Cell
PRESS

The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis

Patrick B. Hamilton[1], Marta M.G. Teixeira[2] and Jamie R. Stevens[1]

[1] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4QD, United Kingdom
[2] Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP 05508-900, Brazil

Recent discussions on the evolution of *Trypanosoma cruzi* have been dominated by the southern super-continent hypothesis, whereby *T. cruzi* and related parasites evolved in isolation in the mammals of South America, Antarctica and Australia. Here, we consider recent molecular evidence suggesting that *T. cruzi* evolved from within a broader clade of bat trypanosomes, and that bat trypanosomes have successfully made the switch into other mammalian hosts in both the New and Old Worlds. Accordingly, we propose an alternative hypothesis – the bat seeding hypothesis – whereby lineages of bat trypanosomes have switched into terrestrial mammals, thereby seeding the terrestrial lineages within the clade. One key implication of this finding is that *T. cruzi* may have evolved considerably more recently than previously envisaged.

Trypanosoma cruzi: Chagas disease, hosts, transmission and evolution

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, continues to rank among the most important public health problems in South America and despite extensive research efforts focusing on both the parasite and the vector over more than 100 years [1], the evolutionary history of this parasite remains to be resolved. *T. cruzi sensu lato* (s.l.) comprises *Trypanosoma cruzi cruzi*, the causative agent of Chagas disease in humans, and *Trypanosoma cruzi marinkellei*, which is found only in South American bats. In addition to humans, the hosts of *T. c. cruzi* include a range of wild animals, including many species of marsupials and xenarthrans (armadillos, sloths, anteaters), a variety of rodents, primates, carnivores, bats and some wild pigs, and a variety of domesticated animals, including dogs, cats, pigs and possibly goats [2]. The parasite is generally transmitted between mammalian hosts by contamination with the feces of infected triatomine bugs and ingestion of infected triatomines by mammals. In addition, vertical transmission between mother and infant via breast feeding or blood contamination, transmission via blood transfusion and mechanical transmission between host animals, for example via anal glands in opossums, have been

documented and may be more or less important depending on local circumstances.

Since the late 1990s, discussion on the evolution of *T. cruzi* trypanosomes has been dominated by the southern super-continent hypothesis [3–5] which suggested that *T. c. cruzi* emerged from trypanosomes that had been isolated in terrestrial mammals of the New World since the break-up of the continental landmass that included Australia, Antarctica and South America approximately 40 million years ago (Figure 1). Although this hypothesis is widely accepted, here we highlight several recent studies that suggest that *T. c. cruzi* was originally a bat parasite that subsequently made the switch into terrestrial mammals.

The southern super-continent hypothesis and its implications

The first comprehensive phylogenetic analyses of disease causing trypanosomes demonstrated that *T. cruzi* was distantly related to another human infective species, *Trypanosoma brucei* s.l., the causative agent of African sleeping sickness, and that both appear to have evolved human parasitism independently [5,6]. Stevens *et al.* [3–5,7] explained the distribution of taxa in 18S rDNA phylogenies using known patterns of host biogeography and geological evidence.

The divergence of African tsetse-transmitted trypanosomes (the Salivaria, also known as the *Trypanosoma brucei* clade) was dated to around 100 mybp, when Africa became isolated from the other continents (Figure 1) [5,7]. The clade consisted predominantly of trypanosomes from African mammals, whereas species from African amphibia (*Trypanosoma mega*) and reptiles (*Trypanosoma grayi*, *Trypanosoma varani*) were unrelated. At this time (100 mybp) the ancestors of many extant mammalian groups were present, but had not begun major diversification [8].

Separation of *T. cruzi* and related trypanosomes from African trypanosomes was similarly dated; the composition of the *T. cruzi* clade at the time included two trypanosomes from South American terrestrial mammals' *T. cruzi* and *Trypanosoma rangeli*, and a trypanosome from an Australian kangaroo [5,9]. This distribution reflects the present day distribution of marsupial mammals; the split of South America from Antarctica and Australia is thought to have occurred later than the separation from Africa [8] (Figure 1). Three bat trypanosomes were also in the *T. cruzi* clade [3]: *T. cruzi marinkellei* from South American bats, and *Trypanosoma dionisii* and *Trypanosoma vespertilionis* that are
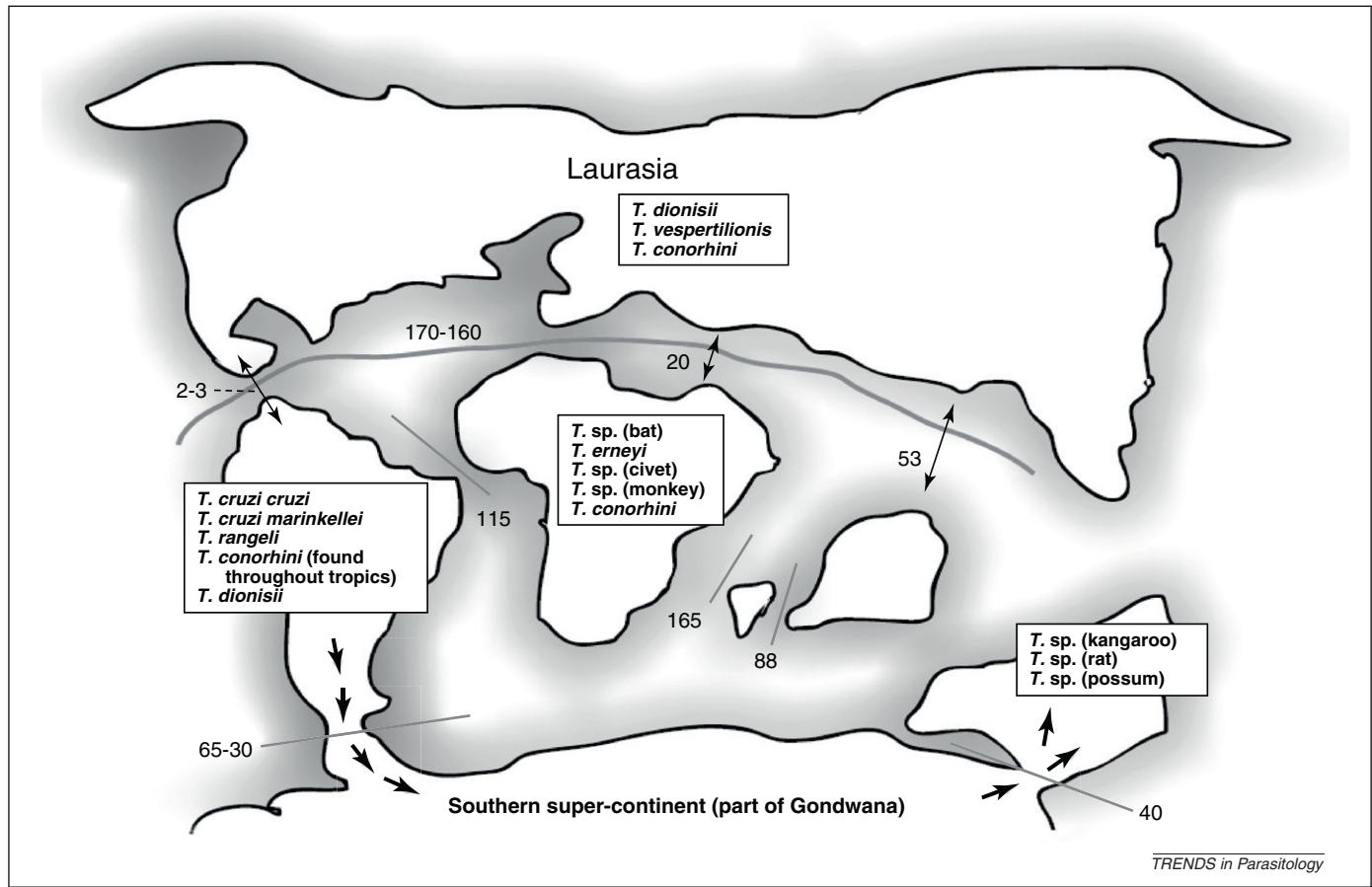
Figure 1. Current geographical distribution of *T. cruzi* clade trypanosomes. Current distribution of trypanosome taxa (characterized by molecular methods) in the *T. cruzi* clade, mapped onto the positions of continents in the late cretaceous; adapted from Cox and Moore [8]. Gray lines show dates of splits between landmasses. Double-headed arrows show dates of collision between continents. Single-headed arrows show the direction of spread of marsupials to Australia from South America via Antarctica [8]. All numbers are millions of years ago. Africa, Antarctica, Australia, India, Madagascar and South America formed the southern super-continent of Gondwana.

present in the Old World. The latter two species were hypothesized to have originated in the New World, and dispersed to the Old World by infected bats flying between the continental landmasses. Latterly, the clade was expanded to include *Trypanosoma conorhini*, a rat trypanosome found throughout the tropics, including South America. At this time, this species was hypothesized to have originated in the New World and spread subsequently to the Old World via rats in ships [10].

Thus, the southern super-continent hypothesis, that is, the idea that *T. cruzi* and its relatives originated when South America, Australia and Antarctica were linked in a single continent – and that at this time marsupials were the only mammals present – has been used to explain these patterns and host distributions, and has dominated discussion of *T. cruzi* evolution for the past decade. For example, it has been used for calibration of molecular trees in a range of studies [7,11,12]. Recently, Flores-López and Machado [13] used 32 genes to date the divergence of extant lineages of *T. cruzi* to within the past 3 million years, with trees calibrated using a divergence date of 100 million years for the split between *T. cruzi* and *T. brucei*. Likewise, Lewis *et al.* [14] used the same date to estimate the emergence of the two hybrid lineages of *T. c. cruzi* that are associated with severe clinical disease to within the past 60 000 years.

New evidence and the southern super-continent hypothesis
New evidence has emerged in recent years that has challenged some of the assumptions of the super-continent hypothesis.

Low diversity of T. cruzi *clade in South American terrestrial mammals*
A wide diversity of trypanosome species within the subgenus *Schizotrypanum* has been described from South American mammals using bloodstream morphology, although it has long been suspected that some of these species were synonyms of *T. c. cruzi* [15]. An increasing number of studies have now characterized trypanosomes from a wide range of South American terrestrial mammals using molecular techniques, revealing high diversity within both *T. c. cruzi* and *T. rangeli*. To date, however, no further *bona fide* species in the *T. cruzi* clade have been discovered in terrestrial mammals from this continent. This apparent low level of diversity does not accord with what might be expected if *T. cruzi*-group trypanosomes had been present in the continent for 40 million years. For example, some parasite cultures identified as *Trypanosoma leeuwenhoeki* (from a sloth, *Choloepus hoffmanni*) and *Trypanosoma minasense* (from a neotropical primate, the squirrel monkey, *Saimiri boliviensis*) on the basis of morphology have

turned out to be *T. rangeli* in 18S rDNA phylogenies [10]. Other recently described parasites from the region have fallen outside the *T. cruzi* clade, for example, isolates morphologically similar to *T. minasense*, which were sequenced directly from blood samples from primates imported into Japan from South America [16] and a trypanosome from a dog [17]. Arguably, further species related to *T. cruzi* have not been detected because many of these studies have first cultured the trypanosomes [18,19], which may have inadvertently screened out species less well suited to growth in culture. For example, trypanosomes with morphology similar to that of subgenus *Megatrypanum* are often encountered in the blood of American primates, bats and didelphids, but cannot be isolated using standard media. The apparently low level of diversity so far detected in Australian *T. cruzi* clade trypanosomes is discussed in Box 1.

The origin of *T. conorhini* is far from clear, but new evidence indicates an Old World origin for this species. Crucially, new phylogenetic studies suggest that its vector, the triatomine bug *Triatoma rubrofasciata*, may have originated in the Old World; it is most closely related to species of the Asiatic triatomine genus *Linshcosteus* [20,21]. This cosmopolitan rat trypanosome has only been reported in domestic rats in Brazil and its vector is strongly associated with rats. Its history and origins are thus probably linked to those of its hosts and vector, both of which now appear to be in the Old World (the brown rat, *Rattus norvegicus*, and the black rat, *Rattus rattus*, are thought to have originated in Asia rather than in South America and were dispersed there by ships [22]).

Thus, based on current evidence, *T. c. cruzi* and *T. rangeli* are the only trypanosomes in the *T. cruzi* clade that we can be confident were in South American terrestrial mammals

before the arrival of humans in the New World. By contrast, over the past decade the known diversity of trypanosomes infecting African tsetse flies has been shown to be considerably higher than previously thought [23,24]. This finding supports the hypothesis that the group (African trypanosomes) has been present in the continent for a considerable period of time [5]; although, as has been shown previously [7], rates of evolution can vary considerably between (and within) the trypanosome clades. Thus, time estimates derived from mutation rates should always be treated with appropriate caution and are not directly comparable between African trypanosomes and the *T. cruzi* group.

T. cruzi *clade in African terrestrial mammals*

The second challenge to the southern super-continent hypothesis comes from a study in which a large range of vertebrates from Cameroon in West Africa were surveyed for the presence of pathogenic African trypanosomes [25]. The species-specific primers used in the study left some trypanosomes unidentified and later phylogenetic analysis placed two trypanosomes from a palm civet and a monkey in the *T. cruzi* clade [26]. The monkey trypanosome was most closely related to a European bat trypanosome, *T. vespertilionis* (Figure 2), whereas the civet trypanosome appeared to be more closely related to *T. conorhini*.

Thus, *T. cruzi* clade parasites are present in terrestrial mammals from the New World, Australia and Africa, questioning the role of geographical isolation in restricting movement of these trypanosomes (Box 1). It is also possible that other *T. cruzi* clade trypanosomes are yet to be discovered elsewhere in the Old World. This possibility has been explored by Weinman *et al.* [27], who found that some monkey trypanosomes from South East Asia developed

---

Box 1. Geographical isolation and trypanosomatid evolution

Vicariance biogeography has been used to explain the current day distribution of many groups of organisms [8]. The approach uses information on plate tectonic movements and the break-up of continents due to continental drift to explain extant species' distribution patterns. Several studies have used vicariance biogeography to explain the distribution of trypanosomatid taxa. Lake *et al.* [42] used the break-up of Africa and South America to date the divergence of *Leishmania* and *Trypanosoma*, whereas Fernandes *et al.* [43] dated the split between Old and New World *Leishmania* to the separation of the continental landmasses.

Several recent studies question the importance of geographical isolation in determining patterns of trypanosomatid biogeography. The position of the kangaroo trypanosome at the periphery of the *T. cruzi* clade was crucial in forming the southern super-continent hypothesis [5]. At the time, an unrelated trypanosome from a wombat was the only other trypanosome from an Australian marsupial included in phylogenies [5]. The diversity of marsupials in Australia largely results from the isolation of the continent 40 million years ago, when it became separated from Antarctica (Figure 1), which allowed diversification of its marsupial fauna. Rodents and bats are the only placental mammals to have entered and successfully colonized Australia before human introductions. Yet, although molecular surveys in the past decade have revealed considerable diversity in trypanosomes of Australian mammals, such studies have not revealed a deep sister clade in Australia as might be expected if trypanosomes within the *T. cruzi* clade had been present within the continent for 40 million years. Trypanosomes from a native Australian rodent, *Rattus fuscipes* [44], and a possum, *Trichosurus vulpecula* [45], appear to be relatively close relatives of the

kangaroo trypanosome. However, other newly discovered trypanosome lineages from Australian marsupials are more closely related to trypanosomes outside of Australia than to each other. Examples include a koala trypanosome, whose closest known relative is a trypanosome from an American bird [46]. *Trypanosoma copemani* and related trypanosomes from wombats, quokkas, potoroos, woylies and ticks from Australia [47,48] have relatives in Japanese ticks [49], Eurasian badgers [5,50] and South American dogs [17]; a wallaby trypanosome is related to trypanosomes found in terrestrial leeches (from Australia and Asian forests) and a primate trypanosome, *Trypanosoma cyclops*, from Southeast Asia [51]. Thus, although the mammals of Australia have been geographically isolated, their trypanosomes have not, casting doubt on the original significance of the kangaroo trypanosome in defining a southern super-continent hypothesis [5].

This pattern mirrors the findings from studies of some other trypanosomatid groups. Within some genera of monogenetic (single host) trypanosomatids, some closely related monoxenous insect parasites have been described from China and the Neotropics, suggesting recent dispersal across substantial distances and biogeographical barriers [52]. Similarly, a recently discovered *Leishmania* taxon from an Australian kangaroo [53,54] appears closely related to a rodent species, *Leishmania enriettii*, a species found infecting rodents in Brazil and another from an AIDS patient in Martinique (West Indies) [55]. Most recently, strains of *T. dionisii* have been discovered in Europe that are very closely related to those in the New World, suggesting recent and probably natural movement, further demonstrating the ability of bat trypanosomes to disperse between geographically separate continents [31].
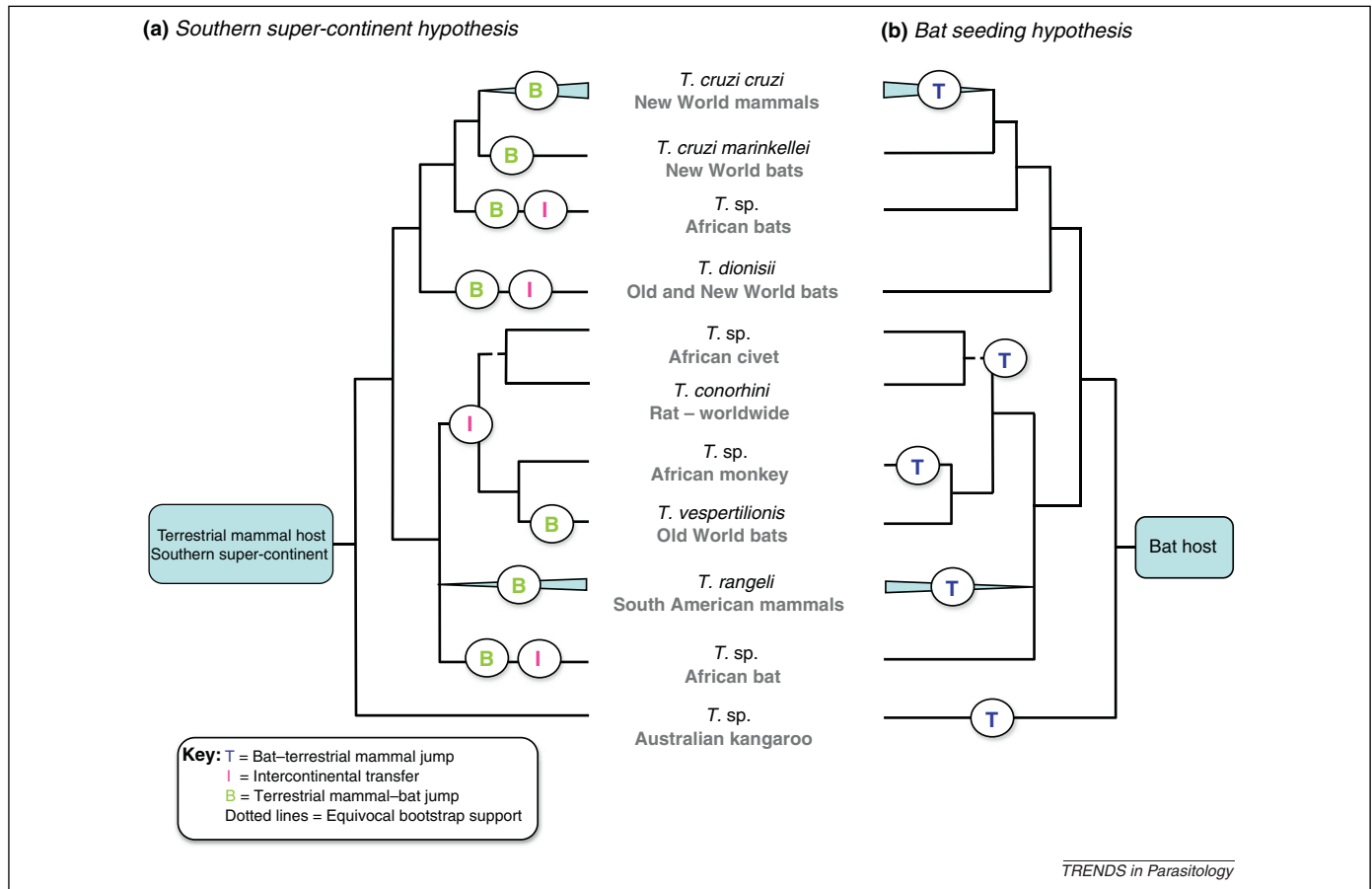
(a) *Southern super-continent hypothesis*

(b) *Bat seeding hypothesis*

T. cruzi cruzi
New World mammals

T. cruzi marinkellei
New World bats

T. sp.
African bats

T. dionisii
Old and New World bats

T. sp.
African civet

T. conorhini
Rat – worldwide

T. sp.
African monkey

T. vespertilionis
Old World bats

T. rangeli
South American mammals

T. sp.
African bat

T. sp.
Australian kangaroo

Terrestrial mammal host
Southern super-continent

Bat host

Key: T = Bat–terrestrial mammal jump
I = Intercontinental transfer
B = Terrestrial mammal–bat jump
Dotted lines = Equivocal bootstrap support

TRENDS in Parasitology

Figure 2. Two hypotheses for the evolution of the *T. cruzi* clade. Relationships within the *T. cruzi* clade, showing two hypotheses for the evolution of the *T. cruzi* clade. **(a)** The southern super-continent hypothesis and **(b)** the bat seeding hypothesis. The two scenarios presented represent (in our opinion) the simplest explanations of the relationships that exist between these taxa. Isolates from Brazilian bats with isoenzyme and RAPD profiles distinct from *T. cruzi* and *T. rangeli*, but more closely related to *T. vespertilionis* and *Trypanosoma hastatus* [40], have been described as a new species *Trypanosoma desterrensis* [41]; however, comparison of the spliced-leader sequences indicates that they are highly similar to Brazilian isolates of *T. dionisii*, which was not compared by these authors (M.M.G. Teixeira *et al.*, unpublished). There is also some doubt as to whether this *T. vespertilionis* is the originally described species in North Africa in 1905 and in several European countries in 1904–1907 [15]. *T.* (*Schizotrypanum*) *vespertilionis* was described in Brazil in 1936 and it has never been clearly ascertained whether descriptions from the New and Old Worlds relate to the same species. To date, moreover, *T. vespertilionis* matching the Old World isolate shown in this figure has not been described from Brazil with molecular methods, whereas molecular techniques have identified *T. dionisii*, which has not been previously reported in South America (M.M.G. Teixeira *et al.*, unpublished); such findings suggest that *T. vespertilionis* as originally described in the subgenus *Schizotrypanum* and *T. dionisii* may be synonyms.

readily in triatomine bugs, whereas others did not. Hoare [15] suggested that the species that developed in triatomines were *T. conorhini*. A refractory species, *Trypanosoma cyclops*, was later found to be more closely related to *Trypanosoma theileri* species than to *T. cruzi* [28], although, to date, no molecular work has been undertaken on *bona fide* isolates of Weinman's unclassified triatomine-infective trypanosome from Indonesian macaques. In addition, uncharacterized trypanosomes from slow loris (*Nycticebus coucang*) from Malaysia were detected in muscle tissue cells [29], a trait associated only with trypanosomes in the subgenus *Schizotrypanum*.

Phylogenetic positioning of bat trypanosomes
Recent phylogenetic studies have included a greater diversity of bat trypanosomes and have resolved relationships within the *T. cruzi* clade using concatenated gene sequences [18,26,30–32]. The most parsimonious explanation for the relationships observed within the group is that the *T. cruzi* clade was primitively a bat clade, which has made multiple jumps into terrestrial mammals, rather than it being a terrestrial clade with multiple lineages

jumping into bats. Thus, it now appears that *T. c. cruzi* evolved from a bat trypanosome (Figure 2), because its closest known living relative is *T. c. marinkellei* (from South American bats), followed by *Trypanosoma erneyi*, a recently described species from bats in Mozambique, Africa [30], and *T. dionisii*, from both Old and New World bats [5,10,18,31,32]. Although a bat origin of the clade requires a minimum of five independent switches from bats to terrestrial mammals, this is – on the basis of currently available taxa – a more parsimonious explanation than a South American terrestrial origin for the clade, which requires six or seven independent switches from terrestrial mammals to bats and four independent movements of the group from the Old World to the New World (Figure 2). As yet, no trypanosomes outside of the *T. cruzi* clade (except *Trypanosoma evansi* in vampire bats [15]) are known to have made the switch into bats, even though bats presumably feed on the vectors of various other trypanosomes, which may indicate that the *T. cruzi* clade trypanosomes have some ancestral adaptations to bat parasitism. Additional surveys will probably further increase our knowledge of the diversity of bat trypanosomes within

the clade, because, although the studies of the past decade have already demonstrated increased diversity, many species of bat remain to be been sampled and several geographical regions, such as Africa, Asia and Oceania, are at this time largely unsampled (note that Hoare [15] includes several descriptions of trypanosomes in the blood of bats from these regions).

Both *T. c. cruzi* [18,33–36] and *T. rangeli* [34] have been detected in South American bats, although the prevalence of *T. c. cruzi* in bats is very low compared to the prevalence of both *T. c. marinkellei* and *T. dionisii*. In addition, one genotype of *T. c. cruzi*, Tcbat, has so far only been found in bats, but its host range may well include other arboreal and terrestrial mammals because it has been shown to infect mice under experimental conditions [33]. Interestingly, Tcbat is most closely related to *T. c. cruzi* TcI, a group that is preferentially associated with didelphids and *Rhodnius* spp. in arboreal niches [2]. Likewise, the *T. rangeli* lineage TrE has only been found in bats and the triatomine *Rhodnius pictipes* from Central and Amazon regions in Brazil, and is probably not restricted to bats, because it was also found in experimentally infected mice [34]. As yet, it is unclear whether these taxa are representative of ancestral lineages or lineages that have been recently derived from terrestrial lineages by, e.g. predation by bats on infected triatomine bugs that share their roosts, such as caves, tree holes, palms and other sylvatic refugia.

The bat seeding hypothesis

The various strands of evidence that have emerged in the past decade do not support the southern super-continent hypothesis and instead can be brought together to form a new hypothesis – the bat seeding hypothesis – a scenario outlined in Figure 2. In this scenario, the common ancestor of trypanosomes in the *T. cruzi* clade (at least the common ancestor of *T. rangeli* and *T. cruzi*) was a bat trypanosome. These bat trypanosomes then diversified and became geographically widespread. Various trypanosomes within this group independently switched from bats into terrestrial mammals, with one such switch giving rise to *T. c. cruzi*. Bat–terrestrial mammal switches occurred at least five times (Figure 2), a number that is likely to increase as the true diversity of trypanosomes in bats and terrestrial mammals becomes known. Such switches would and may continue to be facilitated by the sharing of niches of bats, terrestrial mammals and invertebrate vectors, where a range of arthropod genera will feed on both bats and terrestrial mammals. Establishment of transmission cycles involving terrestrial mammals would have been facilitated by 'host fitting', whereby parasites adapt more readily to novel hosts that are related to their current host [32]. For instance, the only known vectors of *T. c. marinkellei* are triatomines of the genus *Cavernicola*, which are found associated with bat colonies in caverns, hollow trees and palms [37,38]; we envisage that adaptation of the parasite to a vector that feeds on bats may have facilitated transition to other species of triatomines that feed on arboreal and terrestrial mammals.

On the basis of the evidence now available, the *T. cruzi* clade of trypanosomes are likely to have diversified sometime after bats underwent major diversification,

approximately 70–58 million years ago [39] and, moreover, could have done so considerably more recently. The relatively high genetic diversity within *T. c. cruzi*, its wide geographical distribution in the New World and its wide host range (both in mammals and triatomines) point to a pre-human origin of this species. Thus, humans would have first been exposed to *T. cruzi* when they migrated to the New World approximately 30 000 years ago and first came into contact with the arthropod vectors of the parasite. Subsequently, infection would have become prevalent when homes became infested with triatomine species as a natural extension of their preferred habitat.

References

1 Coura, J.R. and Pinto Dias, J.C. (2009) Epidemiology, control and surveillance of Chagas disease – 100 years after its discovery. *Mem. I. Oswaldo Cruz* 104, 31–40

2 Miles, M.A. *et al.* (2009) The molecular epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*: looking back and to the future. *Parasitology* 136, 1509–1528

3 Stevens, J. and Gibson, W. (1999) The molecular evolution of trypanosomes. *Parasitol. Today* 15, 432–437

4 Stevens, J.R. *et al.* (2001) The molecular evolution of Trypanosomatidae. *Adv. Parasitol.* 48, 1–56

5 Stevens, J.R. *et al.* (1999) The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitology* 118, 107–116

6 Maslov, D.A. *et al.* (1996) Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. *Mol. Biochem. Parasitol.* 75, 197–205

7 Stevens, J.R. and Rambaut, A. (2001) Evolutionary rate differences in trypanosomes. *Infect. Genet. Evol.* 1, 143–150

8 Cox, B.C. and Moore, P.D. (2000) *Biogeography: An Ecological and Evolutionary Approach*, Blackwell Science

9 Noyes, H. *et al.* (1999) A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. *Int. J. Parasitol.* 29, 331–339

10 Stevens, J.R. *et al.* (1999) The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*. *Int. J. Parasitol.* 29, 749–757

11 Machado, C.A. and Ayala, F.J. (2001) Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of *Trypanosoma cruzi*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7396–7401

12 Lukes, J. *et al.* (2007) Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9375–9380

13 Flores-López, C.A. and Machado, C.A. (2011) Analyses of 32 loci clarify phylogenetic relationships among *Trypanosoma cruzi* lineages and support a single hybridization prior to human contact. *PLoS Negl. Trop. Dis.* 5, e1272

14 Lewis, M.D. *et al.* (2011) Recent, independent and anthropogenic origins of *Trypanosoma cruzi* hybrids. *PLoS Negl. Trop. Dis.* 5, e1363

15 Hoare, C.A. (1972) *The Trypanosomes of Mammals*, Blackwell Scientific

16 Sato, H. *et al.* (2008) Prevalence and molecular phylogenetic characterization of *Trypanosoma (Megatrypanum) minasense* in the peripheral blood of small neotropical primates after a quarantine period. *J. Parasitol.* 94, 1128–1138

17 Madeira, M.F. *et al.* (2009) *Trypanosoma caninum* n. sp (Protozoa: Kinetoplastida) isolated from intact skin of a domestic dog (*Canis familiaris*) captured in Rio de Janeiro, Brazil. *Parasitology* 136, 411–423

18 Cavazzana, M., Jr *et al.* (2010) Phylogeographical, ecological and biological patterns shown by nuclear (ssrRNA and gGAPDH) and mitochondrial (Cyt b) genes of trypanosomes of the subgenus *Schizotrypanum* parasitic in Brazilian bats. *Int. J. Parasitol.* 40, 345–355

19 Herrera, H.M. *et al.* (2008) The coati (*Nasua nasua*, Carnivora, Procyonidae) as a reservoir host for the main lineages of *Trypanosoma cruzi* in the Pantanal region, Brazil. *Trans. R. Soc. Trop. Med. Hyg.* 102, 1133–1139

20 Hypsa, V. *et al.* (2002) Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. *Mol. Phylogenet. Evol.* 23, 447–457

21 Patterson, J.S. and Gaunt, M.W. (2010) Phylogenetic multi-locus codon models and molecular clocks reveal the monophyly of haematophagous reduviid bugs and their evolution at the formation of South America. *Mol. Phylogenet. Evol.* 56, 608–621

22 Krinke, G.J. (2000) *The Laboratory Rat*, Academic Press

23 Adams, E.R. *et al.* (2010) New *Trypanosoma* (*Duttonella*) *vivax* genotypes from tsetse flies in East Africa. *Parasitology* 137, 641–650

24 Hamilton, P.B. *et al.* (2008) A novel, high throughput technique for species identification reveals a new species of tsetse-transmitted trypanosome related to the *Trypanosoma brucei* subgenus, *Trypanozoon*. *Infect. Genet. Evol.* 8, 26–33

25 Njiokou, F. *et al.* (2004) Infection rate of *Trypanosoma brucei* s.l., *T. vivax*, *T. congolense* "forest type", and *T. simiae* in small wild vertebrates in south Cameroon. *Acta Trop.* 92, 139–146

26 Hamilton, P.B. *et al.* (2009) Phylogenetic analysis reveals the presence of the *Trypanosoma cruzi* clade in African terrestrial mammals. *Infect. Genet. Evol.* 9, 81–86

27 Weinman, D. *et al.* (1978) Triatomines as experimental vectors of trypanosomes of Asian monkeys. *Am. J. Trop. Med. Hyg.* 27, 232–237

28 Stevens, J.R. *et al.* (1998) The evolution of trypanosomes infecting humans and primates. *Mem. I. Oswaldo Cruz* 93, 669–676

29 Kuntz, R.E. *et al.* (1970) *Trypanosoma cruzi*-like parasites in the slow loris (*Nycticebus coucang*) from Malaysia. *Trans. Am. Microsc. Soc.* 89, 304–307

30 Lima, L. *et al.* (2012) Evolutionary insights from bat trypanosomes: morphological, developmental and phylogenetic evidence of a new species, *Trypanosoma* (*Schizotrypanum*) *erneyi* sp. nov., in African bats closely related to *Trypanosoma* (*Schizotrypanum*) *cruzi* and allied species. *Protist* DOI: 10.1016/j.protis.2011.12.003

31 Hamilton, P.B. *et al.* Parasites reveal movement of bats between New and Old Worlds. *Mol. Phylogenet. Evol.* doi:10.1016/j.ympev.2012.01.007

32 Hamilton, P.B. *et al.* (2007) Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Mol. Phylogenet. Evol.* 43, 15–25

33 Marcili, A. *et al.* (2009) A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and histone H2B genes and genotyping based on ITS1 rDNA. *Parasitology* 136, 241–255

34 da Silva, F.M. *et al.* (2009) *Trypanosoma rangeli* isolates of bats from Central Brazil: genotyping and phylogenetic analysis enable description of a new lineage using spliced-leader gene sequences. *Acta Trop.* 109, 199–207

35 Lisboa, C.V. *et al.* (2008) *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) genotypes in neotropical bats in Brazil. *Vet. Parasitol.* 156, 314–318

36 Cottontail, V.M. *et al.* (2009) Habitat fragmentation and haemoparasites in the common fruit bat, *Artibeus jamaicensis* (Phyllostomidae) in a tropical lowland forest in Panama. *Parasitology* 136, 1133–1145

37 Marinkelle, C.J. (1982) Prevalence of *Trypanosoma cruzi*-like infection of Colombian bats. *Ann. Trop. Med. Parasitol.* 76, 125–134

38 Molyneux, D.H. (1991) Trypanosomes of bats. In *Parasitic Protozoa* (Kreier, J.P. and Baker, J.R., eds), pp. 195–224, Academic Press

39 Teeling, E.C. *et al.* (2005) A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* 307, 580–584

40 Steindel, M. *et al.* (1998) Characterization of trypanosomes from the subgenus *Schizotrypanum* isolated from bats, *Eptesicus* sp. (Chiroptera: Vespertilionidae), captured in Florianopolis, Santa Catarina State, Brazil. *J. Parasitol.* 84, 601–607

41 Grisard, E.C. *et al.* (2003) A new species of trypanosome, *Trypanosoma desterrensis* sp. n., isolated from South American bats. *Parasitology* 127, 265–271

42 Lake, J.A. *et al.* (1988) Evolution of parasitism – kinetoplastid protozoan history reconstructed from mitochondrial ribosomal-RNA gene sequences. *Proc. Natl. Acad. Sci. U.S.A.* 85, 4779–4783

43 Fernandes, A.P. *et al.* (1993) Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. *Proc. Natl. Acad. Sci. U.S.A.* 90, 11608–11612

44 Averis, S. *et al.* (2009) The diversity, distribution and host–parasite associations of trypanosomes in Western Australian wildlife. *Parasitology* 136, 1269–1279

45 Paparini, A. *et al.* (2011) Identification of novel trypanosome genotypes in native Australian marsupials. *Vet. Parasitol.* 183, 21–30

46 McInnes, L.M. *et al.* (2009) *Trypanosoma irwini* n. sp. (Sarcomastigophora: Trypanosomatidae) from the koala (*Phascolarctos cinereus*). *Parasitology* 136, 875–885

47 Austen, J.M. *et al.* (2011) Vector of *Trypanosoma copemani* identified as *Ixodes* sp. *Parasitology* 138, 866–872

48 Austen, J.M. *et al.* (2009) Morphological and molecular characterization of *Trypanosoma copemani* n. sp. (Trypanosomatidae) isolated from Gilbert's potoroo (*Potorous gilbertii*) and quokka (*Setonix brachyurus*). *Parasitology* 136, 783–792

49 Thekisoe, O.M.M. *et al.* (2007) A trypanosome species isolated from naturally infected *Haemaphysalis hystricis* ticks in Kagoshima Prefecture, Japan. *Parasitology* 134, 967–974

50 Hamilton, P.B. *et al.* (2004) Trypanosomes are monophyletic: evidence from genes for glyceraldehyde phosphate dehydrogenase and small subunit ribosomal RNA. *Int. J. Parasitol.* 34, 1393–1404

51 Hamilton, P.B. *et al.* (2005) A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). *Int. J. Parasitol.* 35, 431–443

52 Votypka, J. *et al.* (2010) Probing into the diversity of trypanosomatid flagellates parasitizing insect hosts in South-West China reveals both endemism and global dispersal. *Mol. Phylogenet. Evol.* 54, 243–253

53 Dougall, A. *et al.* (2009) New reports of Australian cutaneous leishmaniasis in Northern Australian macropods. *Epidemiol. Infect.* 137, 1516–1520

54 Rose, K. *et al.* (2004) Cutaneous leishmaniasis in red kangaroos: isolation and characterisation of the causative organisms. *Int. J. Parasitol.* 34, 655–664

55 Noyes, H. *et al.* (2002) A previously unclassified trypanosomatid responsible for human cutaneous lesions in Martinique (French West Indies) is the most divergent member of the genus *Leishmania* ss. *Parasitology* 124, 17–24