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Emerging Chagas disease in Amazonian Brazil

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In the Amazon Basin, *Trypanosoma cruzi* infection is enzootic, involving a variety of wild mammals and at least 10 of the 16 reported silvatic triatomine bug species. Human cases of Chagas disease are increasing, indicating that the disease may be emerging as a wider public health problem in the region: 38 cases from 1969 to 1992, and 167 in the past eight years. This article reviews the status of Chagas disease in Amazonian Brazil, including known reservoirs and vectors, and the genetic diversity of *T. cruzi*. At least three subspecific groups of *T. cruzi* – *T. cruzi* Z1, *T. cruzi* Z3 and *T. cruzi* Z3/Z1 ASAT – are present. It appears that *T. cruzi* has an extant capacity for genetic exchange. Attention is also drawn to the risk of domestic endemicity, in addition to the tasks facing the disease control authorities.

The greatest risks underlying the establishment of Chagas disease in the Amazonian region of Brazil are human migration and uncontrolled deforestation. Human migration, with carriage of triatomine vectors from endemic areas, could transport the domestic cycle from established areas of domestic transmission to other areas. Deforestation can encourage adaptation of silvatic triatomine vectors to human dwellings.

Since the beginning of the 1900s, it has been known that there are abundant reservoir hosts for *Trypanosoma cruzi* among wild animals [1,2] and several triatomine insect vector species [3,4] in Amazonian Brazil. However, the first autochthonous human cases of Chagas disease in the region were reported only in 1969, from the city of Belém, Pará State [5]. From a recent survey of case reports assembled at the Instituto Evandro Chagas, a total of 205 cases of Chagas disease have now been registered, between 1968 and 2000 [6] (Fig. 1). Of these, 178 were acute cases, nine of which were fatal, and 27 were chronic. Geographical distribution by State was as follows: 121 in Pará, 53 in Amapá, 14 in Amazonas, nine in Maranhão and eight in Acre. This is considered to be a small proportion of the real

number of cases, as the distribution reflects the research interest in Chagas disease and the presence of local diagnostic facilities in Belém. The national serological survey carried out by the Brazilian Ministry of Health from 1975 to 1980 showed a 1.88% seroprevalence in the State of Amazonas and 2.4% in the State of Acre [7]. Three additional surveys performed in 1991, 1993 and 1997 involving 2254 individuals from Barcelos, State of Amazonas, showed that 2.8–5% of this number had positive

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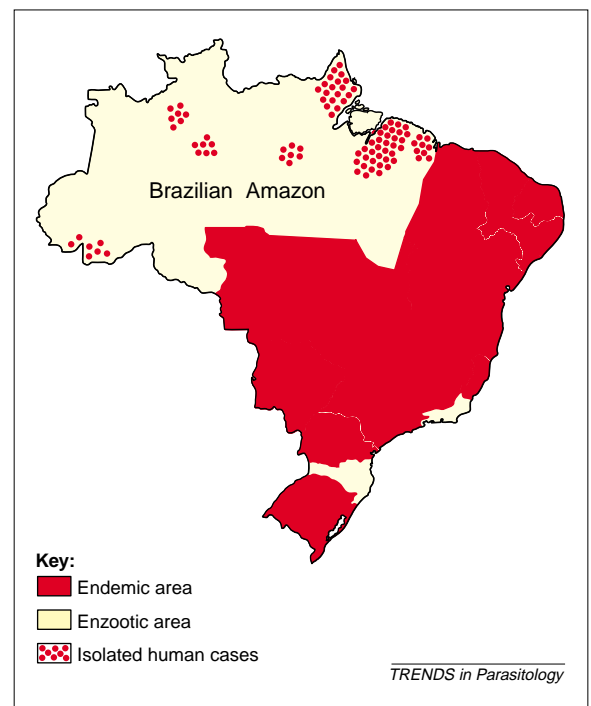


Fig. 1. Distribution of Chagas disease in Brazil: enzootic areas with isolated human cases or small outbreaks and endemic regions [6,8,15].

Table 1. Mammals infected with *Trypanosoma cruzi* in Amazonian Brazil

Order	Species ^a	Refs
Marsupialia	<i>Caluromys</i> spp.	[24,26]
	<i>Didelphis marsupialis</i>	[18,19,23,24,26,27]
	<i>Marmosa cinerea</i>	[18–20,24,27]
	<i>Metachirus nudicaudatus</i>	[16,24,26,27]
	<i>Monodelphis brevicaudata</i>	[26,27]
	<i>Philander opossum</i>	[16,24,26]
Chiroptera (<i>T. cruzi</i> or <i>T. cruzi</i> -like)	<i>Carollia perspicillata</i>	[22]
	<i>Choeronycteris minor</i>	[22]
	<i>Glossophaga soricina</i>	[22]
	<i>Lonchophylla mordax</i>	[22]
	<i>Micronycteris megalotis</i>	[22]
	<i>Molossus major</i>	[18,19]
	<i>Molossus ater</i>	[19]
	<i>Phyllostomus hastatus</i>	[18,19]
	<i>Phyllostomus elongatus</i>	[22]
	<i>Noctilio labialis</i>	[22]
	<i>Saccopteryx bilineata</i>	[22]
Rodentia	<i>Agouti paca</i>	[24]
	<i>Coendou</i> spp.	[24,26]
	<i>Dasyprocta</i> spp.	[17,24,27]
	<i>Echimyus chrysurus</i>	[26]
	<i>Nectomys squamipes</i>	[17]
	<i>Oryzomys capito</i>	[24,27]
	<i>Proechimys guayannensis</i>	[19,24]
	<i>Rattus rattus</i>	[24,26]
<i>Sciurus</i> spp.	[26,27]	
Edentata	<i>Cyclopes didactylus</i>	[26]
	<i>Dasybus novemcinctus</i>	[18,19,23,24,26]
	<i>Tamandua tetradactyla</i>	[23]
Carnivora	<i>Nasua nasua</i>	[24,26]
	<i>Tayra barbara</i>	[18,19,21,23]
Primates	<i>Saguinus midas niger</i>	[26]
	<i>Saimiri sciureus</i>	[2,18]

^aHosts with a high prevalence rate for triatomine vectors are indicated in bold.

Table 2. Triatomines from Amazonian Brazil found infected and not infected with *Trypanosoma cruzi*

Species	Refs	
	Infected	Not infected
<i>Belminus herrerii</i>	– ^a	[32]
<i>Cavernicola lenti</i>	– ^a	[29]
<i>Cavernicola pilosa</i>	– ^a	[22,28]
<i>Eratyrus mucronatus</i>	[31,32]	[23,35]
<i>Microtriatoma trinidadiensis</i>	[32]	[33]
<i>Panstrongylus geniculatus</i>	[23,24,27–29,31,36]	[23,24,30,35,36]
<i>Panstrongylus lignarius</i>	[4,24,27–32]	[24,30,33,35]
<i>Panstrongylus rufotuberculatus</i>	[31]	[23,32]
<i>Rhodnius brethesi</i>	[8]	[3,28,29,31,35]
<i>Rhodnius nasutus</i>	– ^a	[30,35]
<i>Rhodnius neglectus</i>	[30]	[30,35]
<i>Rhodnius paraensis</i>	[31,32]	[31,32]
<i>Rhodnius pictipes</i>	[23,24,27–33]	[23,24,30,35]
<i>Rhodnius robustus</i>	[27,30–33]	[28–30,35]
<i>Triatoma maculata</i>	– ^a	[28,29,31,35]
<i>Triatoma rubrofasciata</i>	– ^a	[24,30,32,35]

^aTriatomines that have not been found infected with *Trypanosoma cruzi* to date.

This article reviews the status of Chagas disease in Amazonian Brazil and the genetic diversity of *T. cruzi*. It also discusses the risk of domestic endemicity because of the transposition of the domestic cycle from endemic areas of Chagas disease to the Amazon, by the human migration from endemic regions and/or by adaptation of the wild cycle to human dwellings within that area [9–15].

Infected mammals

A summary of host records of *T. cruzi* in the Amazon region is shown in Table 1 [2,3,16–27]. Known hosts include marsupials, edentates, rodents, carnivores, primates and bats; although, in bats, infections are often considered to be *T. cruzi*-like and might represent infections morphologically indistinguishable from bat trypanosome species of the same subgenus (*Schizotrypanum*). Systematic surveys to identify *T. cruzi*-infected mammals have been extensive in Pará State but have been limited in those other Amazonian States where human cases have been reported.

Triatomines

Of the 16 species of triatomine found in Amazonian Brazil, ten are infected with *T. cruzi* [8,28–33] (Table 2). A single record of *Rhodnius prolixus* (a species that is difficult to distinguish from *Rhodnius robustus*) [34], and reports of *Triatoma rubrovaria* are not included here as they are questionable and are almost certainly based on equivocal identifications. Note also that the records of *Rhodnius nasutus*, *Rhodnius neglectus* and *Triatoma maculata* are from the State of Maranhão bordering the Amazon basin and not from typical Amazon forest [35].

The only domestic and peridomestic species is *Triatoma rubrofasciata*, apart from the recent description of *Panstrongylus geniculatus* in the peridomicile [36]. Interestingly, *Triatoma rubrofasciata* is strongly associated with the ship's rat, *Rattus rattus*, with which it has spread to tropical ports around the world. Although *Triatoma rubrofasciata* has been known to take a bloodmeal from *Didelphis* and might rarely carry *T. cruzi*, it is reluctant to feed on humans and is not considered a direct health threat. *Triatoma rubrofasciata* transmits *Trypanosoma conorhini* to *Rattus rattus* by the contaminative route [32].

Broad habitats and host associations for some Amazonian triatomine species have been described, either through habitat dissection, especially of palm trees, or after spool-and-line tracking of trapped mammals back to their nest [32]. *Rhodnius brethesi*, *Rhodnius pictipes*, *Rhodnius robustus*, *Rhodnius nasutus* and *Rhodnius neglectus* are all primarily associated with palm trees, often inhabited by *Didelphis*. *Rhodnius paraensis* has been found only once, in a canopy tree hole inhabited by the arboreal spiny rat, *Echimyus chrysurus*. Further examples of habitats are *Panstrongylus geniculatus* in armadillo burrows, *Panstrongylus lignarius* in arboreal tree hole nests of *Didelphis* in forest, *Eratyrus mucronatus* with

indirect immunofluorescence reactions, further confirmed by indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA) and western blot [8].

porcupines (*Coendou*) in hollow trees, *Cavernicola* species in hollow trees with bats, and *Belminus herreri* with lizards under tree bark [32].

Rhodnius brethesi poses a particular occupational hazard to collectors of fronds from the piassaba palm because the palms are often infested by piacava lice, which either cause contamination during collection or possibly attack workers when they are sleeping in their huts locally in the forest [8,37]. *Panstrongylus geniculatus* poses an alarming new threat in that it has been found infesting pigsties adjacent to houses. Formerly, the species was thought to require 100% humidity in armadillo burrows but some populations seem to be able to overcome this limitation [36].

Trypanosoma cruzi

Trypanosoma cruzi isolates from the Amazon rainforest have been characterized since the late 1970s [25,26]. Because of the high prevalence of infection among mammals and silvatic triatomines, and the initial scarcity of isolates from human cases, most of these stocks were from non-human sources in the enzootic transmission cycle. Some isolates were passaged in mice as part of the isolation procedure [26] and a few biological clones have been characterized in experimental models [38]. Nevertheless, further investigation of the behavior of biological clones in comparison with the series of biochemical and molecular markers that are now available could give new insight into virulence and pathogenesis.

The use of multilocus enzyme electrophoresis (MLEE) has demonstrated that the vast majority of Amazonian *T. cruzi* isolates belong to a subspecific division originally described as *T. cruzi* principal zymodeme 1 (Z1), which corresponds to the major phylogenetic lineage now defined as *T. cruzi* I. This subspecific division is distinct from the second major phylogenetic lineage, principal zymodeme 2 (Z2) or *T. cruzi* II, which is more common in traditional Brazilian endemic areas of Chagas disease [25,26,39,40]. There is further phenotypic and genotypic heterogeneity within *T. cruzi* I and, in particular, *T. cruzi* II. Two additional phenotypes have been described from the Amazon region: zymodeme 3 (Z3), a rare phenotype with a wide but sporadic distribution; and a Z3 subgroup with an aspartate aminotransferase (ASAT) phenotype compatible with *T. cruzi* I (Z1) [26]. Of these isolates, *T. cruzi* I (Z1) has been associated with arboreal mammals such as *Didelphis marsupialis*, whereas Z3 stocks have been isolated mainly from terrestrial or burrowing mammals such as the nine-banded armadillo, *Dasybus novemcinctus*, and the short-tailed marsupial *Monodelphis brevicaudata* [26,41]. Human infections, acquired from silvatic foci of transmission, either by vector incursion into houses or by oral transmission, are predominantly *T. cruzi* I with occasional cases of Z3 [26].

Other molecular techniques have since been applied to study the major genotypes of *T. cruzi*

isolates from the Amazon basin. A multiplex PCR, based on a hypervariable site on the non-transcribed spacer of the mini-exon gene, has proved to be a useful technique to type isolates as *T. cruzi* I, II and Z3. It has also been used to distinguish these from the closely related species *Trypanosoma rangeli*, which is widely distributed throughout the Amazon region and is transmitted by triatomine bite, from the infected salivary glands of *Rhodnius* species [42].

The typing of human isolates from the Amazonian region using the mini-exon approach, together with the coding region to the 24S ∞ subunit of the ribosomal gene, has confirmed the isoenzymic findings – that the most prevalent genotype causing Chagas disease in the region is indeed *T. cruzi* I. Clinical and parasitological studies have suggested that the low morbidity in the area and the low parasitemias must be correlated with the *T. cruzi* I genotype present in the Amazon basin, which leads predominantly to the indeterminate form of the disease [43].

Although these genetic markers (mini-exon gene and 24S ∞ rDNA) distinguish the major groups of *T. cruzi*, they do not allow characterization of intra-group polymorphisms, as both regions have proved to be slowly evolving targets. Ribosomal spacers are more-suitable markers for subgroup discrimination. The internal transcribed spacers, flanking the 5.8S rDNA, have therefore been used to investigate genetic relationships among Amazonian *T. cruzi* isolates. PCR amplification, followed by restriction fragment length polymorphism (RFLP) analysis, cloning, sequencing and computer-based phylogenetics inference have confirmed the further diversity of the parasites in the region [44,45] (Fig. 2). Thus, genetic polymorphism was evident within *T. cruzi* I, as expected from the presence of some isoenzyme heterogeneity [26] and from the multiple mammalian host and vector species.

Furthermore, genetic diversity among Z3 isolates from a human case, from *P. geniculatus* and from *R. brethesi*, was revealed using the same methodology and by heterogeneity of ribosomal loci. In fact, two distinct subgroups of Z3 were found. Although it has not yet been confirmed by isoenzyme analysis, it is probable that these two distinct subgroups will prove to correspond to the Z3 and Z3/Z1 ASAT isoenzyme groups.

The affinities of Z3, whether to *T. cruzi* I or to *T. cruzi* II, are controversial. The data have so far suggested closest affinity to *T. cruzi* I, as surmised from the original isoenzyme data. However, Brisse *et al.* [46] have encompassed Z3 and the Z3/Z1 ASAT reference groups within *T. cruzi* II, which they have divided into five sublineages, as their sublineages IIa (Z3) and IIc (Z1/Z3 ASAT).

Genetic exchange in *T. cruzi* I

Research on *T. cruzi* I isolates from the Amazon Basin has indicated the presence of putative parental and hybrid phenotypes and genotypes, based initially on isoenzymes and profiles of randomly amplified polymorphic DNA (RAPD), with isolates from a single

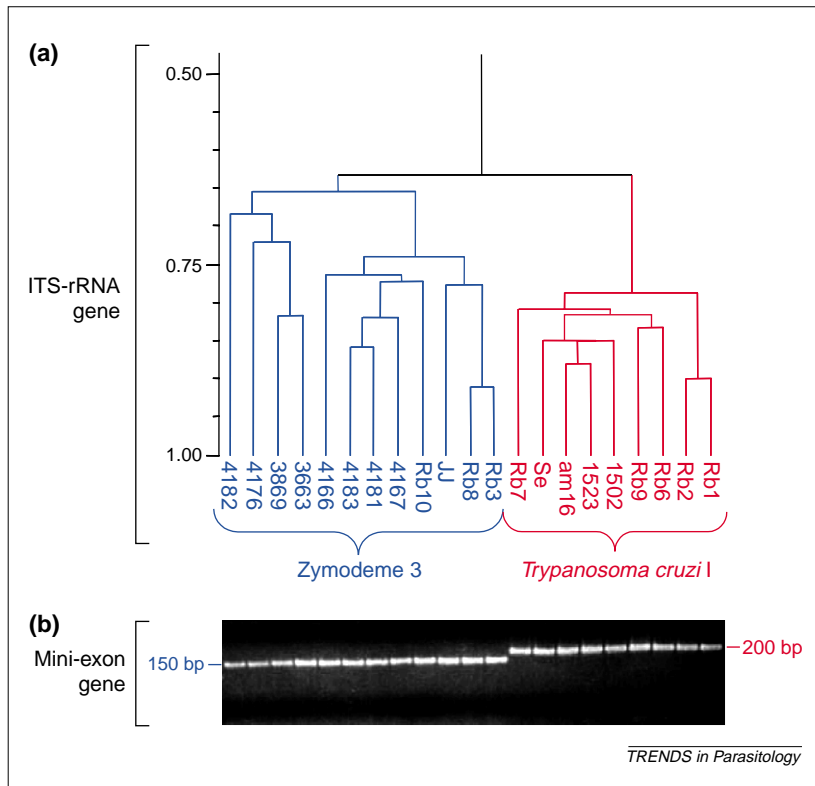


Fig. 2. Genetic diversity of *Trypanosoma cruzi* isolates from the Amazon basin [44,45]. Stocks Rb1, Rb2, Rb6, Rb9, Rb7, 4176, 4166, 4183, 4181, 4176, Rb10, Rb8 and Rb3 were isolated from *Rhodnius brethesi*; 3663 from *Panstrongylus geniculatus*; am16, Se, 3869 and JJ from humans; and 1502 and 1523 from *Didelphis marsupialis*. (a) A phenetic dendrogram was generated after restriction fragment length polymorphism (RFLP) analysis with four enzymes (*Bst*UI, *Rsa*I, *Sau*3AI, *Hae*III) of the internal transcribed spacers of the ribosomal gene (ITS-rRNA) by a numerical methodology. A similarity matrix was constructed using the simple matching coefficient and phenogram generated by the UPGMA algorithm (NTSYS program, Exeter Software, Setauket, NY). Two major clusters can be detected showing the presence of *T. cruzi* I and *T. cruzi* Z3 in the area [44,45]. (b) An agarose gel (3%) electrophoresis of PCR products stained with ethidium bromide and visualized under UV light. The bands correspond to a hypervariable region of the mini-exon gene non-transcribed spacer that is able to characterize *T. cruzi* I (200 bp) and Z3 (150 bp) [42].

undisturbed locality of enzootic transmission in Pará State [47]. Experimental hybrids, displaying shared parental phenotypic and genotypic characters, have since been generated in the laboratory, from genetically transformed biological clones [48]. This might have major implications for understanding the evolution, biology and epidemiology of *T. cruzi*. In particular, it is the first experimental proof of extant hybridization potential in any trypanosomatid apart from the African trypanosome, *Trypanosoma brucei*, although there is circumstantial evidence of naturally occurring hybrid strains in *T. cruzi* II from multiple isoenzyme phenotypes and in *Leishmania* from cross-species hybrid phenotypes and genotypes [49].

Infections in humans

Surprisingly, 111 of the 205 acute cases of Chagas disease recorded between 1968 and 2000 were attributable to microepidemics of orally transmitted infection from contaminated food; the largest of these outbreaks involved 17 cases [6,12]. Potential sources of food contamination are whole triatomine bugs or triatomine feces (containing highly infective

metacyclic trypomastigotes), raw meat from infected wild mammals carrying pseudocysts and blood-form trypomastigotes, and anal gland secretions of infected opossums (*D. marsupialis*), which are occasionally known to harbor metacyclic forms more typically seen in the insect vector [11]. A particular risk for oral transmission has been identified as palm juice presses lit at night and open to contamination by light-attracted triatomine bugs [12].

Thus, at present, fewer than 50% of cases of Chagas disease in Amazonian Brazil are attributable to infection of humans in their houses by contamination with infected triatomine feces as the bugs take a bloodmeal. This is understandable because intensive studies of triatomine habitats have shown that none of the local species is yet domiciliated [32]. No bug colonies, with all life cycle stages, have been reported from inside houses. Individuals infected inside a house must be attacked by adult bugs, which are presumably initially attracted to light. There are numerous records of occasional incursion of adult bugs into houses. Species commonly attracted into houses by light include *R. pictipes*, *R. robustus*, *P. geniculatus*, *P. lignarius* and *E. mucronatus*. A few cases might also result from bringing construction materials or firewood into houses, or from occupational exposure to silvatic triatomines, particularly *R. brethesi* [37].

Three serological screenings using the indirect immunofluorescence method performed in 1991, 1993 and 1997 analyzing 2254 individual samples from Barcelos, located in the Rio Negro region in the State of Amazonas, surprisingly revealed seropositivities of 12.5%, 13.7% and 13.2%, respectively [8]. However, when these results were further confirmed by indirect hemagglutination, ELISA and western blot, the seroprevalence reduced to 2.8% in 1991, 3.5% in the second survey (1993) and 5% in the last serological screening (1997).

The national serological survey carried out by the Brazilian Ministry of Health from 1975 to 1980, showed a 1.88% seroprevalence for *T. cruzi* infection in the human population of the State of Amazonas and 2.4% in the State of Acre [7]. These results were not checked with confirmatory techniques and migrants from endemic regions were not identified. Nevertheless, it indicates a significant human reservoir of infection that could contribute to domestic transmission of *T. cruzi* if a domiciliated triatomine vector arises in the Amazon region. It is conceivable that infection with *T. rangeli* might contribute to seropositivity rates among the few individuals exposed to *Rhodnius* but only low antibody titres to *T. cruzi* antigens are presumed to occur in *T. rangeli* infections. Smaller scale serological studies have suggested additional autochthonous cases of *T. cruzi* infection [50–52], some of which were confirmed by xenodiagnosis and PCR [8,51,52]; the associated rate of electrocardiograph abnormality was low, implying a low chronic morbidity owing to the infection [52,53].

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Box 1. Control measures against local outbreaks of Chagas disease

- Establishment of regional reference centres responsible for:
 - (1) Informing the population to be vigilant and to report any colonies of triatomine bugs, either local, silvatic species or domestic species, imported from elsewhere.
 - (2) Rapidly spraying any dwellings with emergent domestic colonies, using residual pyrethroid insecticide, with follow-up surveillance.
 - (3) Informing the local population of the risk of sporadic outbreaks, particularly oral outbreaks from contaminated palm presses [a].
- Further studies of the habitats, ecology and adaptability of local triatomine species, of risk factors for their incursion and of selective control measures against high-risk silvatic foci [a].
- Reduction and control of deforestation, particularly on the periphery of population centers, and formulation of a global policy for the settlement and colonization of the Amazon, which would simultaneously preserve the ecology of the area while promoting its socioeconomic development [b].

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Surveillance and control Uncontrolled migratory movements of people from south, southeast and northeast Brazil towards the

Amazon region over the past 20 years might have introduced a naïve population that is susceptible to local endemic infectious diseases. In addition, such migration might import new sources of infection such as Chagas disease patients and domestic reservoirs of *T. cruzi* and triatomines [8,9]. Although the establishment of the domestic transmission cycle is a slow process, it does pose a serious threat of transforming this vast region into one that is endemic for Chagas disease. Measures that might reduce the risk, by containing or eliminating local domestic outbreaks and adaptation of triatomines to human dwellings in the Amazon [8,32], are outlined in Box 1.

A new serological survey for Chagas disease in the Brazilian Amazon has been planned by the Ministry of Health to start in 2002. In addition, during the 17th Meeting of Applied Research on Chagas Disease held in Uberaba, Minas Gerais, Brazil, in October 2001, special recommendations were made that reinforced the need to include the microscopic analysis of blood smears for the direct search of *T. cruzi* in febrile patients in the routine survey for malaria parasites in the Amazon basin.

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Trematode life cycles: short is sweet?

Robert Poulin and Thomas H. Cribb

Complex life cycles are a hallmark of parasitic trematodes. In several trematode taxa, however, the life cycle is truncated: fewer hosts are used than in a typical three-host cycle, with fewer transmission events. Eliminating one host from the life cycle can be achieved in at least three different ways. Some trematodes show even more extreme forms of life cycle abbreviations, using only a mollusc to complete their cycle, with or without sexual reproduction. The occurrence of these phenomena among trematode families are reviewed here and show that life cycle truncation has evolved independently many times in the phylogeny of trematodes. The hypotheses proposed to account for life-cycle truncation, in addition to the factors preventing the adoption of shorter cycles by all trematodes are also discussed. The study of shorter life cycles offers an opportunity to understand the forces shaping the evolution of life cycles in general.

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From simple beginnings, the life cycles of parasitic helminths have evolved into complex sequences of improbable events. Both adaptive scenarios and evolutionary contingencies are often invoked to explain the complexity of their life cycles [1–3]. For example, if a new type of predator evolves, the parasite of the prey might add a developmental stage to their life cycle inside the predator. Alternatively, transmission from prey host to predator host could serve to concentrate conspecific parasites and facilitate cross-fertilization [3]. Whatever the origin of complex life cycles, they have

imposed new selective pressures on parasites by being so difficult to complete.

The life cycle of a typical digenean trematode offers a good example of a complex cycle, involving three transmission challenges. First, eggs released from adult worms in the DEFINITIVE HOST (see Glossary) hatch into MIRACIDIA, which must find a suitable mollusc as the FIRST INTERMEDIATE HOST. Second, the free-living, but short-lived, CERCARIAE that emerge from the first intermediate host must locate a suitable SECOND INTERMEDIATE HOST. Third, METACERCARIAE must be ingested, along with the second intermediate host, by an appropriate definitive host for the life cycle to be completed. Natural selection has favored various adaptations in trematodes to counteract the odds stacked against the completion of the cycle. Examples include high adult-fecundity, asexual multiplication within the molluscan first intermediate host, efficient host-finding mechanisms in miracidia and cercariae, and parasite-mediated increases in the susceptibility of the second intermediate host to predation [2,4].

In some species, however, evolution has taken a drastic approach, resulting in the typical three-host life cycle being truncated to two hosts or even to one host [1,2]. The corresponding decrease in the number