11

Protozoa

Trypanosomatidae

Trypanosoma (Genus)

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Phylum Euglenozoa

Class Kinetoplastea

Order Trypanosomatida

Family Trypanosomatidae

Genus *Trypanosoma*

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Chapter 11

Trypanosoma (Genus)

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Introduction

It is difficult to know exactly where to begin the introduction of the flagellated eukaryotic parasites that are classified in the genus *Trypanosoma*. This is because there is so much that can be learned about parasitism from the study of the morphological characters of these organisms; or by studies of how their populations cycle through individual mammals; or how they grow and reproduce in their insect vectors; or how these parasites are maintained in populations of mammals and their arthropod vectors; there is even a species that jumps from host to host without the inconvenience of having to use a vector; it has evolved past the need for a vector and instead uses the behavior of its infected host to transfer to a new potential host. However, starting off with a general classification of the group is generally a good idea, so that students who are just beginning, or even an advanced student, can see where the parasite belongs in the general scheme of life.

The trypanosomes are a **monophyletic** group (meaning, having a common origin from a single ancestral species) of single-celled eukaryotes (**eu** = true, **karyon** = nucleus; Greek), or those organisms that have a true nucleus. Obligate parasites of the genus *Trypanosoma* are included within the superorder Kinetoplastida. One of the current classifications looks like this:

Domain Eukaryota
Phylum Euglenozoa
Class Kinetoplastida
Order Trypanosomatida
Family Trypanosomatidae
Genus *Trypanosoma*

As seen in the list above, all known trypanosomatids are classified in the order Trypanosomatida, family Trypanosomatidae (see Moreira et al., 2004).

Members of this unique family are characterized by the single celled organism having an elongated cell body containing one flagellum emerging at the anterior part of the parasite and a single mitochondrion that is distributed throughout the cell body (see Figure 1 for details about the general morphology). The compressed DNA of this mitochondrion is called a **kinetoplast**, which is composed of **maxi-circles** and **mini-circles**, and is situated close to the base of the **flagellar pocket** (the place in the body from which the flagellum originates). Maxi-circles encode the genes required to perform the physiological function of oxidative phosphorylation that occurs, for example, in the midgut of the tsetse fly, and mini-circles are responsible for mRNA editing (Shlomai, 2004).

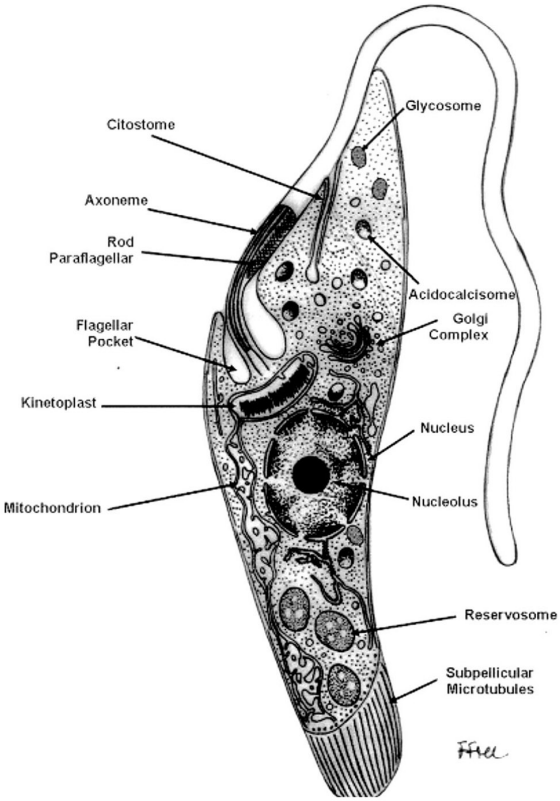

Figure 1. Schematic drawing, based on information obtained with the transmission electron microscope, showing the various structures found in the epimastigote form of *Trypanosoma cruzi*. Source of diagram: Souza, 1999. License: CC BY 4.0.

While species included in the family Trypanosomatidae have variable and distinct life histories, all species are obligate parasites. This family includes predominantly **monoxenic** forms (meaning, living on one host species), but also includes 4 genera with **heteroxenic** species (meaning, living in more than one host species) that are parasites of: 1) Plants (*Phytomonas* spp.); 2) sloths (*Endotrypanum* spp.); 3) other mammals and lizards (*Leishmania* spp.); and 4) all vertebrate classes (*Trypanosoma* spp.). Relatively recently, a fifth genus was proposed (*Porcisia* sp.) to include Neotropical porcupine-infecting species, previously known as *Leishmania hertigi* and *L. deane* (see Espinosa et al., 2018).

Concerning the monoxenic trypanosomatids, currently from 14 to 17 genera of parasites have been recognized, including those parasitizing Diptera, Hymenoptera, Siphonaptera, and Hemiptera. These monoxenic forms are distributed among the genera: *Angomonas*, *Blechomonas*, *Blastocrithidia*, *Crithidia*, *Herpetomonas*, *Kentomonas*, *Leptomonas*, *Lotmaria*, *Novymonas*, *Paratrypanosoma*, *Sergeia*, *Strigomonas*, *Wallacemonas*, and *Zelonia* (see Kaufer et al., 2017), as well as *Jaeinimonas, Lafontella*, and *Rhynchoidomonas* (see D'Ávila-Levy et al., 2015). Although described as monoxenic insect parasites, this trait (occurring in insects) seems not to be strict, since some of them have been reported parasitizing mammals, probably after being transmitted by insects. In fact, co-infections of *Leptomonas seymouri* and *Leishmania donovani* have been reported in patients with visceral leishmaniasis (Ghosh et al., 2012), and records of concomitant infections by *Leptomonas* sp. and *Herpetomonas samuelpessoai* in a HIV-positive human patient (Morio et al., 2008). In addition, there are records of *Herpetomonas* species infecting plants (Borghesan et al., 2013), and reports of *Blastocrithidia* sp. and *Chritidia mellificae* occurring in bats (Hodo et al., 2016; Rangel et al., unpublished data). Interestingly, when experimentally injected in the scent glands of opossums, *Leptomonas* sp. and *Chritidia* sp. not only established the infection, but also multiplied. The parasitism of scent glands of *Didelphis* spp. by monoxenic trypanosomatids was interpreted by Deane et al. (1984) as being the stepping-stone of trypanosomatids on their way to adapt to a mammal host.

Species of the genus *Trypanosoma* are parasites of vertebrates that are, with a few exceptions, transmitted among vertebrates by invertebrate vector hosts (Figure 2). Four primary morphological stages are recognized among trypanosomes: The extracellular trypomastigote, epimastigote, and spheromastiogote forms, and the intracellular amastigote form. These morphological forms occur in various stages among trypanosomatids with different species sometimes manifesting these forms differently. In 1972, the British protozoologist Cecil Hoare, in his foundational monograph, proposed the separation of the genus *Trypanosoma* in 2 **sections** based on the **transmission mode** of these parasites: **Stercoraria** and **Salivaria** (see Hoare, 1972). The divergence between these 2 sections would have occurred in the Cretaceous period (approximately 100 Ma = million years ago), accompanying the breakup of Gondwanaland and the separation of Africa from South America, Antarctica, and Australia. According to this classification that was widely accepted and used up to the current time, there are 3 sub-genera in **Stercoraria section**: *T.* (*Herpetosoma*) sp., *T.* (*Megatrypanum*) sp., and *T.* (*Schizotrypanum*) sp.; and 4 in **Salivaria section**: *T.* (*Dutonella*) sp., *T.* (*Nannomonas*) sp., *T.* (*Trypanozon*) sp., and *T.* (*Pycnomonas*). An eighth subgenus, *Tejeraia*, was proposed at a later date to include *Trypanosoma rangeli,* a South American parasite previously included in the *Herpetosoma* subgenus, but, as it later became clear, it is in fact transmitted by several hematophagous triatomine species (Añez, 1982).

The recent molecular tools with their great discrimination power that have been developed in the last decades have resulted in extensive revisions and descriptions of new genera and species. In fact, classifications up to 3 decades ago were made based on parasite morphology combined with the host species in which trypanosomatids were found. Another important point is the attention that was classically given to trypanosomatids with enzootic potential or potential to affect animals of economic interest. The current awareness of the importance of biodiversity (and parasites as important components of this) has widened the focus of attention in order to increase the interest in parasites not necessarily of humans or of domestic animals (Gardner and Campbell, 1992; Poulin, 2014; Jenkins et al., 2015).

Section Salivaria

Trypanosomes included in this section are highly prevalent in sub-Saharan Africa in the so termed Tsetse-Belt region and represent important health threats for humans and other animals with concomitant economic impacts in the areas of occurrence. Several species of the section Salivaria occur and can be found being transmitted in several countries beyond the African continent and the possibility of even greater geographic dispersion should not be neglected (Osório et al., 2008; Aregawi et al., 2019). Transmission occurs through inoculation of infective metacyclic forms as the insect vector feeds on blood of an infected mammal. For most species of salivarian trypanosomes but one, the only proven insect-vectors are various species of the hematophagous flies in the genus *Glossina* (the infamous and notorious tsetse flies of Africa). The only species that can be transmitted mechanically by other dipetera is *T. vivax*. *Glossina* spp. (Diptera: Glossinidae) (Figures 3 and 4) are the biological vectors of all salivarian trypanosomes and an individual fly may harbor

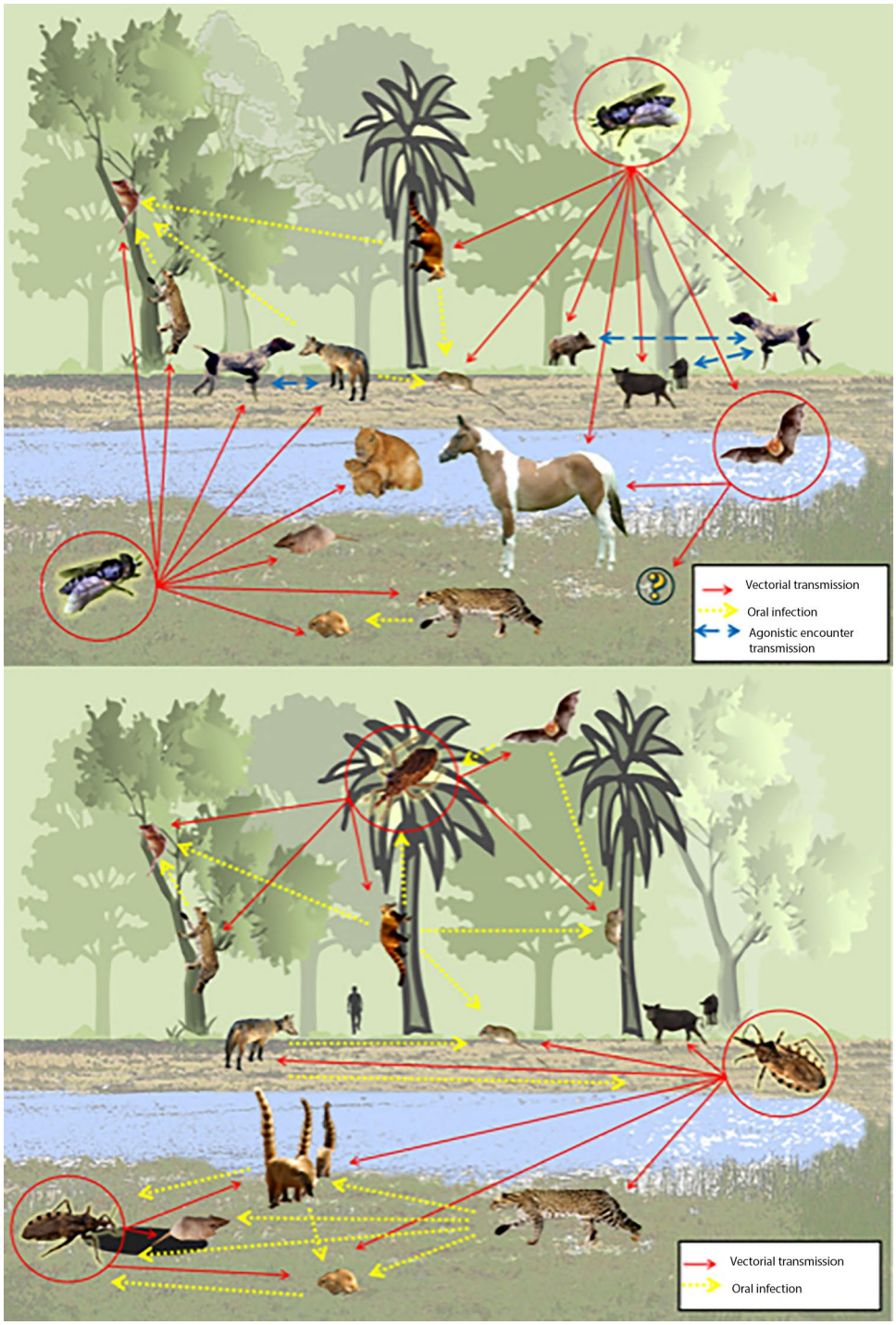

Figure 2. The wild and synanthropic reservoirs of *Leishmania* species represented graphically. Source: Rodrigues Roque and Jansen, 2014. License: CC BY-NC-SA 4.0 International.

more than one trypanosome species (Van den Bossche et al., 2004). All of the approximately 33 known species of *Glossina* that have been tested are able to act as vectors of salivarian trypanosomes; However, in contrast to many species of dipteran vectors, such as the blood-feeding Tabanidae, in which only females take blood meals, both sexes of *Glossina* are hematophagous and are capable of transmitting trypanosomes via saliva during blood feeding. Moreover, *Trypanosoma vivax* (another species of Salivarian trypanosome that lives in African mammals) can be transmitted by *Glossina* as well as by other hematophagous diptera (Fetene et al., 2021). Interestingly, *Trypanosoma evansi* does not appear to

Figure 3. A female tsetse fly (*Glossina morsitans morsitans*) from the Serap Aksoy Lab colony at Yale University School of Public Health. Tsteste flies transmit African trypanosomiasis. (Note: Taken by Geoffrey M. Attardo, this photo was a winner of the 2010 Fogarty Grantee Photo Contest, Fogarty International Center, United States, National Institutes of Health.) Source: G. M. Attardo, 2010. License: CC BY-NC-SA 4.0.

be transmitted by *Glossina* spp. and is mechanically transmitted from ungulate to ungulate by tabanids (order Diptera: family Tabanidae) and other hematophagous vector insects such as species of *Stomoxys*, *Atylotus*, *Lyperosia* (see Brun et al., 1998), and other slash and bite blood feeders such as *Desmodus rotundus* (common vampire bat) or *Diaemus youngi* the white-winged vampire bat in the Americas. *Trypanosoma equiperdum* the causative agent of Dourine in horses is a venereally-transmitted trypanosome and is generally considered to have a cosmopolitan (worldwide) distribution with the parasite generally absent in North America (north of Mexico), Australia, and western Europe (Brun et al., 1998; Gizaw et al., 2017).

The development of salivarian trypanosomes in the tsetse fly may be generally complex among *Trypanosoma* species and may be influenced by the insect immune response modulated by the fly's gut and symbiotic microbiota. Sexual reproduction in trypaonsomes has been reported, but only when the protozoans are actively reproducing within the insect vector-hosts (Gibson, 2015). Three interaction patterns of trypanosomes in the tsetse fly are recognized:

1) *Trypanosoma vivax* group (*Dutonella* subgenus) includes the trypanosomes with the lowest degree of interaction with the insect vector. This species is found across the Tsetse-Belt in Africa as well as in several countries in Latin America, occurring in wild and domestic animals. Recorded hosts for *T. vivax* include water buffalo, cattle, dogs, dromedary camels, horses, suids, and small ruminants. As noted, this species also occurs in wild animals that can serve as reserviors of infection for domestic animals. In this species development of the flagellates in the insect is restricted to the proboscis and cibarium where the parasite passes through 2 forms including both the **epimastigote** and **trypomastigote** stage. The entire life cycle of this species in the tsetse fly-vector is completed in as little as 3 days after initial infection. Forms of *T. vivax* in the blood stream of mammals are only of the monomorphic **trypomastigote** type. Representatives of this subgenus have evolved independently of tsetse flies and have adapted to mechanical transmission as noted earlier.
2) *Trypanosoma congolense* group *(Nannomonas* subgenus). Species included in this subgenus use a larger area of the digestive tract of the tsetse fly relative to species in the *Dutonnela* subgenus: In the gut, the ingested blood trypomastigotes differentiate into long trypomastigotes that migrate to cibarium and the proboscis of the flies where they differentiate into epimastigotes and, then, into metacyclic forms. Like *T. vivax*, mammalian blood stream trypomastigotes are also monomorphic.
3) Except for *T. evansi* and *T. equiperdum*, the representative of *T. brucei* group (*Trypanozoon* subgenus) are pleomorphic and go through a much more complex cycle in the vector. There are 2 proliferative forms in the fly, procyclic trypomastigotes in the gut and epimastigotes in the salivary gland and the entire life cycle can be completed in as little as 3 weeks (Sharma et al., 2009).

Salivarian trypanosomes may be highly pathogenic and lethal for their mammalian hosts, but a wide range of host species are tolerant to several of these trypanosome species. This is the case of native cattle breeds N'Dama and the West African shorthorn (WASH) (Ganyo et al., 2018). The basis of

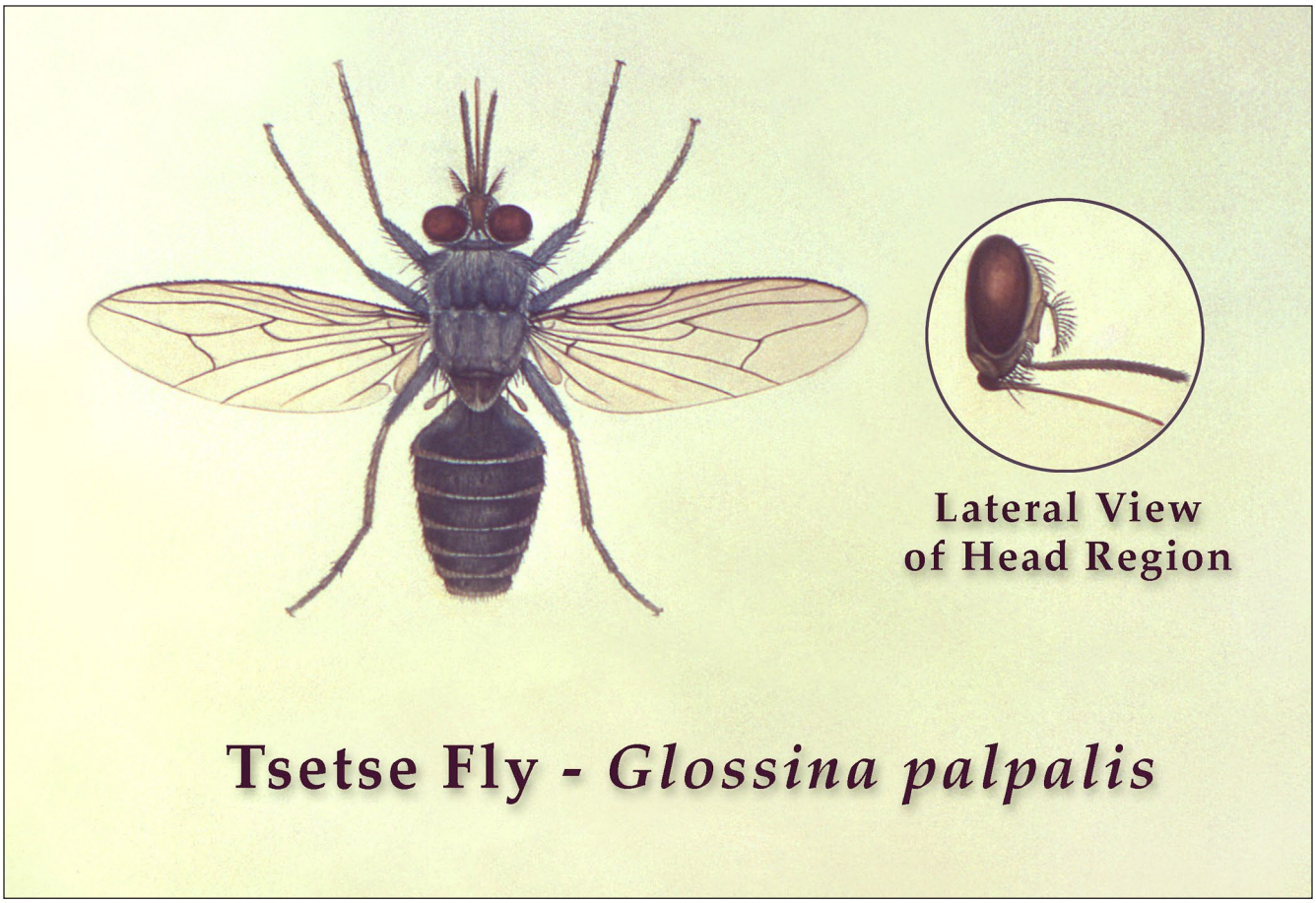

Figure 4. Tsetse fly, *Glossina palpalis*, top view with lateral view of head region. Source: United States Public Health Image library, image 17638; R. Darsie, 1976. Public domain.

this tolerance, although much studied with several hypotheses already formulated, is still a controversial subject. One unquestionable point is that whether the animals are trypanotolerant and trypanosusceptible is defined by their capacity to control anemia, which is the major outcome of the infection, and that determines whether or not host-animals remain competitive and productive.

The salivarian trypanosomes are not able to invade cells, and only extracellular trypomastigote and epimastigote forms are recognized. Trypomastigote forms are maintained in blood and body fluids of the infected mammal host. In the trypomastigote forms, 3 characteristics can be highlighted:

1) Two main types of blood trypomastigotes can be observed in most of the parasites from this section, including: a) Thin or slender forms are the dividing stage that are completely adapted to the mammal host, and b) broad or stumpy forms do not divide and possess well-developed mitochondria throughout the cell, and are adapted to the cycle in the vector dipterous host.
2) In the mammal host, where access to glucose is unrestricted, the mitochondrion is reduced in size and complexity and the parasite performs compartmentalized glycolysis, that is, within glycosomes. In this organelle, glucose is broken down into pyruvate and ATP, which is very effective whereas in the vector, where glucose is much more scarce, the now active mitochondria carries out an oxidative catabolism cycle generating CO_2, H_2O, and ATP oxidative cycle that results in CO_2 and H_2O; which is a more efficient mode of metabolizing glucose. Recently, it was shown that blood forms of *Trypanosoma brucei* are able to perform gluconeogenesis using glycerol as substrate for ATP production. The implications of this metabolic pathway are still unknown, but it demonstrates an important physiological plasticity. This pathway was suggested as being related to the passage of the parasite through tissues of its multiple host species (Kovařová et al., 2018). In the same way, gluconeogenesis was proposed as an important ATP source and is used by the procyclic forms in the midgut of the tsetse fly vector. In this environment, the parasite uses proline as substrate (Wargnies et al., 2018).

3) The ability of trypanosomes to sequentially modify their surface glycoproteins is an efficient escape mechanism from the mammalian host immune system. This variation in the surface coat of the protozoan are known as **VSGs** (**Variable Surface Glycoproteins**) initially described as **VATs (Variable Antigen Types)** (Barry et al., 1979). Salivarian trypanosomes live an extracellular existence in the blood and lymphatic system of the mammalian host and, are therefore, completely exposed to the humoral immune response, these parasites evolved to periodically alter all of their surface glycoproteins, as if changing a coat, in an efficient programmed mechanism where only one VSG is expressed at a time and is never repeated. It is estimated that there are about 10 million molecules anchored on the surface of these trypanosomatids being periodically exchanged, and about 2,000 vsg encoding genes regulating this process (Mugnier et al., 2016; Romero-Meza and Mugnier, 2020). This phenomenon is responsible for the parasitemia waves characteristic of mammals infected by salivarian trypanosomes. Also, their high motility, capacity of supporting non-immune defense mechanisms, and the mechanical forces inherent to blood circulation are important to survival in the mammal host (Stijlemans et al., 2016). The metacyclic forms of *T. brucei* develop in the salivary glands of the tsetse flies and also synthesize VSGs that; however, differ from the VSGs from the blood trypomastigotes (Kolev et al., 2017; Romero-Meza and Mugnier, 2020).

Tsetse flies live in moist savannah and woodlands, regions of more than 500 mm of rain a year, which include more than 30 countries across Africa (Cecchi et al., 2015). In those areas, human sleeping sickness is well-known and is caused by *Trypanosoma brucei rhodensiense* while *T. b. gambiense* causes Nagana, the non-human disease that is generally fatal in livestock and cycles in wild mammals (Büscher et al., 2017).

Humans are protected from infection by most of the African trypanosomes because of their production of a trypanolytic protein named Apolipoprotein 1 (APOL1), which is secreted by 2 protein complexes (TLF-1 and TLF-2) leading the formation of pores in the parasite membrane, resulting in its lysis. However, the 2 subspecies of *Trypanosoma brucei* associated with human sleeping sickness produce substances that are capable of lysing APOL1, the RAS factor in *T. b. rhodesiense* and TgsGP in *T. b. gambiense* (Capewell et al., 2015). Interestingly, baboons and one African human population named G1/G2 present a mutation in APOL1 that confers resistance to RAS and, therefore, to the infection by *T. b. rhodesiense*. The rare cases of humans that become infected with *T. b. brucei* and other species of trypanosomes are usually associated with people with some mutations that result in the absence of APOL1 production.

The genetic exchange that occurs solely in the insect vector of the salivarian trypanosomes, besides their huge repertoire of surface antigens, implies that new genotypes of salivarian trypanosomes may emerge to infect humans, and domestic and wild animals, and pose an important and worldwide health risk that would be magnified due to the absence of vaccines (Gibson, 2015).

Trypanosoma (Nanomonnas) congolense

Nannomonas trypanosomes include species that infect wild and domestic suidae (*Trypanosoma simiae* and *T. godfreyi)*, and *T. congolense*, a parasite that infects a broad spectrum of domestic and wild mammalian species and is the main cause of Nagana in Africa (Hamill et al., 2013; Morrison et al., 2016). These parasites are restricted to areas of occurrence of tsetse flies in sub-Saharan Africa and are described as extremely pathogenic for mammals (Cecchi et al., 2015). Some African breeds (Djallonke sheep, N'Dama cattle, and West African dwarf goats) are trypanotolerant, meaning that they are able to support the infection without anti-therapy and still maintain good health. The mechanisms underlying this tolerance probably depend on a genetic basis but this is still under debate (Yaro et al., 2016).

The trypomastigote forms are characterized by a flagellum that runs through the body of the parasite with a very short free end. The infection often starts with a skin lesion, a canker, where the parasites multiply, before reaching the bloodstream and lymphatic vessels of the host. In the vector, the multiplication takes place throughout the digestive tract before migration to the salivary gland, where the differentiation to metacyclic forms occurs (Dyer et al., 2013).

Human infection by *Trypanosoma congolense* has been only rarely reported (Truc, 1996). Compared to other African trypanosomes that infect livestock, *T. congolense* is considered as the most pathogenic, most prevalent, and most widely distributed trypanosomatid within the area of occurrence of *Glossina* spp. Three different genotypes of *T. congolense* are recognized: 1) The genotype Savannah, which is the most pathogenic and can affect a greater diversity of hosts, including carnivores; 2) the genotype Forest, described as poorly pathogenic and more often observed in cattle, goats, pigs, and dogs; and 3) the genotype Kilifi, considered as non-pathogenic and found infecting domestic ruminants (Rodrigues et al., 2014).

Trypanosoma (Trypanozoon) brucei

Trypanosoma brucei is the etiological agent of sleeping sickness in Africa and one of the few parasites able to cross the human blood-brain barrier, resulting in the nervous symptomatology observed in human disease. This parasite displays 3 subspecies: 1) *T. b. gambiense*, present in West Africa, associated with the more chronic form of human sleeping sickness, reported as less pathogenic; 2) *T. b. rhodensiense*, present in East Africa, associated with the acute form, the most pathogenic form of human disease; and 3) *T. b. brucei*, which infects wild and livestock animals, is associated with Nagana and is only rarely associated with human disease (Büscher et al., 2017).

The initial and recurrent symptoms of sleeping sickness are fever, tremors, muscle and joint pain, lymphadenopathy, malaise, weight loss, anemia, and thrombocytopenia. Later, neurological symptoms and meningoencephalitis can be present associated with mental retardation, convulsions, somnolence, and apathy that can progress to coma and death. This serious human disease is endemic in 36 African countries, including some epidemic areas in Angola, Democratic Republic of the Congo, and Sudan, and affects mainly people living in rural areas where tsetse fly is present. About 95% of human infections are caused by *Trypanosoma brucei gambiense* and commonly associated with *Glossina palpalis*. Those cases have a slower evolution of the disease and the infection can be present by months and even years without clinical signs. However, when the first signs appear, the disease is usually already in an advanced state and with the central nervous system compromised. The other 5% of the infections are caused by the *T. b. rhodensiense* and usually transmitted by *G. morsitans*. In these latter cases, the infection course is much faster and in a few months or even in a few weeks the disease progresses to the central nervous system presenting the characteristic neurological symptoms (Büscher et al., 2017).

In mammals, *Trypanosoma brucei* is always present in the extracellular trypomastigote forms and 2 morphotypes are recognized: 1) The slender forms, the divisionary forms that perform glycolysis in glycosomes and cannot survive in the insect vector; and 2) the stumpy forms, that do not divide in blood vessels, and display well developed mitochondrial ridges that are essential for survival in the vector.

Inside vectors, *Trypanosoma brucei* can colonize the whole digestive tract where both procyclic trypomastigotes and epimastigotes are present and can replicate themselves. The epimastigote forms that reach the salivary glands adhere to the microvilli of glands and perform cell division with subsequent differentiation to pre-metacyclic trypomastigotes, still attached to the salivary gland, but with fewer adhesion plaques. These pre-metacyclic trypomastigotes start to reacquire the coat of glycoproteins that are important in the blood phase of the infection. This form, named nascent metacyclic form, begins to detach itself from the glandular epithelium and will be totally free in the salivary gland when this differentiation is complete. At this point, the glycosomes are reverted to their spherical shape and the mitochondria are reduced to a small structure, which will no longer be functional while this parasite is in its mammalian host. The transmission will take place through the inoculation of these metacyclic trypomastigotes in the following blood meal (Vickerman et al., 1988; Dyer et al., 2013).

Trypanosoma (Dutonella) vivax

Trypanosoma vivax has a wide diversity of ungulate hosts; especially ruminants (order Artiodactyla). The infection occurs in Africa, Asia, Central America, and South America. Both wild and domestic ungulates (including buffaloes and antelopes); as well as equines and camels, are their most common hosts. Infection in laboratory animals has never been established (Osório et al., 2008). This parasite species can be transmitted both in cyclic and mechanical transmission. Cyclic transmission is reported in Africa, in areas where tsetse flies are present, and usually results in a more severe form of the disease. Mechanical transmission occurs in other parts of Africa (apart from the tsetse geographical area), in addition to Asia and the Americas, where the disease tends to be milder in cattle. In Brazil for example, outbreaks have always been associated with low mortality and few economic losses (Silva et al., 1996; Batista et al., 2007; Bastos et al., 2017).

In the mammalian host the trypomastigote forms are found exclusively in the blood. In *Glossina* spp., where the transmission is cyclic, this parasite differs in the replicative epimastigote forms that are restricted to earlier parts of the digestive tract. After the differentiation to trypomastigote forms, parasites migrate from the hypopharynx to the salivary gland where they differentiate into metacyclic trypomastigotes, which are the infective forms inoculated by the tsetse. Once in the salivary gland, *Trypanosoma vivax* can be maintained throughout the whole life of the vector. In mechanical transmission, there is no differentiation and the trypomastigote forms are carried from one mammal host to another by other hematophagous flies, especially those from the *Stomoxys* and *Tabanus* genera.

The first symptoms of *Trypanosoma vivax* infection are usually unspecific, such as anemia, fever, apathy, weight loss, and diarrhea. Diverse reproductive problems are associated with the infection, including transplacental transmission and abortion. When present, the neurological symptoms observed

are incoordination, muscle tremors, transient and/or permanent blindness, meningoencephalitis, and malacia (Batista et al., 2007; 2009).

Trypanosoma vivax was introduced into the Americas, probably with cattle brought from the European colonies in Africa. On this new continent, the parasite adapted to the mechanical transmission by several hematophagous insects, circulating among recently introduced domestic cattle and, perhaps, has spread among wild ungulates such as the cervids, never before exposed to this parasite. In Brazil, the first report of the parasite was in a buffalo in the swampy regions of Marajó Island, in the Brazilian Amazon (Shaw and Lainson, 1972). Later, in the late 1970s, this parasite was reported in sheep and cattle in the State of Amapá, also in Amazon region, and only a decade after this reported outside the northern region of the country, in cattle from the Brazilian Pantanal biome (Silva et al., 1996).

Possibly, the first outbreaks in the Pantanal were due to the increase of the displacement of animals from the north to the center-west of Brazil. In this biome, *Trypanosoma vivax* epidemiology is directly associated with drought and flood periods. In flooding periods, the reduction of pasture area increases the animal density per area, resulting in nutritional problems and resulting in higher susceptibility to infections, including trypanosomiasis (Silva et al., 1996).

Trypanosoma vivax can also be pathogenic for horses (da Silva et al., 2011). In both natural and experimental conditions, asinins were demonstrated to present high infection rates in subpatent and asymptomatic infections (Rodrigues et al., 2015). The infection of domestic ruminants by *T. vivax* results in severe economic losses, especially in South America. In spite of this, *T. vivax* remains poorly studied as a consequence of the inability to grow this trypanosome species in mice or in culture media.

Trypanosoma (Trypanozoon) evansi

The species *Trypanosoma evansi* masterfully exemplifies the genetic plasticity and consequent evolutionary success of salivarian trypanosomes. Together with *T. equiperdum*, *T. evansi* seems to have branched off from *T. brucei* due to the profound alterations of its kinetoplast DNA and, as a consequence, to have gained independence from a biological vector and to be transmitted mechanically, which has resulted in its enormous dispersion throughout Asia, the Americas, and Africa. The distinct kDNA alteration patterns observed in *T. evansi* samples suggest that *T. evansi* arose multiple times from a different *T. brucei* ancestor. These findings point to the necessity of revisiting the nomenclature of the members of the subgenus *Trypanozoon* (Radwanska et al., 2018).

Among all trypanosomes, *Trypanosoma evansi* is the one that is able to infect the largest variety of mammalian hosts, being dispersed in all continents (Desquesnes et al., 2013a). This parasite is the etiological agent of one of the major diseases affecting horses, called **Surra** (in the Old World) or **Mal de Caderas**, **Quebra Bunda**, or **Derrengadera** (in South America). The transmission is mechanical, being carried out normally by hematophagous flies from *Tabanus* spp. and *Stomoxys* spp. (Desquesnes et al., 2013b). The *Trypanosoma evansi* infection has been recently considered by OIE (World Organization for Animal Health) as a mandatory disease (Jaimes-Dueñez et al. 2017). Human cases are rare, but have been reported in Africa and Asia, usually associated with an extremely rare condition termed Tangier disease (Tomlinson et al., 1995).

Trypanosoma evansi belongs to the same *Trypanozoon* subgenus of the *T. brucei* species but is different from *T. brucei* whose transmission is totally dependent on cyclic transmission. *Trypanosoma evansi* is dependent only on mechanical transmission, even by tsetse flies. That is because, at some point on the evolutionary path of these parasites, *T. evansi* differentiated from *T. brucei* and lost the kDNA maxi-circles where most of the genes responsible for the oxidative metabolism and multiplication are located in *Glossina* spp., making *T. evansi* incapable of multiplying in the biological vector. Currently, some researchers suggest that this species comprises total or partial diskynetoplastic *T. brucei* strains. Although still considered to be a different species, some authors propose that *T. evansi* (and *T. equiperdum*) be classified as subspecies of *T. brucei,* or even variant strains of *T. b. brucei* (Carnes et al. 2015; Wen et al. 2016).

Trypanosoma evansi is a monomorphic parasite, and the trypomastigote form is the only recognized morphotype. Its trypomastigotes are the replicative forms observed exclusively in the blood of infected mammals and its morphology is exactly identical to the stumpy forms of *T. brucei*. Infection in mammals usually results in very high parasitemias, favoring mechanical transmission. Besides the mechanical transmission by hematophagous flies and the iatrogenic form through sharing of contaminated fomites, hematophagous bats display a differentiated feature, being able to act both as reservoir and vector of this parasite. That is because, in addition to being infected as all other mammalian hosts (which means that the parasite multiplies in the blood of these animals), it can still transmit the parasite that is easily present in its saliva during a blood meal (Desquesnes et al., 2013b). The oral route and agonistic encounters are also proposed as transmission routes in the wild (Herrera et al., 2011).

The estimated date of entry into South America by *Trypanosoma evansi* is still controversial. Although it is

hypothesized that *T. evansi* was introduced by infected horses of European colonizers (Desquesnes et al., 2013a), other authors propose that their introduction was much earlier, perhaps carried by the first primates and caviomorph rodents that came directly from Africa to South America, about 35–40 Ma (= million years ago). Caviomorph rodents, including capybaras, are considered to be important *T. evansi* reservoirs in the Pantanal region (Herrera et al., 2004) and infected rodents may have entered the Americas through the well described island-hopping or even sweepstakes dispersal processes (Lavocat, 1974; Raven and Axelrod, 1975; Flynn and Wyss, 1998).

In South America, trypanosomiasis caused by *Trypanosoma evansi* is economically very important in the flooded areas of the Brazilian Pantanal and Argentinean Chaco regions. These regions have a great concentration of livestock, and horses are essential for cattle handling. Outbreaks occur sporadically, though more common after a flood period, and result in a diversity of clinical features, from mild to severe fulminant forms (Herrera et al., 2004). In most severe cases, horses may present with anemia, emaciation, and subcutaneous edema of the lower body regions, with reports of abortion in pregnant females, and various types of neurological manifestations. The characteristic symptoms that inspired the name of the horse disease are the atrophy of the great muscular masses of the pelvic limbs, devolving to incoordination and ataxia (Desquesnes et al., 2013a).

The main wild reservoirs in the Pantanal region are the coatis and capybaras because they: 1) Are quite abundant in the region; 2) present high infection rates with long-lasting patent parasitemia; and 3) may remain infected for a long time. Capybaras support infection by *Trypanosoma evansi* without anemia and while maintaining good general health. Coatis, in contrast, when infected by *T. evansi* display anemia (Herrera et al., 2002; 2004). Infected horses may also act as reservoirs because the persistence of the parasite in asymptomatic animals after treatment is not rare (Herrera et al., 2004).

Usually reported in domestic animals around the world, studies on *Trypanosoma evansi* transmission in the wild are generally restricted to the Brazilian Pantanal. In this region, *T. evansi* can be considered a typical enzooty, with several infected wild mammals already found and occurring in sympatry with other trypanosomes, such as *T. cruzi* and *T. rangeli*. Interestingly, most infections observed in the wild seem to be subpatent and anemia is not commonly observed (Rademaker et al., 2009).

Trypanosoma (Trypanozoon) equiperdum

The other species within the *Trypanozoon* subgenus, also considered as a subspecies of *Trypanosoma brucei* or a mutant strain of *T. b. brucei* by some authors (Carnes et al., 2015; Wen et al., 2016), is *Trypanosoma equiperdum.* This species is the causative agent of **equine Dourine**, a disease that affects horses and other equidae. The transmission is exclusively venereal and the parasite is found only in genitals and their secretions. Asinines are usually reported as asymptomatic carriers of the parasite (Gizaw et al., 2017). Although as widespread as *Trypanosoma evansi* in the world, the reports of infection are quite intermittent and most studies are only case reports. The symptoms are similar to other trypanosomiases, such as fever, anemia, and emaciation, besides symptoms more specific to the genital organs, such as edema of the genitalia and mammary glands. More severe forms may progress to incoordination, facial paralysis, and death (Gizaw et al., 2017).

The difficulty in studying *Trypanosoma equiperdum* is that there are almost no available isolates of this parasite, and most isolates are from the beginning of the last century and lack essential information such as isolation site, year, and even host. After a long time without description of new isolates, in 2015, a group from Venezuela obtained the first 2 isolates of this parasite in Latin America (Sánchez et al., 2015). In 2016, another group obtained a new isolate from the urogenital tract of a horse in Mongolia (Suganuma et al., 2016). The lack of knowledge about *T. equiperdum* reinforces the question about the validity of this species (or subspecies).

Trypanosoma (Tejeraia) rangeli

One trypanosome that can be classified as neither Stercoraria nor as Salivaria**,** is *Trypanosoma rangeli* (see Grisard, 2002). Actually, *T. rangeli* is a parasite species transmitted by the saliva of its insect vector but shares numerous characteristics with other species of the Stercoraria section, as it is easily cultivated in axenic media (which is not observed in the Salivarian trypanosomes). It also shares mammalian hosts and vectors with *T. cruzi*. Currently, this parasite species is classified in the subgenus *Tejeraia* that was created in 1982 only to classify *Trypanosoma rangeli*, until then classified within the *Herpetosoma* subgenus, within the Stercoraria section (Añez, 1982).

Trypanosoma rangeli is a multi-host (mammal) parasite found exclusively in the Americas and is capable of infecting humans. The main vector species are triatomines from the *Rhodnius* genus, in which *T. rangeli* differentiates to the infective metacyclic forms in the salivary gland of the insect (Guhl and Vallejo, 2003). Moreover, other triatomine species may also maintain *T. rangeli*, as in the case of *Triatoma vitticeps* (see Dario et al., 2017a). The genetic diversity within *T. rangeli* was first observed based on the difference of a nucleotide sequence from its mini-circles, resulting in

the recognition of 2 separate populations, KP1(+) and KP1(−) (Vallejo et al., 2002). Further studies have shown that this genetic polymorphism is even more extensive and 5 lineages could be described, from A to E (Maia da Silva et al., 2009). It was first proposed that the divergence of these lineages was related to different *Rhodnius* species involved in the transmission, but it is now accepted that this separation of lineages is not strict. In fact, the characterization in distinct lineages is recent and we do not have enough sampling to propose associations between *T. rangeli* lineages and mammal hosts and/or ecotypes (Urrea et al., 2011; Dario et al., 2017a).

In the mammal host, only the blood trypomastigote form is observed and the current consensus in the scientific community is that *Trypanosoma rangeli* does not differentiate in amastigote forms, as occurs in *T. cruzi*. In the *Rhodnius* vector, the predominant and replicative form is the epimastigote that colonizes the vector's gut. Some of these parasites differentiate into trypomastigotes in the final portion of the intestine and are eliminated with the feces, but these forms are not infective for mammals. Most of the epimastigote forms invade the vector's hemocoel, where they can multiply both inside and outside hemocytes. From the hemocoel, some of these parasites reach the salivary gland and differentiate into metacyclic trypomastigotes that are transmitted through the saliva during a blood meal (Guhl and Vallejo, 2003).

Some points of this transmission cycle still need to be clarified. First, only the blood trypomastigote form is described in mammal hosts, which is considered to be non-replicative. Otherwise, this parasite is possibly able to multiply in the mammalian host because there are numerous cases of persistence of parasitism even after long periods after exposure. For instance, *Trypanosoma rangeli* was isolated from Brazilian patients who had been out of risk areas for many years and were being treated as having Chagas disease (de Sousa et al., 2008). These cases suggest that this parasite species can multiply in the mammal host, in a mechanism still not identified.

Another intriguing aspect is the niche occupied by *Trypanosoma rangeli* in mammal hosts. This parasite is always diagnosed in blood, but it has been isolated from bone marrow from an anteater in the Amazon region. The presence of *T. rangeli* in this unorthodox niche probably occurred through blood or lymph circulation, as described for other trypanosomes, and could had been influenced by the coinfection of *T. cruzi* and *Leishmania infantum* that were also diagnosed in the same host (De Araújo et al., 2013).

The *Trypanosoma rangeli* infection in mammals are considered innocuous or non-pathogenic, although due to the unknown mechanism of parasite multiplication in mammals, the discovery of *T. rangeli* in bone marrow and the isolation in Chagas disease patients are aspects that have to be considered in the endorsement of this assumption. On the other hand, the pathogenicity of *T. rangeli* infection in invertebrate hosts is well described and reports of the influence of the parasite load on insect molt and destruction of intestinal epithelium during parasite invasion to the hemocoel are some of the damaging effects usually observed in infected bugs (Ferreira et al., 2010; García et al., 2012).

Section Stercoraria

Trypanosomes of this section include numerous species of *Trypanosoma* that live in the intercellular spaces and/or in the blood of their mammalian hosts. Most of these do not display mechanisms of colonizing the intracellular environment or disguising immune response by using variable surface antigenic variation as observed in some salivarian trypanosomes. This is the case for several species of *Trypanosoma* from this section. Stercorarians include heteroxenous parasites that are transmitted between species belonging to all vertebrate classes and hematophagous invertebrates on all continents. Three main evolutionary stages are recognized in this section, including: 1) Extracellular trypomastigote forms, 2) extracellular epimastigote forms, and 3) intracellular amastigote forms. Interestingly, in the stercorarian trypanosomes, replication by cellular division/binary fission of these trypanosomes in mammals occurs only in either the amastigote or the epimastigote stages; fission of the trypomastigote forms in this group is unknown (Hoare, 1972).

All species from this section possess trypomastigote forms with flagella protruding anteriad of the cell with a large and non-terminal kinetoplast. In the vector insects, the final development of the parasites, which corresponds to the formation of metacyclic trypomastigotes, occurs in the posterior portion of the digestive tract of the insect vector host. The main characteristic, which gives the name to the section (stercoraria or posterior station), is their transmission method which is always via insects' feces by rubbing metacyclic trypomastigotes into a wound, eye mucus membranes, or oral mucus membranes (Hoare, 1972).

Trypanosoma (Herpetosoma) lewisi

The subgenus *Herpetosoma* comprises all the species described in the *Trypanosoma lewisi* group, which is the type species of the subgenus. Species from this subgenus include trypanosomes from many species of rodents, in addition to a lagomorph trypanosome called *T. nabiasi*. Most known vectors are fleas, but for many *Trypanosoma* (*Herpetosoma*) species, the transmission cycle is still completely unknown.

Trypanosoma lewisi is a cosmopolitan parasite of *Rattus* spp. This trypanosomatid species was probably introduced to the different continents and countries due to its association

with *Rattus rattus*, a synanthropic rodent that in turn has accompanied humans since their first voyages. *Trypanosoma lewisi* was previously considered specific to *Rattus* spp. and not capable of infecting the other cosmopolitan rodents (*Mus musculus*) in experimental conditions. Currently, it is recorded in some wild rodent species (Mafie et al., 2019), besides primates, including humans (de Sousa, 2014). The phylogenetic analysis of *T. lewisi* isolates from *Rattus* spp. and primates proposed that *T. lewisi* underwent a process of host switching between rodents and primates through accidental contamination of primates with infected fleas from these rodents (Maia da Silva et al., 2010). Some cases have been reported in Asia, associated with fever, anemia, and immunosuppression (Verma et al., 2011). Some authors consider *T. lewisi* a neglected re-emerging human pathogen (Lin et al., 2015) in spite of the rarity of cases of human infection by *T. lewisi* worldwide and because it always presents in immune incompetent individuals or people living in close contact with rats (Verma et al., 2011; Shah et al., 2011).

The recognized vectors of *Trypanosoma lewisi* are *Xenopsylla cheopis* and *Nosopsyllus* sp., but it is believed that other fleas, such as *Ctenocephalides canis*, *Leptopsylla segnis*, and *Pulex irritans*, can also act as vectors (Hoare, 1972). No cell invasion is reported in vertebrates, and the infection is considered innocuous in immunocompetent hosts, although it can be lethal in newborn rats or increase susceptibility to other parasites when presenting as a co-infection, as with *Toxoplasma gondii* (Ríos Carrera et al., 2009) or *Cryptococcus neoformans* (Gross et al., 2006).

Two stages of the parasite can be observed in a vertebrate's blood, the replicative epimastigote forms, and the trypomastigote forms. Inside the digestive tract of fleas, the trypomastigote forms invade the epithelial cells of the stomach and differentiate into amastigote forms, which are replicative, and differentiate again to trypomastigote forms before returning to the digestive lumen. After reaching the flea's midgut, the trypomastigote forms differentiate into replicative epimastigotes that will differentiate into metacyclic trypomastigotes at the end of the digestive tract. Besides the contaminative route, oral transmission through accidental ingestion of fleas has been demonstrated for *Trypanosoma lewisi* and other species from the same subgenus, namely, *T. microti*, *T. evotomys*, and *T. grosi* (see Maraghi et al., 1995).

In mammals, infection by *Trypanosoma* (*Herpetosoma*) species is characterized by intense and short parasitemias. The infection is easily controlled by the host in about 3 weeks due to a humoral immune response because the parasite has limited capacity for antigenic variation and does not invade mammal cells. A characteristic observed in *T. lewisi*, and also believed to occur in other species from the same subgenus, is the production of an IgG immunoglobulin named ablastin that inactivates the replicative forms of the parasite leading to the abrupt remission of the parasitemia. After this, rodents become resistant to new infections (Drew and Jenkin, 1982). The abrupt remission of parasitemia occurs only when preceded by an initial phase of infection establishment where, most probably, other factors are involved. The passive transfer of ablastin from one infected rodent to another was observed, which resulted in a partial control of the infection, but not as abruptly as observed in natural infections (Drew and Jenkin, 1982).

An intriguing aspect of the transmission cycle has been observed in *Trypanosoma musculi*, a parasite considered restricted to *Mus musculus*. After the phenomenon of ablastin, rodents infected by *T. musculi* become resistant to new infections and parasitemia is no longer observed, but throughout their life, the infected rodents still maintain some parasite forms, including replicative forms, in the vasa recta of the kidneys. These forms are biochemically and molecularly different from blood forms and appear to represent a new evolutionary stage of the parasite that are not inactivated by the host's immune system, due to the high concentration of urea (Monroy and Dusanic, 2000). The consequences of the existence of this distinct stage in the life cycle, the occurrence of this phenomenon in other *Herpetosoma* species, and the evolutionary impact of this parasite persistence are still unknown aspects.

Besides *Trypanosoma lewisi* and *T. musculi,* some other related species described in the same *Herpetosoma* subgenus are: *T. microti,* described in *Microtus* spp. and also directly transmitted through agonistic contact in the reproductive season; *T. evotomys* and *T. grosi* from Old World rodents; *T. kuseli* and *T. otospermophili* from squirrels; and *T. nabiasi,* a parasite species specific of the lagomorph *Oryctolagus cuniculus* present in the New World and Australia, the latter as a result of the human introduction of infected fleas in an attempt to control the rabbits (introduced one century before) with the Myxomatosis virus (Hamilton et al., 2005).

Trypanosoma (Megatrypanum) theileri

The morphology of parasites from the subgenus *Megatrypanum* is very typical: they have very large cells with a visible undulating flagellum that adheres to the entire body of the parasite. Trypanosomes from this subgenus are transmitted by a diversity of vectors, including hematophagous flies, fleas, ticks, and *Pseudolynchia* sp. This subgenus comprises species that infect a wide variety of hosts, including marsupials, rodents, primates, and, mainly, varieties of bovinae cattle (Kingston, 1991; Kelly et al., 2017). The species *Trypanosoma* (*Megatrypanum*) *theileri* is a hemoparasite found in

Artiodactyla species worldwide and is the type species of the subgenus *Megatrypanum*. Recently, a second escape mechanism of modeling the trypanosome cell surface, distinct from the VSG's coat of the salivarian trypanosomes, has been described. Actually, the surface of *Trypanosoma theileri* cells can be modeled by the expression of a mixture of proteins (Kelly et al., 2017).

The transmission cycle of *Trypanosoma theileri* is not completely elucidated yet. Mammals are proposed to have a replicative epimastigote form, but this parasite form is very rarely observed in field studies, probably because these forms quickly differentiate into trypomastigotes, which is the predominant morphological type detected in mammals' blood. In mammals, those trypomastigote forms are capable of invading all body fluids, in addition to some tissues, such as lymph nodes, kidney, spleen, and brain. For long time, it was accepted that *T. theileri* did not differentiate into amastigotes, but the existence of these stage forms was recently demonstrated in vitro, including the ability to differentiate and invade other cells in a process similar to that observed in *T. cruzi* (see Lee et al., 2013). In vectors, the epimastigote forms of the parasite replicate in the gut before differentiating into metacyclic trypomastigotes that are eliminated in the feces. The tabanids are the vectors of *T. theileri,* but a tick species (*Hyalomma anatolicum*) was proposed before as an alternative vector (Latif et al., 2004). As observed for other Stercorarian trypanosomes, oral transmission through accidental ingestion of infected vectors is proposed as an important infection route (Kelly et al., 2017).

Trypanosoma theileri is a cosmopolitan and opportunistic parasite. The parasitemia in bovines is usually associated with some unspecific clinical signs. Infection may remain subpatent and asymptomatic for several years but may result in high parasitaemia and pathogenicity when associated with other factors, such as stress, malnutrition, or pregnancy. This parasite species is recognized in bovines and buffaloes from South America, and at least 10 genotypes are recognized, most of them specific in terms of mammal species (García et al., 2011).

Besides *Trypanosoma theileri,* some other species from the same *Megatrypanum* subgenus are: *T. minasense* from New World primates; *T. melophagium* from sheep*; T. theodori* from goats; *T. cervi* and *T. mazamarum* from cervids; *T. freitasi* that colonize scent glands of *Didelphis* spp.; *T. samueli* and *T. saloboense*, from *Monodelphis* sp. from the Amazon region; *T. peba* from the 6-banded armadillo *Euphractus sexcinctus; T. legeri* from the anteater *Tamandua tetradactyla; Trypanosoma pestanai* isolated from European badgers; and *T. caimani* from alligators from the Brazilian Pantanal; as well as a high diversity of bird trypanosomes. For the less studied *Megatrypanum* species, other vectors (apart from tabanids) are involved in the transmission: keds (*Melophagus ovinus*) are involved in *T. melophagium* transmission (Gibson et al., 2010), *T. pestanai* is transmitted by fleas (Peirce and Neal, 1974; Lizundia et al., 2011), while *T. cervi* are also spread by keds (Böse and Petersen, 1991). It is worth mentioning that many of these parasite species were described based on morphology, before the employment of molecular techniques for parasite identification. As a result, some species may not be valid, as in the case of *T. saimirii* and *T. leeuwenhoeki,* currently recognized as synonyms of *T. rangeli* (See Stevens et al., 1999a; Ziccardi et al., 2005).

Trypanosoma (Schizotrypanum) spp. and Other Species Related to *T. cruzi*

Almost all the *Trypanosoma* (*Schizotrypanum*) species are parasites exclusive of bats. The exceptions are *T. cruzi,* a multihost parasite that may also infect bats, and *T. dionisii,* a parasite species considered specific to bats, but recently observed also in human cardiac tissue and in the marsupial *Monodelphis americana* (See Lima et al., 2015a; 2015b; Dario et al., 2016; 2017a; 2017b). Parasites from this subgenus are morphologically identical and all of them are able to differentiate into metacyclic forms and invade cells, where they differentiate into amastigotes.

Until the 1990s, most *Trypanosoma* infections in bats were described as *T. cruzi*-like because of the difficulties differentiating among *Schizotrypanum* species. Currently, due to advances of molecular techniques, especially since it is easier now to analyze DNA sequences, the variety of *Trypanosoma* species infecting bats has been shown to be enormous, and this diversity has been increasing annually with descriptions of new *Trypanosoma* species infecting bats. Some *Trypanosoma* species reported in bats are: *T. cruzi marinkellei* and *T. wauwau,* described in bats from Central America and South America; *T. vesperitilionis* and *T. dionisii,* described in bats from both the Old World and the New World; *T. pipistrelli* from Old World bats; *T. pteropi*, *T. hipposideri*, and *T. teixeirae* from Australian bats, including the enormous flying foxes; *T. hedricki* and *T. myoti* from bats surveyed in North America; *T. erneyi* and *T. livingstonei* from Africa; and yet others (Lima et al., 2012; 2013; 2015a; 2015b).

The majority of the descriptions of *Trypanosoma* infection derived from insectivorous bats suggest that, besides the vectorial contaminative route, oral infections must also occur (Lima et al., 2015a; 2015b; Dos Santos et al., 2018). The vectors involved in the majority of bat trypanosome transmission are unknown. *Trypanosoma cruzi marinkellei* is currently recognized as a subspecies of *T. cruzi* and reported to be transmitted only by triatomines of the genus *Cavernicola*,

associated with caves (Marinkelle, 1982). Vectors of *T. dionisii* and *T. vespertilionis* are reported to be cimicids, but both *T. c. marinkellei* and *T. dionisii* were recently found infecting *Triatoma vitticeps,* a triatomine bug very common in the Atlantic rainforest (Dario et al., 2017a). The close association of *Schizotrypanum* parasites and bats suggests a long coevolutionary process. However, the association with different vectors points to an independent evolution between species within this subgenus.

Other *Trypanosoma* species more recently described were not classified in any subgenus but are phylogenetically closer to *T. cruzi* and known as trypanosomes of the *T. cruzi* clade. Besides all the above mentioned trypanosomes of bats, this clade includes *T. noyesi,* a trypanosome described from the Australian endangered woylie (*Bettongia pencillata*); 2 isolates from African terrestrial mammals (1 feline and 1 primate); *T. conorhini*, a species taxonomically classified in the subgenus *Megatrypanum*, but phylogenetically in the *T. cruzi* clade; and *T. janseni,* a species recently described in Brazilian opossums that, along with *T. wauwau,* forms a sister group of the trypanosomes found in Australian marsupials (Hamilton et al., 2009; Botero et al., 2016; Lopes et al., 2018).

From these, one of the most studied is *Trypanosoma conorhini.* This species infects *Rattus rattus* a synanthropic rodent and the kissing bug *Triatoma rubrofasciatta* all over the world, and only experimentally was demonstrated to be able to infect *Mus musculus* and *Macaca mulatta* (Deane et al., 1986). The only parasite form observed in the rodent's blood is the trypomastigote, without evidence of cell invasion and differentiation to amastigote forms. The biological cycle in the vector includes the replication of the epimastigote forms in the bug's gut and differentiation in metacyclic trypomastigotes that are eliminated in the feces (Hoare, 1972). The fact that this trio–*Rattus rattus*, *Triatoma rubrofasciatta*, and *Trypanosoma conorhini*–are found together in different parts of the world indicate that their dispersal process occurred together, probably from Asia, which represents a unique joint migration process within the heteroxenous protozoa (Deane et al., 1986).

In general, the vectors involved in the transmission of most of the *Trypanosoma* species from the *T. cruzi* clade are unknown, but all of the vectors described up to now are hematophagous hemipterans. In fact, the knowledge of the diversity of *Trypanosoma* species has been a growing subject of study, especially in the past decade when DNA sequencing tools became more accessible. These studies have shown a much greater diversity than was previously known and that the evolutionary paths within this clade are still not fully understood.

Trypanosoma cruzi

Trypanosoma cruzi is the etiological agent of one of the most important and neglected parasitic disease, Chagas disease (Chagas, 1909). However, the human infection is only a minor trait of this parasite ecology that is a wild animal parasite maintained among dozens of species of mammals and triatomines (family Reduviidae, subfamily Triatomiane). In fact, *T. cruzi* is a multi-host parasite, able to infect virtually any mammal species and, within them, any nucleated cell (Jansen et al., 2015). In spite of presenting a population structure that is basically clonal, mitochondrial introgression events are not unusual and evidence of genetic exchange has already been described in *T. cruzi*. Also, recombinant strains have been described (Lewis et al., 2011).

There are 2 non-mutually exclusive hypotheses that explain the origin of *Trypanosoma cruzi*. The first, called **Southern Supercontinent**, places the origin of this parasite from an ancestral trypanosome of marsupials, which represented the dominant local fauna, in a supercontinent formed by South America, Antarctica, and Australia. This hypothesis is supported by: 1) The presence of a trypanosome related to *T. cruzi*, named *T. noyesi* (Botero et al., 2016), isolated from an Australian woylie (besides other related isolates from Australian marsupials); 2) the estimated divergence between *T. cruzi* and *T. brucei* (approximately 100 million years) that is the approximate time of separation of this supercontinent from Africa (Stevens et al., 1999b); and 3) the recent description of *T. janseni*, which was described in Brazilian opossums and clusters with *T. wauwau* in a well-supported clade, representing a sister group of the trypanosomes found in Australian marsupials (Lopes et al., 2018).

The second hypothesis points to the origin of *Trypanosoma cruzi* from ancestral *Trypanosoma* species of bats and is called the **Bat Seeding Hypothesis** (Hamilton et al., 2012). This hypothesis is supported mainly by: 1) The increasing evidence of the diversity and polyphyly of bat trypanosomes; and 2) the description of infection of trypanosomes from the *T. cruzi* clade in African monkeys and palm civets (which does not represent a single lineage that could have been introduced more recently; that is, post-separation of the continents) (Hamilton et al., 2009). Several processes of trypanosome spillover from bats to terrestrial mammals must have occurred until one of them was successfully established and the evolution of this parasite resulted in a new species currently known as *T. cruzi* (Hamilton et al., 2012).

The contaminative transmission of the parasite, described as the classic form of transmission, occurs when the insect vector eliminates metacyclic trypomastigote forms with feces during a blood meal. These parasites penetrate through lesioned skin or mucous membranes or when the person

scratches the location of the insect bite. In the mammal host, these parasites invade the nucleated cells, in which they differentiate into the replicative amastigote forms. A novel differentiation into blood trypomastigotes occurs before the cellular disruption that results in the release of these forms that can invade other cells or circulate in blood vessels and be ingested by other Triatominae in a new blood meal. In invertebrate hosts, *Trypanosoma cruzi* differentiates into the replicative epimastigote forms and only in the final portion of the insect gut, the parasites differentiate into the metacyclic trypomastigote forms that are the infective forms eliminated in the feces.

This classical route of transmission, however, does not reflect all possible routes of infection in mammals, including humans. From the 4 evolutionary stages of the parasite, only epimastigotes may not be infective, mainly when they are in the exponential growth phase. Moreover, epimastigotes of the stationary phase are already resistant to the complement system and already infective (Kessler et al., 2017). Nutrient impoverishment and starvation of the parasite acts as a trigger for metacyclogenesis (Barisón et al., 2017). Human infections can also occur during blood transfusion and organ transplantation, and these are the most important infection routes in non-endemic countries such as the United States, Spain, and Japan (Gascon et al., 2010). The congenital form, well characterized in humans, seems to have a more regionalized importance, especially in the southern region of South America and non-endemic areas. The oral route has emerged as a very important transmission route in the wild and has been responsible for an increasing number of human infections in the past few decades (Coura, 2015). In the South American Amazonian area, especially in Brazil, this transmission route represents more than 90% of new cases and is usually associated with intake of food contaminated by feces of infected triatomines or infected vectors accidentally crushed along with food, such as sugar cane and açaí juice (Figure 5) (PAHO, 2009). This transmission route requires specific surveillance measures whose operation is still not completely known (Xavier et al., 2014).

Figure 5. The oral route has emerged as a very important transmission route in the wild and has been responsible for an increasing number of human infections in the past few decades. In Amazonia, especially Brazil, this transmission route represents more than 90% of new cases and is usually associated with intake of food contaminated by feces of infected triatomines or infected vectors accidentally crushed along with food. Source: Pan-American Health Organization, 2009. Permissions: Reproduction is permitted with attribution.

In nature, the oral route is probably the oldest and most efficient route for parasite transmission and occurs mainly in 2 situations: 1) Ingestion of triatomine feces when the animal scratches the site of an insect bite with its mouth; or 2) predation of infected bugs or mammals. Other possible routes include fights between mammals (when the oral mucosa of a mammal comes in contact with the infected blood of the other mammal) and fomites contaminated by scent gland material of infected *Didelphis* sp. (Deane et al., 1984; PAHO, 2009), although these are still not empirically confirmed.

This biological plasticity of *Trypanosoma cruzi* results in a parasite widely dispersed in nature and immersed in transmission cycles that can be characterized as multivariable, complex, and peculiar to each locality (Jansen et al., 2015). Being found in the most diverse ecological niches from the southern parts of Argentina to southern United States, its transmission cycles are quite complex as it includes hundreds of mammalian species and vectors in scenarios of transmission that can be independent and overlapping with each other (Jansen et al., 2018).

As pointed out before, *Trypanosoma cruzi* has been circulating among wild mammalian fauna for at least 20 million years (depending on which hypothesis for the parasite's origin is considered). Humans are believed to have existed for no more than 500,000 years. Humans are estimated to have entered into the Americas (and therefore, exposed within this time to *T. cruzi* transmission cycles) only 15,000 to 24,000 years ago (Bourgeon et al., 2017). As such, there is evidence of human infections in mummies long before the arrival of Europeans in the Americas. Reports in paleoparasitological studies demonstrated the infection in 40% of the mummies examined in Chile, including at least one of them dating to 9,000 years. Almost half of the mummies had signs of megasyndromes (especially megacolon) and infections were seen among mummies from different cultural groups (Aufderheide et al., 2004). It is reported in many South American Aboriginal cultures the habit of feeding on raw or undercooked meat or even drinking the fresh blood of hunted mammals, which would result in infection by *T. cruzi* (Guhl et al., 2014). The contaminate vectorial transmission probably also occurred: 1) Inside caves used as human dwellings, due to the presence of kissing bugs associated with rocky environments, as in the case of *Triatoma brasiliensis* (Araújo et al., 2003); and 2) in peridomiciliary environments, as a consequence of the presence of small mammals such as guinea pigs that provided an abundant blood source for bugs in this environment (Aufderheide et al., 2004). These ancient scenarios demonstrate that there are no new or old ways of *T. cruzi* transmission. Humans have been exposed to the infection by both the contaminative and oral routes since they reached the Americas.

The species *Trypanosoma cruzi* is a monophyletic and extremely heterogeneous taxon that presents a multiclonal population structure influenced by mechanisms of gene exchange, epigenetic factors, introgression, and others (Stevens et al., 2001; Leonard et al., 2011). The heterogeneity of the parasite has been observed since its discovery, and thin and broad parasite forms observed in patients' blood were first associated with male and female gametocytes (Chagas, 1909). The first attempts to group *T. cruzi* isolates with similar characteristics dated from the 1970s: the biodemes, based on a distinct infection pattern in laboratory animals (Andrade et al., 1970) and the zymodemes, based on biochemical patterns of isoenzymes (Miles et al., 1977). The advent of molecular techniques and the proposal of distinct molecular targets in the 1980s and 1990s have revealed that this heterogeneity was much higher than previously thought. In the last decade, however, with the availability of gene sequence analysis in research labs, an even higher diversity of *T. cruzi* populations (and other related species) has emerged. In this scenario, the most employed gene targets are the small subunit (18S) ribosomal RNA gene and the nuclear glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) (Lima et al., 2015a; Lopes et al., 2018). These are the ones with the highest discriminatory power and those targets with the highest number of sequences deposited for comparison. Moreover, more easy accessibility to Next Generation Sequence (NGS) will probably uncover even higher trypanosomatid diversity, especially due the capacity to identify and characterize mixed infections directly on blood samples (Dario et al., 2017b; Pronovost et al., 2020).

Independent of the different molecular targets employed, 2 distinct and phylogenetically distant *Trypanosoma cruzi* populations have been recognized and were the basis for the first nomenclature consensus for *T. cruzi* (Luquetti et al., 1999). One year later, Brisse and colleagues (2000) proposed the grouping of *T. cruzi* isolates into 6 genotypes, or 6 **Discrete Typing Units** (**DTU**), named TcI to TcVI (Tc stands for *T. cruzi*). This is the basis of the current consensus of nomenclature: *T. cruzi* is a single species composed of 6 distinct DTUs (Zingales et al., 2009).

In addition to these DTUs, in 2009, a new *Trypanosoma cruzi* genotype, first associated with bats, was described: Tcbat (see Marcili et al., 2009). This genotype is phylogenetically close to TcI but represents a well separated clade. As a *T. cruzi* parasite, Tcbat is able to infect mice in experimental conditions. Although, its development in triatomines is more efficient in cave triatomines, which is the same pattern observed for another trypanosome associated with bats, *T. c. marinkellei* (See Marinkelle, 1982). Although first associated with bats, human infections by Tcbat have been reported in a Chilean mummy (Guhl et al., 2014) and a Colombian patient (Ramírez et al., 2014).

The currently most-accepted hypothesis to explain the establishment of the 6 DTUs points to TcI and TcII as the 2 ancestor lineages, and the emergence of the DTUs TcIII and TcIV as a result of a first hybridization process between them. A second and more recent hybridization process involving TcII and TcIII would have given rise to the current hybrid lineages TcV and TcVI (Westenberger et al., 2005).

The geographic distribution of *Trypanosoma cruzi* DTUs is not completely known and further knowledge of both the geographical and host-expanding data for each DTU will certainly result in distinct distribution maps (Jansen et al., 2015; 2018). However, some aspects can be highlighted: 1) TcI is the most dispersed DTU throughout the Americas; 2) outside South America, mainly TcI and TcIV are detected; 3) TcII occurs in the wild, but in restricted transmission foci and is the genotype associated with human infections in the former endemic areas of the disease (Jansen et al., 2015; 2018); 4) TcIII and TcIV are commonly found in wild mammals and the latter has been associated with oral outbreaks in the Amazon region (Santana et al., 2019); 5) TcV and TcVI are rarer than the others, both in humans and wild mammals; and 6) the subdividing genotypes of the *T. cruzi* population, previously known as zymodeme 2 (TCII in the first nomenclature consensus), are more recent. Because of that, ancient descriptions of TCI are still considered TcI, but TCII was subdivided into the other 5 subpopulations. This means that, except for TcI, all other *T. cruzi* DTUs are actually underestimated on the current distribution maps.

The widespread transmission of *Trypanosoma cruzi* to humans, which led to hundreds of thousands cases per year in the last century, was directly associated with the presence of the domiciliary bug *Triatoma infestans* (See Figure 6). This bug species originates from the Bolivian valleys of the eastern Andes, where it is found both in domiciles and in wild environments (Panzera et al., 2014). The domiciliary process of *Tri. infestans* occurred concurrently with the beginning of human settlements near forest environments. At the same time, the process of domestication of local guinea pigs near residences attracted the insects to the home environment (Aufderheide et al., 2004). After the colonization of the Americas by Europeans, the greater movement of people and materials favored the establishment of the intradomiciliary colonies of *Tri. infestans*, first in Bolivia and subsequently in other countries from South America, especially in those regions south of the Amazon basin. The presence of infected domiciliary bugs in stick houses was what Carlos Chagas found when he described the parasite and the associated vector (Chagas, 1909).

This classical scenario of transmission started to change in the 1970s, with insecticide campaigns to eliminate domiciliary colonies of this kissing bug species (Dias, 2007). In 1991, the governments of Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay created the Southern Cone Initiative aiming to eliminate the intradomiciliary transmission of *Trypanosoma cruzi* by *Triatoma infestans,* which was achieved in 2006 (Dias, 2007). The certified interruption of this method of transmission, however, did not represent the end of contaminative vectorial transmission. This manner of transmission continues to occur, but now in the extradomiciliar environment, when humans expose themselves to wild transmission cycles, or in the intradomiciliar environment, usually associated with the invasion of wild vectors and not to domiciliary colonies (Dias et al., 2016). In both cases, control measures adopted against *Tri. infestans* are not effective. With the virtual elimination of *Tri. infestans* in some countries, including Brazil, the majority of the new reported cases are concentrated in the Amazon region, a previously considered non-endemic area due to the absence of *Tri. infestans* in the area. Only in Brazil, approximately 150–200 new cases of Chagas disease are reported per year (Dias et al., 2016). These cases are always associated with infected sylvatic kissing bugs that came in contact with humans.

These sylvatic bugs are immersed in a huge net of *Trypanosoma cruzi* transmission along with mammals from distinct orders. This transmission net resembles the trophic energy characteristic of food webs (Herrera et al., 2011). Both the oral route, through the predation of mammals and insects, and contaminative transmission are involved in the dispersion of this parasite in nature. Triatomines can be preyed upon by smaller mammals, and these by medium or top-chain carnivores (Herrera et al., 2011; Rocha et al., 2013). Moreover, the different mammal hosts are distributed in distinct forest strata and enable the parasite transmission in the most diverse habitats. This is especially important for mammals with generalist habits, like coatis, opossums, and felids, which frequent different forest strata and, thus, can connect parasite transmission cycles from different environments (Jansen et al., 2018).

Trypanosoma cruzi transmission can occur at all levels of the food web, and each group of mammals also presents varying importance as a reservoir of this parasite. In the pyramid base of consumers are the small terrestrial mammals. These mammals are excellent models of study because they comprise large populations and are usually the group of mammals with the highest biomass in any ecotope (Mills and Childs, 1998). In addition, they have a short lifespan and fast generational turnover, which allows the early identification of environmental changes. In the second level of the pyramid are the bats and medium-sized carnivores. They are classified as mesopredators, since they may be exposed to *T. cruzi* infection by predation of both infected vectors and small mammals. They are usually generalists and capable of exploiting

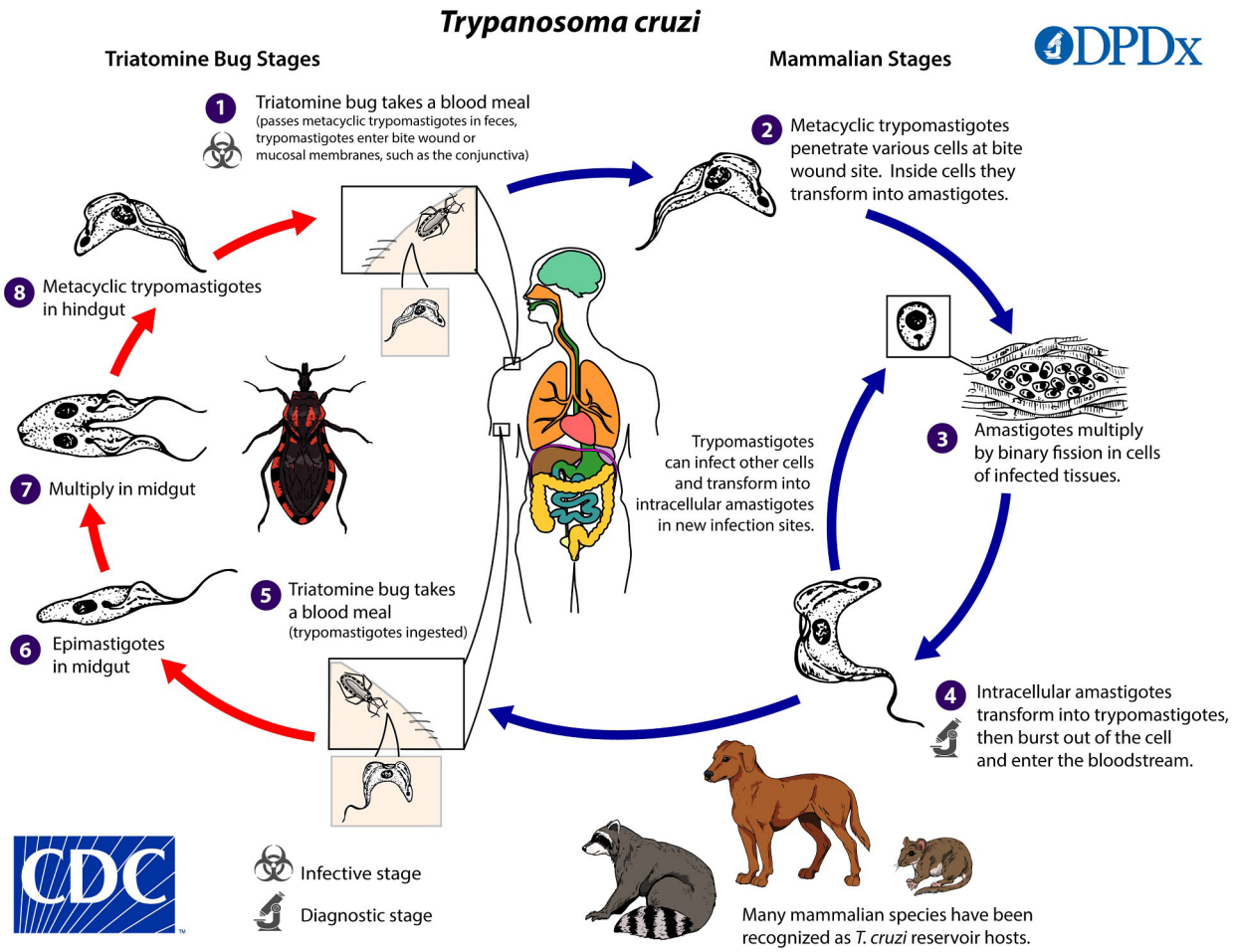

Figure 6. American Trypanosomiasis. *Trypanosoma cruzi*, is a parasitic protozoan that is the causative agent of Chagas disease (American trypanosomiasis). Currently, 6 distinct lineages of *T. cruzi* are classified into discrete typing units (TcI–VI), which vary in their geographic occurrence, host specificity, and pathogenicity. **Life cycle diagram:** An infected triatomine insect vector (or kissing bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the bite wound or intact mucosal membranes, such as the conjunctiva (1). Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes (2). The amastigotes multiply by binary fission (3) and differentiate into trypomastigotes and then are released into the circulation as bloodstream trypomastigotes (4). Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The kissing bug becomes infected by feeding on human or animal blood that contains circulating parasites (5). The ingested trypomastigotes transform into epimastigotes in the vector's midgut (6). The parasites multiply and differentiate in the midgut (7) and differentiate into infective metacyclic trypomastigotes in the hindgut (8). Other less common routes of transmission include blood transfusions, organ transplantation, transplacental transmission, and foodborne transmission (via food or drink contaminated with the vector and/or its feces). Source: United States Centers for Disease Control and Prevention, Global Health, Division of Parasitic Diseases and Malaria, 2021. Public domain.

distinct forest strata. At the top of the pyramid are those animals at the top of the food chain. They are mammals characterized by a wide geographic range and capacity for displacement, which are important conditions for parasite dispersion. In addition, they are considered bioaccumulators of orally transmitted parasites, as is the case for *T. cruzi* (see Herrera et al., 2011; Rocha et al., 2013).

Small mammals are excellent models of study for identifying spatial and temporal variation in *Trypanosoma cruzi* transmission. Concerning the spatial differences, the effect of habitat fragmentation on *T. cruzi* transmission among small mammals has been demonstrated by Vaz et al. (2007). Fragments of forest patches of different sizes (small, medium, and large) were investigated, in addition to a national park, as a

preserved control area. Vaz et al. (2007) observed that the greater the fragmentation of the wild environment, the lower the diversity of small placental mammals and the greater the abundance of marsupials. This different faunal composition was reflected in distinct infection prevalences, especially in the medium and large fragments (Vaz et al., 2007). Temporal differences were observed in the evaluation of infection prevalences in small mammals from the same locality (Jaguaruana, Ceará State, Brazil) during a 4-year follow-up. The anthropogenic devastation of the area resulted in lower species richness of placentals and a higher abundance of opossums. The consequence for *T. cruzi* transmission was an increase from an initial rate of 10% of the mammals infected to a rate higher than 50% after the fourth year (Jansen, unpublished data).

An example of a mesopredator that, throughout several years of study, demonstrated to be an important *Trypanosoma cruzi* reservoir is the coati (*Nasua nasua*) from the Pantanal region of Brazil. Distinct *T. cruzi* DTUs (TcI–TcIV) have been found in single mixed infections and long-term follow-up showed that coatis may present high and long-lasting parasitemias that are influenced by seasonality and gender (Herrera et al., 2008; 2011; Alves et al., 2016; Jansen et al., 2018). Coatis nest in very tall trees and their nests serve as microhabitats for several other mammals and insects, including kissing bugs that have been found to be infected by *T. cruzi* (see De Lima et al., 2015).

Golden lion tamarins—GLT (*Leonthopitecus rosalia*)—are also demonstrated to be competent *Trypanosoma cruzi* reservoirs (Lisboa et al., 2015). This callitrichid is an endangered species restricted to the Atlantic rainforest of Rio de Janeiro State, Brazil. It was during the GLT survey for *T. cruzi* that the presence of TcII (the DTU until then related only to human infections), was observed for the first time in the wild (Lisboa et al., 2000). An 11-year follow-up demonstrated that individuals of *L. rosalia* are able to maintain long-lasting infections and infectivity potential when infected by *T. cruzi* from both DTUs TcI and TcII (Lisboa et al., 2015).

Top-predator mammals are still poorly studied, mainly due to the difficulty in trapping and handling them, but are known as important bioaccumulators of *Trypanosoma cruzi*. In this sense, a positive correlation between the proportion of insects or other mammals in the diet and the infection rates by *T. cruzi* has been demonstrated (Rocha et al., 2013).

The composition of the local fauna may indicate which *Trypanosoma cruzi* DTUs will predominate in an area. *Didelphis* spp., for example, are known to maintain high and long-lasting parasitemias (as expressed by positive blood cultures), but only when infected by DTU TcI (Legey et al., 2003), while GLTs can maintain stable infections also by TcII (Lisboa et al., 2015). In contrast, wild and domestic canids usually exhibit a short period of high parasitemia, exactly as observed for humans (Jansen et al., 2018). This diversity explains the differences in the enzootic cycles of the parasite in different areas. In fact, each area is unique and has its own particularities (Jansen et al., 2015).

Currently, what will impact in the infection rates that have emerged in recent outbreaks of Chagas disease in the Americas include: 1) Control of domiciliary transmission of *Trypanosoma cruzi* by *Triatoma infestans*; 2) control of the parasite in the wild fauna of vectors and mammals; and 3) environmental disturbances that result in faunal modifications. With these in mind, and aiming to identify common risk factors that could be used in surveillance programs, domestic dogs were proposed as sentinels of imminent risk of transmission to humans (Roque and Jansen, 2008). In these outbreak areas, where a well-established wild transmission cycle of *Try. cruzi* was observed, dogs showed high prevalence of infection as diagnosed by serology, but did not contribute to the amplification of the parasite population, as attested by negative hemocultures (Roque et al., 2008; 2013). This proposal was further confirmed by spatial analysis using interpolation and map algebra tools, which also considered the environmental factors that interfered with the transmission. In this study, the authors confirmed the hypothesis that loss of wildlife richness was associated with higher parasitemias in wild fauna and higher serological prevalence in the associated canine population (Xavier et al., 2012).

In fact, the study of a multi-host zoonotic parasite such as *Trypanosoma cruzi* encourages analysis of the transmission to humans using a One Health approach (Thompson, 2013). Additionaly, implementation of the DAMA protocol is urgently needed in order to understand and take action in these and other parasites (Brooks et al., 2014; 2019). Parasite spillover or more properly known as ecological fitting (Janzen, 1985) is a phenomenon frequently amplified by changes in the landscape that: 1) Result in selection of individuals; 2) change the dynamics of transmission; and 3) in the case of zoonosis, will ultimately result in higher or lesser risk of emergence of human cases. Multidisciplinary studies are essential to better understand *T. cruzi* control and transmission, which are key conditions for successful surveillance programs. As a primarily enzootic parasite, *T. cruzi* transmission will never be eliminated, but prediction of new human cases and outbreaks is possible and urgently needed.

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Supplemental Reading

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