

**MOLECULAR PHYLOGENETIC ANALYSIS OF *TRYPANOSOMA EVANSI*
BASED ON SMALL SUBUNIT RIBOSOMAL DNA AND INTERNAL
TRANSCRIBED SPACERS**

A THESIS

BY

SINTAWEE KHUCHAREONTAWORN

**Presented in partial fulfillment of the requirements for the
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AN ABSTRACT

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Trypanosoma evansi is a blood protozoa that causes trypanosomiasis or "Surra" in animals such as horses, beef cattle, dairy cattle, swines, buffaloes and sows. The disease can be acute or sometimes more chronic in some animals. General symptoms of the disease are fever, jaundice and anemia. Due to the wide host range of *T. evansi*, it created a great economic importance in Africa, Asia and South America and the diversity of these protozoa is questionable. At present, taxonomic relationships among trypanosomes are poorly known. Traditionally, all trypanosome species descriptions have been based on morphological characters of bloodstream trypomastigotes and morphometric parameters consisting of total body length including free flagellum, posterior to anterior length, posterior to kinetoplast length, nuclear dimensions and relative positions of nucleus and kinetoplast. However, morphological characters do not clearly provide a mean of distinguishing trypanosome species. In this study, 18S rDNA and internal transcribed spacer (ITS) region were used to elucidate the relationships of *T. evansi* stocks from different hosts and different geographical areas in Thailand. Infected animal blood samples of *T. evansi* were collected and DNA was isolated prior to PCR amplification by using two pairs of specific primers for 18S rDNA and ITS region. The PCR products of 2,100 bp (18S rDNA) and 1,200 bp (ITS) in sizes were obtained. The nucleotide sequences were analyzed and compared to 18S rDNA and the ITS region of the other *Trypanosoma* spp. Maximum likelihood phylogenetic trees were constructed using *Leishmania gerbilli* as the outgroup. The phylogenetic tree of 18S rDNA indicated that *T. evansi* stocks used in this study were closely related to that of Taiwan stock and *T. brucei*. The ITS phylogenetic tree showed some genetic variation among *T. evansi* from different animals. The phylogenetic relationship of 18S rDNA and the ITS region indicated the variation of nucleotide sequences among *T. evansi* stocks in Thailand.

การศึกษาอนุกรมวิธานระดับโมเลกุลของ *TRYPANOSOMA EVANSI* โดยอาศัยข้อมูลลำดับนิวคลีโอไทด์ของ
ยีน 18S rDNA และ INTERNAL TRANSCRIBED SPACERS

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Trypanosoma evansi เป็นปรสิตที่อาศัยอยู่ในกระแสเลือด ทำให้เกิดโรค Trypanosomiasis หรือโรค Surra ในสัตว์ต่างๆ เช่น ม้า วัวเนื้อ วัวนม สุกร กระบือ และ กวาง เป็นต้น อาการของโรคที่เกิดขึ้นมีความรุนแรงแตกต่างกันในสัตว์แต่ละชนิด ซึ่งอาการโดยทั่วไปจะมีไข้ โลหิตจาง และอาจเกิดอาการตี้นขึ้นในสัตว์ที่ได้รับเชื้อดังกล่าว การถ่ายทอดเชื้อและการเกิดโรคสามารถเกิดขึ้นได้ในสัตว์หลายชนิดและการระบาดของโรคยังสามารถเกิดขึ้นได้เป็นบริเวณกว้างโดยพื้นที่ที่มีการระบาดที่สำคัญได้แก่ แอฟริกา เอเชีย และ อเมริกาใต้ ในปัจจุบันการจัดลำดับความสัมพันธ์ทางอนุกรมวิธานของ trypanosome ยังไม่ชัดเจน โดยทั่วไปการจัดอนุกรมวิธานใช้คุณสมบัติทางกายภาพ เช่น ขนาด รูปร่าง ลักษณะของ kinetoplast ตำแหน่งของนิวเคลียส และความสัมพันธ์ของตำแหน่งนิวเคลียส และ kinetoplast อย่างไรก็ตามลักษณะดังกล่าวไม่สามารถที่จะแยกสปีชีส์ของปรสิตได้อย่างชัดเจน ในการทดลองนี้ได้ทำการศึกษายีนบริเวณ 18S rDNA และ internal transcribed spacers (ITS) มาใช้หาความสัมพันธ์ทางวิวัฒนาการของ *T. evansi* สายพันธุ์ต่างๆจากสัตว์แต่ละชนิดที่อยู่ตามพื้นที่ต่างๆในประเทศไทย โดยนำเลือดที่ได้มาสกัด DNA และเพิ่มปริมาณโดยวิธี PCR ซึ่ง primer ที่ใช้มีความจำเพาะต่อยีน 18S rDNA และ ITS ทำให้ได้ PCR product ที่มีขนาด 2,100 bp และ 1,200 bp ตามลำดับ ลำดับนิวคลีโอไทด์ที่ได้ถูกนำมาวิเคราะห์และเปรียบเทียบกับ *Trypanosoma* spp. อื่นๆ โดยใช้โปรแกรม Maximum likelihood โดยใช้ *Leishmania major* เป็น outgroup ผลที่ได้จากการวิเคราะห์ phylogenetic tree ที่ได้จากลำดับนิวคลีโอไทด์ของยีน 18S rDNA พบว่า *T. evansi* ที่พบในวัว กระบือ และกวาง มีความใกล้เคียงกับยีนดังกล่าวที่พบใน *T. evansi* สายพันธุ์ได้หวัน และ *T. brucei* ส่วน phylogenetic tree ที่ได้จากการเทียบลำดับนิวคลีโอไทด์ของยีน ITS แสดงให้เห็นถึงความหลากหลายทางพันธุกรรมบางส่วน of *T. evansi* ที่ติดเชื้อมีสัตว์ชนิดต่างๆ ในการจัดลำดับความสัมพันธ์ทางวิวัฒนาการจากลำดับนิวคลีโอไทด์ของยีน 18S rDNA และยีน ITS แสดงให้เห็นถึงความแปรผันของลำดับนิวคลีโอไทด์ที่เกิดขึ้นภายใน *T. evansi* ที่พบในประเทศไทย

The Thesis Committee and Oral Defense Committee have approved this thesis as partial fulfillment of the requirements of the Master of Science in Molecular Biology of Srinakharinwirot University.

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CHAPTER 1

INTRODUCTION

Trypanosomes and trypanosomiasis have been discovered since the days of Antony van Leeuwenhoek in late of the 17th century. In 1843, Gruby provided the generic name, *Trypanosoma*, to parasites of amphibians. In 1878, Lewis discovered the trypanosome in the blood of rats in India and was later named as *T. lewisi* (1). In 1880, Evans had recognized the parasite that he discovered in the blood of Indian horse and camels suffering from the disease Surra and later named *Trypanosoma evansi* (2). The parasite was pathogenic for domestic animals and this was the first indication that it was capable of causing disease. Evans had described the clinical disease in a number of animals, the gross pathology, transmission experiments and suggested that haematophagous flies transmitted the disease from infected to clean animals. In 1895, Bruce reported that the fly can act readily as a carrier of the Fly Disease from affected to healthy animals (3). The next important discovery was made when Dutton found trypanosomes in the human subject, and named the organism as *T. gambiense* (4). In 1909, Kleine discovered the pathogenic African trypanosomes that undergo a definite cycle of development in the tsetse vector (5).

In 1972, Hoare stated that about 125 different species of trypanosomes had been reported from mammals alone, and that they were distributed among about 400 species of mammalian hosts belonging to 218 genera and 12 orders (6). Trypanosomes were recorded to infect variety of the hosts such as rodents, bats, odd-toed ungulates, even-toed ungulates, primates (including man), carnivores, edentates, marsupials, insectivores, lagomorphs, elephants and monotremes. However, the trypanosomes apparently have not, so far, been found in flying lemurs, pangolins, whales, seals, aardvarks, hyraxes and sirenians.

Morphology of trypanosome

In 1972, Hoare described the morphology of trypanosomes in general, and discussed the appearance of the various organelles of the parasite as revealed by light microscopy of stained preparations and as sections examined under the electron microscopy (6).

Trypanosome identification has been based on the length of the body plus the length of the free flagellum, if present, and on the general morphology of the parasite. The identification demands the investigation of the organism concerning the important points such as the size, shape (round, oval, rod-shaped) and position (terminal, marginal, and subterminal) of the kinetoplast; the point of emergence, direction, convolutions (absence or presence) and length of a free flagellum; the size, shape and location of the nucleus within the body of the trypanosome; the shape of the posterior end of the organism (drawn out, beaked, pointed, obtuse, rounded etc.); the absence or presence of vacuoles and granules and their location, staining reaction and size in the body; the body width (slender, broad); the general appearance (long and slender, short and squat, blade-like, leaf shaped, straight, undulating 'C' shaped etc.).

Electron microscope appearance of trypanosomes

Studies on the morphology of trypanosomes with the aid of the electron microscope (EM) were achieved by Clark and Wallace (7), Rudzinska and Vickerman (8), Vickerman (9-12) and Vickerman and Tetley (13, 14) and others (Figure 1). The following brief description was based largely on the work of these investigators.

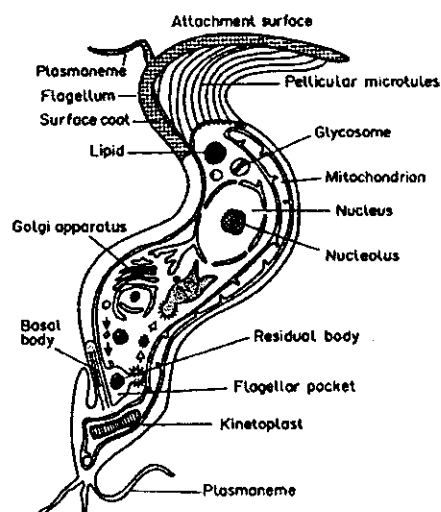


Figure 1 Morphological features of trypanosome revealed by the outline diagram (14).

Plasma membrane

The 'skin' of the trypanosome referred to as the plasma membrane, plasmalemma, or pellicle is a unit membrane about 2 to 4 nm in thickness with maintains the organism's shape. Beneath this membrane, there are cortical or pellicular microtubules sometimes thought of as the cytoskeleton that maintains the elongated shape. Bloodstream forms or forms suitable for development has been shown to be a glycoprotein responsible for variable antigens.

Flagellum and basal body

The flagellum serves to both propel the trypanosome and to provide attachment to other surfaces. It emerges from a flask-like pocket situated at the origin of the flagellum. It begins as a complicated structure called the basal body, or kinetosome, which of the flagellum has a typical arrangement of 9 outer microtubules in doublets and 2 inner separated microtubules. The lining of the flagellar pocket is modified for the intake of nutrient material, and it is capable of pinocytosis. Small pinocytic vesicles become pinched off and enter the cytoplasm where they are digested by lysosomal enzymes. The digestion products and other may be discharged into the flagellar pocket by exocytosis where they are seen as residual bodies which leave the pocket as streamers.

Kinetoplast

The kinetoplast is readily seen in kinetoplastic forms of trypanosomes. It sometime may be inapparent in dyskinetoplastic forms (e.g. so-called *T. equinum*) and is located close to the basal body. A capsule surrounds the kinetoplast that is continuous with the mitochondrial membrane. The kinetoplast looks like a coiled spring and it contains the kinetoplast DNA (k-DNA) that is a slightly concave disc shape. The mitochondrion shows typical cristae on the inner membrane and it is capable of undergoing great changes during the different stages of development of the parasite. The mitochondrion is the organelle of respiratory activity and its function changes depending on whether the parasite is in the fly or in the bloodstream. In the bloodstream forms, the mitochondrion is a simple tube with very few cristae while it is expanded more branched and cristae are abundant in the midgut of the fly as the procyclic trypomastigote. At the metacyclic stage, it again becomes tubular and the cristae are reduced.

Nucleus

The nuclear material is surrounded by the envelope consisted of two membrane layers. The envelope contains nuclear pores that allow the communication between the cytoplasm and the inner contents of the nucleus. Chromatin is seen in clumps attached to the nuclear envelope. The nucleolus is centrally placed.

The outer membrane of the nuclear envelope is continuous with a system of cisternae and tubules (endoplasmic reticulum) that is also associated with the golgi apparatus, subpellicular microtubules and the mitochondrial network. It may be smooth, or granular with ribosomes in the latter case. Ribosomes are concerned with protein synthesis.

Golgi apparatus

The golgi body lies between the nucleus and the flagellar pocket. It appears as a stack of flattened membranes and can produces the surface cote of trypanosomes. It also produces enzymes which are contained in the lysosomes and which are secreted into the flagellar pocket.

Filopodia

Filopodia-like, plasmanemes or micronemata are sometimes seen by light microscopy or in electron microscope photomicrographs. They are fine filaments arising from the surface membrane or flagellum. Their true nature is not well known, but it seems that they are antigenic.

In 1907, Lingard provided the measurement data for *T. evansi* in the blood of cattle (Table 1) (15). He also noted that paroxysms lasted about 5 days and that the parasites increased in length from the early stages of a parasitaemic peak, from 26.8 mm to 30.4 mm and then decreased as the peak diminished.

Table 1 Biomeasural data for *T. evansi* in the blood of cattle (15).

Posterior end to kinetoplast	Kinetoplast to nucleus	Nucleus	Nucleus to Anterior	Free Flagellum	Width	Total Length
2.0 μm (0-3.3)	7.3 μm (5.0-9.0)	2.6 μm (2.0-3.3)	11.6 μm (8.2-16)	4.3 μm	1.6 μm (1.3-1.6)	27.9 μm (19.4-35.3)

In 1956, Hoare carried out biometrical studies on 22 populations of *T. evansi* from 6 species of mammals from 15 locations in the Old World and found that the mean lengths varied considerably varied from 20 mm to 27.4 mm whereas the length distribution curve for 2500 trypanosomes was normal and ranged from 15 mm to 33 mm with mean value at 24 mm (16-18). These data were corresponded to those reported by Bruce (19).

Classification

The classification of trypanosome was introduced by Hoare (6, 20) and that offered by Molyneux and Ashford (21) differ. Hoare and Wallace produced a suggested terminology for the various stages encountered (22). The position of the kinetoplast and point of emergence from the body of the flagellum was used for distinguishing of trypanosome and called 'Mastigote' (from Greek = whip) that was used in combination with appropriate prefixes (6). Five characteristics of mastigotes have been proposed and defined. The first characteristic was 'Amastigote' which formerly referred to as the 'leishmanial' stage. They are rounded or oval bodies. A nucleus and kinetoplast may be distinguished but evidence of a flagellum is seldom observed. The second groups named 'Sphaeromastigote' that is similar to the above but a short flagellum may be distinguishable. The third characteristic represents 'Promastigote' which was formerly referred to as the 'leptomonad' stage. The body is elongated and the kinetoplast is located between the nucleus and the anterior end. The flagellum emerges from the anterior end of the body. The forth set is indicated as 'Epimastigote' to which formerly referred as the 'crithidial' stage. The body is elongated and the nucleus and kinetoplast are in close apposition usually in the posterior portion of the body. The flagellum emerges from the side of the body and runs forward along an undulating membrane. The last characteristic is focused on 'Trypomastigote' that formerly referred to as the 'trypanosome' stage. This is the form seen normally in blood films of infected animals such as the body is elongated, the kinetoplast is situated in the posterior end of the body at some distance from the nucleus, the flagellum emerges from the side of the body and passes forward along the edge of the undulating membrane to end at the anterior tip, or continue as a free flagellum.

In 1964, Hoare recognized that the mammalian species of trypanosomes fell fairly naturally into two sections depending on their development in the vector and in the mammal (23). He suggested the rather descriptive names: Stercoraria and Salivaria for these sections. The section Stercoraria contains genera in which the parasite completes its

developmental cycle in the 'posterior station'. The metacyclic, metatrypanosome, or infective stage for the host mammal, appear in the faeces of the vector and transmission is by the contaminative route. A free flagellum is always present in these trypanosomes. The kinetoplast is large and situated some distance from the posterior end that is generally pointed. Reproduction in the mammal is discontinuous and usually takes place in the amastigote or epimastigote stages. The section Salivaria contains genera whose development cycle in the vector is completed in the 'anterior station' in the salivary medium, and transmission from vector to host occurs by the inoculation of the metacyclic stage.

***Trypanosoma evansi* Balbani 1888 and its epidemiology (24).**

The geographical distribution of surra caused by etiological agent, *T. evansi*, is very extensive and includes vast areas in the New and Old Worlds (Figure 2). The disease ranged from the Western Hemisphere in the section on American Surra to the Eastern Hemisphere in the westernmost parts of Africa until the east of Philippines. In Africa, it occurs in regions around the Sahara Desert, in west, north and east Africa. In the south of the Sahara, it almost merges with the northern limits of the Tse-tse fly areas. In the Arabian Peninsula, surra occurs in Saudi Arabi, Yemen, the Sultanate of Oman and the United Arab Emirates. In the near and far east, it exists in Turkey, Lebanon, Israel, Syria, Jordan, Iraq, Iran, Afghanistan, Pakistan, the central Asian Republics of the U.S.S.R., India, Myanmar, Malaysian Peninsula, Indo-China, southern China, the islands of Indonesia and Philippines including the islands of the Indian Ocean such as Mauritius and Reunion. Throughout much of this area, camels are commonly found and are infected where as they are rare or non-existed in the other regions. The disease tends to be restricted to the other ruminants such as cattle and buffaloes. It is severe in equines wherever it occurs and they probably play a minor part in the maintenance and transmission of the parasite for ruminants. In all areas where surra is a problem, biting flies of the family Tabanidae are prevalent.

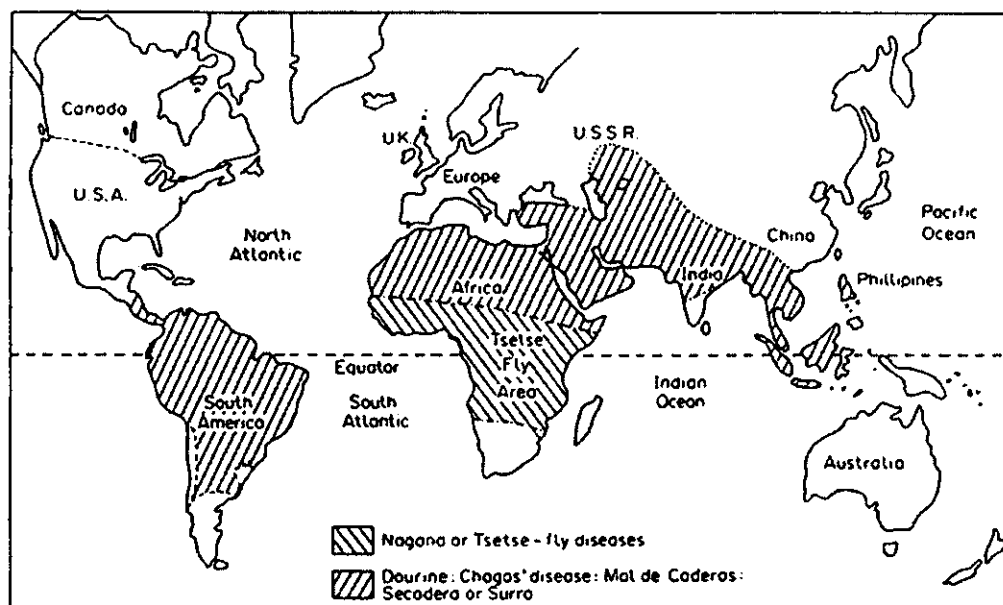


Figure 2 Geographical distribution-animal trypanosomiasis (5).

In Thailand, *T. evansi* are widespread where they cause considerable loss in productivity in domestic animals, such as horses (25), beef cattle (26), dairy cattle (27), buffalo (28-31), and sow (32). Mechanical transmission of these vigorous spindle-shaped protozoa in the blood stream occurs by blood-sucking flies, such as the horse fly (*Tabanus* spp., *Haematopota* spp.), deer fly (*Crysop* spp.) and stable fly (*Stomoxys* spp.). Due to these various vectors, the protozoa spread rapidly and can lead to outbreaks in endemic areas.

A number of factors can influence the number of parasites found in the peripheral blood of the infected animals and these vary from host to host. Animals which recover from an acute disease are often clinically normal with a low number of circulating trypanosomes. Chronic infection can also be characterized by sub-patent or low parasitaemia, and an increase in parasitaemia can take place following stress-induced immunosuppression. Additionally, low parasitaemia can also be due to the localization of parasites in tissues or as a result of the dynamics of antigenic variation.

Life Cycle of *T. evansi*

T. evansi life cycle is similar to the cyclic developmental of *T. brucei* in Tse-tse fly. The cycle of *T. evansi* started from mammals being infected by a bite of insect vector. After sucking the blood, the insect vector spit saliva through the stylum (mouth part which

extended out like a tube) in order to clean it. Then 10^6 cells of metacyclic form were injected into the host skin. In a few days, trypanosomes proliferated and transformed to long slender blood stream form. Size of long slender form is $12-20 \times 1-3$ μ m. It multiplied in mammals with generation time of 6 hours and the antigenic variants would appear with frequency of $1/10^6$ - $1/10^7$ (Figure 3). They have no antigenic determinants in common with their ancestors and thus called 'antigenic variation' so it will be able to escape immune response of host and built up new parasitemia wave (parasitemia = number of parasites per ml of blood). When parasitemia wave declined, the morphology of trypanosomes changed to short stumpy form and reduced growth in mammals but increased in infectivity to insect vector. When the infected blood transferred from multiplied in flies mid-gut epimastigotes stage. This stage multiplied extensively in order to terminate maturity metacyclics. Metacyclics are infective to mammals.

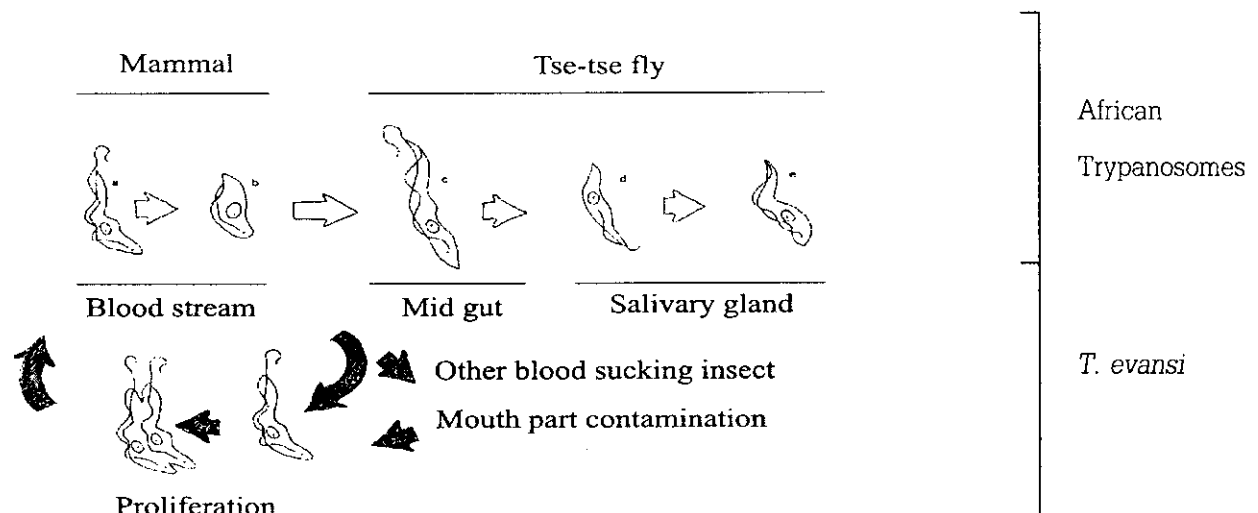


Figure 3 The diagram of stages in life cycle of African Trypanosomes and the diagram of *T. evansi* in blood stream and in blood sucking insect other than Tse-tse fly, without developing the cycle in insect vector. The pathway of *T. evansi* life cycle indicated in black arrow separately from the main pathway (33).

In *T. brucei*, genetic exchange has been showed to occur during passage of mixture of trypanosomes through Tse-tse fly. Apart from this cyclic transmission, *T. brucei*'s mechanical transmission can occur through bite of insects and thus they constitute an animals reservoirs for it. On the other hand, *T. evansi* has no evidence of the cyclical development in insect vector. It transmits mechanically through them. The most important *T. evansi* vectors are the flies of *Tabanus spp.* and the number of blood sucking insects of *Stomoxys spp.*, *Haematopota spp.*, *Chrysops spp.* and *Lyperosia spp.* That is way in the monsoon season, there are increasing incidences of 'Surra' associated with the number of insects.

Host range of *T. evansi*

Among the domestic animals, *T. evansi* cause the disease surra in horses, donkeys, mules, the Indian elephant, cattle, buffaloes, sheep, goats, dogs and cats (Table 3). Among them, equines and horses are most severely affected followed by mules and donkeys. The disease may be serious in working Indian elephants (*Elephas maximus*). Cattle and buffaloes are frequently susceptible with natural infections, but the course of the disease is often undetected. In contrast, there are reports of heavy mortality of these animals in certain areas, particularly in the Far East and Southeast Asian. Sheep and goats are seldom reports to be naturally infected although they can be infected readily by inoculation of infected blood. Dogs are highly susceptible and usually die after a brief period of illness. There are very few reports of surra in cats, although they are highly susceptible to experimental infections. The disease in the camel is usually chronic and the mortality is extremely high. Some acute cases are also reported, but they are quite rare (Table 3).

Most of the commonly employed laboratory animals are susceptible to infection with *T. evansi*, but the infection is usually more acute in the small rodents, than in rabbits and guinea-pigs. There are numbers of animals have been reported as both naturally or experimentally susceptible to infection with *T. evansi* that illustrates the extremely broad host range of the surra parasite.

Table 2 Animals that have been reported as both naturally or experimentally susceptible to infection with *T. evansi* (6).

Natural Infections	Experimental Infections
New world	
deer <i>Odocoileus virginianus</i>)	agouti (Sometimes resistant)
capybara (<i>Hydrochoerus hydrochoerus</i>)	armadillo, bats (various)
wild dogs (<i>Canis azarae</i>)	coati, deer (<i>Mazama</i> sp.)
ocelot (<i>Felis pardalis</i>)	monkeys (various)
vampire bats (<i>Desmodus rotundus</i>)	mongoose (<i>Herpestes palustris</i>)
howler monkeys (<i>Alouatta</i> sp)	opossum
Old world	porcupine
antelope (<i>Saiga tatarica</i>)	collared peccary
deer (<i>Muntiacus</i> , <i>Axis</i> , <i>Cervus</i> , <i>Capreolus</i> sp)	sloth
wild sheep (<i>Ovis ammon</i>)	wild rats
tapir (<i>Tapirus indicus</i>)	wolves
foxes (<i>Vulpes</i> sp)	

Transmission of surra

There is little dispute today that surra is normally transmitted from infected animals to clean animals by mechanical transmission involving bloodsucking Diptera, particularly members of the Tabanidae (Figure 4.1 and 4.2). In 1880, Evans that a tabanid was involved in the transmission of surra in India (2). In 1899, Lingard demonstrated that the 'body fluids' of certain flies, after sucking the blood of infected animals, contained trypanosomes in varying numbers causing the infection in healthy animals (34).

There are numerous reports in the literature of attempts to transmit *T. evansi* by various bloodsucking arthropods (35-38). The haematophagous *Diptera*, most frequently mentioned as mechanical vector of surra, are the Tabanidae and stable flies, *Stomoxys* species. It should be noted that both male and female *Stomoxys* are blood-suckers while only the female tabanids take a blood meal and also that the life cycles of these two groups of flies are quite different (39). In 1909, Leese had reported that haematophagous arthropods are the potential vectors of surra under natural conditions (40). This group contains mosquitoes (*Anopheles*, *Culex*, and *Stegomyia*), sand-flies (*Phlebotomus* spp.), lice,

ticks (*Hyalomma aegyptium*, *Rhipicephalus* spp. and *Haemaphysalis bispinosa*), *Tabanus* spp., *Haematopota* spp., *Chrysops*, *Stomoxys*, *Lyperosia* and *Hippobosca maculata*. In addition, the vampire bat was also reported as it was capable of transmitting *T. evansi* from infected to clean animals (41, 42).

In the case of mechanical transmission by biting flies, the trypanosome is passed from infected to clean host by infected blood in, or on, the mouth parts as the fly moves from one animal to another between interrupted blood meals. The parasite survives for only a short period of time on the contaminated proboscis, and probably undergoes limited multiplication.

Tabanus spp. fed on the horse, ox, buffalo, camels, elephant and man (43). In the horse and camel, alarm is shown at its approach and the animal does its best to dislodge it. This causes the fly to go readily from one animal to another in a short space of time. It bites anywhere on the body but favors the belly, legs and sheath. Blood exudes from the seat of puncture. Its favorite times of biting are 5 to 8:30 AM and 4 to 6 PM. It generally rests in the middle of the day during the great heat.

Haematopota bites the horse, ox, buffalo, camel, elephant, and also man (40, 44-47). Their approach to animal causes no alarm, but when it bites, attempts are made by the victim to dislodge it. Camels, which have only a short tail and a poorly developed panniculus muscle, suffer severely and attempt to defend themselves by kicking, biting and rolling on the acquire a full blood meal, and so may attack several animals. Their favorite times of feeding coincide with those of *Tabanus*, but may also bite after dark. The smaller species may appear in swarms during the rainy season.

Stomoxys bites the horse, ox, buffalo, camel, dogs and man (2, 48-50). It may bite at any time of the day, but is particularly active from 4:30 PM to dusk. It bites on any part of the body, but particularly on the legs below the carpus and hock. It is not located particularly near water since it breeds in manure. Although it will enter a stable to obtain a blood meal, it prefers to feed in the open. The movement of horses' tails, feet, head and skin disturbs their feeding and when the animals are in groups there is movement of flies from one animal to another.

The vampire bat is a nocturnal feeder and gently rasps the skin of its victim with its razor-sharp incisors until blood oozes from the wound. Anticoagulant in the bat's saliva prevent the blood from clotting, and the bat laps up the blood with its tongue. The digestive tract of the vampire bat is adapted to the rapid digestion of blood which is its

only source of sustenance and appears to allow the ready passage of trypanosomes both into the circulation of the bat which becomes infected and develops a parasitaemia, and the movement of the parasites from the bat's blood into the saliva at a subsequent feed. The parasite thus multiplies in the bat host, but does not undergo a cycle of development as it would in a true intermediate host. Bats may succumb to the infection, to survive and recover.

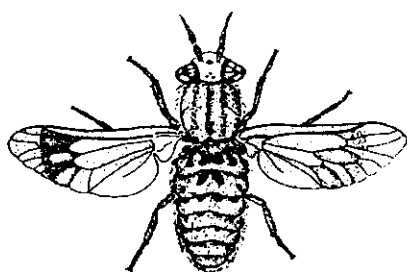


Figure 4.1 Horse Fly - *Tabanus atratus* (51)

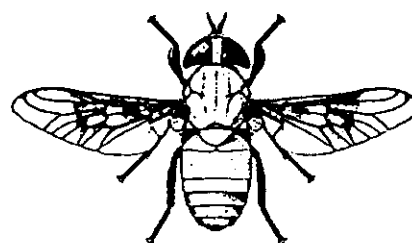


Figure 4.2 Deer Fly - *Chrysops discalis* (52)

Clinical manifestation of the trypanosomiasis in domestic animals

There are many specific trypanosomes since each species of trypanosome may infect several species of animals with the different effects and degrees of severity (Table 2). The clinical signs vary in different mammalian species such as trypanosomiasis in the domestic horse is caused by *T. evansi*. The disease is characterized by massive parasitaemia and lead to death within a period of 24-72 hours. The same trypanosome in the sheep causes a mild disease from which the animal may recover spontaneously.

The disease may be acute, or sometimes more chronic in horses. Generally, the onset of fever and parasitaemia are found. Respiration rate increases with pyrexia, the animal often takes 7 or 8 short, rapid breaths, followed by a sonorous one. Breathing is more abdominal than thoracic. Evidence of jaundice appears early and remains relative constant. Although lymphadenopathy is general, the superficial lymph nodes in the horse are not easily palpable, with exception of the submaxillary nodes which may be swollen and tender. Oedema is evident as plaques on the ventral surface of the body over the sternum swelling of the sheath (prepuce) in horses and in the under of the mare and a hydrocoele in the

stallions were observed. The legs are swollen particularly between the carpus and hock and the fetlock and the swelling pits on pressure being applied. The blanched appearance of all mucous membranes provides clear evidence of the severe anaemia. Death often comes suddenly and quietly, or there may be some signs of delirium, but generally the animal is too weak to rise in the terminal stages (5).

Table 3 Susceptibility of animals to Salivarian Trypanosomes (5).

Animal	<i>T. vivax</i>	<i>T. congolense</i>	<i>T. simiae</i>	<i>T. brucei</i>	<i>T. evansi</i>	<i>T. suis</i>
Horse	* or ***	*	R	***	***	--
Donkey	* or ***	*	R	**	*	--
Camel	* or ***	*	* or ***	***	***	--
Zebu	***	***	R	**	**	R
West African dwarf cattle	**	**	R	**	--	--
Sheep	**	*	**	*	*	R
Goat	***	*	***	*	*	R
Pig	R	*	*****	*	***	***
Dog	R	*short/**long	R	***	**	--
Rabbit	R or *	*	R or *	***	**	--
Monkey	R	R or **	R or ****	****	--	--
Chimpanzee	--	***	--	***	--	--
Rat	R	R or ***	R	*** or ****	***	--
Mice	R	R or ***	R	*** or ****	***	--

***** = virulent ***** = peracute *** = acute ** = chronic

* = mild -- = not well know R = Refractory

The pathogenicity of strains (demes) within a species is another variable character that may affect the clinical signs. Such differences in biological characters, in morphologically identical trypanosomes, have sometimes resulted in their being accorded independent species status.

One of the most important factors that must be considered is the relative susceptibility of different breeds of animals. This is particularly evident in cattle where great variation in the susceptibility of breed is observed. For example, the Zebu cattle of West Africa are much more seriously affected by trypanosomiasis than the dwarf breeds such as the N'dama and Muturu. In East Africa, pure-bred and grade animals react to trypanosome infections quite differently from indigenous cattle.

The clinical manifestations of trypanosomiasis in animals may be greatly influenced by a number of other factors such as the state of nutrition, the occurrence of intercurrent bacterial, viral and helminthic infections and stress due to vaccination regimens, trekking, thirst and temperature changes. The numbers of metacyclic trypanosomes introduced into the animal body in any unit of time, is also an important factor in the character of the resultant infection.

According to the clinical manifestation trypanosomiasis may be described as it occurs in groups of related animals. The disease in horses, donkeys and mules may be described as equine trypanosomiasis. Similarly, sheep and goats form another natural class. Cats and dogs may also be grouped but as cats are seldom naturally infected, and in any case have no commercial value. Some authors group animals as above and then give consecutive descriptions of the disease caused by each trypanosome species.

Pathology and Pathogenesis

In 1880, Evans observed the leukocytosis in *T. evansi* infected animals since there was a remarkable increase in the number of the large granular white corpuscles (2). In 1893, Lingard provided a number of observations on *post-mortem* changes seen in animals dying of surra (15). The observation of heart revealed that myocardium was somewhat abnormal being pale and flabby, and in a state of fatty degeneration. Small ecchymoses were seen under the pericardium, sometimes seen as extravasations. Similar haemorrhages were observed in the endocardium. Fat was replaced by gelatinous exudations (2, 53, 54). There was little change in lungs as well as kidney, bladder, pancreas were fairly normal. The enlargement was observed in lymph nodes, liver and spleen. Oedema in the brain and spinal cord were observed (24). The gross pathology is very similar to that seen in animals dying of *T. brucei* infections. Later, in 1967, Nadim and Soliman reported decreases in erythrocyte counts, haemoglobin concentrations, lymphocyte and basophile counts (55). They noticed the increase in neutrophile, eosinophile and monocyte percentages in natural infections in camels. In 1971, Jatkar and

Purohit had infected 20 camels with *T. evansi* and compared blood values with 20 normal animals (56). They found that packed cell volumes, red cell counts and haemoglobin values dropped in the infected animals but there were increase in the total white cell count, sedimentation rate and reticulocyte counts. They concluded that the anaemia was macrocytic and was due to the destruction of erythrocytes, and not to depression of bone marrow function. Macrocytes, normoblasts and occasional erythroblasts were observed in blood films.

Diagnosis

Since many animals harboring *T. evansi* may have parasites in their peripheral blood only sporadically and in relatively small numbers, a single or even several, examinations may fail to reveal the parasite in thick, thin or wet blood films. Nevertheless, thick and thin blood films are always made. Alternatively, centrifugation techniques are useful for concentrating trypanosomes in the buffy coat using phase-contrast or dark-field illumination (57) as well as the mini-anion exchange chromatography technique (58) is also utilized.

Basically, the inoculation of small laboratory rodents with blood from animals suspected of being infected with surra is an extremely sensitive method for revealing trypanosomes. Godfrey and Killick-Kendrick found trypanosomes in twice as many camels by this technique as they did by blood film examination (59), and Pegram and Scott concluded that it was the best method of detecting surra in camels in Ethiopia (60). A valid objective to this method is the relatively long delay between obtaining blood from the animal being tested and final diagnosis.

In 1978, Luckin and colleagues introduced the application of serological tests for the diagnosis of infections with *T. evansi* (61, 62). The principal of serological tests for trypanosomiasis was the detection of antibody that was produced in response to antigens emanating from pathogenic organisms in the animal's body. However, such antibodies in the serum might indicate that the animal was exposed to a parasite that failed to establish itself or that the animal is currently infected or that the infection has occurred in the recent past but may now be cured. They also showed that antibodies might persist in detectable quantities for up to 100 days, or more, after successful treatment of *T. evansi* infections. In 1960, Weitz pointed out that the cell-free serum of trypanosome infected animals was a source of antigens, which he referred to as exo-antigens (63, 64). In 1984, Rae and Luckins

had altered the micro ELISA test to detect antigens instead of antibody. The test therefore indicates active infection in the animal (65).

Recently, a number of sensitive and specific techniques have been applied in the investigations of *T. evansi* epidemiology such as DNA hybridization (66) and PCR-based methods (67). The development of a reliable and sensitive method for parasite diagnosis and characterization is necessary for proper understanding of the epidemiology of *T. evansi*. Such understanding is as important requirement in designing rational trypanosomiasis control programs.

CHAPTER 2

LITERATURE REVIEW

Trypanosoma spp. consists of a large group of obligated flagellate blood parasites that infect members of every vertebrate class (6). Members of this genus are almost always vectored by blood-sucking invertebrates, primarily arthropods for species infecting mammals and birds and primarily hirudinean annelids for species infecting poikilothermic vertebrates. However, little is known about the life cycles and development of the numerous trypanosome species infecting the latter hosts.

Traditionally, all trypanosome species descriptions have been based on morphological description of bloodstream trypomastigotes and morphometric parameters consisting of total body length, including free flagellum, posterior to anterior length, posterior to kinetoplast length, nuclear dimensions and relative positions of nucleus and kinetoplast. Unfortunately, such descriptions for most species are often confounded by extreme morphological variability, the occurrence by extreme morphological variability, the occurrence of morphologically indistinguishable species in diverse host worldwide, the lack of complete life-cycle descriptions and host-specificity data, or the likelihood that a single species could contain stages resembling those of two or more other regarding the taxonomy of trypanosomes and much confusion in the literature (68-72).

Speciation of polymorphic trypanosomes

The African trypanosomes that cause so-called 'Sleeping Sickness' in the man are *T. gambiense*, the protozoal agent of the Gambian or chronic form of the disease in eastern Africa. A third species of the subgenus *Trypanozoon*, *T. brucei*, is found extensively in domestic and wild animals in whom it causes the disease Nagana. These three trypanosomes are virtually morphologically indistinguishable. Their cycles of development in the tsetse fly, *Glossina*, are identical. Their basic biochemical and immunogenic properties overlap to a large degree as well as their responses to chemotherapeutic agents are very similar. They differ in certain biological properties such as host range, host-parasite relation, susceptibility of development in vector, geographical distribution and certain ecological features. Man is refractory to infection by *T. brucei*

while all three can infect and cause parasitaemia in a wide range of domestic and wild animals.

The Blood Incubation Infectivity Test (BIIT) is performed to differentiate between the polymorphic trypanosomes that are probably infective for man, and those which are not (73). Isoenzyme studies have been used as the indicators for differences that exist between strains of *congolense* organisms, but these have not been related to the biological differences. Such relationships can only be made on organisms isolated from naturally infected normal host animals.

Isoenzyme analysis has widely been used to group morphologically similar or identical forms in a variety of parasitic protozoa, especially among the trypanosomes (74-77). In Africa and the area nearby, it is highly probable that *surra* spread very widely through the camel that act as the infection reservoir and tabanids playing the role of vector. It is difficult to assess the value of attempts to characterize different *T. evansi* stocks. In 1978, Gibson and co-workers (78, 79) compared ten isolates of *T. evansi* based on isoenzymes, soluble proteins, polypeptides and free amino acids. There were no differences between the isoenzymes of the different isolates. However, they reported differences within the species in isoelectric points, in polypeptide sizes and in the free amino acid contents.

Results from isoenzyme analysis of *T. evansi* strains isolated from Asia, West Africa and South America indicate that the majority of *T. evansi* strains worldwide have a single origin (74, 75, 80). Results from biochemical and molecular studies on *T. evansi* support the idea that *T. evansi* may belong to the same species. Twelve strains of *T. evansi* from China were investigated for 16 enzymes using cellulose and thin-layer starch gel electrophoresis. Differences between strains were observed in only two of the 16 enzymes, i.e. in malate dehydrogenase (MDH) and alanine aminotransferase (ALAT). These results indicate that *T. evansi* from China form a homogeneous group with *T. evansi* strains from other parts of the world (76). More recently, analysis of small or large subunit ribosomal RNA sequences (81-83), Restriction Fragment Length Polymorphisms (RFLPs) in DNA (84-89), ribosomal DNA restriction (90), the polymorphisms of PCR amplification products (91, 92) and molecular karyotypes (76, 93, 94) have been successfully used in distinguishing morphologically similar protozoa.

With the exception of a few studies (71, 95-102), the taxonomic relationships among the trypanosomes are poorly known. Morphology often does not provide a clear means of distinguishing trypanosome species. Furthermore, with respect to the characterization of

trypanosomes by host specificity and by ultrastructural, biochemical and biological means as previously mentioned, it is clear that the genetic diversity of trypanosomes is not well reflected in their gross structure. The current system of trypanosome taxonomy does not properly address the species diversity of properly reflect the phylogenetic relationship among these parasites.

Molecular techniques for phylogenetic studies

A number of genes with fundamental biochemical functions are found in all species and they were sequenced, aligned and analysed to study phlogenetic relationships among morphologically indistinguishable (103). This entire of applications, from the origin of life to relatively recent evolutionary events, has been addressed by studying the ribosomal RNA (rRNA) genes and their associated spacer regions, collectively calles ribosomal DNA (104, 105). Some properties of rDNA are so sufficiently distinct from other molecular sequences that a number of special considerations are necessary or desirable when considering the use of rDNA in systematic studies.

Several distinct rRNAs combine with ribosomal proteins to form ribosomes, the organelles that direct protein synthesis from messenger RNA. Ribosomes are composed of two major subunits, each with distinct rRNAs and ribosomal proteins. The small ribosomal subunit contains a single type of rRNA and about 20 proteins in prokaryotes or 30 proteins in eukaryotes. The large ribosomal subunit contains two (prokaryotes) or three (eukaryotes) rRNAs and about 30 (prokaryotes) to 40 (eukaryotes) ribosomal proteins. A single copy of each of the proteins is present per ribosome. Because protein synthesis is a prerequisite for life, ribosomes (and hence rRNAs) are universally present in living systems.

The rDNA array of a eukaryotes nuclear genome typically consists of several hundred tendemly repeated copies of the transcription unit and nontranscribed spacer (Figure 5) (106). The number of copies of this transcription unit, however, may be as few as one (107), or as many as several thousand (108). In prokaryotes, there are one to several copies of the rRNA genes and they may be either organized in a single operon (in which they are usually separated by tRNA genes) or they may be dispersed throughout the genome (109-111). There are usually three or four distinct nonorganellar rRNAs in a species (106, 112) which are often characterized in sedimentation velocity units "S" (Svedburg). Firstly, a large subunit rRNA, which ranges in size from 16S (approximately 1,500 nucleotides) in most prokaryote genomes, and up to 28S (over 4,000 nt) in eukaryote nuclear genomes (113, 114). Secondly, a 5.8S rRNA (~160 nt) in

eukaryotes, which is derived from a part of the 23S rRNA of prokaryotes (115-118) and is still structurally and functionally closely related to the large rRNA. Thirdly, a small subunit rRNA, which ranges from 12S (~900 nt) in vertebrate mitochondria to 16S (~1500 nt) in prokaryotes and up to 18S (~1800 nt) in eukaryotes (119-121). Lastly, a 5S rRNA (~120 nt), the gene of which is closely associated with the other rRNA genes in many prokaryotes but is found elsewhere in the nuclear genome of most eukaryotes (122). Although these are the most common themes, numerous variations have been discovered (123, 124). The large and small subunit rRNA genes of chloroplasts and mitochondria are more similar to those of their eukaryotes hosts (125-131).

In eukaryote, two internal transcribed spacers (ITS-1 and ITS-2) separate the 18S, 5.8S and 28S gene (or their homologs) and an external transcribed spacer (ETS) is located upstream of the 18S gene (Figure 5). The transcribed spacers contain signals for processing the rRNA transcript. Adjacent copies of the rDNA repeat unit are separated by a nontranscribed spacers (NTS), also called an intergenic spacers by some workers. This region contains subrepeating elements that serve as enhancers of transcription (132-136).

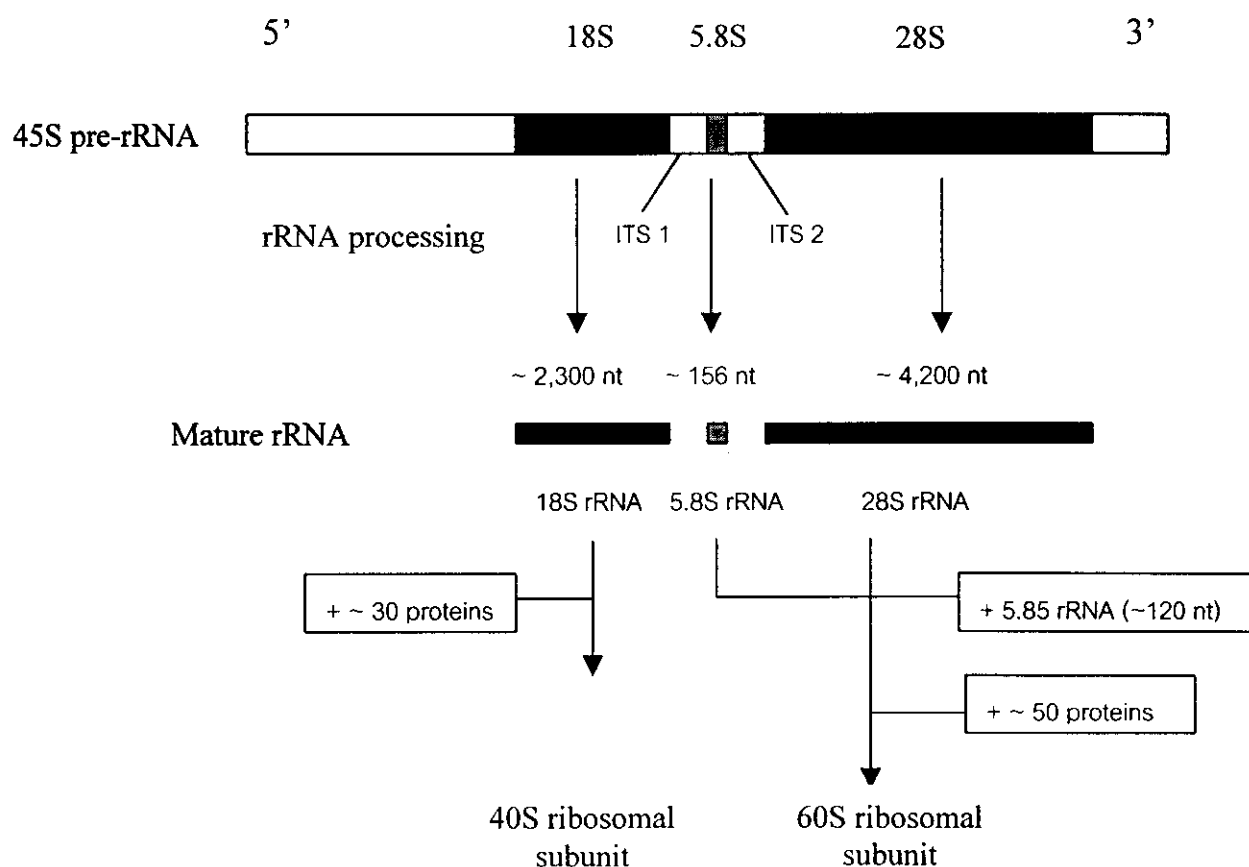


Figure 5 Structure of nuclear ribosomal DNA in eukaryote.

Structure and evolution of rRNA: Implication for phylogenetic analysis.

One of the reasons why rDNA is useful for phylogenetic analysis is that different regions of the rDNA repeat unit evolve at very different rates. Thus, regions of rDNA arrays that are particularly likely to yield informative data for almost any systematic question can be selected for analysis. In addition, the islands of highly conserved sequences within most rRNA genes are very useful for constructing 'universal' primer that can be used for sequencing either rRNA or rDNA from many species, for amplifying regions of interest by use of the PCR, or for use as probes in restriction enzyme analyses (103, 137, 138). Although intraindividual length heterogeneity causes problems for direct sequencing of amplified DNA in some regions of the repeat, the amplified DNA can be cloned and sequenced relatively easily.

The process of choosing a region that is likely to be appropriate for a particular systematic question is perhaps the most critical step in any phylogenetic analysis. If the region chosen is evolutionarily too conserved (i.e., the sequences are nearly the same in all study taxa), then considerable time will be wasted collection invariant data. On the other hand, regions that differ among taxa to the extent that alignments are difficult or questionable also are unlikely to yield robust phylogenies (139). There are two reasons that highly divergent sequences yield less robust results. Firstly, the level of homoplasy (parallelisms, convergences and reversals) increases as the probability for change at each position increases and secondly, the number of possible alignment that are nearly equally good becomes prohibitively large. Since alignment (positional) homologs is an assumption of the phylogenetic analysis, it is best to delete from analyses any regions where the alignment is questionable (139).

The most studied rRNA is the small subunit nuclear gene, 16S-18S rRNA. This gene has been studied most extensively because it is among the slowest evolving sequence found throughout living organisms, and has therefore been very useful for examining ancient evolutionary events. In addition, the slow rate of change permits the construction of many nearly universal primers, which facilitates sequencing efforts from groups that have not been studied previously. Much of the gene is useful for comparisons among phyla of eukaryotes, however, and some comparisons among eukaryotes and prokaryotes are possible. It has been most successfully for reconstruction phylogenetic events from the Precambrian.

The large subunit (23S-28S) nuclear rRNA gene is larger and shows more variation in rates of evolution of its different domains than does the small subunit. Although ancient comparisons are also possible with this gene (140-142), it primarily has been used to examine evolutionary events though the Paleozoic and Mesozoic (143-148). The large subunit rRNA

gene has many divergent domains or expansion segments (149). So, the size of the gene varies considerably among phyla (113). These divergent domains are useful for reconstructing relatively recent divergences of the taxa.

The 5.8S rRNA gene of eukaryotes (and the corresponding region of the large subunit gene of prokaryotes) is similar to the small subunit gene in its useful phylogenetic range, although the shortness of the sequence limits its effectiveness in inferring phylogeny across great time scales. It shows very little variation in comparisons of taxa that diverged after the Paleozoic (150). The 5S gene shows similar levels of variation, but it is even shorter than the 5.8S sequence (151). The shortness of the sequence greatly restricts its phylogenetic usefulness (152, 153).

The mitochondrial rRNA genes evolve much more rapidly than the nuclear rRNA genes, and they can be used for most Cenozoic comparisons. They are easily amplified through use of the PCR (137, 138). The spacer regions of rDNA arrays have been used less frequently for phylogenetic studies (154-156), except in restriction analyses of entire rDNA arrays (105, 157-165). These studies have shown that the spacer regions can be used to infer phylogeny among closely related taxa (taxa that have diverged within the last 50 million years). In addition, variation in the spacer regions has been used to identify species or strains, to study hybridization, and as markers in population genetic studies (148, 162, 166-171). Among the spacers, the NTS evolves most rapidly (172), and the transcribed spacers are somewhat more conserved (173-175). Amplification of the two internal transcribed spacers via the PCR is facilitated by conserved flanking regions of the 18S, 5.8S and 28S genes, so the use of these spacers in studies involving closely related species is increasing.

Since the first broad molecular study of eukaryote evolution (176), phylogenetic analysis of kinetoplastid flagellates has become successively more focused on studies concerning the origins and evolution of parasitism and evolution of RNA editing (177-183). The initial tree of Trypanosomatidae was constructed using kinetoplast ribosomal 9S and 12S rRNA sequences (177, 178). This tree was rooted at the lineage of a monogenetic (single host) insect parasite *Crihidia*, since no outgroup data was available at that time. On that tree, the digenetic (two hosts) parasites *Trypanosoma* and *Leishmania* represented the derived species. An evolutionary scenario deduced from this topology was compatible with the previously proposed notion that monogeneity in insects is a primitive state and digeneity in insects and mammals is a derived state (184) (Figure 6 and 7).

Dating the phylogenetic trees

Phylogenetic trees for trypanosomes are of interest for what they can reveal about the evolution of parasitism and other characteristics, such as antigenic variation or sexual reproduction, in the group. Interpretation of the tree in relation to other events on the evolutionary time-scale depends on conversion of branch points into dates to estimate time of divergence of different clades. The molecular clock approach (185) assumes that changes in a given sequence accumulate at a constant rate, and thus that the difference between two sequences is a measure of the time of divergence. From a post-genomics standpoint these notions look almost quaint and, indeed, the approach has been amply discussed and criticized over the years (186-188). Nevertheless, within given taxonomic groups and defined categories of genetic marker, the concept of a molecular clock can provide a useful tool for dating divergence. For example, using an estimate of 0.85% substitutions per 100 million years (my) derived from rRNA analysis of Apicomplexa (189), the divergence of Salivarian trypanosomes from other trypanosomes was dated at about 300 million years before present (mybp) (190).

A second way in which times of divergence can be estimated relies on congruence of host and parasite phylogenies. Thus, parasite trees can be calibrated by reference to known time points within host phylogenies, which have been independently dated from the fossil record. This assumes that existing associations between hosts and parasites reflect past associations.

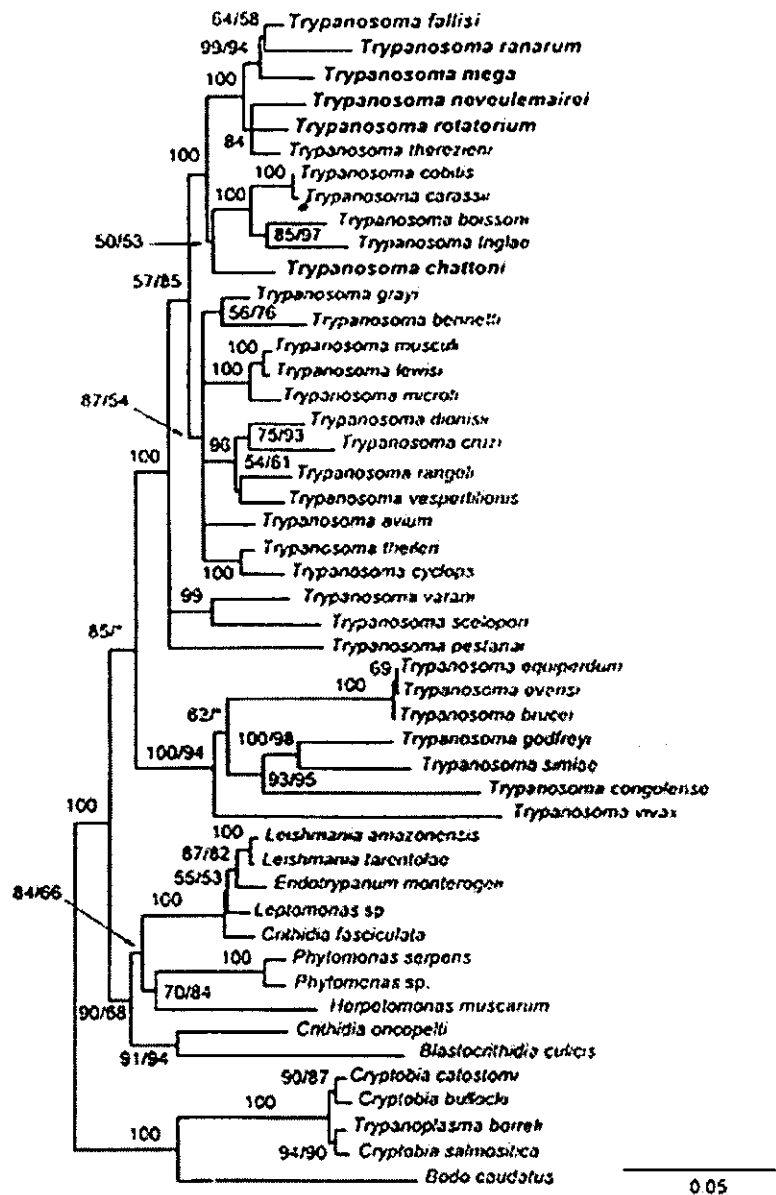


Figure 6 Phylogenetic relationships of the kinetoplastids derived from 18S rRNA evolutionary distances produced by the Kimura 2-parameter correction model (191) and constructed using the least-sequence (LS) and neighbor-joining (NJ) methods. The consensus trees for 1,000 bootstrap resamplings of 18S rRNA gene sequences are included as a percentage at the nodes. However, when NJ bootstrap support value is identical to the LS bootstrap value, only 1 value is show. Bootstrap values less than 50% are indicated by an asterisk (*). Evolutionary distance is represented by the horizontal component separating species in the figure. The scale bar corresponds to 5 changes per 100 positions (192).

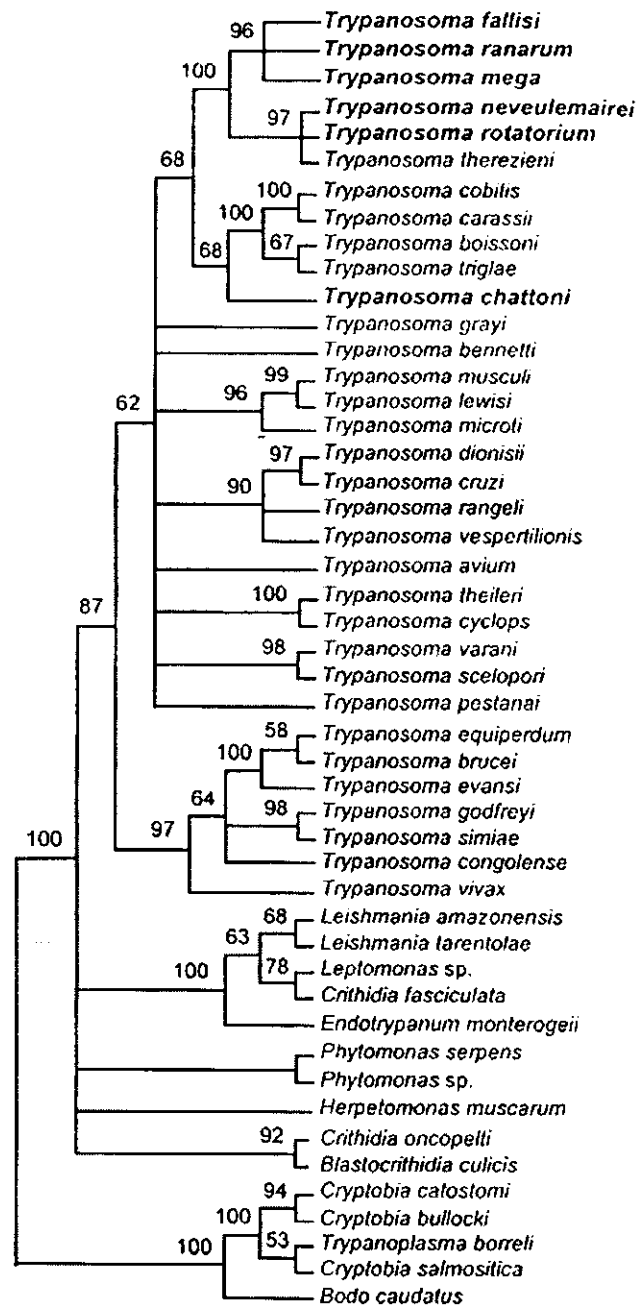


Figure 7 A maximum-parsimony tree of the kinetoplastids produced by the heuristic search of PAUP and infected from complete SSrRNA gene sequences using a bootstrap resampling of the data set. The numbers at the nodes represent the percentage of times the group occurred out of 1,000 trees. Branches with less than 50% bootstrap support were collapsed. No significance is placed on the lengths of the branches connecting the species. The consistency and retention indices were 0.53 and 0.66, respectively (192).

Objectives

1. To determine variations of *T. evansi* isolates from different geographical and different host species.
2. To study evolutionary relationship among *T. evansi* stocks isolates in Thailand.

CHAPTER 3

MATERIALS AND METHODS

Materials

Chemicals and reagents used in this study were listed below;

Chemicals and reagents

Brands

Absolute Ethanol	Merck
Agarose	Seakem
Bacto agar	GibcoBRL
Bacto TM peptone	BDH
Boric acid	Merck
Deep-frozen tube	Nunc
DE 52 DEAE cellulose	Whatman
Deoxynucleotide triphosphates (dNTPs)	Gibco BRL
100 bp DNA Ladder	Gibco BRL
EDTA.Na ₂ .2H ₂ O Ultrapure	BSU
Glucose	BDH
Glycerol	Sigma
Heparin	Leo
Hydrochloric acid (HCl)	Merck
Isoamyl alcohol	Carlo erba
Kanamycin	Sigma
Lambda DNA - <i>HindIII</i> Marker	Promega
Magnesium chloride (MgCl ₂)	Gibco BRL
10x PCR Buffer	Gibco BRL
Phenol	Ultrapure
Phenol/Chloroform/Isoamyl alcohol. pH 7.8	Research organics
Phosphoric acid (H ₃ PO ₄)	Merck
Proteinase K	BRL
SDS (Sodium dodecyl sulfate)	Sigma

Sodium chloride (NaCl)	Merck
Sodium di hydrogen phosphate ((.2H ₂ O) NaH ₂ PO ₄ .2H ₂ O)	Univar
Sodium hydrogen phosphate (Na ₂ HPO ₄ .H ₂ O)	Merck
<i>Thermus aquaticus</i> (Taq) DNA polymerase	Gibco BRL
TOPO [®] XL PCR Cloning Kit	Invitrogen
Tris (Tris(hydroxymethy))	Serva
Tris-acetate EDTA buffer	Sigma
Yeast extract	Gibco BRL

Methods

Parasite collection

Infected animal blood samples of abundant *T. evansi* were collected from the endemic area of Thailand. The samples were collected from deers at Samut Prakan, buffalos at Lad Kra Bang district of Bangkok, and cattles at Khai Bang Rachan district of Sing Buri (Table 4). They were previously screened for *T. evansi* using the traditional blood smear technique before undergoing the parasite infected blood. They were preserved in the Phosphate Saline Glucose buffer (PSG) pH 8.0 (50 mM $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 2 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 36 mM NaCl and 1.5% Glucose) and stored them in liquid nitrogen tank (-196°C).

Table 4. *Trypanosoma evansi* isolates

Name	Location	Host	Source
B15	Lad Kra Bang, Bangkok	buffalos	DNA
B17	Lad Kra Bang, Bangkok	buffalos	DNA
B18	Lad Kra Bang, Bangkok	buffalos	DNA
SAM	Samut Prakan	deer	DNA
KAI	Khai Bang Rachan, Sing Buri	cattles	DNA

Parasite infected blood preservation and isolation

T. evansi from mouse infected blood was obtained from freshed cardiac punctured. If samples came in the form of frozen samples, they should be passaged in mouse before isolation.

Preservation of the infected blood samples

The blood samples were preserved in the PSG buffer pH 8.0 and were kept at -80°C or in liquid nitrogen tank (-196°C). Glycerol anhydrous was added to PSG buffer to make 20% glycerol (v/v). The fresh blood with at least parasitemia of 10^8 cells/ml was mixed at ratio 1:1 with 20% glycerol PSG buffer. Heparin was used as anticoagulant. The mixture was mixed and made to aliquots of 400 μl to 1,000 μl . A deep-frozen tube was used for preservation.

Passage of parasite in mice

Blood samples were thawed at room temperature and then passaged to mice by using 1 ml tuberculin syringe and needle gauge No. 27. Blood smear was examined every day starting from the third day after injection to monitor the parasitemia.

The rapid matching method

At the highest parasitemia (10^8 cells/ml) which is usually in the 3rd-5th day of injection, blood was withdrawn by heart puncture from euthernized mice. Three mice were used for each sample to obtain at least 3 ml of total infected blood further for isolation of parasites.

Parasite isolation

An anion exchange column (DE 52 DEAE cellulose) was used for parasite isolation. The blood components were absorbed onto the DEAE cellulose, and the parasites with lower negatively charge than the blood components were eluted.

Preparation of the DEAE cellulose column

Twenty five gram of preswollen DE 52 was suspended in 200 ml of PSG buffer pH 8.0. The pH of the mixture was lowered to pH 8 with 1.4 ml of 2.5 N HCl. The pH of the column was very critical. It should be exactly 8 not more or less than 0.05. After the exchanger was settled, fine parasites was removed, the supernatant fluid was discarded and replaced with new PSG. The gel suspension was allowed to settle and pH of the supernatant was checked. The equilibrated DE 52 could be kept at 4°C for 24 hours or in -20°C and thawed if required. Plastic syringe was used as column. The flow rate of elution was controlled by screw clip on tubing fitted to the syringe outlet. The column was packed in the conventional manner. Sample of 3 ml blood was diluted to ratio 1:1 in cold PSG buffer and 1-3 volume was loaded onto the packed DE 52 column.

Parasite isolation

The blood was applied on top of the column, then the outlet was opened. When all the blood entered the absorbent, applied the buffer through the column. When parasites passed through, the buffer turned turbid. The outlet elution was collected until parasite fraction had all come out. The better equilibrated column, the sharper the red cell layer. The parasites were precipitated by centrifugation at 5,000 rpm for 10 minutes.

Extraction of high molecular weight of DNA

Parasite DNA was then extracted by conventional phenol/chloroform which responded for deproteinization of the aqueous solution containing the desired nucleic acid. Pellet of parasites was resuspended in 800 μ l of PSG. Then added 200 μ l of 10% SDS and DNA was released from the cells. Parasite lysate solution was clear and moved viscous. One milligram of Proteinase K was added (final concentration was 1 mg/ml) and the solution was incubated at 42°C for 15-18 hours.

For phenol extraction, it was easier to divide DNA solution into four microcentrifuge tubes of 250 μ l. TE buffer was added equal volume to each tube and 500 μ l phenol/chloroform was added to the solution. Centrifuged to separated phases. The upper aqueous phase was transferred to new tubes. The solution was then precipitated with 2 volumes of absolute ethanol and kept at -80°C for 1 hour. The solution was centrifuged at 10,000 rpm for 15 minutes. DNA pellet was collected, washed with 70% ethanol, and redissolved in TE buffer. The DNA concentration was determined at 260 nm using UV Spectrophotometer. Molar extinction of DNA was performed as O.D. 260 nm 20 = DNA concentration of 1 mg/ml.

Primer for PCR amplification

Two sets of primers were used for amplification of the regions encoding for cytoplasmic 18S rDNA (Primer A, forward 5' AAC CTG GTT GAT CCT GCC AGT 3' and Primer B, reverse 5' TGA TCC TTC TGC AGG TTC ACC TAC 3') and ITS region including 5.8S rDNA (Primer ITS/F, forward 5' GGT GAT CGG ACC GTC GCT CGT CT 3' and Primer ITS/R, reverse 5' CCT CTT CGC TCG CCG CTG ACT G 3'). The primers were designed based on the nucleotide sequence comparison of *T. evansi* for 18S rDNA, 5.8S rDNA and 28S rDNA (accession number D89527) in GenBank database. The primers for PCR amplification were synthesized from BSU (Bioservice Unit, Thailand). The PCR products of 2,100 bp and 1,200 bp in sizes were expected from PCR amplification of 18S rDNA and ITS region, respectively.

PCR amplification

PCR amplifications of purified DNA of blood samples were performed using specific primer for each regions. All reactions were manipulated in 25 μ l volume containing 100-200 ng of genomic DNA samples. The 18S rDNA reaction contained 1X PCR buffer, 1.7 mM of each primer, 200 mM of dNTP, 1.5 mM MgCl₂ and 3 units of *Taq* DNA

polymerase. Sterile distilled water was used to make volume to 25 μ l. The PCR amplification was performed using Peltier Thermal Cycler (MJ Research, PTC-200) for 30 cycles. Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute and extension at 72°C for 1 minute. The PCR amplification of ITS region contained 1X PCR buffer, 2 mM of each primer, 400 mM of dNTP, 6 mM MgCl₂ and 1.5 units of *Taq* DNA polymerase. Sterile distilled water was used to make volume to 25 μ l. A PCR cycle was performed using Peltier Thermal Cycler (MJ Research, PTC-200) for 30 cycles. Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 1 minute. PCR products were analyzed using electrophoresis in 1.2% agarose gel at 110 volts approximately 35 minutes prior to staining by ethidium bromide and the gel was observed under ultraviolet light.

Agarose gel electrophoresis

PCR products were electrophoresed in 1.2% agarose gel with a volume 50 ml in 1xTAE buffer (50 mM Tris-acetate, pH 8.0 and 1 mM EDTA). After heating to dissolve completely, the gel was mixed with 50 ml of the 2 mg/ml Crystal Violet solution and was poured onto the gel maker set and a comb of a desired thickness was placed at the top of the it. After the gel became solid, removed the comb and transferred the agarose gel to the chamber gel electrophoresis. DNA samples were mixed in a ratio of 4:1 with 6x crystal violet loading buffer. The gel was run in 1xTAE buffer at a constant voltage of 80 volts at room temperature for 40 minutes or until the crystal violet in the gel was run a quarter of the way up the gel. The PCR product was visualized the thin blue band when the gel was running.

PCR product purification

The PCR fragments were eluted from the gels and were purified by using TOPO[®] XL PCR cloning kit. The procedure of purification was performed according to the instruction manual provided by the company. The DNA fragments were excised from the agarose gel with a clean, sharp scalp. Gel products were bladed with razor on a UV light box into small chunks and transferred to a microcentrifuge tube. The gel slices were weighted in a colorless tube and added 2.5 volumes of 6.6 M sodium iodide to 1 volume and mixed gels. The gel slices were incubated at 42°C until the gel was melted completely. The mixture solution was added 1.5 volume of binding buffer and placed into a S.N.A.P.[™] purification column. The DNA was bind with the column, centrifuged at 3,000 g

for 30 seconds (repeat 3 times), discarded flow-through and placed column back in the same collection tube. After that, the DNA pellet was added with 0.4 ml of 1x Final Wash to S.N.A.P.[™] column and centrifuged at 3,000 g for 30 seconds (repeat 2 times). The DNA pellet was recentrifuged at 10,000 g for 1 minute and column was placed onto a clean 1.5 ml microcentrifuge tube. The DNA pellet was eluted with 50 μ l of TE buffer and centrifuged the column for 1 minute at maximum speed. The products were analyzed using electrophoresis in 1.2% agarose gel at 110 volts approximately 35 minutes prior to staining by ethidium bromide and the gel was observed under ultraviolet light.

Cloning and Transformation

Ligation of PCR fragment and vector

The purified 200 ng of PCR fragment was further ligated to the PCR[®]-XL-TOPO[®] vector using TOPO[®] XL PCR cloning kit. The reaction mixture consisted of 4 μ l of PCR product and 1 μ l of PCR[®]-XL-TOPO[®] vector. The reaction was mixed gently and incubated for 3 minutes at room temperature (25°C). The reaction was involved of 1 μ l of 6x TOPO[®] cloning stop solution for several seconds at room temperature. Then, the reaction was placed on ice and transformed into *E.coli* (One Shot[®] TOP10 Chemically Competent cells).

Transformation

Transformation was manipulated according to the protocols provided by the company. Two microliters of the ligation product was added into a vial containing competent cell, mixed gently and incubated on ice for 30 minutes. The mixture was heat-shocked for 30 seconds at 42°C without shaking and chilled on ice for 2 minutes. Then, the mixture was added with 250 μ l of room temperature SOC medium (2% tryptone, 0.5% yeast extract, 10 mM MgSO₄ and 20 mM glucose) and agitated at 200 rpm at 37°C for 1 hr. A portion of 50-150 μ l from each transformation was spreaded on a pre-warmed LB agar plate (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl and 15 g/l agar) containing 50 mg/ml kanamycin and incubated overnight at 37°C.

Analysis of positive clones

The PCR analyzed positive transformations was used to each PCR primer specific region at same condition to PCR amplification. The colonies were selected for colony PCR amplification. Multiple number of colonies were analyzed. The reaction was incubated for 10 minutes at 94°C to until the cells were lysed and nucleases were

inactivate. Colony PCR amplification was performed as previously described. For the extension, the reaction was incubated at 72°C for 10 minutes and stored at 4°C. The PCR products of positive clones were analyzed by loading 10 µl volume of each reaction into 1.2% agarose gel and electrophoreses at 110 volts for approximately 35 minutes. The gel was stained in ethidium bromide solution and observed under ultraviolet light. Positive clones were picked and transferred to a new LB-agar plate containing 50 mg/ml kanamycin and kept as the stock before proceeding to DNA sequencing.

DNA sequencing

DNA sequencing was achieved by using Big Dye Terminator Cycle Sequencing procedure and analyzed by using ABI PRISM 377 (Perkin Elmer). The 300 ng of sample was used for PCR amplification before proceeding to DNA sequencing using 25 cycles of denaturation at 96°C for 10 secs, annealing at 50°C for 4 secs and extension at 60°C for 4 minutes. The sample was dissolved with dye and formamide solution. The nucleotide sequence data was analyzed by using software of ABI PRISM Model 377 version 3.4.

Detection of the nucleotide sequence data

The nucleotide sequence data was detected by input the data into BLAST program from <http://www.ncbi.nlm.nih.gov/BLAST>. The nucleotide sequences of interested gene fragments were compared with nucleotide sequences of various organisms that have been previously submitted in Gene Bank. The percentage of nucleotide sequence homology in comparison to various organisms was reported. All sequence data were analyzed with Clustal W sequence alignment program for The European Molecular Biology Laboratory (EMBL, U.K.) at <http://www.ebi.ac.uk/>. All alignment data were inspected with sequence data in GeneBank by PAUP program version 4.0 for explained evolutionary relationship of *T. evansi*.

CHAPTER 4

RESULTS

T. evansi genomic DNA extraction

The genomic DNA of *T. evansi* was extracted from the blood of infected mouse (described in Method 3). Agarose gel electrophoresis was used to determine genomic DNA before PCR amplification. A single band of a high molecular weight of more than 23 kb was observed after ethidium bromide staining (Figure 8).

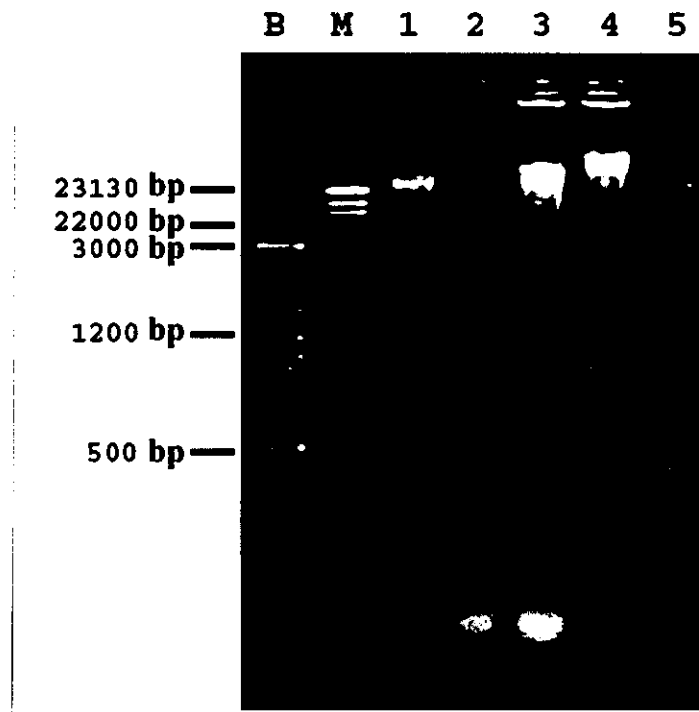


Figure 8 Agarose gel electrophoresis of *T. evansi* genomic DNA.

Lane B = 100 bp marker Lane M = λ Hind III marker

Lane 1 = *T. evansi* genomic DNA from buffalo, B15

Lane 2 = *T. evansi* genomic DNA from buffalo, B17

Lane 3 = *T. evansi* genomic DNA from buffalo, B18

Lane 4 = *T. evansi* genomic DNA from deer, SAM

Lane 5 = *T. evansi* genomic DNA from cattle, KAl

PCR amplification of *T. evansi*

The PCR amplification was performed and analyzed according to the Method 5. Figure 9 showed that the PCR product of 2,100 bp in size was observed when primer A and B were used for amplification of 18S rDNA from B18, SAM and KAI. Similarly, Figure 10 showed the 1,200 bp PCR products of ITS region when primer ITS/F and ITS/R were used.

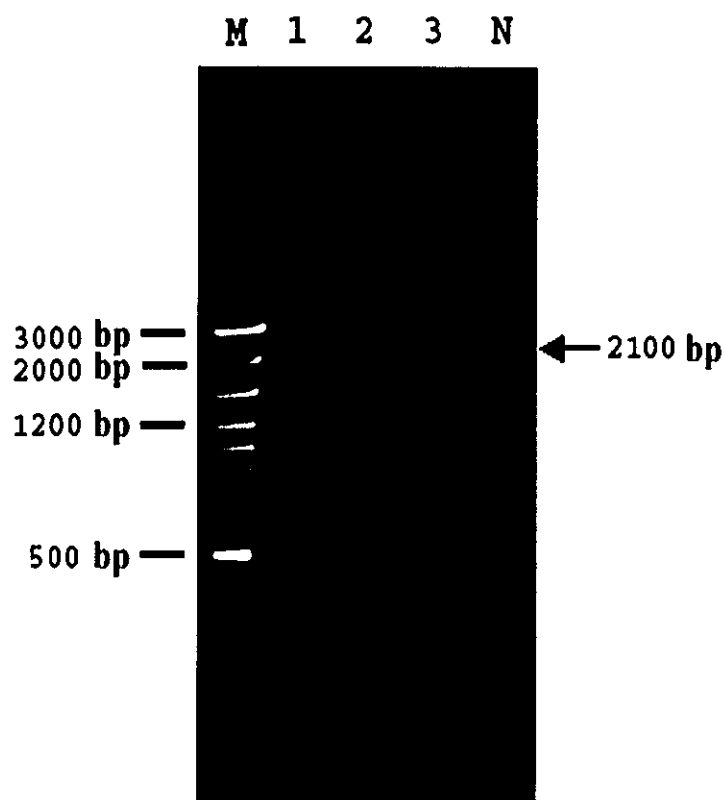


Figure 9 The PCR amplification profile of 18S rDNA from purified *T. evansi* DNA stocks from buffalo (B18), deer (SAM), and cattle (KAI) using primer A and B. The PCR product of 2,100 bp in size was observed on 1.2% agarose gel electrophoresis after ethidium bromide staining.

Lane M = 100 bp marker

Lane N = Negative control

Lane 1 = PCR product of B18

Lane 2 = PCR product of SAM

Lane 3 = PCR product of KAI

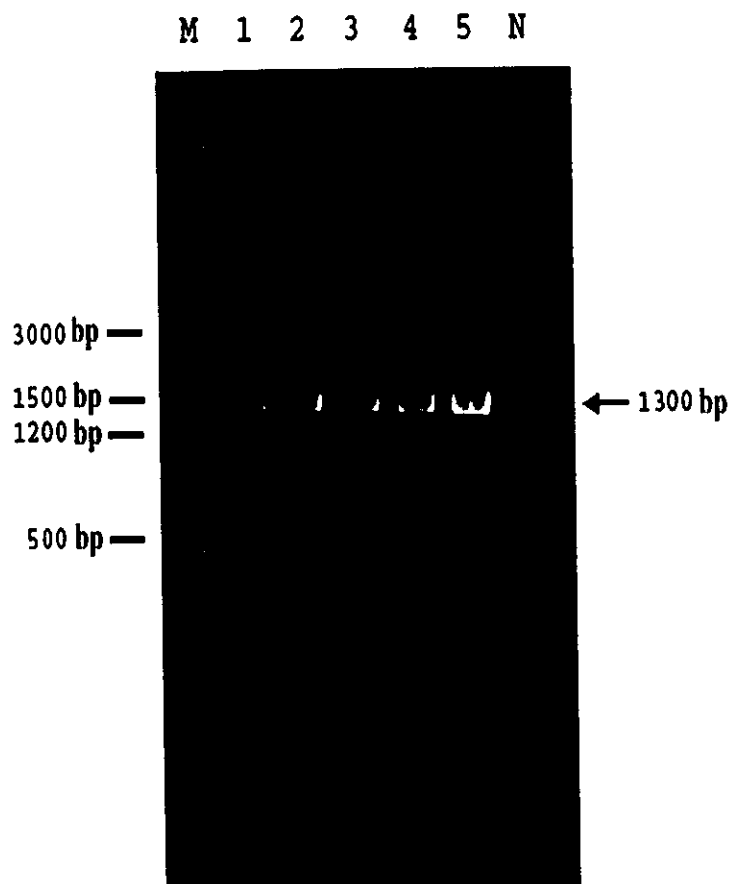


Figure 10 The PCR amplification profile of ITS region from purified *T. evansi* DNA stocks from buffalo (B15, B17 and B18), deer (SAM), and cattle (KAI) using primer ITS/F and ITS/R. The PCR product of 1,300 bp in size was observed on 1.2% agarose gel electrophoresis after ethidium bromide staining.

Lane M = 100 bp marker

Lane N = Negative control

1 = PCR product of B15

2 = PCR product of B17

3 = PCR product of B18

4 = PCR product of SAM

5 = PCR product of KAI

DNA sequencing of 18S rDNA and ITS region from *T. evansi* stocks.

PCR products of 18S rDNA and ITS region was used for cloning and DNA sequencing (described in Method 9). Among 18S rDNA clones, one colony was selected randomly and two colonies of ITS recombinant DNA were chosen for further DNA sequencing. The nucleotide sequences data of 18S rDNA and ITS were shown in Table 5 and 6, respectively.

Table 5 Nucleotide sequence data of 18S rDNA of *T. evansi* stocks from different hosts.

B18 (2188 nt)	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTAGAACTACTGCATTGCAGGAATCTGCCGATGGCTCAT TACATCAGACGTAATCTGCCGCCAAAATTTGCGGTCTCCGCATTACTGGATAACTTGGCGAAACGCCAAGCTAA TACATGAACCAATCGGACGCTCTCTTTTCTATGTCGCGGCTTGTGTTTACGCACTTGTGCTGGCGATGGGACGT CCAGCGAATGAATGAAATTAGAACCAACGCCTCCACCCGGGGGAGTAACACTCAGACGTGTTGACTCAATTCAT TCCGTGCGAAAGCCGAGCCTTGTGCTCGGCGTCTACTGACGAACAACGCCCCTATCAGCCAGTGATGGCCGTGT AGTGGACTGCCATGGCGTTGACGGGAGCGGGGATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTAC CACTTCTACGGAGGGCAGCAGGCGCGCAAATTGCCCAATGTCGAAAAAATACGATGAGGCAGCGAAAAGAAATAG AGCCGACCGTGCCCTAGTGCATGGGTGTTTCAATGGGGGATACTCAAACCCATCCAATATTCGAGTAACAATTG GAGGACAAGTCTGGTGCCAGCACCCGCGGTAATCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTAAAGGG TTCGTAATTGAAGTGTGGGCCACGTAGTTTGTGCGGTGCCAGTCCCGTCCCGCTCCGACGTGTTTTTGACCCAC GCCCTCGTGGCCCGTGAACACACTCAGATACAAAAAACCGGAGCGGGTCCTCTCACTTTCACGCATGTCATGCA TGCGAGGGGGCGTCCGTGAATTTTACTGTGACCAAAAAAGTGGCACCAGCAGTCTGCCGACTTGAGTTACAAA GCATGGGATAACGAAGCATCAGCCCTGGGGCCACCGTTTCGGCTTTTGTGGTTTGAAGTCCTTGGGAGATTA TGGGGCCGCGTGCCCTGGGTGCGTGTTCGTGTCTCATTTTGTGGCGCGCACATTCGGCTCTTCGTGATGTTTT TTTACATTCATTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCCGGGGGAGAACGTACTGGTGCGTCAGA GGTGAAATTCCTAGACCGCACCAAGACGAACACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGAAC CAAAGTGTGGGGATCAAAGATGATTAGAGACCATTTGTAGTCCACACTGCAAACCATGACACCCATGAATTGGGGGA ACATCCATTGGGTGCCGTGTGGCGGCCCTTTGTGCCGACCCTCGGCCCAATTTATTTATCAATTTACGTGCCT ATTCTATCACCCCGGTTCCCTCTTTTGAGGTTCTTCCGGGGTTTTTACGGGAATATCCTCAGCACGTTCTTA CTTCTTCACGCGAAAGCTTGGAGTTACAGTCTCAGGGGGGAGTACGTTTCGAAGAGTGAACTTAAAGAAATTG ACGGAATGGCACCACAAGACGTGGAGCGTGCGGTTAATTTGACTCAACACGGGGAACTTTACCAGATCCCGGAC AGGGTGAGGATTGACAGATGGAGTGTCTTTCTCGATCCCCTGAATGGTGGTGATGGCCGCTTTTGGTGGTGG AGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCAGTAGGTGCGGGGATTGTCCACACAGG ACAGCAGTCCCTCCGGCGGGGATTTTTTCCCCAACGGTGGTCTGTCATCCTTCTTTTACAGGCCCTTCTCTGCG GGATTCCTTGCTTTTCGCGCAAGGTGAGATTTGGGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGA CACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCACTTCACAATGTGCGTCCCGCTTGATCAAAAGA GCGGGGAAACACGGAATCAGTAGACCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGT CTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCGTCGTTGTTCCGA TGATGGTGCAATACAGGTGATCGGACCGTCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA GGAAGCAAAAGTC
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Table 5 (continued)

SAM (2191 nt)	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATTGCAGGAATCTGCGCATGGCTCA TTACATCAGACGTAATCTGCCGCCAAAATTTTGGCGTCTCCGCATTACTGGATAACTTGGCGAAACGCCAAGCTA ATACATGAACCAATCGGACGCTCTCTTTTCTATGTGCGGGCTTGTGTTTACGCACTTGTCTGGCGATGGGACG TCCAGCGAATGAATGAAATTAGAACCAACGCCTCCACCGGGGGCAGTAACACTCAGACGTGTTGACTCAATTCA TTCCGTGCGAAAGCCGAGCCTTGTGTTCCGGCGTCTACTGACGAACAACCTGCCCTATCAGCCAGTGATGGCCGTG TAGTGGACTGCCATGGCGTTGACGGGAGCGGGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTA CCACTTCTACGGAGGGCAGCAGCGCGCAAATTGCCCAATGTCGAAAAAATACGATGAGGCAGCGAAAAGAAATA GAGCCGACCGTGGCCCTAGTGCATGTTGTTTCAATGGGGGATACTCAAACCCATCCAATATCGAGTAACAATT GGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAGCGTATATTAATGCTGTTGCTGTTAAAG GGTTCGTAATTGAACGTGTGGGCCACGTAATTTGTGCCGTGCCAGTCCCGTCCCGCTCGGACGTGTTTTGACCCA CGCCCTCGTGGCCCGTGAACACCCCTCAGATACAAGAAACACGGGAGCGGGTCCCTCCTCACTTTCACGCATGGTCA TGCATGCGAGGGGGCGTCCGTGAATTTTACTGTGACCAAAAAAGTGCAGCAAAAGCAGTCTGCCGACTTGAATTA CAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCCACCGTTTCGGCTTTTGTGTTTGTAGAGTCTTGGGAG ATTATGGGGCCGCTGCCTTGGGTCCGTGTTTCGTGCTCATTTTGTGGCGCGACATTCCGGCTCTTCGTGATG TTTTTTACATTTCATTGCGACGCGCGCTTCCAGGAATGAAGGAGGTAGTTCGGGGGAGAACGTACTGGTCCGT CAGAGGTGAATTTCTAGACCGCACCAAGACGAACACAGCGAAGGCATTCTTCAAGGATACCTTCCCTCAATCAA GAACCAAAGTGTGGGGATCAAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACCATGACACCCATGGAATT GGGGAACATCATTGGGTGCCCGTGTGGCGGCCTTTTGTGCCGACCTCGGCCCAATTTATTTATCAATTTACGT GCCATTCTATCACCCCGGTTCCCTCTTTGAGGTTCTTCCGGGGTTTTTACGGGAATATCCTCAGCACGTTT CTTACTTCTTACGCGAAAGCTTGGAGGTTACAGTCTCAGGGGTTAGTACGTTTCGCAAGAGTGAACCTTAAGAA ATTGACCGAATGGCACCACAAGAGCTGGAGCGTCCGTTAATTTGACTCAACACGGGGAACCTTACCAGATCCG GACAGGGTGAGGATTGACAGATGGAGTGTCTTTCTCGATCCCTGAATGGTGGTGCATGGCCGCTTTTGGTCCG TGGAGTGATTTGTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCAGTAGGTGCCGGGATTGTCCACAC AGGACAGCAGTCCCTCCGGCGGGGATTTTCCCAACGGTGGTCTCATCCTTCTTTTACAGGCCCTTCTCT GCGGGATTCTTGTCTTTTCGCGCAAGGTGAGATTTGGGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGG CGACACGCGCACTACAATGTCAAGTGAAGAACAGAGTCCGAGCGGCACTTCACAATGTGCTCCGCTTGTATCAA AGAGCGGGGAACCAACGGAATCACGTAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCCGCGCAACGAGGAA TGCTTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTGTACACACCGCCCGTCTGTTTTC CGATGATGGTGCAATACAGGTGATCGGACCGTCCGTCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAAT AGAGGAAGCAAAAGTC
KAI (2187 nt)	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTAGAATCACTGCATTGCAGGAATCTGCGCATGGCTCAT TACATCAGACGTAATCTGCCGCCAAAATTTTGGCGTCTCCGCATTACTGGATAACTTGGCGAAACGCCAAGCTAA TACATGAACCAATCGGACGCTCTCTTTTCTATGTGCGGGCTTGTGTTTACGCACTTGTCTGGCGATGGGACGT CCAGCGAATGAATGAAATTAGAACCAACGCCTCCACCGGGGGCAGTAACACTCAGACGTGTTGACTCAATTCA TCCGTGCGAAAGCCGAGCCTTGTGCTCGGCGTCTACTGACGAACAACCTGCCCTATCAGCCAGTGATGGCCGTG AGTGGACTGCCATGGCGTTGACGGGAGCGGGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTAC CACTTCTACGGAGGGCAGCAGCGCGCAAATTGCCCAATGTCGAAAAAATACGATGAGGCAGCGAAAAGAAATAG AGCCGACCGTGCCCTAGTGATGGGTTGTTTCAATGGGGGATACTCAAACCCATCCAATATCGAGTAACAATTGG AGGACAAGTCTGGTGCCAGCACCCGCGTAATTCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTAAAGGGT TCGTAATTGAACGTGTGGGCCACGTAGTTTGTGCCGTGCCAGTCCCGTCCACCTCGGACGTGTTTGACCCACGC CTTCGTGGCCCGTGAACACACTCAGATACAAGAAACACGGGAGCGGGTCCCTCACTTTACGCAATGTCATGCAT GCGAGGGGGCGTCCGTGAATTTTACTGTGACCAAAAAAGTGCAGCAAAAGCAGTCTGCCGACTTGAATTACAAAG CATGGGATAACGAAGCATCAGCCCTGGGGCCACCGTTTCGGCTTTTGTGTTTGTAGAGTCTTGGGAGATTAT GGGGCCGCGTGCCCTTGGGTCCGTGTTTTCGTGCTCATTTTGTGGCGCGCACATTCCGCTCTTCGTGATGTTT TTACATTTCATTGCGACGCGCGCTTCCAGGAATGAAGGAGGTAGTTCGGGGGAGAACGTACTGGTGCCTCAGAG GTGAAATTTCTAGACCGCACCAAGACGAACACAGCGAAGGCATTCTTCAAGGATACCTTCCCTCAATCAAGAAC AAAGTGTGGGATCAAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACCATGACACCCATGAATTGGGGA ACATCATTGGGTGCCCGTGTGGCGGCCTTTTGTGCCGACCTCGGCCCAATTTATTTATCAATTAACGTGCCA TTCTATCACCCCGGTTCCCTCTTTTGTAGGTTCTTCCGGGGTTTTTACGGGAATATCCTCAGCACGTTTTTAT TTTTTACGCGAAAGCTTGGAGGTTACAGTCTCAGGGGGAAGTACGTTCGCAAGAGTGAACTTAAGAAATTGA CGGAATGGCACCACAAGAGCTGGAGCGTCCGTTAATTTGACTCAACACGGGGAACCTTACCAGATCCGAGCA GGGTGAGGATTGACAGATGGAGTGTCTTCTCGATCCCTGAATGGTGGTGCATGGCCGCTTTTGGTCCGTGGA GTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCAGTAGGTGCCGGGATTGTCCACACAGGA CAGCAGTCCCTCCGGCGGGGATTTTCCCAACGGTGGTCTCATCTTCTTTTACAGGCCCTTCTCTGCGG GATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGAC ACGCGCACTACAATGTCAAGTGAAGAACAGAGTCCGAGCGGCACTTCACAATGTGCTCCGCTTGTATCAAAAGAG CGGGGAACCAACGGAATCACGTAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCCGCGCAACGAGGAATGTC TCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTGTACACACCGCCCGTCTGTTTCCGAT GATGGTGCAATACAGGTGATCGGACCGTCCGTCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAG GAAGCAAAAGTC

Table 6 Nucleotide sequence data of ITS region of *T. evansi* stocks from different hosts.

B15.1 (1317 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTGGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTAAACATAGTTCTGTTATTTTCTATCATGTATGTGTGTTAGAGTCTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCTCC CACAACTGTGCGCGATGGATGACTTGGCTTCCATTTTCGTTGAAGAACGCAGCAAAAGTGCATAAGTGGTATCAA TTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCGTG CATGCCACATTTTCTCAGTGTGCAATATAAAACAAAACACACCTATTTTGTGTTGTTCAACGCACGCAAAAAA TCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGGTTTGTGAGTGTGCACATATACGATATCTTTCAACTC TTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTATGGAAGAGAAATGGGAAAGG CATATGTATATATGTATATACATAAATATATATGTGTGTGGATTTGTGTGTTGAGCACATATAAGGAAAAGGGT TGCGTGCATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATATGTATATGTGTGTGTTCCGC TGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCCGTATAG AGATTAAGAAAGAGAGAAAAGTATGCAAAAGAGGCGCGGATAGTGTGTATGTGTGATTACAGCAAGCAAC TATATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTGTGCTTCTTTCGTGTACGCTTCACTTT TTTATATTGTATTTTCAGACCTGAGTGTGGCAGGACCACCGCTAAACTTAAGCATATTACTCAGCGGAGGAAAA GAAACAACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG
B15.2 (1317 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATACATAGTTCTGTTATTTTCTATCATGTATGTGTGTTAGAGTCTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCT CCCACAACGTGTCGCGATGGATGACTTGGCTTCCATTTTCGTTGAAGAACGCAGCAAAAGTGCATAAGTGGTATC AATTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCG TGCATGCCACATTTTCTCAGTGTGCAATATCAAAACAAAACGCACATCTATTTTGTGTTGTTCAACGCACGCAAAA AATCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGGTTTGTGAGTGTGCACATATACGATATCTTTCAA CTCTTTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTATGGAAGAGAAATGGGAA AGGCATATATATATATGTATATACATAATATATATGTGTGTGGATTTGTGTGTTGAGCACATATAAGGAAAAGG TTGCGTGTATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATGTATATGTGTGTGTTCCGCT GTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCCGTATAGA GATTAAGAAAGAGAGAAAAGTATGCAAAAGAGGCGCGGATAGTGTGCATGTGTGATTACAGCAAGCAACT ATATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTGTGCTTCTTTCGTGTACGCTTCACTTTT TTATATTGTATTTTCAGACCTGAGTGTGGCAGGACCACCGCTAAACTTAAGCATATTACTCAGCGGAGGAAAA GAAACAACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG
B17.1 (1315 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATACATAGTTCTGTTATTTTCTATCATGTATGTGTGTTAGAGTCTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCT CCCACAACGTGTCGCGATGGATGACTTGGCTTCCATTTTCGTTGAAGAACGCAGCAAAAGTGCATAAGTGGTATC AATTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCG TGCATGCCACATTTTCTCAGTGTGCAATATCAAAACAAAACGCACATCTATTTTGTGTTGTTCAACGCACGCAAAA AATCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGGTTTGTGAGTGTGCACATATACGATACCTTTCAACT CTTTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTATGGAAGAGAAATGGGAAAG GCATATATATATATGTATATACATAATATATATGTGTGTGGATTTGTGTGTTGAGCACATATAAGGAAAAGG GCGTGTATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATGTATATGTGTGTGTTCCGCTGT GGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCCGTATAGAGA TTAAAAGAGAGAGAAAAGTATGCAAAAGAGGCGCGGATAGTGTGCATGTGTGATTACAGGCAAGCAACTAT ATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTGTGCTTCTTTCGTGTACGCTTCACTTTT ATATTGTATTTTCAGACCTGAGTGTGGCAGGACCACCGCTAAACTTAAGCATATTACTCAGCGGAGGAAAA AACAACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG

Table 6 (continued)

B17.2 (1324 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAGTCGT AACAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACGAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATACATAGTTCTGTTATTTCTATCATGTATGTGTGTTAGAGTGTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCT CCCACAACGTGTGCGGATGGATGACTTGGCTTCCCTATTTCGTTGAAGAACGCAGCAAAGTGGGATAAGTGGTATC AATTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCG TGCATGCCACATTTCTCAGTGTGCAATATCAAAACAAAACGCACATCTATTTTGTGTTGTTCAACGCACGCAAAA AATCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGTTTGTGAGTGTGCACATATACGATATCTTTCAACT CTTTCTACTCGCACAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGAAAG GCATATATATATATGTATATACATAATATATGTGTGTGGATTGTTGTGTTGAGCACATATAAGGAAAAAGGT GCGTGTATATACAGAGAGTCTGTGGCGGTTGAGACGTGTATGTATATATATCTATAATATATGTATATGTGTGTG TTCCGCTGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCC GTATAGAGATTTAAAAAGGAAGAGAAAAAGTATGCAAAAGAGGCGGCGGATAGTGTGCATGTGTGTATTTCACAGCA AGCAACTATATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTTGTGCTTCTTCGTACGCTT CACTTTTTTATATTGTATTTTTCAGACCTGAGTGTGGCAGGACCACCCGCTAAACTTAAGCATATTACTCAGCGG AGGAAAAAGAAAACAACCGTGATTCTCTCAGTCAGCGCGGAGCGAAGAGG
B18.1 (1326 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAGTCGT AACAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGTATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCATT TGACATGGGAGATGAGGGATGTTATATATAGTTCTGTTATTTTCTAATATGTATGTGTGTTAGAGTGTCTGTGT AATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTATAACAAAGAGAATACGTATGTAATGCGTATCT ATCTATCTATATATATATATGTATATATGCTATGTGTATATCAACCTCGTATATTTTCTCCCTGTTGACCACGGC TCCCACAACGTGTGCGGATGGATGACTTGGCTTCCCTATTTCGTTGAAGAACGCAGCAAAGTGGGATAAGTGGTAT CAATTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCC GTGCATGCCACATTTCTCAGTGTGCAATATCAAAACAAAACGCACATCTATTTTGTGTTGTTCAACGCACGCAAAA AAATCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGTTTGTGAGTGTGCACATATACGATATCTTTCAA CTCTTTCTACTCGCACAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGAA AGGCATATATATATATGTATATACATAATATATATGTGTGTGGATTGTTGTGTTGAGCACATATAAGGAAAAAGG TTGCGTGTATATACAGAGAGTCTGTGGCGGTTGAGACGTGTATGTATATATATCTATAATATATGTATATGTGTG TGTTCGCTGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCC CCGTATAGAGATTTAAAAAGGAAGAGAAAAAGTATGCAAAAGAGGCGGCGGATAGTGTGCATGTGTGTATTTCACAG CAAGCACTATATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTTGTGCTTCTTCGTGTACGC TTCACTTTTTATATTGTATTTTTCAGACCTGAGTGTGGCAGGACCACCCGCTAAACTTAAGCATATTACTCAGC GGAGAAAAGAAAACAACCGTGATTCTCTCAGTCAGCGCGGAGCGAAGAGG
B18.2 (1317 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAGTCGT AACAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACGAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATACATAGTTCTGTTATTTTCTATCATGTATGTGTGTTAGAGTGTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCT CCCACAACGTGTGCGGATGGATGACTTGGCTTCCCTATTTCGTTGAAGAACGCAGCAAAGTGGGATAAGTGGTATC AATTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCG TGCATGCCACATTTCTCAGTGTGCAATATCAAAACAAAACGCACATCTATTTTGTGTTGTTCAACGCACGCAAAA AATCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGTTTGTGAGTGTGCACATATACGATATCTTTCAAC TCTTTCTACTCGCACAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGAA GGCATATATATATATGTATATACATAATATATATGTGTGTGGATTGTTGTGTTGAGCACATATAAGGAAAAAGGT TGCGTGCATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATATGTATATGTGTGTTCGCG TGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCCGTATAG AGATTAAAAAGGAAGAGAAAAAGTATGCAAAAGAGGCGGCGGATAGTGTGTATGTGTGTATTTCACAGCAAGCAAC TATATTTTGTGCTTGTGAGTATATGCATATATGTACATTATGTGCTTGTGCTTCTTCGTGTACGCTTCACTTT TTTTATATTGTATTTTTCAGACCTGAGTGTGGCAGGACCACCCGCTAAACTTAAGCATATTACTCAGCGGAGGAAAA GAAACAACCGTGATTCTCTCAGTCAGCGCGGAGCGAAGAGG

Table 6 (continued)

KAI.1 (1315 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAAATATACATATATGTTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTCTACATAGTTCTGTTATTTCTATCATGTATGTGTGTAGAGTGTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCCGTATC TCTCTATATATATTTATGTATATATGCTGTGTGTATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCTCC CACAACGTGTCGCGATGGATGACTTGGCTTCTATTTCGTTGAAGAACGCAGCAAAAGTGGGATAAGTGGTATCAA TTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCGTG CATGCCACATTTCTCAGTGTGCAATATAGAAACAAAACACACACCTATTTTGTGTTGTTCAACGCACGCAAAAAA TCCCGCCACCTCTTCTCCTCGTGTGGTGCATATTCATGGTTGTGAGTGTGCACATATACGATATCTTTCAACTC TTTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGAAAGG CATATGTATATATGTATATACATAATATATATGTGTGTGGATTGTGTGTGAGCACATATAAGGAAAAAGGTTG CGTGCATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATATGTATATGTGTGTGTTCCGCTG TGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTGTACGCATGTATTTGGCGCCCGTATAGAG ATTAAAAAGAGAGAAAAAGTATGCAAAAGAGGCGCGGATAGTGTGTATGTGTATTACAGCAAGCAACTA TATTTTGTGCTTGTGTAGTATATGCATATATGTACATTATGTGCTTGTGCTTCTTTCGTGTACGCTTCACTTTT TATATTGTATTTTTCAGACCTGAGTGTGGCAGGACCACCGCTAACTTAAGCATATTACTCAGCGGAGGAAAAAG AAACAACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG
KAI.2 (1318 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAAATATACATATATGTTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATATATAGTTCTGTTATTTTCTATTATGTATGTGTGTAGGGTGTCTGTGT TAATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCTCC CACAACGTGTCGCGATGGATGACTTGGCTTCTATTTCGTTGAAGAACGCAGCAAAAGTGGGATAAGTGGTATCAA TTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCGTG CATGCCACATTTCTCAGTGTGCAATATAGAAACAAAACGCACACCTATTTTGTGTTGTTCAACGCACGCAAAAAA TCCCGCCACCTCTTCTCCCCGTGTGGGTGCATATTCATGGTTGTGAGTGTGCACATATACGATATCTTTCA ACTCTTTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGA AAGGCATATATATATATGTATATACATAATATATATGTGTGTGGATTGTGTGTGAGCACATATAAGGAAAAAG GTTGCGTGTATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATGTATATGTGTGTGTTCCGC TGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATATGTTGTACGCATGTATTTGGCGCCCGTATAG AGATTAAAAAGAGAGAAAAAGTATGCAAAAGAGGCGCGGATAGTGTGTATGTGTATTACAGCAAGCAAC TATATTTTGTGCTTGTGTAGTATATGCATATATACATTATGTGCTTGTGCTTCTTTCGTGTACGCTTCACTTT TTTATATTGTATTTTTCAGACCTGAGTGTGGCAGGACCACCGCTAACTTAAGCATGTTACTCAGCGGAGGAAAA AGAAAACACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG
SAM.1 (1320 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAAATATACATATATGTTTTACATGTATGTGTTCTATATGCCGT TGACATGGGAGATGAGGGATGTTATATATAGTTCTGTTATTTTCTAATATGTATGTGTGTAGAGTGTCTGTGT AATATACTTTTTAATGCATGCTCTACATAATATACAGTAGTGATAACAAAGAGAATACGTATGGAATGCCGTATCT CTCTACATATATTTATGTATATATGCTATGTGTATATCAACCTCGCATATTTTCTCCCTGTTGACCACGGCTCCC ACAACGTGTCGCGATGGATGACTTGGCTTCTATTTCGTTGAAGAACGCAGCAAAAGTGGGATAAGTGGTATCAAT TGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCCCCGTG ATGCCACATTTCTCAGTGTGCAATATAGAAACAAAACACACACCTATTTTGTGCTTCAACGCACGCAAAAAA CCCGCCACCTCTTCTCCCCGTGTGGTGCATATTCATGGTTGTGAGTGTGCACATATACGATATCTTTCAACTCT TTCTACTCGCACAAATGGTGTATGTACGCATATACGTGTGTGTAGTGAGTGATATGGAAGAGAAATGGGAAAGGC ATATGTATATATATATATACATAATATATATGTGTGTGGATTGTGTGTGAGCACATATAAGGAAAAAGGTTGT GTGTATATACAGAGAGTCTGTGGCGGTTGAGACGTGTATGTATATATATCTATAATATATGTATATGTGTGTGTT CCGCTGTGGAGATTTTATATCTTACGGAGAGTGTTCATATATATGTTGTACGCATGTATTTTGGCGCCCGTAT AGAGATTAAAAAGAGAGAAAAAGTATGCAAAAGAGGCGCGGATAGTGTGTATGTGTATTACAGCAAGCA ACTATATTTTGTGCTTGTGTAGTATATGCATATATGTACATTATGTGCTTGTGCTTCTTTCGTGTACGCTTCACT TTTTATATTGCATTTTTCAGACCTGAGTGTGGCAGGACCACCGCTAACTTAAGCATATTACTCAGCGGAGGA AAAGAAAACACCGTGATTCTCTCAGTCAGCGGCGAGCGAAGAGG

Table 6 (continued)

SAM.2 (1323 nt)	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAAGCAAAAGTCGT AACAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCCATTATACAAAAAGAGCATATTTATG TGCATGTATAAATTGCACAGTATGCAACCAAAAATATACATATATGTTTACATGTATGTGTTCTATATGCCGT TTGACATGGGAGATGAGGGATGCTATACATAGTTCTGTTATTTTCTATCATGTATGTGTGTTAGAGTGTCTGTGT TAATATACTTTTAAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATGCGTATC TCTCTATATATATTTATGTATATATGCTATGTGTATATCAACCTCGCATATTTTCTCCCTGTGACCACGGCTCC CACAAGTGTGCGGATGGATGACTTGGCTTCTATTTTCGTTGAAGAACGCAGCAAGTGCGATAAGTGGTATCAA TTGCAGAATCATTTTCATTGCCCAATCTTTGAACGCAACCGCGCATGGGAGAAGCTCTCTCGAGCCATCCCGTG CATGCCACATTTCTCAGTGTGCAATATAGAAACAAACACACACCTATTTTGGTGTGTTGTTCAACGCACGCA AAAAATCCCGCCACCTCTTTCTCCTCCGTGTGGTGCATATTCATGGTTTGTGAGTGTGCACATATACGATATCTT TCAACTCTTTCTACTCGCACAATGGTGTATGTCACGCATATACGTGTGTGTAGTGAGTGATGGAAGAGAAATG GGAAAGGCATATGTATATATGTATATACATAATATATATGTGTGTGGATTGTGTGTTGAGCACATATAAGGAAA AAGGTTGCGTGCATATACAGAGAGTCTGTGGCGGTTGGGACATGTGTATAAATATATATATGTATGTGTGTGT TCCGCTGTGGAGATTTATATCTTACGGAGAGTGTTCATATATATATGTTTGTACGCATGTATTTTGGCGCCCG TATAGAGATTAAGAAAGAGAGAAAGTATGCAAGAGGCGGGCGATAGTGTGTATGTGTGTATTCACAGCAA GCAACTATATTTGCTGCTTGTGAGTATATGCATGTATGTACATTATGTGCTTGTGCTTCTTTCGTGTACGCTTC ACTTTTTATATTGTATTTTCAGACCTGAGTGTGGCAGGACCCCGCTAAACTTAAGCATATTACTCAGCGGA GGAAAGAAAACAACCGTGATTCTCTCAGTCAGCGCGAGCGAAGAGG
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Identification and analysis of nucleotide sequence data.

BLAST program was used for identification nucleotide sequences of the *T. evansi* Thailand's stocks in comparison to the previously reported data obtained from GenBank. The nucleotide sequences revealed that the *T. evansi* of Thailand stocks were similar to *T. evansi* Taiwan stock (D98527.1) and *T. brucei* (AL929605.1)

Clustal W was used for alignment of the nucleotide sequences of 18S rDNA and ITS region and compared to those of *T. evansi* and other *Trypanosoma* spp. reported in Genbank (Table 7). Upon alignment, some nucleotide regions were deleted to generate the appropriate sequence alignment and the sequences were corrected by eye. The alignment of each region was shown in Table 8 and 9.

Table 7 GenBank accession number of 18S rDNA and ITS region of *Trypanosoma* spp. and other blood parasites.

	Parasites name	specific Host/Lab host	GenBank accession number
18S rDNA	<i>Leishmania major</i>		AC005806.3
	<i>T. brucei</i>		AL929605.1
	<i>T. congolense</i>	<i>Rattus norvegicus</i>	U22319.1
	<i>T. cruzi</i>		AJ009148.1
	<i>T. evansi</i>		D89527.1
	<i>T. rangeli</i>		AJ012416.1
	<i>T. simiae</i>		U22320.1
	<i>T. vivax</i>	<i>Rattus norvegicus</i>	U22316.1
	<i>T. lewisi</i>		AJ223566.1
	<i>T. theileri</i>		AJ009164.1
ITS region	<i>T. congolense</i>	<i>Rattus norvegicus</i>	U22319.1
	<i>T. evansi</i>		D89527.1
	<i>T. brucei</i>		X05682.1
	<i>T. brucei</i> NW2	<i>Homo sapiens</i>	AF306776.1
	<i>T. brucei</i> TH2	<i>Homo sapiens</i>	AF306777.1
	<i>T. brucei</i> Da1972	<i>Homo sapiens</i>	AF306774.1
	<i>T. brucei</i> B8/18	pig	AF306772.1
	<i>T. brucei</i> KP2	<i>Glossina palpalis</i>	AF306773.1
	<i>T. brucei</i> STIB215	Lion	AF306771.1
	<i>T. brucei</i> H3	Lion	AF306770.1

Table 8 Nucleotide sequence alignment of 18S rDNA of *T. evansi* compared to other *Trypanosoma* spp. and *L. major*.

<i>L. major</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATTGCAGGA	60
b18	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCT-AGAATCACTGCATT-GCAGGA	58
Sam	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
Kai	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCT-AGAATCACTGCATT-GCAGGA	58
<i>T. brucei</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. evansi</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. congolense</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. simiae</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. vivax</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. rangeli</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. cruzi</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. theileri</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59
<i>T. lewisi</i>	GTCATATGCTTGTTC AAGGACTTAGCCATGCATGCCTCAGAATCACTGCATT-GCAGGA	59

<i>L. major</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATCTTGCGGTT-TCCGC	119
b18	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTC-TCCGC	117
Sam	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTC-TCCGC	118
Kai	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTC-TCCGC	117
<i>T. brucei</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTC-TCCGC	118
<i>T. evansi</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTC-TCCGC	118
<i>T. congolense</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATCTTGCGGTC-TCCGC	118
<i>T. simiae</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATGTTGCGGTC-TCCGC	118
<i>T. vivax</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCAATCACTTTGCGGTCCTCCGC	119
<i>T. rangeli</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATCTTGCGGTT-TCCGC	118
<i>T. cruzi</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATCTTGCGGTC-TCCGC	118
<i>T. theileri</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATCTTGCGGTT-TCCGC	118
<i>T. lewisi</i>	ATCTGCCGATGGCTCATTACATCAGACGTAATCTGCCGCCAAAATTTTGCGGTT-TCCGC	118

<i>L. major</i>	AAAATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAACCGGGTGTCTCCAC	179
b18	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATCGGACGCTCTCTTT	177
Sam	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATCGGACGCTCTCTTT	178
Kai	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATCGGACGCTCTCTTT	177
<i>T. brucei</i>	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATCGGACGCTCTCTTT	178
<i>T. evansi</i>	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATCGGACGCTCTCTTT	178
<i>T. congolense</i>	ATTATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCGAAGGACGCTCCCTGC	178
<i>T. simiae</i>	ATTATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAATGGACGTTCCCTGC	178
<i>T. vivax</i>	ATTACTGGATAACTTGGCGAAACGCCAAGCTAATACATGAGCC-ACCGGGCGCTCCCTGC	178
<i>T. rangeli</i>	AACATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAACCGGATGTTCTCTGT	178
<i>T. cruzi</i>	AAAATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAACCGGATGTTCTCTGT	178
<i>T. theileri</i>	AACATTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAAGGACGTTCTCTGT	178
<i>T. lewisi</i>	AACAGTGGATAACTTGGCGAAACGCCAAGCTAATACATGAACCAACCGGACGCTCTCTGT	178
* * *****		
<i>L. major</i>	TCCAGA---CGGTGGGCA-----AC-CATCGT---CGTGAGACGCCAGCGAATGA	223
b18	TTCTAT-GTCGCGGCTTGTGTTTACGCACTTGTCTGTGGCGATGGGACGTCCAGCGAATGA	236
Sam	TTCTAT-GTCGCGGCTTGTGTTTACGCACTTGTCTGTGGCGATGGGACGTCCAGCGAATGA	237
Kai	TTCTAT-GTCGCGGCTTGTGTTTACGCACTTGTCTGTGGCGATGGGACGTCCAGCGAATGA	236
<i>T. brucei</i>	TTCTAT-GTCGCGGCTTGTGTTTACGCACTTGTCTGTGGCGATGGGACGTCCAGCGAATGA	237
<i>T. evansi</i>	TTCTAT-GTCGCGGCTTGTGTTTACGCACTTGTCTGTGGCGATGGGACGTCCAGCGAATGA	237
<i>T. congolense</i>	TAC--G-GCCGCGGGGTG-----AC---TCGCGGTACGGGACGTCTCGGAATGA	223
<i>T. simiae</i>	TCC--G-GCGGCGGGGCG-----AC---TCGCGGTACGGGACGTCCGCGAATGA	223
<i>T. T. vivax</i>	TCC--G-GCGACGGGGCA-----AC---CCGCGCTATGGGACGCCCCGCGAATGA	223
<i>T. rangeli</i>	TTCCGGC---GGTGGGGCA-----ACTCGCCGC---CATGGGATGTCAGCGAATGA	223
<i>T. cruzi</i>	TCCGGC---GGCAGGGCA-----ACCTGTCTGC---CATGGGACGTCCAGCGAATGA	223
<i>T. theileri</i>	TCCGGC---GGTGGGGCA-----ACTCACTGT---CATGGGACGTCCAGCGAATGA	223
<i>T. lewisi</i>	TCCGGCTGCGGGGGGCG-----ACCCCTGTGTCATGGGACGTCCAGCGAATGA	229
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<i>L. major</i>	ATGACAGTAAACCAATGCCTTCACT---GGCAGTAACACCCAGCAGTGTGACTCAAT	279
b18	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	296
Sam	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	297
Kai	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	296
<i>T. brucei</i>	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	297
<i>T. evansi</i>	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	297
<i>T. congolense</i>	ATGAAATTAGAACCAACGCCTCCACCCGGGGGCAGTAACACTCAGACGTGTTGACTCAAT	283
<i>T. simiae</i>	ATGGCATTAAACCAACGCCTTCGC---GGGCAGTAACACTCAGACGTGTTGACTCAAT	279
<i>T. vivax</i>	GTGACATTAAACCAACGCCTTCGC---AGGGCAGTACCACACAGACGTGTTGACTCAAT	280
<i>T. rangeli</i>	ATGAAAGTAAACCAATGCCTTCACC---GGCAGTAACACCCAGAGTGTGATTCAAT	279
<i>T. cruzi</i>	ATGAAAGTAAACCAATGCCTTCACC---GGCAGTAACACTCAGAGTGTGATTCAAT	279
<i>T. theileri</i>	ATGAAATAAACCAATGCCTTCACC---GGCAGTAACACCCAGAGTGTGATTCAAT	279
<i>T. lewisi</i>	ATGAAATTAAACCAACGCCTTCACC---GGCAGTAACACTCAGAGTGTGATTCAAT	285
	* * * * *	
<i>L. major</i>	TCATTCCGTGCGAAAGCCGGCTTGT-----CGGCGTCTTTTGACGAACAACCTGC	330
b18	TCATTCCGTGCGAAAGCCGAGCCTTGTGCT-----CGGCGTCTACTGACGAACAACCTGC	351
Sam	TCATTCCGTGCGAAAGCCGAGCCTTGTGCT-----CGGCGTCTACTGACGAACAACCTGC	352
Kai	TCATTCCGTGCGAAAGCCGAGCCTTGTGCT-----CGGCGTCTACTGACGAACAACCTGC	351
<i>T. brucei</i>	TCATTCTGTGCGAAAGCCGAGCCTTGTGCT-----CGGCGTCTACTGACGAACAACCTGC	352
<i>T. evansi</i>	TCATTCCGTGCGAAAGCCGAGCCTTGTGCT-----CGGCGTCTACTGACGAACAACCTGC	352
<i>T. congolense</i>	TCATTCCGTGCGAAAGCCGGCTTCTTCTTGGTGCCGGCGTGTGCTGACGAACAACCTGC	343
<i>T. simiae</i>	TCATTCCGTGCGAAAGCCGGGTCA-----GCCGGCGTGTGTTGACGAACAACCTGC	329
<i>T. vivax</i>	TCATTCCGTGCGAAAGCCGGCTCGTC-----CGGCGTCTTTTGACGAACAACCTGC	330
<i>T. rangeli</i>	TCATTCCGTGCGAAAGCTGGGTTTTTTTAC-----CTGGCGTCTTTTGACGAACAACCTGC	333
<i>T. cruzi</i>	TCATTCCGTGCGAAAGCCGGTTTTTTTAT-----CGGCGTCTTTTGACGAACAACCTGC	333
<i>T. theileri</i>	TCATTCCGTGCGAAAGCTGGATTCTTAT-----CGGCGTCTTTTGACGAACAACCTGC	333
<i>T. lewisi</i>	TCATTCCGTGCGAAAGCCGATTCTTT-----CGGCGTCTTTTGACGAACAACCTGC	337
	***** * *	
<i>L. major</i>	CCTATCAGCTGGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	390
b18	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	411
Sam	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	412
Kai	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	411
<i>T. brucei</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	412
<i>T. evansi</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	412
<i>T. congolense</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	403
<i>T. simiae</i>	CCTATCAGCTAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	389
<i>T. vivax</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	390
<i>T. rangeli</i>	CCTATCAGCCAGCGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	393
<i>T. cruzi</i>	CCTATCAGCCAGCGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	393
<i>T. theileri</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	393
<i>T. lewisi</i>	CCTATCAGCCAGTGATGGCCGTGTAGTGGACTGCCATGGCGTTGACGGGAGCGGGGGATT	397
	***** * *	
<i>L. major</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	450
b18	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	471
Sam	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	472
Kai	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	471
<i>T. brucei</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	472
<i>T. evansi</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	472
<i>T. congolense</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	463
<i>T. simiae</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	449
<i>T. vivax</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	450
<i>T. rangeli</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	453
<i>T. cruzi</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	453
<i>T. theileri</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	453
<i>T. lewisi</i>	AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATAGCTACCACTTCTACGGAGGGCAGCAG	457
	***** * *	
<i>L. major</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGTGT	510
b18	GCGCGCAAATTGCCCAATGTCAAAAAATACGATGAGGCAGCGAAAAGAAATAGAGCCGA	531
Sam	GCGCGCAAATTGCCCAATGTCAAAAAATACGATGAGGCAGCGAAAAGAAATAGAGCCGA	532
Kai	GCGCGCAAATTGCCCAATGTCAAAAAATACGATGAGGCAGCGAAAAGAAATAGAGCCGA	531
<i>T. brucei</i>	GCGCGCAAATTGCCCAATGTCAAAAAATACGATGAGGCAGCGAAAAGAAATAGAGCCGA	532
<i>T. evansi</i>	GCGCGCAAATTGCCCAATGTCAAAAAATACGATGAGGCAGCGAAAAGAAATAGAGCCGA	532
<i>T. congolense</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	523
<i>T. simiae</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	508
<i>T. vivax</i>	GCGCGCAAATTGCCCAATGTCAAGACAGAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	510
<i>T. rangeli</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	513
<i>T. cruzi</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	513
<i>T. theileri</i>	GCGCGCAAATTGCCCAATGTCAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	513
<i>T. lewisi</i>	GCGCGCAAATTGCCCAATGTCTAAAAAACGATGAGGCAGCGAAAAGAAATAGAGCCGA	517
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<i>L. major</i>	CAGTC--CATT-TGGATTGTCATTT-CAATGGGGGATATTTAAACCCATCCAATAT-CGA	565
b18	CCGTG-CCCTAGTGCATGGGTGTTTTCAATGGGGGATACTCAAACCCATCCAATATTCGA	590
Sam	CCGTGGCCCTAGTGCATGGTTGTTTTCAATGGGGGATACTCAAACCCATCCAATAT-CGA	591
Kai	CCGTG-CCCTAGTGCATGGGTGTTTTCAATGGGGGATACTCAAACCCATCCAATAT-CGA	589
<i>T. brucei</i>	CCGTG-CCCTAGTGCATGGTTGTTTTCAATGGGGGATACTCAAACCCATCCAATAT-CGA	590
<i>T. evansi</i>	CCGTG-CCCTAGTGCATGGTTGTTTTCAATGGGGGATACTCAAACCCATCCAATAT-CGA	590
<i>T. congolense</i>	CCGTGCCCTTTTGTGCACGGTCGTTTTCAATGGGGGATATTTAAACCCATCCAACAT-CGA	582
<i>T. simiae</i>	CCTTGCCATTG--GCATGGTCGTTTTCAATGGGGGATATTTAAACCCATCCAATAT-CGA	565
<i>T. vivax</i>	CAGTG----CTACGCATGTGCTGTTTTCAATGGGGGATACCCAAACCCATCCAACAT-CGA	565
<i>T. rangeli</i>	CAGTG--CTT--TGCATTGTCGTTTTCAATGGGGGATATTTAAACCCATCCAAAAT-CGA	568
<i>T. cruzi</i>	CAGTG--CTTT-TGCATTGTCGTTTTCAATGGGGGATATTTAAACCCATCCAAAAT-CGA	569
<i>T. theileri</i>	CAGTG--CCTAGTGCATTGTCGTTTTCAATGGGGGATATTTAAACCCATCCAAAAT-CGA	570
<i>T. lewisi</i>	CAGTG--CCTTTGGCATTGTCGTTTTCAATGGGGGATATTTAAACCCATCCAAAAT-CGA	574
	* * * * *	
<i>L. major</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	625
b18	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	650
Sam	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	651
Kai	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	649
<i>T. brucei</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	650
<i>T. evansi</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	650
<i>T. congolense</i>	GTAACCAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	642
<i>T. simiae</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	625
<i>T. vivax</i>	GTAACATTTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	625
<i>T. rangeli</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	628
<i>T. cruzi</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	629
<i>T. theileri</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	630
<i>T. lewisi</i>	GTAACAATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCAGCTCCAAAAGCGT	634

<i>L. major</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCTGTGCAG-	678
b18	ATATTAAT-----GCTGTTGCTGT-AAAGGGTTCGTAATTGAACTGTGGGCCACGTAGT	703
Sam	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAATTGAACTGTGGGCCACGTAA	705
Kai	ATATTAAT-----GCTGTTGCTGT-AAAGGGTTCGTAATTGAACTGTGGGCCACGTAGT	702
<i>T. brucei</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCCACGTAGT	704
<i>T. evansi</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCCACGTAGT	704
<i>T. congolense</i>	ATATTAAT-----TGCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCGTTGTG--	695
<i>T. simiae</i>	ATATTAATGCTGTTGCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCGC-GAG--	682
<i>T. vivax</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCCGCG--	675
<i>T. rangeli</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGGCCCTTCGAG-	681
<i>T. cruzi</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGAGGGCCTCTAAG-	682
<i>T. theileri</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGAGGGCCTTTGAG-	683
<i>T. lewisi</i>	ATATTAAT-----GCTGTTGCTGTTAAAGGGTTCGTAGTTGAACTGTGGCCCTTTGAG-	687

<i>L. major</i>	GTTTGTTCCTGGTCGTC-CCGTCCATGTCGGATTGGT---GACCCAGGCC-CTTGCAGC	733
b18	TTTGTGCCGTGCCAGTC-CCGTCCCCCTCGGACGTGTTTTTGACCCACGCC-CTCGTGGC	761
Sam	TTTGTGCCGTGCCAGTC-CCGTCCCCCTCGGACGTGTTTT-GACCCACGCC-CTCGTGGC	762
Kai	TTTGTGCCGTGCCAGTC-CCGTCCACCTCGGACGTGTTTT-GACCCACGCC-TTCGTGGC	759
<i>T. brucei</i>	TTTGTGCCGTGCCAGTC-CCGTCCACCTCGGACGTGTTTT-GACCCACGCC-CTCGTGGC	761
<i>T. evansi</i>	TTTGTGCCGTGCCAGTC-CCGTCCACCTCGGACGTGTTTT-GACCCACGCC-CTCGTGGC	761
<i>T. congolense</i>	CAGGTGCCGTGTCGTC-CCACCCGACTCGGGGTGGT---GACCCACGCC-CTCGGCGC	749
<i>T. simiae</i>	TAGGCGC-GTGTCTGTGCTGCCCTCGATTGGGCTGT---GGCGCACGCC-CTCTGCGT	736
<i>T. vivax</i>	CTGCCGCCGCGCTCCGCCCCACCCGAGAGGGTGCGT---GGCACGCGCGCGCGCTGC	731
<i>T. rangeli</i>	GCGCAATGGTT-TAGTC-CCGTCCACTTCGGATT-GGT---GACCCATGCC-CTTGAGGT	734
<i>T. cruzi</i>	GCGCAATGGTT-TAGTC-CCATCCACTTCGGATT-GGT---GACCCATGCC-CTTGAGGT	735
<i>T. theileri</i>	GCGCAATGGTT-T-GTC-CCGTCCACCTCGGATT-GGT---GACCCATGCC-CTTGAGGT	735
<i>T. lewisi</i>	GCGCAATGGTT-TAGTC-CCGTCCACCTCGGATT-GGT---GACCCATGCC-CTTGAGGT	740
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<i>L. major</i>	CCGTGAACA--TTCAAAGAAACAAGAAACACGGGAGTGTTCTTTCTGATTT--ACGC	789
b18	CCGTGAACA--CACTCAGATACAAGAAAAACCGGAGCGGGTCTC-TCACTTTC--ACGC	815
Sam	CCGTGAACA--CCCTCAGATACAAGAAACACGGGAGCGGGTCTCTCACTTTC--ACGC	818
Kai	CCGTGAACA--CACTCAGATACAAGAAACACGGGAGCGGGTCTCTCACTTTC--ACGC	814
<i>T. brucei</i>	CCGTGAACA--CACTCAGATACAAGAAACACGGGAGCGGGTCTCTCACTTTC--ACGC	817
<i>T. evansi</i>	CCGTGAACA--CACTCAGATACAAGAAACACGGGAGCGGGTCTCTCACTTTC--ACGC	817
<i>T. congolense</i>	CCGTGAACA--CA--CAGAAACGAGAAACACGGGAGCGGTCCCTCACTTTC--ACGC	805
<i>T. simiae</i>	CCGTGAACA--CA--CCGATACAAGAACACGGGAATGGTCCCCCAGA-----ACGC	784
<i>T. vivax</i>	CCGCGAACA--CT--CAGACACAAGACAGCGGGAACCGTTCCCCACACC-----ACGC	781
<i>T. rangeli</i>	CCGTGAACA--CTCAGAAACAAAAACACGGGAGTGTTACCTCT-CTGATTT--TCGC	787
<i>T. cruzi</i>	CCGTGAACAGACATTAGAAACAAAAACACGGGAGTGTTACCTTTCTGATTA--TCGC	793
<i>T. theileri</i>	CCGTGAACA--CTCAGAAACAAAAACACGGGAGCGGGTCTCTCT-CTGATTT--TCGC	788
<i>T. lewisi</i>	CCGTGAACA--CTCAGAAACAAAAACACGGGAGTGTTCTTTCTGATTT--TCGC	794
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<i>L. major</i>	ATG-TCATGCATGCCAGGGGGCGTCCGTGATTTTTTACTGTGACTAAAGAAGCGTACTA	848
<i>bl18</i>	ATG-TCATGCATGCGAGGGGGCGTCCGTGA-ATTTTACTGTGACCAAAAAAGTGCACCA	873
<i>Sam</i>	ATGGTCATGCATGCGAGGGGGCGTCCGTGA-ATTTTACTGTGACCAAAAAAGTGCACCA	877
<i>Kai</i>	ATG-TCATGCATGCGAGGGGGCGTCCGTGA-ATTTTACTGTGACCAAAAAAGTGCACCA	872
<i>T. brucei</i>	ATG-TCATGCATGCGAGGGGGCGTCCGTGA-ATTTTACTGTGACCAAAAAAGTGCACCA	875
<i>T. evansi</i>	ATG-TCATGCATGCGAGGGGGCGTCCGTGA-ATTTTACTGTGACCAAAAAAGTGCACCA	875
<i>T. congolense</i>	ATG-TCATGCATGCG--GGGGCGTCCGTGA-TTTTACTGTGACTAAAAAAGTGCACCA	861
<i>T. simiae</i>	ATG-TCACGCATGCG--GGGGCGTCCGTGA-TCTTACTGTGACTAAAAAAGAGCGACCA	840
<i>T. vivax</i>	GTG-TCATACACGCAC--GGGGCGCCCGCGAGTCTTTACTGTGACTAAAAAAGCGCGACCA	839
<i>T. rangeli</i>	ATG-TCATGCATGCCAGGGGGCGCCCGTATTTTTTACTGTGACTAAAAAAGTGTGACCA	846
<i>T. cruzi</i>	ATG-TCATGCATGCCAGAGGGCGCCCGTATTTTTTACTGTGACTAAAAAAGTGTGACCA	852
<i>T. theileri</i>	ATG-TCATGCATGCCAGGGGGCGTCCGTGATTTTT-ACTGTGACTAAAAAAGTGTGACCA	846
<i>T. lewisi</i>	ATG-TCATGCATGCCAGGGGGCGCCCGTATTTTTTACTGTGACTAAAAAAGTGTGACCA	853
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<i>L. major</i>	AAGCAGTCATT-TGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTCTAGGCT	907
<i>bl18</i>	AAGCAGTCTGC-CGACTTGAATTACAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCC	932
<i>Sam</i>	AAGCAGTCTGC-CGACTTGAATTACAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCC	936
<i>Kai</i>	AAGCAGTCTGC-CGACTTGAATTACAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCC	931
<i>T. brucei</i>	AAGCAGTCTGC-CGACTTGAATTACAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCC	934
<i>T. evansi</i>	AAGCAGTCTGC-CGACTTGAATTACAAAGCATGGGATAACGAAGCATCAGCCCTGGGGCC	934
<i>T. congolense</i>	AAGCAGTCCGC-CGACTTGAATTACAAAGCATGGGATAACAAAGCACCAGCCCTGGGGCC	920
<i>T. simiae</i>	AAGCAGTCCAC--GACTTGAATTACAAAGCATGGGATAACAAAGCATCAGCCCCAGGGCC	898
<i>T. vivax</i>	AAGCAGTCCAC-CGACCTGAATTGCAAAGCATGGGATAACAAAGCATCAGCCTCGGGCC	898
<i>T. rangeli</i>	AAGCAGTCATT-CGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTATGGGCC	905
<i>T. cruzi</i>	AAGCAGTCATT-CGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTCGGGCC	911
<i>T. theileri</i>	AAGCAGTCATT-CGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTATGGGCC	905
<i>T. lewisi</i>	AAGCAGTCATTTGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTATAGGCC	913
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<i>L. major</i>	ACCGTTTCGGCTTTTGTGGTTTTAAAGGTCTATTGGAGATTATGGAGCTGTGCGACAAG	967
<i>bl18</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGGCCGCGTGCCCTG	992
<i>Sam</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGGCCGCGTGCCCTG	996
<i>Kai</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGGCCGCGTGCCCTG	991
<i>T. brucei</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGGCCGCGTGCCCTG	994
<i>T. evansi</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGGCCGCGTGCCCTG	994
<i>T. congolense</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGTCCTTGGGAGATTATGGGCGCGCGTGCCCGC	980
<i>T. simiae</i>	ACCGTTTCGGCTTTTGTGGTTTTAGAAAGCCAGGGGAGATTATGGGCGCGCGTGCCG--	956
<i>T. vivax</i>	ACCGATTTCGGCTTTTGTGGTTTTAAACAGTCCGTGGGAGATTATGGGCGCGCGGAGCAC	958
<i>T. rangeli</i>	ACCGTTTCGGCTTTTGTGGTTTTAAAGTCCATTGGAGATTATGGGGCAGTGTGACAAG	965
<i>T. cruzi</i>	ACCGTTTCGGCTTTTGTGGTTTTAAAGTCCATTGGAGATTATGGGGCAGTGTGACAAG	971
<i>T. theileri</i>	ACCGTTTCGGCTTTTGTGGTTTTAAAGTCCATTGGAGATTATGGCGTCGTGCGACAAG	965
<i>T. lewisi</i>	ACCGTTTCGGCTTTTGTGGTTTTAAACAGTCTATTGGAGATTATGGGGCTGTGCGACAAG	973
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<i>L. major</i>	TGCTTTCCCATCG-----CAACTTCGG-----TTCGGTGTGTGGCGC	1004
<i>bl18</i>	GGTCGGTGTTCG-----TGCTCATT-----TTTGTGGCG--CGC	1026
<i>Sam</i>	GGTCGGTGTTCG-----TGCTCATT-----TTTGTGGCG--CGC	1030
<i>Kai</i>	GGTCGGTGTTCG-----TGCTCATT-----TTTGTGGCG--CGC	1025
<i>T. brucei</i>	GGTCGGTGTTCG-----TGCTCATT-----TTTGTGGCG--CGC	1028
<i>T. evansi</i>	GGTCGGTGTTCG-----TGCTCATT-----TTTGTGGCG--CGC	1028
<i>T. congolense</i>	GCTGTGCGCGCC-----CCCTCCTTCCACGGAGGTGTGTGGCGGGCCCGC	1026
<i>T. simiae</i>	-----CGCA-----CACTTCC-----GTGTGCG-----	974
<i>T. vivax</i>	GCACCGCGGCATT-----CCACTCGGA-----ACACGCCGCA-----	990
<i>T. rangeli</i>	CGGCTGGGTGATT-----TCCCTTCAA-----TGGGGTACACTCGTCGC	1005
<i>T. cruzi</i>	CGGCTGGGTGGTTATTCCACACACACTCCTTTTGGAGCTGTGTGGCACTCGTCGC	1031
<i>T. theileri</i>	CGTCTGGGTGATT-----CCCTTTC-----GGGGCACCCTCGC	1000
<i>T. lewisi</i>	CGCTCGAGTGCTC-----TCCTTTTC-----GGTGACACTCGGGCG	1009
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<i>L. major</i>	CTTTG-AGGGGTTTAGTG-CGTC--CGGTG-CGAGCT-----CCGGT	1041
<i>bl18</i>	ACA-----TTCGGCTCT-----TCGTG	1043
<i>Sam</i>	ACA-----TTCGGCTCT-----TCGTG	1047
<i>Kai</i>	ACA-----TTCGGCTCT-----TCGTG	1042
<i>T. brucei</i>	ACA-----TTCGGCTCT-----TCGTG	1045
<i>T. evansi</i>	ACA-----TTCGGCTCT-----TCGTG	1045
<i>T. congolense</i>	CCAGCGTCCTCGTCGTGTGTGCTCTCCTCCTCTT-----TTGAG	1068
<i>T. simiae</i>	-----TT-----TCG--	979
<i>T. vivax</i>	-----CGCC-----CCGCG	999
<i>T. rangeli</i>	CTTTGTGGGAAATCAGTGACGGTAGTGCTGTATTGTTGTGTGTGCGTATGTCTGG	1065
<i>T. cruzi</i>	CTTTGTGGGAAATCCGTG-TGGCACTGTT--TGTTGTGTGGCAGAC-----TTCGG	1080
<i>T. theileri</i>	CTTTGCGGGAAATCCGTGTCGGT--GTTGATGAGCT-----TCGGC	1039
<i>T. lewisi</i>	CTTTGTGGGAAATCCGCGTTGC-----CTCGGAGGCT-----TCGG	1047

<i>L. major</i>	TCGTCCGGCCGTA-----ACGCCCTT-	1061
b18	ATGTTTTTTTAC-----A--TTCA	1060
Sam	ATGTTTTTTTAC-----A--TTCA	1064
Kai	ATGTTTTTTTAC-----A--TTCA	1059
<i>T. brucei</i>	ATGTTTTTTTAC-----A--TTCA	1062
<i>T. evansi</i>	ATGTTTTTTTAC-----A--TTCA	1062
<i>T. congolense</i>	GTGTGTTTGTGT-----C--CGCA	1085
<i>T. simiae</i>	--GCGTTCGCGT-----C-----	990
<i>T. vivax</i>	GCGC-CTCGCGC-----G--T---	1012
<i>T. rangeli</i>	ACATCTTTTCGGTGCCCGCCGACGTTAACGGCGCATTACACACTGCCTTCGCCCTTC	1125
<i>T. cruzi</i>	TCTTGCCCTTCGC-----ATATTTTCATGTGTCATGCCTTC	1116
<i>T. theileri</i>	CCATCTCTCCGG-----CGCCTTC	1058
<i>T. lewisi</i>	CCTCGCAGAGAG-----TGCTTCCGTCTTC	1072
<i>L. major</i>	-TTCAACTCAGGCCCTCTAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGGGCG	1120
b18	TTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1120
Sam	TTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1124
Kai	TTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1119
<i>T. brucei</i>	TTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1122
<i>T. evansi</i>	TTGCGACGCGCGGCTTCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1122
<i>T. congolense</i>	CGGCGACGCGCGG-TGCCAGGAATGAAGGAGGGTAGTTCGGGGAG--ACGTACTGGTGCG	1142
<i>T. simiae</i>	---GACGC-CTG-CGCCGGGAATGAATGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1044
<i>T. vivax</i>	-CGCGGCTCGCCGGC-CCGGGAATGAAGGAGGGCGGCTCGGGGGAGAACGTACTGGCGCG	1070
<i>T. rangeli</i>	CCTCAACTCAGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1185
<i>T. cruzi</i>	CCTCAACTCAGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1176
<i>T. theileri</i>	CCGTGACTCAGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCG	1118
<i>T. lewisi</i>	CCTCAACTCGCGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGTGCG	1132
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<i>L. major</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1180
b18	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1180
Sam	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1184
Kai	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1179
<i>T. brucei</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1182
<i>T. evansi</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1182
<i>T. congolense</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1202
<i>T. simiae</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1104
<i>T. vivax</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1130
<i>T. rangeli</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1245
<i>T. cruzi</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1236
<i>T. theileri</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1178
<i>T. lewisi</i>	TCAGAGGTGAAATTCTTAGACCGCACCAAGACGAACTACAGCGAAGGCATTCTTCAAGGA	1192

<i>L. major</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGAGATCGAAGATG-ATTAGAGACCATTGTAG	1239
b18	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1239
Sam	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1243
Kai	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1239
<i>T. brucei</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1241
<i>T. evansi</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1241
<i>T. congolense</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCAAAGATG-ATTAGAGACCATTGTAG	1261
<i>T. simiae</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGTATCGAAGATG-ATTAGAGACCATTGTAG	1163
<i>T. vivax</i>	CGCCTTCCTCAATCAAGAACCACAAAGTGTGGGATCGAAGATG-ATTAGAGACCATTGTAG	1189
<i>T. rangeli</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCGAAGATG-ATTAGAGACCATTGTAG	1304
<i>T. cruzi</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCGAAGATG-ATTAGAGACCATTGTAG	1295
<i>T. theileri</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCGAAGATG-ATTAGAGACCATTGTAG	1237
<i>T. lewisi</i>	TACCTTCCTCAATCAAGAACCACAAAGTGTGGGATCGAAGATG-ATTAGAGACCATTGTAG	1251

<i>L. major</i>	TCCCACTGCAAACGATGACACCCATG-AATTGGGGATCTTA--TGCGCCG-GCCTGCG	1294
b18	TCCCACTGCAAACCATGACACCCATG-AATTGGGGAACATCCATTGGGTGC-CCGTGTG	1297
Sam	TCCCACTGCAAACCATGACACCCATGGAATTGGGGAACATC-ATTGGGTGC-CCGTGTG	1301
Kai	TCCCACTGCAAACCATGACACCCATG-AATTGGGGAACATC-ATTGGGTGC-CCGTGTG	1296
<i>T. brucei</i>	TCCCACTGCAAACCATGACACCCATG-AATTGGGGAACATC-ATTGGGTGC-CCGTGTG	1298
<i>T. evansi</i>	TCCCACTGCAAACCATGACACCCATG-AATTGGGGAACATC-ATTGGGTGC-CCGTGTG	1298
<i>T. congolense</i>	TCCCACTGCAAACCATGACACCCATG-AATTGGGGACCACC--TGG--C-TTGCACG	1313
<i>T. simiae</i>	TCCCACTGCAAACCATGACACCCATG-AATTGGGGACCATT--TGG--C-CCGTGGG	1215
<i>T. vivax</i>	TCCCACTGCAAACGATGACGCCATG-AATTGGGGACCTGT----GG--C-CAGCGGC	1240
<i>T. rangeli</i>	TCCCACTGCAAACGATGACACCCATG-AATTGGGGAGTTTT--TGGTCGTAGGCAAG	1360
<i>T. cruzi</i>	TCCCACTGCAAACGATGACACCCATG-AATTGGGGAGTTTT--TGGTCGT-AGGCGTG	1350
<i>T. theileri</i>	TCCCACTGCAAACGATGACACCCATG-AATTGGGGAGTTTT--TGGTCGT-AGGCGGG	1292
<i>T. lewisi</i>	TCCCACTGCAAACGATGACACCCATG-AATTGGGGAGTTTT--TGGTCGT-AGGCGGG	1306

<i>L. major</i>	GCAGGG-----TTTACCCTGTGTCAGCACCGCGCCCGCTTTTACCAA-CTT	1339
b18	GCG-GCCT-----TTTGTGCCGACCCCTCGGCCCAATT-TATTTATCAA-TTT	1342
Sam	GCG-GCCT-----TTTGTGCCGACCCCTCGGCCCAATT-TATTTATCAA-TTT	1346
Kai	GCG-GCCT-----TTTGTGCCGACCCCTCGGCCCAATT-TATTTATCAA-TTA	1341
<i>T. brucei</i>	GCG-GCCT-----TTTGTGCCGACCCCTCGGCCCAATT-TATTTATCAA-TTT	1343
<i>T. evansi</i>	GCG-GCCT-----TTTGTGCCGACCCCTCGGCCCAATT-TATTTATCAA-TTT	1343
<i>T. congolense</i>	GCACGGCG-----CTTGCGCGGATTGTTCTGTGCAGTCACAAACAACTT	1360
<i>T. simiae</i>	GTGCTGCG-----CTTGCGTAGCCCTCCC---ACGGCCAATCCACAAA-CTT	1259
<i>T. vivax</i>	GCC-GCCG-----GGCTTGCCGCGCGCGCTCGCTC-CACCAACCA---TC	1283
<i>T. rangeli</i>	GTGAG-----TTCACCTCGC-TCCTGTCTCGCAATGAATATCAA-TTT	1404
<i>T. cruzi</i>	GTGCGGCTTGATTATTATTTTCATCCCGT-TCCTCGTCTCGCAATGAATATTAATTT	1409
<i>T. theileri</i>	GTGAG-----TTCATCTCGC-TCCTCGCTCGCAATGGATATCAA-TTT	1336
<i>T. lewisi</i>	GTGCGG-----TTCATCTCGC-TCCTCGTCTCGCAATGTATATCAA-TTT	1350
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<i>L. major</i>	ACGTATCTTTTCTAT-----TCGGCCTTTA-----CC-----GGCCA	1371
b18	ACGTGCCTATTCTATCAC----CCCCGGTTCCT---CTTTTGAGGTTCTTCCGGGGTTT	1395
Sam	ACGTGCCTATTCTATCAC----CCCCGGTTCCT---CTTTTGAGGTTCTTCCGGGGTTT	1399
Kai	ACGTGCCTATTCTATCAC----CCCCGGTTCCT---CTTTTGAGGTTCTTCCGGGGTTT	1394
<i>T. brucei</i>	ACGTGCCTATTCTATCAC----CCCCGGTTCCT---CTTTTGAGGTTCTTCCGGGGTTT	1396
<i>T. evansi</i>	ACGTGCCTATTCTATCAC----CCCCGGTTCCT---CTTTTGAGGTTCTTCCGGGGTTT	1396
<i>T. congolense</i>	ACGTGTCTATTCTGCTGCCGCGCGTTGGCCCTTATTTCTTAAGGGGTTTGTGCGGTTT	1420
<i>T. simiae</i>	ACGTGCCTATTCTGC-----CGTGGGTGCG-----CTGCGCGCGCCGCAT-----	1300
<i>T. vivax</i>	ACGTGCATATTCTTT-----ATGGTCTCT-----CGCTCTCGCGG---GAGACCT	1447
<i>T. rangeli</i>	ACGTGCATATTCTTT-----TTGGTCTCG-----TTTTTTTACGGCGAGGGCCT	1454
<i>T. cruzi</i>	ACGTGCATATTCTTT-----TCGGTCTCG-----CA-----AG---GGGGCCT	1372
<i>T. theileri</i>	ACGTGCATATTCTTT-----TTGGTCTCG-----CA-----AG---AGGTCT	1386
<i>T. lewisi</i>	ACGTGCATATTCTTT-----TTGGTCTCG-----CA-----AG---AGGTCT	1386
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<i>L. major</i>	CCCACGGGAATATCCTCAGCAGCTTTTCTG--TTTTTTCACGCGAAAGCTTTGAGGTTAC	1429
b18	TTTACGGGAATATCCTCAGCAGCTTTTCTTAC--TTCTTCACGCGAAAGCTTTGAGGTTAC	1453
Sam	TTTACGGGAATATCCTCAGCAGCTTTTCTTAC--TTCTTCACGCGAAAGCTTTGAGGTTAC	1457
Kai	TTTACGGGAATATCCTCAGCAGCTTTTCTTAT--TTTTTCACGCGAAAGCTTTGAGGTTAC	1452
<i>T. brucei</i>	TTTACGGGAATATCCTCAGCAGCTTTTCTTAC--TTCTTCACGCGAAAGCTTTGAGGTTAC	1454
<i>T. evansi</i>	TTTACGGGAATATCCTCAGCAGCTTTTCTTAC--TTCTTCACGCGAAAGCTTTGAGGTTAC	1454
<i>T. congolense</i>	A--ACGGGAATATCCTCAGCAGCTTGTTTACATTTTTCACGCGAAAGCTTTGAGGTTAC	1478
<i>T. simiae</i>	---ACGGGAATATCCTCAGCAGCTTGTTTAC--TCATTCACGCGAAAGCTTTGAGGTTAC	1355
<i>T. vivax</i>	---ACGGGAATATCCTCAGCAGCT--CTCAC---TGCCACGCGAAAGCTTTGAGGTTAC	1373
<i>T. rangeli</i>	TTAACGGGAATATCCTCAGCAGCTTATCTGA-CTTCTTCACGCGAAAGCTTTGAGGTTAC	1506
<i>T. cruzi</i>	TTAACGGGAATATCCTCAGCAGCTTATCTGA-CTTCTTCACGCGAAAGCTTTGAGGTTAC	1513
<i>T. theileri</i>	TTAACGGGAATATCCTCAGCAGCTTATCTGA-CTTCTTCACGCGAAAGCTTTGAGGTTAC	1431
<i>T. lewisi</i>	TTTACGGGAATATCCTCAGCAGCTTATCTGA-CTTCTTCACGCGAAAGCTTTGAGGTTAC	1445

<i>L. major</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1489
b18	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1513
Sam	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1517
Kai	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1512
<i>T. brucei</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1514
<i>T. evansi</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1514
<i>T. congolense</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1538
<i>T. simiae</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1415
<i>T. vivax</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1433
<i>T. rangeli</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1566
<i>T. cruzi</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1573
<i>T. theileri</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1491
<i>T. lewisi</i>	AGTCTCAGGGGGAGTACGTTTCGCAAGAGTGAACTTAAAGAAATTGACGGAATGGCACC	1505

<i>L. major</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1548
b18	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCCCGG	1573
Sam	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1576
Kai	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGG	1572
<i>T. brucei</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1573
<i>T. evansi</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1573
<i>T. congolense</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1597
<i>T. simiae</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1474
<i>T. vivax</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1492
<i>T. rangeli</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1625
<i>T. cruzi</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1632
<i>T. theileri</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1550
<i>T. lewisi</i>	ACAAGACGTGGAGCGTGCAGTTAATTTGACTCAACACGGGGAACTTTACCAGATCC-GG	1564

<i>L. major</i>	ACAGGATGAGGATTGACAGATTGAGTGTCTTTCTCGATTCCCTGAATGGTGGTGCATGG	1608
b18	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1633
Sam	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1636
Kai	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1632
<i>T. brucei</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1633
<i>T. evansi</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1633
<i>T. congolense</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1657
<i>T. simiae</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1534
<i>T. vivax</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1552
<i>T. rangeli</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1685
<i>T. cruzi</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1692
<i>T. theileri</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1610
<i>T. lewisi</i>	ACAGGGTGAGGATTGACAGATTGAGTGTCTTTCTCGATCCCCGAATGGTGGTGCATGG	1624

<i>L. major</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1668
b18	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1693
Sam	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1696
Kai	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1692
<i>T. brucei</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1693
<i>T. evansi</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1693
<i>T. congolense</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1717
<i>T. simiae</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1594
<i>T. vivax</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1612
<i>T. rangeli</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1745
<i>T. cruzi</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1752
<i>T. theileri</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1670
<i>T. lewisi</i>	CCGCTTTTGGTCGGTGGAGTGATTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGC	1684

<i>L. major</i>	TGCCCAGTAGAATTCAGAATTGCCCAT--AGAATAGCAAACATCATC-----GGCGGGT	1719
b18	TGCCCAGTAGGTGCCGGGATTGTCCACACAGGACAGCAGTCCCTCCGGCG--GGGATTT	1750
Sam	TGCCCAGTAGGTGCCGGGATTGTCCACACAGGACAGCAGTCCCTCCGGCG--GGGATTT	1753
Kai	TGCCCAGTAGGTGCCGGGATTGTCCACACAGGACAGCAGTCCCTCCGGCG--GGGATTT	1749
<i>T. brucei</i>	TGCCCAGTAGGTGCCGGGATTGTCCACACAGGACAGCAGTCCCTCCGGCG--GGGATTT	1750
<i>T. evansi</i>	TGCCCAGTAGGTGCCGGGATTGTCCACACAGGACAGCAGTCCCTCCGGCG--GGGATTT	1750
<i>T. congolense</i>	TGCCCAGTAGGGCCCGTGATTGTCCACACAGGACAGCCTACCGTCGTGGGCACGGTGTGC	1777
<i>T. simiae</i>	TGCCCAGTAGGGCCCGTGATTGTCCAC--AGGACAGCATCCGTGC-----ATGGCGTTC	1647
<i>T. vivax</i>	CGCCCAGTAGGCCCCAGGTTACGCAC--AGGACAGCAGCAGCGTGG-----CGGGCCCT	1665
<i>T. rangeli</i>	TGCCCAGTAGGATTTCAGAATTGCCCAT--AGGATAGCAATCCCTTC-----CGCGGGT	1796
<i>T. cruzi</i>	TGCCCAGTAGGATTTCAGAATTGCCCAT--AGGATAGCAATCCCTTC-----CGCGGGT	1803
<i>T. theileri</i>	TGCCCAGTAGGATTTCAGAATTGCCCAT--AGGATAGCAATCCCTTC-----CGCGGGT	1721
<i>T. lewisi</i>	TGCCCAGTAGGATTTCAGAATTGCCCAT--AGGATAGCAATCCCTTC-----TGCGGGT	1735

<i>L. major</i>	TTTACCCAA-----CGGTGGGCGG---CATTCGGTC-----GAATCTTCTCTGC	1761
b18	TTTCCCAA-----CGGTGGTCGTATCCTTCTTTTACA---GGCCCTTCTCTGC	1799
Sam	TTTCCCAA-----CGGTGGTCGTATCCTTCTTTTACA---GGCCCTTCTCTGC	1802
Kai	TTTCCCAA-----CGGTGGTCGTATCCTTCTTTTACA---GGCCCTTCTCTGC	1798
<i>T. brucei</i>	TTTCCCAA-----CGGTGGTCGTATCCTTCTTTTACA---GGCCCTTCTCTGC	1799
<i>T. evansi</i>	TTTCCCAA-----CGGTGGTCGTATCCTTCTTTTACA---GGCCCTTCTCTGC	1799
<i>T. congolense</i>	TCCTTCGGGGTATCTCGGTGTTCATGGCGGCGTATCACAG--GGGTCTTCTCTGC	1835
<i>T. simiae</i>	GCGCGCCG-----TCGGCGG-----AGCCTATCACAGAAGGGCCCTTCTCTGC	1692
<i>T. vivax</i>	CGGGCCCAA-----CGCGTCCG-----CATCCGGTC-----GGGGCCCTTCTCTGC	1706
<i>T. rangeli</i>	TTTACCTAA-----GGGGGGGCGG---TATTCGTTT-----GTATCCTTCTTGC	1838
<i>T. cruzi</i>	TTTACCAA-----GGGGGGGCGG---TATTCGTTT-----GTATCCTTCTTGC	1845
<i>T. theileri</i>	TTTCCCAA-----GGAGGGGCGA---TATTCGTTT-----GTATCCTTCTTGC	1763
<i>T. lewisi</i>	TTTACCAA-----TGGGGGGCGG---TATTCGTTT-----GTATCCTTCTTGC	1777
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<i>L. major</i>	GGGATTCCTTTGTAATTGCACAAGGTGAATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1821
b18	GGGATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1859
Sam	GGGATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1862
Kai	GGGATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1858
<i>T. brucei</i>	GGGATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1859
<i>T. evansi</i>	GGGATTCCTTGCTTTTCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1859
<i>T. congolense</i>	GGGATTCCTTGCC--CCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1893
<i>T. simiae</i>	GGGATTCCTTGCC--ACGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1750
<i>T. vivax</i>	GGGATTCCTTGC---ACGCGCAAGGTGAGATGCTGGGCAACAGCAGGTCTGTGATGCTCC	1763
<i>T. rangeli</i>	GGGATTCCTTGTT--TCGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1896
<i>T. cruzi</i>	GGGATTCCTTGTT--TTGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1903
<i>T. theileri</i>	GGGATTCCTTGTT--TTGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1821
<i>T. lewisi</i>	GGGATTCCTTGTT--TTGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGATGCTCC	1835

<i>L. major</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGA---AAAACGACTT	1878
b18	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCAC	1919
Sam	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCAC	1922
Kai	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCAC	1918
<i>T. brucei</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCAC	1919
<i>T. evansi</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTCCGAGCGGCAC	1919
<i>T. congolense</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGAGTTACCGCGGCGT	1953
<i>T. simiae</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGA---CACGCGGCAT	1807
<i>T. vivax</i>	TCGATGCTCTGGGCGACACGCGCACTACAATGTCAGTGGAACAAGA---CACGCGGCTC	1820
<i>T. rangeli</i>	TCAATGTTCTGG-CGACACGCGCACTACAATGTCAGTGAGAACAAGA---AAAACGACGC	1952
<i>T. cruzi</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGA---AAAACGACTC	1960
<i>T. theileri</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGA---AAAACGACTT	1878
<i>T. lewisi</i>	TCAATGTTCTGGGCGACACGCGCACTACAATGTCAGTGAGAACAAGA---AAAACGACTT	1892
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<i>L. major</i>	TT-----GTCGAACCTACTTGATCAAAAGAGTGGGGAACCCCGGAATCACATAGACCC	1932
b18	TTCACAATGTCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1979
Sam	TTCACAATGTCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1982
Kai	TTCACAATGTCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1978
<i>T. brucei</i>	TTCACAATGTCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1979
<i>T. evansi</i>	TTCACAATGTCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1979
<i>T. congolense</i>	TT-----GCCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	2007
<i>T. simiae</i>	CT-----GCCGCTCCCGCTTGATCAAAAGAGCGGGGAAACCACGGAATCACGTAGACCC	1861
<i>T. vivax</i>	-----GCCGCGCCCGCTGACCAAAAGGGCGGGGAAGCACCAGGAATCACGTAGACAC	1872
<i>T. rangeli</i>	TT-----GTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCCGGAATCACGTAGACCC	2006
<i>T. cruzi</i>	TT-----GTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCCGGAATCACGTAGACCC	2014
<i>T. theileri</i>	TT-----GTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCCGGAATCACATAGACCC	1932
<i>T. lewisi</i>	CT-----GTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCCGGAATCACATAGACCC	1946
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<i>L. major</i>	ACTTGGGACCGAGGATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	1992
b18	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2039
Sam	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2042
Kai	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2038
<i>T. brucei</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2039
<i>T. evansi</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2039
<i>T. congolense</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2067
<i>T. simiae</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	1921
<i>T. vivax</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	1932
<i>T. rangeli</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2066
<i>T. cruzi</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2074
<i>T. theileri</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	1992
<i>T. lewisi</i>	ACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAATGTCTCGTAGGCGCAGC	2006

<i>L. major</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2052
b18	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2099
Sam	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2102
Kai	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2098
<i>T. brucei</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2099
<i>T. evansi</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2099
<i>T. congolense</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2127
<i>T. simiae</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	1981
<i>T. vivax</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	1992
<i>T. rangeli</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2126
<i>T. cruzi</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2134
<i>T. theileri</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2052
<i>T. lewisi</i>	TCATCAAACGTGCCGATTACGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCG	2066

<i>L. major</i>	ATGATGGTGCAATACAGGTGATCGGACAGCG-GTGTTTATCCGCCGAAAGTTCACCG	2111
b18	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2159
Sam	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2162
Kai	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2158
<i>T. brucei</i>	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2159
<i>T. evansi</i>	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2159
<i>T. congolense</i>	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2187
<i>T. simiae</i>	ATGATGGTGCAATACAGGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCG	2041
<i>T. vivax</i>	ATGCTGGCGCAATACAGGTGATTGGACCGCGGGCGCCTCGCCG-CGGGAAGTTCACCG	2051
<i>T. rangeli</i>	ATGATGGTGCAATACAGGTGATCGGACAGTCGAGTGTCTCATTGACCGAAAGTTCACCG	2186
<i>T. cruzi</i>	ATGATGGTGCAATACAGGTGATCGGACAGTCGAGTGTCTCATTGACCGAAAGTTCACCG	2194
<i>T. theileri</i>	ATGATGGTGCAATACAGGTGATCGGACAGTCGAGTGTCTCATTGACCGAAAGTTCACCG	2112
<i>T. lewisi</i>	ATGATGGTGCAATACAGGTGATCGGACAGTCGAGTGTCTCATTGACCGAAAGTTCACCG	2126
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<i>L. major</i>	ATATTTCTTCAATAGAGGAAGCAAAAGTC	2140
b18	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2188
Sam	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2191
Kai	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2187
<i>T. brucei</i>	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2188
<i>T. evansi</i>	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2188
<i>T. congolense</i>	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2216
<i>T. simiae</i>	ATATTGCTTCAATAGAGGAAGCAAAAGTC	2070
<i>T. vivax</i>	ATATTGCCTCATTAGAGGAAGCAAAAGTC	2080
<i>T. rangeli</i>	ATATTTCTTCAATAGAGGAAGCAAAAGTC	2215
<i>T. cruzi</i>	ATATTTCTTCAATAGAGGAAGCAAAAGTC	2223
<i>T. theileri</i>	ATATTTCTTCAATAGAGGAAGCAAAAGTC	2141
<i>T. lewisi</i>	ATATTTCTTCAATAGAGGAAGCAAAAGTC	2155
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Table 9 Nucleotide sequence alignment of ITS region of *T. evansi* compared to different stocks of *T. brucei* and *T. congolense*.

<i>T. congolense</i>	GGTGATCGGACCGTCGCTCGTCTCACGTGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b15.1f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b15.2f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b17.1f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b17.2f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b18.1f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
b18.2f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
sam.1f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
sam.2f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
kai.1f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
kai.2f	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. evansi</i>	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i>	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> NW2	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> TH2	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> Da1972	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> B8/18	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> KP2	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> STIB215	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGA	60
<i>T. brucei</i> H3	GGTGATCGGACCGTCGCTCGTCTCGGGCGACCGAA-GTTCACCGATATTGCTTCAATAGA	59
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<i>T. congolense</i>	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGCTCATTTTCCGAT	120
b15.1f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
b15.2f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
b17.1f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
b17.2f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
b18.1f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
b18.2f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
sam.1f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
sam.2f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
kai.1f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
kai.2f	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. evansi</i>	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i>	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> NW2	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> TH2	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> Da1972	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> B8/18	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> KP2	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> STIB215	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	120
<i>T. brucei</i> H3	GGAAGCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGAT	119

<i>T. congolense</i>	G-----ATAAAAAAGTATACATACATATGTGTACGTGTAGGTGTAGGT-GTGTGCTATCG	173
b15.1f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
b15.2f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
b17.1f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
b17.2f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
b18.1f	ATCCATTATACAAAAAGAGCATATTTATGTGTATGTATAA-TTGCACAGTATGCAACCA	179
b18.2f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
sam.1f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAA-TTGCACAGTATGCAACCA	179
sam.2f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
kai.1f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
kai.2f	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. evansi</i>	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i>	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAA-TTGCACAGTATGCAACCA	179
<i>T. brucei</i> NW2	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i> TH2	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i> Da1972	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i> B8/18	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i> KP2	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCA	180
<i>T. brucei</i> STIB215	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAA-TTGCACAGTATGCAACCA	179
<i>T. brucei</i> H3	ATCCATTATACAAAAAGAGCATATTTATGTGCATGTATAA-TTGCACAGTATGCAACCA	178
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<i>T. congolense</i>	AAGGTTGTTGTGTGCTTGTGTGCCCTCGCTCATGCGCATCCCCATCCCGCACGCCC	233
b15.1f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
b15.2f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
b17.1f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
b17.2f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
b18.1f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	211
b18.2f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
sam.1f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	211
sam.2f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
kai.1f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
kai.2f	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. evansi</i>	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i>	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	211
<i>T. brucei</i> NW2	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i> TH2	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i> Da1972	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i> B8/18	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i> KP2	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	212
<i>T. brucei</i> STIB215	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	211
<i>T. brucei</i> H3	AAAAT-----ATACATATATGTTTACATGTATGTGT-----	210

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<i>T. congolense</i>	CAGTGTTTTGTGTGCTGTGCGGATGCGGTTGTGTGTTGGGTCGCGCATTGTCGGCGCTGTGA	293
b15.1f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTAAACA--TAGTTCTGTGA	265
b15.2f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
b17.1f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
b17.2f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
b18.1f	-----TTCTATATGCCATTGACATGGGAGATGAGGGATGTTATATA--TAGTTCTGTGA	264
b18.2f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
sam.1f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGTTATATA--TAGTTCTGTGA	264
sam.2f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
kai.1f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTCTACA--TAGTTCTGTGA	265
kai.2f	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATATA--TAGTTCTGTGA	265
<i>T. evansi</i>	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i>	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGTTATATA--TAGTTCTGTGA	264
<i>T. brucei</i> NW2	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i> TH2	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i> Da1972	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i> B8/18	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i> KP2	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGCTATACA--TAGTTCTGTGA	265
<i>T. brucei</i> STIB215	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGTTATATA--TAGTTCTGTGA	264
<i>T. brucei</i> H3	-----TTCTATATGCCGTTTGACATGGGAGATGAGGGATGTTATATA--TAGTTCTGTGA	263

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<i>T. congolense</i>	TGTGCCGGCCACGAACCTTGAACACCTTGAAGCAGCTCTCGCGAAACACACGTTGTCCAAG	353
b15.1f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
b15.2f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
b17.1f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
b17.2f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
b18.1f	TTTTCT-----AATATGT-----ATGTGTGTTAGAG	290
b18.2f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
sam.1f	TTTTCT-----AATATGT-----ATGTGTGTTAGAG	290
sam.2f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
kai.1f	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
kai.2f	TTTTCT-----ATTATGT-----ATGTGTGTTAGAG	291
<i>T. evansi</i>	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i>	TTTTCT-----AATATGT-----ATGTGTGTTAGAG	290
<i>T. brucei</i> NW2	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i> TH2	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i> Da1972	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i> B8/18	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i> KP2	TTTTCT-----ATCATGT-----ATGTGTGTTAGAG	291
<i>T. brucei</i> STIB215	TTTTCT-----AATATGT-----ATGTGTGTTAGAG	290
<i>T. brucei</i> H3	TTTTCT-----AATATGT-----ATGTGTGTTAGAG	289

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T. congolense      CACGCGTCTCCATGTCGCTGTCTCTCTCTTGTGTTGCGAAGATGCTTACTGTCGTTGTTG 413
b15.1f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
b15.2f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
b17.1f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
b17.2f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
b18.1f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
b18.2f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 320
sam.1f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
sam.2f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 320
kai.1f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
kai.2f            -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. evansi         -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei         -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei NW2     -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 320
T. brucei TH2     -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei Da1972  -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei B8/18   -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei KP2     -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 321
T. brucei STIB215 -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 320
T. brucei H3      -----TGTC-----TGTGTT---AATATACTTTTAA--ATGCATG 319
                ****          *****  ** * * * *  *   * * * *

T. congolense      CTCCGCCAGGCGGCGCGGCCGTGACGTATGTGTCCGCAAGACAAAAGAAGGGTGTGGAG 473
b15.1f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
b15.2f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
b17.1f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
b17.2f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
b18.1f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
b18.2f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 366
sam.1f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
sam.2f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 366
kai.1f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
kai.2f            CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. evansi         CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei         CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 366
T. brucei NW2     CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei TH2     CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei Da1972  CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei B8/18   CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei KP2     CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 367
T. brucei STIB215 CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 366
T. brucei H3      CTCTACATAATA-TACAGTAGTAAT-----AACACAGAGAATACGTATGGAA 365
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T. congolense      GAGACGCGGTGTTCTTATGCCGCTGACGCTTTTGTGTGCGCACCGGCTCGGCTCATT 533
b15.1f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
b15.2f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GTGTATAT 415
b17.1f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GTGTATAT 415
b17.2f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GTGTATAT 415
b18.1f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 416
b18.2f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GTGTATAT 415
sam.1f            T-----GCGTATCTCTCT---ACATATA-TTTATGTATATATGCTATGT--GT--ATAT 412
sam.2f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
kai.1f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
kai.2f            T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
T. evansi         T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GTGTATAT 415
T. brucei         T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 406
T. brucei NW2     T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
T. brucei TH2     T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
T. brucei Da1972  T-----GCGTATCTCTCT---ATATATA-TTTATGTATATATGCTATGT--GT--ATAT 413
T. brucei B8/18   T-----GCGTATCTCTCT---ATATATA-TATGTGTATATATGCTATGT--GT--ATAT 413
T. brucei KP2     T-----GCGTATCTCTCT---ATATATA-TATGTGTATATATGCTATGT--GT--ATAT 413
T. brucei STIB215 T-----GCGTATCTCTCTAT--ATATATA-TATATGTATATATGCTATGT--GT--ATAT 414
T. brucei H3      T-----GCGTATCTCTCTATCTATATATA-TATATGTATATATGCTATGT--GT--ATAT 415
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<i>T. congolense</i>	TTTCCTCCCTCTCTCTTTTCTCTCTTCTTTTCCCCGCCTTCCACGTGTGTTGGGAG	593
b15.1f	CAACCTC---GCATATTTTCTCCCT-----	435
b15.2f	CAACCTC---GCATATTTTCTCCCT-----	437
b17.1f	CAACCTC---GCATATTTTCTCCCT-----	437
b17.2f	CAACCTC---GCATATTTTCTCCCT-----	437
b18.1f	CAACCTC---GTATATTTTCTCCCT-----	438
b18.2f	CAACCTC---GCATATTTTCTCCCT-----	437
sam.1f	CAACCTC---GCATATTTTCTCCCT-----	434
sam.2f	CAACCTC---GCATATTTTCTCCCT-----	435
kai.1f	CAACCTC---GCATATTTTCTCCCT-----	435
kai.2f	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. evansi</i>	CAACCTC---GCATATTTTCTCCCT-----	437
<i>T. brucei</i>	AAACCTC---GCATATTTTCTCCCT-----	428
<i>T. brucei</i> NW2	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. brucei</i> TH2	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. brucei</i> Da1972	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. brucei</i> B8/18	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. brucei</i> KP2	CAACCTC---GCATATTTTCTCCCT-----	435
<i>T. brucei</i> STIB215	CAACCTC---GCATATTTTCTCCCT-----	437
<i>T. brucei</i> H3	CAACCTC---GCATATTTTCTCCCT-----	437

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<i>T. congolense</i>	AGGACAGAGGGCAAGAAGGCGTGGTGTATTGGAGGAATGTGCGGGGTGAAATGGTGAAA	653
b15.1f	-----	
b15.2f	-----	
b17.1f	-----	
b17.2f	-----	
b18.1f	-----	
b18.2f	-----	
sam.1f	-----	
sam.2f	-----	
kai.1f	-----	
kai.2f	-----	
<i>T. evansi</i>	-----	
<i>T. brucei</i>	-----	
<i>T. brucei</i> NW2	-----	
<i>T. brucei</i> TH2	-----	
<i>T. brucei</i> Da1972	-----	
<i>T. brucei</i> B8/18	-----	
<i>T. brucei</i> KP2	-----	
<i>T. brucei</i> STIB215	-----	
<i>T. brucei</i> H3	-----	

<i>T. congolense</i>	GCGTGCGTGCGTGCGCAGGCGTTGGTCACGGCTCTCACACGTGTCGCGATGGATGACTT	713
b15.1f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
b15.2f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
b17.1f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
b17.2f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
b18.1f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	478
b18.2f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
sam.1f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	474
sam.2f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
kai.1f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
kai.2f	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. evansi</i>	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
<i>T. brucei</i>	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	468
<i>T. brucei</i> NW2	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. brucei</i> TH2	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. brucei</i> Da1972	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. brucei</i> B8/18	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. brucei</i> KP2	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	475
<i>T. brucei</i> STIB215	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477
<i>T. brucei</i> H3	-----GTTGACCACGGCTCCCACAACGTGTCGCGATGGATGACTT	477

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<i>T. congolense</i>	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	773
b15.1f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
b15.2f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
b17.1f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
b17.2f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
b18.1f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	538
b18.2f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
sam.1f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	534
sam.2f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
kai.1f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
kai.2f	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. evansi</i>	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
<i>T. brucei</i>	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	528
<i>T. brucei</i> NW2	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. brucei</i> TH2	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. brucei</i> Da1972	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. brucei</i> B8/18	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. brucei</i> KP2	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	535
<i>T. brucei</i> STIB215	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537
<i>T. brucei</i> H3	GGCTTCCTATTTTCGTTGAAGAACGACGCAAAGTGCATAAGTGGTATCAATTGCAGAATC	537

<i>T. congolense</i>	ATCACATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	833
b15.1f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
b15.2f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	597
b17.1f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	597
b17.2f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	597
b18.1f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	598
b18.2f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	597
sam.1f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	594
sam.2f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
kai.1f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
kai.2f	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
<i>T. evansi</i>	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	597
<i>T. brucei</i>	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	588
<i>T. brucei</i> NW2	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
<i>T. brucei</i> TH2	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
<i>T. brucei</i> Da1972	ATTTTCATTGCCCAATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	595
<i>T. brucei</i> B8/18	ATTTTCATTG----ATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	591
<i>T. brucei</i> KP2	ATTTTCATTG----ATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	591
<i>T. brucei</i> STIB215	ATTTTCATTG----ATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	593
<i>T. brucei</i> H3	ATTTTCATTG----ATCTTTGAGCGCAAACGGCGCATGGGAGAAGCTCTCTCGAGCCATCC	593
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<i>T. congolense</i>	CCGTGCATGCCACATT-CTCAGTGTGACCAACATAAGAACAACAACAGTGCCCTCTTCC	892
b15.1f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	642
b15.2f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	644
b17.1f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	644
b17.2f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	644
b18.1f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	645
b18.2f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	644
sam.1f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	641
sam.2f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAGAAACAA-AACA-----CAC	642
kai.1f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAGAAACAA-AACA-----CAC	642
kai.2f	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACG-----CAC	642
<i>T. evansi</i>	CCGTGCATGCCACATTCTCAGTGTGCA---ATATCAAAACAA-AACG-----CAC	644
<i>T. brucei</i>	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	635
<i>T. brucei</i> NW2	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	642
<i>T. brucei</i> TH2	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACG-----CAC	642
<i>T. brucei</i> Da1972	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	642
<i>T. brucei</i> B8/18	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACG-----CAC	638
<i>T. brucei</i> KP2	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACG-----CAC	638
<i>T. brucei</i> STIB215	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACA-----CAC	640
<i>T. brucei</i> H3	CCGTGCATGCCACATTCTCAGTGTGCA---ATATAAAACAA-AACG-----CAC	640

<i>T. congolense</i>	CTCTGTTTCTCTCTCTGTTGATGGGCAGGGTGTGGGGTGTGTGCTGTGTATATGTTGTTG	952
b15.1f	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTCTT-CTCCTC-	695
b15.2f	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTCTTCTCCTC-	698
b17.1f	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	697
b17.2f	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	697
b18.1f	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	698
b18.2f	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	697
sam.1f	ACCTATTTTGTG-----TGTCGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCCC-	694
sam.2f	ACCTATTTTGTGTTGTTGTTTCAACGCACGCAAAAAATCCCGCCACCTCTTCTCCTCC	702
kai.1f	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTCTT-CTCCTC-	695
kai.2f	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTCTTCTCCCC	697
<i>T. evansi</i>	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	697
<i>T. brucei</i>	ACCTATTTTGTG---TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	690
<i>T. brucei</i> NW2	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	695
<i>T. brucei</i> TH2	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	695
<i>T. brucei</i> Da1972	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	695
<i>T. brucei</i> B8/18	ACCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	691
<i>T. brucei</i> KP2	ATCTATTTTGTG-----TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	691
<i>T. brucei</i> STIB215	ACCTATTTTGTG---TGTTGTTCAACGCACGCAAAAAATCCCGCCACCTC-TTCTCCTC-	695
<i>T. brucei</i> H3	ACCTATTTTGTG---TGTTGTTAAACGCACGCAAAAAATCCCGCCCTCTC-TTCTTCTC-	695
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<i>T. congolense</i>	TCGTGGCGTGTGTGAAAGTGGTGTGGTGGGGCGACGCGCATGTGCGCATCGTTGTTACGT	1012
b15.1f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	749
b15.2f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	752
b17.1f	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATACCTTTCAACT	750
b17.2f	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	750
b18.1f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	752
b18.2f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	751
sam.1f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	748
sam.2f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	756
kai.1f	GTGTGG--TGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	749
kai.2f	GTGTGGGGTGCATATTCATGGTTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	753
<i>T. evansi</i>	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	750
<i>T. brucei</i>	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	743
<i>T. brucei</i> NW2	GTGTGG--GGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	748
<i>T. brucei</i> TH2	GTGTGG--GGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	748
<i>T. brucei</i> Da1972	GGGGGG--GGCATATTCATG-TTTG--AGTGTG---CACAAACACG-ATATCTTTCAACT	746
<i>T. brucei</i> B8/18	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	744
<i>T. brucei</i> KP2	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	744
<i>T. brucei</i> STIB215	GTGTGG--TGCATATTCATG-TTTGTGAGTGTG---CACATATACG-ATATCTTTCAACT	748
<i>T. brucei</i> H3	GTGTGG--TGCATATATGTG-TGTGAGAGTGCA---CACATATACG-ATAT-TTTCACCT	747
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<i>T. congolense</i>	ACGTACATTTACGCACACACACGTGTGCTGCGTGTGTATGTGTTTGTGTTCCCATATT	1072
b15.1f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	802
b15.2f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	805
b17.1f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	803
b17.2f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	803
b18.1f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	805
b18.2f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	804
sam.1f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	801
sam.2f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	809
kai.1f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	802
kai.2f	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	806
<i>T. evansi</i>	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	803
<i>T. brucei</i>	CTTTCTACTCGCACA-ATT-GTATATGTCACGCATATACGTGTGTGTAGTG-----AGTG	796
<i>T. brucei</i> NW2	CTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	801
<i>T. brucei</i> TH2	CTTTCTTCTCGCACA-ATT-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	802
<i>T. brucei</i> Da1972	CTTTATACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	799
<i>T. brucei</i> B8/18	GTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	797
<i>T. brucei</i> KP2	CTTTCTCTCTCGCACA-ATT-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	797
<i>T. brucei</i> STIB215	GTTTCTACTCGCACA-ATG-GTGTATGTCACGCATATACGTGTGTGTAGTG-----AGTG	801
<i>T. brucei</i> H3	GTTTCTACTCGCACA-ATG-GTGTGTGACACGCATATACGTGTGTGTAG-----AGAG	798
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<i>T. congolense</i>	ATTTTTCATGCCGTCAGGAAGGAGAGAGTCCGGTGTGTGCATAGTGGTGTGTGGCGTGC	1132
b15.1f	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATACC---ATAAATATATAT	859
b15.2f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	860
b17.1f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	858
b17.2f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	858
b18.1f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	860
b18.2f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	859
sam.1f	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	856
sam.2f	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	864
kai.1f	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	857
kai.2f	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	861
<i>T. evansi</i>	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	858
<i>T. brucei</i>	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	849
<i>T. brucei</i> NW2	ATATGCAACAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	856
<i>T. brucei</i> TH2	ATATGAAAGAGAAATGGGAAAGGCATATGTATATATGTATACAC----ATAA-TATATAT	857
<i>T. brucei</i> Da1972	ATATGGAAGAGAAATGGGAGAGGCATATGTATATATGTATATAC----ATAA-TATATAT	854
<i>T. brucei</i> B8/18	ATATGGAAGAGAAATGGGAAAGGCATATGTATATATGTATATAC----ATAA-TATATAT	852
<i>T. brucei</i> KP2	ATATGGAAGAGAAATGGGAAAGGCATATATATATATGTATATAC----ATAA-TATATAT	852
<i>T. brucei</i> STIB215	ATATGGAAGAGAAATGGGAAAGGCATAT--ATATATGTATATAC----ATAA-TATATAT	854
<i>T. brucei</i> H3	ATATGGAAGAGAGA-GGAAGGGGCATATATATATATACACACA-----AA-----ATAT	846
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<i>T. congolense</i>	GTGTGAAGGGTT-GTGT--TTGTGTTCTATATGAGTGTGTGGCATGTGTGAGTGGTGTCT	1189
b15.1f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----CAT	909
b15.2f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	910
b17.1f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	908
b17.2f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	908
b18.1f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	910
b18.2f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----CAT	909
sam.1f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	906
sam.2f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----CAT	914
kai.1f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----CAT	907
kai.2f	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	911
<i>T. evansi</i>	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	908
<i>T. brucei</i>	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	898
<i>T. brucei</i> NW2	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	906
<i>T. brucei</i> TH2	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	907
<i>T. brucei</i> Da1972	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	904
<i>T. brucei</i> B8/18	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	902
<i>T. brucei</i> KP2	GTGTGTGGATTTTGTGTTGTTGAGCAC-ATATAAG-AAAAAGGGTGGCGTG-----TAT	903
<i>T. brucei</i> STIB215	GTGTGTGGATTT-GTGT-GTTGAGCAC-ATA---GGAAAAGGGTGGCGTG-----TAT	900
<i>T. brucei</i> H3	ATGTGTGGATTT-GTGT-GTTGAGCAC-ATATAAGGAAAAGGGTGGCGTG-----TAT	896
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<i>T. congolense</i>	GTGCAAGAAGGTGTGGTGTCTTGTGGCGGTGCTTTTGCATGTTGTTTGTCCCGCTTGA	1249
b15.1f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATATGTA	959
b15.2f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	958
b17.1f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	956
b17.2f	ATACA-GAGAGTCTGTGGCGGTTG-AGACGTGTATGTATATAT-ATCTATAATATATGTA	965
b18.1f	ATACA-GAGAGTCTGTGGCGGTTG-AGACGTGTATGTATATAT-ATCTATAATATATGTA	967
b18.2f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATATGTA	959
sam.1f	ATACA-GAGAGTCTGTGGCGGTTG-AGACGTGTATGTATATAT-ATCTATAATATATGTA	963
sam.2f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATATGTA	964
kai.1f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATATGTA	957
kai.2f	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	959
<i>T. evansi</i>	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	956
<i>T. brucei</i>	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	946
<i>T. brucei</i> NW2	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	954
<i>T. brucei</i> TH2	ACACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	955
<i>T. brucei</i> Da1972	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	952
<i>T. brucei</i> B8/18	ACACA-GAGAGTCTGTGGCGGTTG-GCACATGTGTATAAATAT-AT-----ATGTA	950
<i>T. brucei</i> KP2	ATACA-GAGAGTCTGTAGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	951
<i>T. brucei</i> STIB215	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	948
<i>T. brucei</i> H3	ATACA-GAGAGTCTGTGGCGGTTG-GGACATGTGTATAAATAT-AT-----ATGTA	944
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<i>T. congolense</i>	GACGGCCCCGCTTTTCTCCCTCACTTTCTCTCCCGTGCTGCCATCACTACACTTCCTT	1309
b15.1f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1015
b15.2f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1014
b17.1f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1012
b17.2f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1021
b18.1f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1023
b18.2f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1015
sam.1f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1019
sam.2f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1020
kai.1f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1013
kai.2f	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1015
<i>T. evansi</i>	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1012
<i>T. brucei</i>	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1002
<i>T. brucei</i> NW2	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1010
<i>T. brucei</i> TH2	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1011
<i>T. brucei</i> Da1972	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATAGATAT	1008
<i>T. brucei</i> B8/18	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGACGTGTTTCATATATAT	1007
<i>T. brucei</i> KP2	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1007
<i>T. brucei</i> STIB215	TATGTGTGTGTTCCCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1004
<i>T. brucei</i> H3	TATGTGTGTGTTCCGCTGTGGAGATTTTATATCT---TACGGAGA-GTGTTTCATATATAT	1000
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<i>T. congolense</i>	TCGGCCATAGCAGTAGAACACCCTTGCCCTCCCCCGGTGTGTTTACCTCGAAGCCATTGA	1369
b15.1f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1072
b15.2f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1071
b17.1f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1069
b17.2f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1078
b18.1f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1080
b18.2f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1072
sam.1f	--GTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAGGAGAAAAA	1074
sam.2f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1077
kai.1f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1070
kai.2f	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1072
<i>T. evansi</i>	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1069
<i>T. brucei</i>	--GTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1057
<i>T. brucei</i> NW2	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1067
<i>T. brucei</i> TH2	ATGTTTGAACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1068
<i>T. brucei</i> Da1972	ATGTTTGAACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1065
<i>T. brucei</i> B8/18	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1064
<i>T. brucei</i> KP2	ATGTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1064
<i>T. brucei</i> STIB215	--GTTTGTACGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1059
<i>T. brucei</i> H3	--GTTTGTAGGCATG---TATTTTGGCGCCCCGTATAGAGATTAAAAAGGAAGAGAAAAA	1055
	* * * * *	
<i>T. congolense</i>	GCTAAAGAGACAAAGTGGGGAATGATCTTGGAGAGAGGCTCTTGTCTTCTCTCACCTCCA	1429
b15.1f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1129
b15.2f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGCATGTGTGTATTCA-CAGCAAGCAACTATA	1128
b17.1f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGCATGTGTGTATTCA-CGGAAGCAACTATA	1126
b17.2f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGCATGTGTGTATTCA-CAGCAAGCAACTATA	1135
b18.1f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGCATGTGTGTATTCA-CAGCAAGCAACTATA	1137
b18.2f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1129
sam.1f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1131
sam.2f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1134
kai.1f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1127
kai.2f	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1129
<i>T. evansi</i>	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1126
<i>T. brucei</i>	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTAT-CA-CAGGAAGCAACTATA	1113
<i>T. brucei</i> NW2	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1124
<i>T. brucei</i> TH2	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1125
<i>T. brucei</i> Da1972	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1122
<i>T. brucei</i> B8/18	G-TATGCAAAACAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1121
<i>T. brucei</i> KP2	G-TATGCAAAAGAGGCGGCGGATAG-TGTGTATGTGTGTATTCA-CAGCAAGCAACTATA	1121
<i>T. brucei</i> STIB215	G-TATGCAAAAGAGGCGACGGATAG-TGTGTATGTGTGTAT-CA-CAGGAACCAACTATA	1115
<i>T. brucei</i> H3	G-TATGCAAAAGAGGCGGCGGATAGTGTGTATGTGTGTAT-CA-CAGCAAGCAACTATA	1112
	* * * * *	

<i>T. congolense</i>	CATAACTG-TTGTGC-TTTG-TGCATCCCGT-CACGCGATGTACGCACGCTAACCAAAGT	1485
b15.1f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1186
b15.2f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1185
b17.1f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1183
b17.2f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1192
b18.1f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1194
b18.2f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1186
sam.1f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1188
sam.2f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1191
kai.1f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1184
kai.2f	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1186
<i>T. evansi</i>	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1183
<i>T. brucei</i>	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1170
<i>T. brucei</i> NW2	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1181
<i>T. brucei</i> TH2	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1182
<i>T. brucei</i> Da1972	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1179
<i>T. brucei</i> B8/18	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1178
<i>T. brucei</i> KP2	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1178
<i>T. brucei</i> STIB215	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTCAGT	1175
<i>T. brucei</i> H3	TTTTGCTGCTTGTGAGTATA-TGCA-TATATGTACATTATGTGCTTGTGCTTCTTTTC-GT	1169
	* * * * *	
<i>T. congolense</i>	TCAT-----ATGTATGTACATG----TTGACAGACCTGAGTGTGGCAGGACCACCCGCT	1535
b15.1f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1244
b15.2f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1244
b17.1f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1242
b17.2f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1251
b18.1f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1253
b18.2f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1244
sam.1f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1247
sam.2f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1250
kai.1f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1242
kai.2f	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1245
<i>T. evansi</i>	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1241
<i>T. brucei</i>	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1229
<i>T. brucei</i> NW2	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1240
<i>T. brucei</i> TH2	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1241
<i>T. brucei</i> Da1972	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1238
<i>T. brucei</i> B8/18	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1238
<i>T. brucei</i> KP2	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1237
<i>T. brucei</i> STIB215	GTACGGCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1235
<i>T. brucei</i> H3	GTAC-GCTTCACCTTTTTTATATTGTATTTT-CAGACCTGAGTGTGGCAGGACCACCCGCT	1228
	* * * * *	
<i>T. congolense</i>	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1577
b15.1f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1286
b15.2f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1286
b17.1f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1284
b17.2f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1293
b18.1f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1295
b18.2f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1286
sam.1f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1289
sam.2f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1292
kai.1f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1284
kai.2f	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1287
<i>T. evansi</i>	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1283
<i>T. brucei</i>	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1271
<i>T. brucei</i> NW2	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1282
<i>T. brucei</i> TH2	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCT	1283
<i>T. brucei</i> Da1972	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1280
<i>T. brucei</i> B8/18	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1280
<i>T. brucei</i> KP2	AAACTTAAGCATATTACTCAGCGAAGGAAAAGAAAAGTTCCG	1279
<i>T. brucei</i> STIB215	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1277
<i>T. brucei</i> H3	AAACTTAAGCATATTACTCAGCGGAGGAAAAGAAAACAACCG	1270

Phylogenetic analysis of *T. evansi*

The phylogenetic relationship of *T. evansi* based on 18S rDNA and ITS nucleotide sequences was achieved by PAUP program version 4.0. The cladograms were created by using likelihood method and bootstrap 50% majority rule consensus tree to show stability for the trees. The likelihood parameters were set as followed; the number of substitution types = 2 (HKY85 variant), transition/transversion ratio = estimate, distribution of rates at variable sites = gamma (discrete approximation) and shape parameter (alpha) = estimate. The bootstrap was performed by heuristic search with the number of bootstrap = 100 replicates, number of trees held at each step during stepwise addition = 1, initial 'MaxTrees' setting = 100, addition sequence = as-is and Branch-swapping algorithm = tree-bisection-reconnection (TBR). The percentage of nucleotide homologous when compared to the outgroup was shown in Table 10-13. The phylogenetic trees of 18S rDNA and ITS region were shown in Figure 11-14, respectively.

Table 10 The percentage of nucleotide homologous of 18S rDNA among *Trypanosoma* spp. when compared to *L. major*. (outgroup).

<i>Trypanosoma</i> spp.	The percentage of nucleotide homologous (%)
B18	85.127
SAM	85.218
KAI	85.002
<i>T. brucei</i>	85.405
<i>T. congolense</i>	84.640
<i>T. cruzi</i>	89.755
<i>T. evansi</i>	85.453
<i>T. rangeli</i>	89.655
<i>T. simiae</i>	86.349
<i>T. vivax</i>	83.582
<i>T. theileri</i>	90.247
<i>T. lewisi</i>	90.168

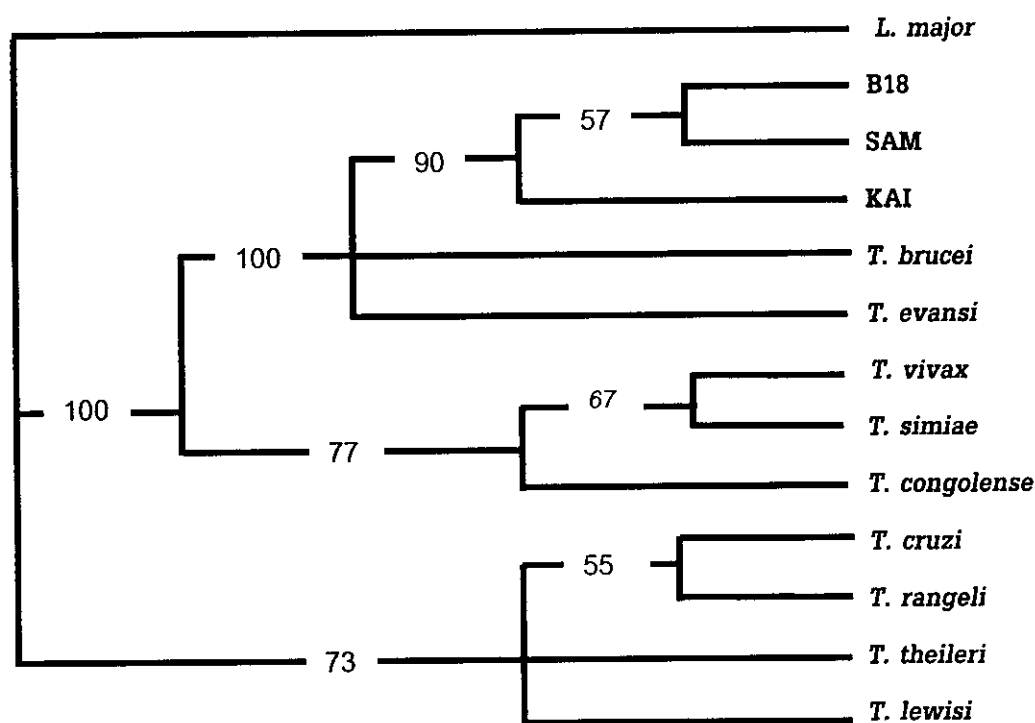


Figure 11 Phylogenetic relationship of 18S rDNA obtained from the likelihood methods between *Trypanosoma* spp. and *L. major* (outgroup). The tree was constructed based on the heuristic search using PAUP version 4.0 and inferred from complete 18S rDNA gene sequences using a bootstrap resampling of the data set. The number at the nodes represent the percentage of times the group occurred out of 100 trees. Branches with less than 50% bootstrap support were collapsed. No significance is placed on the lengths of the branches connecting the species.

Table 11 The percentage of nucleotide homologous of ITS region among different stocks of *T. evansi* and *T. brucei* when compared to *T. congolense* (outgroup).

<i>Trypanosoma</i> spp.	The percentage of nucleotide homologous (%)
B15.1	62.230
B15.2	62.217
B17.1	62.157
B17.2	61.959
B18.1	61.882
B18.2	62.085
SAM.1	62.544
SAM.2	62.019
KAI.1	62.335
KAI.2	62.087
<i>T. evansi</i>	62.290
<i>T. brucei</i>	62.398
<i>T. brucei</i> NW2	62.397
<i>T. brucei</i> TH2	61.738
<i>T. brucei</i> Da1972	62.194
<i>T. brucei</i> B8/18	61.890
<i>T. brucei</i> KP2	61.917
<i>T. brucei</i> STIB215	61.962
<i>T. brucei</i> H3	62.389

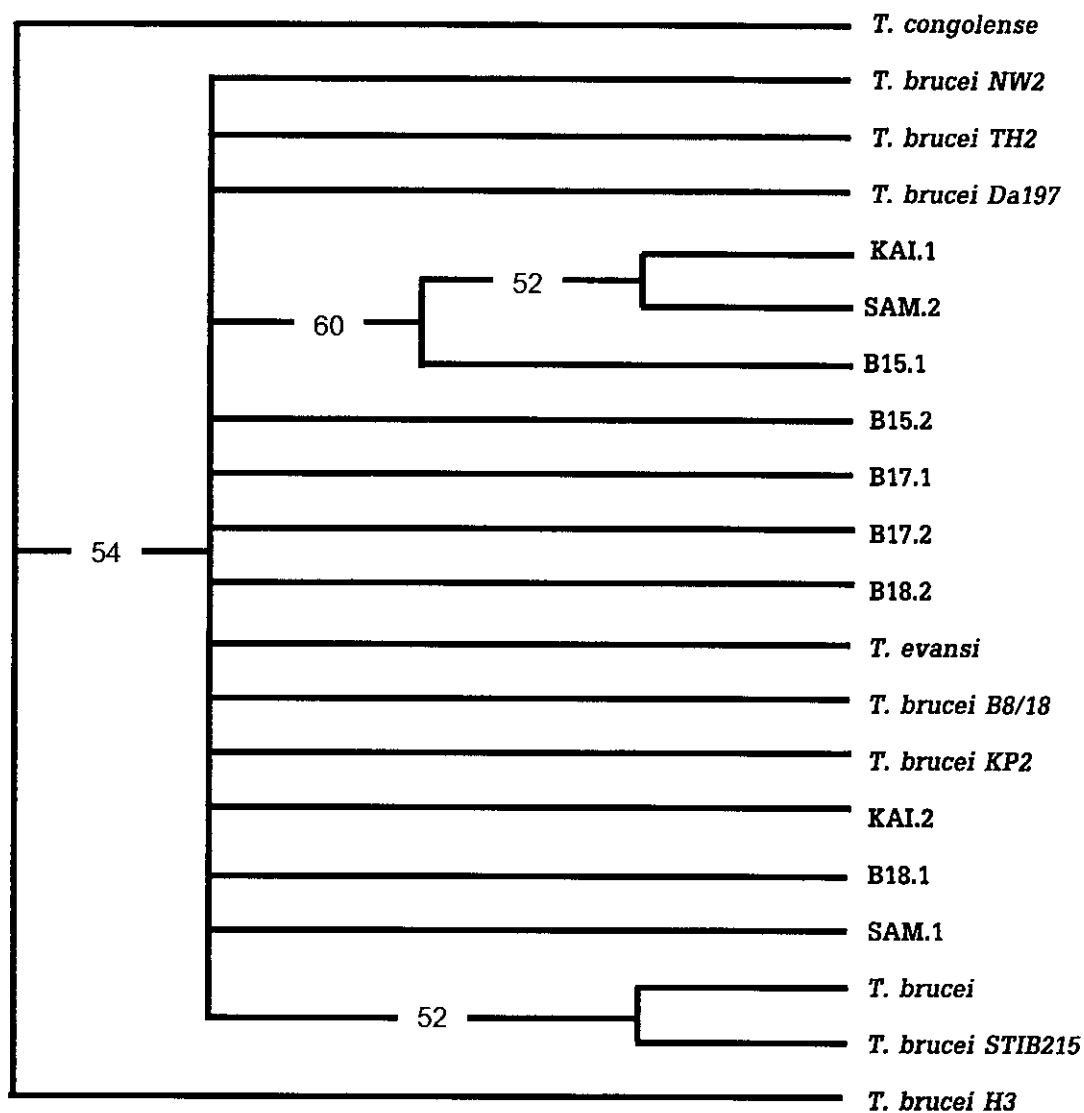


Figure 12 A likelihood cladogram (phylogram usually shows branchlengths.) of ITS region among *T. evansi*, *T. brucei* and *T. congolense* (outgroup) produced by the heuristic search of PAUP version 4.0 and inferred from complete ITS region using a bootstrap resampling of the data set. The number at the nodes represent the percentage of times the group occurred out of 100 trees. Branches with less than 50% bootstrap support were collapsed. No significance is placed on the lengths of the branches connecting the species.

Table 12 The percentage of nucleotide homologous of ITS1 from different stocks of *T. evansi* in Thailand when compared to *T. brucei* (outgroup).

<i>Trypanosoma</i> spp.	The percentage of nucleotide homologous (%)
B15.1	97.550
B15.2	97.777
B17.1	97.557
B17.2	97.777
B18.1	98.427
B18.2	97.777
SAM.1	98.217
SAM.2	97.996
KAI.1	97.771
KAI.2	98.217
<i>T. evansi</i>	98.000

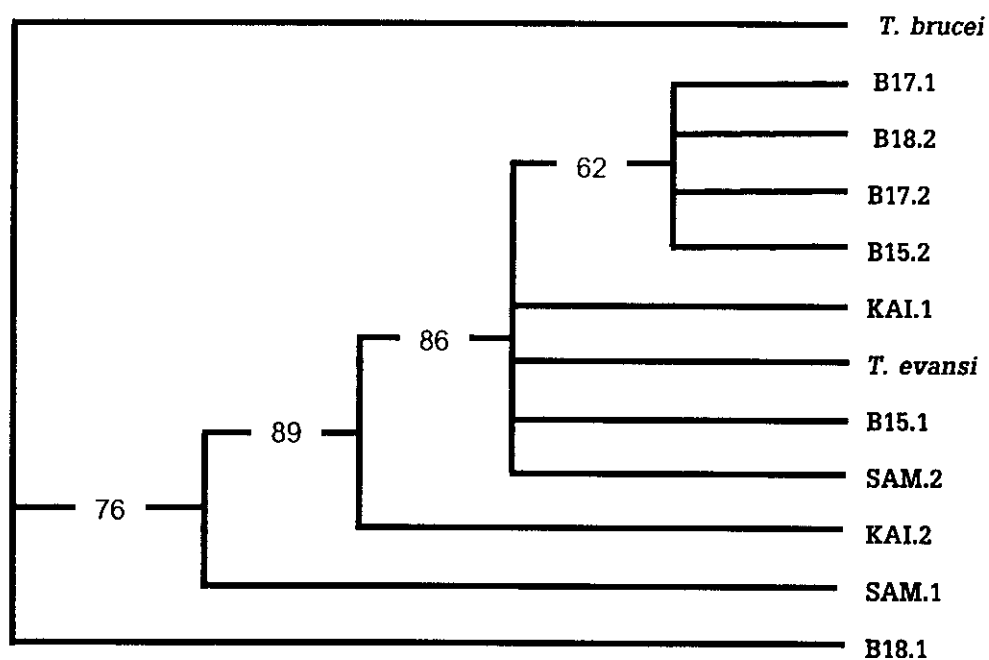


Figure 13 A likelihood methods of ITS1 among *T. evansi* (Thai stocks) and *T. brucei* (outgroup) produced by the heuristic search of PAUP version 4.0 and inferred from complete ITS region using a bootstrap resampling of the data set. The number at the nodes represent the percentage of times the group occurred out of 100 trees. Branches with less than 50% bootstrap support were collapsed. No significance is placed on the lengths of the branches connecting the species.

Table 13 The percentage of nucleotide homologous of ITS2 from different stocks of *T. evansi* in Thailand when compared to *T. brucei* (outgroup).

<i>Trypanosoma</i> spp.	The percentage of nucleotide homologous (%)
B15.1	98.009
B15.2	97.555
B17.1	97.256
B17.2	96.803
B18.1	96.801
B18.2	97.709
SAM.1	97.408
SAM.2	97.875
KAI.1	98.012
KAI.2	97.558
<i>T. evansi</i>	97.860

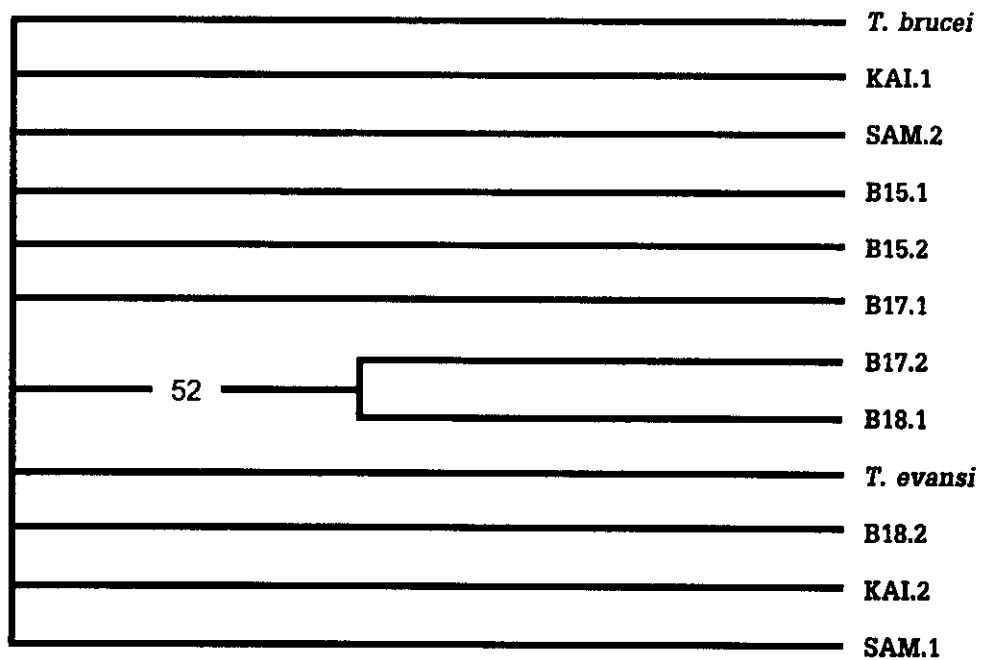


Figure 14 A likelihood methods of ITS2 among *T. evansi* (Thai stocks) and *T. brucei* (outgroup) produced by the heuristic search of PAUP version 4.0 and inferred from complete ITS region using a bootstrap resampling of the data set. The number at the nodes represent the percentage of times the group occurred out of 100 trees. Branches with less than 50% bootstrap support were collapsed. No significance is placed on the lengths of the branches connecting the species.

CHAPTER 5

DISCUSSION

T. evansi is one of the most common haemoflagellate blood parasite found in larger mammals, which is of great economic importance in Africa, Asia and South America (193). Although several reports have suggested that *T. evansi* could cause epidemics of disease called 'Surra', the certain pathogenicity of this organism need to be defined. The life cycle of *T. evansi* has been proposed (5), however, its validation has to be confirmed experimentally. It is assumed that *T. evansi* is transmitted by the blood sucking insects but the transmission route was not exclusively identified yet. This study has used molecular phylogenetic as a tool to explore these *T. evansi* from different local and perceived the gene variation of *T. evansi* in different hosts in Thailand. Molecular phylogenetic provides the tools that have predictive significance and that developed the methods to the epidemiologists can use to better define the epidemiology of *T. evansi*, and to towards their control. The comparison of *T. evansi* Thai stocks to *T. evansi* and other *Trypanosoma* spp. in GenBank database was achieved. The data perceived the molecular genetic differences of *T. evansi*.

Phylogenetic relationship of 18S rDNA among *Trypanosoma* spp. and *L. major*.

The major problem for classification and identification of the trypanosomes, in particular within the *Trypanozoon* subgenus (*T. brucei brucei*, *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi* and *T. equipedium*) are morphologically identical and are classified according to host type of disease and geographical distribution (192). In many cases, exceptions to these criteria were observed (194), necessitating the development of biochemical and genotype criteria such as isoenzyme patterns (74, 77, 195-199), restriction fragment length polymorphisms (RFLP) (86) in variant surface glycoproteins (VSGs) (200, 201) or ribosomal RNA genes (194), conserved antigenic repertoires (202) and specific kinetoplast DNA probes (203) have been used to determine genetic heterogeneity among *Trypanozoon*. The results suggest that several strains of this parasite are existing. Although, more experiments reported the strain variation of *T. brucei* and other trypanosomes but it is unknown whether *T. evansi* have a strain variation of nucleotide

sequences from different host and different locals. This paradoxical observation is explained by the hypothesis that strain variation of *T. evansi*, exhibiting similar morphological features but with a different nucleotide sequences, may exist. If this present really, it could be link to the uncertainty over the role of this organism regarding to the disease and host specifics.

The experiment of 18S rDNA analysis nucleotide sequencing of 3 stocks of *T. evansi* in Thailand compared to 7 stocks of *Trypanosoma* spp. and one stock of *L. major* in GenBank, The result showed percentage homologous nucleotide sequencing (shown in Table 11) and cladogram (shown in Figure 11) when analyzed by using PAUP program version 4.0. The comparison of 18S rDNA sequences from different *Trypanosoma* spp. stocks had enabled to confirm phylogenetic position of *T. evansi* in the *Trypanosomatids* family and showed that *T. evansi* stocks from different hosts in different areas in Thailand belong to a single genus based on the molecular phylogenetic analysis. Within the *Trypanosomatids* when rooted cladogram (phylogram usually shows branchlengths.) with *L. major*, *T. evansi* seems closely related *T. brucei*. To confirmed this relationship (high bootstrap value (100%)), more sequence data of other significant gene are required. However, the cladogram showed that the clade of *T. evansi* in Thailand were separated from the *T. evansi* and *T. brucei* clade relationship (high bootstrap value (91%)) of which the nucleotide sequences were previously reported in GenBank. This indicated the variation of 18S rDNA nucleotide sequences among *T. evansi* stocks.

Although, morphological studies proposed that the *Trypanosoma* sp. in Thailand is *T. evansi* but the results of phylogenetic analysis showed that it was closely related to both *T. evansi* and *T. brucei* (high bootstrap value (100%)). This cladogram may be proposed a new hypothesis to reclassify *T. evansi* and *T. brucei* and it is possible to incorporate twice species to generate a new species to replace as former ones.

Phylogenetic relationship of ITS region among *T. evansi* and *T. brucei*.

The phylogenetic relationship of ITS region of 5 stocks of *T. evansi* from different areas in Thailand were compared to 8 stocks of *T. brucei*, one stock of *T. evansi* and *T. congolense* from Genbank using phylogenetic analysis with PAUP program version 4.0. The result showed percentage homologous nucleotide sequencing (shown in Table 12) and cladogram (shown in Figure 12).

The comparison of ITS region from different hosts of *T. evansi* and *T. brucei* when rooted cladogram with *T. congolense* indicated that the relationship of *T. evansi* are very closely related to *T. brucei* relationship (low bootstrap value (54%)). The deep relationships within the clades of *T. evansi* and *T. brucei* were classified within the same clade excepted that some clades were separated. Both of species from monophyletic groups that are lower supported by bootstrap. However, some stocks of *T. evansi* are separated from groups of branches, indicating groups of *T. evansi* stocks (KAI.1, SAM.2 and B15.1) (moderate bootstrap value (60%)) and *T. brucei* stocks (*T. brucei* and *T. brucei* STIB215) (low bootstrap value (52%)). This data was corresponded to the previously reports that these parasites are closely related and they have a few variation of nucleotide sequences (193). In addition, the ITS cladogram supported the 18S rDNA cladogram to confirm the hypothesis of classification of the *T. evansi* and *T. brucei* species.

Phylogenetic relationship of ITS region among *T. evansi* form different host.

The phylogenetic relationship of ITS region of 5 stocks of *T. evansi* from different hosts and different areas in Thailand were compared to *T. brucei* from Genbank and the trees were determined using phylogenetic analysis with PAUP program version 4.0. The ITS divided into 3 regions, ITS1, 5.8S rDNA and ITS2, and were separately analyzed using PAUP program.

The cladogram of ITS region indicated the variation of nucleotide sequences especially the ITS1 and ITS2 regions but the 5.8 rDNA sequences are homologous the sequences for all strains of *T. evansi* in Thailand (Figure 13 and 14). The cladogram of ITS1 region was enable to show the relationship of *T. evansi* from different hosts in Thailand. According to the ITS1 trees, 5 groups of the parasites were obtained when rooted with *T. brucei*. Group 1 belonged to *T. evansi* stocks from buffalo B15.2, B17.1, B17.2 and B18.2 (moderate bootstrap value (62%)). Group 2 consisted of *T. evansi* stocks from buffalo B15.1, deer SAM.2, cattle KAI.1 and *T. evansi* from GenBank (high bootstrap value (86%)). Group 3 contained *T. evansi* stock from cattle KAI.2 (high bootstrap value (89%)). Group 4 possessed *T. evansi* stock from deer SAM.1 (high bootstrap value (76%)). Group 5 was *T. evansi* stock from buffalo B18.1. The cladogram was able to show the variation of *T. evansi* stocks in Thailand. In contrast, the cladogram of ITS2 when rooted with *T. brucei* showed a single clade of *T. evansi* stocks from buffalo B17.2, B18.1 against the rest (low bootstrap value (52%)). This revealed that the ITS2 is the non-informative data for phylogenetic

analysis. It can be proposed that the ITS1 region could be used as the genetic marker for classification of *T. evansi* strains that will be beneficial for the epidemiological studies of *T. evansi* in Thailand. In addition, the virulence patterns were described distinct in a parasite species in which no molecular or biochemical heterogeneity. Therefore, the correlation of genotypic and phenotypic characteristics of parasites with epidemiological features remains a challenge and the multiple influences of environmental factors on both, parasites and hosts, should be taken into account.

From several studies, it is apparent that extensive genetic diversity exists among *T. evansi* stocks. The genotypes generated from each study can be useful references for studying taxonomic relationships among morphologically identical parasite. However, comparisons of genotypes among each study are difficult due to differences in methodology. To date, there is no phylogenetic analysis of 18S rDNA and ITS region to evaluate variation in *T. evansi* from Thailand. Hence, phylogenetic analysis of 18S rDNA and ITS region can be used as a tool for definition of among define genetic variation *T. evansi* stocks.

In conclusion, phylogenetic and sequence analysis of complete 18S rDNA and ITS region of *T. evansi* were performed for evaluation of the subgenus of *Trypanozoon*. However, more sequences regions such as mtDNA and kinetoplast DNA will be utilized to confirm the evolutionary relationships of *T. evansi* in Thailand stocks from different hosts.

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