

## First Finding of Melanic Sylvatic *Triatoma infestans* (Hemiptera: Reduviidae) Colonies in the Argentine Chaco

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**ABSTRACT** *Triatoma infestans* (Klug), the most important vector of Chagas disease in southern South America, is a highly domiciliated species with well-known sylvatic foci only in the Bolivian Andean valleys and in the Bolivian Chaco, where melanic insects designated as “dark morphs” were found. After the tentative identification of two melanic bugs collected from parrot nests in a forest reserve in the Argentine Chaco as *T. infestans*, we conducted an intensive search there using mouse-baited sticky traps in summer 2006 and 2007. Four live *T. infestans* bugs were collected in trees without parrot nests in 288 trap-nights, whereas no bug was collected from inside trees with active parrot nests in 51 trap-nights. To increase bug captures, hollow tree trunks that recently had had *Amazona aestiva* (Berlepsch) and *Aratinga acuticaudata* (Vieillot) parrot nests were treated with insecticide fumigant canisters exhibiting strong knockdown power. Four (22%) of 18 trees were positive for *T. infestans* with a dark phenotype. A fragment of the mitochondrial gene *COI* of 8 of the 14 triatomine bugs collected was successfully sequenced and confirmed as *T. infestans*. Most of the bugs were captured from *Aspidosperma quebracho-blanco* (Schlechter) hollow tree trunks harboring parrot nests. All of the *T. infestans* collected from the nearest house located at 10 km from the sylvatic foci displayed normal chromatic characters. The repeated finding of *T. infestans* in sylvatic habitats, albeit at very low density, shows that this species is capable of maintaining viable sylvatic foci in the absence of human hosts and immigration from domestic populations. These are the first confirmed findings of sylvatic *T. infestans* colonies in Argentina and of dark morphs in the Argentine Chaco.

**KEY WORDS** *Triatoma infestans*, parrots, Chagas disease, vector control, reinfestation

*Triatoma infestans* (Klug), the main domestic vector of *Trypanosoma cruzi* (Chagas) in the southern cone of South America, is the target of a regional elimination program launched in 1991. This program successfully interrupted vector-mediated transmission to humans by *T. infestans* in Chile, Uruguay, and Brazil and in sections of Argentina and Paraguay (Silveira 2002, Schofield et al. 2006). However, only limited success has been achieved in the Gran Chaco (a 1.3 million-km<sup>2</sup> ecoregion crossing over Argentina, Bolivia, and Paraguay) even in areas under intensive, professional vector control efforts because of recurrent reinfestations (Gürtler et al. 2007). Possible underlying causes include the uneven and discontinuous coverage of control actions; the frequent occurrence of infested peridomestic structures housing domestic animals; the

limited effectiveness of pyrethroid insecticides in peridomestic structures; and the emergence of resistance to pyrethroid insecticides in northwestern Argentina and Bolivia (Gürtler et al. 2004, Picollo et al. 2005, Cecere et al. 2006, Toloza et al. 2008).

*Triatoma infestans* is a highly domiciliated species, and for a long time, sylvatic foci were thought to be present only in the Andean Valleys of Bolivia (Schofield 1988, Dujardin et al. 1996, Noireau et al. 1997). The occasional findings of *T. infestans* in sylvatic habitats in Argentina, Paraguay, and Brazil up to the early 1980s were considered rare and of little relevance because the ecotopes were relatively close to human dwellings and the vector species may have been misidentified (Abalos and Wygodzinsky 1951, Usinger et al. 1966). The highly domiciliated status of *T. infestans* implied that (peri)domestic reinfestation after residual insecticide spraying originating from sylvatic foci could be ruled out. By the mid-1990s, however, wild populations of *T. infestans* were discovered in terrestrial and epiphytic bromeliads, parrot nests, and hollow trees in the Bolivian Chaco (Noireau et al. 1997, 2000b). These bugs were much darker and had thinner yellowish spots on the connexivum (i.e., “dark morphs”) than the typical *T. infestans*. They occurred in isolated dry forest very far from human settlements and occasionally flew to the lights of local

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hunters. Another melanic form found previously in peridomestic trees in the humid forest of Misiones (northeastern Argentina, not part of the Gran Chaco) was first considered a subspecies of *T. infestans* and later raised to species rank as *Triatoma melanosoma* (Martínez, Olmedo & Carcavallo) (Martínez et al. 1987, Lent et al. 1994). Several methods confirmed that dark morphs and *T. melanosoma* were chromatic variants of *T. infestans* differing in color, head and wing morphometry, antennal sensilla patterns, chromosome banding, random amplified polymorphic DNA, and ribosomal internal transcribed spacer regions (Monteiro et al. 1999, Noireau et al. 1997, 2000a, Gumiel et al. 2003, Bargues et al. 2006). Outside of Bolivia, *T. infestans* was collected in sylvatic habitats in Argentina (see Discussion), Brazil (Barretto et al. 1963), and Chile (Bacigalupo et al. 2006). Nymphs of *T. infestans* were recently found in the interphase between peridomestic and sylvatic habitats in the Paraguayan Chaco (Yeo et al. 2005). These reports indicate that populations of *T. infestans* in sylvatic habitats may be much more widespread than previously assumed and point to the need for further research on their role in the process of recolonization of insecticide-treated villages (Noireau et al. 2005, Bacigalupo et al. 2006).

During the course of field research on the reproductive biology of the parrot *Amazona aestiva* (Berlepsch) ("loro hablador") in a Forest Provincial Park in northern Argentina (Berkunsky and Reboreda 2008), researchers frequently collected triatomine bugs from nests located in hollow tree trunks between 2003 and 2006. One of these triatomines brought to our attention was preliminarily identified as a dark morph *T. infestans* male based on morphological characters. This fortuitous finding prompted this study on the occurrence of sylvatic populations of *T. infestans* in the Argentine Chaco, where it had not been found before.

### Materials and Methods

**Study Site.** Field work was conducted 40 km south of Fuerte Esperanza (25°30' S, 61°50' W), General Güemes Department, Chaco Province, Argentina. This area is located in the Dry Chaco Ecoregion and belongs to "El Impenetrable," an extended and old hardwood forest of "quebracho blanco" (*Aspidosperma quebracho-blanco* [Schlechter]) and "quebracho colorado" (*Schinopsis lorentzii* Griseb). A dense thorn scrub also includes smaller trees such as "algarrobo" (*Prosopis* sp.), "guayacán" (*Caesalpinia paraguayensis* Parodi), and "mistol" (*Ziziphus mistol* Griseb).

Temperatures are high during summer (maximum 50°C), and there is a pronounced deficit of rainfall (mean annual rainfall, 500–600 mm), which usually occurs as heavy, sporadic showers during summer. The study area is surrounded by protected forest lands belonging to the provincial and federal governments and is under a high conservation status. It preserves most of the original fauna, including anteaters (*Myrmecophaga tridactyla* L.), "guazunchos" (*Mazama gouazoubira* Fischer), "pumas" (*Felis con-*

*color* L.), peccaries (*Catagonus*, *Tayassu*, *Pecari*), the extremely rare "tatú carreta" (*Priontontes maximus* Kerr), and "yaguaretés" (*Panthera onca* L.). The area is difficult to access, lacks water, and only has six houses within a 30-km radius from the center of our sampling location.

**Vector Collection.** Searches for triatomine bugs were conducted with mouse-baited sticky (Noireau) traps placed in tree holes (Noireau et al. 1999) in February and December 2006 and January 2007. The sampled area approximately covered 135 km<sup>2</sup>. Most of the traps were placed in nests of the blue-fronted parrot *A. aestiva* and of the blue-crowned conure ("calancate," *Aratinga acuticaudata* [Vieillot]) located within tree trunks that were ≈2 km from each other. These nests had been located and regularly inspected for parrot presence during their reproductive season since 2005 (Berkunsky and Reboreda 2008). Nests and chicks were accessed by a small window carved out of the tree at the nest's height (Fig. 1A); the occurrence of other vertebrates in the nest was recorded occasionally. Because collections of triatomine bugs with the baited traps in parrot nests were scarce or null in December 2006 and January 2007, another collection method that took advantage of the knockdown and flushing-out effects of pyrethroid insecticides (Gürtler et al. 1993) was attempted. After recovering Noireau traps from parrot nests where fledglings had left or where the clutch had failed, the bottom of each nest was covered with a plastic sheet, and the hollow tree was treated with an insecticide fumigant canister (Chemofog; Chemotecnica, Carlos Spegazzini, Argentina) containing permethrin and tetramethrin. The plastic sheet was recovered 6–24 h later, and the knocked-down insects were inspected for triatomine bugs. The nearest houses to the forest sampling locations were visited in February 2006 to search for triatomine bugs in peridomestic and domestic sites using 0.2% tetramethrin as a dislodging agent (Icona, Buenos Aires, Argentina). One skilled person conducted the searches for 30 min per house.

All collected bugs were kept in plastic vials with folded filter paper and identified to species following Lent and Wygodzinsky (1979), and the live bugs were examined microscopically for *T. cruzi* infection at ×400. Three nymphs were reared on laboratory mice until they reached the adult stage to record its color and allow a more accurate taxonomic identification.

**Genetic Characterization of *T. infestans*.** DNA from bugs assigned to *T. infestans* was obtained, polymerase chain reaction (PCR) amplified, and sequenced for a 661-bp fragment of the mitochondrial gene cytochrome oxidase I (*COI*) as described in Cortez et al. (2007). Sequences from sylvatic bugs were compared with *Triatoma* spp. sequences available at GenBank (García and Powell 1998, Cortez et al. 2007) and from a previous survey on *T. infestans* intraspecific variability (Piccinali et al. 2009). Phylogenetic analyses were performed with maximum parsimony and Bayesian approaches. The sequence of *Panstrongylus megistus* (Burmeister) (García and Powell 1998; GenBank

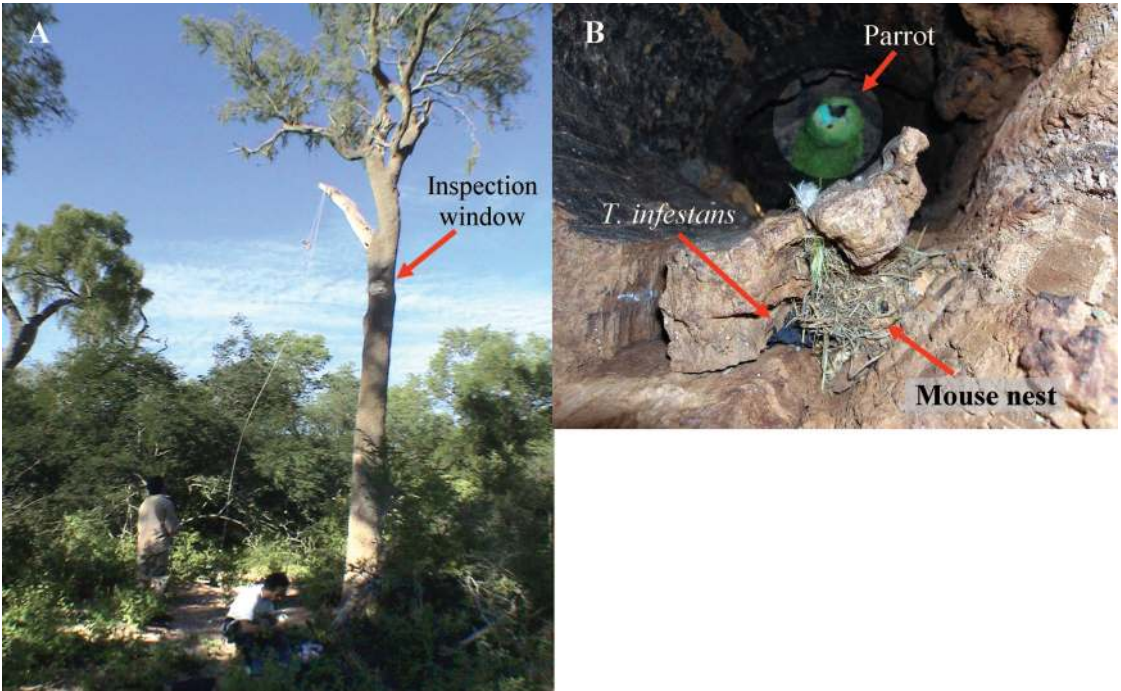


Fig. 1. (A) Nest of *A. aestiva* within a hollow quebracho blanco tree. The inspection window on the tree trunk points to the approximate location of the nest. (B) Nest of *A. aestiva* within a hollow quebracho blanco tree occupied by a dark morph adult of *T. infestans*.

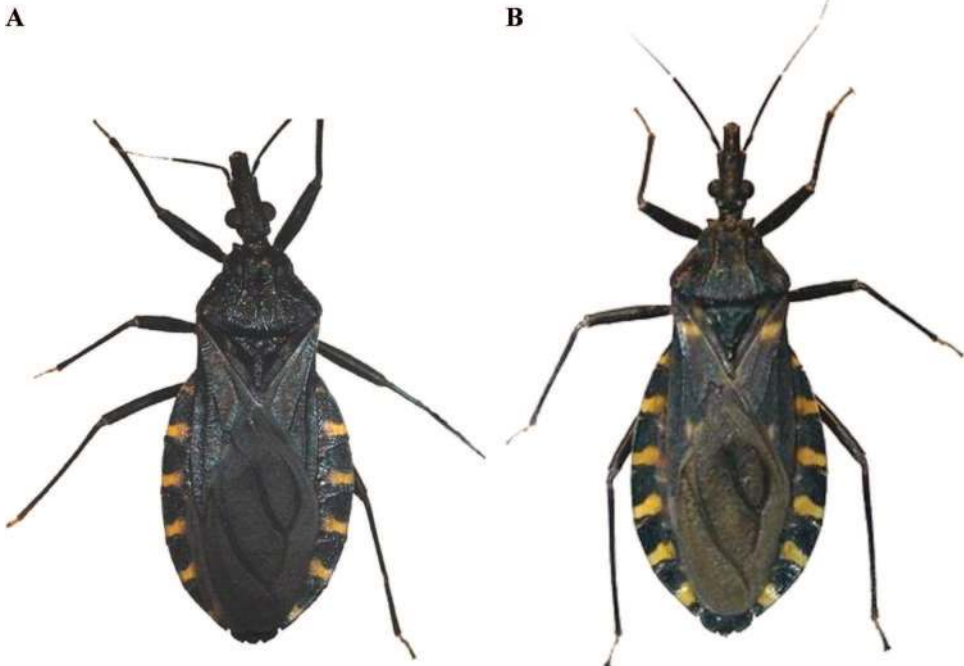


Fig. 2. Chromatic differences between dark morph and normal *T. infestans* captured from sylvatic (A) and peridomestic sites (B) in the Argentine Chaco.

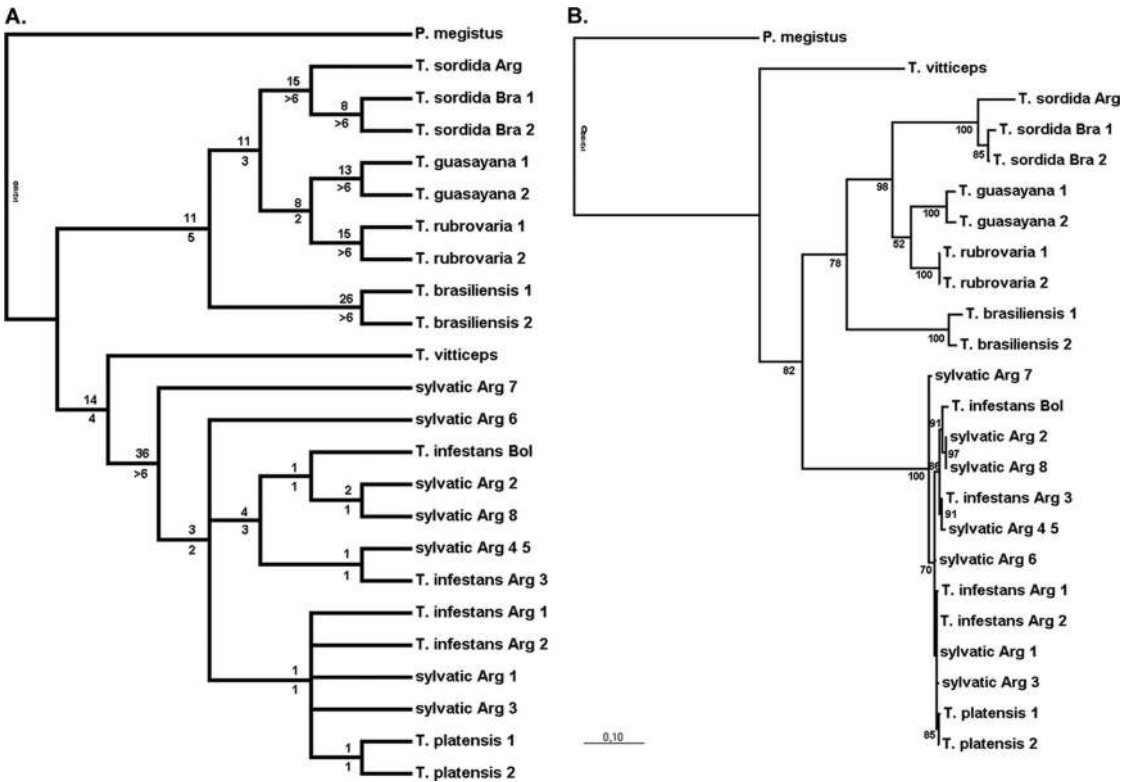


Fig. 3. Phylogenetic relationships between Chaco melanic *T. infestans* and other species of Triatominae. (A) Most parsimonious tree. Length: 462 steps. Numbers above and below branches are unambiguous changes and Bremer support values, respectively. (B) Bayesian 50% majority rule consensus tree. Numbers on branches are clade credibility values. The scale indicates expected substitutions per site. Arg, Argentina; Bol, Bolivia; Bra, Brazil. GenBank accession numbers—*P. megistus*: AF021179, *T. vitticeps* (Stål): AF021219, *T. brasiliensis* (Neiva) 1: AF021184, *T. brasiliensis* 2: AF021186, *T. sordida* Arg: AF021210, *T. sordida* Bra 1: AF021213, *T. sordida* Bra 2: AF021216, *T. rubrovaria* (Blanchard) 1: AF021204, *T. rubrovaria* 2: AF021206, *T. guasayana* 1: AF021193, *T. guasayana* 2: AF021195, *T. platensis* 1: AF021202, *T. platensis* 2: FJ811849, *T. infestans* Bol: EF451010, *T. infestans* Arg 1: EF451012, *T. infestans* Arg 2: EF451013, *T. infestans* Arg 3: EF451011, sylvatic Arg 1–8: FJ811842–FJ811848.

accession number AF021179) was used to root the phylogenetic trees. The two *T. platensis* (Neiva) sequences used came from laboratory colonies originally captured at Córdoba and La Rioja, Argentina. The shortest phylogenetic trees were found with the implicit enumeration search option implemented in TNT (Goloboff et al. 2008). All characters were regarded as unordered and unweighted. Statistical support for clades was assessed by Bremer support values (Bremer 1994). Bayesian analyses were performed with MrBayes 3.1 (Huelsenbeck and Ronquist 2001). Clade support was estimated with a Markov chain Monte Carlo (MCMC) algorithm. Parameters were set to  $10^7$  generations, and trees were sampled every 100th generation using the general time reversible model of DNA substitution (nst = "6"; rates = "invgamma") and the random tree option to begin the analysis. Log-likelihood values from four simultaneous MCMC chains (three hot and one cold) stabilized at  $\approx 334,000$  generations, resulting in the first 3,340 trees being discarded (burnin = 3,340). Phylogenetic trees were drawn with MrEnt 2.0 (Zuccon and Zuccon 2008).

## Results

A total of 24 triatomines were captured during the three surveys, including 14 *T. infestans* (three fourth instars, four fifth instars, and seven males), 6 *Triatoma sordida* (Stål) nymphs, 3 *Triatoma guasayana* (Wygodzinsky & Abalos) nymphs, and an unidentified first-instar nymph. None of the live bugs examined microscopically was found to be infected with *T. cruzi*.

All the 14 bugs identified as *T. infestans* had melanic coloration (Fig. 2A), and 8 of them were successfully sequenced for a fragment of the *COI* gene (Genbank accession numbers FJ811842–FJ811848). Sylvatic bugs from Chaco together with other *T. infestans* from Argentina and Bolivia and *T. platensis* from Argentina formed a monophyletic group defined by 36 synapomorphies (unambiguous optimization) in the most parsimonious phylogenetic tree (Fig. 3A). A similar result was obtained with the Bayesian analysis, where the clustering of sylvatic bugs with *T. infestans* sequences had 100% clade credibility in the 50% majority rule consensus tree (Fig. 3B).

**Table 1.** Distribution of *T. infestans* catches in natural ecotopes in the Argentine Chaco

Survey date	No. sites positive for <i>T. infestans</i> (no. inspected with Noireau traps)				No. trees with parrot nests inspected with fumigant canisters (% positive)
	Trees with parrot nest	Trees without parrot nest	Fallen tree	Total	
Feb. 2006	0 (44)	0 (28)	1 (23)	1 (95)	—
Dec. 2006	—	2 (69)	0 (11)	2 (80)	8 (25)
Jan. 2007	0 (7)	1 (152)	0 (5)	1 (164)	10 (20)
Total	0 (51)	3 (249)	1 (39)	4 (339)	18 (22)
Percent positive	0	1.2	2.6	1.2	

Four live *T. infestans* bugs were collected in 288 trap-nights placed in standing live (148) or dead trees (101) or in fallen (39) trees without parrot nests, whereas no *T. infestans* was collected from inside trees with parrot nests inspected with a total effort of 51 trap-nights (Table 1). Among the 18 trees that recently had parrot nests and were treated with insecticide fumigant canisters, 4 (22%) were positive for *T. infestans*, including 3 trees with an *A. aestiva* nest (Fig. 1B) and 1 tree with an *A. acuticaudata* nest. The percentage of inspected parrot nests that were found positive for *T. infestans* was significantly higher with fumigant canisters (22%) than with Noireau traps (0%; Fisher test,  $P = 0.004$ ). All *T. infestans* adults, three fourth-instar nymphs, and one fifth-instar nymphs were captured in parrot nests located inside quebracho blanco trees. Another fourth-instar nymph was collected from a dry quebracho blanco tree that had fallen the previous year when it had a woodpecker nest. The remaining fifth-instar nymphs of *T. infestans* were captured in two quebracho colorado standing trees (one dry) and a mistol tree. The average distance between sites with dark morph catches was 3.9 km (SD, 3.2; median, 2.9; range, 0.3–10.5).

In the nearest house located at 7 km from the sampling site and at 10 km from the nearest site where a sylvatic dark morph *T. infestans* was collected, a total of 49 *T. infestans* (8 males, 15 females, 21 fifth instars, and 5 fourth instars) were collected, all from a peridomestic storeroom; no bug was collected in domestic sites. All the bugs showed the normal coloration of *T. infestans* (Fig. 2B), and only one of the bugs was microscopically positive for *T. cruzi*. Among other five houses inspected for infestation, only eight *T. sordida* (microscope-negative for infection) were captured in peridomiciles of three houses.

### Discussion

To the best of our knowledge, these are the first confirmed findings of sylvatic *T. infestans* colonies in Argentina and of dark morphs in the Argentine Chaco, outside their only known locations in the Bolivian Chaco. Morphological and DNA sequence information showed that the melanic sylvatic bugs from the Chaco belong to *T. infestans*. In the phylogenetic analyses, sylvatic bugs clustered with Bolivian and Argentinian peridomestic *T. infestans* and *T. platensis* COI sequences, in agreement with previous studies showing great genetic similitude between these two species

using isozymes (Pereira et al. 1996), patterns of chromosome C-banding (Pérez et al. 2005), and mitochondrial (García and Powell 1998, Sainz et al. 2004) and nuclear DNA (Bargues et al. 2006). However, none of the sylvatic bugs carried the T > C change at position 556, which defined the *T. platensis*-nested clade. This change seems to be characteristic of this species and absent in *T. infestans*, because it was not found in a broader survey of COI variability based on 244 *T. infestans* from Argentina, Bolivia, Peru, and Uruguay (Piccinini et al. 2009).

The first records of *T. infestans* in sylvatic habitats apparently were provided by Mazza and others (Mazza 1936, Mazza et al. 1936, Mazza and Schreiber 1938), who found colonies with nymphs or eggs in tree holes, under bark of trees, cacti, bird (*Pseudoseisura lophotes* Reichenbach), and opossum (*Didelphis* sp) nests, sometimes far from houses, in Salta and Mendoza provinces (Argentina). Several other such findings by Mazza and others within the Argentine Chaco, Paraguay (1942) and Bolivia (1946), reviewed by Bejarano (1967), led this author to conclude that (1) *T. infestans* bugs found in sylvatic habitats may have originated from nearby houses or were introduced by passing vehicles and (2) the finding of nymphs in sylvatic habitats indicated that *T. infestans* could develop there, but it was unclear whether they may persist and eventually be a source of (peri)domestic reinfestation after insecticide spraying. Numerous incidental findings of *T. infestans* in sylvatic habitats (including colonies with nymphs in bird and rodent nests, under bark of trees, and in palm trees) within most of the geographic range of *T. infestans* in Argentina were recorded later (Viana and Carpintero 1977, Brewer et al. 1978, Ronderos et al. 1980, Cichero et al. 1984, Carpintero 1986, Carcavallo et al. 1988, Wisnivesky-Colli et al. 1992). The vast majority of these incidental findings, particularly those made before the mid-1960s before the start of intense use of insecticides in Argentina, occurred in highly infested areas—in arboreal habitats at distances from houses compatible with the estimated flight range of *T. infestans*. Exact locations were frequently unclear or unspecified; only a fraction of these findings provided evidence of colony development in sylvatic areas, and no further inquiry into the persistence of such foci over time was conducted. None of the cited reports on *T. infestans* collected in sylvatic habitats by experienced entomologists specified an unusual melanic form. The only exception known to us was the report

of a melanic form as a new species of *Triatominae* (*Triatoma mazzae* [Jörg]) based on a male adult bug collected in a house together with normal phenotypes of *T. infestans* in Chungara, Jujuy Province (Jörg 1937). *T. mazzae* was later synonymized to *T. infestans* (Lent and Wygodzinsky 1979). Because the dark morph feature is recessive (F. Noireau, personal communication), the occurrence of dark morph colonies in a well-defined area indicates complete reproductive isolation from other *T. infestans* populations with normal phenotype. In the original collection site of *T. melanosoma* in Misiones, "The specimens were collected in various opportunities in peridomestic sites, especially close to chicken houses" (Martínez et al. 1987). Based on this review of the evidence, we conclude that ours is the first confirmed finding of entirely sylvatic *T. infestans* colonies in Argentina and of dark morphs in the Argentine Chaco.

The chromatic appearance of the sylvatic *T. infestans* collected as adults or as nymphs reared to the adult stage was radically different from adult *T. infestans* collected from peridomestic sites at the nearest local houses and elsewhere in the Argentine Chaco. In agreement with findings in the Bolivian Chaco (Noireau et al. 1999), the local sylvatic *T. infestans* had an overall darker coloration than (peri)domestic *T. infestans*, smaller yellow spots in the connexivum, and yellow/orange spots in the posterior coxa. This coincidence in the chromatic pattern might be explained by (1) a single population that underwent regional expansion and (2) the melanic phenotype became fixed independently in two different populations (i.e., Bolivian and Argentine Chaco) by genetic drift (because sylvatic populations have very small population sizes), or more probably, by natural selection because melanic coloration could be an adaptive character or be linked to other adaptive traits (i.e., pleiotropic effects). The first alternative seems the least likely given the large distance ( $\approx 800$  km) between the Bolivian (Izozog) and Argentine locations of dark morphs. In addition, *T. infestans* dark morphs have not been found or reported in intermediate locations, although this may be because of insufficient searching efforts. Distinguishing between these two hypotheses is beyond the scope of this work and would require more genetic information. The occurrence of peridomestic *T. infestans* with normal phenotype at 10-km distance from the parrot nests with dark morphs strongly suggests that these dark morphs might represent truly sylvatic populations and not a derivative (spill-over) of peridomestic or domestic populations.

Our study also identified the main habitat of *T. infestans* dark morphs in parrot nests located inside hollow trees. Noireau et al. (1997) reported the collection of two *T. infestans* in 1 of 11 nests of *Myopsitta monacha* (Boddaert) monk parrots ("cotorra"), although at first these nests were misidentified as belonging to *A. araticaudata* (F. Noireau, personal communication). Later, four *T. infestans* were collected in 3 of 46 nests of *M. monacha* (Noireau et al. 2000b). Unlike all other parrot species, *M. monacha* builds the nests with twigs or thatch on tree branches and not

inside hollow trunks. The finding of different developmental stages in hollow tree trunks with parrot nests suggests that this is a suitable ecotope for the development of *T. infestans* colonies. Hollow tree trunks maintain a dry, dark environment that dampens oscillations of ambient temperatures, similar to a mud-and-thatch house; they also harbor a wide diversity of hosts, such as birds, rodents, bats, lizards, and snakes (I.B., unpublished data).

The apparent density of sylvatic *T. infestans* in our study area was lower than in other sylvatic foci in Bolivia investigated with Noireau traps. The bug catch per unit effort during the hot season in the Argentine Chaco (1.2 bugs per 100 trap-nights, 4 bugs in 339 trap-nights) was much lower than those recorded in the Bolivian Chaco (17.3 bugs per 100 trap-nights; Noireau et al. 2000b) and in the Andean valleys, including Cotapachi (122.8 bugs per 100 trap-nights, 582 in 474), Mataral, and Jamach'uma (8.3 bugs per 100 trap-nights, 5 in 60) (Cortez et al. 2007). Although the underlying reasons for such differences are unclear, it is likely that the rocky outcrops in the Bolivian Andean valleys provide more suitable refuges and more hosts (rodents) than the sparse hollow tree trunks with unstable resident hosts in the Argentine Chaco. Unlike in the Bolivian Andean valleys (Cortez et al. 2007), no bug was infected with *T. cruzi*, but this is probably related to the very small sample size and the fact that the bugs were mainly associated with birds (insusceptible to *T. cruzi*). Dark morph populations of *T. infestans* from the Bolivian Chaco also had a very low (2.5%) prevalence of *T. cruzi* infection (Noireau et al. 2000b).

Pyrethroid-based fumigant canisters were an efficient method for collecting triatomine bugs hidden inside hollow tree trunks. Bug collections were likely increased by directing sampling efforts to tree holes that at least had been occupied by parrots, on the assumption that the occurrence of triatomine bugs was more likely when a host was or had been present shortly before sampling. If mouse-baited traps are deployed where a resident host from which the bugs normally feed is present (as in our initial trapping efforts) or if the host is more accessible than the mouse bait, the bugs would probably be well nourished and not motivated to attack the mouse-baited trap suspended from a string. The main disadvantage of insecticide fumigant canisters is that the insects collected are invariably dead, unlike what happens with mouse-baited sticky traps.

Reinfestation specimens of *T. infestans* found 6 mo after house spraying with pyrethroid insecticides were survivors of the original bug population in the Bolivian Chaco (Dujardin et al. 1996). The repeated findings of *T. infestans* in sylvatic habitats show that this species may occur far from the human environment; may occur in more than one region and in different types of environment; and is capable of maintaining viable sylvatic colonies at very low density. A current challenge is to elucidate the exact role of the sylvatic foci of melanic and typical *T. infestans* as sources for domestic or peridomestic reinfestation after insecticide

spraying campaigns in the Gran Chaco ecoregion (Noireau et al. 2005).

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