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Trypanosoma cruzi – The Vector-parasite Paradox

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Trypanosoma cruzi *and the majority of its insect vectors (Hemiptera, Reduviidae, Triatominae) are confined to the Americas. But while recent molecular studies indicate a relatively ancient origin for the parasite (~65 million years ago) there is increasing evidence that the blood-sucking triatomine vectors have evolved comparatively recently (<5 mya). This review examines the evidence for these ideas, and attempts to reconcile the apparent paradox by suggesting that marsupial opossums (Didelphidae) may have played a role, not just as original reservoir hosts, but also as original vectors of the parasite.*

Key words: *Trypanosoma cruzi* - Triatominae - Didelphidae - evolution

Trypanosoma cruzi, causative agent of Chagas disease, occurs exclusively in the Americas (except for occasional human infections that have travelled elsewhere). Similarly, the majority of its insect vectors – blood-sucking Reduviidae of the subfamily Triatominae – also occur primarily in the Americas (except for the aberrant Indian genus *Linshcosteus*, and the tropicopolitan *Triatoma rubrofasciata* and its Asian relatives). Parasite and vector are associated, but a paradox arises from evidence that *T. cruzi* is a relatively ancient parasite, whereas the Triatominae appear to have evolved comparatively recently (Stevens et al. 2000). Such interpretations may be erroneous, or they may be reconcilable. This review seeks to analyse the available evidence.

EVIDENCE FOR THE ANTIQUITY OF *TRYPANOSOMA CRUZI*

T. cruzi is widespread in the Americas, from the Great Lakes of the USA to the southern Patagonia of Argentina (roughly 42°N to 46°S). Throughout this region it is a common parasite of small mammals, especially nest-building species of rodents and opossums which are commonly associated with silvatic species of Triatominae. Human infections are rare in the USA, but regrettably frequent in Latin America where rural houses are often infested with domestic species of Triatominae. It is clear that most transmission of *T. cruzi* to humans is via faecal deposits from infected Triatominae, although other routes of transmission are also possible, notably by blood transfusion from infected donors, transplacental transmission from infected mothers, and oral route transmission by eating infected material (for review see Pipkin 1969). But even for transmission via infected triatomine faeces, there is still doubt about the frequency of parasite passage across intact human skin. Many authorities consider that transmission most commonly occurs when the infected faecal deposits are inadvertently passed to the mucosa of eye, nose or mouth, across which the parasite can pass quite readily. Similarly, transmission to small mammals would seem most likely to occur when the mammal eats an infected bug, or licks triatomine faecal deposits while grooming the haircoat (Diotaiut et al. 1995). Oral route transmission would seem to be the primitive state.

If the first line of evidence for the relative antiquity of *T. cruzi* comes from its wide distribution in the Americas, the second comes from comparative clinical studies. Human infections are often virulent, with some mortality during the acute parasitaemic phase of infection, and severe tissue lesions developing in up to 30% of chronic human infections. Similar pathology can be seen in domestic animals such as cats and dogs, and also in some strains of sinanthropic murid rodents (especially laboratory rats and mice). In contrast, severe pathology has not been recorded in commonly infected wild hosts such as opossums, armadillos, and cricetid rodents. This comparison suggests that those mammals of greater antiquity in the Americas have had a longer association with *T. cruzi*, compared to those mammals imported more recently from the Old World, allowing time for some degree of co-evolution leading to attenuated virulence.

The third line of evidence comes from genetic studies. Phylogenetic analysis based on ssu rRNA sequence data indicate that the genus *Trypanosoma* is monophyletic (Stevens & Gibson 1999, Stevens et al. 1999a,b, 2000) and this conclusion is

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endorsed by studies based on the GAPDH gene (Wiemer et al. 1995, Alvarez et al. 1996, Hannaert et al. 1998), and on 9S and 12S mitochondrial rRNA genes (Lake et al. 1988), elongation factor 1α (Hashimoto et al. 1995), trypanothione reductase and α-tubulin (Alvarez et al. 1996) and phosphoglycerate kinase (Adjé et al. 1998). All trypanosomes are parasitic, so the idea of of a monophyletic origin suggests an ancestral parasitic form that gave rise to the mammalian trypanosomes of Africa, America, and Australasia, as well as the various forms that parasitise fish. The implied connection between these groups is offered by plate tectonic theory, suggesting that the original parasitic forms developed prior to splitting up the continents during the mezozoic era (~230 mya). However, the ssu rRNA data also indicate monophyly for the 'cruzi clade', where this group includes the various forms of *T. cruzi*, together with *T. rangeli*, various trypanosomes of bats, and an unamed species isolated from an australian kangaroo (Stevens et al. 2000). Development of the cruzi clade is thus suggested to have initiated prior to splitting up the southern supercontinent (Gondwanaland) in the cenozoic era. The earliest forms of *cruzi* itself are deduced to have been associated with marsupial opossums at the time of separation of South America from Gondwanaland about 40 mya.

Within *T. cruzi*, there is strong evidence for at least two main lineages, originally denoted as Z1 and Z2 on the basis of their isoenzyme profiles (Miles 1979), subsequently denoted as lineage 2 and lineage 1, repectively, on the basis of molecular markers: RAPDs (Tibayrenc et al. 1993, Souto et al. 1996, Brisse et al. 1998), miniexon gene sequences and 24S lsu rRNA sequences (Souto et al. 1996, Zingales et al. 1998, Fernandes et al. 1998), cytochrome-b sequences (Brisse 1997) and the topoisomerase locus (Dos Santos & Buck 1999). These two lineages are now denoted as *cruzi* 1 (= Z1of Miles 1979, and lineage 2 of Souto et al. 1996) and *cruzi* 2 (= Z2 of Miles 1979, and lineage 1 of Souto et al. 1996) (*Memórias* 1999). Of these, *cruzi*1 seems the more homogenous and, from studies throughout the Americas, seems primitively associated with opossums (*Didelphis* spp.). By contrast, *cruzi* 2 shows a number of well-characterised natural clones found mainly in southern cone countries, and seems primitively associated with rodents. *T. cruzi* 2 in human infections is also associated with chronic intestinal lesions (eg. megaoesophagus, megacolon) as well as the cardiopathy that characterises *cruzi* 1 infections. *T. cruzi* Z3 of Miles (1979) is also heterogeneous, and seems by analysis of miniexon gene sequences to have affinity with *cruzi* 1, although it can generally be recognised as a sub-clade characterised by a ~50bp insertion in the non-transcribed spacer region of the miniexon (Fernandes et al. 1998). It is almost invariably silvatic, with current evidence suggesting a primitive association with armadillos. *T. cruzi marinkellei* (sometimes referred to as *T. cruzi* Z4) is strongly associated with bats.

Other parasites grouped within the cruzi clade of Stevens et al. (2000) are *T. dionisii* and *T. vespertilionis* of bats, *T. conorhini* of rats, *T. rangeli* which can transiently infect a wide range of mammals but seems primarily associated with *Rhodnius* species, *T. leeuwenhoeki* and *T. minasense* which seem similar to *T. rangeli*, and the unamed trypanosome species from an Australian kangaroo. Their data incompletely resolve the position of the flea-transmitted rodent trypanosomes *T. lewisi*, *T. musculi*, and *T. microtis*, but place them close to the cruzi clade.

EVIDENCE FOR THE ANTIQUITY OF TRIATOMINAE

The Triatominae are defined as Reduviidae (Hemiptera, Heteroptera) that suck vertebrate blood (Jeannel 1919, Lent & Wygodzinsky 1979) in contrast to the other 30 or so reduviid subfamilies[1] that prey on invertebrates. The Reduviidae themselves are clearly an ancient family, with the fossil record suggesting that the earliest predatory forms may have derived from phytophagous Hemiptera during the Permian/Triassic periods some 230 mya (Evans 1956, Wootton 1981). Such forms would clearly predate haematophagy since the earliest mammals and birds seem to have arisen during the Jurassic period some 50 million years later. Predatory reduviids are now of worldwide distribution, with well over 6,000 described species (Maldonado Capriles 1990). All reduviid subfamilies now seem to be represented in the Americas, and at least three subfamilies of predatory reduviid are represented in Oligocene and Eocene amber (25-65 mya) from Mexico and the Caribbean – Apiomerinae (Capriles et al. 1993a), Emesinae (Thomas 1992, Capriles et al. 1993b) and Holoptilinae (Poinar 1991). In contrast, there appears to be no evidence of fossilised haematophagous forms.

The evolution of feeding habits within the Heteroptera has been subject to considerable discussion (see: Cobben 1978, 1979, Sweet 1979, Schuh 1986,). Blood-sucking most probably derived from

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[1] At the time of writing, there is no universal agreement on the number of subfamilies within the Reduviidae. Of the 32 named subfamilies, 22 are accepted by Davis (1969), 24 by Putshkov and Putschkov (1985), 25 by Maldonado Capriles (1990), and 23 by Schuh and Slater (1995).

a predaceous habit, with the intermediate stages perhaps being predation on guilds of nest-dwelling invertebrates followed by facultative blood feeding from the vertebrates occupying the nests (Schofield 1988, Schofield & Dolling 1993). Within the heteropteran Hemiptera, there are many species that suck vertebrate blood to a greater or lesser degree (Table I). Even some phytophagous species will probe vertebrates and may imbibe blood on occasion. The frequency of haematophagous behaviour suggests that facultative blood-sucking is a relatively simple step within the Heteroptera, and seems to be particularly frequent amongst predatory groups of Reduviidae and Lygaeidae, and also amongst the Anthocoridae which are believed to have given rise to the obligate haematophagous families of Cimicidae and Polyctenidae (see: Southwood & Leston 1959, Schuh & Slater 1995). In the converse sense, the predatory state is reflected in many species of Triatominae. Examples include *T. rubrofasciata*, which was considered beneficial in some huts in SE Asia because of preying on caterpillars that would otherwise damage the palm thatch roofs (Kalshoven 1970), *T. rubrovaria* which can be fed on spiders and silkworm larvae in the laboratory (Abalos & Wygodzinsky 1951, Lent & Wygodzinsky 1979) and, along with *T. circummaculata*, has been shown to complete its entire life cycle on a diet of either vertebrate blood or cockroach haemolymph (Lorosa et al. 2000). Young nymphs of *Eratyrus mucronatus* seem preferentially to feed on invertebrates, while older nymphs and adults preferentially feed on vertebrate blood (Miles et al. 1981). A further transitional stage between predator and blood-sucker is indicated by cannibalistic behaviour (‘cleptohemodeipnonism’ of Ryckman 1951) where, under crowded laboratory conditions, triatomine nymphs that cannot reach the vertebrate host will penetrate feeding nymphs and take blood through them. Some triatomine species seem to have predominantly cleptohaematophagic behaviour, for example *Belminus herreri* that preferentially takes bloodmeals by feeding from recently engorged *Rhodnius* (Sandoval et al. 2000). These observations not only suggest that Triatominae have evolved the blood-sucking habit relatively recently, but could be interpreted to indicate that some are still in the process of doing so.

Morphological features used to distinguish Triatominae from other Reduviidae, such as the straight rostrum adpressed to the gula, and the ability of the third rostral segment to flex upwards, seem to have been derived in association with adaptations for feeding on vertebrate hosts (Cobben 1978, Schofield & Dolling 1993), and more stringent differences between the haematophagous and predatory forms of Reduviidae are not apparent. Even characters that can be helpful in determination, such as the laterally inserted antennae, absence of dorsal abdominal scent glands, and features of the external genitalia and wing venation, are all shared with one or more of the predatory reduviid groups. Indeed, the morphological similarities between some predatory Reduviidae and blood-sucking Triatominae are so striking that at least one predator has been erroneously described as a new species of Triatominae (see Lent 1982). Carcavallo et al. (1999) offer several other examples of these similarities – predatory reduviids that are morphologically almost indistinguishable from one or other group of blood-sucking Triatominae, and often occur in the same type of habitat. We must conclude that the blood-sucking Triatominae are generally poorly differentiated from predatory reduviids, both in body form and habitat, which is again suggestive that they have evolved the blood-sucking habit relatively recently.

TABLE I

Blood-sucking Hemiptera

Lineage	Facultative blood-suckers	Obligate blood-suckers
Lygaeidae	Cleradini	
Reduviidae	Emesinae Harpactorinae Peiratinae Reduviinae Triatominae	Triatominae
Anthocoridae	Doufouriellini Xylocorini	
		Cimicidae Polyctenidae

In contrast to the Triatominae however, both the Cimicidae and Polyctenidae are highly evolved as blood-suckers, with an almost ectoparasitic habit and highly specialised morphology (see Schofield & Dolling 1993). Most of the 93 species of Cimicidae occur in the Middle East and Africa (Table II) and those that occur in the Americas are generally species associated with humans and/or domesticated or sinanthropic animals – suggesting that they have been exported to the Americas in recent, postcolombian times. In Africa, the niche occupation of Cimicidae is very similar to that of the Triatominae in the Americas, suggesting that both have followed a similar evolutionary route. But the complete absence of Triatominae from Africa [except for *T. rubrofasciata* exported in sailing ships to some African ports (see Gorla et al. 1997)] tempts the idea that evolution of blood-sucking reduviids in Africa was inhibited by prior evolution of those blood-sucking anthocorids, now known as Cimicids, that had already occupied the niches available for that type of evolutionary progression. The high degree of morphological specialisation of the Cimicidae suggests that they evolved earlier than the Triatominae, so that it would appear that the Triatominae evolved independently in the Americas, after separation of the Atlantic divide, rather than having arisen also in Africa but subsequently becoming locally extinct. Blood-sucking reduviids have also evolved independently in the Indian subcontinent (a biogeographic island for most of its existence) to give the aberrant triatomine genus *Linshcosteus*. However, neither Cimicidae nor Triatominae appear to have arisen elsewhere in Asia, which may relate to the proliferation of the Polyctenidae in the Asia-Pacific region (Table II) and may also explain why *T. rubrofasciata* was able to differentiate quite rapidly on arrival in east Asia during the 17-18th centuries (Gorla et al. 1997).

TABLE II

Biogeographical diversity of blood-sucking Hemiptera. Number of genera and species in Africa, Asia and the Americas

	Africa	Asia	Americas
Cimicidae			
Genera	**11**	8	14
Species	**76**	11	20
Polyctenidae			
Genera	3	**4**	1
Species	7	**10**	16
Triatominae			
Genera	1	2	**14**
Species	1	13	**116**

Within the Americas, it is clear that the Triatominae could not have followed the proposed evolutionary route from predator to nest-dwelling blood-sucker prior to extensive development of nest-dwelling vertebrates. Adaptation to vertebrate nests would only be warranted if these represented a reasonably abundant resource, but would offer important advantages in terms of protection from climatic extremes (allowing reproduction to proceed with less dependence on seasonal climate) and more abundant proteinaceous food sources. However, exploitation of vertebrate blood requires important physiological and behavioural changes, especially in terms of avoiding predation by the vertebrate host and becoming physiologically capable of ingesting and digesting vertebrate blood. To avoid host predation requires behavioural adaptations – cryptic behaviour and inverse activity patterns, feeding when the host is asleep – and also physiological adaptions of biting and salivary function to avoid undue host disturbance when feeding. For mammals, the bite of predatory reduviids tends to be extremely painful, and at least one species of Apiomerinae is known (*Apiomerus lanipes*) that will readily feed on laboratory mice, but generally kills them by anaphylactic shock within a few minutes of beginning to feed (MH Pereira & L Diotaiuti, pers. commun.). But the bite of many blood-sucking Triatominae is also very painful (Ryckman & Bentley 1979). Bites of *Panstrongylus geniculatus* on pigs and humans in the Amazon region leave painful lesions that resemble cutaneous leishmaniasis (Valente et al. 1998) and there is at least one record of a person succumbing to anaphylactic shock after being bitten by *T. rubrofasciata* (Teo & Cheah 1973). We must conclude that many Triatominae remain imperfectly adapted for feeding on vertebrates – another indication of recent evolution as blood-suckers.

At the physiological level also, there is strong evidence for imperfect adaptation to a diet of vertebrate blood. All obligate blood-sucking insects seem to require symbionts, which are believed to provide vitamins such as folate that may be scarce in vertebrate blood (see Nyirady 1973). But whereas all other obligate blood-suckers carefully store specific symbionts either intracellularly or in a special organ, the mycetome, the Triatominae invariably have a wide range of bacteria (not all of which may be important) living freely in the gut lumen (Table III). This would suggest them to be at an early stage in adaptation to obligate haematophagy.

TABLE III

Location of intestinal symbionts of obligate blood-sucking insects (excluding facultative blood-sucking groups, and those that make use of other fluids as well as vertebrate blood)

In specialised Mycetome	Intracellular	Free in intestinal lumen
	Glossinidae	Triatominae
	Streblidae	
Nycteribiidae	Nycteribiidae	
Hippoboscidae	Hippoboscidae	
Cimicidae		
Polyctenidae		
Anoplura		
Rhynchophthirina	Mallophaga	

There is also evidence for rapid evolutionary change within the Triatominae. For example, comparison of derived populations of *Rhodnius prolixus* in Central America, with their putative ancestral populations in Venezuela and Colombia, shows them to morphometrically distinguishable and also to have a reduced genome as indicated by far fewer RAPD bands. And yet historical reconstruction indicates separation of the Central American and South American forms to be due to accidental human intervention a mere 85 years ago (Dujardin et al. 1998a). Similarly, comparison of domestic *T. infestans* from Uruguay, with their original silvatic populations in Bolivia, again shows clear morphometric and genetic differentiation over a time scale that appears to be little more than 100 years (Dujardin et al. 1998a,b). A further well-studied example is that of *T. rubrofasciata*, exported from a New World origin on sailing ships to port areas throughout the tropics, but speciating in eastern Asia – to give seven species defined by morphological characteristics – over a period that cannot have been much more than about 200 years (Gorla et al. 1997, Patterson 1999). Even in the space of a few generations in laboratory colony, morphological changes leading to apparent differentiation can be demonstrated (eg. Dujardin et al. 1999, Galindez-Girón et al. 2000).

All the evidence presented above points in the same direction. As blood-suckers, Triatominae seem capable of evolving rapidly, and appear to have done so quite recently. And their current geographical distribution accords fully with this idea, since the distribution of species and species groups matches modern American geography, rather than ancient scenarios. In almost all cases so far examined, species groups, complexes, and the smaller genera, occupy discrete geographic areas constrained by post-pleistocene geographic features (Schofield 1988, Schofield & Dujardin 1999). The only exceptions are discontinuities in the distribution of *R. prolixus*, *T. infestans*, and *T. rubrofasciata*, which are consistent with human intervention, and in the distribution of *T. maculata* (Venezuela) and *T. pseudomaculata* (Northeastern Brazil) which is consistent with their association with migrating birds. However, there is strong evidence to suggest that the Triatominae represent a polyphyletic grouping, with the smaller genera and many of the species groups of *Triatoma* assumed to have arisen from different predatory ancestors (Schofield & Dujardin 1999, Dujardin et al. 2000). Thus, although the evidence presented here suggests recent evolution of haematophagy in the Reduviidae to give the various forms now grouped as the Triatominae, there is no reason to suggest that such adaptations were simultaneous, nor that some form of haematophagy could not have arisen in some earlier groups that have since become extinct.

RECONCILING THE PARADOX

In contrast to infection of a tsetse fly with *T. brucei*, which leads to a very complex system of defence and counter-defence mechanisms by fly and parasite (see Welburn & Maudlin 1999), infection of a triatomine with *T. cruzi* leads to an extremely modest interaction (see Brener 1979). This is reflected in the resulting infection rates, which tend to be extremely low in Glossinidae, but can often exceed 50% in Triatominae – simply mirroring the cumulative probability of taking an infected blood meal (Schofield 1994). Indeed, *T. cruzi* seems a relatively non-specific parasite, able to develop, at least partially, in a wide range of invertebrates including leeches, ticks, bedbugs, and even experimentally-infected caterpillars (eg. Brumpt 1912, Mazzotti & Osorio 1943, Goldman 1950, Marsden & Pettit 1969) (none of these acts as vector however, since there is no adaptation for subsequent transmission). We can infer that association between glossinids and *T. brucei* seems to be relatively ancient, while the association between Triatominae and *T. cruzi* may be be relatively recent. But as we have seen, there is evidence that *T. cruzi* itself may be relatively ancient, arising in association with marsupials when South America was still connected to Antartica and Australia over 65 mya. Here is the apparent paradox – ancient parasite and recent vector.

A study by Deane et al. (1984) offers a way by which these divergent evolutionary scenarios might be reconciled. These authors demonstrated the vectorial part of *T. cruzi* development within the anal glands of opossums (*Didelphis marsupialis*). Their original findings were not serendipitous. They had infected the opossums by subcutaneous inoculation, but then failed to recover the parasites from any tissues, except until careful sectioning revealed the parasites in the anal glands (Lenzi et al. 1984). Subsequent studies also showed *T. cruzi* in the anal glands of naturally infected *D. marsupialis* (Naiff et al. 1987, Steindel et al. 1988) and *D. albiventris* (Fernandes 1989, Fernandes et al. 1987, 1989, 1991) and in experimentally infected *Lutreolina crassicaudata* (Steindel & Pinto 1988). The bloodstream and anal glands of some of these opossums were also infected by *T. freitasi* (Deane & Jansen 1986, 1990, Fernandes et al. 1987). *T. freitasi* is a larger trypanosome (subgenus Megatrypanum) which is considered by Hoare (1972) to be similar to *T. binneyi* of Australian platypus – again reflecting the proposed evolutionary link between Australian and New World trypanosomes. However, neither *binneyi* nor *freitasi* are known to develop in insects, and it may be that *freitasi* is transmitted directly between opossums, for example via their anal gland secretions. Moreover, subsequent studies of naturally infected opossums appeared to reveal not just *T. cruzi* and *T. freitasi* but also other flagellates in the anal glands (MP Deane, pers. commun. 1985, Deane & Jansen 1988, Jansen et al. 1988). Opossums are omnivorous, readily feeding on a wide range of vegetable and animal tissues, so the finding of trypanosomes and other flagellates in their anal glands suggests that these organisms were able to penetrate the oral mucosa and pass in the bloodstream to the glands. In other words, the capacity for mucosal penetration and subsequent survival in the blood would seem to be shared innately by a number of protozoan flagellates. The monophyletic origin of extant Trypanosomatidae may have been a flagellate of plants or primitive insects, that first entered mammals by being eaten by an omnivorous marsupial and migrating to the anal glands. Subsequent adaptation to a further bloodstream form would then make the adapting parasite available for transmission by blood-sucking insects.

So far in this hypothesis, we have a primitive trypanosomatid associated with didelphids in the southern supercontinent (Gondwanaland) during the early tertiary period about 65 mya. Then, around 40 mya, South America becomes separated from Antartica, and the fore-runners of modern American didelphids commence their northerly spread. We can propose that during this time, the trypanosomatid is being transmitted directly between opossums via their anal gland secretions (as demonstrated by Jansen & Deane 1985) and/or urine (as demonstrated by McKeever et al. 1958, and Olsen et al. 1964). But by the late tertiary or early pleistocene, some 2-5 mya, opossums would be common throughout South America, along with other nest-building vertebrates such as furnariid and psittacid birds, armadillos, and various forms of cricetid rodent. We can imagine that the habitats offered, and their associated guilds of nest-dwelling invertebrates, would be attractive to a wide range of reduviid predators – many of which would develop as facultative blood-suckers, capable of imbibing opossum blood infected with the primitive trypanosome.

But a feature of opossum lodges is that they also offer refuges to other vertebrates, just as opossums themselves may utilise abandonned nests of birds or rodents, or even armadillo burrows. So the advent of blood-sucking Reduviidae, today classified as Triatominae, would have provided the precise vehicle for spreading the trypanosome to new hosts. In birds it would not survive – killed by a form of complement-mediated lysis in bird blood (Kierszenbaum et al. 1976). But in rodents, armadillos, and bats, it would not only survive, but encounter profound selection pressures leading to the development of new forms distinguishable today by a range of genetic markers.

This theory predicts that the opossum form of *T. cruzi* (known as *cruzi* 1) represents the original stem, from which the diverse rodent forms (collectively known as *cruzi* 2) and their variants in armadillos (zymodeme Z3) and the bat forms (*T. cruzi marinkellei*) have been derived at various times by being vectored by Triatominae from the opossum to the alternative hosts. But there is no reason to suppose that such changes occurred at the same time. The Triatominae are clearly polyphyletic (Schofield 1988, Schofield & Dujardin 1999) and the different groupings of Triatominae currently recognised may well have arisen at different times. At the tribal level for example, there is clear morphometric evidence that the Cavernicolini are the most divergent and may be amongst the earliest Triatominae (Dujardin et al. 2000). The Cavernicolini are strongly associated with bats, and it may be that the bat-associated relatives of *T.cruzi* were amongst the earliest of the divergent lines – which is consistent with their enzymatic and ssu rRNA sequence differences. And the theory can go further by suggesting that *T. conorhini* is simply another form derived from the original *cruzi* stem, vectored into murid rodents by species of the *T. lecticularia* complex including *T. rubrofasciata* with which it has remained associated as this species has been spread into the Old World ports. The other rodent forms such as *T. lewisi*, *T. microtis* and *T. musculi* (= *T. duttoni*) may be further derivatives from *conorhini* through the selection pressure of subsequent adaptation to their flea vectors, although there is no genetic evidence to suggest a particularly close relationship between *conorhini* and the other rodent forms (see Stevens et al. 1999b). Similarly, the bat trypanosomes such as *T. dioniisi* and *T. vespertilionis* can be envisaged as derivatives of *cruzi* 1, via or parallel to *cruzi marinkellei*, that have been spread to the Old World by the bats themselves and then undergone further differentiation through the intervention of their Old World cimicid vectors. *T. rangeli* may also be seen as a derivative from the original *cruzi* stem (Stevens et al. 1999b) representing a form that seeks to improve its transmission efficiency by attempting the anterior route via the salivary glands of its vectors. That such a step is evolutionarily difficult is suggested by the high mortality often provoked by parasite invasion of the bugs' salivary glands, and also by the fact that only the specialised nitrophorin-containing glands of *Rhodnius* can permit this type of anterior development [observations that heavily infected salivary glands of *Rhodnius* tend to loose their characteristic red colouration (eg. Añez 1983) suggest that the parasites may be utilising the red nitrophorins, which could explain both their predilection for *Rhodnius* species, as well as contributing to vector mortality]. And perhaps the evolutionary cost of this adaptation is mirrored by reduced survivorship in the mammalian hosts, possibly due to loss of membrane neuraminidase which seems to be involved in passage through the vector haemolymph by *rangeli*, and in penetrating vertebrate cells by *cruzi* (D'Alessandro & Saravia 1992).

This theory reconciles the apparent disparity between the antiquity of *T. cruzi*, and the relatively recent development of blood-sucking Triatominae. It is consistent with the relative homogeneity of *cruzi* 1, compared to the multiple clones of *cruzi* 2 and other forms proposed as derivatives from the *cruzi* stem. But the theory implies that the putative derivatives from the original *cruzi* stem have radiated in comparatively recent times in response to being vectored into new hosts by blood-sucking insects, which is not at all consistent with current attempts to date the divergence of *cruzi* using molecular clocks. On the basis of 18S rDNA sequences, for example, the divergence between *cruzi* 1 and *cruzi* 2 has been dated at 88 mya (before the arrival of opossums and rodents in South America[2]) and divergence between *T. cruzi* and *T. rangeli* was put at 475 mya (Briones et al. 1999). This is difficult to reconcile with the geological record, since 475 mya was the time of ammonites and early fish. Early insects arose during the upper Paleozoic about 330 mya, with early mammals and birds arising in the Jurassic period about 200 mya, so there is no obvious clue as to how *cruzi* and *rangeli* could have diverged, or even existed as such, at 475 mya. Perhaps part of the problem is the assumption of strict homology between 18S sequences, whereas at least two divergent 18S types have been demonstrated in the *cruzi* genome (see Stothard 2000), and the assumption of a relatively low rate of sequence divergence (Briones et al. 1999) together with the idea that molecular clocks would run at a constant rate, whereas it may be that the intense selection pressure associated with adaptation to a completely new host – as in the proposed step from *cruzi* 1 to *cruzi* 2 – may distort the clock speed even of apparently 'neutral' genes such as 18S.

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REFERENCES

Abalos JW, Wygodzinsky P 1951. Las Triatominae Argentinas (Reduviidae, Hemiptera). *An Inst Med Regional, Tucuman, Monografia 2*: 1-179

Adjé CA, Opperdoes FR, Michels PAM 1998. Molecular analysis of phosphoglycerate kinase in *Trypanoplasma borreli* and the evolution of this enzyme in Kinetoplastida. *Gene 217*: 91-99.

Alvarez F, Cortinas MN, Musto H 1996. The analysis of protein coding genes suggests monophyly of *Trypanosoma*. *Mol Phylog Evol 5*: 333-343.

Añez N 1983. Studies on *Trypanosoma rangeli* Tejera, 1920. VI. Developmental pattern in the haemolymph of *Rhodnius prolixus*. *Mem Inst Oswaldo Cruz 78*: 413-419.

Brener Z 1979. O parasito: relações hospedeiro-parasito. In Z Brener & ZA Andrade (eds), *Trypanosoma cruzi e Doença de Chagas*, Guanabara Koogan, Rio de Janeiro, p. 1-41.

Briones RSM, Souto RP, Stolf BS, Zingales B 1999. The evolution of two *Trypanosoma cruzi* subgroups inferred from rRNA genes can be correlated with the interchange of American mammalian faunas in the Cenozoic and has implications to pathogenicity and host specificity. *Mol Bioch Pathog 104*: 219-232.

Brisse S 1997. *Phylogénie Moléculaire des Clones Naturels de Trypanosoma cruzi, Agent de la Maladie de Chagas: Évolution Clonale, Recombinaison Génétique, et Relations Phylogénétiques avec d'Autres Espèces du Sous-genre Schizotrypanum*, PhD Thesis, Université de Montpellier II, Montpellier, France.

Brisse S, Barnabé C, Tibayrenc M 1998. *Trypanosoma cruzi*: how many relevant phylogenetic subdivisions are there? *Parasitol Today 14*: 178-179.

Brumpt E 1912. Le *Trypanosoma cruzi* evolue chez *Conorhinus megistus, Cimex lecularius, Cimex boueti* et *Ornithodorus moubata*. Cycle evolutif de ce parasite. *Bull Soc Pathol Exotique 5*: 360-367.

Capriles JM, Santiagoblay JA, Poinar GO 1993a.

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[2] The earliest marsupial fossils, in Asia-America, have been dated at 80 mya. Fossil marsupials in South America have been dated at 55 mya, in Antarctica at 65 mya, and in Australia at 27 mya. It is deduced that marsupials evolved in Asia-America to spread southwards across the Paleocene land bridge into South America and Antartica about 65 mya. Marsupial opossums however, seem to have evolved in the southern supercontinent formed by Australia, Antarctica and South America, about 40-65 mya, and then spread northwards into South America to cross the Pleistocene land bridge into Central and North America about 1-2 mya (Clemens 1966). The earliest rodent fossils in South America have been dated at 30-55 mya.

Apicrenus fossilis gen and sp-n (Heteroptera, Reduviidae, Apiomerinae) from Dominican amber (lower oligocene-upper eocene). *Entomol Scandinavica 24*: 139-142.

Capriles JM, Santiagoblay JA, Poinar GO 1993b. *Paleoploiariola venosa*, a new fossil Emesinae (Heteroptera, Reduviidae) genus and species from Dominican amber. *J Agric Univ Puerto Rico 77*: 95-100.

Carcavallo RU, Jurberg J, Lent H 1999. Phylogeny of the Triatominae. In *Atlas of Chagas Disease Vectors in the Americas*, Vol. 3, Fiocruz, Rio de Janeiro, p. 925-969.

Clemens WA 1966. Origin and early evolution of marsupials. *Evolution 22*: 18.

Cobben RH 1978. Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. *Mededlingen Landbouwhogeschool, Wageningen 78*: 1-407.

Cobben RH 1979. On the original feeding habits of the Hemiptera (Insecta): a reply to Merrill Sweet. *An Entomol Soc America 72*: 711-715.

D'Alessandro A, Saravia NG 1992. *Trypanosoma rangeli*. In JP Kreier & JR Baker (eds), *Parasitic Protozoa*, 2nd ed., Vol. 2, Academic Press, San Diego, p. 1-54.

Davis NT 1969. Contribution to the morphology and phylogeny of the Reduvioidea. Part IV. The Harpactaroid complex. *An Entomol Soc Am 62*: 74-94.

Deane MP, Jansen AM 1986. Another *Trypanosoma* distinct from *Trypanosoma cruzi* multiplies in the lumen of the anal gland of the opossum *Didelphis marsupialis*. *Mem Inst Oswaldo Cruz 81*: 131-132.

Deane MP, Jansen AM 1988. From a mono to a digenetic life-cycle: how was the jump for flagellates of the family Trypanosomatidae. *Mem Inst Oswaldo Cruz 83*: 273-275.

Deane MP, Jansen AM 1990. Developmental stages of *Trypanosoma* (*Megatrypanum*) *freitasi* Rego, Magalhães & Siqueira, 1957 in the opossum *Didelphis marsupialis* (Marsupialia, Didelphidae). *J Protozool 37*: 44-47.

Deane MP, Lenzi HL, Jansen AM 1984. *Trypanosoma cruzi*: vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis*. *Mem Inst Oswaldo Cruz 79*: 513-515.

Deane MP, Lenzi HL, Jansen AM 1986. Double development cycle of *Trypanosoma cruzi* in the opossum. *Parasitol Today 2*: 146-147.

Diotaiut L, Pereira AS, Loiola CF, Fernandes AJ, Schofield CJ, Dujardin JP, Dias JCP, Chiari E 1995. Interrelation of sylvatic and domestic transmission of *Trypansoma cruzi* in areas with and without domestic vectorial transmission in Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz 90*: 443-448.

Dos Santos WG, Buck GA 1999. Polymorphisms at the topoisomerase II gene locus provides more evidence for the partition of *Trypanosoma cruzi* into two major groups. *J Eukar Microbiol 46*: 17-23.

Dujardin JP, Muñoz M, Chavez T, Ponce C, Moreno J, Schofield CJ 1998a. The origin of *Rhodnius prolixus* in Central America. *Med Vet Entomol 12*: 113-115.

Dujardin JP, Schofield CJ, Tibayrenc M 1998b. Population structure of Andean *Triatoma infestans*: allozyme frequencies and their epidemiological relevance. *Med Vet Entomol 12*: 20-29.

Dujardin JP, Schofield CJ, Panzera F 2000. *Les Vecteurs de la Maladie de Chagas. Recherches Taxonomiques, Biologiques et Génétiques*, Académie Royale des Sciences d'Outre Mer, Brussels, 162 pp.

Dujardin JP, Steindel M, Chavez T, Machane M, Schofield CJ 1999. Changes in the sexual dimorphism of Triatominae in the transition from natural to artificial habitats. *Mem Inst Oswaldo Cruz 94*: 565-569.

Evans JW 1956. Palaeozoic and mesozoic Hemiptera (Insecta). *Austral J Zool 4*: 165-258.

Fernandes AJ 1989. *Importancia de Didelphis albiventris e Panstrongylus megistus na Interacão dos Ciclos de Transmissão do Trypanosoma cruzi no Municipio de Bambuí, Minas Gerais*, Thesis, UFMG, Belo Horizonte, 116 pp.

Fernandes AJ, Chiari E, Rodrigues RR, Dias JCP, Romanha AJ 1991. The importance of the opossum (*Didelphis albiventris*) as a reservoir for *Trypanosoma cruzi* in Bambuí, Minas Gerais state. *Mem Instit Oswaldo Cruz 86*: 81-85.

Fernandes AJ, Diotaiuti L, Chiari E, Dias JCP 1987. Natural infection of *Didelphis albiventris* by *Trypanosoma cruzi* and *Trypanosoma freitasi*. *Mem Instit Oswaldo Cruz 82* (Suppl.): 65.

Fernandes AJ, Diotaiuti L, Dias JCP, Rnmanha AJ, Chiari E 1989. Infecção natural das glandulas anais de gambas (*Didelphis albiventris*) pelo *Trypanosoma cruzi* no município de Bambui – MG. *Mem Inst Oswaldo Cruz 84*: 87-93.

Fernandes O, Souto RP, Castro JA, Pereira JB, Fernandes NC, Junqueira ACV, Naiff RD, Barrett TV, Degrave W, Zingales B, Campbell DA, Coura JR 1998. Brazilian isolates of *Trypanosoma cruzi* from humans and triatomines classified into two lineages using mini-exon and ribosomal RNA sequences. *Am J Trop Med Hyg 58*: 807-811.

Galindez-Girón I, Torres E, Galvão C, Magalhães dos Santos C, Lizano E, Barazarte R, Marquez J 1999. Influencia da manutenção em laboratorio sobre o fenotipo de *Rhodnius robustus* Larrousse, 1927 (Hemiptera, Reduviidae, Triatominae). *Entomol Vect 6*: 677-703.

Goldman M 1950. The experimental infection of pupae of *Philosamia cynthia* Drury (Lepidoptera: Saturniidae) with *Trypanosoma cruzi*. *J Parasitol 36*: 1-8.

Gorla DE, Schofield CJ, Dujardin JP 1997. Biosystematics of Old World Triatominae. *Acta Trop 63*: 127-140.

Hannaert V, Opperdoes FR, Michels PAM 1998. Comparison and evolutionary analysis of the glycosomal glyceraldehyde-3-phosphate dehydrogenase from different Kinetoplastida. *J Mol Evol 47*: 728-738.

Hashimoto T, Nakamura Y, Kamaishi T, Adachi J, Nakamura F, Okamoto K, Hasegawa M 1995. Phylogenetic place of kinetoplastid protozoa inferred from a protein phylogeny of elongation factor 1α.

Mol Bioch Parasitol 70: 181-185.

Hoare CA 1972. *TheTtrypanosomes of Mammals,* Blackwell Scientific Publications, Oxford.

Jansen AM, Deane MP 1985. *Trypanosoma cruzi* infection of mice by ingestion of food contamined with material of the anal glands of the opossum *Didelphis marsupialis*. XII Reunião Anual sobre Pesquisa Básica em Doença de Chagas, Caxambu, BI-09.

Jansen AM, Carreira JC, Deane MP 1988. Infection of a mammal by monogenetic insect trypanosomatids (Kinetoplastida, Trypanosomatidae). *Mem Inst Oswaldo Cruz 83*: 271-272.

Jeannel R 1919. Henicocephalidae et Reduviidae. In *Voyage de Ch. Alluaud et R. Jeannel en Afrique orientale (1911-1912). Resultats scientifiques – Hemiptera,* vol. 3, Paris, p.131-314.

Kalshoven LGE 1970. Observations of the blood-sucking reduviid *Triatoma rubrofasciata* (De Geer) in Java. *Entomologische Berichten, Amsterdam 30*: 41-47.

Kierszenbaum F, Ivanyi J, Budzko DB 1976. Mechanism of natural resistance to trypanosomal infection. Role of complement in avian resistance to *Trypanosoma cruzi* infection. *Immunology 30*: 1-6.

Lake JA, De La Cruz VF, Ferreira PCG, Morel C, Simpson L 1988. Evolution of parasitism: Kinetoplastid protozoan history reconstructed from mitochondrial rRNA gene sequences. *Proc Nat Acad Sci USA 85*: 4779-4783.

Lent H 1982. *Microtriatoma pratai* Sherlock & Guitton, 1982 e sinonimo do hemiptero predador *Aradomorpha championi* Lent & Wygodzinsky, 1944 (Reduviidae, Reduviinae). *Mem Inst Oswaldo Cruz 77*: 449-451.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. *Bul Am Mus Nat Histo 163*: 123-520.

Lenzi HL, Jansen AM, Deane MP 1984. The recent discovery of what might be a primordial escape mechanism for *Trypanosoma cruzi*. *Mem Inst Oswaldo Cruz 79*: 13-18.

Lorosa ES, Jurberg J, Souza ALA, Vinhaes MC, Nunes IM 2000. Hemolinfa de blatideos na manutenção do ciclo biológico silvestre de *Triatoma rubrovaria* (Blanchard 1843) e *Triatoma circummaculata* (Stal, 1859). *Rev Soc Bras Med Trop 35* (Supl.1): 172.

Maldonado Capriles J 1990. Systematic catalague of the Reduviidae of the world (Insecta: Heteroptera). *Caribbean J Sci* (Special edition) x + 1-694.

Marsden PD, Pettitt LE 1969. The survival of *Trypanosoma cruzi* in the medicinal leech (*Hirudo medicinalis*). *Trans R Soc Trop Med Hyg 63*: 414-415.

Mazzotti L, Osorio MT 1943. Experimentos de transmisión de *Trypanosoma cruzi* en cuatro especies de *Ornithodorus. Rev Inst Salub Enferm Trop, Mexico 4*: 163-165.

McKeever S, Gorman GW, Norman L 1958. Occurrence of a *Trypanosoma cruzi*-like organism in some mammals from southwestern Georgia and northwestern Florida. *J Parasitol 44*: 583-587.

Memórias 1999. Recommendations from a satellite meeting. *Mem Inst Oswaldo Cruz 94* (Suppl.1): 429-432.

Miles MA 1979. Transmission cycles and the heterogeneity of *Trypanosoma cruzi*. In WHR Lumsden & DA Evans (eds), *Biology of the Kinetoplastida,* Vol. 2, London, Academic Press, p. 117-196.

Miles MA, Souza AA de, Povoa M 1981. Chagas disease in the Amazon basin. III. Ecotopes of ten triatomine bug species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. *J Med Entomol 18*: 266-278.

Naiff RD, Barrett TV, Arias AR 1987. *Trypanosoma cruzi* nas galndulas anais de *Didelphis marsupialis*: primero registro de infecções naturais. X Congresso da Sociedade Brasileira de Parasitologia, Salvador, BA, abstract 165.

Nyirady SA 1973. The germfree culture of three species of Triatominae: *Triatoma protracta* (Uhler), *Triatoma rubida* (Uhler) and *Rhodnius prolixus* Stal. *J Med Entomol 10*: 417-448.

Olsen PF, Shoemaker JP, Turner HF, Hays KL 1964. Incidence of *Trypanosoma cruzi* (Chagas) in wild vectors and reservoirs in east-central Alabama. *J Parasitol 50*: 599-603.

Patterson J 1999. *A Morphometric Investigation of the Relationships between Triatoma rubrofasciata (Hemiptera: Reduviidae: Triatominae), Old World Triatoma and Representatives of the New World Species,* Thesis, LSHTM, London, 44 pp.

Pipkin AC 1969. Transmision of *Trypanosoma cruzi* by arthropod vectors: anterior versus posterior route infection. *Internl Rev Trop Med 3*: 1-47.

Poinar GO 1991. *Praecoris dominicana* gen n, sp n (Hemiptera, Reduviidae, Holoptilinae) from Dominican amber, with an interpretation of past behavior based on functional morphology. *Entomol Scandinavica 22*: 193-199.

Putshkov VG, Putshkov PV 1985. A catalogue of Assassin-bug genera of the world (Heteroptera, Reduviidae). *Vestnik Zoologica,* 112 pp.

Ryckman RE 1951. Recent observations of cannibalism in *Triatoma* (Hemiptera: Reduviidae). *J Parasitol 37*: 433-434.

Ryckman RE, Bentley DG 1979. Host reactions to bug bites (Hemiptera, Homoptera): a literature review and annotated bibliography. *Calif Vect Views 26*: 1-49.

Sandoval CM, Joya MI, Gutierez R, Angullo VM 2000. Cleptohaematophagy of the triatomine bug *Belminus herreri*. *Med Vet Entomol 14*: 100-101.

Schofield CJ 1988. Biosystematics of the Triatominae. In M Service, *Biosystematics of Haematophagous Insects,* Clarendon Press, Oxford, p. 284-312.

Schofield CJ 1994. *Triatominae - Biology & Control,* Eurocommunica Publications, West Sussex, UK, 80 pp.

Schofield CJ, Dolling WR 1993. Bedbugs and kissing-bugs (bloodsucking Hemiptera). In RP Lane & RWCrosskey (eds), *Medical Insects and Arachnids,* Chapman & Hall, London, UK, p. 483-516.

Schofield CJ, Dujardin JP 2000. Theories on the evolution of *Rhodnius*. *Actual Biol* (*Medellin*) *21*: 183-197.

Schuh RT 1986. The influence of cladistics on

Heteropteran classification. *Ann Rev Entomol 31*: 67-93.

Schuh RT, Slater JA 1995. *True Bugs of the World (Hemiptera: Heteroptera) Classification and Natural History*, Cornell University Press, New York, 336 pp.

Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B 1996. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. *Mol Bioch Parasitol 83*: 141-152.

Southwood TRE, Leston D 1959. *Land and Water Bugs of the British Isles*, Frederick Warne, London, xi + 436 pp.

Steindel M, Pinto CJC 1988. *Trypanosoma cruzi* development in the anal glands of experimentally infected *Lutreolina crassicaudata* (Marsupialia, Didelphidae). *Mem Inst Oswaldo Cruz 83*: 387.

Steindel M, Scholz AF, Toma HK, Schlemper Junior BR 1988. Presence of *Trypanosoma cruzi* in the anal glands of naturally infected opossums (*Didelphis marsupialis*) in the State of Santa Catarina, Brazil. *Mem Inst Oswaldo Cruz 83*: 135-137.

Stevens J, Gibson W 1999. The evolution of salivarian trypanosomes – Second Internet Conference on Salivarian Trypanosomes. *Mem Inst Oswaldo Cruz 94*: 225-228.

Stevens JR, Noyes HA, Dover GA, Gibson WC 1999a. The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitology 118*: 107-116.

Stevens JR, Noyes HA, Schofield CJ, Gibson W 2000. The molecular evolution of Trypanosomatidae. *Adv Parasitol 48*: 1-53.

Stevens JR, Teixeira MMG, Bingle LEH, Gibson WC 1999b. The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*. *Internl J Parasitol 29*: 749-757.

Stothard JR 2000. Trypanosome trees and homologies. *Parasitol Today 16*: 173.

Sweet MH 1979. On the original feeding habits of the Hemiptera (Insecta). *Ann Entomol Soc America 72*: 575-579.

Teo SK, Cheah JS 1973. Severe reaction to the bite of the triatomine bug (*Triatoma rubrofasciata*) in Singapore. *J Trop Med Hyg 76*: 161-162.

Thomas DB 1992. A fossil *Empicoris* Wolff (Reduviidae, Heteroptera) from Mexican amber with remarks on the phylogenetic status of the fossil genus *Alumeda* Popov. *J NY Entomol Soc 100*: 535-539.

Tibayrenc M 1995. Population genetics of parasitic protozoa and other microorganisms. *Adv Parasitol 36*: 47-115.

Tibayrenc M, Neubauer K, Barnabe C, Guerrini F, Skarecky D, Ayala FJ 1993. Genetic characterization of six parasitic protozoa: parity between random-primer DNA typing and multilocus enzyme electrophoresis. *Proc Natl Acad Sci USA 90*: 1335-1339.

Valente VC, Valente SAS, Noireau F, Carrasco HJ, Miles MA 1998. Chagas disease in the Amazon Basin: association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with domestic pigs. *J Med Entomol 35*: 99-103.

Welburn SC, Maudlin I 1999. Tsetse-trypanosome interactions: rites of passage. *Parasitol Today* 15: 399-403.

Wiemer EAC, Hannaert V, Van Den Ijssel PRLA, Van Roy J, Opperdoes FR, Michels PAM 1995. Molecular analysis of glyceraldehyde-3-phosphate dehydrogenase in *Trypanoplasma borreli*: an evolutionary scenario of subcellular compartmentation in Kinetoplastida. *J Mol Evol 40*: 443-454.

Wootton RJ 1981. Palaeozoic insects. *Ann Rev Entomol 26*: 319-344.

Zingales B, Souto RP, Mangia RH, Lisboa CV, Campbell DA, Coura JR, Jansen A, Fernandes O 1998. Molecular epidemiology of American trypanosomiasis in Brazil based on dimorphisms of rRNA and mini-exon gene sequences. *Internl J Parasitol 28*: 105-112.