMPPPSEVRTFQFPSGATYTGQYFGTK
KHGRGYWLHPEGDSYEGQFDNNHHSGWGVYVIGRSGKKYVGHWRNGKMNGIGVYFFNPQE
TEYYVGLYRDDVKNGRGMYHFAESGASMVQMWENGVLRQEMEADKATEKAYEAAIRRIVE
VVKPYAPNYEPVTFGF

Parasites	L.mex
Gene ID	LmxM.28.2740a
Gene name	Kinase C receptor
Feature	Trypanosomads only

Sequencing length	312 aa
Molecular weight (MW)	34403 Da
Isoelectric Point (ISP)	6.5
Signal peptide	No
Gene copy	Multiple

DNA sequence

TATAATCGATCTCCTATAGGGGATATCAGCTGGATGGCAATAATGATTTTATTTTGACTG
ATAGTGACCTGTTCGTTGCACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGT
ACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTACGGTAACGGAAAACAT
AAAATAACATTGAAAAAAAAAGCGCTGCAACGGGGATGGTGGGCCGACACACACGCATCA
CATGGCGTTGTTGCCGGATTTGTTGTGCGCGAGTTGCGGCTCACCTTTATTCCTGTTGTC
TATAACTGTGATATATAACATTAATGTGTATTAATTGTAACAATAGACTTAACATCCTTA
CTAAACATTTATACTTTTAAACAAGACCATTTTTGGGGGGGNCTCATTTTCTCCGGTCCT
GNCCACAGNAACAANCATCTATTGTTCCGTCTTGCCTGGCCACCNTTTGTTACNTTCTCT
TTCTCNATGAAATTCTACCCCCCCCCCCGTATTTNCTCTTGGCNTAATCCCTGTTTCTGT
CCTTTTTTCCTCCTCTTTTAANCCTTTGGGCCTNCTCATTCTCCTCTCCTATTGTTTTTG
GGTTCCTGTTTAATTTTTGGTGGGGTGTTTTTTGTGTGGTAAAGTGGGTGTGGTGGGTCC
CGGAAAAACATTTTGGGTTTTGGGTAAATGTGTGGCCTTATTTTTTGGTTTTTTGTAAAA
GTAAAAAGGGTGGTTTTATTTTGAAAATTTTGGGGGGGGGGAAAAGGGGGGAAAAGGGGG
GTTTTTGGGGGGGGTGTCCTTTTTTTGGGTGGTA

Protein Sequence

MNYEGHLKGHRGWVTSLACPQQAGSYIKVVSTSRDGTAISWKANPDRHSVDSDYGLPSHR
LEGHTGFVSCVSLAHATDYALTASWDRSIRMWDLRNGQCQRKFLKHTKDVLAVAFSPDDR
LIVSAGRDNVIRVWNVAGECMHEFLRDSHEDWVSSICFSPSLEHPIVVSGSWDNTIKVWN
VNGGKCERTLKGHSNYVSTVTVSPDGSLCASGGKDGAALLWDLSTGEQLFKINVESPINQ
IAFSPNRFWMCVATERSLSVYDLESKAVIAELTPDGAKPSECISIAWSADGNTLYSGHKD
NLIRVWSISDAE

Parasites	L.mex
Gene ID	LmxM.08_29.1740
Gene name	histone H2A, putative
Feature	Trypansomads only

Sequencing length	132 aa
Molecular weight (MW)	13961 Da
Isoelectric Point (ISP)	11.61
Signal peptide	no

DNA sequence

GTNATAATCGATCTACCTATAGGGGATATCAACTGGATGGCAATAATGATTTTATTTTGA
CTGATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACT
TTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGCCCGAGCCTACC
CTGACTCGTACCCTCTGCTGAGGGGCGGAGAGAAACAGAAGAAAGGAAGGAATCAAGTGG
TCATCTGAGAAGGCATAAAACGAAAGATAAGGTCTCTATGGAGGTATGATGCGCCTCATC

GCCGGCCCCACAGTGGGGGCCCGCGCACACGCGCACATACCAGCGGCGCGTGTGCGCTGT
CAAGACGGAGGGTTTATCTTCGCTCGGCGTCGCCTTGAGCTTCTTGCCACCCTTCTTCTT
CGACATCGCCTTGCTGACGCTTGGCACAACGCCGCTGTGAGACAGGGTCACGTTCTTCAG
AAGCGAGCTGATGTCGTCGTCGTGGCGCGCAGCCAGCATCACGGTGCGCGGGATCAGGCG
GCACCGCTTCTTCCCGCCTCTGCGCGGCCGCCTTCACGGACAGCTCCAGCAGCTCCGCGG
TCAGGTACTCCCAGCACAGCCGTCATCTACTCGG

Protein Sequence

MATPRSAKKASRKSGSKSAKCGLIFPVGRVGGMMRRGQYARRVGASGAVYMTAVLEYLTA
ELLELSVKAAAQSGKKRCRLIPRTVMLAARHDDDISSLLKNVTLSHSGVVPSVSKAMSKK
KGGKKLKATPSA

Parasites	L.mex
Gene ID	LmxM.03.0640
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	580 aa
Molecular weight (MW)	63626 Da
Isoelectric Point (ISP)	7.48
Signal peptide	No
Gene copy	Single

DNA sequence

TAATCGATTCACTATAGGGGATATCAGACTGGATGGCAAATAATGATTTTATTTTGACTG
ATAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTG
TACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTAGAGCGCCAAGTGGTT
CACCTGCGACGCGCGCGCGTGTGCACCGCCCCCCTCCCCCCCCGGAAACGACTTCGCCGCC
ACGTGTAACCGCACTGTCACTGGCATCACACCACCAGCACCCACCCACCCTTCCGTATTT
CATGAGTAGCACCAGACGTAGGGAGAGGAAGAGGGGGAGGCAACGCAGGAAGACCTGATG
GACCACATGCATCTCCCCTCTCCCCCTAAAATATCACCAGGACCAACAAATGACGCACAC
ACGAACACATGCGTGCGCACCAGCATTCCCCCTCTCTCTCACCACTGGAAAGCCAGCAGC
ACTAGCCCGACCTCGGCTCGGTAGAGGGAAGGTGAATGTGTGGGTGGGTAGGTGGGGGGA
GAGGGGGAGTCAGTAACATCGACGGAAACGCGTTGACCTCCACAGAGACCCGACATACCC
GCCACACACGTGCCCGCATGTGCGCGCTAGCGCATATACATACACCACGACGTGTGTCCG
CACCGTTTCGGCTGGCCAGACAACCCCCCCGAAGAGCTCCAAACGCCCGCGCCGAGGCCA
CCCCCCGGCCCCTGGCCCCCCCGGGGACCCCATCGGCGGTGGGTGCCCCAAGCCCACCCG
TAAAACCCCCCGCCC

Protein Sequence

MQHPVEMAFYFPRFHPDTHVNPAGIPELIDAVGLMTRHSRPSRAESTASASTSTAESAMS
RALPPTRWTTTVSATAQPTTDASVAEAQKDNAKSLLLLERGIDILTSVGGVRSPAVANLL
RPLYAARFEMLNGSEHLPRSEYAKRMHVHKAILQQAAPLLYYDTWDKSDVHQLDTSCVLV
AHFMKAFCALHPRPFHPVPAPPTSGGASSSQTSTTTTASSSTVTSSPPKDAFNPAEWKMA
VGDDGKGAHLTLSIVQDRVEELLRLATAAYGKHGSTHPELRWLRPKLLVLKGLLTIPLTG
HLLHAQRCIEEAANYVDAMTKRKNLLLDGLKARTHEPELGLYMLLQAEIAARVFGWDMAP
GQVDGDVVNMFTDAAGYYADPPNTTLDGDAIMSERKLAEQRFEAEAYTCCLRSYAAFLLG
APRPKATTTRDSPVFLSKQLFSLNPLLTVATPSSLIFSDVRNVSELPLDMCRRRTGEALD
RALKLNRMLYPDFRKNAPAAATLMTMACMYADTRDYLYATGLFESANKAVTCNFGDTSLE
HVFLQKLRYEFLAGVGSEQEAKTASHEVVHLLKRLDALPC

Parasites	L.mex
Gene ID	LmxM.27.1350
Gene name	hypothetical protein, unknown function
Feature	Leishmania only

Sequencing length	271 aa
Molecular weight (MW)	29159 Da
Isoelectric Point (ISP)	10.64
Signal peptide	no

DNA sequence

TATCTATAAACCTATAGGGGATATCAAACTGGATGGCAATAATGATTTTATTTTGACTGA
TAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGT
ACAAGAAAGTTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTATATCCCTCATGTTCGC
GCGCATCTTCGTGACCTCCCCTTCTGCTCTTCTCCGAAGGAGGTCATGCCGCAACATCTG
CACAGATTGCGTCGCGCGCACATACACAAACCTTGCACAGATGAAACAAGCTGACAGCCG
GCGTCATTAAACACACCACAAGCAGGCCAGCCCACGTAAGACCTACACAGAGATTGACTG
CCACTACAGCAGCACCGATGAAGGCACCAAAAAACGAAGTTCGAAGGCACAGAAAGGATG
CTGTGGAGAGAACAAGAAGGACGAGCGAGAGATGGGAGAAAGGTAGACNAGCNACAACAG
CACGGAAATTCAACAAGAGGCAAGACAGTGGGAGAGGCCGCGGTCCCTTTTCAGTTATGG
CCAGTCGAACAGCTACCCGAAAGCCACTGCCGCCGACACACCTTCACTTTCCTAACCAAA
CGATATAACGTTGTGCTCAGTTTCCAAACAACTCGTGTACGAGTGCCGTTGGCACGAAAT
CCTTCCCCGCTCCCGTATCCACGTTGCNTGTTGAGTATATCTCGTTAGATCTTGCTNGCC
NCCCAGATCTGCCCTTGAGGCGGTCCCTCTTGGCTCCGCCTTAGCTCGTTCTACCCTCCG
CCCCCTCTCTTCGAAGGGCTCCCCTCCTCCCGGAATTGTTTCCGCCCTCCCCAGGGATTT
TTGGCCTTCCCTT

Protein Sequence

MLSTRLQRPPPPRRAPPAATLVKDVSSDAYISSHVILRLSGAALISQQLLQMKDWVSDIK
MAVITADDSLKAIWTSPAVQLPSLSDGATTGGDDVQSLAFASVTLSKLVEEQETQDAFYT
KLSETAVVRAEVQKGSQDARLQRYKQQVECQLASSMADGLLPQQQRKNSVPPEKRQILLN
PYSTGHRKLPQTASQTGNRPLPSVSTSMPPTMTSPYRTSSSSAVSAPQSPSSSTPVAANG
NTLGTSPIPLARQAHRVKGPHSAVQRLRRSQ

Parasites	L.mex
Gene ID	LmxM.09.1220
Gene name	AAA family ATPase, putative
Feature	trypanosomads only

Sequencing length	1274 aa
Molecular weight (MW)	139649 Da
Isoelectric Point (ISP)	6.62
Signal peptide	No /3

DNA sequence

AAAAAAAGTCATATAAAAATAAGTTCAAAAAAAAAAACCTCAATAAGGGGGGGAAATAAT
AAAAAAAAATCGGAATGGGAAACAATAAATTGATATTTAATAATTTNGAAACTGAATAAG
TAGAAAACGTTACGTTGAACAAAACAAAATTGAATAAGAAACAATGACTTTACTTAATAA
ATGAACACAAACTTTGTAACAAAGAAAAGTATGGGTGAATTACTTTTTTTTTTTTTTTT
TTATNTTTTTTTTTTTTTTTTTCGTCGGGGAAAGACCTTTTTTCCTACACGTCTTGTGAC
ATTAGACGTGCGCAAGATCTGAGTATCCACCGTGACTTTGATGGGGNCACGCTTCCGCCC
GAATTCCTCGGCAGCGGACAAGACGGCTGTGTCCCCGTTCCTCTGCCCGTGACTTGATCC

TGCGCTTCTTCGCTTTTCTTTTCCGTCCTTGCCGCTCTGAAGAAGTGGCTGCGTGTGCCG
TCCCCGAAGGGTACGTACGCACAAACATGTCGTTCGGGCCTCCCCCCCTACCCCCTCCGT
GTGGCGCGCCCTCGGAGAGCGGAGTGCACCGCTTAAGCGTCTCTGTCGTTATTTTCCGCG
GTGCGGGAGGAGCGAGTCGGCGGGGCGGGGCCCCCCNCCCGCNGAGAAGCCCCCGCCNTT
CNCCCGTTCCCCGAAGGGGGTGGTGGGGANGAATGTTTTTGGAACGAACTTCCGNCCGNC
TCCCTCCCTCCGCCCCTTGGCCCCCCCCCCCCCCCTTTCTATNGNCCCTTACTTTTCGNC
GCCTCCGCCCCGGGGNACCGGGGGCGGGTGGCCGCCCTCCCCCGCCCGTTTTCCCCCGGT
TCCCCTGGTTTTCCCTTTCTTTCTGGGCCCCTCCGCAATTTNCCTTGGGGGCCCCTTTTC
GGTTTCCACCGAAACACCCCGCCCTTCCCGCAAAGATCCCTGGGGCCCCAACCCGGGCCG
CC

Protein Sequence

MDLLRLYVEFFLLCVQPTVPCRTRTAAVVAGAGCNGSGTAAKPVAVDVATQLTTLTFISV
PHCVRPFFEGVALLRSDNEAAPAVADKAAGKVAGRKRHRGSIERNGTKSAAADHPVVEGG
EDSDFPYQSSVMLQRLREYGEVQVPGFESDSAYLAAKDVLVYLRHYTYLVSGMTLSAAVL
RNIELVGLDFRQQCVPCMAAVTQLASHLIDAQLHKMQLRPRVVRLSERLCLSAKAARTFE
YLLVCHCGRYAPGNIAEPLRPANIAYHNDLTPQELLSILSESGVLWKQGLIFSDIKMRTT
FMECKYALPMETIAALSGDQLSEEQLIKLERTALLEVLNEERNAKTTSTAAATPAPQAEA
HVGGSRSVSPSPKRGDEESDVDDEEALDSVLADIDPAVLASKDQDAIYEAVSHRLSNTLA
RRTATSSSARAVAAAPAAKNSAATETEEAVKDGKRSREESAMVTPSTAPPTAPPSRATDT
TPRPEDGEEAATATSAPRKTRLVLADINPLAQRYRCTGTGAGAAAASSSPSATSHTDSSM
RGVPYESDIEYMDAAFRILANMIRIRYAEGDMKDEEDSYTPKSKVEASIRELKGKVRVAA
AVHESRLQATLSARTFTPRIEQLAQRLQLTEMEKQIMLFMVGNVISHDMLVAVNGRYVMR
DGQRLITVGYMLFVLCESLEERVVARRAFYQSSPLVSNGVLSLTLDAVGRSCFNTDLMDY
LVDIDRKIVDDVMGITAETAEMVPGSQLYFPKVELANVVLPTSTMERVLSTIEHYSLFEQ
CKKSSGFGDGLGTSKGGLVMLFHGPSGTGKTMLANAVAHHLKKRILLVSVSQFRSSTKAE
ADALRFLFREAKLSDAILFFDECESLFEDRTSNGTVTALLSEFERYDGLIILATNRAQNF
DEAMNRRISLMMEFRPPDHQLRLRIWRSHIPKQLSMSEDVCLEKLALNYELSGGLIRNAV
LAALSRAVAREKSATPKLTMRDLDEGARLQLRGFFLAAELPEGMSEFYLAPKRTLAELVV
EPNLAKKLEGIASSAKSRSTLYTEWGFSEDANDDCGALYLFQGVSGTGKSLAAEGIAYEC
GATIRLCNVAELLLREEMRVHVVFEEGRRLGAIIVFDEAQVLFNESPKSLQLSQLIQYHA
RRYPRPVIVIATTVHRDGSGGRHLFSTTNINSRSSCMLFQAELTFALPCRPLREQLWRKA
FPERVPTSSDVDYGRLSATSISPKLIRTIAFNVCCTAALLPVSERVVTMAMIEAEMDRTV
TRERTAVSASAMFA

Parasites	L.mex
Gene ID	LmxM.31.0180
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	116 aa
Molecular weight (MW)	13129 Da
Isoelectric Point (ISP)	9.95
Signal peptide	No
Gene copy	Single

DNA sequence

TACAATACTACAAATAANTNAAGAAGGGAGATAATNCANAAACTAGGGATAAGAGACAAA
AATAANATAAAGAATNATATNTATATATAGACATAAGATAAAGATAGNCACAAAAGTNTA
CAGAATTAGCAAAACNAAAATTTAAGATAAAGCNAAATAGACATATATNCTAATAATAAN
ATAGACACAAAAACTAATATNGTTAAACAAAAAGAAAAAAAGTATAGGGGGGTTAGAAAA
TTAACTTAATATATATATATTATTATTTTTTATATTTTATATTTTAATTGTTCTACGACA
ATCCAANCGAAAAGCTACTACGACCAGCCAAGGGTAGCCAGACGAGGTTGCCGATTGCCA
TACTACCAAAATACATCCGAGGGGGCAATCAGAGTTCTATGTTTATCTTTTTTACTACTA

GATCTACTTCCCCTACGAGCGAGCGAGCGTCTGTGGNGAAACACGTCNTTGGCTGCATGC
GCACCCACCACACCACCNCCACNCCNCACCTCCCCACGGGGGTTCACGNGAGGGGCAAGG
GGGAAGNCAGTTAGGCCACAAAAGGGCTCTGCCGCCTCCACGGGGCAGGCAGTGTGCTAG
GTTTGTNGCAGTCTCCCTCGAGNCCCGTTCGCATGCCGTTTGCCCGCGGCAGCTTGCGGC
AGGTGTCCAAGGTGGCCGGCGGTCCCCATCCTCGTGCCCCCTTTCGTTCCGNGNCGGGTG
NCTTGGCGCTCACTCACTCCTCCGCGCACTTCTTGGTGTTGNTGCGACGGTNGCCCTTCC
GCTCCCCGTGGGCACCGTNGTTGGGCTCTAGAGTGTTCTCGCGCCTGTCCTGCTTGTGCG
GGGTCGGCCCCTGTGTGGCCTGCTCCGCTGGGTTTGGGGNGGGGGGGTTTTGGGTTGGGC
GGCACGCCCAAATTGTTGGTTTGGGGGAGAGCCCAGACGGGGGCCCAGGG

Protein Sequence

MSASEDTAPAPTAKPRRSVANYTEPPKKNPYLVKFSRFYSTKIPMGVREFVFTGPLLLIT
FGVAYFIPSLIPAEQFTQGLTPHKQDMREYTLEPIYGAHGELKGYRRINTKKSAEE

Parasites	L.mex
Gene ID	LmxM.31.1090
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	1629 aa
Molecular weight (MW)	178719 Da
Isoelectric Point (ISP)	5.84
Signal peptide	No
Gene copy	Single

DNA sequence

TATCGTACTCACTATAGGGGATATCAGCTGGATGGCAAATAATGATTTTATTTTGACTGA
TAGTGACCTGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGT
ACAAGAAAGTTGGGTGAATTCTTTTTATTTTTTTTTTTTTTTCATTGTAGCCGGAGATG
CACCGTGAGAGAGTGCGATAAAATACGTTGATTGTCGCCGGTAAACTAACCAGGAGGGAG
AAAGAACTGGTAGCGGGCACCACCGCACAGCCTCCCAGTCAAAACGTTTCCTCCCCCGCC
TCGACCAGCAACACGAGAAGACGGCCACCAAAGAGGTCTGCCGAAGAGGCTTCCACGGCA
GCAGCGTCAGACGAGACAATATACAGAACCAATACCGCACGAAGAGTATCCACAACAACG
AAATCAGCGAAGAGCAAGTCGAGAAACCAGCACACTCCGCAACAAACGTCTATACGGTCA
CATATAGCTGCTTTGACAGCCTGTGGCCACCGCAGAGGTACCTAGGAGAGCAAGAACACG
CCGCCGCCTTCGGTTATGGCGAAGCGAAGCTTCTTCTCCATCTCCTCTTTCGTGTTGTAC
TGCGGTAGCTTCAAGTAATTCTGACACGTCATGGCGGATGGTAGCATGTTCTCTTCCTTG
ATGCTGGCATTCTCGGACGTTTTCCGCACATTCGTAAACGACGGCCGCAGTCGACCCAGT
CGCTACAGGGAGGTGCGCCGACCGGTGAGGAGAAAAAAACGCCTCTGCTCCTCCACGGTG
AGGCGGGCAGTATTCTAAAGAGCCACTGTACATGCTTGCCACCTTAGGTTTTATCGTTAN
TCGCCCTGTCGTATCTTTTAAGGTTGTGGCGCGTATTGCGCTTCTTGGACACATAGACCG
GTTACCTCTTCCCCCGACATCTAGTACGTAGCAGACCG

Protein Sequence

MDGFRFYMEGVGLPEGSMRRVNAELIGAINSPDPMVQHSGLQMLCDQLTMSSFISPSTMA
TIPLVLPSLLRCIASSQVREVFITAARALTYIIDAFPRTFETFPTRDTLMEVLLQHLRSI
QDVELSEQCITCLEMITRSQMGSRELLRNDGVEAVLGFADFFTLHKQRQIWTIVQRLVGE
MDESSVRHITACLPTLRIGMTNNDSEIRQKAIATLAQAIEGVKTDRATVETVFGDAADRI
AVLLHERDVNDDTMSSALSLLYAGVQWSAGIAASVIQSDLFNTLLSLLQPAPLPAVVAEQ
YTAPSAAGRAGHRGAVAEMVVGGDSGRDSGGDAGTEPAAARTTMLTSHQRTIVCRLLASL
LIPYRAGAAEELERLEMLTQRPLLTGAPAHGNRSGMDDGDGYDEDYTTEEDEDGEEMHDF
DDDSDADTTLTHLRQRVAQEQGLKIETKPAYARCRASGFMCDGCGKNCTPGDWYRCNECP
DKDYCTACVLEHYKDDHDGQHSYTDMEQVVGAAARNRDKLELYRKSPQLLQRVLEAIPTV
VGVCISSELVAVRTSCLDFLVSAVDMASTEQLLASEITKVSLGESLNNNLRGADLLCNAL
AVALAGRLLQKLSDIYQVQFVREGVKLSLQVLKQRCKVKGRTTLTREARAALITCTAGWG
TIIGTEASILLQRFLDVEDEQATESLRRVVEELRQDHFSTAVGLLRDVLAGDVTAFEFFS
SGVVRELRSCLSRQQNIYAVMHLVATLSTSPATTRGAKAKKSAGVAPTLAATTAGRVGHG

SGSLLSHFVHHLHTILTLLDDFAVPTYDFIGGVHNYFVVSFEPHRASVAVAGDTTAGTSP
PSVRGDGDSAVSPRQCIKARIRPISSVSAMAQVLQQEVLQQESEEGDNADGEEGEAVTNV
LPDLHPRHSPTSRDAEAPVPAGPSSPASAPENSSIWIRYGTHVLPLSMTMLQIMEHLVLP
AAAASEQEARGSHHDNAERRAKPPLQQRGKRDDTTASEEGAHEEEGARHGAHSRPAPLAT
STAAGYSLQRPVVLYYSTAPYNPQYYSLFKVPNAFPASGNPQAPLQVRLPSEDRKPRAVW
DVQEQLAGAFPYSKHVLSDSQRDVLGLLGTLYAAVANWAVLLNYVRAQAAAESKHLRGID
AACILSTFAPAISIAEFQHAKLNNKAIQQCSQMLLAGQQRGTWAVKLALDCTYLFSYSTR
KFLFDVGFTSTDRCLIQMRKYRELFGLNELRMSSEQMRGIYGQLKKETKRVWREDVLECA
KKVLSAQDARARNRVWSFHFYNENGWGDGPTREFYTLVSQELRQRKLGLWRDGNEAAGDT
EYSTAAYGLFPKPVLPGSTQEEDHLVPFFRLIGRFIGRALVDEHVPGLPLSPVFLRLLRG
DVCGVHDVQDLSEEVGRLLVAMAGAAASGHARVQLPGQTKAVEVQDLGLDFTLPGDDSVE
LCVNGAKTAVTANNMMAYCDAVTSFLLDRGVAAAVHALREGFHWYIPLVALQMLSVDELY
RLIAGHETAITRDDFEKYSKANYGYTLSCKHVQWLFDILAAFTVEEQRQFFFFLTGSAHL
PVGGLGRLRPSFTIVRKTSEDASIKEEDMLPSAMTCQNYLKLPQYNTKEEMEKKLRFAIT
EGGGVFLLS

Parasites	L.mex
Gene ID	LmxM.20.1290
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	280 aa
Molecular weight (MW)	31358Da
Isoelectric Point (ISP)	4.17
Signal peptide	no
Gene copy	multiple

DNA sequence

NNNNNNNNNNNNNNNNNNTGNNNGNNNATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTT
TTTTTTTTTGAAGTGTGCGCCCGTGCAAACATGTGTATGTGTGTGTGTAGCGGTGTCTGTGCATCGGTA
AGTGTGTGTGTATATGTGCGTCTCTCATTACACCGTGGCCCCTCCCCTCTCCCCTGACTCCCTTTTCTT
TGAGTTTGTTTCACATGTTCCGCGTCCGTTTCTTCTTTGGGTCCCCTTCCCTCCTCCCCCCTCCCCCTC
CCCCCGCCTGGGGTGTACCGTCGATCACAGACGAGGAAAAGAAAAGAAAACGAAAGAGGAATGGACCGA
CAGACAAACAGCGCGAACACAGCAACGTAAAACATTAAGTGAAAACAAGAAGAAACACGCGCACAGCCA
CACACACACACACGTATGCATGCAGGCACACGGACACACCTTCGAACAATACCTCTCGCTCTTTCCCGT
CTCTAGCTACTCGAGGACGTGGTTCCGTTCGACTTTATTTCGATTTCATTGTCCACTTTGCCTTGGCTT
TCTCCTTGCTATGTAGGTGTGTGTGTGTATGTGTGTGGCTGTGGTCGTTGATACATGCTTGCTCAGCTC
TTTTGCCGTCTCCCTCCCTCTTCCCCTAAACGTCTATATATATATATGTTCCTTCCCCACCCCCGTCCC
TTTTTTTTCGCTCTCTCTCCAGTGTTCCCCACTTTTTCTTTTTCCATCTTTTCTTCGTTCTTTTCGTTT
GGNCACGCCCTCTCNNAACTGANTGCCCTGTCTTTACTCGACTGCACGCACACACACACACACATGNNA
GTGNTCTCGTGNGTGTCTGTGCGTNCGCTTNNTTNNGNNNCGTCTTTTGNTTTGCGTTGNTTCCNGNTN
CTTCCTTTTTGNTTTTTTTTCCCCNNNNCCCCNNNCCNCCCCTTNNNTGANGGGNNNANGNTNGGNNNN
NGNNNNNAANANNNNAAANGNNNNNNNNNNANCNNNNNNNANNNNNTNNNNNNNNGNNNNNNNNCNNNN
NNNANANNNNANNNNNNNNNNNNANACNTNNNNNNNGNCTTTTTNNNNNNNNNNNNNNNNNNNNNTNC
CCTNNNNNNNNTNTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

Protein Sequence

MEKAREIQSKLDSLQQEMHAKVEACDVKYNKEKNAIFAARRAIVAELIAKKEMPANFWAL
ALIALLQMKDRESTTTPHFLGPYDDELLKTYLEDIEVLYTEKGHRITLRFKPNPFFEETE
LWAQASEIMNDEADEEDDMPPVEESWGFSGVTWKKGHGPQLDEDEDEEDDGAPGKKRPHP
GIGDLAASASSSTQGPSVLEVFSEMPPHPEEDEEMDEEDDDAVADAIEEWEEEMADRKML
LRMVELFVHHNPVSALRDGGAATAGASNGEEAAAKKAKVE

Parasites	L.mex
Gene ID	LmxM.10.0405 (99%)
Gene name	GP63, leishmanolysin
Feature	In other organisms

Sequencing length	644 aa
Molecular weight (MW)	68738 Da
Isoelectric Point (ISP)	7.75
Signal peptide	No/2
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNTCNGCTGGATGGNNNTAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTCATGTGCGTCGCCATCTCTTGTGGCTCGCGGCATGCTCTGCTGCGCCTATTTCT
TCGTCCGGTCGTCGCCTCTTCTTGCTCGTGTGACGTTTTCCTCTGGTCCTGCCCGCGCACCTCTCCGGC
TCCAGCCCTTGCCGGTCCTCAATGCTGCGTGCGTGCGTGCGTGTGCTGGGTGCATGGCGACGCCGTCGA
CAGCGCAGCTGCCCTGTCGCTCCGCTGTGTGCTGCCATTCGGTGGGCGATGATGCACGATGACGTGCGC
CCACAAAGTGCGCATTGTTGTCGGGCGAACAGCACAACCGCCGCACCACGAGCGGCGGAGAAAAGGGTA
GAAGAGAGAACACAGCGCGAGAAGCAGGCGGCTCCCCCTCCCCTCCCCCGACTGGCGGCATGGAATCAC
GAGCGAGGCAGAAGGGGGAGCGTGGGGGAGGGGAAGCGTGACGCAACGCAAAGAGGGGATCCGCATAGG
TGCGGACAACAGAGCAGCACGAGCACATCACCAAAGTACAAGAAGCCACCTGCCTCTCGCCTGGGCGCT
GGCCAACTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGG
TCACTATCAGTCAAAATAAAATCATTATTTGGGGCCCNAGCTTAAGACTGGCCGNCGNTTTACAACGTC
GTGACTGGGAAAACATCCGTGCTAGCGTTAACGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGA
AGGCTCAGTCGGAAGACTGGGCCCTTTCGNTTTATCTGTTGTTTGNCGGTGAACGCTCTCCTGAGTAGG
ACAAATCCGCCGGGAGCGGNNTTGAACGTTGTGAAGCACGGCCCNGANGTGGCGGGCAGGNNNCCGCCN
TAAACTGCNGGNNTCAATTANNNGAAGGNCNTCNTGNNNGANGGN

Protein Sequence

MSVDSSSTHRNRCVAARLVRLAAAGAAVTVAVGTAAAWAHAGAPQHRCIHDAMQARVRQS
VAAQRMAPSAVSAVGLPHVTLDAADTAAGADPSTGTPRNVVRAANWGALRIAVSAEDLTD
PAYHCARVGQRISARDGRFAVCTAEDILTDEKRDILVKHLVPQALQLHRERLKVRQVQGK
WKVTDMAADVCSYFKVPPAHVTGGVSNTDFVLYVASVPSEESVLAWAMTCQVFPDGHPAV
GVINIPAANIASRYDQLVTRVVAHEMAHALGFSGTFFEAVGIVQEVPGIRGKTFTVAVIT
SSTAVAKAREQYGCNSLEYLEIEDQGGAGSAGSHIKMRNAKDELMAPAASAGYYTALTMA
VFQDLGFYQADFSKAEEMPWGRNVRCAFLSEKCMAKNVTKWPAMFCNESAATIRCPTDRL
RVGTCGITAYNTSLATYWQYFTNASLGGYSPFLDYCPFVVGYRNGSCNQDASTTPDLLAA
FNVFSEAARCIDGAFTPKNRTAADGYYTALCANVKCDTATRTYSVQVRGSNGYANCTPGL
RVKLSSVSDAFEKGGYVTCPPYVEVCQGNVKAAKDFAGDTDSSSSADDAADKEAMQRWSD
RMAALATATTLLLGMVLSLMALLVVRLLLTSSPWCCCRLGGLPT

Parasites	L.mex
Gene ID	LmxM.31.0920 (99%)
Gene name	vacuolar proton-ATPase-like protein, putative
Feature	In other organsims

Sequencing length	893 aa
Molecular weight (MW)	101197 Da
Isoelectric Point (ISP)	5.29
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTGCACTGACGGTGATGTCTAAAGAGTGGGAAAGAAGAGGAGAAAGAATGGAAAAC
ACAGAAGGGAACGGAAGAGATGAGAAGCGCGATGCACAAACGCCGTATTGAGCTGGAGCTGATGAAAAA
AAAAACACGAAAACGAAAACAAAAGAGAAAGAAGGGCAGAAAGCACAAAATTGACCAGCGCTACGGGTA
CAGAGAGTAACACACCCACGCAACAGCAAGGCACATATGCAGAAGAAGAAAGGAAAGGTGTATGTGAAT
TAGCACACACACACACAATGAAATATATATATATAACTGCAGGCAAAACTACCACAAAAAAAGGAACGG
CGGGAAAGCGAAAAGTGGAACACCAAACAGCAGCGACGAATGAGAACCACAGCCGCTGTGTATATGGCG
AAAGCAAGAGAATTACGAAAACGAAAGGGATACTTCGATCGACACTGTGCCGGAGAGGCGCGACAGGGG
TTGTTCTAAGAGGGGCGCAGAGAAATGGAATCGACAACCACAGACGCGGGGCAAAACAAGAGCGAGACA
GCTGCAGAAAAGTCTCGGGACGTTGGATATGTCGACCGCGGTCTGATGGAGGTGTTGCAAGGACAGTGA
CAATGACAACAGGCGAAAGGAGGGGAGTCCTAAAAAAGAAGAGGTGTATGCGTGAACGCTTTGTGTATG
TTACTGATCTTGTGTGTTTTCTTATGTGATTCAATGAGCTGGAACGACGCGTCGACTCAATAAAGTACA
GAAACCCAACTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAAC
AGGTCACTATCAGTCAAAATAAAATCATTATTTGGGGNCCNAGCTTAANACTGGNNGTCGNTTTACNAC
GTCGTGACTGGGNAAACATCCATGCTAGCGTTAACGCGAGAGTNGGGAACTGCCAGGCATCAATAAAAC
GAAANGNTCAGTCNNANNACTGGNNNTTCNTTTATCTGNNGNTNNNNGNNANGNNCNCNNNNNGNNAAT
CNNNNNNNGNNTGACNTNNNANNACGNCNNNANNGNGNGNNGNNANNNCNCNNCNNAACNNNNNGNNNN
ANTANCNAANNNNNCNNNNNNNGNNTNNTNNNNNANNNNCNNGNNNNNNNNCNNNNTCTNANNNNNNAA
CNNAANNNNNNNNNNNTNNANNNNNTNNNNNNNNNNNNAN

Protein Sequence

MPWRQEHCSGLWRSEDMIRVNIILQREVLYDTMYEVGMLGRVQFLDMNEGITTFARPFTE
ELRRCEELQRKLHFIEESMRKDADLLDRYPGDVNMSATVEEMRSSLLRGQMHMIDDRIES
TVNELTAMLTSLEGFQHEMNQNQEMTLLYYKYRLLVETPSDMTMGNSSFAHQSAAVSSEA
FSRLASLFGFIDSKLSEELYRLCYRITRGNAIVEISSEPAMFVDVQTGERNVAKTPFVVL
CSSPTMIVRLKKLMIGLGAGVYTLDEVQSRGIELTTSTTAHDVEETIEGVERRKRDVLTQ
WYEEHRLYKTYLKVEKVVLTAMNMCAMSGSTCTASAWVPLRHEQSLRRALQDAVASANGS
VESIVTLHAEQQHPPTFFETNRFTESFQGIVDSYGMARYKEVNPGVFTIITFPYLFGIMY
GDIGHGFLLLFIALFFISKEKAWRTAQLNEIVAMVFGGRYLLLLMSLFAIYMGVLYNDFF
GFSLNLFSSGYTWAPISEQKGTTYPTMPSGRPSVKPPHVYAMGLDAAWAETDNKLEFYNS
VKMKHAVIVGVAQMFAGLFLSLNNSIYEKNWYKIAFLFVPEFVFLLCTFGYMSILIMVKW
CRTWENTNKAPSILEIMTNFFLQPGSVPNPLFSGQAGLQVFLLLAASSMVPFMLLGMPYI
EMRDYKRWQQRRQVGGSRRRHGGAQRASVATIEASDYTDAFLNEPSASLQPQPANYSGDD
SAHRNLMSDDDDASNIFGDDNMHPFGVSSANSEDGATATVIERENEKFEHFDVSELLIHY
VIHTIEYVLSSVSNTASYLRLWALSLAHAQLSEVFFSFTVTKTLDIDNNSGFVIAIGVLL
WLGATLGVLVGMEALSAFLHALRLHWVEFQNKFYAGDGRAFDPMDLISLNMQN

Parasites	L.mex
Gene ID	LmxM.34.3870 (99%)
Gene name	nucleoside diphosphate kinase, putative
Feature	In other organisms

Sequencing length	337 aa
Molecular weight (MW)	37055 Da
Isoelectric Point (ISP)	5.63
Signal peptide	No
Gene copy	single

DAN sequence

NNNNNNNNNGNNANNNGCTGGATGGCNNTAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTT

GTTCTTGCGTGGCAAGGGAGAGGAAGGAATCAACACGCACACACAGGAGAAAATGTGCCGGCTCTTGCA
AAGCATGTGAAGTAGCGGTTCGAAAGGAGTAGCCCTCCGTCGTCTACAGTAGCCTGCAATCAGCTTGCC
CTTCGCACGTCAGGCTATTTACATCCAAGAGCACCGAGGGAGGAAAAAACGAAACAGGAATGTCGTGTG
CTGGGCACGTCCTGGCTGTCGCCGGAGCACGTGCATCGCGAGGCGAGCAGAAAGAGGGTGAGAAACAGA
GACAAGGCATGATGAGTATTCCAGTGCGTCGGTCCCTCTCGTGCCTTTGGTGTCCCGACACACAGTTCG
TCCGCCTTCTCCGGTCATCTTGCGATAAGCAAGATCACTGCTTGTTCTGCAGCAAAGAAAAGAAGAACT
CTGATTCAAGGGGACCATCCTCTTCCAGGTCGGTACAATGGACAGCGTTCCGGATGCGGTCCACGCCGT
ACATGGAGCGGAGCGTGTGAGGGAAAAGAACGTGGCACACTTCTGGGTCATGCGGGCCACACACCGCCC
GCAGCGCCGGCACGGCGTTCTCGGCGCAAACCTCAATGGCCCAGCACGGGCCGGACGACATCTGCTCCA
CCAGCTTCTTGTACTCCGGAAGAACTCCGTTGTAAACCTCTAAAAAGTCCTCAGCATCTGCCACTGTCA
GCTGGTAAGAACCAAGGGCACTAATGTAAAACCCCTCCTCAACCAAGCGATGAAGAATGGGACCTTGAT
AGCCGCTAGTGATCGCGTGTGGCTTGATGACGCACACGGCGCAGTTCTTCAGCGCCGCTGGGGACTCGC
CTGGCCCCATGACTGCCTCCTTCAGCTCCTGCACAGCACTCTCGCTCGAAATCTGCGTCACGGCAGTGG
AAANNNNGGCCGCCACCGAGGCCACCTTTTCACGGGCTCCGGCGCCGCTCGCAAGCACNACTAGGCANC
NNCTGCGCCTTTGATGCACTCGGNAGCGAGGCTCTGCGAAAGCTCTACTANNGNTGANGTATCNCNNNN
NGCGTTGTCNNNNCTNANCANGANCNGCTNCNNNTNTNNNCTGCATGTCANNGTATCATGCNNNNNCCC
NANTNNNGNNNNNNNNNNNNNNNNNCNNCNCCNTNNNCNNNANGNNNNNNNNNNNNAANACNTNNANCNN
NNNCCNNNNNNANNNNNNNNNNNNNNNNNNNNNNNCGCANNNNNNNNNNNANNNNNNNCTNGNNNNNNNN
NNNNANNN

Protein Sequence

MVGEQRDTFVVEYFDPQASLSRTYQFCYFTDDKTIEMYNLKTKRLFLKRCAYPSLSPNEL
YVGATINVFSRPLRIVDYGDDATRKRLTANSGECMITVDMQHHSAAAGSVIEGLTTQGLR
ITFIRLVELSQSLATRVASKAQRCLVVLASGAGAREKVASVAASFSTAVTQISSESAVQE
LKEAVMGPGESPAALKNCAVCVIKPHAITSGYQGPILHRLVEEGFYISALGSYQLTVADA
EDFLEVYNGVLPEYKKLVEQMSSGPCWAIEVCAENAVPALRAVCGPHDPEVCHVLFPHTL
RSMYGVDRIRNAVHCTDLEEDGPLESEFFFSLLQNKQ

Parasites	L.mex
Gene ID	LmxM.36.1610 (99%)
Gene name	Nucilirdie binding
Feature	In other organisms

Sequencing length	264 aa
Molecular weight (MW)	29756 Da
Isoelectric Point (ISP)	4.79
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNANNNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTT
TTTTTTTTTTTTTTCCGATGTCGGCATGGGACAAGAAACACAACAGAACAAAAAAAAGGCACAAAGACA
ATACGAAAAAAAAAAGGAAGTGACACACACGCACACACACACACACGCACACACGCACGCACGCACACAA
CACCAAAAAGAAGACGCGTCGTTGTTTGGAGGCGTTGCCGAATGGCGTGCCGCACTCCTATCTGATTTT
TCTTTCTTTAGTGGGCCTTCCTTCGTTTCGTCTTGTTGGATCTCCGTTCTCTCGCTCTCTGTGTGTGTA
TTGATGTGTGTATTGATGTGTGTGCGTGTGTGTGTGTGTGTGTGTCGGTTCTGTCTTGGTGATCATTCC
TCCTCCGGAACGCTTTTCGTTGTGCGTCGCCTACCTTGTCTTTTTCTCATGTTTGTTTTTTTTCCTTGT
TGCCCTTGTGTGTATTTGAGGCGGTGTTCTCAAGAATGCTTCTCTTTTCCTTCTTTCATGTTTTAAACA
ACAAAAAAAAGGCGACTCACATTAGCGTACACAAAGACATACACCGAATCATGTGCGCGTAGTGAACGG
GGCACAAACACATTGCTTGATACGGGCGAGGGTCTTGCAAGGTGGGACGAGCAAGGAGCTAATTCTCTT
GAAGAGAGACACGCAAACATACGCACTAGCCAGCAAACCTACCAACAACACGCATAGCAAGATAATCAG
GAGTTGCTCTTGAGTTACTGAGCATGTCGAAAGTACTCTGTTTCGAGCCTTGCGGTTCAGAATACATGC
CATTGCGTATGTCTGTCCATCTGCCTATAGGTATGGCTGTTTGTATCTGTGTATGTGTGTGTACGTGTG
CCTGTGTCTATCTGCGTGCAGGGCAAGGTGTCTCATAGACCTTTGGTCAGCCCATCTCGCCGTCCCCCT
TCTCTTGCGCTTTTTTCCCACGTGTTCCTTATTGTCTCCCGTCCCGCACGTCTTTTTGTTTGTTTGTTT

GTTTCTTCCTCTCCACTTCCCGGAGGGGCGTGTTTCCANCANCAAACNGGCGCANNAGAAANAAAAAAA
NAANGAAAAAANANNNAAAAAAGAACGNAANAGNNACACNANTAAATNNTATATATCACTTTTTGTACN
AGTGNATNNAAANNCATGCTCATNNNNNNGCACNANNGTCNNNNTCNGTNAANNAANCNTTANNTNGNN
NNNNNNTNNACNNNNNNNNNNNNNNNNNGNCNGNNNANCNCNNNNTNNNNANNNNNNNNGNNNNNCNN
NNNNNANNNNGNNNNNNNGNAANNGNNNNNNTNNNNNN

Protein Sequence

MFKNEYDSDITTWSPTGRLFQIEYANEAVNNGSATVGVKGRDYVVLTALKRSPVAELSSY
QEKVFEIDEHVGMSISGLVADGRVLARFLRTECMNYRYMYSDGMPMNQMADMVGEKHQRH
IQFGGKRPFGVGLLLAGYDRQGPHLYQTVPSGDVYDYKATAMGVRSQASRTYLEKHFVHF
TDCTLDELVVHALKALASATSEGVELNVKNTTIAIVGKDTPFTIFEEESARRYLDEFKMR
PEDRVAAADDEEEVLHEQPLDVEE

Parasites	L.mex
Gene ID	LmxM.34.1890 (92%)
Gene name	60S ribosomal protein L5, putative
Feature	In other organisms

Sequencing length	305 aa
Molecular weight (MW)	34030 Da
Isoelectric Point (ISP)	10.69
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAACGGGGAAGCTCTGACCTTTCCCCCCCG
CTAGGGCCCCATCAGGGGGGAGAGAGGGGGAGCAAACCCGACAAGAGGCCGAAAAAAAAA
CCCCCAGAAAATTAGCAATGAAAACAAAAAAAAAAAAAAATATTTGAGGTTCCCTCAGCG
GCACGTGCGCNCGCGGTTGCCGCCAGCGCGCCTCNCTGTCCCTGTTCCAAGACAACCATG
GGGATGCACTTCTTTCNNCCAAAGGAACAGGAATCAAAAAAGAGGCGTCAAANCAGGAGA
CCAAAANAGACTCGCGTATTCAGAGGTGAGCGCTCGACTTACTTGCCGAGGCGCTCGCGG
ATGGCCGCGACCTTCGCCAACTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTC
ATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAAAATAAAATCATTATTTGGGGCCC
GAGCTTAANACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACATCCATGCTAGCGT
TAACGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCNCNGTCGGAAGACT
GGGCCTTTCGTTTNNTCNGTTGNTNGTCGGTGAACNNNNNTCCTGANTAGGANAAATCCN
CCGGGANCGGATTTGAACGTTGNGNANCNANCGGCCCGGANGGGTGGGCGGGNNNGACCC
CCGCCNNNAACTGNCAGGCATCAAACTAAGCNNNAGGCCATCCTGACGGATGGNCTTTNN
NNTTCTANNAANNCTTNCNGGNTAGNGGTACNNNCGTTCCCNGANNNNNNANCCNGTAGA
AAAANNNCAAAGGANNNNNNNNNGNATCNNNTNTNNNNNNNNGNANNNNNNNNNNTNGNA
NNNAANNNCNNNNNCCNNNNNGNNNNNNNNNCNNGNNNANANNNNCCANNNNNTTTNCNA
NNNNNNNGGNNTNNNNNNNNNNNCNANNNNGNNNTNNNNNGGNANCNNNNTNGNCNCNTN
NNNANCNNNNNNNANNCNCNNNNNNNNNNNGNNNNANNNNNNNCNNNNNNCNNGGNNN

Protein Sequence

MPFVKVVKNKAYFKRFQVKYRRRREGKTDYHARRQMVLQDKTKFGSPKYRLVVRITNKDI
IAQIVQAKIVGDEVVMAAYAHELPAFGIEHGLTNYAAAYATGLLLARRTLAKLGIADKFQ
GAKEADGSYSAVRTKKDDEGDDEERFPFKAILDVGLARTTTGARVFGVLKGAVDGGMAVP
HRPNRFPGYNKEKSSLDAKVHRDRIFGKHVAEYLKQVKEEASSNPDEKCVQFSKYMAAKV
LPESIEGMYKKAHAAIRADPSKSLPKKAKKESVAHKSYKTKKLSGAEKRAAAKAKVAAIR
ERLGK

Parasites	L.mex
Gene ID	LmxM.36.3590 (99%)
Gene name	cysteine synthase, putative
Feature	No information

Sequencing length	467 aa
Molecular weight (MW)	53542 Da
Isoelectric Point (ISP)	6.73
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNANGNNNNNNGCTGGATGGCNNTAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTT
TTTTTTTTTTTTTTGCCTTGCGTGCGCCTCCACCTCCTCCCAGGGTTGGCGTTTCTCTGTGTCGCTGCC
TTGCCGGTTGTGTAAGGTCCCCAGTTGGGAATTGGTGTCCCCTTTTTCCACTGTCGAGACGAGTGGCAA
TGCAACAGTGAAAGAACACACACACGCACACCCACACACACAAAAAAATCAATGGCACGTTCACCGATG
GACATTGAAAGGGATGAGAAGACAGAGCAGGGAGTCACAGTCGATGCGTATGGACAAGGAAGGGGCACG
CGGCCCTGGCCTCGCCTCCTCCCCCCCCCCCTCCTCTCCTCCTCTCCACACTACCTTCCCTTCCCCCCC
ATCTACTCGTTAATCTCTTCGACGCCGACTCGAGCCAACCTACTCATCTGTTTCGTCGACTCTGCCGCA
CTACGCGCGCACGCCGCGAGAAACGAACTTTTCACATTCAGAGCCTCCGTGGGGAGTGTGTGTGTGTGT
CAACATGCGGCAACACCTCCGCGACGGTTTCACACAACCATAGAGAGAGGGGAGTCCGGTGGAACCGGT
AGGAGGAAGCACTAGGAAACCACACACAAAAACGTCTCGCAGAACACGGCGAAAAAGAGAAGTAAAGAA
ACCAAGCACGAAAGGAGAAGCGCGCATGCTGATATATAGACACGTAAGCCCATGCGCGTGGAGATGGAC
AACCCGAGACAAGACGTAGTGTGCCGCGTCAACTGTATGCATATAGAGTTTGATGGAAACACGTGAGGG
ANGACAAGGGACAAGGAGACACCTGATGTACTCCCCTCCTTTCTTTCTCAGTGTATGTATGTATGTATG
TGTGTGTGTGTGTGTGTTTCTGTGCGGTAGATGTGTGNNNNNGNNNNNNNNGGAAGTTNACNTTTTNNT
ACCNCGGATAAAGGAGGAANNNNGNAGNNTNNCGNANGCAANANNNAAANANNNNTGGNANNNGCNNCN
NNNTCNCNTCNNCNCCNNCANNNNNAAAATANNCGCTNNNCGNNNNGGTGACNGNTANNNCNGCTAANC
ACTAANNNGGCGGNGGCAGCNNANNNNNNNNANCGGNAAAAAAANNNANCNGNTNNNNNNNNNAATGANN
ANAANNTGNGCNNCNTNCCNNNNNNNNNCGGNNNNNNNNCNNNNCCNNCNNNNGNNNNTTGNNNNNNN
NNTNAAATANANNCNGNNNNNNGNNNANNNNNNNNNGNNNNGNNCNNNNNNNNNNNNANAANNNNNTNG
NNNNNNTNNNNNNNCNNNNNCNNNNNCNNNCCNNNNN

Protein Sequence

MHFSQYPLRLTDLERQKLQLIVAALKVSEYTDDVDDFMRPYGKEGRMEVAMREFIDIVVG
LAIASDAIPRSVKNSFLAGEVKVATVVPLLEDLFEIMRRHKRLNPFSHRGEFGKLMMMLQ
DVQKQSIQRALAIQSTLVIPVRTVEAALSSIHCETLADDEVVRTDYLKRKGAEKQAGMQS
LIERYGKGDGHRKEVIEHCLRSIDDVYSFIQFNTRPLRTLRRWLSRDFESLPSDDVYSIS
IRHGRGGACFTHSHATHCQYVAESLLLWENVQKNILNLWEAAEDDMLVEGQGQYVVSNTG
QGFHRMCSAPRSYGVMSRLVRDTEQRMGGWVGIKVIHLGDRDVPNPLVFIDKYTVIPRLV
KPVVQTLHALRYVFHEEDEEEEGQPQVAHEYDNYPGLRNLLRSKYHSYAELMMMILSDFF
KHAFDGSGDDGGSCIDGRLTSAWNWCHQLHKKKYYDAFVLTGFAGFD

Parasites	L.mex
Gene ID	LmxM.13.0390 (78%)
Gene name	Alpha tubuline
Feature	In other organisms

Sequencing length	548 aa
Molecular weight (MW)	60185 Da
Isoelectric Point (ISP)	5.55
Signal peptide	No/3
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTCCACCTACCAACGACGGGGAGGGAGAGGGAA
GGCCCCCCCCCCACAAAAACNCAGCGCTCCGGCTACCCGCGCGGGGGCNTGCNCGGCAAAC
CCCCCCCCNAGGAAGGAAAGGNNGGCAAACGGAAAANCCCCGGAAAGCGGGGCCCCCCCC
CCCTCCCCCCCCCCCGCCCCCCNNAAAAAAAAAANCCCCCACNAANGCNAAAAAAAAAAAA
AAAAGGGGGNCATCGCANGCACNNGCNCNCTCCNCCCCCNCCCTCNNGCCGGTCGNNNNG
CNCNCCCNCACNNNGNCNGGGGGGNAGGGGNTCCCCTGCNNCNCACCCACNCGGGCNCCT
ACTTCATCCCCGGGGGGNNCNAGGGNNCCTTANNNNTCCNCNACNNNNTNNTCNNNNTGN
CGCCGNNNNATNCGNCCCNNNNNNNNNGNANTCNTNNNNNNNNNANNNANCCANNNTTTN
NNNNNNNNNNNNNNNNAAANNNNNNNNNNNTNNNNTTNNNNNNNCNNNNNNNCNNNNNNNNN
NNNANNNNNNTNNGNNNNNNNNNNNNNNNNNNNCNNNTNNNANNNNNNNNNNANNNNNN
NNNNTANCNNNNNNNNNGTNNNNNNNNNNNNNNNNNNNNNANNNNNNNNNNANNNNNNNNN
NNNNNCTNNNTNNNGNNNNNNNNNNNNNNNNNAANCNNNNNNNNNTNANNNNNANNNNNNN
NNNGNGNGNN

Protein Sequence

MGLMRTDTHVHAPPSSRSAALLLLLLPCAGRLPRVSSPHLALSLLSSPLPSPPLLADTQH
ARCTHAHAHRNPRSSSLSFFEQTPLNRLLTPLPSFSAMREAICIHIGQAGCQVGNACWEL
FCLEHGIQPDGSMPSDKCIGVEDDAFNTFFSETGAGKHVPRCIFLDLEPTVVDEVRTGTY
RQLFNPEQLVSGKEDAANNYARGHYTIGKEIVDLALDRIRKLADNCTGLQGFMVFHAVGG
GTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTAVVEPYNCVLSTHSLLEHTDVATM
LDNEAIYDLTRRSLDIERPSYTNVNRLIGQVVSSLTASLRFDGALNVDLTEFQTNLVPYP
RIHFVLTSYAPVVSAEKAYHEQLSVADITNSVFEPAGMLTKCDPRHGKYMSCCLMYRGDV
VPKDVNAAIATIKTKRTIQFVDWCPTGFKCGINYQPPTVVPGGDLAKVQRAVCMIANSTA
IAEVFARIDHKFDLMYSKRAFVHWYVGEGMEEGEFSEAREDLAALEKDYEEVGAESADDM
GEEDVEEY

Parasites	L.mex
Gene ID	LmxM.14.0650 (99%)
Gene name	fatty acid elongase, putative
Feature	In other organisms

Sequencing length	375 aa
Molecular weight (MW)	42481 Da
Isoelectric Point (ISP)	10.09
Signal peptide	No/3
Gene copy	multiple

DNA sequence

NNNNNNNNNNGNNNNNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTT
TTTTTTTTTTTTTTCCGGCCTTGTCCCCAAAACAGCTGCCCCCCGCTGTGGGTGCGGCAACACAGAGCG
ATGCAGGACTAAGAAAAACACACTCAATCCGCACTCACCCACACCTGCAGGACGTTGCACCAGCGACAA
GCAGGCGGTCCATCTCTGCAAGCTTTTTTTCTTGCCTTTTTTCGTTTCCGACTTCTTTGTGGCTCGTCG
ATACTTATGTATGTCTGTGTGTGTGTGTGGGTGGGTGTAAGCATGCAGGTGTTCAAGTGCGCGTGTGCG
TGCATCCATCATTGTTGTTGGCTTCCACCCCCTCTCCGTACACCTCCACACACAACCATACAAAGTACG
CATGCGCACACTGGTGCGCCATCACACAGATGGAAAGAAAAAAGAGAGAGACGATTGTATGACCTGCCA
CATACCCGAGTACGCACATCAACACACACACACACACACACACAAGCAAGGCACTTCAAGGAAAGAAAT
CAGAAGGCAAGTAGCTGATACGTGGATACACGCACGCACACGCACGGATTGGTGGTCGTGAACTCAGGA
GGAGGAATACAAAGACGAACAGCTAAAACACGTTCGAAGTCGATCCTTGAGGGAGAGGGGATGAAGAAG
GGAGCATAACACGCACACACGCACACACACACAGACACACAATAGGGGGAAAGAAGGAGACGATGTGCC
TGTGCGCGTGCGTTGGTGCGGGTGTATGAGGGGAGGAGGGGGGGGGGGGGAGGAAGGGGAGGGGAGGAA
GGGGGGGANGGGGTTGGACTCGCACAANANTAGACNAANAACGTGCCTCGCTACTTGTACNAGCCCGGA

AACGTTCAAAGAAATAGAAACAGANAACAAACANAGCCCTNCCCCCNNNANTACGAAAAGATAANCATC
ATANAAGC

Protein Sequence

MRACVCVCVLSLCLFRWPLPPPSAPPPPIALARLTQPAGWVAEIGFTRLSAHTVFFVLRK
GLQLTLTDVLPLRRLSFPYRIYLARRKQSNTRRDKMQWLDNYGDHHYNGHAVRMWLASNV
DVCAYIAGAYLAFVFTGPRLMEAIFHGKPPIGVKKVWALWNLGLSVFSLYGWLRVGPPLL
RHLMNDGLHNTLCTFHEDEFYTTKVGFAIGMFAISKVPEFIDTVFLLMSGVKLGFLSWFH
HVTTYLFAWYSYQQGTSIFICAAAMNYFVHSIMYTYFALAEAGFKKLVKPFAMYITLLQI
TQMVGGLFVSGYVLAKKLTDDPSSPCPGTSMSAARTQLVIYIFNFYLFSEMFIKAYVLPR
KAGAAPRRPSPSKRA

Parasites	L.mex
Gene ID	LmxM.20.1290 (97%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	280 aa
Molecular weight (MW)	31358Da
Isoelectric Point (ISP)	4.17
Signal peptide	no
Gene copy	single

DNA sequence

NNNNNNNNNNNNNNNNNNTGNNNGNNNATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTT
TTTTTTTTTTGAAGTGTGCGCCCGTGCAAACATGTGTATGTGTGTGTGTAGCGGTGTCTGTGCATCGGT
AAGTGTGTGTGTATATGTGCGTCTCTCATTACACCGTGGCCCCTCCCCTCTCCCCTGACTCCCTTTTCT
TTGAGTTTGTTTCACATGTTCCGCGTCCGTTTCTTCTTTGGGTCCCCTTCCCTCCTCCCCCCTCCCCCT
CCCCCCGCCTGGGGTGTACCGTCGATCACAGACGAGGAAAAGAAAAGAAAACGAAAGAGGAATGGACCG
ACAGACAAACAGCGCGAACACAGCAACGTAAAACATTAAGTGAAAACAAGAAGAAACACGCGCACAGCC
ACACACACACACACGTATGCATGCAGGCACACGGACACACCTTCGAACAATACCTCTCGCTCTTTCCCG
TCTCTAGCTACTCGAGGACGTGGTTCCGTTCGACTTTATTTCGATTTCATTGTCCACTTTGCCTTGGCT
TTCTCCTTGCTATGTAGGTGTGTGTGTGTATGTGTGTGGCTGTGGTCGTTGATACATGCTTGCTCAGCT
CTTTTGCCGTCTCCCTCCCTCTTCCCCTAAACGTCTATATATATATATGTTCCTTCCCCACCCCCGTCC
CTTTTTTTTCGCTCTCTCTCCAGTGTTCCCCACTTTTTCTTTTTCCATCTTTTCTTCGTTCTTTTCGTT
TGGNCACGCCCTCTCNNAACTGANTGCCCTGTCTTTACTCGACTGCACGCACACACACACACACATGNN
AGTGNTCTCGTGNGTGTCTGTGCGTNCGCTTNNTTNNGNNNCGTCTTTTGNTTTGCGTTGNTTCCNGNT
NCTTCCTTTTTGNTTTTTTTTCCCCNNNNCCCCNNNCCNCCCCTTNNNTGANGGGNNNANGNTNGGNNN
NNGNNNNNAANANNNNAAANGNNNNNNNNNNANCNNNNNNNANNNNNTNNNNNNNNGNNNNNNNNCNNN
NNNNANANNNNANNNNNNNNNNNNANACNTNNNNNNNGNCTTTTTNNNNNNNNNNNNNNNNNNNNNTN
CCCTNNNNNNNNTNTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

Protein Sequence

MEKAREIQSKLDSLQQEMHAKVEACDVKYNKEKNAIFAARRAIVAELIAKKEMPANFWAL
ALIALLQMKDRESTTTPHFLGPYDDELLKTYLEDIEVLYTEKGHRITLRFKPNPFFEETE
LWAQASEIMNDEADEEDDMPPVEESWGFSGVTWKKGHGPQLDEDEDEEDDGAPGKKRPHP
GIGDLAASASSSTQGPSVLEVFSEMPPHPEEDEEMDEEDDDAVADAIEEWEEEMADRKML
LRMVELFVHHNPVSALRDGGAATAGASNGEEAAAKKAKVE

Parasites	L.mex
Gene ID	LmxM.22.0030 (100%)
Gene name	60S ribosomal protein L11 (L5, L16)
Feature	In other organisms

Sequencing length	188 aa
Molecular weight (MW)	21676 Da
Isoelectric Point (ISP)	10.68
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNTCNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTGAGAAACAAGAAATGACATGGAAATCCGTTATCTGTTCAGTAGACCCGAGGACGT
CTGTGTGGTGAAGTATGCGAGCGCGTGCGAGCCGCCAGAAATGAGGGGGCGGCGTACAGGCTTAGCGGC
GGCGGCGGCGGATCATGTTCTTCTTCTTCTTGGCCTGGAAGATGATACCGTCGTGGACTTTCTCGAACC
ACTTCATCGCCTCCTCCTTGGTGACGTGGTGGGAGTGGCCGACGCGGCTGCACTTGCGCTTGCGGTGCG
CCACTCGCTCGCCACGGCGACCGAGGACGACGTAGAAGTCCATGCCGTAGATACCGGTGGAGGGGTCGT
ACTTGATGCCGAGGTCGATGTGCTCGTCGATGCCGAAGCCGAAGGAGCCCGTGTCGGAGAAGTTGTAGC
TCTTCAGCTCAAACTCCCGCACCTTGAGGCCCTTCTCCAGCAGCTCCTCGGCCTTCTTGCCGCGGACGG
TGCAGTGCACGGCGATCTTCTCGTTACGACGGATGCCGAACGTGCGCACGGTCAGGCGAGCGCGGGAGA
GCACCGGGGTCTGCTCGCACAGCTGCTCGAGCACCTTCGAGGCACGCGTCAGGCGATCGCCGCTCTCAC
CAACGCAAATGTTGATGCAGAGCTTCTTCACGACGATCTCCCGCATGGGGTTCGCAGCCTTGCTCTCGG
CGACCATTTTCGCAGTCTTCGGTCTGATGAACAATAAAGCCAACTTTTTTGTACAAAGTTGGCATTATA
AAAAAGCATTGCTCATCANTTTGTTGCANCGAACAGGTCACTATCAGTCAAAATAAAATCATTATTTGG
GGCCCGAGCTTAAGACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACATCCATGCTAGCGTTAAC
GCGAGAGTAGGGNACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGNCTTTCGTTT
TATCTGTTGTTGTCGNGAACGCTCTCCTGANTAGGACAAATCGNNNAGCGGATTGANNNTNTNANCACG
CNNNGNGNGGNNGNCNCCGCNTNAACTNCAGCATNANTANCNNAAGCATCNGANGNNGNNNNNNNNNNN
NNNTNNNGNTANNGNACNNCNTCNNGNNNNNNNNCNNNNAANANNANGANNTNTGNNNTTTTNNNNNNN
NNNNNNNNANAANNNCNNNNNNNNNNTNCGNNNNNNNNNNNNN

Protein Sequence

MVAESKAANPMREIVVKKLCINICVGESGDRLTRASKVLEQLCEQTPVLSRARLTVRTFG
IRRNEKIAVHCTVRGKKAEELLEKGLKVREFELKSYNFSDTGSFGFGIDEHIDLGIKYDP
STGIYGMDFYVVLGRRGERVAHRKRKCSRVGHSHHVTKEEAMKWFEKVHDGIIFQAKKKK
NMIRRRRR

Parasites	L.mex
Gene ID	LmxM.12.0905 (73%)
Gene name	Hypothetical protein, putative
Feature	Leishmania only

Sequencing length	267aa
Molecular weight (MW)	28461 Da
Isoelectric Point (ISP)	11.98
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNNNNGCTGGATGGNNNTAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

TTTCCAACAGGGAAAAAAAAAATAAAAAAACACAAAGGAGCCCCCGCCCGGTGCAGGGCCCCCCCCCGG
GGCCCCCCGCCAGGGGGCCCACAGGGGGGGACACCCACAGGGGAGGGGGGAAAAAAAGGGGGGGGCAAC
ACGCGGGCNCNNNCACCCTAATATACCCCCCCNCNTTTTTTTTTTTTTTTTTTTTTTGGGGGGGNNCG
CCCCCCCGGCCCCCCCCCTGGGGGTTTGCNTGCCCCCCCCCNTCCTTCNATGCNTGCCTGCCCCCCCCA
CAAACGTGCAAAACCCGNGGGANCGANCACAGGGGNNGGGGGGNNCCAATGNCCCCCCCCCCCGGGGAT
GCACCCNTCNNCGGNTTTGTNANANNAAGGGNGGGGGCAGGNGCGGGCAANCAAGGNCNTACATAAAAA
AANCGNGNNGGGNCCCCCCCNNNNNNGGGGNCNCNCCGGGAGNATGCNNGCNCANCGGCNCTAACNTCC
GGGGGGNGGCNCACCCNAACAAAAATTTACGNAANAGGCNNGNAGGCAATGCNNAAATAAGGTCCNNGN
NNNNNANAANCANNACNNGNNNAGGGGNAGGNNNAANGNAANAGGNNATNNNCCNNCNNNCCNTGAAAG
GGGGGGGGGGGCACAANNNNNNNNAANANNNNAANNNNNNNCCNNNNGNGNNNCCCNNNNAAAANGGNN
NNNAANNNNANNNTANNTCNNTCNNNNGNNNNANNNNAAANNNNGGGNANNCNGGNNAGGGGNGGGCNN
CCNNTNCNNNNNNNANGNNANNNNNNCNNNNNGNGCANNNNNNNNNNNNNNNGGNNNCNNNNNGNTNNNN
NNNNNNNNNNNNNNNANAGNNGGANNNNNNNNNNNATGNNNNCCTNCNNNTGNCCNTNNTNNNNNNNGGNN
NNNNNNGNNNAANNNANNNNNNCNNNNNNNNNNNNNNNNNNNNNTNNCNNNNCCNNNNNNNNNGNNANA
NNNNNNNNNCNNCNGGNNNNNNANNNNNNNNNNNNNNNANNNCNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNGNNNCNTNNTNNNNNNNCNNNNNNNNGNNNGNNNNNNTNNNCNNNNNNN

Protein Sequence

MKAARPRAPAPCRGRGMCCGSDDDAWHCAGRRAAAVTMVACSAVRWTRVRAVSRHRCSSR
CVPRRLLSLLALSRIARAPPRPAHCLPHCVQVCVSPCLQRTAHSPGGRCRRQHCDRCHVR
TCRPRACAHTGGDGAGRQHPWAEPRRVASGCCFSAPTLPCRGKVAATAAALVHPRLAALR
TARRCQRMVRASSAAGAVDAGLLGMTRVTAPHRTRCDRGGGGRPGGCSGGGCGSCPVALF
VRCGEVLRRCGGLRRTRRSSACARTFF

Parasites	L.mex
Gene ID	LmxM.31.0020 (99%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads o nly

Sequencing length	494 aa
Molecular weight (MW)	56540 Da
Isoelectric Point (ISP)	8.29
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNGNNNNNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTTACGGGGGAAACGAGCGATAAGGACAAAT
CGCAGCTACTGCTCGAGAGAAGGAAAAGGGTTCGCAAGAACGCAAGCGACAGCAGCATCC
ATGCAATAACAGCCTCACACTCTCTTTCTCTCTCTTTCTCCTGTGGTACGCTCATGTGCC
TGGGAGGTAGCATATCCGCCGCGAAGCGGCGGGAAAGCATGCACCCAGGGAATGACACCG
GAGCACAGACGCAGAAACAAGGATGCACACACACAAAAACAATTTCGGCAGGTGCACTCG
CCGATGACATTACCGCAAAAAAAAAAAAACTGAGAGACTTGCAAATAGAAGTTCTCCATC
AAAAGGAAGGTCAGTGGCGTCACGCAGACGATTCCCGTCTCTCTTCTCCCTCACCAACTC
TTTCCAAACTAAGGCCACAAGACTCAATGAACTATGGTTACCGGGAGCGTAGGGGCTAGG
CAGCCTCCTCAGTCGCCTTCGTCCCCGGCGTCTCCGCATCCTCTGGAGCCTTTGGCGAGG
GCGTGTCACCCCCGTCGTCCGGCGCCTCCTCGTCTTCGTCGTCGCCACTAGAGAGCACCA
ACTCGCCCGTCTGAATGTGTGACTCATACTGGCTTTTCAGCACCTCAATGTGGCGAATCT
TGTCGTCGATGGACGCAAGCGACAGTACCGGATCAACCTCTATCATCTGGAAAAGCCGTG
TCTTGTCTTCCGGGTGCTGCAGAACGCGATCCTGCTGGGGGTTCGCCACGCTGCTCGGCT
TCACGGTGGGGGTATTCTCATTTTTCTTTTGCTGATGCTTCTGTTGCTTCTTTGCAGCTT
TGCCCAGGGGCTTGTTTTCTGACCACTTGCTCTCGCTCACGACCACACAACCTGCGGGTG
CCATCTGCGAGGGGTCAAAGTGTGCCGCAGCCTCNCGCCTCTTGNNNNCCTCCTCGTCCA
GCTGAANNTCATCTTCTTCTGCTTCAGGTACTGAATGANCGTCNAGCAGNNGCTAATCTC
GGCGGCGTACGGNNNTGACGCTTCGCANCTCGNANACGCTTCNNNGNCGATCTTNNNNGC
NNNCGNCNNNGTCANACNNNNNGGNNGNNNTNNCGNGNNNCTGNNNNANCNNNTNAAGTA

NTNNNGNNGGNNNNNNNCCNNNCNNCNNNCNANNANNTTNNNNGNNNTNNNNNNNNNNNC
NNNNNNNNNNNNNNANNNCNNANNCNNNNNANNNNGNANNNGTNNNNNNNNNNNCNNNNAN
NNNNNNNNNN

Protein Sequence

MSVQEETTTARPAAKKVPWQSMPGAPTRPNFAQYGPKLAALADQRRILIDEIKKLQSSVQ
NDAVTQARNAERNAFFEELNEIDARRKVQRDRRAAQDAEIAKLRKRRGEISDKLREVQAE
VGGFTNVREIEEAIDYMMRKMESSGGGLGAERRNQQRLHKLEDAKTHLLRLQPLADAIKE
ITEQEVILQQEYHAICEQIGILNREYEEKLQKKRAKDKETQADGAKRADVYKQCDELRIR
VSEITQSMESLRAEREKVSSEWDAWNKEARKKYFEQLEQQREKRRKEYDERRNAHKIAAK
RVRAAKRQNPYAAEISACSTLIQYLKQKKMMVQLDEEDRKRREAAAHFDPSQMAPAGCVV
VSESKWSENKPLGKAAKKQQKHQQKKNENTPTVKPSSVANPQQDRVLQHPEDKTRLFQMI
EVDPVLSLASIDDKIRHIEVLKSQYESHIQTGELVLSSGDDEDEEAPDDGGDTPSPKAPE
DAETPGTKATEEAA

Parasites	L.mex
Gene ID	LmxM.33.3790 (100%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	1301 aa
Molecular weight (MW)	139827 Da
Isoelectric Point (ISP)	7.01
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNNGGNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTCTCGGGCTCTCTCTGCCGAAGCAGTC
ACAGGGCGCACAAACATTCCTACAAGAGCGCAAGCATGATCGAAGCCGCAACCAATATC
GTTGTGTTGCGGCTTCTCCCTCGCCTCCGCACCGCAACCGCACGTCACGCGAGTGAGAGA
GGCAGCAGAGAAGGTGTGCGGTAGAAAGATCATGCTTCACCGCCGTCACAACAGTCAGTC
CATCCCTGTGTGCAGCGGTCCCTAATTAGTCCCCGCCTTCATCGCTGTCGTCCTTGGCAG
CTTGCCGCATGGACACCTTCAGCGCTGCAGCCTGCCGCAGCACGACCTCTTCGGCCGCCA
TGTTCCCAACTGTGTGCAGGAGGCCGCTGTAGTCGGCCAAGGCGTGCAGCACAGTGTCCA
TTATGGCACTGGCACTCACGGGCTGCCCTGGTGAGGTGTCCGAAGAGTGGGCGGGTGATA
TAGACGACCACGCCGCTGCAGCCGTCTCTGGCCAGCCAAGAATGGCCTCGTGGTACTGGG
CCAGCACGAGCGCCAGGAGCCCAGCCACATCCGTGAAATGAGCCGACTCTATCAGCAGGG
ACAAAGCTGCCAGTGGAGGGGGCTGCGTCTCACGAGGAGTCGGTCCACCGACGGCGAGTA
GAAGAGCGGCGCTAAGCAGGCGGTAGCCGCGCTTCACTGAGAGCGCTGACCAGCGCTGCA
GCACCTCCGCCAGCAAGGGCCGCTGCACTTGAGGCACCGCCTTGACAAGAAGCACAGCAT
CGCGCCACCGACCTTTCGACTGGAGTGTGACGCAGGCGCTGCGGTACTCGCCAGCCAGCG
CTAGCNTCTCTACAGCTGCCTCCACGTCGCCCTGCGCTAGCAACATTGCCGCCGCACGCT
TCNCCGAGGCGGCGAAGANCGCCGACCCAACTTTTTTGTACAAAGTTGGCATTNTAAAAA
GCATTGCTCATCAATTTGTTGCAACGAACAGGTCNCTANCAGTCAAATAAAATCATTATT
TGGGGNCCGAGCTTANACTGNNNNCNTTTTACNACGTCNNNNCNGGNNAANNNNNCATGC
TANCGNNACNNNNGANNNNNNNACTNCNGNATNNANNAAANNAANGNTCANNNGNNANTGN
NNNTTCNNTTNNNNGNNNNNTTNNNNNGNNNNNNNNNNNCNGNANNNNNNAANNCNNNNN
NNNNNNTTNANNTNNANNNGNCNNNNNNNNNNGNNGNNNNNNNCNATNAGNNNNNNNNNC
NNNANNNNTNGNNNNNNNNNNNNNNNNNNNNNNANANNNNNNNNNN

Protein Sequence

MSSVSAPPQLSPKVIPGCSNTHSNSGLPTILSDLGTFAYQSACYGPQQLLACSSAVTSAG
EVIFVYDMNSMLLTQSLSGHKAAVTALLWRRSPASYLQTGLFLWSGDTSGALMYWDVVEG
VALTSIQTPCRALVQSLTLLTDEHLLVVTQEGTGYVFNSHLVDNIPLPPGRLLLEATQST
LLGNISRPSLVACSRLNDQHCCAVVLGDRLRILTAIQLETGAAPPVAKDLIYDSCDGAPT
VIDVVFSDAQEEVLYFATRNTVGCYDWKLGLMLNESLLWLPDEVEFRRIFRSCSSGAAVH
AAAMRSPESTASSSGDAAAAQVPLMYSFGSDQRLVAWHVTRHDRFTAVSTDVRGVRINSK
LCVNVAQSELDSSLFAVLFEDGSIVHWQYTPLARRWKLLDCWMAPVVRPVTFCAVGAHHC
CVALESGHLALMDITHSMAVRRFNLVYSGGTQIVLLCGYKWSDLVWIVSDRIQQYRHHHQ
VSLIDTRTGEVVRVLRKPSSAPEQTRMKEITMDPTATYVLLTFWNGTFEVWTAADSRLVH
IHSGLGVANVSWAPPLMRRCLSGVQGTPQLLAVLFSEGTLSLWSAYKDRVVVSRDAIPLF
HPSVVEGSVRSAVVVDTLVMWDGQGNGVVLRAQGTRLAVRRLRDAPPNSGAVMCLGGPSP
PSRATEFMSFSSPLAWTSSGSFSHHEDSDNAANLINTGEAGLSMPSAAGFSAARAVAVLF
ESGVFAVWDIATGERRALSSAGMAAEVRALSLYWTGGSLCVLGADGCLYVLDTYLTEMNS
SVRYRALRRPMKNVAFLLPAHRTYVQVALELGSPPSGVRDEAQRASHSLSDAPSAVMEAV
PVSARPCRGPFGQLLTEGIRTLQDEVELYRTTMVPRDVLLRLSRCGSTAGPVSPLTWLRR
AAVVADFLGQSAKQRFMRLAVNVLRHWQPSAVARRGVGGCPSAASSPADAVLAAESADCP
PASPNSESLGEDSFIVPHTYPCADAYSEALAPSHIVRRNRILLNEQRTAALLVNARNHRA
RDDSMSRLALARDWLRLGHRQSVIEVLLDAPPQSTTYNELATLSMAVAASTAVQSSSVDS
ATSALFVASAKRAAAMLLAQGDVEAAVEKLALAGEYRSACVTLQSKGRWRDAVLLVKAVP
QVQRPLLAEVLQRWSALSVKRGYRLLSAALLLAVGGPTPRETQPPPLAALSLLIESAHFT
DVAGLLALVLAQYHEAILGWPETAAAAWSSISPAHSSDTSPGQPVSASAIMDTVLHALAD
YSGLLHTVGNMAAEEVVLRQAAALKVSMRQAAKDDSDEGGD

Parasites	L.mex
Gene ID	LmxM.36.0370 (99%)
Gene name	phosphatidylinositol-4-phosphate 5-kinase-like protein
Feature	Trypanosomads only

Sequencing length	455 aa
Molecular weight (MW)	51239 Da
Isoelectric Point (ISP)	9.53
Signal peptide	No/3
Gene copy	single

DNA sequence

NNNNNNNNGNNANNNGCTGGATGGNNNTAATGATTTTATTTTGACTGATAGTGACCTGTCGTTGCAACA
AATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTTT
TTTTTTTTTTTTCGAGCAATCAGTCTATGTGAAGCAGCCGCAGCACCCTTTGCAGTAGGGCACTACGAA
AGCAGGAGAGGGTGTGGAAGAGCAACGAGGCTAGTGTAGGCGGTACATTCGGCTGGCGTGCTTTTAGTT
TCAAAAAAGGGAGGGCGCATGGAGAGGAAAGCCTGGGCCGGTCGTGACGGACAGCACCTCAGACACCAC
CGTGCGTACGAATGGCACAAAGGGGGAGACGGCGAAGAACAACTAAAAAAAAAATGAAGGTCTGCTGTG
GAGTGCGAACAGTGGGGTGCGTACACACCAGCACGCTAGCATGAACACTCATCAAATGTAGCAGATCAA
TAGCAAACGGCCCCAGCGTCGGCGTGGGAAGAGAGGACTGCTTAGGGCACCGGCACATTGCACACGCAG
CAGCTCCCCCTTCATGACGGCGCTCGGCCAGACGCTGCCTCTTCTTTCAATGATGAAGAAAGCGCGATG
ATTACCCGCCGTTCGCCTCCTCCCTCACGTCCGGGAAGATCAGCCCGGTGAACGATGTGATGCGGCGCG
CGTAGCGGTCGGGCGGGATGGTGGAGAGCGTTTTTTGCTCCCACAAAAGCGACTTGAAGAAGTTGGCAC
TCCTTTTCTTCAACGTGTACGTGGTGAGAACGTCGATTATGCCGATGTAGTACACCTCCTGATCGTCGA
AGCTGTGCACGCCGTTGGCAAACTCTGGAAACGTCGTTAGCGTCGTCATCTCCCCGGACGGGTGATTGT
CCTGGGCAAGAGAGGCGGGCCTCACCTCGGCCGACGTCGATGCAGGCGCGACGCCGGACGAGGATGTCA
TATTCGTGCTCTCAATACCGTACCGCTTGTCTGCCGGCTGCGTCTTGTCATCCTGGNAGGCGACGCCAA
TGAGCAGCGAGTAGTCCATCATGCCCGCATTTTTGAGAAAGTCATAGTCGTGAAGCAGCTCCTCCACGA
GTCGCTTACGTATGGNGCGTGNNACCAAAAGCGCNNCGTCAGGTCCTNGTCGNGCAGCGTGGGCAGTCN

NNCGCGTCNCNTTNCCCCTNNNCACGACNGCNNNNNCCNNTCGGNNNCTGCTGAGNNNCATCNATNGCT
GNNNNNANNANCGNGNNNNNTTNNNNNNNNNNNNNNNGNTNNNNNNNNANCNCNNNNNNNANGNGNNNN
NGGGNGNNNNNNNNNNNNNNNNNNNNNNNNNNTTTCGNNNNGNNNNNNNNNANNNN

Protein Sequence

MGQVGGTATSGRGLTALQVAVALKVTAAAWLKKAEAPTSSSQAMHSTHCSPDEQFKAIKP
EDCSTLQQLTVLSTDHKGKQVKVRVTEYAPNVFSFLRHLKGVTESQFADEWSLPENRLKM
EMGEGRSQALFLKSRTMLFMCKTISAEEVHALLRVLRAYTLHIIAHPSSLLMRFYRLLKV
SVRSEVGYILCFNNVFDVAAVLHEKWDIKGRVPKNRKHPHYSHLIRSGYEPDPHVTKTRK
NRHVIANPAIDAPQQHPDREGAVVVRGNEDAQRLPTLHDKDLTRRFWLPRTIRKRLVEEL
LHDYDFLKNAGMMDYSLLIGVAYQDDKTQPADKRYGIESTNMTSSSGVAPASTSAEVRPA
SLAQDNHPSGEMTTLTTFPEFANGVHSFDDQEVYYIGIIDVLTTYTLKKRSANFFKSLLW
EQKTLSTIPPDRYARRITSFTGLIFPDVREEANGG

Parasites	L.mex
Gene ID	LmxM.25.0920 (100%)
Gene name	Hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	873 aa
Molecular weight (MW)	93052Da
Isoelectric Point (ISP)	9
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNNTCNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAAC
AAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTTT
TTTTTTTTTTTTGTGTTTCTCTATACGGGCGAGGAAGAGAGCGAGAGATGCGGCGCATCTGCCGTGATC
ACCGGAACACACCACTACACACCCACACACACACAGACCCACACACACACACACACCCAGGCAGACA
CAGACAGCAGCTCATGCACAAACATGTACGTATATGCGTGGCGTAAATCCGGAAAAAAAATAGTGTGTG
CGTGTGGTGGTGCGCGTGTGTCATTTTCTTGTCTTCCTTTTCTTTTGTAAATCATATATTTTTTTCGAT
TTTTTCGTCATCTTTTAAGAGGCTGTGGTGGTGGTGGTGGTGACGTGTGTGTCAGGGAGTCCTGAAGGC
TGAAGAGCGAGAGGGGGCAAGGATGGGGGAGGACACTAGCCACAGAGTCGCAACAGTGGAGAGTCGTTC
GAGACGCGTCCCGCCCTTTCCCCCCTCCCCCTTCCTTGCTTTCTTTTTTTTTCTTCACGCGCCTCGACAC
ACCCGTTTCGCACCCAGGCCGCCTCCCAGTCACCAAAAGCGTGTGTAGAGACACACAGTGATGTTGGCC
GAGAGAGGCGCAGGTACCCCCAGTAGATCGGTTCGTGAGTGCGCGTGTGACCGATTATGTACCGAAAGC
ACAGACGTGGAACGAGAAGGTGACCGAGCCCATTCATGCAGAGAGGCACGGAAAGGCGTGCAGACACGC
AACCTCCACGCTGGGGTTGCCCCCTTTCACACAACATACGGCGACGAGGACGACGACGACGACCACCAC
CACCACCAACGAACAAAGGATAAAGGAATGTCTCCCTTTCCTGTCCTCGCTTCCCGCCCTTCTCTCCTG
TAGGGCACGTGAGGAGTGCTGCTCGCTGCGGACCGAGCGCGAGACCGATGGGTCCCGCANAAACAGACA
CACAGACACGCGTAGTCAGTGGGTGGGTGGNNNNNANNNNNNGGGGNNGGNAGGGNAAAAAAANNNGCN
ANNANNGNNATNCNNNGCGAAAGGGNNNGGGNNAGNANAANNNTCANNNAATANNNNNAGANGNTGNGN
NNNCTANNAGGGNNNAGGNNGGCGNGNNNANGGGGANNGNANN

Protein Sequence

MKQQQQSLTMRQRAPRDDGSASHVKECVGQTPANCGVLLMGSRSLSPAPCFPSDDISPSA
RPQLQQDHHLAGTPSRNRRPPSSQPRESSHAPQRRQPHKQQQELSPGDNPRSHRGDGERG
VSGSGAPNDWWQRPVPSNSKVSSFGGNTRPRKLSPSRNSPDEDRRRSQREKQIQFGYVTD
GYTNMKRLIAHDPLLRSGGILPLSPPDVVKGSKRLWDIQLRKWRRALHMFDYVFIDGEDH
PETRAKVLEEQRRQWVSEAFNEMPREARLKIDLDTLRGVQHSSAVPSRIPIEEDLRCILR
SDDCYESVRSVVPQSASSLTKGTDISPLEAGIKIHIAPSAAVLQRQQAQLEVQQRLSSLH

QQQQHQHVLATEVAPESMETGSHVPSAPPVLGATVESAAVEPPVELSPSPRRPPLPLLST
QSPSNFSCPSQPQKAFETTPHRQLCCSPRDEQRCSTLSSSPLPRAAAAPAPTAFGSTETP
PGISASQPRTLSLSGSSARVQGASLSLRGVSATAAVLAPPPPFANPTPCIVPNVWMPGVT
VSSTPAATAAPATMAATAQPLMPWCSHCGHCIGLPAPTITGTPYGGMRASTAGNRGGVLA
PAQVPPQHVNPVAIGGTNGAMMPPSMQFPGAMPYMSHPTDSMAAAAVATSPLYWQMLTPP
VVERIGGGGTFTAHHGSSRSQYRQSDARQDAFATMPRSAAAAAGRSCPRDGHCRSSGAVA
TGGGTATPQTVPRFVAQLSTSPNEVRLGERQSAERLYSAEVASGDVSAATKPRATDTKAA
AAAPPPLRQPSSAFDAAADAARLEKSQSALAVTPERKLFGTGIIAEVTTESRSVVVEALQ
QHETQQGTPSGKLRPTLEVEDAPVGPLVFDGDE

Parasites	L.mex
Gene ID	LmxM.32.0720 (99%)
Gene name	60S ribosomal protein L6, putative
Feature	In other organisms

Sequencing length	195 aa
Molecular weight (MW)	21142 Da
Isoelectric Point (ISP)	11.26
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNGGNNNNTCNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTG
CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTT
TTTTTTTTTTTTTTTTTAAAGGAAAGCGAAGAAACCAAAAAATAGAAAAGACGCCCACGCCTGCGGGAG
AACCGAGAAACACGTCGGCACGCGCCTCACCAGAGAGGCTGTAGAGGTAGCGAGGCTACAACACGAGTA
CGTGCTGCGAGAGCGCGTACGGGTATCCGGTGCTTACCAGTTCCAGCGGTGCGGCGCATCACCGGGCTT
CACCGTGAAGACGGAGCGCAGGTAGCCGGCCTTCTCCTTGCCCTGAGCGTCCTTCTTGATGGCGGCGAT
GAGGGCGGCGTCGATCTTCTTCTGCAGCTGGGCGCGCGCGTCCGACACGGTGGTCTTCTTCCCCGCCTT
GGAGGTCTTCTTGGCAGCCTTCTCCGCCTTGGCCTTCTGCTTGTCGCCCATGAAGTCGCCCTCGCTCTT
GGTCGGCTTCTCCGCCTTGGGGCGCTGGAACACCTCAGGGGTGATGGGCGCCGTGTCAACGCTGGAGAT
GTCGACCTTGGTGCTGGTGGCGATCACGTANCGGGAGTCAATGCGGCGGATGGGGACGCCATTGTACTT
CACCAACTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGG
TCACTATCAGTCAAAATAAAATCATTATTTGGGGCCCGAGCTTAANACTGGCCNGCGTTTTACAACNNN
NGACTGGNAAAACATCCATGCTANCGTTAACNCNAGAGTAGGGAACTGCCAGGCATCANATAAAACGAA
AGGNTCAGTCGAANANTGGGCCTNTCGTTTTATCTGTTGNTGTCGGNGAACGCTCTCCTGAGTAGACAA
ATCCNCNGGGAGCGGATTTGAACGTTGGAAGCAACGCCCGGAGGNGGTGGNNNGANNCCGCNNTAANNN
NNNNTNNCANNNANNNGAAGGCNNCGNGANATNGNCTTTTTGCGTTCNANNANCTTCCTGNNANCGNNC
CGTCGGNNCNGCNN

Protein Sequence

MAATKSAASAAKRKSAKKVSRKSPEYTTLRKSCAPGTIAIILAGRFRGRRAVILKQLPHN
GPLVVSGPMKYNGVPIRRIDSRYVIATSTKVDISSVDTAPITPEVFQRPKAEKPTKSEGD
FMGDKQKAKAEKAAKKTSKAGKKTTVSDARAQLQKKIDAALIAAIKKDAQGKEKAGYLRS
VFTVKPGDAPHRWNW

Parasites	L.mex
Gene ID	LmxM.25.0910 (100%)
Gene name	cyclophilin a
Feature	In other organisms

Sequencing length	177 aa
Molecular weight (MW)	18794 Da
Isoelectric Point (ISP)	7.94
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNGNNNNNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAA
CAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTTT
TTTTTTTTTTTTTTTCTCTATACGGGCGAGGAAGAGAGCGAGAGATGCGGCGCATCTGCCGTGATCACCG
GAACACACCACTACACACCCACACACACACAGACCCACACACACACACACACCCAGGCAGACACAGA
CAGCAGCTCATGCACAAACATGTACGTATATGCGTGGCGTAAATCCGGAAAAAAAATAGTGTGTGCGTG
TGGTGGTGCGCGTGTGTCATTTTCTTGTCTTCCTTTTCTTTGTAAATCATATATTTTTTTCGATTTTTT
CGTCATCTTTTAAGAGGCTGTGGTGGTGGTGGTGGTGACGTGTGTGTCAGGGAGTCCTGAAGGCTGAAG
AGCGAGAGGGGGCAAGGATGGGGGAGGACACTAGCCACAGAGTCGCAACAGTGGAGAGTCGTTCGAGAC
GCGTCCCGCCCTTTCCCCCCTCCCCCTTCCTTGCTTTCTTTTTTTTCTTCACGCGCCTCGACACACCCG
TTTCGCACCCAGGCCGCCTCCCAGTCACCAAAAGCGTGTGTAGAGACACACAGTGATGTTGGCCGAGAG
AGGCGCAGGTACCCCCAGTAGATCGGTTCGTGAGTGCGCGTGTGACCGATTATGTACCGAAAGCACAGA
CGTGGAACGAGAAGGTGACCGAGCCCATTCATGCAGAGAGGCACGGAAAGGCGTGCAGACACGCAACCT
CCACGCTGGGGTTGCCCCCTTTCACACAACATACGGCGACGAGGACGACGACGACGACCACCACCACCA
CCAACGAACAAAGGATAAAGGAATGTCTCCCTTTCCTGTCCTCGCTTCCCGCCCTTCTCTCCTGTAGGG
CACGTGAGGAGTGCTGCTCGCTGCGGACCGAGCGCGAGACCGATGGGTCCCGCAGAACAGACACACAGA
CACGCGTAGTCAGTGGTGGTGGNNANAAAANNNNNGGGNAAANGNNAAGNNAAAAAAAAGCANGCAANA
ANNGGACATCAACAGCGAAAGGNNGGGGGANANNAANAAATCATCANNANAATNCAAGGAAAANAGGCT
GNGNNNNCTATNNAGGGNANAGGNNGNNNNNNNNNGGGGNANNAGGNNNNNTNNNNNNNNTCNNTNNN
NNNNNNANNNNTCNNNNNANNNNNANANANANNNNNNGANNNNNN

Protein Sequence

MPYKPYYPVVESNPKVWMDIEIGGKSAGRVTMELFADAVPQTAENFRVLCTGEKGFGYSN
SPFHRVIPDFMCQGGDFTAGNGTGGKSIYGSKFADESFVGKAGKHFGPGTLSMANAGPNT
NGSQFFLCTAPTSWLDGKHVVFGQVLEGYDVVKAMEAVGSRSGVTSKPVRVSACGQL

Parasites	L.mex
Gene ID	LmxM.18.1620 (100%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	143 aa
Molecular weight (MW)	15848 Da
Isoelectric Point (ISP)	10.15
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNGNNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTG
CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTT
TTTTTTTTTTTTTTTTTGATGCCGCGGCAGCACGCTTAAACGCAGTATCGGAGAAGATGTGTCAGCGAC
AGAGAGACGCTTGGCCGTGTGTGAGCGTACCTTGCTTGCGCATACACGCACCACAGCTCTTGTGTATAG
ATGTTCGCATGCGTCATGCACGCGAGCACGGAGGAAGGTGGACGCCCGATCTGCGATACCAGAGAACAG

CATCAAAGAAGGAATAGCATACAACCCACGGGCGTACACAGACACCCGGCCAGTCCTCACCCACCCCGC
GATGGAGACCTCATGTACAAGCGAGCAAGGAGGCAATGAAGAAGTGAGGCGACGAAGGAGACGCACATG
GACGCTGCAGGGCACAAAGAGTCTTGCATCGCACTGCGTATCAGTGTCTGAAGAGTCTTGCGCGACCGT
TCTCTTCAAAGCCTGCATCGCAATCGTGCGACTCAAGAATCCGCACCTTTCCGCGCCTGCCGGACTGCC
AGACTCCATCACCGCACTGCTGCTCTCCTTCCACCCCTCTCTCAAATCGACTTGACGTCGCAGTGCTGC
AACTGTCAGTCACCTAGGAGGGGGGCAGAGAGGGCACGCTCACAGCTCTAAACATTCCGCAGGTTTCGC
ACATCGTTGACATGCATGAGAGACAACACTTTCTGGACCGGGGCGGCCAACTTTTTTGTACAAAGTTGG
CATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAAAATAAAATCAT
TATTTGGGGCCCGAGCTTAAGACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACATCCATGCTAG
CGTTAACGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAANGNTCAGTCGGAAGACTGGGNCT
TTCGTTTTATCTGTTGTTTGTCGGTGAACGCTNTCCTGAGTAGGACAAATCCGCCGGNAGCNGATTTGA
NNTNTNNNNANNNNCNNNNNNNNNGNNGACNNCGCNTAACTGCAGNATNAACTAGNNNNNATCNNANGA
TGGCTTTNNNNNTANANTNTNCNGNTANCGNNCNNNNNNTNCNNGANNNNNNCCGTNAAAANATNANNN
NTNTNNANTCNNTTNNGNCNNNNCGNNNNNNGNANAAANNNCNNNNCNNNGNNNNNNCNNNNNNNTCNN
NTTCNAGNANNNNNNNNNNNNNANNGNCTNNNN

Protein Sequence

MDSLYNWNDSTKIGVAFTGLGMFFTFMGIVMLLDSILLTMGNFLFVAGVAMVMGPRRCKA
FFIARQRASACFFLGILFVVLRWCFIGLCIQGFGALNLFGNFFPVLVRVLESAPVIGPII
LSAPVQKVLSLMHVNDVRNLRNV

Parasites	L.mex
Gene ID	LmxM.29.0860 (95%)
Gene name	surface protein amastin, putative
Feature	Trypanosomades only

Sequencing length	198 aa
Molecular weight (MW)	21152 Da
Isoelectric Point (ISP)	8.8
Signal peptide	No/3
Gene copy	multiple

DNA sequence

NNNNNNNNNGGNNNNNNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCA
ACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTTT
TTTTTTTTTTTTTTTCGGTCTGCTCTATGGCATGAATCACCCGATTCGCTCCCTTTCTATTTTTTTCGT
TGTATGACGTCCATTCTTCTCTTTACAGGGTTGTGGGGGGAGTTCCCGTGTTTACGTATGCGTGTGCCT
TCGTCTTCGGTACGTGGGAAAGGAGTCGTCGGCGGCAAGAGGGAGGTGCGGCTTCTTGGAGCGGAAACA
GACGAAGAGGGACGCTCATGCGGTCATCTTCGTTCTTCCCTACCACCCCCCCCATGTGAATAAAGAACG
ACAAAGACAAGTGAAAACGAAAAAATTGGGTAGCACACAGGCACACGCGCGCGTAAGCAGACGTGTGTG
TGTGTGTGGTCTGCTAGCCAAGCAATGAAGAGAGAGCTAGCGAGCGAAGCCTGAAAGAGGGCGAGGGGA
TTACCAGCGCTCAGCACAAAACAAAAGAGAAGAAATAGAAAAACTCAAAGATGACGCACGAGCATAGAG
CACCGCATATGGGGTATAAAAAGTAGGGGAAAAAGCGGTGGGTTGGGGGAGAGAAAAAAAAAGAGAGCG
AACAGGAGCAGGAATTGGGGGAAATTCGGCAGGACGAAGAAGGGGCACCACACGCGAAAAATTCACATC
AACGTAAGAAAGCAGAGTAGCCAAGGAGGATGCAGCATTTTTCTCCGCCCCCCCCCCACACACACGGTG
ATTATACCGTTTCTCTTCTGGTTTTTGTTTGGTTTTTTTGGGGGGGTGGGGGACATTGACTCCCCTCCTA
CTTTTTTCTGTCACGTCGATGTGCAGGTCTCTTCTCCATTCACGCCGTCCTTGTCTGGCGTTCGCTCCT
CCCTTCCGCTGTGCATTCCCTGGCCAGACACCCAAAAACGTGTGTATTTTTTTTTCGCTCGCCCTCTCT
CACAGCATGAAGCAAATCCATCTGTTTCCATACTCTGAACCTCGTCCCCGNNGNNAGCTGCGTGCTTAT
GCGTGNGTGTTGNGTGTTGTGTGCATGTGTGTGTGTGTGNAGGGGGGGGGNACNACTCCNTNCCNNNNA
NGANGGACGCNNGGNNNCCCNCCACNNNANNCNNNGNNAAAANGNAANCNANNNNANNATCNNNNGNNN
NANGNNGNNNNANTGNNANNNGGCNTNNNNTNCNNNNNNNNNNNNNNNNNTANNNNTNNTNNNNNGNN
NTNNNNNNGANNTNNNNNNCTTNNTNCNNNNGCNGNNNNNCNNNCNTCCNTCNNNNNNN

Protein Sequence

MGFEALRGRMDVALSMLCSCIVFMFLVTSAPISQFRGRGINASATGGASKLSCVTAWGLK
NDCNSNNYDYRPTSIGCARAKQLFQVSEAFYIVAVIVSFLSCLMSGLYFIGMKAKVLLVL
LAVLEVVVALIPWVCMTAVWHGNYCGGSTVKINTSNGKADGVPYGSVLRESFKASAGYGL
TVAAWCTQVIGLVLLIIM

Parasites	L.mex
Gene ID	LmxM.32.0940 (99%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	1177 aa
Molecular weight (MW)	122803 Da
Isoelectric Point (ISP)	8.07
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNNNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGC
AACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGGGTGAATTCTTTTTT
TTTTTTTTTTTTTTTTCTCGTGCGGCGGACAGCCATCTAAACAGCACCGTCATCGACACACCGGCCCAC
ACACACACAGACACACACACACACGCATAACAACGGAGTTGGAGAGCGCGCCTAATGAAAGTCGTCC
GATGCCATGAGCACCTGCGTGATTGCTTTTAGTGCCATCATCATATCGCTCACAGACTTGTCGGTCGCC
ATGTGGTCGTCTCGCGTCAGCACTGTCAGCTGGCGCCACGCACAGGGAAGGGCTGCGAGCAACATGCAA
AGCTCGCGCAGATACCGCTTGTCGATCACTTTCAGCACCATGTCACTTACCGGGACGGCAGGGACGACG
TCAAGGCAGGCAGGGGCTGTGCCGTCAATGAAGAAAGCGCGCCGCCGCTATTCGGCGAAGACCCACCCG
CCATGGGCCGAGCCGCAGCCTCACCGTGCCCGTTTCCTGCGTCCGCGTCCCCGTCGGTGGGCTCGTCCT
CTGACGCGCAACCGCGACTTCCCAGCGCAGACTGCAGCGGCGACGAGGCAAAGCCTCCGCTTGCGGGGG
CGTTATGGGCAGCGATGGCCGCCGTCAAGGGCAGCAGAACGCTGGCAGGCTGAAGCAAAGAGGTGTTCA
CCACCGTGGATACCGCGTAGGAGAACGGCGCGCTCGCGGCCATGCCGTCATCAACGCAACCGCTCCCTC
CTCCACCGGTGCGAAGGTTGGTGGCGCTTGCGACTGTCAGCGTGGACATCGAGGCCGCATTACAACCTC
CTCTCCCGGAGGCTTGACGGGAAACATGTCCAGAAGACGCCACACGAGTGAGGCCTGACCGGAGCGGTC
TGCCGAGGTTTGTGCCACCGCGCGACAAAGGGGTCAACGACGAGGATGAACCTTGGGGCGGCGGTATCG
GGGCACCAGCTGGCGCCATCGCATTTCCGCTGTCAGCCGNGGTGTAGCTCAAGTCACCATCGCTCTGTA
CGCNGNCACCGTACGGCGGCAACGGCGGTCGACNGGCGNGCTACGGACGTGCCGATACCGGGNGCAGCN
CACACNNCACNACCCNNNNCTNNNNATAGGNGCTNCANCCCCCNNTTTTTTGTACAAGTGNNTNNAAAA
GCATGCTCATCNNNNNNACNANNNGNNNNNNNCNNNNAANNNAANNATTNTTGGNNNNNNNNANACNGN
CNTCNNTTNNNNNNNNGNCNGNNNNNNNCNNNNTNNNNNNNNNNNNGGNNNNNCNGNNNNNANNNNG
GNNNCNNNNNANNNCNTNNNNN

Protein Sequence

MPVSVSMHACSAWPVSIDFVAFRFFLAGYSVEETVEQLMALQSETPAASPRRRTSSASLS
SSRSSDLGKASHAGSESSSRSATRSKAHRRHRRQQRTSQQRQHSLSVMHNAQQQTEDRRL
EDEDENPQEIAVATTAAALPRNTSISSSASGAHVPATATAPSTTTVVDNSNNNVSASSSM
AVAVTPSSAAFHPAQGGARTHHHRHRHRHHQRLDKSPGGSEHKKSGHVAPHSSRSRIAAT
SPGAATRTSFALPSTLNTEYSNSSLPSVDLSSFRRLTDDPTAAATAEVAAAVITGGGSGA
RAGGASSSHHLHVNFGDTKASSSPQQRREKHRKDSAERQCFFVSESYMKFQRQLQEFEAI
GGVGVGSSSLQSRYLFEEVTEQYQVFRELSREEHLGSPYAFLSNYYIPIPVAARLQLLEM
YYETDASVFRWAFGDKLSRFDLPAALSAAREENVCGTLSAGFGGVAGLGSGGGNSSSAAD
GSVTAASFWTSRWKGSPGKLAAMDAATVTRLSDQHVHALRRQWENIKHICVTVAALYRGK
GDMCVPLDMPLLTTIQQCFGLRHEQALDYATAAFGFEHRLETRLFEHLHNFNEYGVICSI
VASLWCDCSGYFLSGSFRDGCRRLGRLLDEYRILSELHLIVFGEAMRPRWQVQLDEVQRV
ITTSSHVEKNAMASATAAVSATSPVTPSVSGGGGGLATPTTMSAGILPTLSSSATGVGSN
HFGSPLCGNGHLGGATGGAAADAGNTSNFTSPVFPTTGGAFSTNNVHSPFSGNGQYSRPF

LMEFPSLMKHLLRVCVALSSNGGVNDGLDIFFTRIYSYLELLSSRTAPAIVSRMINAAAS
GASTMTGAAAAVGTGGIGSTLSGQLGGSVGGGGAAPGIGTSVAPPGRPPLPPYGDRVQSD
GDLSYTTADSGNAMAPAGAPIPPPQGSSSSLTPLSRGGTNLGRPLRSGLTRVASSGHVSR
QASGRGGCNAASMSTLTVASATNLRTGGGGSGCVDDGMAASAPFSYAVSTVVNTSLLQPA
SVLLPLTAAIAAHNAPASGGFASSPLQSALGSRGCASEDEPTDGDADAGNGHGEAAARPM
AGGSSPNSGGALSSLTAQPLPALTSSLPSQVSDMVLKVIDKRYLRELCMLLAALPCAWRQ
LTVLTRDDHMATDKSVSDMMMALKAITQVLMASDDFH

Parasites	L.mex
Gene ID	LmxM.27.1130 (63%)
Gene name	intraflagellar transport protein IFT88, putative
Feature	Trypanosomads only

Sequencing length	811 aa
Molecular weight (MW)	91083 Da
Isoelectric Point (ISP)	6.24
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNNNGNANNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAATTTC
AAAACGGCCCCCTTTTCCCCCCCCCCGGGGGGGGCCAACCCCCAAAACCCCCCAAAAAAN
AACCNAAAAAAAAAAAAGGGGGGGTTTTTTGGGGGGGGGAAAGGGGGAANCCNGGGGGGGG
GGGGGGGNNCCCCCCCCCCCAAAAAAAANNAACCCCCCCCCCCTTTNAANAAAAAAAAAA
NNGGGGGGGGGGGGCCCNAANNCCCCGGGCCCCCCTNNNNTTTTTTNNCCCCCCTTTTTT
NCNAANNCCCCTTGGGNANNNGGGGNNNACCCCCCNGGGGAAANNGGGNCCCTTTTTTNN
AAANNAANTTTTTTTTCCCCCCGGGGGGCCNNCCCNNNANCCGGGGGGGGGGGGANNGGGG
GGNNCCCTTTTNNCCCNNTTTGNNNNNCCCTTTNNNGGNNNNCNNNNANNCCNNNGGNNN
GNNCCCCNNNNNNNNNNNNGGNNNGGNCNNNNGNCCCNNNNNNNNNNNNNGNNNCNNNN
NNNNNCCNNNNNNNNCNNNNAAAAANNNNNNNNNNNNNNNNNNNNNCNNNNNNNGNNNNN
NNNNNNNNNNNNNNNNNNNNCNNNNNNNNNNNNNNNNGGNNNNNNNNNNAANNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNANAAANNGGNNNNNNNNNNNNNNNNTTNNNNNNN
NNNNNNAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCNNNNNNACNNCNNNNNNANA
NAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNANNNNNNNNNTNNCTNNA
ANNNNNNNNNGNNNN

Protein Sequence

MNGNDEDIYAAFQTPDIVSNPWTTNTNPFEAPAQEAMAGNPLMQAPPSQWGRAGMGSAWG
LPGSRMGTRGCGILGAARPMTSNRAVGFNSASTGAAAALFDPTGQACMANMAMGPAPPLK
KRSENSQEEELAEMEKQVNRLIEESAMLALQKDYGAALEKAKDAGKLERSLCKKREQYGL
AEQINVDLTYAVHFNLAVQYQNHQLYTEALNTYNLIIRNVQFPQAGRLRVNMGNIYLAQQ
NYLLAIKMYRKVLDETPTAGKELRYHLCRNIANAFVKLGQYRDAANSYETVVEGNGDANA
TFNLILCYYALGETEKMKRTFTRLMNCRLAGLDDEEDFEEEEKRKDVLVDDSLSRMCKER
RARYLKYIITAARLIAPVLHKDWCVGYDYIISQLRTYEMRDPTSHVASELEMCKNLNYLK
HKRYQEAINGLKEFEKKDRSLRARAATNLAYLYFLEGDYENGEQYSDLSLVANQYNAKAL
VNKGNFSFVKKDYEKAKELYNKALAVEADNVEAIYNLGLAAKKLGLYEEAVRMFKRVQAL
VDSSEVLYQIADLSDLVGDPSALEWFNRLIGRVPTDPNALARIGSLYARDGDDVQAFHYY
LEAYRYYQVNMDVISWLGAYFVKNEVYDKAVQFFERASHIQPQEVKWQLMVASCHRRRGD
YAQAKLLYEQVHRKYPDNIECLNYLVQLCKDAGLNEEANEWFKATKKVERQQFHSSSSSV

GGESGDDDLESSVEGGNNINGHHRRRTSGTAAPDTAVAGRRAGGGAVADKDFSVGLSDDD
IVDGKAKQNGAKRLKKAKSSDSDDEIDLPGI

Parasites	L.mex
Gene ID	LmxM.13.0280 (100%)
Gene name	alpha tubulin
Feature	In other organisms

Sequencing length	548 aa
Molecular weight (MW)	60185 Da
Isoelectric Point (ISP)	5.55
Signal peptide	No/3
Gene copy	multiple

DNA sequence

NNNNNNNNNNGNNNNNNGCTGGATGGNNNTNATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCATCTATCAACGACGGGGAGGGAGAGG
CAAGGCCCTCCTCCACAGATACACAGCGCTACGGCTACGCGCGCGTGTGCATGCGCGGCA
GACGCACGCACGAGGAAGGAGAGGACGGCGAACGGAGAGACGCCGGAGAGCGTGGCCGCT
CCGCCATCCGCCCCGCCGTCCTCTCAGAAAAAAAAAACGCCGACGAGAGCGAAAAAAAAA
AAGAGAAGGGGCGCATCGCACGCACACGCGCGCTCCTCCTCCTCCCTCGAGCCGGTCGTC
ACGCGCACACACACACAGTCGCGGTGGCAGCGGCTCCGCTGCAGCACACGCACGCGTGCA
CCTACATCATCACCGTGGGGCACGAGTGCACCTTAGTACTCCTCGACGTCCTCCTCACCC
ATGTCGTCGGCGGATTCGGCGCCAACCTCCTCGTAGTCCTTCTCCAGCGCAGCGAGATCC
TCGCGCGCCTCGGAGAACTCGCCCTCCTCCATGCCCTCACCCACGTACCAGTGCACGAAC
GCGCGCTTGCTGTACATCAGATCGAACTTGTGGTCGATGCGGGCAAACACCTCAGCGATC
GCGGTCGAGTTGGCAATCATGCACACGGCGCGCTGCACCTTCGCGAGGTCACCGCCGGGC
ACAACGGTCGGCGGCTGGTAGTTGATGCCGCACTTGAAGCCAGTCGGGCACCAGTCCACG
AACTGAATTGTCCGCTTCGTCTTGATCGTCGCAATCGCGGCGTTGACATCCTTCGGCACG
ACATCACCGCGGTACATGAGGCAGCACGACATGTACTTGCCGTGGCGAGGATCGCACTTG
GTCAGCATGCCGGCAGGCTCAAACACCGAGTTCGTGATGTCCGCGACGGACAGCTGCTCG
NNNACGCCTTCTCGGCAGACACCACCGGGGCGTAGCTCGTCAGCACGAAGNGGATGCGCG
GGTACGGCACNNNTTCGTCTGGNNCTCCGTCAGGTCNCNTCAGCGCNCATCGAAGCGCAG
CGACGCCGTCAGANACGANNNNNCTNNNGATCNGNNNNACGTCGNGTACNANGGNNNTCA
NNNNCNANAGAACNGNNNNGAGNCGTANANNNNTCNNNNCANCATCNNCNNNNCATCGNN
NGNNNNANNCAGCNANNGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTTTGTNNNANN
GNNTNNANNNNTNNNNNCNNNTNNNNNNACGNANNNNNNNNNNNNNNNANNAANCNNNN

Protein Sequence

MGLMRTDTHVHAPPSSRSAALLLLLLPCAGRLPRVSSPHLALSLLSSPLPSPPLLADTQH
ARCTHAHAHRNPRSSSLSFFEQTPLNRLLTPLPSFSAMREAICIHIGQAGCQVGNACWEL
FCLEHGIQPDGSMPSDKCIGVEDDAFNTFFSETGAGKHVPRCIFLDLEPTVVDEVRTGTY
RQLFNPEQLVSGKEDAANNYARGHYTIGKEIVDLALDRIRKLADNCTGLQGFMVFHAVGG
GTGSGLGALLLERLSVDYGKKSKLGYTVYPSPQVSTAVVEPYNCVLSTHSLLEHTDVATM
LDNEAIYDLTRRSLDIERPSYTNVNRLIGQVVSSLTASLRFDGALNVDLTEFQTNLVPYP
RIHFVLTSYAPVVSAEKAYHEQLSVADITNSVFEPAGMLTKCDPRHGKYMSCCLMYRGDV
VPKDVNAAIATIKTKRTIQFVDWCPTGFKCGINYQPPTVVPGGDLAKVQRAVCMIANSTA
IAEVFARIDHKFDLMYSKRAFVHWYVGEGMEEGEFSEAREDLAALEKDYEEVGAESADDM
GEEDVEEY

Parasites	L.mex
Gene ID	LmxM.36.1040 (99%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	286 aa
Molecular weight (MW)	32514 Da
Isoelectric Point (ISP)	7.25
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNNGNNNNNNNGCTGGATGGCNNTAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGATGACACTATCGTCACACGAGGAAGG
GCAGCGTCAACGCGCGAACGTTCAAGGACGCAAACACACACACACACTCATGCTCAGT
TACTCTGAGAAGCGCAAGAGCATAAGAAAACAAGGCATTCTGCTAGTTTACATAGCAAAC
AACGACTGGCACAACGTGGGCGGCATTCCCGCACGCACGACGGCGGATACCAACCCGGCA
GCAGCGGTGTCACCTGCACAGTGCCGCCGTTACGCAGAGGGTAAGGCAGAAGAAGGGGGA
TGGGGCCACGGTGCGAGCTGTGAAGGGATCAGAGGGCGAAGAGAGAGAGAGAAGGCTGCA
GATGGAGCGCGCAGCGGGTAGGTAGCGACAGCGCAGCCGAGACGCTCGCTTCACCGCAGT
GCACCGCTCCCCTTGGCGTATTCCGTTGCCGCATGCGCCCATATGCGGTCACTTCTTCTT
CGACGACCCTCTGGAGCCCTGACGACTACGATTGCCACTACTCTGCGCCTCATTCACCTT
GGCCTCAATAGCCGTGAGTTTCTCCTCGATGAAGTCTAGTCGTTCGCTGGACTTTGTCGA
CACGGCGTCGCGAATCGCCTCAAGCTGCACGCGAATGCCGGCCATAAATCTGGAATTGTC
AAGCGTGGCCGCGTTCTTCTGCGCCTCAGACACCGCATCTGAGACGATCAGCTCCTCCTC
CATCTCGGCCTCCTCGAGCGTGCGCACCTTCTCCTGCATCTTCTGCTTCCAAGCATCCTC
TTTAATGGCAGCGGACAGAGGCGGGAGTTGAGCTGGCGGTACCTCGGCGGTGTCGTTCAG
CATGACTGTCCGAAGGCTCGCCACCTCGCGTGGTACAAAGCAGTACACGTACATTTTATA
GTGGCGGTAGTAGNNGCTCATCATGTACGTGTCGATATCCTGCACTTCTCGCACGTTGAA
CACCATCGTCGAGTANNNNGGCGGTGNACGCTGNNCCGCACCACNAGCTCCCNGAGAGNC
GTACGAGTGAAAGCNGGAANGCGATCNACGATGCTCGCTTCNNGCANNCNATGCACGATN
NNNNANCGTACTTGNNNTNNNTGGGAAACTGNNNGCTTTGNANNNANANNAGATGNNTNN
GNCNGCANNNTTCNCTGNNNNNNNNNNNNNNNCNACNNNNGCANNNCCNNCNNNNTCNNCN
NCNNTGNNCNTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCNNNCNNNNNNNGNNNNN
NNTTTTTNN

Protein Sequence

MAQCLTWQVLDQRQTGECLDAVDLEALRVVIKRIDDDHGGALLQSGKRTATQVNVLLDLY
GHLVLFAKASQFSQFKTSTLFGIVHRVHEASIVDRLSRFHSYDLLRELVVRHSVHRPPYS
TMVFNVREVQDIDTYMMSTYYRHYKMYVYCFVPREVASLRTVMLNDTAEVPPAQLPPLSA
AIKEDAWKQKMQEKVRTLEEAEMEEELIVSDAVSEAQKNAATLDNSRFMAGIRVQLEAIR
DAVSTKSSERLDFIEEKLTAIEAKVNEAQSSGNRSRQGSRGSSKKK

Parasites	L.mex
Gene ID	LmxM.34.1890 (98%)
Gene name	60S ribosomal protein L5, putative
Feature	Trypanosomads only

Sequencing length	305 aa
Molecular weight (MW)	34030 Da
Isoelectric Point (ISP)	10.69
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNGNNANTNNGCTGGATGGNNNTAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGAGGAAGCTCTGACCTTTCCGCCTCGCT
AGCGCCCCATCAGTGGTGAGAGAGGGGGAGCAGACGCGACAAGAGGCCGAGAAAAAGACA
CACAGAAAATTAGCAATGAAAACAAAAAAAAAGAGAAATATATGAGGTTCCCTCAGCGGC
ACGTGCGCACGCGGTTGCCGCCAGCGCGCCTCGCTGTCCCTGTTCCAAGACAACCATGGG
GATGCACTTCTTTCAGCCAAAGGAACAGGAATCAAAAAAGAGGCGTCAGAGCAGGAGACC
AAAAGAGACTCGCGTATTCAGAGGTGAGCGCTCGACTTACTTGCCGAGGCGCTCGCGGAT
GGCCGCGACCTTCGCCTTCGCGGCGGCCCTCTTCTCCGCGCCGCTCAGCTTCTTCGTCTT
GTANCTCTTGTGCGCGACGCTCTCCTTCTTCGNCTTCTTCNGNAGCGACTTCGACGGGTC
CGCGCGGATCGNCGCGTGCGCCTTCTTGTACATGCCCTCGATGCTCTCCGGCAAAACCTT
CGCGGCCATGTACTTCNAGAACTGCACGCACTTCTCGTCNGGGTTCGAGCTCNCCTCCTC
CTTCACCTGCTTCAGGTACTCCGCAACGTGCTTGCCAAAGATGCGGTCGCGGTGCACCTT
CNCGTCCAGTGAGCTCTTCTCCTTGTTGTANCCGGGGAAGCGGNTGGGGCGGTGCGGCAC
ANCCATACCGCCGTCCACCGCGCNCTTCAGCACGCCNAACACGCGGGCACCGGTCGTCGT
GCGCGCGAGGNCGACGTCCAGGATCNCCTTGAACGGGAAGCGCTCCTCGTCNTCGCCCTC
GTCGTCCTTCTTCGTGCGCACAGCANAGTACGAGCCGTCCGCCTCCTTCNCGCCNNGNAA
CTTGTCCGCGATGCNNANNTTCNCCAGCATGCGGCGCGNCNGCAGCAGCCCANTCGCGNA
CNCNNCNGCGTAGTTGTCNNNCCGNGCTCAATCCNNAACGNNGCANNTCGAGCGCGTANN
NGNCCATNANCACCNTCGTCNNCGNCNATCTTCNNNNNACGANNNGCGGNATGANNNCTT
NNNNTNATGNNCNNNNCANNGGNGGNACTNNGNNANNCNANNNNNNNNNNNNTNNNGCANC
ANNNTCTGGNNGNNNNNNNNNNANNCNNNNNNNCNNNNNNNNNNNGNNNNNNTNNNNNNN
NNNNNTGNNNNNNCNNNNNTNNNANCNNNNNNANNNNAANNNCGNNNNNNNNNNGNNNNN
NNNNNANNCNNNNNNNNAN

Protein Sequence

MPFVKVVKNKAYFKRFQVKYRRRREGKTDYHARRQMVLQDKTKFGSPKYRLVVRITNKDI
IAQIVQAKIVGDEVVMAAYAHELPAFGIEHGLTNYAAAYATGLLLARRTLAKLGIADKFQ
GAKEADGSYSAVRTKKDDEGDDEERFPFKAILDVGLARTTTGARVFGVLKGAVDGGMAVP
HRPNRFPGYNKEKSSLDAKVHRDRIFGKHVAEYLKQVKEEASSNPDEKCVQFSKYMAAKV
LPESIEGMYKKAHAAIRADPSKSLPKKAKKESVAHKSYKTKKLSGAEKRAAAKAKVAAIR
ERLGK

Parasites	L.mex
Gene ID	LmxM.23.0890 (99%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	470 aa
Molecular weight (MW)	53143 Da
Isoelectric Point (ISP)	8.27
Signal peptide	No/2
Gene copy	single

DNA sequence

NNNNNNNNGNNNNTNNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTACGGGGTAGGGGCTTGATGAAGCCTACG
TCACAGGCAGTGGCAGAGGCGGCGGCCACGTGACCATCGGTACGTACTTGTGTGCACAAC
GGACAAGATACGGAGAATGTTGCTCCTCTACGAGAGGATCGAGGATGCATACTCCTCGTT
GTGGTATGTGCGAGGCTCCCTCCCACTCCTACCTACACATATGCATACACAAGCGGGTAT
GTGTGTGTGTGTGTGTGCTCTCACTCTCCCCACTCAGCTAGGCACATCCCTCTGCGGCAGCGGA
AAGGGTGTCCCCCAACGCTGCATGCATCCGGTCTACCGGCCATCCCTCTGCGGCAGCGGA
GGCAGAGGCATCTCAGCAAAGAAAGGCGACACGTTCGGAAAGTTTCGGGTCTGAAAGGCG
AACACCTTGTAGCTGTCTCCCATCTCCTCCTTATCCATCAAGCGCCGGTAGTTCTGCAGC

ACCTTCATTGCTGCTTTCGTCTCCTCGTCCTTGATCACGTGCGCCAGCCGAATGTCAATG
CCGTTCTCGAGCAGGAAGTCGTGCTGCGTCATCACCGGGAACCACTTGAGGTGCCGTCGC
GCCACCTCCAGCCGCTCAAGGGCCCAACGCAGCTGCCGGAAGCTCACCCAGCAGGAGAGA
TCCACCTCGCCGGGCGACAGCAGCGGGTCGACGAAGCGGTGGCCACGGATTCCGCGGAGC
GTGCTGTGCATGTGATCATCCTTGCCGTAGTCAATCAGCAGGCACGCAGCCTTCTGGCAG
TCTATCATCTTTTTCATGAGCGTCTCCATCGTCTGCATCCCGACCGTGTTCACCTCGACA
CAGTCGCCCTTCTTGCCCTTGGTGCGAATGTCGTCAGGGATGAGATAGGCGGACATCGAC
CCGGACGGGGCATGGNCAAGGCGGAAGTGCGCCTCCGTGCCGGGGTCGGTGTCCACCTCC
ACACACGTCTCCACCCACCCGCGCTCGGNGTAGCNGANTGGGCCANCNGCAGCGCATCAA
GTACTCATTCGCATGANNNNGNGNTCGAGCGAAANGNAGCNATNNGNCATCNNCNNTGAT
NTGNCNTGNNNNNNNGNNNGNNNANNTCNNTNTGNTNCTCGNGGNNNNNNNNCCNANCNN
NNNNNNNNATCTGCNNAAANNNNANNNNNGNNNNNNNNNNNNNNNNNCTNNNCANTNNNT
NATNANNNNCNNNNNNNNNNNNNNNNNNCANANNNNGGNNCNNNNTNGCAGGTNNNTNNN
NN

Protein Sequence

MQQCLARRCSVAAAASVLGVSTPVGPSSSGSSVAVPFMCHHRAGLLRSTPVRLAMNLHAS
DLSTPDRATVNSANKKEFKTALCNELVNKITAQGYYPMSQFVKDCLTHPQYGYYAAKKNV
IGSEKADFITAAEIPFFGDVLAAWVMDAWQKMGTPRVLHLVEMGPGRGTLMRTMLKQIQY
SNPHLLHFLQIHLVEVGAARREEQKRALAEFQTAQGKIKWWMDLESLPFSLEPTVFIANE
YFDALPVAQFRYTERGWVETCVEVDTDPGTEAHFRLVHAPSGSMSAYLIPDDIRTKGKKG
DCVEVNTVGMQTMETLMKKMIDCQKAACLLIDYGKDDHMHSTLRGIRGHRFVDPLLSPGE
VDLSCWVSFRQLRWALERLEVARRHLKWFPVMTQHDFLLENGIDIRLAHVIKDEETKAAM
KVLQNYRRLMDKEEMGDSYKVFAFQTRNFPNVSPFFAEMPLPPLPQRDGR

Parasites	L.mex
Gene ID	LmxM.25.0910 (100%)
Gene name	cyclophilin a
Feature	In other organisms

Sequencing length	177 aa
Molecular weight (MW)	18794 Da
Isoelectric Point (ISP)	7.94
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNGNNNTCNGCTGGATGGNNNTNATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTCTCTATACGGGCGAGGAAGAGAGCGAG
AGATGCGGCGCATCTGCCGTGATCACCGGAACACACCACTACACACCCACACACACACAG
ACCCACACACACACACACACCCAGGCAGACACAGACAGCAGCTCATGCACAAACATGT
ACGTATATGCGTGGCGTAAATCCGGAAAAAAAAATAGTGTGTGCGTGTGGTGGTGCGCGTG
TGTCATTTTCTTGTCTTCCTTTTCTTTTGTAAATCATATATTTTTTTCGATTTTTTCGTC
ATCTTTTAAGAGGCTGTGGTGGTGGTGGTGGTGACGTGTGTGTCAGGGAGTCCTGAAGGC
TGAAGAGCGAGAGGGGGCAAGGATGGGGGAGGACACTAGCCACAGAGTCGCAACAGTGGA
GAGTCGTTCGAGACGCGTCCCGCCCTTTCCCCCCTCCCCCTTCCTTGCTTTCTTTTTTTT
CTTCACGCGCCTCGACACACCCGTTTCGCACCCAGGCCGCCTCCCAGTCACCAAAAGCGT
GTGTAGAGACACACAGTGATGTTGGCCGAGAGAGGCGCAGGTACCCCCAGTAGATCGGTT
CGTGAGTGCGCGTGTGACCGATTATGTACCGAAAGCACAGACGTGGAACGAGAAGGTGAC
CGAGCCCATTCATGCAGAGAGGCACGGAAAGGCGTGCAGACACGCAACCTCCACGCTGGG
GTTGCCCCCTTTCACACAACATACGGCGACGAGGACGACGACGACGACCACCACCACCAC
CAACGAACAAAGGATAAAGGAATGTCTCCCTTTCCTGTCCTCGCTTCCCGCCCTTCTCTC
CTGTAGGGCACGTGAGGAGTGCTGCTCGCTGCGGACCGAGCGCGAGACCGATGGGTCCCG
CANAAACAGACACACAGACACGCGTAGTCAGTGGGTGGNCAACTTTTTTGNNNNAGTTGG
CATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCCCTANCAGTCAAAT

AAAATCATTATTTGGGNNNNNNCTTANNACTGGNCCGTCGGTTNNNACGNCNTGNCTGNN
NANNNTCNNNGCTAGCGTNACNCNNNAGTAGGGNACTGCCAGGCATNANTAAACGANNNT
CANTCGANNNNTGGGCNTNNTTTATCTNNNNNTNNCGNNNCNNNNNNCNGANNGNNNNAA
TCCNCGGNANCNGATTNNANNTNNNNANNCNNNNNNNCGNANNGNNNGNNNG

Protein Sequence

MPYKPYYPVVESNPKVWMDIEIGGKSAGRVTMELFADAVPQTAENFRVLCTGEKGFGYSN
SPFHRVIPDFMCQGGDFTAGNGTGGKSIYGSKFADESFVGKAGKHFGPGTLSMANAGPNT
NGSQFFLCTAPTSWLDGKHVVFGQVLEGYDVVKAMEAVGSRSGVTSKPVRVSACGQL

Parasites	L.mex
Gene ID	LmxM.34.3700 (92%)
Gene name	Gim5A protein, putative,glycosomal membrane protein
Feature	In other organisms

Sequencing length	225 aa
Molecular weight (MW)	24881 Da
Isoelectric Point (ISP)	8.74
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNGNNNNTNNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTCAAGTGCTTTATTTTCTTGAGTGTCCAC
GTTATCATGTCGCTGCTGCATAAATGCATTGAACTCGATGGCCCTTGAAGAACAGCGCTG
CTTTTCATTTTTCCGGAATTACTGTTCTTGCCTGTATACACCTCAGCTACCTTTTCACAC
CAACAGCACCCCCCCCACACACACACAAACATGCATGCACGTCACAACGACGAGGGCGGC
GTTTCGTTTTTATAATGGTGAGAGAGGACGGCGAGAGAGTGAGGTCGAAGACATGTGTGT
GTGTGTGTGTTGGTTTACCAGTGAATGCAACATGATTCCACAACATATTTGTACACATCC
GCACCTCAAGACACGCACACACTTACTCTTACGCCCACACACCCCCGGCAGCAACGCACG
TGCGCACGCCTCTTTTTTGTTTTTCGTTGCTTGTGCTCCTCTGCTGCCTGAACTACAAGA
GTCTCTGAAACACACACCATACACCACACACCACACACCACACGCACACCGCCATCAACA
GACATGAGACGACGACAACACACCGGCACACGAAAGAGGGAAGATGGGAAAAACAAAACA
AAAAATGCATAAGAAGAGCATTTGTAGCGCGCGTGTTTGTGTTCGAGTTTTTTTTCTTTG
GGGGAGGGANGGGGAGGTTCTTTTGGAGAGCCCCCCATCACACGCAACTCCCGAGCACGC
CCAGACCCACACAAGCACAAATCCATACACGCACGGCAGCACGACCATGCAAACGAAAAA
AAAGTGGGTCCGCTAGCCCCTCTCGACCACTCACTGGCGTCTCCGTCAAGCGGATGGAGG
CAGGCACGCTTCCCCATTCGCCTCACGTTTGTCCTCCATGTAATGTGTGTTGTGTGGGCG
CTGGTCGTTGTAAGCGCAAAAAGAACCACATCGAGGGAGATGAAAAGAAAAAGATAAATG
CGGCAAACTGTTTCACGTGCNTCTCTCCTCTCTCCCTNNNGTGCGTGNNGTGTGNGCCCT
GCACTGTCGCTNNNCNGCTCNTCTCTCCTCGCCCCNTNNNNNNNNNGCACGTACTGNNNN
NGANNAGGNNNNNGAGACGTCANGAAGCNNNCNAGANNNCANGAANAANCNNNAANCNNN
NNANNNNCNNGGNNNNCATGCANNATNNNNNNNNNNAANNNCNNGNNNGNNNNNNNNCNA
NNNNNATCNANNNNNAANNNCANNNNNNNNNNNNNNNNNCNNTNNNNCCNNGNNAANN

Protein Sequence

MSAAVFEYLGNTGDRDKVMAIVQFLPMALAGPANDAGCIALSKSLKSLSSMADGYRAITR
LALLFNALSKPTLEALSKPKGDVLLDRVDQLSHFFHVCFCFFENTAVLSSHNVYPNRFVR
LGGCAVTCWFYTLMLGLMRQAYVMTQKKNTPEEQKRQMITTVKLGCFLIFSLTCFPKGGP
QLLEDVNGPLVPLHKTLQLIAPKHLALNETIRGVLGFIASMCDFY

Parasites	L.mex
Gene ID	LmxM.10.1225 (99%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	148 aa
Molecular weight (MW)	16160 Da
Isoelectric Point (ISP)	9.04
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGCCGTGCACAGCTACTTGCCCTCAATA
GCCGGTGACACCTCAGTGAATAACGTCACGATCCACAACTCACTCTGTTGGAAAGCCAAG
CAGTCTCCCTAACCTTACCAATACCGGACCACTTCTGACGATGCTAGTGACAATCACCCA
CGCCGTTGGGCATGTCAGGGCGATGTGTGGCTGCTGATGTCGGCGGTGAGGTGGTGGATG
GCGTTGCGTTGGAGCGGCCTGTGACAGCGAACACGCTTGTGCCATCCGTATCATGGGCAA
GGTGTCAACGCCACTCGCACGCCTCTCACCCCGCCCTGACACTGCCTGCAGGTGTGGTGG
CGCCTGAGCCACCTGGAGGGGTGGCGCATGAGGCGGGTCCCGGCACCATGGGAGCGGCTG
TGAGGCGACCCGGCCTGCGAGGCAGGGGCGGGTAGAGTGTGAGGCAGAGGCTGTGCTGCG
ATGTCTGAGTCGGCACTGCTGTCGCACTGGTCTGCTGCTGCTTGGGCCCACGCGGTGGGG
ACTCTGTGACGAGGTCGGGTAGAGTGGAGCTGGATTCTCGCGCTATAGCACAATGGGCAG
TGAAAAAAAGCAAATATATATCGCTAAATAGTTCGCCGAGATTGTGACAACCAAGGCGAA
AAAGCTCGAAATCGAAGAGGTTAAAAATTCATTTTCCAGGTCCGCCACGGTAGCCCTCAG
GGAAGAGAAAGGCAAGCAGGTCCTGTGTCTTGCGCCACGCCTCGCGAGAGGTCTGCAGTC
CTATTTGGGCCATCGATAAATGGCGTGGTGTTGCCTCGCGCTTGTTCTCCGGGCGAAATG
ACTTGGGGTCTATTGCATNAAGGATGGCGGCGCAGAACTGCAGACCTTCGCATTGCTTGA
AGTATGATATCCAGCTGCGGTACTCACAGTCTTGAAACGGAGCCAACTTTTTTNGTACAA
AGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCACTATCAG
TCAAAATAAAATCATTATTTGGGGNCCGNNCTTANACTNNNGTCNNTTTNNANNTNNNNN
CTGNNNAAACNTCNTGCTAGCNTNNNNNNNANTNGNNNTNCAGCANNNAATAAANNAANG
NNNANNNGANACTNNNTNNNTTTATCTGNNNNTNNNCNNNNNNNNNCTGNNNNNANNANN
CNNNNNANNNNNTNANNNTNGNANNNNNCCNNNNNNGGNGNGGNNNNNNNNNNNCCNNAN
NNCGGNNNNNNNGNNNGNNCNNNNNNNNN

Protein Sequence

MEFLILHSVVNHARTMVFIDKLKTIVNRSVGLRPGNALATSSGAAPPTFAEAWNQAKAMT
NKLFEEREWQCPCGHKFRAAGEWVACAPIYCEVPSCPNPKYYIDGPGRALLEANPSGVKL
EEVNNTGSRVTSQPDSLKKPGGVGSRFR

Parasites	L.mex
Gene ID	LmxM.36.5120 (99%)
Gene name	40S ribosomal protein SA, putative
Feature	In other organisms

Sequencing length	246 aa
Molecular weight (MW)	27542 Da
Isoelectric Point (ISP)	7.6
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNTCNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTGTTTGAAGTTGTGGAAAGTGATAAAAG
CAAAGGCGCTTTTTGAGGGTGTGAGGACCACCGAAAGGGCGCTGCTGTGGGCATACCGTT
GTCAGAAAGTCGATTCCCTCGTCGAACGTCAGACACCCAGCACACGCATGTGAACCGTCT
AGGCGGCATCACTCACCCCTTCAAGCCCCGAAAAAAAAAAAAAGAAATGACTAGACCAGT
TTGTTAAAGAGCACCTTGCCGTGTGCGTAGAGCACAGCCTGACCTCACTAGGGACGCTGC
ACGCGCGAACAAAGGAATGTCGTAAAGAATGCAAGGACGAACACGGGCAGAGTGGCGCGG
CAGAGCTGCACAAGGTCACCAGCCTGCCATTTCCCCACCGCTCCTCTCGTCCACTCCGTC
TCCACAACGCCTTACGCCTCCCACGCGTTGTCGTCGTTGCGCTCCACCCAGCCGAAGCCC
TCCTCCGCCTCCGCGACGGGCACCGCAGCGGCCGCAGCGGCCTTCTCCTCCGCAGCCTCG
TTGGGGTCGCGGTAGAAGAACAGGTCCACCTTCTCCTCCCACGGCACAGAGCGCACAATA
GTGCCGCGCAGGCGCAGCACCTCGCGCGCAAGCAGCCAGTACATCATGCCGATCGACTTG
ATGCCGCGGTTGTTGCACGGGATCGCAATGTCCACGTACTCCAGCGGCGCGTCCGTGTCG
CACAGCGCAATCACAGGGATGTTCACCAGCGACGCCTCGCGGATGGCCTGGTGGTCCGTG
CGCGGGTCCGTCACCACAAGCACGCGCGGCTGCACGAACTTCTTCTGGATCTGGTTCGTG
AACGTACCAGGGATGAAGCGGCCGCCGTGGAAGCTCGTGCCCACGTGCTGCGAGAACTTG
TAGATGGCGCGTGNGCCGTACAGGCGCGACGAGCACACGCACACGTCCTTCGGGTTNNCN
ACAGCCGCGATCACGCGAGCAGCGAGGATCAGCTTNNNCCACATCATGNNGCACGTCGAT
GANNNNNTNCCNNCNNNNGNNNNTAGATTNNCTTCCNNTTTTTTNNNCNAAGTTGGCATT
NNTAAAAAGCATTGCTCATCATTTGTTGCACNANNNNNNNNNNTCAGNNNAAATNAANNN
TNATTTGGNNNNNNNTNANACTNNNNCGTTNNANNNNNNNCTGNNNNANNNNCNNNNNNN
NNNNNNNNNNNNNANNNCNGNNNNANNANNANNNNNNNNNNAANNGNNNNNN

Protein Sequence

MTAVESGSKVLRMKESDAQKLLAMRCHIGTRNQSSAMKKYIYGRTAEGSHIIDVHMMWEK
LILAARVIAAVENPKDVCVCSSRLYGTRAIYKFSQHVGTSFHGGRFIPGTFTNQIQKKFV
QPRVLVVTDPRTDHQAIREASLVNIPVIALCDTDAPLEYVDIAIPCNNRGIKSIGMMYWL
LAREVLRLRGTIVRSVPWEEKVDLFFYRDPNEAAEEKAAAAAAVPAAEAEEGFGWVERND
DNAWEA

Parasites	L.mex
Gene ID	LmxM.34.3670 (100%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	225 aa
Molecular weight (MW)	26516 Da
Isoelectric Point (ISP)	7.58
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNGGGNANNNGCTGGNTGGCNNTAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGGTTTGTGCAACATCGCCTTCCGCTTTC
CTCATCTCCCTGCGGCCGAACTGTGATGGACGAGAGGACAAAGTGCACGCGTCATCGCCT
AGGCCTCCCATCAAGTCGAAGAGCACAAACCACTTGCGAGCTATGCGCTGCATTTTTTTT
ACTCTTCGTTGTATCGCTTCTTTCGAAGGTCTTTTGAGCTTCCACACCGGTTCCGGCAAA
CTCGTCAACTACCCAATCCGCCGCGTATTGCGCGCCCCTCACCAGTTCCTCAGCAGACGC
ATCAAGTAACCAACGCCACCCCCAACACCGTCAGAGGCGTTCGAAGTTTTTCTCCGTCGA
ATTCGCGTTGGCATTCCCCTTCAACTCACCAAGCAGCAAACCGATCGCGAAACCGCGAAT
GAAGGAAGAGAAAGCGTCAAAGGTAAGAACAACACTCACCGGGATGCAGAGGCCACTTGG
CGCTGTTGATCTTGTCCCTGGGCCCTCGTGTGTGTGTGTGTGTGTCACAAATACTTCTCA
CTAATTTGAGCTCTCTGTTTATTTCCTTACAGGCAGTGAAACACCGCGAAGCTGGCGTTG
CGGGGGGTGAGCGGCAGTCTCGGAGAAAGGGGAAGGCCTTGAGAGAGACGATGCAGATCC
TCCGTTTATCTTTTGAGTTTTCGTGAAGCTGAGAGAAATAGATTTCGCTTTTTGCTTGTC
TGCTGGCCTAATCTCGCCTTCTCCTGTTGGTTGNTGGNTGCTGCCTTTCTCTCGTGTTCA
TCCCTGCGTGAGAAAAAGTGGGTGTCCGAGGGAGATGAAAGTTGCTGTGATGGGCTGGTG
AGACAGGAGGCCCCTGTGCCTCTAGGTGAAAAGTAGAAATGGGGATGTGGCGGAGGCCCA
AAAACAAATTACACCAATCCGTGAGGAACCCCAATCGTCAGTTTTACAGTGTGTAAAGGG
AGACGNNTCNCNNANANATGCGCAGTNNGGGTNGTTTTTCCCCCATTTTANNGANGGGCN
GCAGATGCNTTTTCATAAGTNCAGCANTTTTNGNANAAGTGNATNNNAAAANCATGNNNA
TCANNNTGCACNANNGNNNNNNANCNNNNANTAANCNTNNTNGNNNCNNNNANACNNNNNN
CNNTTNNANGNNNGNCNNNNNNNCNNNNTANNNNNNNNNNNNGNANNNCNNNNNNANNNN
AGNNNNNNNNNNNNNNNNNNNCNGNNNTGNGNNNNNNNCNN

Protein Sequence

MTEYLKDLASVKPGEPMPPVNLGPYDNPLLWNKLDPFGADRGHQRRPMTVSRDFMELNQV
PIVYRDHCVHRWIPFNRCMRNLKPVTWGTVNCHEFEESWMICRAHETYRLQLLKSKFMEL
TKDYTAEDKKFFPSILYLGIPYYMPSFYWNLAASQRLSGWDEKDPANPTMWREPNRSLMR
SEFSPTNWEKGTMTSAYGHKLIPDEIVHDMVPGFPLPEEKRPQAA

Parasites	L.mex
Gene ID	LmxM.36.1640 (99%)
Gene name	universal minicircle sequence binding protein (UMSBP), putative
Feature	Trypanosomads only

Sequencing length	298 aa
Molecular weight (MW)	30849 Da
Isoelectric Point (ISP)	7.82
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNNNNANNNGCNNNNNNNAATAATGATTTTATTTTGACTGATAGTGACCTGTTC
GTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTGG
GTGAATTCTTTTTTTTTTTTTTTTTTTTTCTTTTTTGCTTTTGCCTTTCTTTTTCATCTC
CGCACATGATAGCTGCTCGTTACTCGATCTCACAGAGGAATAAAAACGACATGTACACAC
ACACACACGCACACACACAACCCCATCGTGAGAGCGAAAGATGGTCGAATGAAAAGGGGC
GAAGGTCTTTCAAAAAACAAAGTCATACAAACAAAAACTCTTGTTAAATAAAATAAAATA
AAACAGAAAAACGCAGACAGAGACAGAGAGGCTGATGAATAAACGTGCGTGCATGTCTGT
GCTTGTCACAAAGAGCTTGTCGGCACTTCACGGTGCAGACAGGAAGCGGAAGAAAAACAA
AAGAAAGGCGATGCGCCGCAAGACACACACAGAGAGAGACACAGACAAGCACCGGGTTTT
TGTACTGTTTTTTGTTTTCCTCTACCTCCCTGCCGGTAAAGCAGAGAGGAGAAGTGAGCA
GGAGAGAGCCATCTCGACTCCTTCGATCACACCCTCACACGCGTGGTGGGCCGTGCTTTT

TTTTTGGTTTTGGAATCGACAGTCGCCTTCTGCTCTCCTACACTGGCACCGAAAAAGGAA
AAGGCGCATTCTCACCCGCGCACTGTCGCTGCCTTTTTTTTCCAATGCGCGCAGGCGCAG
GCGTGTGTTCGCACCCGCTTTTTGTTAATGGGAGAGGGGGTCTGATCTTCGTTTTTCGTG
AAAAGGAGTGAAGGGTGCAATGACGTGGACGCAGAGAGAGTCCGACATGGCATGTCTCTA
AGTGTTGAAACAACAACAAGAAAAGAGGGCTACGCGGGCGTGTTGCACGCCACTCTTCCC
TTCTCCTCACCGCCTCGTCTGTGACCTCCATTTGGTTTGCGGAGCGACCACGCTCTTCCT
TCTCATCTTGCTGCCTCCTCCCCTGACAATTGTGGTTTTCTCTTCCAAAGTCCAGTCNGN
NCTTGCTGACGCTACGCACGCAGTACNACCCACCCCCGTCGTCGTCATGGNCGGACTGNN
CTTTCNNGNTTANTNNNNTTTTTTTAACCCNCCNTCNCGCCNNCNTTTCCTTTTNNNNNN
NGNNNNGAATATCGANNGGNNNCATGAAANNAAGANNAAAAANCATCNNCTTTTNNNTNN
NNNNNAGNNNNNNNNNGATCNNNNNNNNNNNNNNNNNNNNNNNNAANGNNNNGGNNGNNNA
NNNNNNNCCCNNNNNNNANNNNNNNNNNNNNNNGATNCNNNNNNNAANNTCNTTNTNTCN
NCNCNCNNTN

Protein Sequence

MSETEDVKRPRTESSTGCRNCGKEGHYARECPEADSKGDERSSTCFRCGEEGHMSRECPNEARSGAAGA
MTCFRCGEAGHMSRDCPNSAKQGAAKGFECYKCGQEGHLSRDCPSSQGGSRGGYGQKRGRNGAQGGYGG
DRACYKCGDAGHISRDCPNGQGGYSGAGDRTCYKCGDAGHISRDCPNGQGGYSGAGDRKCYKCGESGHI
SRECPSAGSTGSGDRTCYKCGKPGHISRECPEAGGSYGGSRGGGSDRTCYKCGEAGHMSRECPSAGGTG
SGDRACYKCGEAGHISRDCPSS

Parasites	L.mex
Gene ID	LmxM.36.1635 (99%)
Gene name	poly-zinc finger protein 2, putative
Feature	Trypanosomads only

Sequencing length	135 aa
Molecular weight (MW)	14578 Da
Isoelectric Point (ISP)	8.18
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNGNNNNCNGCTGGANGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTT
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTG
GGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTCTTTTTTGCTTTTGCCTTTCTTTTCATCT
CCGCACATGATAGCTGCTCGTTACTCGATCTCACAGAGGAATAAAAACGACATGTACACA
CACACACACGCACACACACAACCCCATCGTGAGAGCGAAAGATGGTCGAATGAAAAGGGG
CGAAGGTCTTTCAAAAAACAAAGTCATACAAACAAAAACTCTTGTTAAATAAAATAAAAT
AAAACAGAAAAACGCAGACAGAGACAGAGAGGCTGATGAATAAACGTGCGTGCATGTCTG
TGCTTGTCACAAAGAGCTTGTCGGCACTTCACGGTGCAGACAGGAAGCGGAAGAAAAACA
AAAGAAAGGCGATGCGCCGCAAGACACACACAGAGAGAGACACAGACAAGCACCGGGTTT
TTGTACTGTTTTTTGTTTTCCTCTACCTCCCTGCCGGTAAAGCAGAGAGGAGAAGTGAGC
AGGAGAGAGCCATCTCGACTCCTTCGATCACACCCTCACACGCGTGGTGGGCCGTGCTTT
TTTTTTGGTTTTGGAATCGACAGTCGCCTTCTGCTCTCCTACACTGGCACCGAAAAAGGA
AAAGGCGCATTCTCACCCGCGCACTGTCGCTGCCTTTTTTTTTCCAATGCGCGCAGGCGCA
GGCGTGTGTTCGCACCCGCTTTTTGTTAATGGGAGAGGGGGTCTGATCTTCGTTTTTCGT
GAAAAGGAGTGAAGGGTGCAATGACGTGGACGCAGAGAGAGTCCGACATGGCATGTCTCT
AAGTGTTGAAACAACAACAAGAAAAGAGGGCTACGCGGGCGTGTTGCACGCCACTCTTCC
CTTCTCCTCACCGCCTCGTCTGTGACCTCCATTTGGTTTGCGGAGCGACCACGCTCTTCC
TTCTCATCTTGCTGCCTCCTCCCCTGACAATTGTGGTTTTCTCTTCCAAAGTCCAGTCAG
GTNCTTGCTGACGCTACGCACGCAGTACAACCCACCCCNGTCGTCGTCATGGNNGGACTG
NNCTTTCGTGNTNATNNNNTTTTTTNNCCCACCNTCACGCCTTNTTTNNTTCNNNNGNNT
AGAANATCNNNNNNNNCATNNNNNNANNNNNCATNNNCNTTTNNNNGNCNGNAGNNNNNN
NGNNNNTNNNANNNNNNNNNNNNNGNNNNNNANTGNGNNNNNTGNANNNNNNNNNNCNNA
NNNGNNNNAANNNNNNGNNNATNNNANNNNC

Protein Sequence

MVCYRCGGVGHQSRECTSAADSAPCFRCGKPGHVAKECVSTITAEEAPCFYCQKPGHRAR
DCPDAPPKSETVMCYNCSQKGHIASECPNPAHCYLCNEDGHIGRSCPTAPKRSVAEKSCR
KCGKKGHLRKDCPEA

Parasites	L.mex
Gene ID	LmxM.17.0083 (99%)
Gene name	elongation factor 1-alpha
Feature	No information!

Sequencing length	208 aa
Molecular weight (MW)	23169 Da
Isoelectric Point (ISP)	8.45
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNNNNNNCNGCNNNNGGCNNNNATGATTTTATTTTGACTGATAGTGACCT
GTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAG
TTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCAGGGGCGCCATCGCGCGCGTGGCCT
ACCTGCCTGGGGGTCGCTTCACGGCCCAGTCACGTTACCTTCACTTTCTCAGGGGAATGT
CATTGCTATACCTTTTCCTTCCGCGCACCGCCGCGTCGCGCAGGGTGCACGCACAGGCAC
GCGCACAGCCGCACGCACGCCTGCACGTCCCCGCGCGCCGCCGCGGAAGTGGTGCTTACA
CCCACCTCGGCTCTGCGCCTTACAGGGTGGCTTACGAGACACGCACGCGCACACGCTTAT
AGAGAGAGAGGGGCGTGTGCGCGTGCGCGGGTTTGGGGGAAAAGAGGGGAGGTCCCGCGG
GACGCATGGGGGAGATGGAAACAAAAAGAAAGGGGCGGCGGGCGGCGGAGCACCGCACCG
CCAGCGCAGCCACGGCACGCTTACTTCTTCGCAGCCTTCGTGGCCGCCTTGGTCACCTTC
CCGCCGCTGCTCTCCTTCTTGTTCACGCCCTTGATGATGCCCACGGCGACCGTCTGCCGC
ATGTCGCGCACGGCAAAGCGGCCCAGCGGCGCGTAGTCGTTGAACACCTCCACGCACATC
GGCTTCTGCGGCACCATCTTCACGATCGCGGCGTCGCCAGACTTGATCGCCTTGGGGTTC
TTCTCCAGCTCCTTGCCGGAGCGGCGGTCGATCTTGGACTCGATCTCCGCGAAGCGGCAC
GCGATGTGGCTCGTGTGGCAGTCCAGCACCGGCGCGTAGCCGTTGCTGATCTGGCCGGGG
TGGTTCAGCACGATCACCTGCGCCGTGAAGTCGGCCGCCTCCTTCGGCGGGTCGTTCTTC
GAGTTGCCGCACACGTTACCACGGCGGATGTCCTTCACCGACACGTTCTTCACGTTGAAG
CCGACGTTGTCGCCGGGCTGCGCCTCCGCCAGCTGCTCGTGGTGCATCTCGATCGACTTC
ANCTCAGTCGTCACGTTGGCGGGCGCGAACGTCACCACGTCGNCCNGGNTNATGATCCNN
GTCTCCNCNNCGNNNANGGGCACGTCCCGANNNCCNNNATNNNNNNNNGTCNGCNGGGNN
GNNCAGCGGNNTNNTCNANCGGGNNCNNCGNNGNTCNGCANGTNNAGNNNGNCCAGNANC
NNGGNNNNNNNNCNNNNNNNTNNNNCGACNTNNNATCANNTNNNNNNNNNNCNNNNNNNN
NNNNANNNNNNNTNCGNNNNNCNNNNNNTNAGNNNNNNNNNNNNNNNNNANNNNNNGN

Protein Sequence

MGKDKVHMNLVVVGHVDAGKSTATGHLIYKCGGIDKRTIEKFEKEAAEIGKASFKYAWVL
DKLKAERERGITIDIALWKFESPKSVFTIIDAPGHRDFIKNMITGTSQADAAILMIDSTH
GGFEAGISKDGQTREHALLAFTLGVKQMVVCCNKMDDKTVMYAQSRYDEISKEVSAYLKR
VGYNPEKVRFIPISGWQGDNMIDKSDNM

Parasites	L.mex
Gene ID	LmxM.29.1100 (70%)
Gene name	DREV methyltransferase, putative
Feature	In other organisms

Sequencing length	303 aa
Molecular weight (MW)	34394 Da
Isoelectric Point (ISP)	7.28
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGNNNNCNGCTNNNGGNAATAATGATTTTATTTTGACTGATAGTGACCTGTT
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTTG
GGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGCGTAAAAGTTAGTTTCCGATATTAAAAT
AATATAGCTATTATTTTTGTAGTAAAAAGTCAACTAAAACAAATAACACACATATAAAAC
AAAAAAATTAATATAAACGCTTGTGCTGTTGTAATTTTTAATCTATAACCAATTACTCTA
GGAAAAAAAAATCCAATTATTAAAATAATTATAGCTTTAAAACATATAAATAATCCACCA
AAGCATAAGCTAGAAAATAATAAGGTTGCTAATAAAATATGGTTAATTTCAAGTACTGAG
TATACTATAAAAAAAAATCCAGATAATTCTGTAATCAATCCAGCAACTAACTCACTTTCA
CATTCCATATAGTCAAATGGCAGTTTCAACCCGTCCAATAACATACTTATTCAAAATAAA
CTTACAAATAAAATTCCTGCTATATATAAATTTGTAATATAAATCTGCCCCACACAAACG
TCTTTAATACAAAAAAAGCTAAAGTAATCTAAAGAATAAACAGTCGTATATAGTAGAAAT
ATGCCACTTTCTGAAATAATGCTAAAGAACATCGTTCTCATTGCAGATAAATATACAAAG
CAACTAGAAAATAAAAAGCAACCTACAAAAAACGTGCTAAACATACTACTAAAAACATGT
ACGCATAGCATTATTGTAAGCGTAAATCCAGTATCTAATAATAATATAAAACCTATAGGA
AAGTAAAACCAACCAATAAAAATACAACATGCTGTAACTAAAATTGCACCTATTAAATAA
AAAATTTCAAAACTAATTACAAATATTATAAATTTAATAAATAGTTTTACTCCGTCTGAA
ATAGGTGTTAATAAACCNAAAAAGCATAGAGCAGGGCCTACTCTTATTTGAACTAATGCC
NAAATTCTTCTTTCGCAAAGACTTACAAATCCCGGTCAAGANAGAACAACTAAAATGTNA
TAATAATAATAATNATAATATCGATATTTANCATATAAAATGGCTTTTATCCAACTTTTT
GTACNAAGTTGGCATTATAAAAAAGCATNCTCATCNNTNNNNCACNANAGGTCACTATCA
GTCNAANNAAATCNTNNTTGGGGNCCGANCTANACTNNNTCNNNNNACGTNNNNCTGNNN
AANNNTCNTGCTNNNNNNNNNNNNNNNGGNNNNCCNGNNTNNNNAANNNNNNNNNNNGNN
ACNNNNTNNNNNNNNNNNNNNNANNNNNCTGANNNNNANCNNNNNNNNNNNANNNNNNNA
NNNNNN

Protein Sequence

MQGLSKDLQLYISGHRGRPALIYEPRASLVSPSLLQLFEACNCDAETTAFLIRSRNMSVW
KLMLADFLSVFLSRTTAQGMVGRGMMFVYSTEQIRRLLRPPQAPLTSPLPPDFQFDSLLD
IGAGDGGVTEKIAPLFKKVYVTEFSASMRWRLRRRGYEVLPHDDPFHMNTAEKLLDRRYF
DVIACNNVLDRADMPETLLREMRDSLKPNGLLVLAVVLPWCPFVEDGPRQKRPSEILPME
GGECCRGASFEESMSKLVENVLVPMGFEVVRWTRLPYLCEGNLRIEYAVLSDAVLILRNR
GDV

Parasites	L.mex
Gene ID	LmxM.13.0570 (99%)
Gene name	40S ribosomal protein S12, putative
Feature	In other organisms

Sequencing length	141 aa
Molecular weight (MW)	15592 Da
Isoelectric Point (ISP)	4.49
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNNNTCNGCTGGATGGNAATAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTGTGTGGGTCATCGAAAAACGAAAAAATC
GAAACATTTTACCGAAAAGAGCAAATGAAGTTAGCGATTTCGTAGATGTGCAGCAGTGCT
GCCCTCTTCCTCGTCTCCTCGCTCTGCCATCTGCCATCTGCCCCCCCAACACACACACAC
ACTCATCATCATCTCTCGCTTTCGCTCGCCACCGAGCCTGCACACGCGCGCACGCATGCG
CACATGTGTGTGTGTGTGTGTGTGTGTCCGAGCACGAGAGAGCACAGAGAAGAGTAGG
AGAGAGCGAGAGCGGGCAGCGCGAGGAGACAAGAGGATGAAGGAGTAGGTGAAGCGTAGG
AGAAATCTGCTCCCCGCCGCGCGCATACATGCGCACACACACACACATACACACTGCATA
CCGTGACGAGTAGCTCGCGAAAAGCGTGCGCAGGCATACACGAGAAAAAAAACAAAACAC
GCACGGATACACGAGCCCCTTCTTCTGCATGCGCAGCATGCATGCGTGTGTGCCTGGCAC
GTTCCTTGCAAGCCACTGTCTGTGCATCTCCTCGCACGTCTTTCGTTTCTTTAGAAAAAG
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CCGAAGTCNCGAATGGCGACAGAGGAGCACTTGAGAGTCTTCGTCACCTCACCAGCCACA
TCGCGGCGCACGANGCCGGNCCACTGGGCGAGCTTCNNNGCGCTCNTCAACGTTGATCAG
ATCAATGNTGCCCTGCTNGNGAGCGNNNGACGAGCTTNTNGTANTNNNATCNNNCNNNCN
TCAGCNNAATGNACATGTGCNCNTCNTGCAGTCANCNNGCGNNNNCCTCAGANNNNNNNG
NNGANNNNNNNNNNNNCNNCNGNTNNANGNGNNGNATCTCAGCTTTCNGGGNNNNNNNNC
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ANNANNGGAANCNGNNGNNNNNNNNANNNNNNNNNNNNNNNNN

Protein Sequence

MAEETVRVEVPAVEENVVVDVAPESLEDAVRIVIQKALEANGLVRGLSEVARTLDCKTAH
MCILADDCEDEEYKKLVTALAKQGNIDLINVEEREKLAQWAGLVRRDVAGEVTKTLKCSS
VAIRDFGERTKALDYLLSQLH

Parasites	L.mex
Gene ID	LmxM.29.0180 (100%)
Gene name	2-hydroxy-3-oxopropionate reductase, putative
Feature	No Information!

Sequencing length	299 aa
Molecular weight (MW)	31027 Da
Isoelectric Point (ISP)	5.16
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNGNNNNNCNGCTGGATGGNNNTAATGATTTTATTTTGACTGATAGTGACCTGT
TCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGTT
GGGTGAATTCTTTTTTTTTTTTTTTTTTTTCGATGTCAATACAGCCAAGACACTCACA
GAAAGTGAAAATAGGGAGGCAGGCAAGCTGCAAGCGCGAGAGGGGCCCGCAGGCAACGAC
CGGAGAAGAGCTACAGCGGCCGATGCGCTACTTCTCGGCAGCCACGCACGCACCGCACAC
ACACAAGCACACCTCCGCCCTAAATACCCCCCATCTCCTTCAGGGGTGCTACACCAGACA
CGCGGTCGCCGTTCCATCTGCACTTCACCGAGCACCAGTGAACAGTGTCCACAAGTGCCC
GCGCATATGCACTATTCCTTCTTCACCTCCGAGATGTGGACGTTGGCCATGCGTTCGAGC
ACGCTGACGACAGCGGAGTTGTCGCGGTCTCCATCGCCGTTCTGAACCATCGCCTGGAAG
AGCTGCGTATTGACGGCGGTGGAGGGCAGCGGGACGCCAAACTCCTTGGCCGAGTCCATC
ACGATGCCCATGTCCTTGCTCTGCAGGGCAGCCTTGAAACCGGGCTCGCGGTTGCCGGCA
AAGAGGCGGTCCGGCTTCACATCGAGTGCCCAGCACTGTGCCGAACCACTCTTGATCGCC
TCGATAACTGTCGACCCCGACACGCCGCACTTCTCAGAGAAGACAAGGATCTCGCCAAGC
GCGACCATCTGGGCAGCCACCATGATCTGGTTTGCCGCCTTGCACACCTGCCCCGCCCCG
CAGTCGCCAATGTGGGTCACCTTCTTACCCACTGCGAGCAGCACAGGCAAAACAGTCTCA

AGCGCAGCAGCGTCACCGCCGACCATGACGGTCAAGGTGCCGTTGCGCGCTCCAATGTCG
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ATGCCGGCTGCCACGCCGTTGGTGCCGAACACGATCTNCATCACGTCAGAGNANTCCNAN
ANNNGTGAAGACNACGTCAGAGCNGCCGCCAGCTCNNNNGTCNCNNNCGNCNNGNNCNNC
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GNGNATGGNNTGCNTNNNNNNACANNNNCCGANNNNNATNNCNANAGNNNNNCNNNGGNN
CNNNNNNNNNNNNNNANGNNNNNCNGNNNANNNNNNANNNNNNNNNTNNNNNNNTNNNNG
NNNNNNCANNNN

Protein Sequence

MRVGYIGLGLMGKPMAANILKAGFPVCVWNRTASKCDDLVTAGATACATPAELAAASDVV
FTNLSDSSDVMEIVFGTNGVAAGIREGAIFVDNSTIKPSVAQEIARRLWEEKKVRALDAP
VSGGDIGARNGTLTVMVGGDAAALETVLPVLLAVGKKVTHIGDCGAGQVCKAANQIMVAA
QMVALGEILVFSEKCGVSGSTVIEAIKSGSAQCWALDVKPDRLFAGNREPGFKAALQSKD
MGIVMDSAKEFGVPLPSTAVNTQLFQAMVQNGDGDRDNSAVVSVLERMANVHISEVKKE

Parasites	L.mex
Gene ID	LmxM.04.0750 (99%)
Gene name	60S ribosomal protein L10, putative
Feature	In other organisms

Sequencing length	213 aa
Molecular weight (MW)	24557 Da
Isoelectric Point (ISP)	11.37
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGGNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGGTGAAACAAGAAATAAAAGGCGGCCG
TCACGCCGGTGCCCGCAGAAAACAAAAAAAATACGAAAGAGGACCGGATAAACCGCCCAC
TGGTGACATCACGCCCCATCGACGAGGAGGTCCTTCACCCCAGCAATGTCCCCCTCCCCC
CCTGTGCACGGGGGGCGCGCGGCATGCTGCGGATGTCGTTAAACGCCGTAGCACACACAC
ACACACACACACACACAAAGCACCCGTTTCCCCACGCGTTTCACGCTGCTGCGAGAGCTT
ACGCCATCACGTTGCGCATCGTGATTTTGCCCTTGGGGGCGATCAGCTTGCAGTGGGTAC
CGCGCTGCTCGAGCTTGCCGGCGTCGCGCAGAGCCTCGTACTCGGTGCGGAGGATGTTGG
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GGCGCAGGGCCTCGAAGGCCTGCGGCACGTACGCCTCCTTGGTGCGCATGGACAGCAGGA
TCTGGCCAATGCGGACGCGGGCACACACGCCGTTCGGCTTGCCGAAGGCACCACGCATAC
CGGTCTGCAGACGATCAGCGCCGGCGCACGAGAGCATCTTGTTGATGCGCAGCACGTGGA
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TGTTGGCCTGAATGCGGGCAGCCTCCAGCGCCTCGGACGCGATCTGCTCCAGCTCGCGGG
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NCGATCCAACTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCNNTTGTTG
CAACGAACAGGTCACTATCAGTCAAAATAAAATCATTATTTGGGGNCCNNNNCTTANACN
NNNTCNNNNNANNTCNNGNCTGNNNNNACNNCNTGCTAGCNTACGCNNNAGTAGGANTNC
NGNATNANTAANNNANGNNCANNCGANACTGGNNCTNCNTTTNNTNNTNNTGNTGNCGNN
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NNNNNNNNNNNNCCNNNNNNNGTNCNGNNNNNN

Protein Sequence

MARRPSRCYRFCKNKPYPKSRFCRGVPDPKIRNFDIGRRRATVDEFPVCIHVVSRELEQI
ASEALEAARIQANKYMVKRANKDVFHMRTRAHPFHVLRINKMLSCAGADRLQTGMRGAFG
KPNGVCARVRIGQILLSMRTKEAYVPQAFEALRRAKMKFPGRQIIVMSKYWGFTNILRTE
YEALRDAGKLEQRGTHCKLIAPKGKITMRNVMA

Parasites	L.mex
Gene ID	LmxM.33.3790 (100%)
Gene name	hypothetical protein, conserved
Feature	Trypanosomads only

Sequencing length	1301 aa
Molecular weight (MW)	139827 Da
Isoelectric Point (ISP)	7.01
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGGNNNTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTCTCGGGCTCTCTCTGCCGAAGCAGTCC
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GTTGTGTTGCGGCTTCTCCCTCGCCTCCGCACCGCAACCGCACGTCACGCGAGTGAGAGA
GGCAGCAGAGAAGGTGTGCGGTAGAAAGATCATGCTTCACCGCCGTCACAACAGTCAGTC
CATCCCTGTGTGCAGCGGTCCCTAATTAGTCCCCGCCTTCATCGCTGTCGTCCTTGGCAG
CTTGCCGCATGGACACCTTCAGCGCTGCAGCCTGCCGCAGCACGACCTCTTCGGCCGCCA
TGTTCCCAACTGTGTGCAGGAGGCCGCTGTAGTCGGCCAAGGCGTGCAGCACAGTGTCCA
TTATGGCACTGGCACTCACGGGCTGCCCTGGTGAGGTGTCCGAAGAGTGGGCGGGTGATA
TAGACGACCACGCCGCTGCAGCCGTCTCTGGCCAGCCAAGAATGGCCTCGTGGTACTGGG
CCAGCACGAGCGCCAGGAGCCCAGCCACATCCGTGAAATGAGCCGACTCTATCAGCAGGG
ACAAAGCTGCCAGTGGAGGGGGCTGCGTCTCACGAGGAGTCGGTCCACCGACGGCGAGTA
GAAGAGCGGCGCTAAGCAGGCGGTAGCCGCGCTTCACTGAGAGCGCTGACCAGCGCTGCA
GCACCTCCGCCAGCAAGGGCCGCTGCACTTGAGGCACCGCCTTGACAAGAAGCACAGCAT
CGCGCCACCGACCTTTCGACTGGAGTGTGACGCAGGCGCTGCGGTACTCGCCAGCCAGCG
CTAGCNTCTCTACAGCTGCCTCCACGTCGCCCTGCGCTAGCAACATTGCCGCCGCACGCT
TCNCCGAGGCGGCGAAGANCGCCGACCCAACTTTTTTGTACAAAGTTGGCATTNTAAAAA
GCATTGCTCATCAATTTGTTGCAACGAACAGGTCNCTANCAGTCAAATAAAATCATTATT
TGGGGNCCGAGCTTANACTGNNNNCNTTTTACNACGTCNNNNCNGGNNAANNNNNCATGC
TANCGNNACNNNNGANNNNNNACTNCNGNATNNANNAAANNAANGNTCANNNGNNANTGN
NNNTTCNNTTNNNNGNNNNNTTNNNNNGNNNNNNNNNNNCNGNANNNNNNAANNCNNNNN
NNNNNNTTNANNTNNANNNGNCNNNNNNNNNNGNNGNNNNNNNCNATNAGNNNNNNNNNC
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Protein Sequence

MSSVSAPPQLSPKVIPGCSNTHSNSGLPTILSDLGTFAYQSACYGPQQLLACSSAVTSAG
EVIFVYDMNSMLLTQSLSGHKAAVTALLWRRSPASYLQTGLFLWSGDTSGALMYWDVVEG
VALTSIQTPCRALVQSLTLLTDEHLLVVTQEGTGYVFNSHLVDNIPLPPGRLLLEATQST
LLGNISRPSLVACSRLNDQHCCAVVLGDRLRILTAIQLETGAAPPVAKDLIYDSCDGAPT
VIDVVFSDAQEEVLYFATRNTVGCYDWKLGLMLNESLLWLPDEVEFRRIFRSCSSGAAVH
AAAMRSPESTASSSGDAAAAQVPLMYSFGSDQRLVAWHVTRHDRFTAVSTDVRGVRINSK
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CVALESGHLALMDITHSMAVRRFNLVYSGGTQIVLLCGYKWSDLVWIVSDRIQQYRHHHQ
VSLIDTRTGEVVRVLRKPSSAPEQTRMKEITMDPTATYVLLTFWNGTFEVWTAADSRLVH
IHSGLGVANVSWAPPLMRRCLSGVQGTPQLLAVLFSEGTLSLWSAYKDRVVVSRDAIPLF
HPSVVEGSVRSAVVVDTLVMWDGQGNGVVLRAQGTRLAVRRLRDAPPNSGAVMCLGGPSP

PSRATEFMSFSSPLAWTSSGSFSHHEDSDNAANLINTGEAGLSMPSAAGFSAARAVAVLF
ESGVFAVWDIATGERRALSSAGMAAEVRALSLYWTGGSLCVLGADGCLYVLDTYLTEMNS
SVRYRALRRPMKNVAFLLPAHRTYVQVALELGSPPSGVRDEAQRASHSLSDAPSAVMEAV
PVSARPCRGPFGQLLTEGIRTLQDEVELYRTTMVPRDVLLRLSRCGSTAGPVSPLTWLRR
AAVVADFLGQSAKQRFMRLAVNVLRHWQPSAVARRGVGGCPSAASSPADAVLAAESADCP
PASPNSESLGEDSFIVPHTYPCADAYSEALAPSHIVRRNRILLNEQRTAALLVNARNHRA
RDDSMSRLALARDWLRLGHRQSVIEVLLDAPPQSTTYNELATLSMAVAASTAVQSSSVDS
ATSALFVASAKRAAAMLLAQGDVEAAVEKLALAGEYRSACVTLQSKGRWRDAVLLVKAVP
QVQRPLLAEVLQRWSALSVKRGYRLLSAALLLAVGGPTPRETQPPPLAALSLLIESAHFT
DVAGLLALVLAQYHEAILGWPETAAAAWSSISPAHSSDTSPGQPVSASAIMDTVLHALAD
YSGLLHTVGNMAAEEVVLRQAAALKVSMRQAAKDDSDEGGD

Parasites	L.mex
Gene ID	LmxM.09.1340 (98%)
Gene name	histone H2B
Feature	In other organisms

Sequencing length	107 aa
Molecular weight (MW)	11908 Da
Isoelectric Point (ISP)	11.82
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNGGNNNTCNGCTGGATGGCNNTAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTGGGAGGGGTGGAGGAGGACGTTGGTGT
CTAGACGACAACGAAAACCAAGAAGCGAAGACGAAGAAGGGGAAGAAGAAAACGTGTGTG
TCACCTGTTCTGAGAGAAGACAAAGCGTGCACGCCTCTGTGCAGCGTGGCGCGGACGGCG
ACTGGAAGACGGAGAAGCGCATCTGTGCTTTCACCCACCTCTCCTCTTCTTCAGCGTCTC
GCCGCCGAACCCGCCACGCACTCCAGCAAAGGACGAGCCATCACCTTCAAGGAAATACAA
TCGAAAAGCAAACAATAGATCTCTATGAAAGCCCACGGCATAGGCGGGGAGGGGGAAGGC
GAGGGCCCCCCCCCACACACACACCCGACACACGCGCGAGGCGTCGACACACCCTCTAAC
TGAGTCACTTAACGGGACGCGTTCGACACGGCCTTCGTGCCCTCAGCCATGGCGTGCTTC
GCGAGCTCCGCCGGCAGCACAACGCGCACCGCCGTCTGCACCTCGCGCGCACCCAGCGTG
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TTCACGTACGAGTTCACGATCTTCATCGTGCGACCCGACATCGACATCTGGGAGTTGATC
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AAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCACTATC
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ATAAAACGAAANGNTCAGTCNGAAGACTGGGNNTTTCGTTTATCTGNNNNGTCGGTGANG
CTNTNCTGANTNGANAATCNNNGNGCGATTGANNTNNNANNACGNNNNNNGGNGNNNNNN
NNNTNNNNNCNNNNNNCNNGNNNNNACNNNNNNNAGNNATNNNNNNGNNGNCNNNNNNNN
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NNNNN

Protein Sequence

MASSRKTSHAHKSHRKPKRTWNVYVNRSLKAINSQMSMSGRTMKIVNSYVNDVMERICTE
AASIVRANKKRTLGAREVQTAVRIVLPAELAKHAMAEGTKAVSNASR

Parasites	L.mex
Gene ID	LmxM.17.0860 (80%)
Gene name	hypothetical protein, conserved
Feature	Leishmania only

Sequencing length	147 aa
Molecular weight (MW)	16877 Da
Isoelectric Point (ISP)	9.65
Signal peptide	No
Gene copy	single

DNA sequence

NNNNNNNNNNNNNNANNCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACC
TGTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAA
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Protein Sequence

MSFSTKFAMWWGSITTKTEKLFNKEKERLVTHEYYDNPNPMSARPKSIHSIRGSMRESASSKGLQNSND
NAKRPRSFRQQEANPSGNYADQMPMGSNPGYGGARQAPCMQNYNDMQQYPQGQQYMPGPQYIHQQHQGH
SNQGYDQV

Parasites	L.mex
Gene ID	LmxM.06.0410 (95%)
Gene name	60S ribosomal protein L19, putative
Feature	In other organisms

Sequencing length	247 aa
Molecular weight (MW)	28202 Da
Isoelectric Point (ISP)	11.95
Signal peptide	No
Gene copy	multiple

DNA sequence

NNNNNNNNNNNGNANTCNGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCT
GTTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAG
TTGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTTTGTTTTTTTTCGGGGAGAAAATGACCA
AAAAATGAGAGCGAAAACCTAAAACACAAAAAACAGAGAAAGGGAGACAGTGCAGAACAA
CGCACGAGGAGAAGAGAGAATCGGCACGCGACGCATCACCGAAGAAGCGGCGAAGAGATG
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GGCAGCAGCAGCGGCCTTCGCGGCGGGGGCAGCAGCCTTCGCGGCAGGCGCGGCGGACCA
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TGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGCCTTTCGTTTTATCTG
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Protein Sequence

MVSLKLQARLASSILSCGRARVWLDPNEAMEIQNANSRKSVRKLIKDGFIIRKPVKVHSR
ARWRKMKEAKDMGRHNGVGRREGSREARMPSKELWMRRLRILRRLLRKYRADKKIDRHVY
RDLYMRAKGNVFRNKRNLVEYIHKIKNEKKKARQLAEQLAAKHLRDEQNRNKARKQELRK
REKERERAKRDDAAAAAQKKKADAAKKSAAPAAKSAAPAAKAAAPVAKAAAAAPAAKGAA
PVKKSKK

Parasites	L.mex
Gene ID	LmxM.19.1640 (77%)
Gene name	hypothetical protein, unknown function
Feature	No information!

Sequencing length	749 aa
Molecular weight (MW)	79477 Da
Isoelectric Point (ISP)	8.1
Signal peptide	No/3
Gene copy	multiple

DNA sequence

ANNNNNNNNGNNANTNAGCTGGATGGCAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGT
TGGGTGAATTCTTTTTTTTTTTTTTTTTTTTTTGNTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGGNCCCCCC
CCCCCCCCCAAAAAAANNCCAAAAAAAAANANCCCCCCCCCCCCCCCCCCCCCANGG
GGGGGGGGAAAGGGGGGNTGGGGGGNCCGGGNCCCCCAAANAAAAANNGGGANNCCCCCC
CCAGGGGGGGNNAAAATGGGGGNNGAAAAANAAAAAAAAAAAAAAANNGGGGNGGGNNN
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Protein Sequence

MLCRPPSFNLLGLLHFSRFVSLITAHQPPFPIRCLYPVASGSSPPHRHHDSLQPILHQHL
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DMSMHRSFLCYVCCEPLFRAMSDSVCGHHVCEAYCLKMSISGAATCLLCGWNESWSRDTA
HSQCVRDAVEARLRESVAVLLRDA*PVLSSDCVGDVVDSCFDRSCRTVHLHQAQQYLAAV
PPDGVGAVCTALAAPDTIPNKAAVFSLRVEGPWLTSCDLVRSFLQLQVLVLCDCADLVSL
RGVECAPLLERLTVERCGLLDTLGINACQCLESLQRRECPRLSHLGWNQISNSQSSLRES

SGQEDGCAALCSVSVFFLLPLLGHRLPLCRSAPARAPCTVCSHQQPGRVA*VPPPQVA*C
WRVSAGVLH*SPARCKGAKVS*PLQHGCLRHRCAFTVHSAREGEPERVPPAALSR*S*VL
YGAQRAAGVSNEQSDADRSAPVPCPQESECQRLRGAERRRGPHASLRTHTCRSFLHGTGW
CWLPCLLPRGGERPSARMSTHTRLLATAQLRGCAAATLSRPLQHKCLQHLRVGSLPT*AA
DAPHERLHGALRHFGPGVCNPAEGGGFGQYQCTERVAAAVGCV*AGGASHQWCHLPERGA
SLPIHWGAAAV*RDKHRCVELITVICATRAHVWLHCW*M*ANTHAAFHALRIVYLLSM*L
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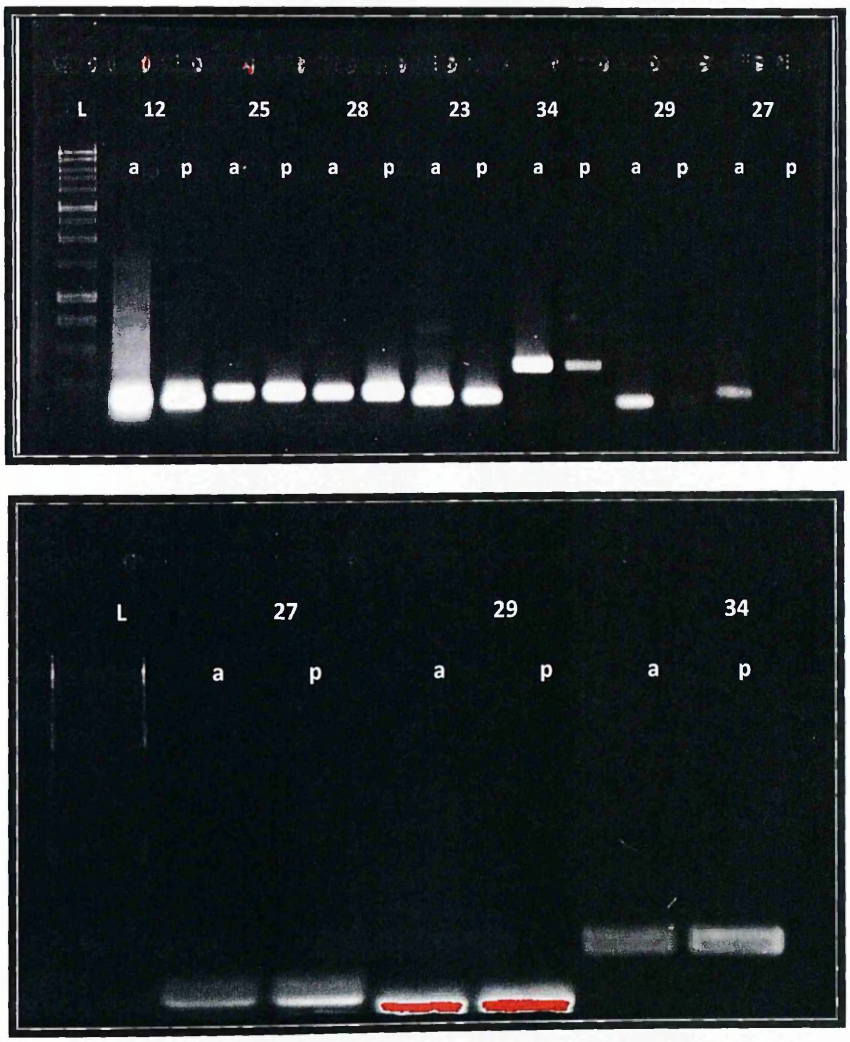
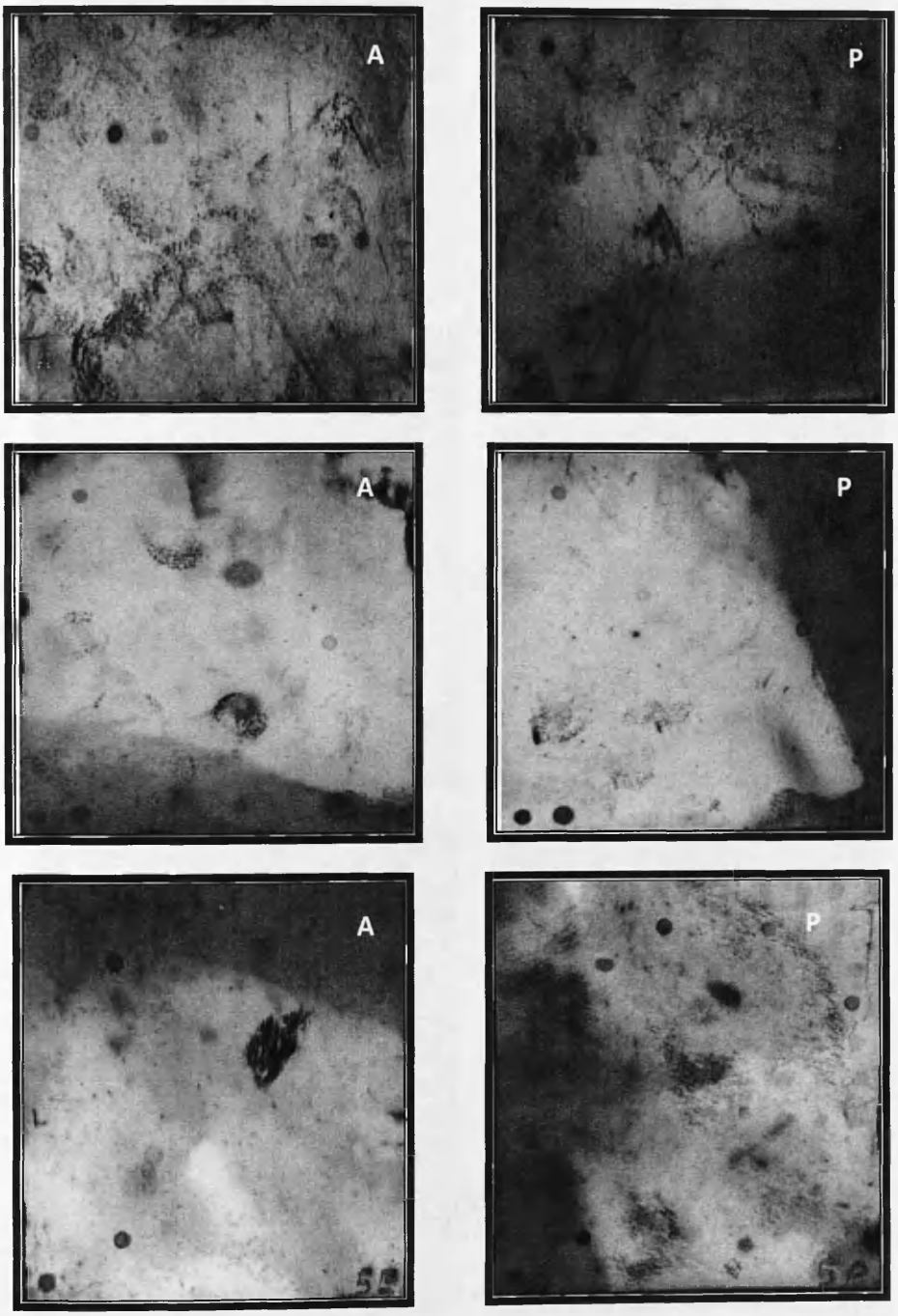
6.5 Appendix

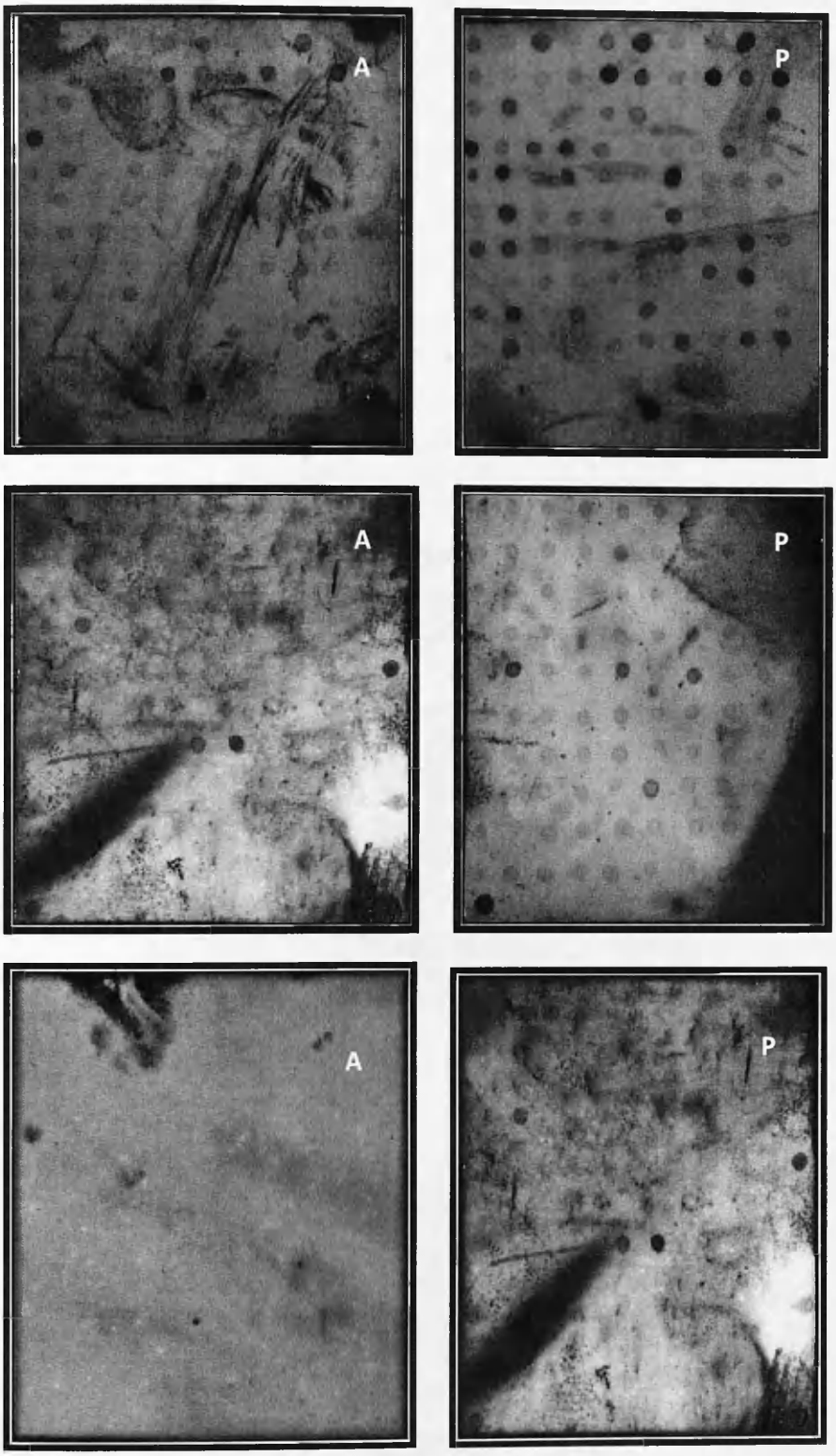
1% agarose gels showed *Leishmania* genes only analyzed by RT-PCR.



6.6 Appendix

Screened nylon membrane hybridized by log-phase prmastigotes and attached promastigotes probes. (A) attached promastigotes, (P) free log-phase promastigote




A
P
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P
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