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Allozyme relationships among ten species of Rhodniini, showing paraphyly of *Rhodnius* including *Psammolestes*

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Abstract. Genetic relationships among 10 species of bugs belonging to the tribe Rhodniini (Hemiptera: Reduviidae), including some important vectors of Chagas disease, were inferred from allozyme analysis of 12 enzyme loci (out of 21 enzyme systems examined), using agarose gel electrophoresis. These species formed two clusters: one comprising *Rhodnius brethesi*, *R. ecuadoriensis*, *R. pallescens* and *R. pictipes*; the other with *Psammolestes tertius*, *Rhodnius domesticus* and the *Rhodnius prolixus* group comprising *R. nasutus*, *R. neglectus*, *R. prolixus* and *R. robustus*.

The resulting tree was [((*R. ecuadoriensis*, *R. pallescens*) *R. brethesi*) *R. pictipes*], [*R. domesticus* (*P. tertius* {(*R. nasutus*, *R. neglectus*) (*R. prolixus*, *R. robustus*)})]. *Rhodnius nasutus* and *R. neglectus* differed by only one locus, whereas no diagnostic loci were detected between *R. prolixus* and *R. robustus* (22 loci were analysed for these four species), despite considerable DNA sequence divergence between species in each of these pairs. Allozymes of the *R. prolixus* group showed greater similarity with *Psammolestes tertius* than with other *Rhodnius* spp., indicating that *Rhodnius* is paraphyletic and might include *Psammolestes*.

Key words. *Psammolestes*, *Rhodnius*, Chagas disease, Hemiptera, Reduviidae, Rhodniini, Triatominae, isoenzymes, molecular systematics, paraphyly, phylogeny, vector, Brazil, Colombia, Ecuador, Peru.

Introduction

Haematophagous bugs (subfamily Triatominae of the family Reduviidae) are classified as five tribes, of which the Triatomini Jeannel 1919 and Rhodniini Pinto 1926 include species of medical importance as vectors of Chagas disease. The tribe Rhodniini, containing two recognized genera, *Rhodnius* Stål 1859 and *Psammolestes* Bergroth 1911, is well defined by three synapomorphies: postocular callosities, the distinctive second antennal segment, and the modified basal plate struts (Lent & Wygodzinsky, 1979). *Psammolestes* comprises three species inhabiting Funariid birds' nests east of the Andes (from Venezuela to Argentina), morphologically distinguished from *Rhodnius* by the femoral width and characteristic shape of the head and apical segment of the rostrum (Lent & Wygodzinsky, 1979). *Rhodnius* comprises 14 species, also mainly arboreal, often found in palm tree crowns, epiphytic bromeliads and hollow trees. Although not found so far south in South America, *Rhodnius* has a much more extensive geographical distribution (from southern Mexico through Central America to southern Brazil) and a wider range of hosts, including birds, rodents, marsupials and sloths. Several species of *Rhodnius* (marked † in Table 1) are well adapted to living in human dwellings and serve as vectors of Chagas disease to humans, a major public health problem in Latin America (World Bank, 1993).

Monophyly of Rhodniini is indicated by the distinctive presence of heme proteins, giving salivary glands a characteristic cherry colour (Ribeiro *et al.*, 1998; Soares *et al.*

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Table 1. Triatomine species examined.

Species	Code	Origin	Field/Colony
R. prolixus	PRO	Cojedes, Venezuela, 1995	Colony #5 at Fiocruz
R. robustus	ROB	Napo, Ecuador*	Colony #3 at Fiocruz
R. neglectus	NEG	Itambaracá, Paraná, Brazil*	Colony #2 at Fiocruz
R. nasutus	NAS	Teresina, Piauí, Brazil*	Colony #1 at Fiocruz
R. domesticus	DOM	Santa Catarina, Brazil, 1985	Colony #1 at Fiocruz
R. pallescens	PAL	Vegachi, Colombia, 1989	Colony #1 at Fiocruz
R. ecuadoriensis	ECU	Peru, 1979	Colony #3 at Fiocruz
R. pictipes	PIC	Belém, Pará, Brazil, 1989	Colony #2 at Fiocruz
R. brethesi	BRE	Amazonas, Brazil, 1995	Colony #1 at Fiocruz
P. tertius	PSA	Goiás, Brazil, 1997	Field

*Year of collection not available. Bold type = important vectors of Chagas disease to humans.

2000). Mitochondrial and nuclear DNA sequence data corroborate monophyly but also suggest that *Rhodnius*, together with *Psammolestes coreodes* and *P. tertius*, is a paraphyletic assemblage (Lyman *et al.*, 1999; Monteiro *et al.*, 2000; Marcilla *et al.*, 2001).

In this paper we investigate the monophyly and paraphyly of Rhodniini using another type of genetic marker, allozymes, that have been employed in several previous studies of triatomine systematics (Dujardin *et al.*, 1987; Harry *et al.*, 1992; Pereira *et al.*, 1996; Solano *et al.*, 1996; Costa *et al.*, 1997; Panzera *et al.*, 1997; Monteiro *et al.*, 1998; Noireau *et al.*, 1998). In addition, we assess the practical issue of relationships among the *R. prolixus* group of species, namely *R. nasutus*, *R. neglectus*, *R. robustus* and the important vector *R. prolixus* itself. These allozyme analyses support the paraphyly of Rhodniini (i.e. *Rhodnius* plus *Psammolestes*), and show minimal interspecific divergence of allozymes between species of contrasted importance: the allopatric *R. nasutus* and *R. neglectus* from Brazil and the more widespread, primarily domestic *R. prolixus* and sylvatic *R. robustus*.

Materials and methods

Specimens were obtained from the International Triatomine Reference Laboratory at the Oswaldo Cruz Foundation (Fiocruz) in Rio de Janeiro, Brazil. Origins of strains and numbers of insects used in this study are listed in Tables 1 and 2, respectively. Agarose gel electrophoresis of allozymes followed Momen & Salles (1985), with minor modifications, using a buffer system of Tris-Citrate pH 8.0 (Ward & Beardmore, 1977). The head and thorax of each specimen were homogenized in 150 µL of lysis buffer (500 mM Tris HCl, 26 mM EDTA, 10 mM DTT and 10 mM ε-amino-n caproic acid) and 3–5 µL of the homogenate was applied to the gels. From 21 enzymes tested, the following 11 produced scorable bands for all species analysed: aconitase (EC 4.2.1.3, ACON); fumarase (EC 4.2.1.2, FUM); α-glycerophosphate dehydrogenase (EC 1.1.1.8, α-GPD); hexokinase (EC 2.7.1.1, HK); isocitrate dehydrogenase (EC 1.1.1.42, IDH); malate dehydrogenase (EC 1.1.1.37, MDH); malic enzyme (EC 1.1.1.40, ME); mannose-phosphate isomerase (EC 5.3.1.8, MPI); phosphogluconate dehydrogenase (EC 1.1.1.44, PGD); glucose-6-phosphate isomerase (EC 5.3.1.9, PGI) and phosphoglucomutase (EC 5.4.2.2, PGM).

In addition, in order to assess the degree of genetic differentiation within the *prolixus* group of species (*R. prolixus*, *R. robustus*, *R. neglectus* and *R. nasutus*), nine other enzymes: adenilate kinase (EC 2.7.4.3, AK); catalase (EC 1.11.1.6, CAT); α-esterases (EC 3.1.1.1, α-EST); glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PDH); glutamate ehydrogenase (EC 1.4.1.4, GDH); glutamate oxaloacetate transaminase (EC 2.6.1.1, GOT); leucine aminopeptidase (EC 3.4.11.1, LAP); peptidases (EC 3.4.1.1, PEP); and superoxide dismutase (EC 1.15.1.1, SOD), could be reliably scored for this smaller set of species. For this set, sample sizes varied from three to seven insects per species per enzyme (data not shown).

Genotype frequencies were obtained by visual interpretation of bands on the gels. From these, gene frequencies, genetic variation, unbiased genetic identities (*I*) and distances (*D*) (Nei, 1978) were calculated and a UPGMA tree (Sneath & Sokal, 1973) constructed with the BIOSYS-1 program, version 1.7 (Swofford & Selander, 1981).

Results

For the complete set of species, 12 loci were observed in the 105 samples analysed. Mean heterozygosity levels (*H*) were very low, with only *R. pictipes* ($H = 0.05$), *R. domesticus* ($H = 0.03$) and *R. prolixus* ($H = 0.01$) showing variability. Secondary bands observed for IDH in *R. brethesi* and *R. pictipes* (Fig. 1) were ignored for the genetic analyses.

The 10 species analysed were grouped in two clusters: one formed by *R. brethesi*, *R. ecuadoriensis*, *R. pallescens* and *R. pictipes*; the other by the *R. prolixus* group of species (*nasutus*, *neglectus*, *prolixus*, *robustus*) plus *R. domesticus* and *Psammolestes tertius*. Greater genetic similarity was found between *P. tertius* and all four species of the *R. prolixus* group than between any of these and other *Rhodnius* species (Table 3, Fig. 2).

Table 2. Allozyme frequencies for the 10 species of Rhodniini examined (see Table 1 for code of species names): n = number of individuals scored; most frequent alleles per locus per species are in bold.

		Species									
Locus	Allele	ECU	PAL	DOM	BRE	PIC	NAS	NEG	PRO	ROB	PSA
Acon	1	0	0	0	0	0	0	0	0	0	**1.00**
	2	0	0	**1.00**	0	0	**1.00**	**1.00**	**1.00**	**1.00**	0
	3	0	0	0	**1.00**	0	0	0	0	0	0
	4	**1.00**	**1.00**	0	0	0	0	0	0	0	0
	5	0	0	0	0	**1.00**	0	0	0	0	0
	(n)	(3)	(4)	(3)	(3)	(4)	(4)	(4)	(4)	(2)	(3)
Fum	1	**1.00**	**1.00**	0	0	0	0	0	0	0	0
	2	0	0	0	**1.00**	0	0	0	0	0	0
	3	0	0	**1.00**	0	0	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	4	0	0	0	0	**1.00**	0	0	0	0	0
	(n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(6)	(4)	(3)
α-Gpd	1	0	0	0	**1.00**	0	0	0	0	0	0
	2	0	0	0	0	0.03	0	0	0	0	0
	3	**1.00**	**1.00**	**1.00**	0	**0.97**	0	0	**1.00**	**1.00**	0
	4	0	0	0	0	0	0	0	0	0	**1.00**
	5	0	0	0	0	0	**1.00**	**1.00**	0	0	0
	(n)	(16)	(16)	(3)	(5)	(15)	(8)	(12)	(10)	(5)	(3)
Hk	1	0	0	0	**1.00**	0	0	0	0	0	0
	2	0	**1.00**	0	0	**1.00**	0	0	0	0	0
	3	**1.00**	0	0	0	0	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	4	0	0	**1.00**	0	0	0	0	0	0	0
	(n)	(3)	(6)	(3)	(3)	(4)	(2)	(2)	(2)	(2)	(3)
Idh	1	0	0	0	0	0	**1.00**	**1.00**	**1.00**	**1.00**	0
	2	0	**1.00**	0	0	0	0	0	0	0	0
	3	0	0	**1.00**	0	0	0	0	0	0	0
	4	**1.00**	0	0	**1.00**	0.12	0	0	0	0	0
	5	0	0	0	0	**0.88**	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	**1.00**
	(n)	(5)	(5)	(3)	(4)	(4)	(9)	(8)	(11)	(7)	(3)
Mdh	1	0	0	0	**1.00**	0	0	0	0	0	0
	2	0	0	**0.83**	0	0	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	3	**1.00**	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	**1.00**	0	0	0	0	0
	5	0	**1.00**	0	0	0	0	0	0	0	0
	6		0	0.17	0	0	0	0	0	0	0
	(n)	(17)	(17)	(3)	(5)	(16)	(9)	(9)	(12)	(7)	(3)
Me-1	1	0	0	0	0	0	**1.00**	**1.00**	0	0	0
	2	0	0	0	0	0	0	0	**1.00**	**1.00**	0
	3	0	0	0	0	0	0	0	0	0	**1.00**
	4	0	0	**1.00**	0	0	0	0	0	0	0
	5	0	0	0	**1.00**	0.12	0	0	0	0	0
	6	**1.00**	**1.00**	0	0	0	0	0	0	0	0
	7	0	0	0	0	**0.88**	0	0	0	0	0
	(n)	(5)	(5)	(3)	(4)	(4)	(7)	(9)	(10)	(7)	(3)
Me-2	1	**1.00**	**1.00**	**1.00**	0	0	0	0	0	0	0
	2	0	0	0	0	0	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	3	0	0	0	**1.00**	**1.00**	0	0	0	0	0
	(n)	(4)	(4)	(3)	(5)	(4)	(7)	(9)	(10)	(7)	(3)
Mpi	1	0	0	0	0	0	**1.00**	**1.00**	**1.00**	**1.00**	0
	2	0	0	0	0	**1.00**	0	0	0	0	0
	3	**1.00**	**1.00**	**1.00**	**1.00**	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	**1.00**
	(n)	(4)	(4)	(3)	(6)	(4)	(2)	(2)	(4)	(2)	(3)
Pgd	1	0	0	0	0	**1.00**	0	0	0	0	0
	2	0	0	0	**1.00**	0	0	0	0	0	0

Table 2. Continued.

		Species									
Locus	Allele	ECU	PAL	DOM	BRE	PIC	NAS	NEG	PRO	ROB	PSA
	3	**1.00**	**1.00**	0	0	0	0	0	0	0	**1.00**
	4	0	0	0	0	0	**1.00**	**1.00**	**1.00**	**1.00**	0
	5	0	0	**1.00**	0	0	0	0	0	0	0
	(n)	(6)	(6)	(3)	(5)	(5)	(6)	(9)	(11)	(8)	(3)
Pgi	1	0	**1.00**	0	0	0	0	0	0	0	0
	2	**1.00**	0	0	0	0	0	0	0	0	0
	3	0	0	0	**1.00**	0	0	0	**0.92**	**1.00**	0
	4	0	0	0	0	**1.00**	0	0	0	0	0
	5	0	0	**1.00**	0	0	0	0	0	0	**1.00**
	6	0	0	0	0	0	**1.00**	**1.00**	0.08	0	0
	(n)	(15)	(16)	(3)	(5)	(18)	(9)	(11)	(12)	(8)	(3)
Pgm	1	0	0	0	0	0	0	0	**1.00**	**1.00**	0
	2	0	0	0	0	0	0	0	0	0	**1.00**
	3	0	0	**1.00**	0	0	0	0	0	0	0
	4	**1.00**	0	0	**1.00**	0	**1.00**	**1.00**	0	0	0
	5	0	0	0	0	**1.00**	0	0	0	0	0
	6	0	**1.00**	0	0	0	0	0	0	0	0
	(n)	(6)	(6)	(3)	(5)	(5)	(6)	(9)	(9)	(5)	(3)

Among members of the *R. prolixus* group, no diagnostic locus (*sensu* Ayala, 1983) was detected between *R. prolixus* and *R. robustus*, and only one (*α-Est*) out of 22 loci examined was found to separate *R. nasutus* from *R. neglectus*. However, these species pairs were distinguishable at five loci (*α-Est*, *α-Gpd*, *Me-1*, *Pgi*, *Pgm*).

Discussion

Placement of *P. tertius* as the sister taxon of the *R. prolixus* group, based on allozyme data (Fig. 2), agrees with mitochondrial and nuclear DNA sequence data (Lyman *et al.*, 1999; Monteiro *et al.*, 2000) and supports the idea that *Rhodnius* is paraphyletic. This indicates that the morphological characters (mentioned in the Introduction) currently used to separate *Psammolestes* from *Rhodnius* are unlikely to be derived (apomorphies), and are therefore of little phylogenetic value. *Rhodnius paraensis* has been considered to be one of the most primitive species of *Rhodnius*, because it shares with *Psammolestes* the short head and stout legs and antennae (Lent & Wygodzinsky, 1979). However, should *Psammolestes* be placed within *Rhodnius*, the short head and appendages could in fact represent derived characters and thus make *R. paraensis* a good candidate for a sister species of *Psammolestes*. Unfortunately, due to its rarity, *R. paraensis* has not yet been subjected to molecular analyses. Another possible indication of the close genetic relationship between *Psammolestes* and the *R. prolixus* group comes from ecological observations. All three species of *Psammolestes* are associated with birds (Funariidae and Psittacidae); *R. prolixus* and *R. nasutus* have also been found in bird nests (Dujardin *et al.*, 2000) and *R. neglectus* occasionally colonizes nests of Funariidae (Lent & Wygodzinsky, 1979).

To date, the only study comparing Rhodniini species based on nuclear markers (allozymes) used a cladistic approach, and was unable to resolve the position of *Psammolestes* (Dujardin *et al.*, 1999). Initially, using *Triatoma infestans* as the outgroup, we tried the same approach, hoping that the identification and exclusion of symplesiomorphies would reduce the 'phylogenetic noise' in the study. In consequence of the deep splitting between Triatomini and Rhodniini (Monteiro *et al.*, 2001), however, no shared alleles were found between the ingroup and the outgroup at any of the loci tested (data not shown), precluding the use of a cladistic approach. Distance-based approaches generally rely on the assumption that if the data set is big enough, the informative characters will outnumber the homoplasies, and thus lead to a good estimate of the relationship between the examined taxa (Ridley, 1993). This is a weakness of the present study, as for all 10 species we obtained reliable resolution for only 11 out of 21 enzymes examined (Fig. 1). Nevertheless, the close similarity between our results and published data based on other markers, suggests that the small number of loci used was not a critical factor. The fact that only very few colony specimens were analysed for some loci could also be a problem. Alleles may become fixed or be lost very rapidly as a consequence of the colonization process distorting the real relationship between species. However, the very low proportion of polymorphic loci in natural populations of triatomines (Harry *et al.*, 1992; Pereira *et al.*, 1996; Dujardin *et al.*, 1998; Noireau *et al.*, 1998) minimizes this problem, as genetic drift can only produce detectable changes when there is gene variation.

Rhodnius pictipes appears to be the most basal species of its cluster (comprising *brethesi*, *ecuadoriensis*, *pallescens* and *pictipes*: Fig. 2), instead of forming a pair with *R. brethesi* as suggested by other allozyme and DNA work (Chavez

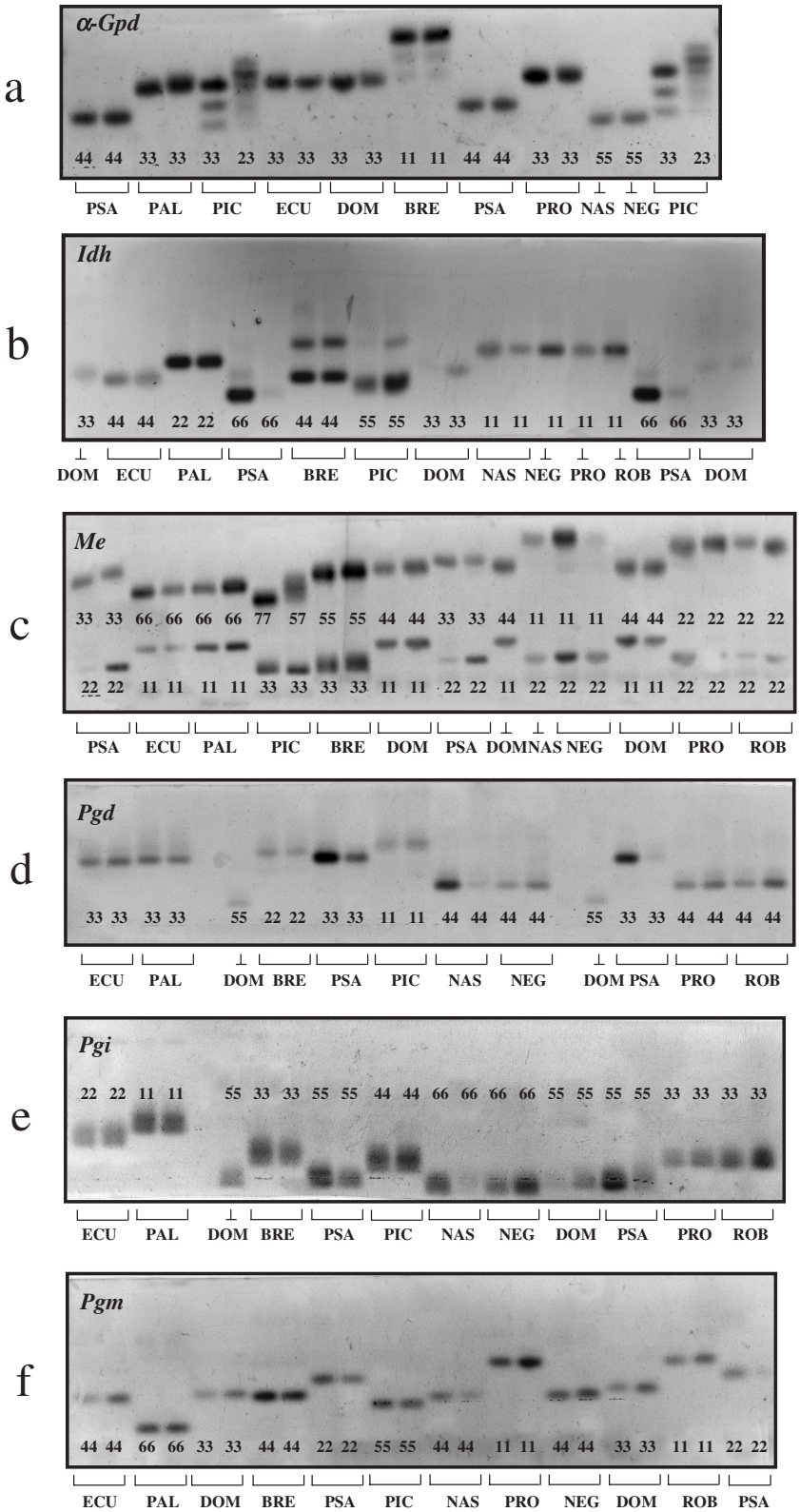

Fig. 1. Agarose gels showing the allozyme banding pattern and individual genotypes for the enzymes α-GPD, IDH, ME, PGD, PGI and PGM (a, b, c, d, e and f, respectively) of 10 species of Rhodniini (see Table 1 for species name code).

Table 3. Unbiased genetic identities (*I*, above diagonal; *not calculated) and genetic distances (*D*, below diagonal) (Nei, 1978) between 10 species of Rhodniini analysed.

Population	1	2	3	4	5	6	7	8	9	10
1. *R. ecuadoriensis*	xxxxx	0.583	0.254	0.250	0.093	0.167	0.167	0.168	0.167	0.167
2. *R. pallescens*	0.539	xxxxx	0.254	0.083	0.168	0.000	0.000	0.084	0.083	0.083
3. *R. domesticus*	1.372	1.372	xxxxx	0.085	0.084	0.239	0.239	0.326	0.324	0.239
4. *R. brethesi*	1.386	2.485	2.471	xxxxx	0.107	0.083	0.083	0.077	0.083	0.000
5. *R. pictipes*	2.373	1.784	2.481	2.238	xxxxx	0.000	0.000	0.083	0.083	0.000
6. *R. nasutus*	1.792	*	1.429	2.485	*	xxxxx	1.000	0.678	0.667	0.333
7. *R. neglectus*	1.792	*	1.429	2.485	*	0.000	xxxxx	0.678	0.667	0.333
8. *R. prolixus*	1.785	2.478	1.120	2.565	2.488	0.388	0.388	xxxxx	1.000	0.336
9. *R. robustus*	1.792	2.485	1.127	2.485	2.495	0.405	0.405	0.000	xxxxx	0.333
10. *P. tertius*	1.792	2.485	1.429	*	*	1.099	1.099	1.092	1.099	xxxxx

et al., 1999; Dujardin *et al.*, 1999; Lyman *et al.*, 1999; Monteiro *et al.*, 2000). This could be a consequence of a random increase in frequency of rare alleles due to genetic drift. The fact that *R. pictipes* presents, in low frequency, two alleles that are also shared with *R. brethesi* (Idh^4 and $Me\text{-}1^5$, Table 2) could be an indication of this process. These two species present sub-bands of α-GPD (Fig. 1), further indicating their similarity.

Previous workers (Harry *et al.*, 1992; Solano *et al.*, 1996) could not find diagnostic allozyme loci between *R. prolixus* and *R. robustus*, nor between *R. neglectus* and *R. nasutus*. From this lack of differentiation, Harry (1993) inferred conspecificity, whereas Solano *et al.* (1996) took it to show recent speciation arising from ecological separation. It has also been suggested, for the *prolixus*/*robustus* pair, that the lack of diagnostic loci could be due to misidentification of samples (Garcia *et al.*, 1998; Stothard *et al.*, 1998). However, sequence analyses of these four taxa have shown that they are genetically very different, with pairwise distances ranging from 0.064 to 0.111 (Kimura 2-parameter distance for a cytochrome *b* gene fragment) and should thus be regarded as distinct species (Monteiro *et al.*, 2000). In order to shed light on the issue of high allozymic similarity within the two species pairs, we here analyse samples from the same laboratory colonies used by Monteiro *et al.* (2000). Surprisingly, given the high levels of sequence divergence reported previously, we were unable to detect any diagnostic locus between the *prolixus*/*robustus* pair, and found only one diagnostic locus (α-*Est*) between the *nasutus*/*neglectus* pair, among 22 loci examined. This finding supports the hypothesis of recent speciation (Solano *et al.*, 1996). High allozyme similarity in spite of significant divergence of mitochondrial genes has been observed in other arthropods (Langor & Sperling, 1997; Gusmão *et al.*, 2000), indicating that allozymes might sometimes be too conserved to detect recent speciation events, particularly when levels of gene variation are low. Other molecular markers, such as single-strand conformational polymorphism (SSCP) and randomly amplified polymorphic DNA (RAPD), have also been used to investigate similarity between these species. Although SSCP could not distinguish *R. nasutus* from *R. neglectus* (Stothard *et al.*, 1998), preliminary data on RAPD seem to indicate its

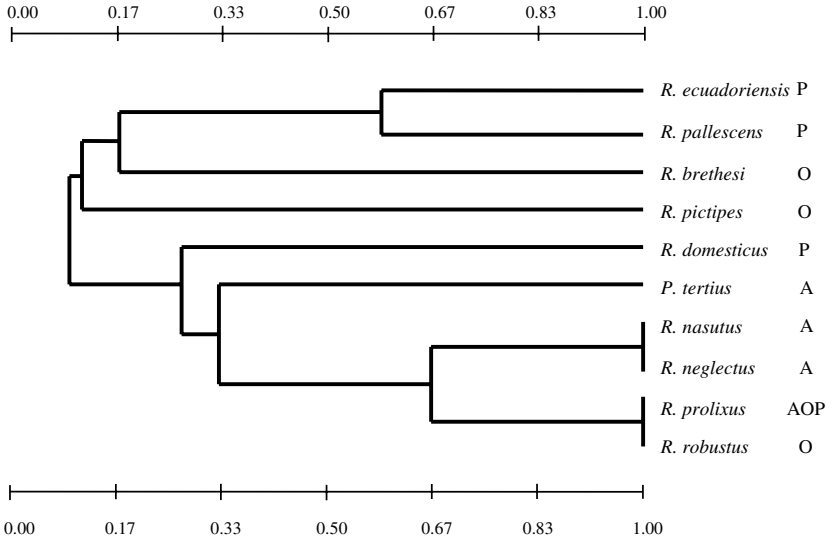

Fig. 2. UPGMA dendrogram for 10 species of Rhodniini, based on genetic identity values (Nei, 1978) of 12 loci (not including the extra 10 loci analysed for *Rhodnius prolixus* group, see text). A, Atlantic; P, Pacific; O Amazon-Orinoco.

usefulness in separating all four species (Garcia *et al*., 1998). Alternatively, non-molecular approaches such as electrophoresis of salivary heme proteins have also distinguished *R. prolixus* from *R. robustus*, and *R. nasutus* from *R. neglectus* (Soares *et al*. 1998, 2000). These findings and their interpretation are of public health importance, as they will ultimately be used to guide vector-control campaigns. For example, the specific distinction between *R. prolixus* and *R. robustus* has operational significance because, in areas where the former occurs in houses and the latter occurs in nearby palms, vector control based on insecticide treatment of infested houses would be effective without re-colonization of treated houses by sylvatic *R. robustus* (Monteiro *et al*., 2001). Finding *R. robustus*-infested palms near villages does not apparently pose a risk of domestic *Rhodnius* infestation.

It has been suggested that there is good correlation between the present geographical distribution of species belonging to the tribe Rhodniini in South America and their phylogenetic relationships (Chavez *et al*., 1999; Dujardin *et al*., 1999; Schofield & Dujardin, 1999). Allozyme data presented here support that hypothesis, showing a 'Pacific group' occurring west of the Andes, represented by *R. ecuadoriensis* and *R. pallescens*; an 'Amazon-Orinoco group' including *R. brethesi* and *R. pictipes*, and an 'Atlantic group' with *R. domesticus*, *R. nasutus* and *R. neglectus*, plus *P. tertius* in central Brazil. The only species not fitting these geographical groups are the pair *R. robustus* and *R. prolixus*, which are genetically closer to the 'Atlantic group' and spread around the Amazon-Orinoco region: *R. robustus* in palm trees and the primarily domestic *R. prolixus*. The latter's widespread distribution across northern South America and in parts of Central America may be readily attributed to passive transport by man. This study confirms the lack of diagnostic allozymes for separating *R. prolixus* from *R. robustus*, despite the evidence above for their recognition as distinct species derived from the Atlantic lineage of paraphyletic Rhodniini.

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