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# Phenetic Analysis of *Panstrongylus megistus* Burmeister, 1835 (Hemiptera: Reduviidae: Triatominae) in the State of Paraná-Brazil

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## ABSTRACT

Panstrongylus megistus *is an important Chagas Disease vector and is said to be one of the species that might replace* Triatoma infestans *as the main vector of that disease in Brazil. The different degrees of* P. megistus *domiciliation in Brazil and its epidemiological relevance draw forth the need for the development of genetic studies that make it possible to analyze and understand the interchange of individual and gene fluxes among different populations. Thus, the present work aimed at studying the genetic variability of* P. megistus *in the State of Paraná – south of Brazil- and at comparing it with populations of the same species from five other states in Brazil (SP, MG, SC, RS, SE). In order to attain the proposed objective, 25 populations were studied using fifteen isoenzymatic systems (6PGD, G6PD, ME[1], ME[2], ICD, PGM, GPI, GOT[1], GOT[2], NP[1], NP[2], DIA, MPI, F, and MDH). The phenetic analysis allowed the individuation of 22 electromorphs and five zymodemes. The G6PD enzyme was the only polymorphic one presenting four electromorphs for the studied populations, all of them described for the State of Paraná-BR. The* P. megistus *populations from other states grouped with those from Paraná-BR, evidencing a low genetic variability in that species. Despite the existing geographic barriers, sub-samples – away from one another by at most 570km - were grouped in one and the same zymodeme. The epidemiological implications of such results are discussed in the present work.*

**Key words**: *Panstrongylus megistus*, isoenzymes, genetic variability, triatomines

## INTRODUCTION

Among the triatomine species, *Panstrongylus megistus* Burmeister, 1835 has epidemiological relevance as a *Trypanosoma cruzi* Chagas, 1909 disease constitutes one of the serious health problems in Latin American countries, showing bigger incidence in rural areas and in economically disadvantaged human populations. The distribution of this vector includes Uruguay, Argentina, Bolivia, Paraguay, and Brazil (Lent and Wygodzinky, 1979). It is largely distributed throughout Brazil: in the

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south it is found in the wild whereas in the State of Minas Gerais and in the city of Salvador (BA), it is found in intra-domicile environments; and in the remaining regions it is present both in the wild and in intra- and peri-domicile environments (Miles, 1976, Forattini et al, 1977; Dórea et al, 1982). Nowadays, *P. megistus* is receiving increasing attention from Chagas Disease's epidemiology it is considered the species that might replace the *Triatoma infestans* Klug, 1834 after its intra-domicile elimination in Brazil (Ramos Jr and Carvalho, 2001).

In the State of Paraná, south of Brazil, *P. megistus* is largely distributed throughout the state (Luz and Borba, 1966) showing heavy infection by *T. cruzi*, (Luz, 1976; Toledo et al, 1997; Thomaz-Soccol et al, 2002). According to the program for decentralizing public health policies implemented by the Brazilian Government, the municipalities must take responsibility for the epidemiological surveillance over transmissible diseases. But many municipalities do not have enough technical resources to maintain control programs for Chagas's Disease. In Brazil, the different degrees of *P. megistus* domiciliation and its epidemiological relevance draw forth the need for genetically based studies that make it possible to analyze and understand the interchange of individuals and gene fluxes in different populations (Moura et al, 1969; Forattini, 1980; Silveira and Vinhaes, 1998). Based on enzymatic differentiations, the present work aimed at characterizing *P. megistus* populations from the State of Paraná and from other areas in Brazil, thus contributing to the understanding of the domiciliation process of this species.

## MATERIAL AND METHODS

The *P. megistus*'s samples included in the present study were collected in different regions of the State of Paraná-BR and kept in the Molecular Parasitology Laboratory of the Department of Basic Pathology of the *Universidade Federal do Paraná* (SCB-UFPR). A total of 19 populations collected from Paraná were analyzed (Table 1). Insects from Minas Gerais, Rio Grande do Sul, Sergipe, Santa Catarina, and São Paulo were sent by researchers from those areas, and *Dipetalogaster maxima* Uhler (1894) was used as an outgroup (Table 1).

Only negative samples of *T. cruzi* were used for isoenzyme electrophoresis. The thoracic musculature of the insects selected for the present work was submitted to electrophoresis in thick starch gel (Dujardin and Tibayrenc, 1985a,b). For obtaining the protein extracts, the prothorax of adult insects or $5^{th}$-instar nymphs was used. For obtaining the extracts, the material was macerated in a mortar kept on ice in order to maintain the viability of the extract. For grinding each insect, 200 µl of a lysis hypotonic enzyme-stabilizer solution (500 mM Tris- HCl, 26 mM EDTA, 10 mM DTT, 10 mM amino-n-caproic acid - Sigma®) were added to the medium. The homogenized sample was spun down at 10,000 x g for 30 minutes at 4°C. The supernatant was subsequently placed into 'pellet'-form micro tubes and frozen in liquid nitrogen at -196°C. Before carrying out electrophoresis, the pellets were centrifuged for 5 minutes at 10,000 x g, at 4°C, for subsequent deposit of extracts on support. Fifteen enzymatic systems were used in the present study: glucose phosphate isomerase (E.C.5.3.1.9, GPI), phosphoglucomutase (E.C.5.4.2.2, PGM), purine nucleoside phosphorylase (E.C.2.4.2.1, $NP^1$ and E.C.2.4.2.*, $NP^2$), mannose-6-phosphate isomerase (E.C.5.3.1.8, MPI), NADH diaphorase (E.C.1.6.2.2, DIA), isocitrate dehydrogenase (E.C.1.1.1.42, ICD), fumarate hydratase (EC 4.2.1.2 FH), glucose-6-phosphete dehydrogenase (E.C.1.1.1.49, G6PD), 6-phosphate gluconate dehydrogenase (E.C.1.1.1.44, 6-PGD), malic enzyme (E.C.1.1.1.40, $ME^1$, $ME^2$), malate dehydrogenase (E.C.1.1.1.37, MDH), and glutamate oxalacetate transaminase (E.C.2.6.1.1, $GOT^1$, $GOT^2$). All enzymes used were supplied by Boehringer® and the protocols used for isoenzymes resolution and electrophoresis were previously described by Kopp et al, 2005. For studying the genetic variability, the numeric analysis of individualized electromorphs, genetic distance (Nei, 1987), polymorphism and population differentiation by exact tests (Raymound and Rouset, 1995) were used. In order to study the genetic variability in those populations, 22 electromorphs were individualized, and the zymodemes were labeled 'Lpm' followed by Arabic figures from 1 to 4 (Table 2), and these increasing-order figures were defined as corresponding to a faster migration by smaller molecular weight or greater isoelectric potential, and '0' as complete absence (Table 2). Phenograms were constructed by analyzing each electrophoretic band as a unit character, by construction of a matrix with the presence or absence of electrophoretic bands using the interactive molecular evolutionary genetics analysis (NTSYs programs pc version 2.1k –

grouping by Unweighted Pair Group Method - UPGMA- with Arithmetic mean (Rohlf, 1985).

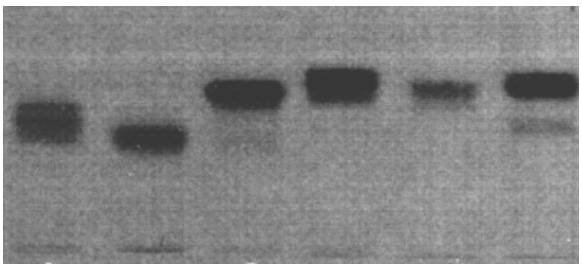


**Figure 1** - Electromorphs observed for isoenzyme G6PD.

**Table 1**- Origin of *Panstrongylus megistus* submitted to enzymatic study.

| Population ID code | Collected samples | Origin | Region | Loci | Collection date |
|---|---|---|---|---|---|
| Pr.Ara.1 | 10 | Arapongas | North/PR | Peri-domicile | 02/1999 |
| Pr.Ara.2 | 8 | Arapongas | North/PR | Peri-domicile | 06/1999 |
| Pr.Ara.3 | 20 | Arapongas | North/PR | Peri-domicile | 06/1999 |
| Pr.Ara.4 | 200 | Arapongas | North/PR | Peri-domicile | 11/2001 |
| Pr.Ara.5 | 72 | Arapongas | North/PR | Peri-domicile | 11/2002 |
| Pr.Ara.6 | 4 | Arapongas | North/PR | Peri-domicile | 11/2001 |
| Pr.Cam.1 | 4 | Cambé | North/PR | Peri-domicile | 08/2000 |
| Pr.Cam.2 | 30 | Cambé | North/PR | Peri-domicile | 08/2000 |
| Pr.Rol.1 | 10 | Rolândia | North/PR | Peri-domicile | 08/2000 |
| Pr.Rol.2 | 6 | Rolândia | North/PR | Peri-domicile | 08/2000 |
| Pr.Lon.1 | 5 | Londrina | North/PR | Peri-domicile | 08/2000 |
| Pr.Fax.1 | 3 | Faxinal | North/PR | Peri-domicile | 07/2000 |
| Pr.Aru.1 | 3 | Araruna | North/PR | Peri-domicile | 11/2000 |
| Pr.Nau.1 | 3 | Nova Aurora | West/PR | Peri-domicile | 12/2000 |
| Pr.Pal.1 | 4 | Palmitópolis | West/PR | Peri-domicile | 12/2000 |
| Pr.Rbs.1 | 1 | Rio B.do Sul | Met.Reg.Curitiba | Peri-domicile | 02/2000 |
| Pr.Rbs.2 | 2 | Rio B.do Sul | Met.Reg.Curitiba | Peri-domicile | 01/2001 |
| Pr.Alt.1 | 2 | A.Tamandaré | Met.Reg.Curitiba | Peri-domicile | 01/2001 |
| Pr.Alt.2 | 2 | A.Tamandaré | Met.Reg.Curitiba | Peri-domicile | 02/2001 |
| MG | 10 | Minas Gerais | MG | Peri-domicile | 03/2000 |
| RS | 10 | Rio G. do Sul | RS | Not informed | 04/2000 |
| SE | 10 | Sergipe | SE | Wild | 10/2000 |
| SC | 10 | S. Catarina | SC | Wild | 10/2000 |
| SP | 10 | São Paulo | SP | Domicile | 10/2001 |
| *D.maxima* | 10 | Mexico | Mexico | Wild | 07/2000 |

## RESULTS

### Isoenzyme Electrophoresis Analysis

Four different electromorphs were observed for isoenzyme G6PD (Fig. 1 and Table 2).

Zymodeme is an expression used to define individuals with similar biochemical and enzymatic characteristics. A reference population was assigned to each zymodeme, having the *P. megistus* population collected first as rule criterion.

### Zymodemes

*Zymodeme Lpm1*: it consisted of 14 populations, having the Pr.Ara.1 population as reference. A group having the same profile was formed by the following populations: Pr.Ara.1, Pr.Ara.2, Pr.Ara.3, Pr.Rbs.1, Pr.Rbs.2, Pr.Pal.1, Pr.Lon.1, Pr.Fax.1, Pr.Rol.2, Pr.Aru.1, Pr.Alt.1, Pr.Alt.2, SC, and SP (G6PD4). In relation to *Zymodeme Lpm2*, a group of four populations was found, three of them from the State of Paraná (Cam2, Ara4, Ara6) and one from Minas Gerais, having population Cam2

(G6PD1) as reference. *Zymodeme Lpm3*: it consisted just of the Pr.Cam.1 population, characterized by the G6PD2 electromorph. *Zymodeme Lpm4*: grouping five populations, three of them from the state of Paraná (Pr.Nau.1, Pr.Rol.1, Pr.Ara.5) and another two from the States of Rio Grande do Sul and Sergipe, respectively. It had Pr.Rol.1 as reference population by the fact that it presented a better development and maintenance. *Zymodeme Lpm5*: it individualized the *D. maxima* population presented differentiation in seven isoenzymatic systems (GPI, PGM, ICD, FH, G6PD, 6PGD, and $GOT^1$) of the *P. megistus* populations under study. The G6PD3 electromorph also characterized *D. maxima* (Table 2).

**Dendrogram**

Dendrogram construction was based on the individualized electromorphs in the 15 isoenzymatic systems from 24 *P. megistus* populations and one *D. maxima* population. Two clusters were clearly individualized (Fig. 2): the first characterizes the *P. megistus* populations and the second is formed by the outgroup (*D. maxima***)**. Inside the cluster formed by the *P. megistus* populations, one could observe that there was a clear separation of the Cambé population – labeled PrCam1 – from the remaining populations under study. In the cladogram, one could observe that the *P. megistus* populations from the State of Paraná were distributed in all different clusters, as well as those coming from the remaining states. Besides representatives from the North and West of Paraná, the higher cluster included all populations from the east region of Paraná and those from the states of Santa Catarina and São Paulo. The population from Minas Gerais was in a cluster just with representatives from the north of Paraná. Group 1 consisted of four groups with different isoenzymatic profiles of *P. megistus* populations.

**Table 2** - Isoenzymatic profiles displayed by the *P. megistus* and *Dipetalogaster maxima* (outgroup) populations, corresponding zymodemes and electromorphs for isoenzyme G6PD.

| Isoenzyme Population | GPI | PGM | $NP^1$ | $NP^2$ | MPI | DIA | ICD | FH | G6PD | 6PGD | $ME^1$ | $ME^2$ | MDH | $GOT^1$ | $GOT^2$ | Zymo-deme | Electro-morph |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pr.Ara.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Ara.2 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Ara.3 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Ara.4 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm2 | G6PD1 |
| Pr.Ara.5 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm4 | G6PD3 |
| Pr.Ara.6 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm2 | G6PD1 |
| Pr.Cam.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm3 | G6PD2 |
| Pr.Cam.2 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm2 | G6PD1 |
| Pr.Rol.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm4 | G6PD3 |
| Pr.Rol.2 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Lon.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Fax.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Aru.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Nau.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm4 | G6PD3 |
| Pr.Pal.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Rbs.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Rbs.2 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Alt.1 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| Pr.Alt.2 | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| MG | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm2 | G6PD1 |
| RS | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm4 | G6PD3 |
| SE | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm4 | G6PD3 |
| SC | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| SP | 1 | 1 | 1 | 0 | 1 | 1 / 1 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 0 | Lpm1 | G6PD4 |
| *D.maxima* | 2 | 2 | 1 | 0 | 2 | 1 / 1 | 2 | 2 | 3 | 2 | 1 | 0 | 1 | 2 | 0 | Lpm5 | G6PD3 |

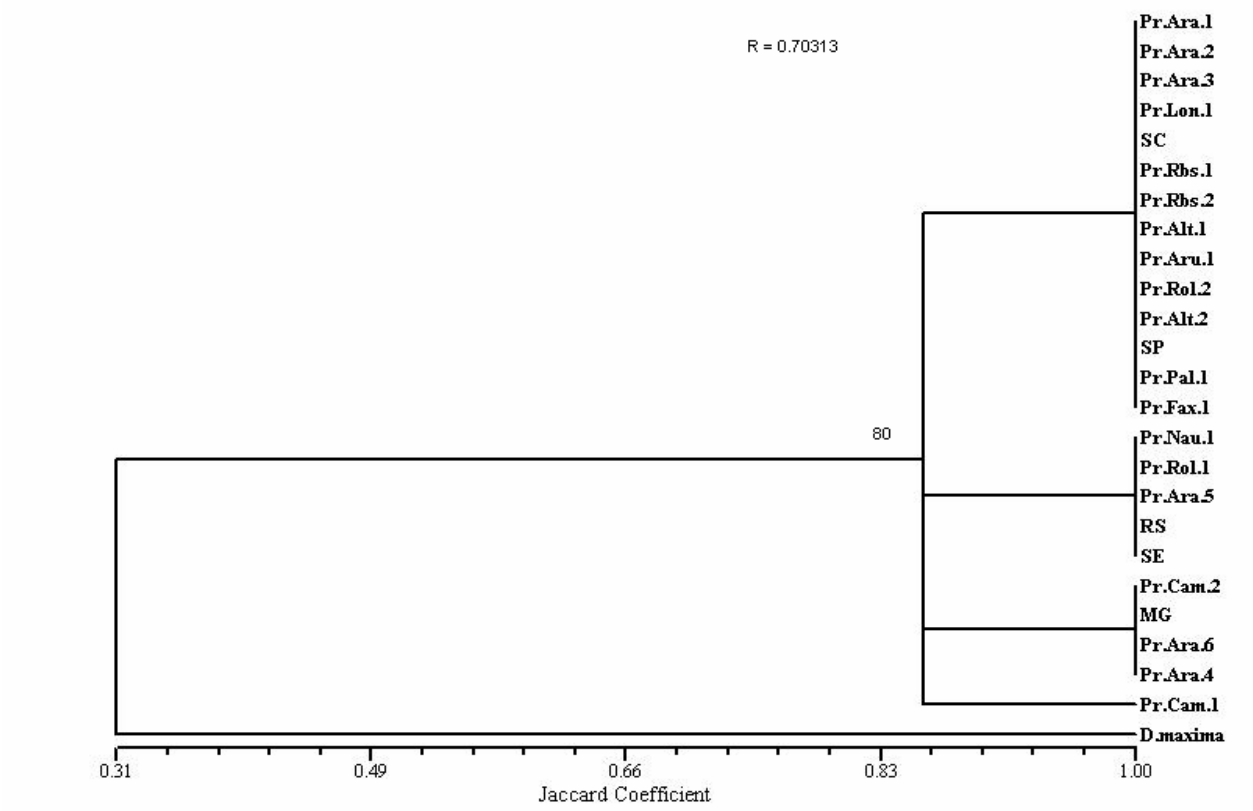


**Figure 2** - Dendrogram obtained by grouping through the Unweighted Pair Group Method with Arithmetic mean UPGMA for isoenzymes from *Panstrongylus megistus* and *Dipetalogaster maxima* populations.

Nei's genetic distances were estimated through data matrix and the result was a genetic distance equal to 0.08 and a 0.91 identity between these sets. The similarity index obtained through the Jaccard index for these sets was 0.55 (Table 3).

In cluster 2, one could observe the isolation of the *Dipetalogaster maxima* population, with a different profile as compared to that displayed by the previous group. The genetic distance and identity presented by it - in relation to groups 1, 2, and 3 of the first cluster - was 0.50; and 0.41 and 0.58, respectively, in relation to group 4. The geographic distribution of zymodemes on the different plains and plateaus of the State of Paraná is presented in Fig. 3.

**Polymorphism**

In the analysis of the loci provided by the isoenzymes, heterozygotes could only be observed when related to G6PD isoenzyme (Table 2). The results corresponded to four alleles, of which the frequency of allel 1 (Lpm2) was equal to 0.16, allel 2 (Lpm3) was equal to 0.04, allel 3 (Lpm4) was equal to 0.21, and allel 4 (Lpm1) was equal to 0.58. The probability of occurrence of heterozygotes for these loci was 0.58 and an average of 0.05 for all populations.

**Analysis of grouping by UPGMA through Nei's minimal distances**

The constructed dendrogram consisted of 24 insertion branches for a set of 25 populations, and these branches had a minimal distance varying between zero and 0.48. The consistency index on each insertion point varied between 6.67 and 80%.

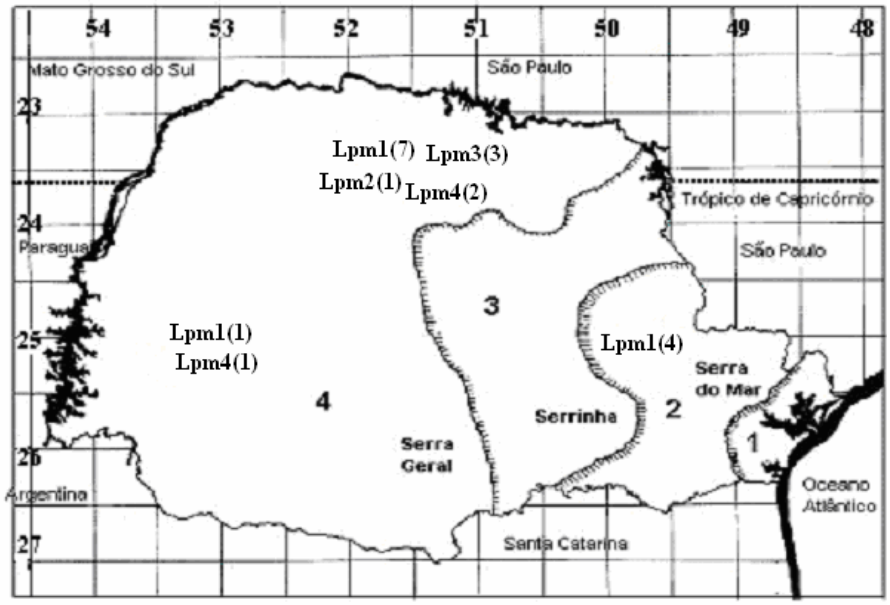


**Figure 3** - Geographic distribution of zymodemes of *Panstrongylus megistus* in the State of Paraná-Brazil. 1- Coastal plain, 2- First plateau, 3- Second plateau, and 4-Third plateau. The number of populations for each zymodeme is given in brackets.

**Population differentiation using exact tests**

When the outgroup was not taken into account, one can observe differences in the frequency of allels when the natural populations from the State of Paraná were compared to those from other States, and which resulted – only in the locus constituted by the G6PD - in a P equal to 0.31 with a standard error of 0.009, which was not significant.

## DISCUSSION

Finding *P. megistus* in peri-domicile environments both in the rural area and in peri-urban areas - in the State of Paraná-BR - may be a step toward its domiciliation, since these insects have a high invasive power in relation to the human environment (Steindel et al, 1994). Falavigna-Guilherme et al, (2001) have already found this species in domicile, in Paraná-BR. Another characteristic of it is its well-developed dispersing capacity, for its colonization in inhabited houses has constantly been observed in other regions of Brazil (Guilherme et al, 2001). This was the reason why the present compared the populations found in the State of Paraná and studied its genetic diversity, comparing it to those of other Brazilian states.

For the 24 analyzed populations of *P. megistus* coming from six states, the only enzyme that proved to be polymorphic was G6PD, presenting four electromorphs, all with representation in the State of Paraná. Barbosa et al, (2003) analyzed three populations of *P. megistus* and found polymorphism for the PGM enzyme, for which no polymorphism was observed in the present study. In the 1950's, the north region of the State of Paraná received immigrant groups from various places in Brazil due to the coffee plantations expansion (LUZ – personal communication) and *P. megistus* could have come with the human population, and this could explain such diversity. Among the five places under study in the north of Paraná, three of them present more than one zymodeme: for Arapongas, for example, three different electromorphs were described for G6PD. The Lpm3 zymodeme was only described for Cambé, for which the zymodeme Lpm2 was also described. In the present work, the enzyme G6PD proved to be a good molecular marker for *P. megistus.*

Taking a closer look at the dendrogram, it was possible to observe that the twelve populations of *P. megistus* in Paraná were gathered in the same cluster as those from the states of São Paulo and Santa Catarina (Lpm1). When compared to the *P. megistus* populations from Minas Gerais, Rio

Grande do Sul, and Sergipe, the genetic distances were bigger (Table 3).

However, the natural population from Minas Gerais grouped with both populations from Paraná (Lpm3), and that from Rio Grande do Sul grouped with that from Sergipe and all three from Paraná (Lpm4). The *P. megistus* population from Minas Gerais was isolated from that from Santa Catarina had been previously also observed by other authors (Barbosa et al., 1999, 2001). However, the grouping of the populations from Rio Grande do Sul with that from Sergipe and some from Paraná demonstrated the existence of sister-populations separated by vast geographic expanses. A genetic distance of 0.08 and similarity above 90% were found, reinforcing the hypothesis of identical populations standing out in different geographical regions, and different populations in one and the same region (Carpintero et al., 2003). This was also observed in a study carried out on *P. megistus* samples from Pernambuco, Bahia, and Rio de Janeiro with 15 isoenzymatic systems in which no isoenzymatic difference among the three populations was found (Barbosa et al., 2001). *Panstrongylus megistus* is considered a triatomine bug coming from forested and humid regions, owing its dispersion to the adverse conditions of temperature and humidity (Foratini, 1980, Foratini et al., 1977, 1984); the anthropic action would also afford such condition. Thus, the hypothesis that these could explain the presence of different lines of *P. megistus* in Paraná-BR was raised.

In relation to the polymorphism found for *P. megistus* in the northern area of the state in the present study, it allowed to suggest that the still preserved Atlantic rain forest – '*Mata Atlântica*' – at the '*Serra do Mar*' and small sites of natural forests in the remaining areas of Paraná, but with a larger proportion of secondary forest associated to the favorable weather conditions, might have allowed the arrival of lineages from São Paulo, which, later on, might have migrated to Santa Catarina, thus explaining the present grouping with populations from Paraná, São Paulo, and Santa Catarina (Lpm1).

**Table 3** - Genetic distances among knots using Nei's grouping (1978).

| Nº | DISTANCE | POPULATIONS |
|---|---|---|
| 1 | 0.0000 | 23,24 |
| 2 | 0.0000 | 17,23,24 |
| 3 | 0.0000 | 16,17,23,24 |
| 4 | 0.0000 | 14,16,17,23,24 |
| 5 | 0.0000 | 13,14,16,17,23,24 |
| 6 | 0.0000 | 12,13,14,16,17,23,24 |
| 7 | 0.0000 | 10,12,13,14,16,17,23,24 |
| 8 | 0.0000 | 8,10,12,13,14,16,17,23,24 |
| 9 | 0.0000 | 5,8,10,12,13,14,16,17,23,24 |
| 10 | 0.0000 | 4,5,8,10,12,13,14,16,17,23,24 |
| 11 | 0.0000 | 3,4,5,8,10,12,13,14,16,17,23,24 |
| 12 | 0.0000 | 2,3,4,5,8,10,12,13,14,16,17,23,24 |
| 13 | 0.0000 | 1,2,3,4,5,8,10,12,13,14,16,17,23,24 |
| 14 | 0.0000 | 21,22 |
| 15 | 0.0000 | 18,21,22 |
| 16 | 0.0000 | 9,18,21,22 |
| 17 | 0.0000 | 7,9,18,21,22 |
| 18 | 0.0833 | 1,2,3,4,5,7,8,9,10,12,13,14,16,17,18,21,22,23,24 |
| 19 | 0.0000 | 19,20 |
| 20 | 0.0000 | 15,19,20 |
| 21 | 0.0000 | 11,15,19,20 |
| 22 | 0.0833 | 1,2,3,4,5,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24 |
| 23 | 0.0833 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24 |
| 24 | 0.4826 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25 |

Such hypothesis would explain the presence of a single zymodeme (Lpm1) for the east region of Paraná, the same described for the samples from São Paulo and Santa Catarina. The presence of this

zymodeme in the north region of Paraná might have happened after its active or passive dispersion via the east of Paraná or via São Paulo.
*Panstrongylus megistus* probably constituted a monophyletic group with a low genetic variability, and a more including sampling could confirm these data. New molecular markers must be applied to this group of triatomimes in an attempt to understand the domiciliation and dispersion processes of this species. The continuity of such genetic studies for *P. megistus* could bring about useful information for the epidemiology of the Chagas disease in the State of Paraná and in the other states of Brazil, considering the high natural infection rate by *T. cruzi* in those insects (Thomaz-Soccol et al., 2002).
The *P. megistus* grouping from Minas Gerais and Paraná could explain the introduction of the insects following the migration of the population from Minas Gerais, making the implantation of other lineages possible in the state. Likewise, the identical lineages of *P. megistus* in Paraná, Rio Grande do Sul, and Sergipe favors the dispersion starting from the '*Serra do Mar'* and spreading toward inland areas, or passive migrations following human immigrations.

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## RESUMO

*Panstrongylus megistus* é um importante vetor da Doença de Chagas e é apontado como uma das espécies com potencial para substituir *Triatoma infestans* como principal vetor desta doença no Brasil. Os diferentes graus de domiciliação por *P. megistus* - no Brasil - e sua importância epidemiológica evocam a necessidade de estudos com bases genéticas que possibilitem analisar e compreender os intercâmbios de indivíduos e os fluxos gênicos entre as distintas populações. Assim, o presente trabalho tem como objetivo estudar a variabilidade genética de *P. megistus* no Estado do Paraná e compará-los com populações da mesma espécie de cinco estados do Brasil (SP, MG, SC, RS, SE). Para atingir o objetivo proposto, 25 populações foram estudadas empregando quinze sistemas isoenzimáticos (6PGD, G6PD, $ME^1$, $ME^2$, ICD, PGM, GPI, $GOT^1$, $GOT^2$, $NP^1$, $NP^2$, DIA, MPI, FH e MDH). A análise fenética permitiu a individualização de 22 eletromorfos e quatro zimodemas. A enzima G6PD foi a única polimórfica que apresentou quatro eletromorfos para as populações estudadas, todas descritas para o Estado do Paraná. As populações de *P. megistus* procedentes dos outros estados agruparam-se com as do Paraná, demonstrando haver baixa variabilidade genética na espécie. Apesar das barreiras geográficas existentes, sub-amostras - distantes entre si por até 570 km - ficaram reunidas num mesmo zimodema. As implicações epidemiológicas destes resultados são discutidas no presente trabalho.

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