Chapter 9
Interactions of *Trypanosoma cruzi* and Triatomines

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Abstract Triatomine bugs are vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease in Latin America. The flagellate colonizes the intestinal tract of the insect, especially the rectum. *T. cruzi* changes the composition of amino acids and proteins/peptides in the rectum and affects the intestinal innate immune homeostasis. Since it induces only adverse effects on larval developmental times and mortality rates if starvation as a second stressor is present, the flagellate is classified as "subpathogenic" for the vector. Effects of the vector on the flagellate are obvious in the differing competence for different strains of *T. cruzi*. In addition, the development of the flagellate is affected by different nutritional stages of the vector, i.e. starvation and feeding induce changes in the population density and the percentages of the different developmental stages, especially of spheromastigotes and giant cells which usually occur rarely. Compounds in the urine which is secreted rapidly after feeding induce the development of metacyclic trypomastigotes, the human-infectious stage.

9.1 Introduction

Trypanosoma cruzi is the etiologic agent of Chagas disease, one of the "Big six" of tropical diseases (Schaub and Wülker 1984). It is endemic in Latin America and mainly transmitted by triatomines (Coura 2007). These insects are night-active, and especially poor living conditions, for example houses in rural areas made of adobe bricks or wooden frames covered with mud, offer hiding places during the daytime (Schaub 2009). Of the 140 species of triatomines, only some are strongly adapted to houses, especially *Triatoma infestans*, *Rhodnius prolixus*, *Panstrongylus megistus* and *T. dimidiata* (Fig. 9.1). These species are in the focus of control campaigns,

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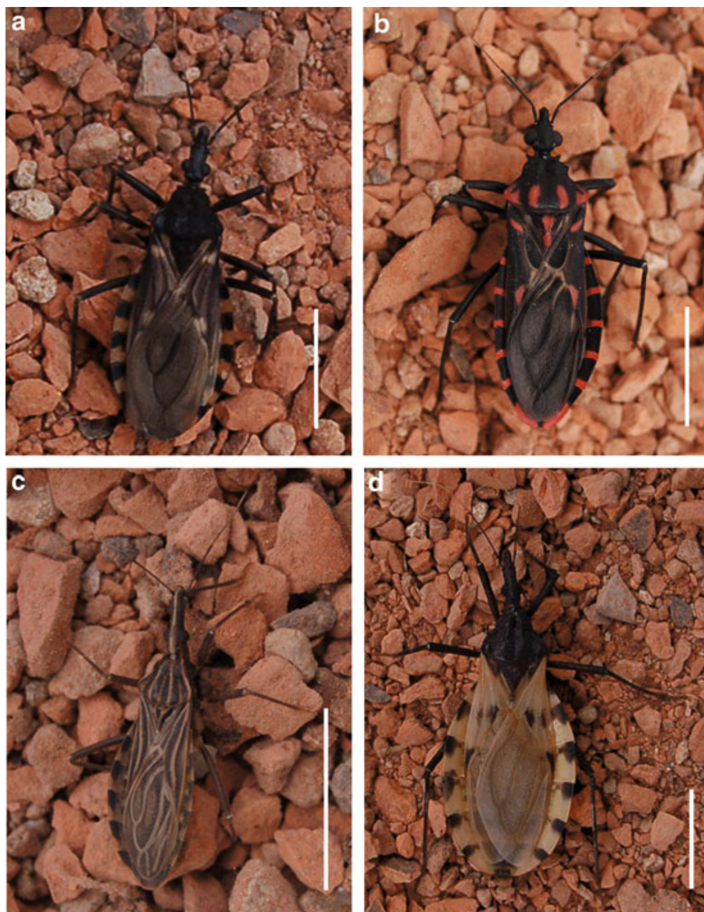


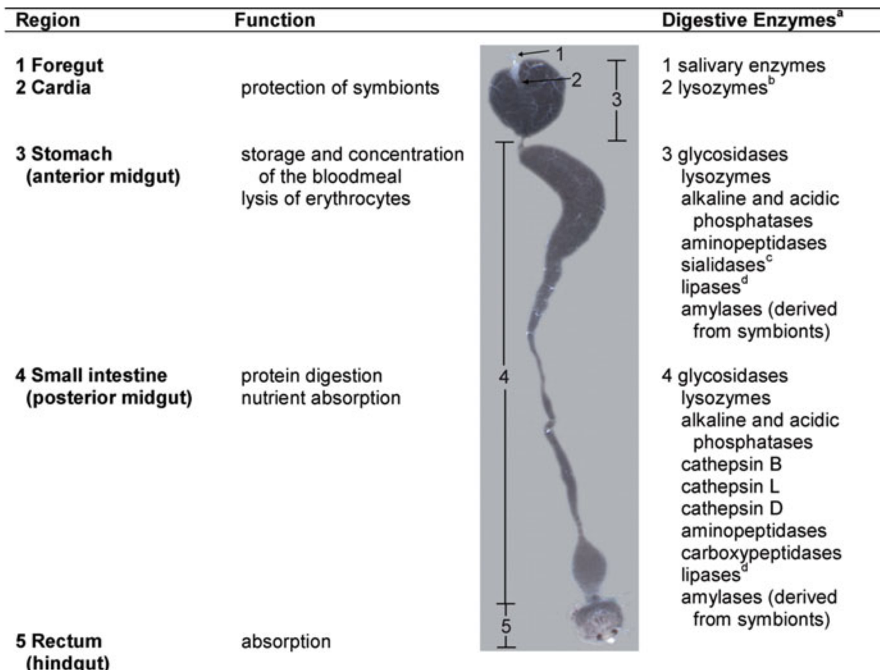
Fig. 9.1 Adults of *Triatoma infestans* (**a**), *Panstrongylus megistus* (**b**), *Rhodnius prolixus* (**c**), and *Triatoma dimidiata* (**d**), (Scale bar: 1 cm)

since only two compounds for chemotherapy which were developed about 1970 are still available, often inducing severe side effects in the patients. Beside house improvements and education, mainly intense insecticide campaigns against the domestic populations of the most important vector *T. infestans* are performed. This has strongly reduced the prevalence from about 20 million chronically infected people in 1982 to about 8 or 12 million in 2007, the latter estimations differing according to the source (WHO 1982, 2007; Dias 2007). Eradication is impossible since Chagas disease is an anthropozoonosis circulating also in many wild mammals as reservoir hosts. In addition, after eradication of domestic *T. infestans* sylvatic populations or other species of triatomines invade the houses (Abad-Franch and Monteiro 2005). Therefore, vector surveillance is of high priority. A suitable and promising immunologically based monitoring technique was recently developed to screen the sera of peridomestic and domestic animals for antibodies against saliva of the vectors (Schwarz et al. 2009, 2010).

9.2 Triatomines

Triatomines are the biggest blood-sucking insects (Schaub 2008), ingesting about 6–12-times their own body weight. Thereafter, some larval instars can starve for up to about 1 year. One full engorgement or several smaller volumes of blood are necessary for the development to the next larval instar or adult stage. The blood is stored in the distensible stomach, concentrated by withdrawal of ions and water and only processed by a lysis of erythrocytes and resorption of sugars (Schaub 2008) (Fig. 9.2). Small portions of blood are digested in the following small intestine where perimicrovillar membranes develop to thick staples after feeding (Billingsley and Downe 1986). Digestion of haemoglobin starts immediately, as indicated by the colour change from red to brown.

The digestion of triatomines is different from that of other blood-sucking insects, for example mosquitoes and lice. Whereas the latter have an alkaline intestinal pH and digest the blood mainly by the serine proteases trypsin and chymotrypsin, Hemiptera and therefore triatomines have an acidic pH in the lumen of the gut and digest mainly by at least three different cathepsins (Kollien et al. 2004a, b). Originally, the activity of cysteine proteases was attributed to cathepsin B (summarized by Terra 1990), but according to molecular biological identifications, also cathepsin L is synthesized in the gut (Lopez-Ordoñez et al. 2001; Kollien et al.



Region	Function	Digestive Enzymes[a]
1 Foregut **2 Cardia**	protection of symbionts	1 salivary enzymes 2 lysozymes[b]
3 Stomach (anterior midgut)	storage and concentration of the bloodmeal lysis of erythrocytes	3 glycosidases lysozymes alkaline and acidic phosphatases aminopeptidases sialidases[c] lipases[d] amylases (derived from symbionts)
4 Small intestine (posterior midgut)	protein digestion nutrient absorption	4 glycosidases lysozymes alkaline and acidic phosphatases cathepsin B cathepsin L cathepsin D aminopeptidases carboxypeptidases lipases[d] amylases (derived from symbionts)
5 Rectum (hindgut)	absorption	

Fig. 9.2 Digestive tract of triatomines: regions, functions and digestive enzymes. [a]Modified from Kollien and Schaub (2000); [b]Araújo et al. (2006); [c]Amino et al. (1995); [d]Canavoso et al. (2004); Grillo et al. (2007)

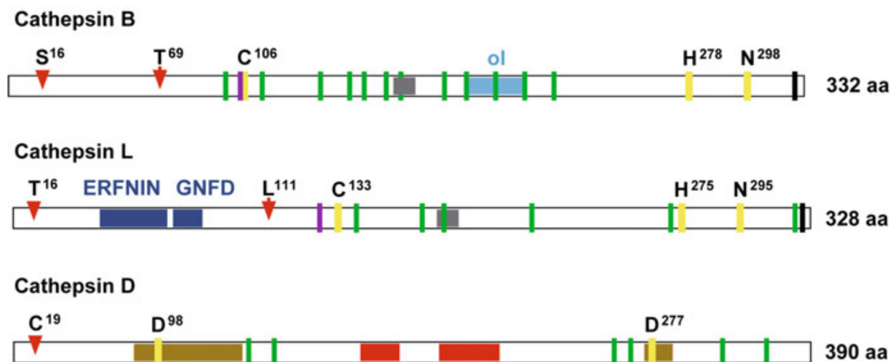


Fig. 9.3 Scheme of primary structures of cathepsin B, L and D from *Triatoma infestans*. Putative cleavage sites of signal peptide and activation peptide are indicated by *red arrow-heads* and *arrows*, respectively. Active site residues and cysteine residues forming disulphide bonds are marked in *yellow* and *green*, respectively. *Black bars* represent substrate specificity determining amino acid residues in cathepsin B and L. The "oxyanion hole" in cathepsin B and L is built by a glutamine residue (*purple*) and specific conserved regions classifying the different cathepsins are highlighted in *light blue* (occluding loop in cathepsin B, ol) and *dark blue* (ERFNIN and GNFD motif in cathepsin L). The common GCDGG motif of cysteine proteases cathepsin B and L is indicated in *grey*. Conserved regions of cathepsin D proteases are indicated by *red* and *brown boxes*, the latter containing the catalytic aspartate residues

2004b). This cathepsin shares many conserved regions in the deduced amino acid sequence with cathepsin B, but can be identified by the ERFNIN and GNFD motifs (Rawlings and Barrett 1994; Sajid and McKerrow 2002) (Fig. 9.3). Whereas both cysteine proteases possess an activity optimum at about pH 5, the aspartic protease cathepsin D has its major activity at about pH 3 (summarized by Garcia et al. 2010). Carboxypeptidases and aminopeptidases continue the digestion of the blood proteins (summarized by Garcia 1987).

After absorption of the nutrients in the small intestine, the remains of the blood are stored in the rectal sac before being defaecated (Terra 1990). Blood ingestion induces a rapid 1000-fold increase of the diuresis rate by the Malpighian tubules (Maddrell 1991). The urine sweeps off the remains of digestion, changing the conditions in the rectum rapidly (Kollien et al. 2001). Between 1 and 10 days after feeding, yellow-white urate crystals from the Malpighian tubules predominate in the rectum, followed by dark-brown remains of digestion.

9.3 *Trypanosoma cruzi*

9.3.1 *Strain Peculiarities*

Investigations of isoenzymes of strains of *T. cruzi* indicate a predominantly clonal genetic structure of the populations and only restricted genetic recombinations

(Tibayrenc et al. 1986). Until last year, strains of *T. cruzi* were classified into 2–3 groups (Anonymous 1999). Strains of the two major groups differ strongly in terms of biological characteristics, for example virulence and pathogenicity for mice, multiplication rate in in vitro cultures or metacyclogenesis rate in the vector (summarized by Schaub 2009). In some regions, lineages of *T. cruzi* are associated with infections in domestic vectors and humans, sylvatic vectors and humans, and sylvatic vectors and wild mammals (summarized by Vallejo et al. 2009; Schaub 2009). In vectors and mammalian hosts, often mixed infections occur. After experimental infections of mice and triatomines with strains belonging to *T. cruzi* I and *T. cruzi* II, a host-dependent selection towards one of the groups seems to occur (summarized by Schaub 2009). Recently, six genetic subgroups were classified based on molecular markers, named TcI–VI (Zingales et al. 2009). The characteristics of strains of these subgroups have to be determined.

9.3.2 Developmental Cycle of T. cruzi

Infectious stages in the faeces of the vector initiate the infection of the mammalian host. These metacyclic trypomastigotes are phagocytized, transform to amastigotes and multiply intracellularly in all cells of the mammalian host, except erythrocytes, because the parasites require purines from the host cell. If the host cell is exhausted, the amastigotes transform to non-dividing blood trypomastigotes. After rupturing the host cell they infect new cells or circulate in the blood (Schaub and Wunderlich 1985).

If a bug ingests blood containing blood trypomastigotes, these are aggregated, and some seem to fuse, enabling a genetic exchange (Brener 1972). They transform in the stomach to a-, sphero- and epimastigotes that multiply and colonize the whole intestinal tract and the Malpighian tubules (Fig. 9.4). The development of non-dividing metacyclic trypomastigotes seems to be restricted to the rectum. In the vector, *T. cruzi* reaches high population densities: 3 months after infection of second instars of *T. infestans*, the small intestine of fifth instar larvae contains about 500,000 parasites and the rectum about 1,500,000 (Schaub 1989a).

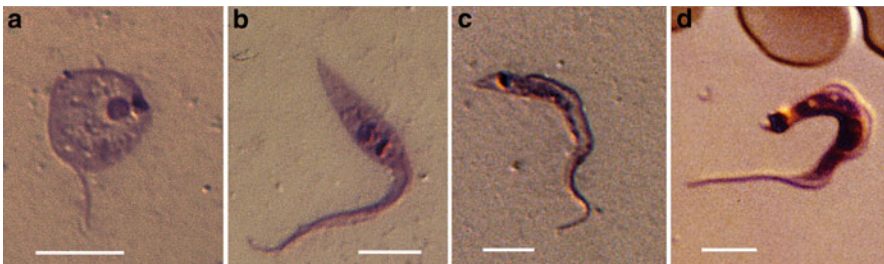


Fig. 9.4 Different developmental stages of *Trypanosoma cruzi*: spheromastigote (**a**), epimastigote (**b**), metacyclic trypomastigote (**c**), blood trypomastigote (**d**); Giemsa stained. (Scale bar: 5 μm)

9.4 Effects of *T. cruzi* on Triatomines

9.4.1 Effects on the Development of Larvae and Starvation Capacity

Effects of *T. cruzi* on the developmental rates of larvae were found rarely (summarized by Schaub 1992). Using a system in which vector and the *T. cruzi* strain originate from the same village, no retardation of development of regularly fed larvae occurs. Also the mortality rates are not affected by *T. cruzi* if the groups are maintained under optimal conditions (summarized by Schaub 2009). Under such conditions, the volume of ingested blood seems to be sufficient to compensate the metabolite losses of triatomines to the parasite.

This compensation theory is supported by investigations of the starvation resistance. After an infection in the first instar and the last feed in the second, third and fourth instar, the resulting third, fourth and fifth instar larvae survive about 5, 11 and 9 months, respectively, and the mean starvation resistance is reduced respectively by 3%, 14% and 32% relative to uninfected bugs (Schaub and Lösch 1989). Since more food remains are present in the intestine of infected than in uninfected bugs not the availability of proteins from the blood determines the starvation capacity, but the concentration of essential metabolites for which *T. cruzi* and vector compete. An accumulation of toxic products by *T. cruzi* seems to be unlikely since many flagellates die in starved bugs (Schaub and Böker 1986; Kollien and Schaub 1998a). Since no effects are obvious under optimal conditions and infections induce only adverse effects if starvation as a second, synergistic stressor is present, *T. cruzi* is classified as “subpathogenic” (Schaub 1989b, 1992).

9.4.2 Effects on Behaviour

Disturbances of probing in infected bugs have been found several times (summarized in Schaub 1992, 2009). However, also long-term starved bugs probe more often before engorging and ingest lesser amounts of blood than short-term starved bugs (Schaub, unpublished). If infected and uninfected bugs are compared at the same time after the last feeding, the infected bugs are in a more progressed state of starvation and thus the effects attributed to *T. cruzi* might have been effects of starvation and of minor relevance under natural conditions.

9.4.3 Effects on the Intestinal Border Face, Digestion and the Composition of the Intestinal Contents

In the small intestine, no effects on the perimicrovillar membranes or the cells of the intestinal wall are evident (Kollien et al. 1998). In the rectum, the flagellates attach

to the rectal cuticle and insert the flagellum between the layers of wax, also at the rectal pads, which are suggested to absorb metabolites from the remains of blood digestion (Schmidt et al. 1998). The disturbance of the wax layer might affect absorption processes, but more important effects should result from the colonization density, a carpet of three or four layers on the rectal pads (summarized by Kollien et al. 1998).

The composition of the contents of stomach, small intestine and rectum of uninfected and infected bugs has not been compared in detail, for example by two-dimensional electrophoresis, but the haemolysis of erythrocytes in the stomach and the protein digestion by cathepsin B in the small intestine are not affected by the flagellate (summarized by Schaub 2009). However, more detailed investigations are necessary, since molecular biological assays also identified another cysteine protease, cathepsin L (Lopez-Ordoñez et al. 2001; Kollien et al. 2004b). Such investigations should also consider that the flagellate possesses cysteine proteases, cruzipains, not only intracellularly but also as membrane-bound proteases and also the respective inhibitors (summarized by Schaub 2009). The activity of the third protease, cathepsin D, is increased at 1 and 3 days after an experimental infection with epimastigotes (Borges et al. 2006).

In the rectum, the strong colonization should induce effects. Therefore, it is not surprising that in infected bugs the composition of the rectal contents is changed: the concentrations of free amino acids are reduced and the composition of protein/peptide bound amino acids is changed, indicating that proteins or peptides from the rectal contents are hydrolyzed in the rectum also by surface proteases of *T. cruzi* or intracellularly in the flagellate (Kollien and Schaub, unpublished).

9.4.4 Effects on the Immune System and Intestinal Microorganisms

Ingestion of trypomastigotes stimulates immune reactions in the intestine of the triatomine *R. prolixus*. The expression of the gene of the more intestinally active lysozyme RpLys-A is increased after an infection and not that of RpLys-B, the gene of which is primarily expressed in the fat body (Ursic-Bedoya et al. 2008). The production of the only known antimicrobial peptide of triatomines, defensin, has not been investigated in infected triatomines, but another factor, nitric oxide, is also synthesized in the intestine after an infection. This has been shown by the upregulated expression of the gene of the nitric oxide synthase in the stomach after an infection with blood trypomastigotes, but also by the increased concentrations of a metabolite of nitric oxide, nitrite, in the small intestine (Whitten et al. 2007). In addition to lysozymes, defensins and nitric oxide, several unidentified bacteriolytic compounds are present, which are visible in zymograms using *Micrococcus luteus* (syn *M. lysodeikticus*) (Waniek et al. 2009). Since the expression rates of the genes of lysozymes, defensins and nitric oxide synthase are also increased after blood ingestion (Araújo et al. 2006; Whitten et al. 2007), these short-term reactions to

T. cruzi can partly be a general defence reaction against microorganisms and the increase of cathepsin D activity (see 4.3) could also be a part of this concerted action.

Effects of *T. cruzi* are also evident in long-term infected *T. infestans*. After an experimental infection via a mixture of blood and different microorganisms, high numbers of fungi and bacteria develop only in infected bugs not in uninfected controls, indicating an immune suppression in the intestine (Eichler 1998). The number of symbionts is not affected in these long-term infected larvae.

9.5 Effects of the Triatomine on *T. cruzi*

9.5.1 Susceptibility and Refractoriness

So far, all species of triatomines which were maintained in the laboratory could be experimentally infected with *T. cruzi*. Therefore, probably all species are potential vectors (Schofield 1994). However, susceptibility varies depending on various factors, and not all infections remain established (Garcia and Azambuja 1991). Usually natural infections of triatomines are not lost, indicating a strong co-evolution of *T. cruzi* and the respective species/strain of the insect host (summarized by Schaub and Lösch 1989). In endemic regions, the infection rates of triatomines vary strongly, for example between 79% in a local survey in Bolivia and 5% in a general survey in Brazil (Medrano-Mercado et al. 2008; Dias 2002). The latter value is not only due to a limited access of triatomines to an infected host, but to refractoriness mechanisms in the gut of the vector (summarized by Garcia et al. 2010). This is especially important in xenodiagnosis: Blood of patients suspected to be infected with *T. cruzi* in the chronic phase of the disease, in which the parasite cannot be found in microscopical blood examinations, are fed to laboratory-bred uninfected triatomines. Low numbers of parasites in the blood multiply in the triatomine and can more easily be found after some weeks (Dias 1940). In such diagnoses, sometimes parasites do not multiply. Therefore, the use of local vectors is suggested (summarized by Meiser and Schaub 2011).

9.5.2 Effects of Factors in the Stomach – Components of the Saliva of Triatomines and Host-Derived Factors

The saliva of triatomines contains many different pharmacologically active compounds that inhibit blood coagulation and enable rapid blood ingestion. For the majority of these compounds the function is unknown. In some of them the activity is controlled by the cleavage of the activation peptide via serine proteases in the saliva (Amino et al. 2001; Assumpção et al. 2008; Meiser et al. 2010b).

Usually such serine proteases act very specifically. Their activity in the stomach should be limited by Kazal-type inhibitors of serine proteases, which usually inhibit the activation of thrombin and thus blood coagulation (summarized by Meiser et al. 2010a). Possible interactions of these proteases and inhibitors with the surface proteins of *T. cruzi* have not been investigated.

One of these proteolytically activatable proteins, the alkaline, lysine-rich protein trialysin of *T. infestans*, lyses bacteria and mammalian cells, but also the cell culture-derived trypomastigotes and epimastigotes of *T. cruzi* (Amino et al. 2002, Martins et al. 2008). In addition to other factors, it seems to impair the transmission of *Trypanosoma rangeli* by *T. infestans*, since the main vector, *R. prolixus,* does not possess this lytic activity in the salivary glands (Gregório and Ratcliffe 1991).

Another protein of the saliva of *T. infestans*, a sialidase, targets sialic acids that are involved in blood coagulation as well as in inflammatory processes (Amino et al. 1998). A sialidase activity is not only found in the saliva, but also in the stomach contents, the enzyme rapidly desialylating the ingested blood cells and also the epimastigotes in the midgut (summarized by Amino et al. 1995). Therefore, sialic acid on the surface is not required for the development of epimastigotes in the intestine. Since stationary phase epimastigotes and metacyclic trypomastigotes possess high levels of a unique transsialidase to transfer sialic acid to their surface, sialic acids appear to be important for the survival of trypomastigotes in the mammalian host (Amino et al. 1995).

Another component of the saliva, an inhibitor of the complement activation, is suggested to be necessary for the protection of the intestinal cells of the vector against this host-derived system (Cavalcante et al. 2003; Barros et al. 2009). However, the activity of the complement in the stomach is not totally, but only strongly reduced within 2 h after blood ingestion and remains slightly active up to 1 day later (Garcia, unpublished). Within this period of time ingested blood trypomastigotes are agglutinated and transform to epimastigotes. This stage of *T. cruzi* is lysed by the complement. The sensitivity of epimastigotes is evident in the initial phase of an infection of humans: If faeces of the vector with the different stages of development of *T. cruzi* gets access to the mammalian host, all stages except metacyclic trypomastigotes are killed by the complement system. The sensitivity is also evident in established infections in which the stomach has been re-colonized from the population in the small intestine. After the larval moults, nearly all blood passes to the small intestine, and the red colour of the remaining blood in the stomach changes to brown, indicating a re-flux of contents with digestive enzymes from the small intestine into the stomach. Via this re-flux, also *T. cruzi* re-establishes in the stomach. Then the ingestion of blood from chickens and rats but not from mice (which possess a weak complement system) results in a lysis of epimastigotes (Schaub 1988; Schaub, unpublished). After such a feeding on mice, another factor from mammalian blood, plasminogen, binds to the epimastigotes (Rojas et al. 2008). The advantage or disadvantage of the presence of this inactive, usually fibrinolytic serine protease remains to be investigated.

A third host-derived factor, antibodies against *T. cruzi*, is also ingested together with the blood of the host. They can be a reason for the failure of the development of *T. cruzi* in xenodiagnosis triatomines (see Sect. 9.5.1), but an experimental proof is required. An indication is offered by in vitro assays in which, after an immunization of mice or rabbits with homogenates of epimastigotes, the decomplemented sera agglutinate the flagellates and induce ultrastructural damage (Fernández-Presas et al. 2001).

9.5.3 Effects of Factors in the Stomach – Agglutinins and Hemolysins

Whereas the general tasks of agglutinins/lectins in the stomach are unknown, hemolysins have to lyse the erythrocytes in the stomach. Lectins were suggested to be involved in the development of the different stages of *T. cruzi* in the vector because they differed between the stomach and the small intestine of *R. prolixus* and the respective receptors were present in epimastigotes but not in blood trypomastigotes (Pereira et al. 1981). The respective lectins in the small intestine were not verified, but the levels of lectins seem to be affected by the blood source (Ratcliffe et al. 1996). Whereas the effects of agglutinins on the long-term development of the different stages require new investigations, the initial establishment of *T. cruzi* in the vector is determined by agglutinins and hemolysins. After ingestion of *T. cruzi* they determine the susceptibility or refractoriness of the respective species or population of triatomines but also for a specific strain of *T. cruzi*. In the initial transformation of *T. cruzi* the blood trypomastigotes are often agglutinated, but not in all strains of *T. cruzi*. In incubations in stomach extracts of *R. prolixus*, epimastigotes of the strain Cl and the clone Dm28c are agglutinated but not lysed and develop in the bug. However, epimastigotes of strain Y are not agglutinated but lysed and thereby are unable to develop in *R. prolixus* (Mello et al. 1996). The agglutination is not induced by a reaction of a peanut-like lectin with the disaccharide D-Gal-β(1 → 3)D-GalNAc, since not only epimastigotes of strain Y but also of strain Cl do not react with this lectin (Schottelius 1982). The purified hemolytic factor of *R. prolixus* lyses the majority of epimastigotes of strain Y, but much less of the clone Dm28c (Azambuja et al. 1989).

9.5.4 Effects of Factors from Small Intestine and Rectum – The Border Face

Although the digestive small intestine contains higher concentrations of nutrients than the rectum, in regularly fed fifth instars of *T. infestans* only about 0.5 million flagellates/bug develop there, about one third of the population in the rectum (Schaub

and Lösch 1988; Schaub 1989a; Kollien and Schaub 1998a, b). After infecting fourth instars of *T. brasiliensis* and feeding the fifth instar, the small intestine even contains only one tenth of the rectal population (Araújo et al. 2008). Using two other strains, only one strain mainly colonized the small intestine of *T. brasiliensis* (Araújo et al. 2007). Except in the last investigation, the concentration of nutrients seems not to be relevant for these differences in the colonization. Since small intestine and rectum are of different ontogenetical origin – entodermal or ectodermal – the border face is different, i.e. the cells of the midgut possess apical microvilli that are covered by perimicrovillar membranes, whereas the rectal cells are covered by a cuticle.

In transmission electron microscopy of infected small intestines, all epimastigotes are in intimate contact with the perimicrovillar membranes (Kollien et al. 1998; Gonzalez et al. 1999). No ultrastructural modifications of the flagellum of trypanosomatids are evident, and the epimastigotes attach via glycoinositol phospholipids at their surface (Alves et al. 2007; Nogueira et al. 2007). Since the development of perimicrovillar membranes is hormonally regulated, 10 days after decapitation of *R. prolixus*, *T. cruzi* is present only occasionally in the gut; the same effect occurs after the feeding of blood containing antiserum against the membranes and midgut tissue (Alves et al. 2007; Gonzalez et al. 2006).

In the rectum, *T. cruzi* prefers to attach to the cuticle and initially colonizes the four rectal pads (summarized by Schaub and Böker 1986). In established infections, about one third of the rectal population is attached to this region, which covers – roughly estimated – only about 20% of the rectal surface (Schaub and Lösch 1988; Kollien and Schaub 1997, 1998b). Another third is attached to the remaining rectal wall and the final third colonizes, unattached, the rectal lumen. The preference for the rectal pads might have a physiological or mechanical basis (summarized by Schaub 2009). As a mechanism in the attachment by the flagellum originally lectins at the surface of the flagellum and chitin residues were supposed to be involved (summarized by Schmidt et al. 1998). Especially, the use of wheat-germ lectin-gold conjugates for the detection of chitin in transmission electron microscopy verifies that chitin is not accessible for *T. cruzi* and that the superficial layer at the luminal surface of the rectum is covered by a wax layer (Schmidt et al. 1998). The attachment of hexadecane droplets to a small region near the tip of the flagellum of epimastigotes identifies a hydrophobic interaction as attachment mechanism (Kleffmann et al. 1998). After the initial attachment to the rectal wall, the flagellum is modified. At the attachment site to the rectal cuticle, epimastigotes develop enlargements of the flagellum (summarized by Kollien et al. 1998).

A species-specific composition of the rectal border face is indicated by different attachment rates of epimastigotes of *T. cruzi* strain Y and strain Berenice: Epimastigotes of the latter strain adhere better to recta from *Rhodnius neglectus* than to recta from *Triatoma pseudomaculata* (Carvalho-Moreira et al. 2003). This corresponds with a higher metacyclogenesis rate in vivo. The epimastigotes of the *T. cruzi* strain Y show no differences in the attachment rate to the rectum of the two triatomines in vitro, but also develop more metacyclic trypomastigotes in *R. neglectus*. In general, attachment strongly enhances the transformation of epimastigotes to trypomastigotes in vitro (summarized by Kleffmann et al. 1998).

9.5.5 Effects of Factors from Small Intestine and Rectum – Effects of Proteases

All parasites developing in digestive regions of the intestine must possess a refractory surface and/or a rapid shedding or inactivation of attached proteases and/or inhibitors of digestive enzymes. The surface coat of trypanosomatids is refractory against many adverse compounds, and specific mucins are suggested to protect the epimastigotes against proteases (Acosta-Serrano et al. 2001). In addition, *T. cruzi* possesses a cysteine protease inhibitor, chagasin, at the surface (Monteiro et al. 2001, 2008; Ljunggren et al. 2007). The target of this inhibitor can be cathepsins in the direct neighbourhood of the flagellate and/or in the lumen of the gut (see Sect. 9.2). However, tissue culture-derived trypomastigotes possess higher levels of this inhibitor than epimastigotes, the latter producing inversely correlated more papain-like cysteine proteases, cruzipains. Therefore, in epimastigotes chagasin might mainly regulate the endogenous and not the surface bound cruzipain (Monteiro et al. 2001).

Direct or indirect effects of proteases have only been considered once. After feeding blood supplemented with the cathepsin D inhibitor pepstatin, the number and metacyclogenesis rate of *T. cruzi* in the gut of *R. prolixus* is not affected compared to bugs fed solely on blood (Garcia and Gilliam 1980).

9.5.6 Effects of Factors from Small Intestine and Rectum – Influence of Starvation

An effect of starvation on *T. cruzi* in a field population of triatomines was first detected in *Triatoma dimidiata*, of which more starved than regularly fed larvae lost the infection (Vargas and Zeledón 1985). In experimental infections and a scanning electron microscopical follow-up of the colonization density on the rectal wall (Schaub and Böker 1986), throughout the first 16 weeks after feeding of fifth instar larvae, no changes in the colonization pattern were evident: minimal colonizations around the entrance into the rectum, highest on the rectal pads and at a similar level in the other three regions. At 20 weeks after feeding, many regions were free of flagellates, but a residual population always remained attached to the rectal pads.

According to quantifications of the population density in small intestine and rectum, a starvation period of 3 or 4 weeks kills many flagellates in the small intestine of fifth instar larvae of *T. infestans* and reduces the population density (Schaub 1989a; Schaub and Lösch 1989). However, if infected triatomines die of starvation, some flagellates are still alive (Schaub and Lösch 1989). Longer starvation periods of 2 months, eliminate the population in the small intestine, but not in the rectum, which still contains about one third of the population of short-term

starved bugs (Kollien and Schaub 1998a, b). Four months after the last feeding, only 1% of the initial population are present, but in all bugs some parasites are alive.

Not only the number of flagellates is affected, also the composition of the population changes (Kollien and Schaub 1998a). The percentages of the respective intermediate forms and of spheromastigotes which only are present up to 2% of the total population in well-fed bugs increase during starvation to about 20% at 2 and 3 months after the last feeding (Kollien and Schaub 1998a).

9.5.7 Effects of Factors from Small Intestine and Rectum – Influence of Blood Ingestion and Excretion

Blood ingestion also affects *T. cruzi*, not only under certain circumstances in the stomach (see Sect. 9.5.2). In the small intestine, the population density increases after feeding (Schaub 1989a). Considering the whole intestine, this increase in the number of epimastigotes is correlated to the volume of blood ingested by the triatomine (Asin and Catalá 1995).

Initially opposite effects of blood ingestion are evident in the rectum. There, a low percentage of the attached population, but nearly all of the population in the lumen – about one third of the total population – is washed out by urine (summarized by Kollien and Schaub 1997). Identifying the different stages in the deposited drops of faeces and urine (see Sect. 9.2), the percentage of metacyclic trypomastigotes is low in the first drop of faeces, and the urine often contains pure populations of metacyclics (summarized by Schaub and Lösch 1988; Zeledón 1997). This is presumably based on the inability of trypomastigotes to attach, and especially metacyclic trypomastigotes of *T. cruzi*, lying on the carpet or in the upper layers of the carpet, are washed out. In addition, metacyclogenesis is induced (see Sect. 9.5.8). Thereby, in the remaining population the percentages of epi- and trypomastigotes are changed. The percentages of spheromastigotes and their intermediate forms are reduced from about 20% – the starvation effect – to about 2–3%, the level of regularly fed bugs (Schaub and Lösch 1988; Kollien and Schaub 1997).

After feeding of long-term starved bugs the population in the rectal lumen is also washed out, but an interesting phenomenon is evident in the composition of the population (Kollien and Schaub 1998b). In fifth instars which have starved for 60 days, the rectum contains about 20–30% spheromastigotes and the respective intermediate stages, about 20% epimastigotes and 40–50% trypomastigotes. One day after feeding, these forms represent 2%, 70% and 10%, respectively. However, about 10% are the so far only occasionally seen giant cells, containing many nuclei, kinetoplasts and flagella. In the following 2 days, the percentages of this form increase to 30–50% of the total population, and then decrease to 0%. These giant cells in long-term starved bugs originate from epimastigotes, those in the initial development after the infection from blood trypomastigotes (summarized by Schaub 2009).

9.5.8 Effects of Factors from Gut and Malpighian Tubules – Induction of Metacyclogenesis

Of all stages of *T. cruzi* deposited in the faeces/urine, only metacyclic trypomastigotes can survive after invasion through mucous membranes or skin lesions and initiate an infection in the mammalian host (Schuster and Schaub 2000). Therefore, elucidation of the mechanisms inducing the development of this stage has been the topic of many investigations. However, the investigations mainly focus on in vitro assays, simplified by the easy cultivation of *T. cruzi* in different media and cell cultures. In axenic in vitro cultivations first metacyclics appear after about 5 days (Chiari and Camargo 1984).

Using extracts of the small intestine or stomach of adult *T. infestans*, dissected 24–48 h after feeding, as a supplement to Grace medium, metacyclogenesis is induced after 4–7 days (de Isola et al. 1981). Extracts of adults fed 3 weeks before the dissections are inactive. Extracts of the rectum induce metacyclogenesis already within 15 min after incubation, and an additional supplementation with inhibitors of the ADP-ribosyltransferases inhibits this induction (de Isola et al. 1986, 1987). The inductory factor, which is present in the rectum of fifth instars and adults 2 days after feeding on chicken, increases the activity of the adenylate cyclase of *T. cruzi* and thereby induces metacyclogenesis. This factor is a 10-kDa peptide fragment from the amino terminus of chicken $\propto^{D}$-globin (Fraidenraich et al. 1993); pure chicken haemoglobin is inactive. A decrease of the concentration of this peptide in the rectum during the subsequent days after feeding explains the failure of induction using extracts of adults 3 weeks after feeding (see above). The reaction is not restricted to chicken $\propto^{D}$-globin since after feeding on mice, the rectum of *T. infestans* contains a similar active compound. Since 1–2 days after feeding, only urine or uric acid granules are present in the rectum, but no dark remains of digestion of haemoglobin (Schaub 2009), these data indicate a rapid passage of minor amounts of small molecules along the whole small intestine or an excretion of such compounds via the Malpighian tubules. However, the $\propto^{D}$-globin should also be present in the small intestine in which haemoglobin is digested, but extracts of this region induce metacyclogenesis much more slowly than extracts of the rectum (see above). In addition, metacyclic trypomastigotes rarely develop in the small intestine (Schaub 1989a).

According to in vitro incubations with synthetic peptides, which cover different parts of the respective $\propto^{D}$-globin fragment, the peptide corresponding to residues 1–40 at the amino terminus possesses the highest activity at concentrations higher than 10^{-10} M (Fraidenraich et al. 1993). In vivo, i.e. after feeding blood or plasma with different concentrations of haemoglobin and the synthetic peptides to *T. cruzi*-infected *R. prolixus,* higher concentrations of haemoglobin but not pure blood increase metacyclogenesis (Garcia et al. 1995). Although not all peptides show identical individual or synergistic effects in vitro and in vivo, the $\propto^{D}$-globin fragment seems to be a biologically relevant factor.

However, not only the usual adenylate cyclase pathway is activated during metacyclogenesis. In an induction via free fatty acids, especially oleic acid, and at concentrations similar to those found in the intestinal tract, protein kinase C isoenzymes are translocated to the membrane of culture-derived epimastigotes (Parsons and Ruben 2000; Wainszelbaum et al. 2003; Belaunzarán et al. 2009). It remains to be investigated whether or not one of these metacyclogenesis-inducing factors interacts with GP72, a major surface glycoprotein of *T. cruzi*; monoclonal antibodies against this glycoprotein strongly inhibit metacyclogenesis of epimastigotes (Snary 1985).

Whereas these assays identified specific factors, another approach uses nutritional stress. Epimastigotes in the late exponential growth phase of axenic in vitro cultures, shortly before an increase in the number of metacyclics, are incubated for 2 h in saline, named "artificial urine", and then in this saline supplemented with glutamate, aspartate, proline and glucose (Contreras et al. 1985a, b). This procedure strongly increases metacyclogenesis rates (Contreras et al. 1988), but most intensively only in one specific clone of *T. cruzi* (Dm28c) (Schaub, unpublished). Compared to the urine excreted by *T. infestans*, the "artificial urine" has other ionic strengths, another pH and contains neither amino acids nor peptides (Kollien et al. 2001). In addition, in the supplemented saline glucose is necessary for the transformation (Tyler and Engman 2001), but the presence of glucose in the rectum of a triatomine is doubtful. However, the respective clone and the specific conditions enable well reproducible studies, and the good timing of events enables the identification of steps in this cAMP-mediated process and of genes specifically expressed at each step of metacyclogenesis (e.g. Krieger and Goldenberg 1998; Gonzales-Perdomo et al. 1988; Ávila et al. 2003). The changes in the concentrations of the mRNA of the respective genes can also be found in *T. cruzi* populations in the vector (Cordero et al. 2008).

Can these in vitro assays be correlated to the metacyclogenesis in the vector? In the triatomines, metacyclogenesis seems to be restricted to the rectum. Although intermediate stages sometimes develop in the small intestine, only in the rectum do concentrations of metacyclic trypomastigotes increase up to 50% of that population, in part being enhanced by the attachment (summarized by Schaub 2009) (see Sect. 9.5.4). Metacyclogenesis already starts about 1–2 weeks after infection, some days after colonization of the rectum. After infection of second instar larvae and regular feeding, the percentages of trypomastigotes remain at this level for about 8 weeks (e.g. Schaub 1989a). Then in fifth instar larvae, the percentages increase to a higher level. However, an identification of factors acting 8–9 weeks after infection is difficult.

A better chance is offered by the induction of metacyclogenesis after blood ingestion (Schaub and Lösch 1988; Kollien and Schaub 1997) since it occurs in a short period of time, about 20 min after blood ingestion. Such a rapid induction is important for the population of *T. cruzi*. Urine washes out the majority of the population in the lumen. However, only metacyclic trypomastigotes or intermediate stages which possess the surface of the metacyclic trypomastigote can survive and develop in the mammalian host. Therefore, a rapid induction of metacyclogenesis increases the chance for survival. Since the surface coat does not change rapidly in

total (Schaub et al. 1989), a quantification of stages via these changes in a cell sorter is difficult. Easier, but more time-consuming is a morphological quantification in stained smears, also enabling a quantification of the four different ways of metacyclogenesis. Metacyclic trypomastigotes develop from spheromastigotes via drop-like or rarely ring forms and from epimastigotes as slender forms via a translocation of the kinetoplast to the posterior end or after unequal divisions resulting in an epi- and a trypomastigote daughter cell (Brener and Alvarenga 1976; Schaub 1989a). Before blood ingestion of larvae starved for 6 weeks, unequal divisions and ring forms are rarely found in the rectal lumen, drop-like and slender forms each make up about 50% of the intermediate forms. Within the first four drops of faeces/urine, only the percentages of slender intermediate forms increase, afterwards remaining at 100% (Schaub and Lösch 1988). Since this also occurs in vitro in incubations of the isolated complex of the rectum and the four Malpighian tubules after induction of diuresis by the artificial diuretic hormone 5-hydroxytryptamine (Kollien and Schaub 1997), the inducing factors seem to originate from the urine. This hypothesis is supported by incubations of pieces of recta with attached *T. cruzi* either in saline with different pH, or in faeces or urine of triatomines. Using different salines, the strong changes of pH, osmolality and ionic composition in the rectum (Kollien et al. 2001) do not induce metacyclogenesis. Also a mixture of remains of digestion and urine deposited in the first drop after feeding does not induce metacyclogenesis. Metacyclogenesis is increased only in incubations with urine (Kleffmann 1999).

Summarizing these investigations, they indicate the presence of different factors that induce metacyclogenesis. A factor in the urine enhances rapid metacyclogenesis in those epimastigotes which have already started to transform. About 1 or 2 days after blood ingestion, the $\propto^{D}$-globin fragment and oleic acid induce metacyclogenesis in the population remaining in the rectum after blood ingestion. This population might be sensitive to the enriched “artificial urine” which requires a previous nutritional stress that also occurs in the vector. It should be emphasized that all these factors are relevant for the metacyclogenesis of epimastigotes via a translocation of the kinetoplast. So far there is no indication which factors might induce metacyclogenesis of epimastigotes via unequal divisions and of spheromastigotes via drop and ring-like forms.

9.5.9 Effects of Microorganisms and Antimicrobial Compounds

The intestinal tract of triatomines is colonized by many different fungi and bacteria, the latter containing non-symbiotic and symbiotic species (summarized by Vallejo et al. 2009). Effects of non-symbiotic bacteria on *T. cruzi* have only been investigated for *Serratia marcescens,* which produces a red pigment, prodigiosin, and has been found in wild populations of *R. prolixus.* It is apathogenic for the triatomine (Azambuja et al. 2004). In *S. marcescens*-infected larvae, a subsequent infection with epimastigotes of *T. cruzi* strain Y and clone Dm28c results in strain/

clone-specific differences: the population density of strain Y decreases, whereas the density of clone Dm28c in the stomach remains unchanged (Azambuja et al. 2004, 2005). In incubations of the bacterium with epimastigotes of the strain/ clone in vitro, the same differences arise. The bacteria develop long filamentous structures that connect the bacteria with the epimastigotes and kill them (Castro et al. 2007a, b). Using different strains of *S. marcescens*, only the strain which produces the red pigment kills *T. cruzi* strain Y. However, recent data exclude a direct effect of the pigment, since promastigotes of *Leishmania* are also lysed, if the production of prodigiosin is inhibited by changes in the growth conditions (Moraes et al. 2009). For lysis, attachment of the bacterium alone is sufficient. Lectin interactions seem to be involved since the effect is excluded if the epimastigotes are incubated in D-mannose (Castro et al. 2007a, b).

Effects of symbionts on the development of *T. cruzi* have only been considered in one investigation (Mühlpfordt 1959). The initial development of *T. cruzi* is stronger in bugs containing symbionts, but after a longer period is stronger in aposymbiotic bugs. However, the composition of the population, i.e. the percentages of the broad and slender epimastigotes, amastigotes and metacyclic trypomastigotes, remains unaffected. Since symbionts multiply in triatomines after blood ingestion and are suggested to produce vitamin B, the short-term results with *T. cruzi* correlate with results of another flagellate infecting triatomines, *Blastocrithidia triatomae*. A supplementation of blood with B-group vitamins (folic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine) supports the initial development of *B. triatomae* in the small intestine of young larvae (Jensen and Schaub 1991).

Possible effects of antibacterial compounds of triatomines on *T. cruzi* have not been investigated. The RNAi technique for a knockdown of genes of antimicrobial factors and the incubation with heterologously synthesized antimicrobial compounds like lysozymes and defensins or enzymes of the immune system like the prophenoloxidase or nitric oxide synthase can elucidate possible effects on *T. cruzi*. Antibacterial compounds not belonging to the humoral response of triatomines affect the development of *T. cruzi* in vitro, for example mellitin, magainin, dermaseptin and tachyplesin (summarized by Löfgren et al. 2008). However, such in vitro assays usually use higher concentrations of peptides than those present in the respective insect. They might be usable in other approaches: The production of the lepidopteran cecropin by transformed symbionts kills all *T. cruzi* in the gut (Beard et al. 2002). Although *T. cruzi* induces the synthesis of several antibacterial factors, an effect on *T. cruzi* remains to be investigated.

9.6 Conclusions

Investigations of the interactions of *T. cruzi* and triatomines will be strongly supported by the genome project of *R. prolixus*. Although species of the tribe Rhodniini possess many differences in comparison to species of the other tribes (summarized by Schaub 2009), the increasing number of expressed sequence tags

and the RNAi technique offer many possibilities. Investigations of the effects of *T. cruzi* on triatomines mainly focus on the intestinal innate immune reactions, representing a second model system beside the Diptera. Two important open questions remain to be clarified on the effects of triatomines on *T. cruzi*: reactions inducing the initial killing of some strains of *T. cruzi* in the stomach and the molecular basis of metacyclogenesis in the rectum of the vector.

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References

Abad-Franch F, Monteiro FA (2005) Molecular research and the control of Chagas disease vectors. An Acad Bras Cienc 77:437–454

Acosta-Serrano A, Almeida IC, Freitas-Junior LH, Yoshida N, Schenkman S (2001) The mucin-like glycoprotein super-family of *Trypanosoma cruzi*: structure and biological roles. Mol Biochem Parasitol 114:143–150

Alves CR, Albuquerque-Cunha JM, Mello CB, Garcia ES, Nogueira NF, Bourguingnon SC, de Souza W, Azambuja P, Gonzalez MS (2007) *Trypanosoma cruzi* attachment to perimicrovillar membrane glycoproteins of *Rhodnius prolixus*. Exp Parasitol 116:44–52

Amino R, Serrano AA, Morita OM, Pereira-Chioccola VL, Schenkman S (1995) A sialidase activity in the midgut of the insect *Triatoma infestans* is responsible for the low levels of sialic acid in *Trypanosoma cruzi* growing in the insect vector. Glycobiology 5:625–631

Amino R, Porto RM, Chammas R, Egami MI, Schenkman S (1998) Identification and characterization of a sialidase released by the salivary gland of the hematophagous insect *Triatoma infestans*. J Biol Chem 273:24575–24582

Amino R, Tanaka AS, Schenkman S (2001) Triapsin, an unusual activatable serine protease from the saliva of the hematophagous vector of Chagas' disease *Triatoma infestans* (Hemiptera: Reduviidae). Insect Biochem Mol Biol 31:465–472

Amino R, Martins RM, Procopio J, Hirata IY, Juliano MA, Schenkman S (2002) Trialysin, a novel pore-forming protein from saliva of hematophagous insects activated by limited proteolysis. J Biol Chem 277:6207–6213

Anonymous (1999) Recommendations from a satellite meeting. Mem Inst Oswaldo Cruz 94:429–432

Araújo CAC, Waniek PJ, Stock P, Mayer C, Jansen AM, Schaub GA (2006) Sequence characterization and expression patterns of defensin and lysozyme encoding genes from the gut of the reduviid bug *Triatoma brasiliensis*. Insect Biochem Mol Biol 36:547–560

Araújo CAC, Cabello PH, Jansen AM (2007) Growth behaviour of two *Trypanosoma cruzi* strains in single and mixed infections: In vitro and in the intestinal tract of the blood-sucking bug, *Triatoma brasiliensis*. Acta Trop 101:225–231

Araújo CAC, Waniek PJ, Jansen AM (2008) Development of a *Trypanosoma cruzi* (TcI) isolate in the digestive tract of an unfamiliar vector, *Triatoma brasiliensis* (Hemiptera, Reduviidae). Acta Trop 107:195–199

Asin S, Catalá S (1995) Development of *Trypanosoma cruzi* in *Triatoma infestans*: influence of temperature and blood consumption. J Parasitol 81:1–7

Assumpção TCF, Francischetti IMB, Andersen JF, Schwarz A, Santana JM, Ribeiro JMC (2008) An insight into the sialome of the blood-sucking bug *Triatoma infestans*, a vector of Chagas' disease. Insect Biochem Mol Biol 38:213–232

Ávila AR, Dallagiovanna B, Yamada-Ogatta SF, Monteiro-Góes V, Fragoso SP, Krieger MA, Goldenberg S (2003) Stage-specific gene expression during *Trypanosoma cruzi* metacyclogenesis. Genet Mol Res 2:159–168

Azambuja P, Mello CB, D'Escoffier LN, Garcia ES (1989) *In vitro* cytotoxicity of *Rhodnius prolixus* hemolytic factor and mellitin towards different trypanosomatids. Braz J Med Biol Res 22:597–599

Azambuja P, Feder D, Garcia ES (2004) Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite, *Trypanosoma cruzi*, in the vector. Exp Parasitol 107:89–96

Azambuja P, Garcia ES, Ratcliffe NA (2005) Gut microbiota and parasite transmission by insect vectors. Trends Parasitol 21:568–572

Barros VC, Assumpção JG, Cadete AM, Santos VC, Cavalcante RR, Araújo RN, Pereira MH, Gontijo NF (2009) The role of salivary and intestinal complement system inhibitors in the midgut protection of triatomines and mosquitoes. PLoS One 4(6):e6047. doi:10.1371/journal.pone.0006047

Beard CB, Cordon-Rosales C, Durvasula RV (2002) Bacterial symbionts of the Triatominae and their potential use in control of Chagas disease transmission. Annu Rev Entomol 47:123–141

Belaunzarán ML, Lammel EM, Giménez G, Wainszelbaum MJ, de Isola ELD (2009) Involvement of protein kinase C isoenzymes in *Trypanosoma cruzi* metacyclogenesis induced by oleic acid. Parasitol Res 105:45–55

Billingsley PF, Downe AER (1986) The surface morphology of the midgut cells of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) during blood digestion. Acta Trop 43:355–366

Borges EC, Machado EMM, Garcia ES, Azambuja P (2006) *Trypanosoma cruzi*: effects of infection on cathepsin D activity in the midgut of *Rhodnius prolixus*. Exp Parasitol 112:130–133

Brener Z (1972) A new aspect of *Trypanosoma cruzi* life-cycle in the invertebrate host. J Protozool 19:23–27

Brener Z, Alvarenga NJ (1976) Life cycle of *T. cruzi* in the vector. In: Pan American Health Organization (ed) New approaches in American trypanosomiasis research, Sci. Publ. No. 318. Pan American Health Organization, Washington, DC, pp 83–88

Canavoso LE, Frede S, Rubiolo ER (2004) Metabolic pathways for dietary lipids in the midgut of hematophagous *Panstrongylus megistus* (Hemiptera: Reduviidae). Insect Biochem Mol Biol 34:845–854

Carvalho-Moreira CJ, Spata MC, Coura JR, Garcia ES, Azambuja P, Gonzalez MS, Mello CB (2003) In vivo and in vitro metacyclogenesis tests of two strains of *Trypanosoma cruzi* in the triatomine vectors *Triatoma pseudomaculata* and *Rhodnius neglectus*: short/long-term and comparative study. Exp Parasitol 103:102–111

Castro DP, Moraes CS, Garcia ES, Azambuja P (2007a) Inhibitory effects of D-mannose on trypanosomatid lysis induced by *Serratia marcescens*. Exp Parasitol 115:200–204

Castro DP, Seabra SH, Garcia ES, de Souza W, Azambuja P (2007b) *Trypanosoma cruzi*: ultrastructural studies of adhesion, lysis and biofilm formation by *Serratia marcescens*. Exp Parasitol 117:201–207

Cavalcante RR, Pereira MH, Gontijo NF (2003) Anti-complement activity in the saliva of phlebotomine sand flies and other haematophagous insects. Parasitology 127:87–93

Chiari E, Camargo EP (1984) Culturing and cloning of *Trypanosoma cruzi*. In: Morel CM (ed) Genes and antigens of parasites. A laboratory manual, 2nd edn. Fundacao Oswaldo Cruz, Rio de Janeiro, pp 23–26

Contreras VT, Morel CM, Goldenberg S (1985a) Stage specific gene expression precedes morphological changes during *Trypanosoma cruzi* metacyclogenesis. Mol Biochem Parasitol 14:83–96

Contreras VT, Salles JM, Thomas N, Morel CM, Goldenberg S (1985b) In vitro differentiation of *Trypanosoma cruzi* under chemically defined conditions. Mol Biochem Parasitol 16:315–327

Contreras VT, Araújo-Jorge TC, Bonaldo MC, Thomas N, Barbosa HS, Meirelles MNL, Goldenberg S (1988) Biological aspects of the Dm28c clone of *Trypanosoma cruzi* after metacyclogenesis in chemically defined media. Mem Inst Oswaldo Cruz 83:123–133

Cordero EM, Gentil LG, Crisante G, Ramírez JL, Yoshida N, Añez N, da Silveira JF (2008) Expression of GP82 and GP90 surface glycoprotein genes of *Trypanosoma cruzi* during *in vivo* metacyclogenesis in the insect vector *Rhodnius prolixus*. Acta Trop 105:87–91

Coura JR (2007) Chagas disease: what is known and what is needed – a background article. Mem Inst Oswaldo Cruz 102(suppl 1):113–122

Dias E (1940) Xenodiagnosticos seriados em cães infectados com amostras Venezuelanas de "*Schizotrypanum cruzi*". Bras Med 54:859–861

Dias JCP (2002) Controle da doença de Chagas no Brasil. In: Silveira AC (ed) Controle da doença de Chagas nos países do cone sul da América. Pan American Health Organization, Washington, DC, pp 145–239

Dias JCP (2007) Eradication of Chagas disease: What are its possibilities? In: Pan American Health Organization/World Health Organization (PAHO/WHO) and Sociedade de Pediatra do Estado de Rio de Janeiro (SOPERJ) (eds) Update of American Trypanosomiasis and leishmaniasis control and research: Final Report, pp 65–72

Eichler S (1998) Interaktionen von Triatominen mit ihren Symbionten und Trypanosomatiden. Ph. D. thesis, Fak Biol, Ruhr-Universität Bochum, Bochum

Fernández-Presas AM, Zavala JT, Fauser IB, Merchant MT, Guerrero LR, Willms K (2001) Ultrastructural damage of *Trypanosoma cruzi* epimastigotes exposed to decomplemented immune sera. Parasitol Res 87:619–625

Fraidenraich D, Peña C, Isola EL, Lammel EM, Coso O, Díaz Añel A, Pongor S, Baralle F, Torres HN, Flawiá MM (1993) Stimulation of *Trypanosoma cruzi* adenylyl cyclase by an α^D-globin fragment from *Triatoma* hindgut: effect on differentiation of epimastigote to trypomastigote forms. Proc Natl Acad Sci USA 90:10140–10144

Garcia ES (1987) The digestion of Triatominae. In: Brenner RR, Stoka A (eds) Chagas' disease vector, vol 2, Anatomic and physiological aspects. CRC Press, Boca Raton, pp 47–59

Garcia ES, Azambuja P (1991) Development and interaction of *Trypanosoma cruzi* within the insect vector. Parasitol Today 7:240–244

Garcia ES, Gilliam FC (1980) *Trypanosoma cruzi* development is independent of protein digestion in the gut of *Rhodnius prolixus*. J Parasitol 66:1052–1053

Garcia ES, Gonzalez MS, de Azambuja P, Baralle FE, Fraidenraich D, Torres HN, Flawiá MM (1995) Induction of *Trypanosoma cruzi* metacyclogenesis in the gut of the hematophagous insect vector, *Rhodnius prolixus*, by hemoglobin and peptides carrying alpha D-globin sequences. Exp Parasitol 81:255–261

Garcia ES, Genta FA, de Azambuja P, Schaub GA (2010) Interactions of intestinal compounds of triatomines and *Trypanosoma cruzi*. Trends Parasitol 26:499–505

Gonzales-Perdomo M, Romero P, Goldenberg S (1988) Cyclic AMP and adenylate cyclase activators stimulate *Trypanosoma cruzi* differentiation. Exp Parasitol 66:205–212

Gonzalez MS, Nogueira NFS, Mello CB, de Souza W, Schaub GA, Azambuja P, Garcia ES (1999) Influence of brain and azadirachtin on *Trypanosoma cruzi* development in the vector, *Rhodnius prolixus*. Exp Parasitol 92:100–108

Gonzalez MS, Hamedi A, Albuquerque-Cunha JM, Nogueira NFS, de Souza W, Ratcliffe NA, Azambuja P, Garcia ES, Mello CB (2006) Antiserum against perimicrovillar membranes and midgut tissue reduces the development of *Trypanosoma cruzi* in the insect vector, *Rhodnius prolixus*. Exp Parasitol 114:297–304

Gregório EA, Ratcliffe NA (1991) The distribution of agglutinins and lytic activity against *Trypanosoma rangeli* and erythrocytes in *Rhodnius prolixus* and *Triatoma infestans* tissue extracts and haemolymph. Mem Inst Oswaldo Cruz 86:181–186

Grillo LA, Majerowicz D, Gondim KC (2007) Lipid metabolism in *Rhodnius prolixus* (Hemiptera: Reduviidae): role of a midgut triacylglycerol-lipase. Insect Biochem Mol Biol 37:579–588
de Isola ELD, Lammel EM, Katzin VJ, Gonzalez Cappa SM (1981) Influence of organ extracts of *Triatoma infestans* on differentiation of *Trypanosoma cruzi*. J Parasitol 67:53–58
de Isola ELD, Lammel EM, González Cappa SM (1986) *Trypanosoma cruzi*: differentiation after interaction of epimastigotes and *Triatoma infestans* intestinal homogenate. Exp Parasitol 62:329–335
de Isola ELD, Lammel EM, González Cappa SM (1987) *Trypanosoma cruzi*: differentiation to metacyclic trypomastigotes in the presence of ADP-ribosyltranserase inhibitors. Exp Parasitol 64:424–429
Jensen C, Schaub GA (1991) Development of *Blastocrithidia triatomae* (Trypanosomatidae) in *Triatoma infestans* after vitamin B-supplementation of the blood-diet of the bug. Europ J Protistol 27:17–20
Kleffmann T (1999) Mechanismen der Anheftung und Induktion der Metazyklogenese von *Trypanosoma cruzi* in *Triatoma infestans*. Ph.D. thesis, Fak Biol, Ruhr-Universität Bochum, Bochum
Kleffmann T, Schmidt J, Schaub GA (1998) Attachment of *Trypanosoma cruzi* epimastigotes to hydrophobic substrates and use of this property to separate stages and promote metacyclogenesis. J Eukaryot Microbiol 45:548–555
Kollien AH, Schaub GA (1997) *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion of the vector and artificial diuresis. Parasitol Res 83:781–788
Kollien AH, Schaub GA (1998a) The development of *Trypanosoma cruzi* (Trypanosomatidae) in the reduviid bug *Triatoma infestans* (Insecta): influence of starvation. J Eukaryot Microbiol 45:59–63
Kollien AH, Schaub GA (1998b) *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion by the starved vector. Am J Trop Med Hyg 59:166–170
Kollien AH, Schmidt J, Schaub GA (1998) Modes of association of *Trypanosoma cruzi* with the intestinal tract of the vector *Triatoma infestans*. Acta Trop 70:127–141
Kollien AH, Schaub GA (2000) The development of *Trypanosoma cruzi* in Triatominae. Parasitol Today 16:381–387.
Kollien AH, Grospietsch T, Kleffmann T, Zerbst-Boroffka I, Schaub GA (2001) Ionic composition of the rectal contents and excreta of the reduviid bug *Triatoma infestans*. J Insect Physiol 47:739–747
Kollien AH, Waniek PJ, Pröls F, Habedank B, Schaub GA (2004a) Cloning and characterization of a trypsin-encoding cDNA of the human body louse *Pediculus humanus*. Insect Mol Biol 13:9–18
Kollien AH, Waniek PJ, Nisbet AJ, Billingsley PF, Schaub GA (2004b) Activity and sequence characterization of two cysteine proteases in the digestive tract of the reduviid bug *Triatoma infestans*. Insect Mol Biol 1:569–579
Krieger MA, Goldenberg S (1998) Representation of differential expression: a new approach to study differential gene expression in trypanosomatids. Parasitol Today 14:163–166
Ljunggren A, Redzynia I, Alvarez-Fernandez M, Abrahamson M, Mort JS, Krupa JC, Jaskolski M, Bujacz G (2007) Crystal structure of the parasite protease inhibitor chagasin in complex with a host target cysteine protease. J Mol Biol 371:137–153
Löfgren SE, Miletti LC, Steindel M, Bachère E, Barracco MA (2008) Trypanocidal and leishmanicidal activities of different antimicrobial peptides (AMPs) isolated from aquatic animals. Exp Parasitol 118:197–202
Lopez-Ordoñez T, Rodriguez MH, de la Cruz Hernández-Hernández F (2001) Characterization of a cDNA encoding a cathepsin L-like protein of *Rhodnius prolixus*. Insect Mol Biol 10:505–511
Maddrell SHP (1991) The fastest fluid-secreting cell known: the upper Malpighian tubule cell of *Rhodnius*. Bioessays 13:357–362

Martins RM, Amino R, Daghastanli KR, Cuccovia IM, Juliano MA, Schenkman S (2008) A short proregion of trialysin, a pore-forming protein of *Triatoma infestans* salivary glands, controls activity by folding the N-terminal lytic motif. FEBS J 275:994–1002

Medrano-Mercado N, Ugarte-Fernandez R, Butrón V, Uber-Busek S, Guerra HL, de Araújo-Jorge TC, Correa-Oliveira R (2008) Urban transmission of Chagas disease in Cochabamba, Bolivia. Mem Inst Oswaldo Cruz 103:423–430

Meiser CK, Piechura H, Werner T, Dittmeyer-Schäfer S, Meyer HE, Warscheid B, Schaub GA, Balczun C (2010a) Kazal-type inhibitors in the stomach of *Panstrongylus megistus* (Triatominae, Reduviidae). Insect Biochem Mol Biol 40:345–353

Meiser CK, Piechura H, Meyer HE, Warscheid B, Schaub GA, Balczun C (2010b) A salivary serine protease of the haematophagous reduviid *Panstrongylus megistus*: sequence characterization, expression pattern and characterization of proteolytic activity. Insect Mol Biol 19:409–421

Meiser CK, Schaub GA (2011) Xenodiagnosis. In: Mehlhorn, H. (ed) Parasitology research monographs. Vol. 1, Nature helps… How plants and other organisms contribute to solve health problems. Springer, Heidelberg, pp 273–299

Mello CB, Azambuja P, Garcia ES, Ratcliffe NA (1996) Differential in vitro and in vivo behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. Exp Parasitol 82:112–121

Monteiro ACS, Abrahamson M, Lima APCA, Vannier-Santos MA, Scharfstein J (2001) Identification, characterization and localization of chagasin, a tight-binding cysteine protease inhibitor in *Trypanosoma cruzi*. J Cell Sci 114:3933–3942

Monteiro ACS, de Oliveira Neto OB, Del Sarto RP, de Magalhaes MTQ, Lima JN, Lacerda AF, Oliveira RS, Scharfstein J, da Silva MCM, Valencia JWA, Jimenez AV, Grossi-de-Sal MF (2008) A recombinant form of chagasin from *Trypanosoma cruzi*: inhibitory activity on insect cysteine proteinases. Pest Manag Sci 64:755–760

Moraes CS, Seabra SH, Albuquerque-Cunha JM, Castro DP, Genta FA, de Souza W, Brazil RP, Garcia ES, Azambuja P (2009) Prodigiosin is not a determinant factor in lysis of *Leishmania* (*Viannia*) *braziliensis* after interaction with *Serratia marcescens* D-mannose sensitive fimbriae. Exp Parasitol 122:84–90

Mühlpfordt H (1959) Der Einfluß der Darmsymbionten von *Rhodnius prolixus* auf *Trypanosoma cruzi*. Z Trop Med Parasitol 10:313–327

Nogueira NFS, Gonzalez MS, Gomes JE, de Souza W, Garcia ES, Azambuja P, Nohara LL, Almeida IC, Zingales B, Colli W (2007) *Trypanosoma cruzi*: involvement of glycoinositolphospholipids in the attachment to the luminal midgut surface of *Rhodnius prolixus*. Exp Parasitol 116:120–128

Parsons M, Ruben L (2000) Pathways involved in environmental sensing in trypanosomatids. Parasitol Today 16:56–62

Pereira MEA, Andrade AFB, Ribeiro JMC (1981) Lectins of distinct specificity in *Rhodnius prolixus* interact selectively with *Trypanosoma cruzi*. Science 211:597–600

Rawlings ND, Barrett AJ (1994) Families of cysteine peptidases. Methods Enzymol 244: 461–486

Ratcliffe NA, NigamY MCB, Garcia ES, Azambuja P (1996) *Trypanosoma cruzi* and erythrocyte agglutinins: a comparative study of occurrence and properties in the gut and hemolymph of *Rhodnius prolixus*. Exp Parasitol 83:83–93

Rojas M, Labrador I, Concepción JL, Aldana E, Avilan L (2008) Characteristics of plasminogen binding to *Trypanosoma cruzi* epimastigotes. Acta Trop 107:54–58

Sajid M, McKerrow JH (2002) Cysteine proteases of parasitic organisms. Mol Biochem Parasitol 120:1–21

Schaub GA (1988) Direct transmission of *Trypanosoma cruzi* between vectors of Chagas' disease. Acta Trop 45:11–19

Schaub GA (1989a) *Trypanosoma cruzi*: quantitative studies of development of two strains in small intestine and rectum of the vector *Triatoma infestans*. Exp Parasitol 6:260–273

Schaub GA (1989b) Does *Trypanosoma cruzi* stress its vector? Parasitol Today 5:185–188
Schaub GA (1992) The effects of trypanosomatids on insects. Adv Parasitol 31:255–319
Schaub GA (2008) Kissing bugs. In: Mehlhorn H (ed) Encyclopedia of parasitology, vol 1, 3rd edn. Springer, Heidelberg, pp 170–173
Schaub GA (2009) Interactions of trypanosomatids and triatomines. Adv Insect Physiol 37:177–242
Schaub GA, Böker CA (1986) Colonization of the rectum of *Triatoma infestans* by *Trypanosoma cruzi*: influence of starvation studied by scanning electron microscopy. Acta Trop 43:349–354
Schaub GA, Lösch P (1988) *Trypanosoma cruzi*: origin of metacyclic trypomastigotes in the urine of the vector *Triatoma infestans*. Exp Parasitol 65:174–186
Schaub GA, Lösch P (1989) Parasite/host-interrelationships of the trypanosomatids *Trypanosoma cruzi* and *Blastocrithidia triatomae* and the reduviid bug *Triatoma infestans*: influence of starvation of the bug. Ann Trop Med Parasitol 83:215–223
Schaub GA, Wülker W (1984) Tropische Parasitosen im Programm der Weltgesundheitsorganisation. Universitas 39:71–80
Schaub GA, Wunderlich F (1985) Die Chagas-Krankheit. Biol unserer Zeit 15:42–51
Schaub GA, Grünfelder C, Zimmermann D, Peters W (1989) Binding of lectin-gold conjugates by two *Trypanosoma cruzi* strains in ampullae and rectum of *Triatoma infestans*. Acta Trop 46:291–301
Schmidt J, Kleffmann T, Schaub GA (1998) Hydrophobic attachment of *Trypanosoma cruzi* to a superficial layer of the rectal cuticle in the bug *Triatoma infestans*. Parasitol Res 84:527–536
Schofield CJ (1994) Triatominae: biology and control. Eurocommunica Publications, West Sussex
Schottelius J (1982) The identification by lectins of two strain groups of *Trypanosoma cruzi*. Z Parasitenkd 68:147–154
Schuster JP, Schaub GA (2000) *Trypanosoma cruzi*: skin-penetration kinetics of vector-derived metacyclic trypomastigotes. Int J Parasitol 30:1475–1479
Schwarz A, Helling S, Collin N, Teixeira CR, Medrano-Mercado N, Hume JCC, Assumpção TC, Marcus K, Stephan C, Meyer HE, Ribeiro JMC, Billingsley PF, Valenzuela JG, Sternberg JM, Schaub GA (2009) Immunogenic salivary proteins of *Triatoma infestans*: development of a recombinant antigen for the detection of low-level infestation of triatomines. PLoS Negl Trop Dis 3(10):e532. doi:10.1371/journal.pntd.0000532
Schwarz A, Medrano-Mercado N, Billingsley PF, Schaub GA, Sternberg JM (2010) IgM-antibody responses of chickens to salivary antigens of *Triatoma infestans* as early biomarkers for low-level infestation of triatomines. Int J Parasitol 40:1295–1302
Snary D (1985) Receptors and recognition mechanisms of *Trypanosoma cruzi*. Trans R Soc Trop Med Hyg 79:587–590
Terra WR (1990) Evolution of digestive systems of insects. Annu Rev Entomol 35:181–200
Tibayrenc M, Ward P, Moya A, Ayala FJ (1986) Natural populations of *Trypanosoma cruzi,* the agent of Chagas' disease, have a complex multiclonal structure. Proc Natl Acad Sci USA 83:115–119
Tyler KM, Engman DM (2001) The life cycle of *Trypanosoma cruzi* revisited. Int J Parasitol 31:472–481
Ursic-Bedoya RJ, Nazzari H, Cooper D, Triana O, Wolff M, Lowenberger C (2008) Identification and characterization of two novel lysozymes from *Rhodnius prolixus*, a vector of Chagas' disease. J Insect Physiol 54:593–603
Vallejo GA, Guhl F, Schaub GA (2009) Triatominae-*Trypanosoma cruzi/T. rangeli*: vector-parasite interactions. Acta Trop 110:137–147
Vargas LG, Zeledón R (1985) Effect of fasting on *Trypanosoma cruzi* infection in *Triatoma dimidiata* (Hemiptera: Reduviidae). J Med Entomol 22:683
Wainszelbaum MJ, Belaunzaran ML, Lammel EM, Florin-Christensen M, Florin-Christensen J, Isola ELD (2003) Free fatty acids induce cell differentiation to infective forms in *Trypanosoma cruzi*. Biochem J 375:705–712

Waniek PJ, Mendonça-Lima L, Menezes GB, Jansen AM, Araújo CAC (2009) Recombinant expression and characterization of a lysozyme from the midgut of *Triatoma brasiliensis* (Hemiptera, Reduviidae) in comparison with intestinal muramidase activity. Physiol Entomol 34:309–317

WHO (1982) Chagas' disease. In: UNDP/World Bank/WHO (eds) Sixth programme report. Special programme for research and training in tropical diseases. WHO, Geneva, pp 137–190

WHO (2007) New global effort to eliminate Chagas disease. Wkly Epidemiol Rec 82:259–260

Whitten M, Sun F, Tew I, Schaub GA, Soukou C, Nappi A, Ratcliffe NA (2007) Differential modulation of *Rhodnius prolixus* nitric oxide activities following challenge with *Trypanosoma rangeli*, *T. cruzi* and bacterial cell wall components. Insect Biochem Mol Biol 37:440–452

Zeledón R (1997) Infection of the insect host by *Trypanosoma cruzi*. In: Carcavallo RU, Galindez I, Jurberg J, Lent H (eds) Atlas of Chagas' disease vectors in the America, vol 1. Editora Fiocruz, Rio de Janeiro, pp 271–287

Zingales B, Andrade SG, Briones MRS, Campbell DA, Chiari E, Fernandes O, Guhl F, Lages-Silva E, Macedo AM, Machado CR, Miles MA, Romanha AJ, Sturm NR, Tibayrenc M, Schijman AG (2009) A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz 104:1051–1054