Immigration and establishment of urban *Trypanosoma cruzi* populations

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Abstract

Changing environmental conditions, including those caused by human activities, reshape biological communities through both loss of native species and establishment of non-native species in the altered habitats. Dynamic interactions with the abiotic environment impact both immigration and initial establishment of non-native species into these altered habitats. The repeated emergence of disease systems in urban areas worldwide highlights the importance of understanding how dynamic migratory processes affect the current and future distribution and abundance of pathogens in urban environments. In this study, we examine the pattern of invasion of *Trypanosoma cruzi*—the causative agent of human Chagas disease—in the city of Arequipa, Peru. Phylogenetic analyses of 136 *T. cruzi* isolates from Arequipa and other South American locations suggest that only one *T. cruzi* immigrant established a population in Arequipa as all *T. cruzi* isolated from vectors in Arequipa form a recent monophyletic group within the broader South American phylogeny. We discuss several hypotheses that may explain the limited number of established *T. cruzi* lineages despite multiple introductions of the parasite.

Author Summary

Human-associated pests and pathogens, who benefit from the abundance of humans and human-associated hosts or vectors, commonly invade environments altered by human activities. As the number and size of human-disturbed environments increase, so does the importance of identifying ecological and environmental factors that affect the probability that disease systems immigrate to, subsequently establish populations in, urban environments. We examined the number and timing of immigration and establishment events of *Trypanosoma cruzi*, the causative agent of Chagas disease, in a currently endemic area. Phylogenetic analyses of 136 *T. cruzi* isolates suggests that the current population descended from a single, recent immigration event. We discuss historical and ecological hypotheses that can explain the limited *T. cruzi* diversity in this region.

Introduction

Habitat alterations are transforming biological communities worldwide [1-3]. The current and future geographic distributions of many species in disturbed environments depends upon their interactions with novel biotic and abiotic features during immigration and while establishing a growing population [4]. Although many species fail to establish thriving populations in altered habitats, others are well-suited to migrate to, and prosper in, these novel environments. For example, several populations of plant [5-7], insect [8-11], mammal [12,13], and bird [14,15] species are severely diminished or suffer local extinctions in recently urbanized environments [1], while several microbial species benefit from the abundance of humans and human-associated hosts or vectors in similar habitats [16]. Although conservation efforts have focused primarily on the impacts of environmental changes on native plant and animal species, establishment or population growth of disease-causing microbial populations can have a strong negative impact on populations of native flora and fauna [17] in addition to their impact on human health and economy [18]. The rate or impacts of invasions of infectious microbes may be mitigated through public health programs based on an understanding of the dynamic processes determining immigration and establishment rates. The regularity at which disease systems are emerging in many urban and urbanizing areas underscores the importance of understanding how disease-causing microbes migrate to, and establish in, urban environments [16], one of the most dramatic examples of habitat alteration [19,20]. In this study, we examine the patterns of invasion of *Trypanosoma cruzi*—the causative agent of Chagas disease in humans—into the city of Arequipa, Peru.

Invasion of a new environment by a pathogen occurs in three stages: (1) immigration, or the movement of an individual to the new environment; (2) establishment of a population via

reproduction and population growth; and (3) local dispersal [4]. The many studies focusing on outbreaks of disease systems have generated a wealth of knowledge concerning factors affecting population growth [21,22] and considerable progress in understanding local dispersal [23]. For example, prior studies concluded that human-created containers increase the abundance of standing water that provide breeding habitats for the mosquitos that spread dengue virus [24]. Relatively few studies, by contrast, have investigated the early stages of invasion, the immigration and establishment processes, due to the practical difficulties of collecting the necessary data before a novel species is established.

The Chagas disease system in Arequipa, Peru, provides an ideal system in which to study the early invasion processes in urban environments. Arequipa has experienced rapid urbanization and human population growth in the previous half century with a concurrent population of *T. cruzi* [25]. The expansion in population sizes and geographic range of humans and their domestic animals provide hosts for both *T. cruzi* and its primary insect vector, *Triatoma infestans* [26-31]. The population history of the *T. cruzi* currently in Arequipa - including the geographic locations of the migrants that established the current population, the rate at which migrants enter and establish in the area, and the age of each established lineage - have not been investigated. Here, we performed phylogenetic analyses of maxicircle DNA, a non-recombining circular element analogous to mitochondrial DNA, to estimate the number of independent *T. cruzi* lineages established in Arequipa and to estimate the timing of each establishment event. We assessed whether extant *T. cruzi* in Arequipa form a single monophyletic clade, indicative of the establishment of a single migrant lineage, or multiple diverse clades, indicative of multiple independent immigration and establishment events.

Results

The maxicircle sequence is useful for population genetic and phylogenetic analyses because it is conserved among diverse *T. cruzi* lineages and is likely non-recombining [32]. While there was substantial maxicircle sequence diversity among samples across South America, almost no diversity was observed among the samples from Arequipa. Over 13% (2055/15367bp) of the maxicircle sites were polymorphic among all 136 samples while only 16 sites were polymorphic (0.1%) among the 123 samples collected within Arequipa (Table 1). Similarly, estimates of diversity among the samples from Arequipa derived from population genetic statistics were substantially lower than the total diversity across all samples ($\pi=6.8*10^{-5}$ vs $8.18*10^{-3}$; $\theta=1.93*10^{-4}$ vs $2.44*10^{-2}$; average pairwise distance 1.04 vs 126). In contrast to the limited genetic diversity within Arequipa, other locales from which multiple isolates were sampled contain substantial genetic diversity among limited samples ($N<3$; Fig 1).

Table 1. Population Genetic Statistics.

	Average Pairwise Distance	π	θ	Segregating Sites
All samples (N=136)	126	$8.18*10^{-3}$	$2.44*10^{-2}$	2055
Arequipa (N=123)	1.04	$6.80*10^{-5}$	$1.93*10^{-4}$	16
South America (N=13)	728	$4.73*10^{-2}$	$4.24*10^{-2}$	2022
La Esperanza (N=3)	643	$4.18*10^{-2}$	$4.18*10^{-2}$	964

The monophyletic group containing all 123 samples derived from *T. infestans* and domestic animals collected in Arequipa coalesce in the very recent past, despite collection sites extending throughout Arequipa and surrounding towns across 7 years (Fig 1). One sample derived from an infected human in Arequipa (Fig 1C) belongs to a lineage that is more closely related to samples

collected in Rio Grande, Brazil than to the other samples collected in Arequipa (Fig 1). This sample is distinct from all other samples in Arequipa suggesting that *T. cruzi* can immigrate to Arequipa but may not establish in the vector population. The population size of an unsampled *T. cruzi* lineage – if present in the *T. infestans* population – must be at least 42 times smaller than the dominant population in Arequipa to have remained undetected by chance ($p < 0.05$).

In contrast to the monophyletic ancestry found in Arequipa, genetic diversity was apparent in the samples collected from other regions, despite limited numbers of samples ($N < 3$). Population genetic diversity among the three samples collected La Esperanza, a town of 57 houses in the Cutervo Province of Cajamarca, Peru ($\pi = 4.18 \times 10^{-2}$; $\theta = 4.18 \times 10^{-2}$), are much larger than those in Arequipa despite the limited number of samples [33]. There is no statistical correlation between genetic relatedness and geographic distance among South American samples (Table 2). For example, while proximal towns La Esperanza and Campo Florido, Peru have closely related *T. cruzi*, isolates from cities around Brazil encompass nearly the total genetic diversity.

Table 2. Distance matrix showing average pairwise SNP distance between samples (bottom triangle) and Euclidean distance between sample collection locations (top triangle).

	(A) Arequipa, Peru	La Esperanza, Peru	La Esperanza, Peru	(B) La Esperanza, Campo Florido, & Naranjal, Peru	Bolivia	Bolivia	(C) Arequipa, Peru - Human	São Paulo, Brazil	Para State, Brazil	Bahia State, Brazil	Rio Grande, Brazil
(A) Arequipa, Peru		1400km	1400km	1400km	700km	700km	0km	2700km	2500km	3200km	2500km
La Esperanza, Peru	95		0km	60km	2000km	2000km	1400km	4000km	3000km	4100km	3900km
La Esperanza, Peru	95	0		60km	2000km	2000km	1400km	4000km	3000km	4100km	3900km
(B) La Esperanza, Campo Florido, & Naranjal, Peru	970	966	966		2000km	2000km	1400km	4000km	3000km	4100km	3900km
Bolivia	137	137	137	957		0km	700km	2000km	1800km	2500km	2000km
Bolivia	137	137	137	958	2		700km	2000km	1800km	2500km	2000km
(C) Arequipa, Peru - Human	975	971	971	89	967	968		2700km	2500km	3200km	2500km
São Paulo, Brazil	1044	1045	1045	1033	1045	1046	1058		2100km	1300km	1100km
Para State, Brazil	216	216	216	984	205	205	992	1059		1500km	2800km
Bahia State, Brazil	1284	1289	1289	1234	1287	1287	1258	68	1308		2500km
Rio Grande, Brazil	1005	1003	1003	82	1001	1001	77	1052	1026	1284	

(A) 123 samples isolated from bugs, dogs, and guinea pigs in Arequipa are represented here.

(B) 4 samples collected in Campo Florido, Naranjal, and La Esperanza are represented here.

Euclidean distances (top triangle) are displayed for Santa Cruz.

(C) The only sample isolated from a human in Arequipa is represented here.

Discussion

The invasion of recently altered environments by non-native species impacts the population health of native species as well as human health and economy. Investigation of the dynamic process of immigration and establishment of non-native species into these disturbed habitats has the potential to mitigate the impacts of pests and pathogens that are detrimental to human populations as well as agricultural and native species [17]. The analyses presented here suggest that the population of *T. cruzi* in Arequipa, Peru, descended from a single recent invasion. This conclusion is supported by the extremely limited genetic diversity observed among *T. cruzi* isolates sampled within and around the city, in contrast with considerable genetic diversity observed regionally and at other locales (Table 1; Fig 1). Several non-exclusive hypotheses may explain these results, including a low immigration rate, that immigration is common but immigrants rarely establish populations as a result of the low transmission rate between hosts and vectors, or that there is a high turnover rate among *T. cruzi* lineages.

Successful invasion of a novel geographic area is a function of the rate of immigration, the temporal duration that a habitat has been suitable for establishment, and the probability that an immigrant can reproduce and establish a population. The influx of humans and associated products and domesticated animals into Arequipa over the last ~60 years due to rapid urbanization and economic growth [25,34] has provided many opportunities for *T. cruzi* immigration. However, many migrants to Arequipa come from areas in which they were not exposed to *T. cruzi*, such as the neighboring regions of Puno and parts of Cusco which are beyond the range of *T. infestans* [35]. Nevertheless, one *T. cruzi* lineage that does not appear to be circulating within the *T. infestans* population was detected in an infected human (Fig. 1), suggesting that *T. cruzi* immigration through human movement can occur. These data suggest

that multiple *T. cruzi* lineages may have immigrated to Arequipa with all but one failing to transmit sufficiently to establish a population. While the data presented here are consistent with the hypothesis that *T. cruzi* in Arequipa originated in Bolivia [36,37], future studies will be necessary to identify the source, rate, and potential mechanism of *T. cruzi* immigration, independent of establishment probability, through analyses of the genomic diversity in human infections.

Prior studies across multiple species suggest that the majority of immigrants in most species that reach a novel geographic area fail to establish a population due to both inhospitable local environmental conditions [1,38] and stochasticity [39,40]. Environmental factors that can reduce establishment probabilities include unfavorable abiotic conditions, limited food resources or vectors, or an abundance of predators or competitors. The establishment probability of immigrant *T. cruzi* in an urban environment is likely depressed by a low transmission rate from infected humans to vectors [41-44]. For example, many human immigrants moved to locations in the city without established *T. infestans* populations [25,35], which may have resulted in few opportunities for *T. cruzi* transmission from infected immigrant humans to insect vectors, thus curtailing establishment probabilities. Further, the transmission probability of *T. cruzi* from *T. infestans* that do acquire the parasite to novel hosts is low [42,45,46], thus reducing the probability that a recently-immigrated *T. cruzi* lineage will establish a population. Both a low probability of establishing a population due to limited contact between *T. infestans* and infected immigrant humans or due to the limited probability of transmission from vectors to host are consistent with the observation of only a single established *T. cruzi* lineage in vectors in Arequipa.

The observed establishment probability is likely independent of competition among *T. cruzi* lineages. Competitive exclusion—where an existing population prevents the invasion of new immigrants—appears unlikely as the majority of city blocks do not contain *T. cruzi* [25,47] despite substantial vector populations [48]. Under the competitive exclusion hypothesis, one might expect different *T. cruzi* lineages establishing in different areas of the city. Indeed, multiple *T. cruzi* lineages do co-circulate within the same locality [44,49-53], as seen in the samples sequenced here from La Esperanza (Fig 1), and even within the same host [54,55], suggesting that competition is not preventing the establishment of multiple lineages in Arequipa.

The limited genetic diversity within Arequipa could result from a recent replacement of a previously dominant lineage through natural population processes. While the continuous substitution of a dominant strain through natural selection or drift is common in well-mixed populations, geographic structure within populations tends to result in the persistence of genetic diversity [56]. The absence of samples deriving from a previously established *T. cruzi* lineage in the fragmented urban and inter-district landscapes, much of which contains an active vector population but no *T. cruzi* [25,47,48], is suggestive that no previous lineages dominated this area. Additionally, no other *T. cruzi* lineage circulating in the vector population was detected despite the temporal range of samples in our dataset (7 years).

In conclusion, all relevant data suggest that the vast majority of, if not all, *T. cruzi* circulating in vector populations prior to the recently-controlled epidemic in the city of Arequipa descended from a single immigrant. The single divergent lineage found in a human patient suggests that *T. cruzi* may regularly immigrate to the city but that immigrants rarely establish populations. Low genetic diversity, coupled with minimal gene flow into the city, could limit the phenotypic diversity that results in variable effectiveness of treatment options and diagnoses regularly

associated with Chagas disease [57,58]. However, a potential downside of the limited diversity is that many currently available diagnostics may be ineffective against the local lineage [59,60], as seen in Arequipa [61]. It may be necessary to use locally-optimized diagnostics in endemic regions, which highlights the difficulties in diagnosing *T. cruzi* infection in immigrants to non-endemic countries such as the United States [62,63].

Methods

Ethics Statement

The Institutional Animal Care and Use Committee (IACUC) of Universidad Peruana Cayetano Heredia reviewed and approved the animal-handling protocol used for this study (identification number 59605). The IACUC of Universidad Peruana Cayetano Heredia is registered in the National Institutes of Health at the United States of America with PHS Approved Animal Welfare Assurance Number A5146-01 and adheres to the Animal Welfare Act of 1990 [64].

Sample collection

DNA from 133 *T. cruzi* isolates collected over 7 years (2008 to 2015) were analyzed to determine phylogenetic relationships (Fig 2; Fig 3; Fig S1). The majority of samples were isolated from *T. infestans* bugs collected from houses throughout Arequipa (N=114). Three of these samples were obtained using xenodiagnosis as described in Chiari & Galvão (1997). An additional ten samples from Arequipa were isolated from the blood of guinea pigs (N=7), dogs (N=2), and a human (N=1). Six isolates were derived from *Panstrongylus lignarius* (N=5) – also known as *P. herreri* [65] - and one guinea pig (N=1) collected in the small towns of La Esperanza, Campo Florido, and Naranjal in northern Peru [33]. Cultures of three previously established strains isolated from humans in Bolivia (Bol-SH001 and Bol-DH29) and São Paulo,

Brazil (TC-y) were provided by the Infectious Diseases Research Laboratory at Universidad Peruana Cayetano Heredia (Fig 3).

T. cruzi was isolated from vertebrates (N=8) using an adaptation of an artificial feeding system that was originally described in Harington (1960) [66]. Briefly, each blood sample was collected with citrate-phosphate-dextrose, transferred into a small plastic jar, and covered with a latex membrane fitted tightly with a rubber band. The jars were placed into an incubator and gradually heated to 35° C. Once the temperature was reached, the jars were inverted to allow uninfected *T. infestans* to feed through the membrane for 15 minutes. *T. cruzi* from the eight laboratory-infected *T. infestans*, all 114 naturally-infected *T. infestans*, and five naturally-infected *P. lignarius* were passaged through guinea pigs or mice in order to avoid isolating other microbes present in the vector, as described in Castillo-Neyra *et al.* (2016) [67]. Feces from infected vectors were injected into guinea pigs or mice and *T. cruzi* was isolated from the blood of each experimentally-infected mammal. *T. cruzi* were directly isolated in LIT culture media from the blood samples of three naturally-infected guinea pigs collected in Arequipa without passage through *T. infestans*.

Reference sequences of three *T. cruzi* isolates obtained from NCBI database were used in subsequent analyses: Silvio, isolated from a human in Para State, Brazil; Esmeraldo, isolated from a human in Bahia State, Brazil; and CL Brener, isolated from a human in Rio Grande, Brazil [68] (Fig 3).

Sequencing

DNA from all laboratory cultures was extracted using Qiagen DNEasy DNA Purification Kit. 150bp single-end read libraries were prepared using TruSeq Nano kit and sequenced to an average depth of >50X using Illumina's NextSeq500. Six *T. cruzi* isolates were prepared in

duplicate, and one in triplicate, to allow estimation of sequencing error. Low quality bases were trimmed from raw reads using trimmomatic-0.32 [69].

Sequence assembly

Bowtie2 [70] was used to assemble maxicircle sequences to the most closely related reference sequence, Silvio (gi|225217165|gb|FJ203996.1), obtained from NCBI [68]. Duplicate reads were removed from the assembly using Picard's MarkDuplicates functionality [71]. The assembly had an average depth of >600X across all maxicircles. Maxicircle consensus sequences were determined using VarScan [72], ensuring highly-confident base calls by requiring a 60% match to call each SNP.

Maxicircle alignment

All assembled maxicircle sequences and the reference were aligned to the Silvio partial maxicircle sequence (gi|225217165|gb|FJ203996.1), Esmeraldo strain complete maxicircle (gi|85718082|gb|DQ343646.1), and the CL Brener complete maxicircle (gi|85718081|gb|DQ343645.1) downloaded from the NCBI database. The sequences were aligned using MUSCLE as implemented in MEGA7 [73]. The ends were trimmed so that all sequences started and ended on the same nucleotide, resulting in a final alignment of 15357bp.

Phylogenetic analyses

Phylogenetic analyses of the 15357bp maxicircle sequence from all samples and reference strains were performed using BEAST 1.8.4 [74]. Phylogenetic analyses assumed a model of sequence evolution in which the rates of A→T, C→G, and G→T are equal (123343) with γ -distributed rate heterogeneity. An Extended Bayesian Skyline tree prior [75] with constant evolutionary rates across lineages (strict clock) was chosen based on BEAST Model Test implemented in BEAST2 [76]. Starting with a UPGMA tree and running one Markov chain

Monte-Carlo chain for each of five independent runs of 20 million iterations sampling every 2000 iterations ensured sufficient mixing after a 10% burn-in (ESS values >200 in Tracer v1.6.0) [77]. Tree files were combined using LogCombiner1.8.4, excluding a 10% burn-in for each. A Maximum Clade Credibility tree was generated from the combined tree file using TreeAnnotator 1.8.4 and FigTree v1.4.2 was used to visualize tree files (available at <http://beast.bio.ed.ac.uk>). Phylogenetic analyses were performed using the BEAGLE library to increase computation speed [78,79].

Statistical Analyses

Metrics of population genetic variation, π and θ , were calculated using MEGA7 [73]. Assuming the sample from 123 infected *T. infestans* is representative of the *T. cruzi* population in vectors in Arequipa, the probability that a distinct lineage could be co-circulating but not detected by chance can be calculated using a binomial distribution. The minor lineage must constitute less than 2.41% of the total population in order for there to be a statistically significant chance that a distinct lineage was not detected in any of 123 sampled *T. cruzi*.

Figures and Tables

Figure 1. The extant *T. cruzi* population in Arequipa arose from a single, recent introduction. Maximum clade credibility (MCC) tree shows that (A) all 123 *T. cruzi* isolated from bugs and domestic mammals in Arequipa form a monophyletic clade with a single, recent common ancestor, indicative of a single immigration event in the recent past. Despite substantial genetic diversity among *T. cruzi* throughout South America, those collected in Arequipa show little diversity. (B) Three samples collected in Campo Florido and Naranjal, Peru and one sample from La Esperanza, Peru have nearly identical maxicircle sequences and form a monophyletic clade. La Esperanza, Peru contains at least two distinct *T. cruzi* lineages, suggesting multiple independent introductions. (C) The only *T. cruzi* sample isolated from a human in Arequipa is distinct from all other samples from Arequipa, suggesting that this introduction has not established in the city. All tips represent a single sample except (A) (N=123) and (B) (N=4). All nodes have strong support (posterior probability ≥ 0.99). Nodes are collapsed when the samples contained have nearly identical maxicircle sequences.

Figure 2. Spatial distribution of samples collected in (A) Arequipa, Peru and (B) South America. (A) The names of ten districts from which *T. cruzi* samples were collected are labeled. Houses from which isolates were collected are represented by red dots. Lines represent major roadways. Densely populated areas appear grey due to the density of roads. Map of Arequipa with sample locations was generated using QGIS v. 2.1 [80]. (B) The sites where isolates were collected are represented by blue dots. Neighboring towns of La Esperanza, Campo Florido, and Naranjal are represented by a single blue dot labeled “Northern Peru”. Sequences obtained from

NCBI database are represented by red dots. Map of South America was modified from https://commons.wikimedia.org/wiki/Atlas_of_the_world.

Figure 3. Number of samples collected from each host species per location. Most samples (N=124) were collected in Arequipa and 6 from small towns in northern Peru. 3 isolates were provided by the Infectious Diseases Research Laboratory at Universidad Peruana Cayetano Heredia. Maxicircle sequences for 3 *T. cruzi* lineages were downloaded from the NCBI database.

Figure S1. Sample collection locations and sources.

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374 Literature Cited

- 375 1. McKinney ML. Urbanization, biodiversity, and conservation. *BioScience*. 2002;52: 883–
376 890.
- 377 2. Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, et al. Global
378 consequences of land use. *Science*. American Association for the Advancement of
379 Science; 2005;309: 570–574.
- 380 3. Seto KC, Güneralp B, Hutyrá LR. Global forecasts of urban expansion to 2030 and direct
381 impacts on biodiversity and carbon pools. *Proceedings of the National Academy of*
382 *Sciences*. National Acad Sciences; 2012;109: 16083–16088.
- 383 4. Theoharides KA, Dukes JS. Plant invasion across space and time: factors affecting
384 nonindigenous species success during four stages of invasion. *New Phytol*. 2007;176:
385 256–273.
- 386 5. Kowarik I. On the role of alien species in urban flora and vegetation. *Plant Invasions-*
387 *General Aspects and Special Problems*. 1995; 85–103.
- 388 6. Hahs AK, McDonnell MJ, McCarthy MA, Vesik PA, Corlett RT, Norton BA, et al. A
389 global synthesis of plant extinction rates in urban areas. *Ecol Lett*. Wiley Online Library;
390 2009;12: 1165–1173.
- 391 7. Aronson MF, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, et al. A global
392 analysis of the impacts of urbanization on bird and plant diversity reveals key
393 anthropogenic drivers. *Proc R Soc B*. The Royal Society; 2014;281: 20133330.
- 394 8. Denys C, Schmidt H. Insect communities on experimental mugwort (*Artemisia vulgaris*
395 *L.*) plots along an urban gradient. *Oecologia*. 1998;113: 269–277.
- 396 9. McIntyre NE. Ecology of urban arthropods: A review and a call to action. *an*. 2000;93:
397 825–835.
- 398 10. Deguines N, Julliard R, De Flores M, Fontaine C. Functional homogenization of flower
399 visitor communities with urbanization. *Ecology and evolution*. Wiley Online Library;
400 2016;6: 1967–1976.
- 401 11. New TR. Promoting and developing insect conservation in Australia's urban
402 environments. *Austral Entomology*. Wiley Online Library; 2018.
- 403 12. Mackin-Rogalska R, Pinowski J, Solon J, Wojcik Z. Changes in vegetation, avifauna, and
404 small mammals in a suburban habitat. *Polish Ecological Studies*. 1988;14: 293–330.
- 405 13. Pekin BK, Pijanowski BC. Global land use intensity and the endangerment status of
406 mammal species. *Diversity and Distributions*. Wiley Online Library; 2012;18: 909–918.

- 407 14. Blair RB. Birds and butterflies along urban gradients in two ecoregions of the United
408 States: Is urbanization creating a homogeneous fauna? *Biotic Homogenization*. 2001;: 33–
409 56.
- 410 15. Strohbach MW, Haase D, Kabisch N. Birds and the city: urban biodiversity, land use, and
411 socioeconomics. *Ecology and Society*. JSTOR; 2009;14.
- 412 16. Alirol E, Getaz L, Stoll B, Chappuis F, Loutan L. Urbanisation and infectious diseases in a
413 globalised world. *The Lancet Infectious Diseases*. Elsevier; 2011;11: 131–141.
- 414 17. Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE. The spread of invasive
415 species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and*
416 *the Environment*. Wiley-Blackwell; 2008;6: 238–246.
- 417 18. Vitousek PM, D Antonio CM, Loope LL, Westbrooks R. Biological invasions as global
418 environmental change. *American Scientist*. Research Triangle Park; 1996;84: 468–478.
- 419 19. Seto KC, Fragkias M, Güneralp B, Reilly MK. A meta-analysis of global urban land
420 expansion. *PLoS ONE*. Public Library of Science; 2011;6: e23777.
- 421 20. Beninde J, Veith M, Hochkirch A. Biodiversity in cities needs space: a meta-analysis of
422 factors determining intra-urban biodiversity variation. *Ecol Lett*. Wiley/Blackwell
423 (10.1111); 2015;18: 581–592.
- 424 21. Deplazes P, Hegglin D, Gloor S, Romig T. Wilderness in the city: the urbanization of
425 *Echinococcus multilocularis*. *Trends in Parasitology*. 2004;20: 77–84.
- 426 22. Bradley CA, Altizer S. Urbanization and the ecology of wildlife diseases. *Trends in*
427 *Ecology & Evolution*. Elsevier; 2007;22: 95–102.
- 428 23. Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, et al.
429 Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *The*
430 *American Journal of Tropical Medicine and Hygiene*. 2005;72: 209–220.
- 431 24. Adalja AA, Sell TK, Bouri N, Franco C. Lessons learned during dengue outbreaks in the
432 United States, 2001–2011. *Emerg Infect Dis*. 2012;18: 1–7.
- 433 25. Levy MZ, Barbu CM, Castillo Neyra R, Quispe-Machaca VR, Ancca Juárez J, Escalante-
434 Mejia P, et al. Urbanization, land tenure security and vector-borne Chagas disease. *Proc*
435 *Biol Sci*. The Royal Society; 2014;281: 20141003–20141003.
- 436 26. Levy MZ, Bowman NM, Kawai V, Waller LA, Cornejo del Carpio JG, Benzaquen EC, et
437 al. Periurban *Trypanosoma cruzi*-infected *Triatoma infestans*, Arequipa, Peru. *Emerg*
438 *Infect Dis*. Centers for Disease Control and Prevention; 2006;12: 1345–1352.
- 439 27. Levy MZ, Kawai V, Bowman NM, Waller LA, Cabrera L, Pinedo-Cancino VV, et al.
440 Targeted screening strategies to detect *Trypanosoma cruzi* infection in children. *PLOS*
441 *Neglected Tropical Diseases*. Public Library of Science; 2007;1: e103.

28. Levy MZ, Small DS, Vilhena DA, Bowman NM, Kawai V, Cornejo del Carpio JG, et al. Retracing micro-epidemics of Chagas disease using epicenter regression. PLoS Comput Biol. Public Library of Science; 2011;7: e1002146.
29. Bowman NM, Kawai V, Levy MZ, Cornejo del Carpio JG, Cabrera L, Delgado F, et al. Chagas disease transmission in periurban communities of Arequipa, Peru. CLIN INFECT DIS. 2008;46: 1822–1828.
30. Hunter GC, Borrini-Mayorí K, Ancca Juárez J, Castillo Neyra R, Verastegui MR, Malaga Chavez FS, et al. A field trial of alternative targeted screening strategies for Chagas disease in Arequipa, Peru. PLOS Neglected Tropical Diseases. Public Library of Science; 2012;6: e1468.
31. Foley EA, Khatchikian CE, Hwang J, Ancca Juárez J, Borrini-Mayorí K, Quispe-Machaca VR, et al. Population structure of the Chagas disease vector, *Triatoma infestans*, at the urban-rural interface. Molecular Ecology. 2013;22: 5162–5171.
32. Gaunt MW, Yeo M, Frame IA, Stothard JR, Carrasco HJ, Taylor MC, et al. Mechanism of genetic exchange in American trypanosomes. Nature. Macmillian Magazines Ltd. SN ; 2003;421: 936–939.
33. Alroy KA, Huang C, Gilman RH, Quispe-Machaca VR, Marks MA, Ancca Juárez J, et al. Prevalence and transmission of *Trypanosoma cruzi* in people of rural communities of the high jungle of Northern Peru. PLOS Neglected Tropical Diseases. Public Library of Science; 2015;9: e0003779.
34. Pedersen D, Tremblay J, Errázuriz C, Gamarra J. The sequelae of political violence: assessing trauma, suffering and dislocation in the Peruvian highlands. Social science & medicine. Elsevier; 2008;67: 205–217.
35. Bayer AM, Hunter GC, Gilman RH, Cornejo del Carpio JG, Naquira C, Bern C, et al. Chagas disease, migration and community settlement patterns in Arequipa, Peru. PLOS Neglected Tropical Diseases. Public Library of Science; 2009;3: e567.
36. Dujardin J-P, Muñoz M, Chavez T, Ponce C, Moreno J, Schofield CJ. The origin of *Rhodnius prolixus* in Central America. Medical and Veterinary Entomology. 1998;12: 113.
37. de Arias AR, Schofield CJ. *Triatoma infestans*: pasado, presente y futuro del principal vector de la Enfermedad de Chagas en el Cono Sur. Reunión Intergubernamental INCOSURChagas. XIIa. Santiago, Chile; 2003.
38. Thomas CD. Translocation of species, climate change, and the end of trying to recreate past ecological communities. Trends in Ecology & Evolution. Elsevier Ltd; 2011;26: 216–221.

- 477 39. Pitelka LF, Group PMW. Plant migration and climate change: a more realistic portrait of
478 plant migration is essential to predicting biological responses to global warming in a
479 world drastically altered by human activity. *American Scientist*. JSTOR; 1997;: 464–473.
- 480 40. Lockwood JL, Cassey P, Blackburn T. The role of propagule pressure in explaining
481 species invasions. *Trends in Ecology & Evolution*. 2005;20: 223–228.
- 482 41. Cohen JE, Cecere MC, Lauricella MA, Chuit R, Segura EL. Influence of humans and
483 domestic animals on the household prevalence of *Trypanosoma cruzi* in *Triatoma*
484 *infestans* populations in northwest Argentina. *The American Journal of Tropical Medicine*
485 *and Hygiene*. ASTMH; 1998;58: 748–758.
- 486 42. Cohen JE, Gürtler RE. Modeling household transmission of American trypanosomiasis.
487 *Science*. American Association for the Advancement of Science; 2001;293: 694–698.
- 488 43. Gürtler RE, Cecere MC, Lauricella MA, Cardinal MV, Kitron U, Cohen JE. Domestic
489 dogs and cats as sources of *Trypanosoma cruzi* infection in rural northwestern Argentina.
490 *Parasitology*. Cambridge University Press; 2007;134: 69–82.
- 491 44. Llewellyn MS, Miles MA, Carrasco HJ, Lewis MD, Yeo M, Vargas J, et al. Genome-scale
492 multilocus microsatellite typing of *Trypanosoma cruzi* discrete typing unit I reveals
493 phylogeographic structure and specific genotypes linked to human infection. *PLOS*
494 *Pathogens*. Public Library of Science; 2009;5: e1000410.
- 495 45. Rabinovich JE, Wisnivesky-Colli C, Solarz ND. Probability of transmission of Chagas
496 disease by *Triatoma infestans* (Hemiptera: Reduviidae) in an endemic area of Santiago del
497 Estero, Argentina. *Bulletin of the World Health Organization*. World Health Organization;
498 1990;68: 737.
- 499 46. Nouvellet P, Dumonteil E, Gourbière S. The improbable transmission of *Trypanosoma*
500 *cruzi* to human: the missing link in the dynamics and control of Chagas disease. *PLOS*
501 *Neglected Tropical Diseases*. Public Library of Science; 2013;7: e2505.
- 502 47. Levy MZ, Tustin A, Castillo Neyra R, Mabud TS, Levy K, Barbu CM, et al. Bottlenecks
503 in domestic animal populations can facilitate the emergence of *Trypanosoma cruzi*, the
504 aetiological agent of Chagas disease. *Proc R Soc B. The Royal Society*; 2015;282:
505 20142807.
- 506 48. Billig E. Detecting and controlling insect vectors in urban environments: novel Bayesian
507 methods for complex spatial data. *Publicly Accessible Penn Dissertations*. 2017.
508 Available: <https://repository.upenn.edu/edissertations/2193>
- 509 49. Oliveira RP, Broude NE, Macedo AM, Cantor CR, Smith CL, Pena SDJ. Probing the
510 genetic population structure of *Trypanosoma cruzi* with polymorphic microsatellites.
511 *Proceedings of the National Academy of Sciences of the United States of America*. The
512 *National Academy of Sciences*; 1998;95: 3776–3780.

- 513 50. Higo H, Miura S, Horio M, Mimori T, Hamano S, Agatsuma T, et al. Genotypic variation
514 among lineages of *Trypanosoma cruzi* and its geographic aspects. *Parasitology*
515 *International*. 2004;53: 337–344.
- 516 51. Llewellyn MS, Lewis MD, Acosta N, Yeo M, Carrasco HJ, Segovia M, et al.
517 *Trypanosoma cruzi* IIc: phylogenetic and phylogeographic insights from sequence and
518 microsatellite analysis and potential impact on emergent Chagas disease. *PLOS Neglected*
519 *Tropical Diseases*. Public Library of Science; 2009;3: e510.
- 520 52. Curtis-Robles R, Snowden KF, Dominguez B, Dinges L, Rodgers S, Mays G, et al.
521 Epidemiology and molecular typing of *Trypanosoma cruzi* in naturally-infected hound
522 dogs and associated triatomine vectors in Texas, USA. *PLOS Neglected Tropical*
523 *Diseases*. 2017;11: e0005298–18.
- 524 53. Hodo CL, Wilkerson GK, Birkner EC, Gray SB, Hamer SA. *Trypanosoma cruzi*
525 transmission among captive nonhuman primates, wildlife, and vectors. *EcoHealth*.
526 Springer; 2018;: 1–11.
- 527 54. Perez CJ, Lymbery AJ, Thompson RCA. Chagas disease: the challenge of polyparasitism?
528 *Trends in Parasitology*. Elsevier Ltd; 2014;30: 176–182.
- 529 55. Dumonteil E, Ramirez-Sierra M-J, Pérez-Carrillo S, Teh-Poot C, Herrera C, Gourbière S,
530 et al. Detailed ecological associations of triatomines revealed by metabarcoding and next-
531 generation sequencing: implications for triatomine behavior and *Trypanosoma cruzi*
532 transmission cycles. *Scientific Reports*. Springer US; 2018;8: 1–13.
- 533 56. Kerr B, Riley MA, Feldman MW, Bohannan BJ. Local dispersal promotes biodiversity in
534 a real-life game of rock–paper–scissors. *Nature Publishing Group*. *Nature Publishing*
535 *Group*; 2002;418: 171.
- 536 57. Revollo S, Oury B, Laurent J-P, Barnabé C, Quesney V, Carrière V, et al. *Trypanosoma*
537 *cruzi*: impact of clonal evolution of the parasite on its biological and medical properties.
538 *Experimental Parasitology*. Elsevier; 1998;89: 30–39.
- 539 58. Veloso VM, Carneiro CM, Toledo M, Lana M, Chiari E, Tafuri WL, et al. Variation in
540 susceptibility to benznidazole in isolates derived from *Trypanosoma cruzi* parental strains.
541 *Memorias do Instituto Oswaldo Cruz*. SciELO Brasil; 2001;96: 1005–1011.
- 542 59. Longhi SA, Brandariz SB, Lafon SO, Niborski LL, Luquetti AO, Schijman AG, et al.
543 Evaluation of in-house ELISA using *Trypanosoma cruzi* lysate and recombinant antigens
544 for diagnosis of Chagas disease and discrimination of its clinical forms. *The American*
545 *Journal of Tropical Medicine and Hygiene*. ASTMH; 2012;87: 267–271.
- 546 60. Abras A, Gállego M, Llovet T, Tebar S, Herrero M, Berenguer P, et al. Serological
547 diagnosis of chronic Chagas disease: is it time for a change? *J Clin Microbiol*. *American*
548 *Society for Microbiology*; 2016;54: 1566–1572.

- 549 61. Verani JR, Seitz A, Gilman RH, LaFuente C, Galdos-Cardenas G, Kawai V, et al.
550 Geographic variation in the sensitivity of recombinant antigen-based rapid tests for
551 chronic *Trypanosoma cruzi* infection. The American Journal of Tropical Medicine and
552 Hygiene. 2009;80: 410–415.
- 553 62. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United
554 States. CLIN INFECT DIS. The University of Chicago Press; 2009;49: e52–e54.
- 555 63. Leiby DA, Nguyen ML, Proctor MC, Townsend RL, Stramer SL. Frequency of
556 *Trypanosoma cruzi* parasitemia among infected blood donors with a potential association
557 between parasite lineage and transfusion transmission. Transfusion. Wiley Online Library;
558 2017;57: 1426–1432.
- 559 64. Castillo Neyra R, Barbu CM, Salazar R, Borrini K, Naquira C, Levy MZ. Host-seeking
560 behavior and dispersal of *Triatoma infestans*, a vector of Chagas disease, under semi-field
561 conditions. PLOS Neglected Tropical Diseases. Public Library of Science; 2015;9: e3433.
- 562 65. Cáceres AG, Troyes L, Gonzáles-Pérez A, Llontop E, Bonilla C, Heredia N, et al.
563 Enfermedad de Chagas en la región nororiental del Perú. I. Triatominos (Hemiptera,
564 Reduviidae) presentes en Cajamarca y Amazonas. Revista Peruana de Medicina
565 Experimental y Salud Publica. Instituto Nacional de Salud; 2002;19: 17–23.
- 566 66. Harington JS. A simple apparatus for the artificial feeding of *Rhodnius prolixus*
567 (Hemiptera, Reduviidae). Parasitology. Cambridge University Press; 1960;50: 273–277.
- 568 67. Castillo Neyra R, Borrini-Mayori K, Salazar Sánchez R, Ancca Suarez J, Xie S, Náquira
569 Velarde C, et al. Heterogeneous infectiousness in guinea pigs experimentally infected with
570 *Trypanosoma cruzi*. Parasitology International. 2016;65: 50–54.
- 571 68. Geer LY, Marchler-Bauer A, Geer RC, Han L, He J, He S, et al. The NCBI BioSystems
572 database. Nucleic Acids Research. Oxford University Press; 2010;38: D492–D496.
- 573 69. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
574 data. Bioinformatics. Oxford University Press; 2014;30: 2114–2120.
- 575 70. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods.
576 2012;9: 357–359.
- 577 71. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The
578 Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA
579 sequencing data. Genome Research. 2010;20: 1297–1303.
- 580 72. Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER, et al. VarScan:
581 variant detection in massively parallel sequencing of individual and pooled samples.
582 Bioinformatics. Oxford University Press; 2009;25: 2283–2285.
- 583 73. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis
584 Version 7.0 for Bigger Datasets. Molecular Biology and Evolution. 2016;33: 1870–1874.

- 585 74. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti
586 and the BEAST 1.7. *Molecular Biology and Evolution*. Oxford University Press; 2012;29:
587 1969–1973.
- 588 75. Heled J, Drummond AJ. Bayesian inference of population size history from multiple loci.
589 *BMC Evol Biol*. 2008;8: 289–15.
- 590 76. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, et al. BEAST 2: A
591 software platform for Bayesian evolutionary analysis. *PLoS Comput Biol*. Public Library
592 of Science; 2014;10: e1003537.
- 593 77. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA, Susko E. Posterior
594 summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*. 2018;22
- 595 78. Suchard MA, Rambaut A. Many-core algorithms for statistical phylogenetics.
596 *Bioinformatics*. Oxford University Press; 2009;25: 1370–1376.
- 597 79. Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, et al. BEAGLE: An
598 application programming interface and high-performance computing library for statistical
599 phylogenetics. *Systematic Biology*. Oxford University Press; 2012;61: 170–173.
- 600 80. QGIS Development Team. QGIS Geographic Information System [Internet]. 2nd ed.
601 Open Source Geospatial Foundation Project. Available: <http://qgis.osgeo.org>
602

Figure 1. The extant *T. cruzi* population in Arequipa arose from a single, recent introduction. Maximum clade credibility (MCC) tree shows that **(A)** all 123 *T. cruzi* isolated from bugs and domestic mammals in Arequipa form a monophyletic clade with a single, recent common ancestor, indicative of a single immigration event in the recent past. Despite substantial genetic diversity among *T. cruzi* throughout South America, those collected in Arequipa show little diversity. **(B)** Three samples collected in Campo Florido and Naranjal, Peru and one sample from La Esperanza, Peru have nearly identical maxicircle sequences and form a monophyletic clade. La Esperanza, Peru contains at least two distinct *T. cruzi* lineages, suggesting multiple independent introductions. **(C)** The only *T. cruzi* sample isolated from a human in Arequipa is distinct from all other samples from Arequipa, suggesting that this introduction has not established in the city. All tips represent a single sample except (A) (N=123) and (B) (N=4). All

Figure 2. Spatial distribution of samples collected in (A) Arequipa, Peru and (B) South America. (A) The names of ten districts from which *T. cruzi* samples were collected are labeled. Houses from which isolates were collected are represented by red dots. Lines represent major roadways. Densely populated areas appear grey due to the density of roads. Map of Arequipa with sample locations was generated using QGIS v. 2.1 [80]. **(B)** The sites where isolates were collected are represented by blue dots. Neighboring towns of La Esperanza, Campo Florido, and Naranjal are represented by a single blue dot labeled “Northern Peru”. Sequences obtained from NCBI database are represented by red dots. Map of South America was modified from https://commons.wikimedia.org/wiki/Atlas_of_the_world.

Figure 3. Number of samples collected from each host species per location. Most samples (N=124) were collected in Arequipa and 6 from small towns in northern Peru. 3 isolates were provided by the Infectious Diseases Research Laboratory at Universidad Peruana Cayetano Heredia. Maxicircle sequences for 3 *T. cruzi* lineages were downloaded from the NCBI database.

Table S1. Sample collection source and location.

Sample ID	Region	District	Collection Year	Source
TC001	Arequipa	La Joya	2008	<i>T. infestans</i>
TC002	Arequipa	La Joya	2008	<i>T. infestans</i>
TC003	Arequipa	La Joya	2008	<i>T. infestans</i>
TC004	Arequipa	La Joya	2008	<i>T. infestans</i>
TC010	Arequipa	Mariano Melgar	2008	<i>T. infestans</i>
TC014	Arequipa	La Joya	2008	<i>T. infestans</i>
TC015	Arequipa	La Joya	2008	<i>T. infestans</i>
TC016	Arequipa	La Joya	2008	<i>T. infestans</i>
TC019	Arequipa	La Joya	2008	<i>T. infestans</i>
TC020	Arequipa	La Joya	2008	<i>T. infestans</i>
TC022	Arequipa	La Joya	2008	<i>T. infestans</i>
TC023	Arequipa	La Joya	2008	Guinea pig
TC026	Arequipa	La Joya	2008	<i>T. infestans</i>
TC027	Arequipa	La Joya	2008	<i>T. infestans</i>
TC029	Arequipa	La Joya	2008	<i>T. infestans</i>
TC031	Arequipa	La Joya	2008	<i>T. infestans</i>
TC033	Arequipa	La Joya	2008	<i>T. infestans</i>
TC034	Arequipa	La Joya	2008	<i>T. infestans</i>
TC035	Arequipa	La Joya	2008	<i>T. infestans</i>
TC036	Arequipa	La Joya	2008	<i>T. infestans</i>
TC037	Arequipa	La Joya	2008	<i>T. infestans</i>
TC038	Arequipa	La Joya	2008	<i>T. infestans</i>
TC039	Arequipa	La Joya	2008	<i>T. infestans</i>
TC040	Arequipa	La Joya	2008	<i>T. infestans</i>
TC041	Arequipa	Mariano Melgar	2010	Dog
TC042	Arequipa	Mariano Melgar	2010	Guinea pig
TC043	Arequipa	Mariano Melgar	2010	Guinea pig
TC044	Arequipa	Mariano Melgar	2010	Dog
TC045	Arequipa	Mariano Melgar	2010	Guinea pig
TC046	Arequipa	Mariano Melgar	2010	<i>T. infestans</i>
TC047	Arequipa	Mariano Melgar	2010	<i>T. infestans</i>
TC048	Arequipa	Mariano Melgar	2010	<i>T. infestans</i>
TC049	Arequipa	Mariano Melgar	2010	<i>T. infestans</i>
TC050	Arequipa	J.L.B. y Rivero	2009	Human
TC051	Arequipa	Miraflores	2010	<i>T. infestans</i>
TC052	Cajamarca	La Esperanza	2010	<i>P. herreri</i>

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