

THEORIES ON THE EVOLUTION OF *Rhodnius*TEORÍAS SOBRE LA EVOLUCIÓN DE *Rhodnius*Christopher J. Schofield¹ and Jean-Pierre Dujardin²**Abstract**

Current methods of Chagas disease control rely mainly on elimination of the domestic vector populations. Domestication of these insects has clearly been a recent event in evolutionary terms, associated with a series of genetic and phenetic changes, and this paper reviews current knowledge about the sequence of events leading to the domestication of species of *Rhodnius* that are important vectors of Chagas disease in the Andean pact and Central American countries. Available evidence suggests that species of *Rhodnius* have radiated from an ancestral source in the Amazon region, giving three main adaptive lines: southwards into the cerrados of Brazil, northwards into the llanos of Venezuela, and northwestwards through the Andean Cordillera into the Magdalena valley of Colombia. There has also been specialisation within the Amazon forest itself. The form of radiative adaptation is predicted from morphological and biogeographic characters, and subsequently supported by a series of morphometric and genetic markers including mtDNA sequence analysis.

Key words: Chagas disease, *Rhodnius*, *Psammolestes*, evolution, population genetics.

Resumen

Actualmente, el control de la enfermedad de Chagas depende principalmente de la eliminación de las poblaciones de vectores domésticos. La domesticación ha sido muy reciente en términos evolutivos y está asociada con cambios fenéticos y genéticos. Este trabajo revisa el conocimiento actual sobre los eventos involucrados en la domesticación de las especies de *Rhodnius* importantes como vectores de la enfermedad de Chagas en los países del Pacto Andino y América Central. Las evidencias indican que las especies actuales de *Rhodnius* radiaron desde una fuente ancestral en la región amazónica y produjeron tres líneas evolutivas principales: al sur, hacia la región del Cerrado brasilero; al norte, hacia los llanos venezolanos; y al noroeste, pasando la cordillera de los Andes hacia el valle del Magdalena en Colombia. Simultáneamente se produjeron especializaciones en la selva amazónica. La forma de radiación adaptativa está indicada por factores morfológicos y biogeográficos, y se apoya en estudios de varios marcadores morfométricos y genéticos incluyendo análisis de secuencias de DNA mitocondrial.

Palabras clave: enfermedad de Chagas, *Rhodnius*, *Psammolestes*, evolución, genética poblacional.

INTRODUCTION

The genus *Rhodnius* is one of seventeen genera comprising the subfamily Triatominae (Hemiptera: Reduviidae) —notorious as blood-sucking household pests and vectors of Chagas disease throughout Latin America. Because of their epidemiological importance, control of Triatominae has become a public health priority in affected regions, with three multinational initiatives launched

to eliminate the most domesticated species. The Southern Cone Initiative launched in 1991 involves Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay and (since 1996) southern Perú, in a regional control programme directed primarily at the eradication of domestic and peridomestic populations of *Triatoma infestans* (Schofield and Dias, 1998). The Central American Initiative, primarily involving El Salvador,

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Guatemala, Honduras and Nicaragua, was launched in 1997 with the initial aim of eradicating *Rhodnius prolixus* and suppressing populations of *T. dimidiata*, while the Andean Pact Initiative—including Colombia—has similar aims (WHO, 1997).

In Central America and the Andean Pact countries, species of *Rhodnius* are the primary targets of the Chagas disease vector control initiatives—particularly *R. prolixus*, but also *R. ecuadoriensis* in parts of Ecuador and northern Perú, and *R. pallescens* in Panamá and parts of Colombia. Other species of *Rhodnius* have local epidemiological importance, including *R. neglectus* and *R. nasutus* in central and northeastern Brazil, *R. stali* in Bolivia, and *R. brethesi* in some parts of the Brazilian Amazon. This review is therefore designed to summarise current knowledge about the distribution and importance of *Rhodnius* species, and to propose a general theory about the evolution of the genus. The theory is based first on phenetic characters and biogeography, and then tested as far as possible with reference to recent genetic studies.

TAXONOMY AND DISTRIBUTION

The genus *Rhodnius* was erected by Stal (1859) to contain two species, *prolixus* and *nasutus*, distinguished from other Triatominae mainly by their longer heads and apical insertion of the antennae. By 1979, twelve species had been included in the genus (Lent and Wygodzinsky, 1979) with a further species added by Lent *et al.* (1993). The thirteen currently described species are grouped with the three species of *Psammolestes* to form the tribe Rhodniini (table 1) which is customarily considered to be monophyletic (Lent and Wygodzinsky, 1979; Schofield, 1994; Schofield and Dolling, 1993). Similarities between these two genera, which are not shared with other Triatominae, include the apically inserted antennae and general body form, post-ocular callosities (Lent and Wygodzinsky, 1979), characters of the male genitalia (such as lack of phallosome supports—except in *R. pictipes* and the closely-related *R. stali*) (Lent and Jurberg, 1969; Jurberg, 1996), and the presence of nitrophorins giving a characteristic red colour to the salivary glands

(Soares *et al.*, 1998). *Rhodnius* and *Psammolestes* are also of primarily arboreal habit, compared to the terrestrial habit of most other Triatominae.

At its zenith, the genus *Rhodnius* was distributed from southern México to southern Brazil, although for various reasons (see below) it seems to be diminishing in many parts of Central America. The current distribution of species is given in synoptic form in table 1, although there are doubts about the distribution of several species—especially *R. prolixus*, which appears to have been misidentified on some occasions (Dujardin *et al.*, 1991). Part of the confusion arises from the close morphological similarity between *prolixus*, *robustus*, *neglectus* and *nasutus*—grouped by Barrett (1991) as the 'prolixus complex'. As Barrett (1996) points out, the original description of *R. robustus* (Larrousse, 1927) is hardly diagnostic, being based on two females considered larger and darker than a reference collection of *prolixus*. Moreover, populations of *robustus* from Perú were shown to be fully inter-fertile with authentic domestic populations of *prolixus* from Honduras, Colombia and Venezuela (Barrett, 1996) and no consistent morphometric differences could be found between these populations (Hurtado Guerrero, 1992). Harry (1992, 1993a) also found a lack of morphometric differentiation between a *robustus* population from a palm tree in Trujillo, Venezuela, and domestic populations of *prolixus* from Trujillo and other states of Venezuela; she also found a lack of isoenzyme differentiation or reproductive isolation between these populations (Harry *et al.*, 1992; Harry, 1993b). Isoenzymic similarity and a lack of reproductive isolation between *robustus* and *prolixus* was also shown by Solano *et al.* (1996). Thus, although *R. prolixus* may be conveniently described as a domestic species in Central America, Venezuela and Colombia, its relationship with morphologically similar populations in palm trees in the latter countries remains unclear.

Prior to Lent (1954), the distribution of *prolixus* was also considered to include large regions of the cerrado of central and southern Brazil. The cerrado form was then distinguished as *R. neglectus* by Lent

(1954), although there are more recent reports of *prolixus* from Tocantins (northern Goiás) in the southern Amazon region of Brazil (Diotaiuti *et al.*, 1984), and also from Teresópolis in the state of Rio de Janeiro, Brazil (Pinho *et al.*, 1998). Descendants of the Tocantins (Tucuruí) collection were shown to have consistently different salivary nitrophorin profiles compared to populations of *R. prolixus* from Venezuela and Honduras, but these profiles were also unlike those of a population of *R. robustus* from Venezuela (Soares *et al.*, 1998). Morphometric examination of specimens from the Teresópolis collection (courtesy of dra. Ana Maria Jansen) shows them to be consistent with the general morphometric profile of *R. prolixus* from other areas (Dujardin *et al.*, unpublished) and partial sequencing of the cytochrome-B gene also reveals no significant

differences between the Teresópolis specimens and other populations of *R. prolixus*, although these populations do differ from *R. robustus* (F. Monteiro, personal communication).

The distribution of *R. prolixus* in the Guyanas is also unclear. Although *prolixus* has been reported from Guyana (Haviland, 1931) and more recently from French Guiana (Chippaux *et al.*, 1985; Didier *et al.*, 1989) these determinations were based on overt morphology and cannot be assumed to exclude other species such as *R. robustus*. Moreover, since true domestic populations of *Rhodnius* have not yet been described in the Guianas, the presence of *R. prolixus* in this region must currently be considered as unconfirmed (WHO, 1988, 1991).

Table 1. Synopsis of the tribe Rhodniini

Tribe **RHODNIINI** Pinto, 1926

Genus **PSAMMOLESTES** Bergroth, 1911

Ps. arthuri (Pinto, 1926)

holotype F, allotype M: FIOCRUZ, Rio de Janeiro

type locality: Venezuela (no further details given)

Synonyms: *Eutriatoma arthuri* Pinto, 1926

Ps. salazari Aldana *et al.*, 1996 (cf. Lent, 1997)

Distribution: Colombia, Venezuela

Biology: (S, bird nests) Tc

Ps. coreodes Bergroth, 1911

type: Universitats Zoologiska Museum, Helsinki

type locality: unknown

Distribution: Argentina (Catamarca, Chaco, Córdoba, Corrientes, Entre Ríos, Formosa, Jujuy, La Rioja, Salta, Santa Fe, Santiago del Estero, Tucumán), Bolivia, Paraguay

Biology: (S, bird nests)

Ps. tertius Lent & Jurberg, 1965

holotype M, allotype F, paratypes: FIOCRUZ, Rio de Janeiro

type locality: Carmo de Rio Claro, Minas Gerais, Brazil

Synonym: *P. coreodes* Costa Lima, 1934

Distribution: Brazil (Bahia, Ceará, Goiás, Mato Grosso)

Biology: (S, bird nests) Tc

Genus **RHODNIUS** Stal, 1859

R. brethesi Matta, 1919

type: unknown

type locality: Amazonas, Brazil (from piassaba palm)

Distribution: Brazil (Amazonas, Pará), Colombia, Venezuela

Biology: (S, piassaba palms) Tc

R. dalessandroi Carcavallo & Barreto, 1976

holotype M, allotype F, paratypes: FIOCRUZ, Rio de Janeiro

type locality: Hacienda Guarapito, Barbasal, El Meroy, San Martín, Prov. Meta, Colombia

Distribution: Colombia

Biology: (S) This species was considered of doubtful validity by Lent and Wygodzinsky (1979) although its identity has been defended by Martínez (1984)

R. domesticus Neiva & Pinto, 1923

holotype F, paratypes MM: FIOCRUZ, Rio de Janeiro

type locality: Angra dos Reis, Rio de Janeiro, Brazil

Distribution: Brazil (Bahia, Espirito Santo, Rio de Janeiro, Paraná, Santa Catarina, São Paulo)

Biology: (S, bromeliads) Tc

R. ecuadoriensis Lent & León, 1958

holotype M, allotype F, paratypes MF: FIOCRUZ, Rio de Janeiro

type locality: La Toma, Valle de Catamayo, Prov. de Loja, Ecuador

Synonym: *R. pallescens* Cornejo, 1958 (not Barber)

Distribution: S. Colombia, Ecuador, N. Perú

Biology: (S palms, P,D) Tc

R. nasutus Stal, 1859

type: Zoologisches Museum, Berlin

type locality: Rio Grande do Norte, Brazil

Synonym: *R. brumpti* Pinto, 1925 (holotype M, paratypes MF: FIOCRUZ, Rio de Janeiro)

Distribution: Brazil (Ceará, Piauí, RGN)

Biology: (S palms and birdnests, P,D) Tc

R. neglectus Lent, 1954

holotype M, allotype F, paratypes MF: FIOCRUZ, Rio de Janeiro

type locality: Fazenda Aguas Limpas, Uberaba, Minas Gerais, Brazil

Distribution: Brazil (Bahia, DF, Goiás, Mato Grosso, Minas Gerais, Paraná, São Paulo)

Biology: (S palms, P,D) Tc

R. neivai Lent, 1953

holotype F: FIOCRUZ, Rio de Janeiro

type locality: Camacaro, C.Parajara, Est. Lara, Venezuela

Distribution: Colombia, Venezuela

Biology: (S)

R. pallescens Barber, 1932

type: National Museum of Natural History, Smithsonian Institution, Washington DC

type locality: Panamá (no further details given)

Synonym: *R. durni* Pinto, 1932 (holotype F, paratype F: FIOCRUZ, Rio de Janeiro)

Distribution: Colombia, Panamá

Biology: (S,P, palms, D) Tc

R. paraensis Sherlock, Guitton & Miles, 1977

holotype M, allotype F: FIOCRUZ, Rio de Janeiro

type locality: Utinga, Belem, Pará, Brazil (in nests of *Echinmys*)

Distribution: Brazil (Pará)

Biology: (S arboreal rodent nests) Tc

***R. pictipes* Stal, 1872**

type: Naturhistoriska Riksmuseet, Stockholm

type locality: Amazonas, Brazil

Synonyms: *Conorhinus limosus* Walker 1873 (part)

(type: Natural History Museum, London)

Rhodnius amazonicus Almeida, Santos & Sposina, 1973

(type: INPA, Manaus, Brazil)

Distribution: Bolivia, Brazil (Amazonas, Goiás, Mato Grosso, Pará), Colombia, Ecuador, Fr. Guiana, Guyana, Perú, Suriname, Trinidad, Venezuela.

Biology: (S, palms, L) Tc

***R. prolixus* Stal, 1859**

type: Zoologisches Museum, Berlin

type locality: La Guayra, Venezuela

Synonym: *Conorhinus limosus* Walker, 1873 (part) (type: Natural History Museum, London)

Distribution: Colombia, Guatemala, Honduras, Nicaragua, El Salvador, Venezuela

[note: *R. prolixus* has also been recorded from parts of Costa Rica, Fr. Guiana, Guyana, Suriname, and México (Chiapas, Oaxaca), but now seems to be largely absent from these regions]

Biology: (S,P,D) Tc

***R. robustus* Larrousse, 1927**

type: Faculté de Médecine, Paris

type locality: Cayenne, Fr. Guiana

Distribution: Brazil (Amazonas, Pará), Colombia, Ecuador, Fr. Guiana, Perú, Venezuela

Biology: (S, palms & Bromeliads) Tc

***R. stali* Lent, Jurberg & Galvão, 1993**

holotype M, allotype F: FIOCRUZ, Rio de Janeiro

type locality: Salobra, Mato Grosso, Brazil

Synonym: *R. pictipes* Stal, 1872 (part)

Distribution: Bolivia, Brasil (Mato Grosso)

Biology: (S,P,D) Tc

S: silvatic; P: peridomestic; D: domestic; Tc indicates natural infection with *Trypanosoma cruzi* has been reported.Note: *R. jacundaensis* Serra *et al.*, 1980, is declared a *nomen nudum* under Article 9 of the International Code of Zoological Nomenclature (cf. Carcavallo *et al.*, 1998).**EVOLUTION OF THE RHODNIINI**

Our working hypothesis considers the Triatominae to represent a polyphyletic assemblage of predatory reduviids that have adapted towards particular habitats offering shelter from climatic extremes together with a more abundant supply of proteinaceous food. First discussed by Schofield (1988), this idea has since received support from a number of studies that highlight the differences between the two main tribes of Triatomini and Rhodniini. Not only do these tribes diverge in terms of overt morphology (Lent and Wygodzinsky, 1979),

male genitalia (Jurberg, 1996), eggshell architecture (Barata, 1996), and antennal sensilla patterns (Catalá and Schofield, 1994; Catalá, 1997), they also diverge significantly in terms of biochemical markers such as isoenzymes (Dujardin *et al.*, 1999), cuticular hydrocarbons (Juárez, 1996), salivary proteins (Pereira *et al.*, 1997; Soares *et al.*, 1998) and RAPD banding patterns (García *et al.*, 1998). Partial sequencing of mitochondrial DNA genes also supports the separation of Triatomini and Rhodniini into two distinct clades (Stothard *et al.*, 1998; Lyman *et al.*, 1999) and their 18S ribosomal gene sequences indicate that their ancestral forms probably diverged

long before development of haematophagy (A. Marcilla *et al.*, unpublished). In fact, the Triatomini and Rhodniini appear to derive from different reduviid lineages, and now have little in common other than their basic reduviid form overlaid with convergent characters associated with their bloodsucking habit.

In primitive terms, we can envisage an adaptive process from a free-living predatory form to a nest-dwelling haematophagous form, and we can suppose that this has happened several times within the Reduviidae to give rise to the various tribes of Triatominae. Such a trend has been associated with various morphological, biochemical and reproductive changes, but can also be envisaged as a typical evolution of demographic strategy (*sensu* Rabinovich, 1974) from *r*-strategists (free-living predators adapted to relatively unstable conditions of habitat and food-supply) to *K*-strategists (nest-dwelling predators exploiting a more stable habitat and food-supply) (Schofield and Dolling, 1993). Similar adaptations are thought to have occurred in other Hemipteran families, for example amongst the predatory Anthocoridae to give rise to the haematophagous Cimicidae and Polycetenidae (see Leston *et al.*, 1954; Schuh and Slater, 1995), and also amongst the predatory Lygaeidae to give rise to the Cleradini, of which several species seem to be at least facultatively haematophagous (Lent, 1939; Harrington, 1990; Torres *et al.*, 1999). However, the fact that Triatominae do not appear to have evolved in Africa, and remain very similar to their original predatory form, suggests that Triatominae have evolved much later than the Cimicidae (Gorla *et al.*, 1997).

Within the Triatominae there are several lines of evidence to suggest that this adaptive process has been relatively recent. The absence of autochthonous species from Africa (Gorla *et al.*, 1997) dates them post continental separation, and they could not have followed our proposed evolutionary route before the advent of nest-building mammals and birds in the neotropics, which already places them in the post-cretaceous period. Their frequent association with rodents would suggest them to have evolved well after the mid tertiary period and, from their relative lack of divergence from the basic reduviid form, we see no reason to suggest that they would have evolved even prior to the quaternary period. Apart from characters directly associated with their blood-sucking habit, such as mouthparts, digestive system, and sensilla patterns, their body form is little changed from that of their putative reduviid ancestors. Several are still known to feed facultatively from invertebrates, and species of Bolboderini and at least one species of Triatomini —*Eratyrus mucronatus*— seem to preferentially feed on invertebrates during the earlier developmental stages (Miles *et al.*, 1981; M. Sandoval and V. M. Angullo, unpublished). The bite of many Triatominae remains painful to the vertebrate host (cf Ryckman and Bentley, 1979) which recalls the painful bite of most predatory reduviids. Also, like all obligate bloodsuckers, Triatominae require intestinal symbionts to provide essential vitamins that are lacking in their blood diet, but while other obligate blood-suckers carefully store their symbionts either intracellularly or in a special organ —the mycetome— the symbiotic fauna of Triatominae is inconsistent and lives haphazardly in the gut lumen (table 2).

Table 2. Location of intestinal symbionts of obligate blood-sucking insects (excluding facultative blood-sucking groups, and those that make use of other fluids as well as vertebrate blood)

In specialised Mycetome	Intracellular	Free in intestinal lumen
Nycteribiidae	Glossinidae	Triatominae
Hippoboscidae	Streblidae	
Cimicidae	Nycteribiidae	
Polycetenidae	Hippoboscidae	
Anoplura	Mallophaga	
Rhynchophthirina		

GENUS *Rhodnius*

Developing the evolutionary hypothesis for *Rhodnius*, we would suggest that the genus arose during the quaternary from a predatory form of reduviid occupying arboreal habitats in the Amazon-Orinoco rainforest. From overt morphological similarities, an ancestral form similar to extant Stenopodinae has been suggested (T.V. Barrett, personal communication). For such a predator, vertebrate nests in the region would offer shelter from climatic extremes together with more abundant supplies of invertebrate prey than could be captured by opportunistic ambush. Moreover, within the nests, the diet of invertebrate fluids could be supplemented by vertebrate blood – progressively more so as the organs involved in feeding on vertebrates became more appropriately adapted. Eventual adaptation to obligate haematophagy would be associated with several physiological and behavioural changes (Schofield, 1988) including greater reliance on the hosts for dispersal. This implies that the current species distribution of the genus would be strongly influenced by the distribution and migratory patterns of key host species.

Our hypothesis suggests that within the genus, the form closest to the original would be best represented by a geographically widespread and relatively generalist species such as *R. pictipes*. This species occupies a wide range of palm tree and bromeliad habitats from French Guiana to Colombia, and from Venezuela to Bolivia and Perú, and is frequently recorded flying into forest dwellings – presumably attracted by light. *R. pictipes* has the widest geographical distribution within the genus, and also shares characteristics of the male genitalia with other Triatominae and predatory reduviids. Phallosome supports for example, are generally absent in the Rhodniini, but present in *R. pictipes* and the closely related *R. stali*, and in most other species of Triatominae (Jurberg, 1996). *R. stali* is now a recognised form of *R. pictipes*, with which it was previously included (Lent *et al.*, 1993) which is found in palm tree crowns and occasionally invading peridomestic and domestic habitats in the southern part of the Brazilian Amazon into northeastern Bolivia (A. Matias *et al.*, unpublished).

Other species of *Rhodnius* within the Amazon region tend to be more highly specialised, and may be considered specialist derivatives from the *pictipes*-like ancestor. *R. brethesi* for example, is known only from Piassaba palms (*Leopoldinia piassaba*) in a restricted area covering the border region of Brazil, Venezuela, and Colombia. It is known locally as the piassaba louse ('piolho da piassaba') and has been recorded flying to attack palm nut collectors (Coura *et al.*, 1994). *R. dalessandroi* shares some similarities with *R. brethesi* (Martínez, 1984) and appears restricted to a few palm stands in the forests of eastern Colombia. *R. neivai* is known from fallen tree trunks and from the crowns of 'palma de llanera' (*Copernicia tectorum*) over a limited area of central Venezuela into northern Colombia, and *R. paraensis* is known only from nests of the arboreal rodent *Echimy*s in the state of Pará, Brazil (Sherlock *et al.*, 1977).

From the Amazon forest region, there appear to have been three main radiations of *Rhodnius* species towards the surrounding savannas – to the north into the llanos of Venezuela, to the northwest into northern Colombia, and to the south into the cerrados of Brazil. For both the northern and southern route the result seems to be represented by palm-tree dwelling species similar to *R. robustus*. Specimens determined as *R. robustus* have been recorded from various sites within the Amazon forest (eg. Miles *et al.*, 1983) and from the forest fringes of northern Brazil (states of Pará and Amazonas), French Guiana, Venezuela, Colombia, Ecuador, Perú and Bolivia. Morphologically similar specimens (generally determined as *R. prolixus*) have also been collected from palm tree habitats along the southern fringe of Amazonia, in the state of Goiás (now Tocantins) Brazil. It is far from clear that all these reports refer to the same taxonomic entity, but they are at least morphologically indistinguishable from *robustus*, and those that we have examined also conform to the general morphometric pattern that we attribute to this species. For the purposes of developing our hypothesis however, we refer to these as the northern form of *robustus* (specifically represented by palm tree populations from the llanos of Venezuela) and the southern form of *robustus* (specifically

represented by palm tree populations from the states of Tocantins and Pará, Brazil). We believe that the northern form of *robustus* has given rise to a domestic form, now known as *prolixus* (see below), and that the southern form has adapted to give rise first to *neglectus*, the common *Rhodnius* species of the *Acrocomia* and *Mauritia* palms of the Brazilian cerrados, and subsequently to *nasutus* which is common in the Carnauba palms (*Copernicia prunifera*) of the arid caatinga of northeastern Brazil. This, to a very large extent, would explain the close morphological similarity between the four species of the *prolixus* complex (*prolixus*, *robustus*, *neglectus* and *nasutus*). A further adaptation from *neglectus* into the coastal Atlantic forest of Brazil appears to have given rise to *R. domesticus*. In spite of its name, this species is exclusively found in bromeliad habitats of the coastal forest, and, unlike most other *Rhodnius* species, is both difficult to find and hard to rear in the laboratory (Guarnieri *et al.*, 1998).

The northwestern adaptation, rather than being a diffuse outflowing along the forest fringes, seems to have proceeded through the geographic bottleneck represented by the Sierra Nevada and the northern tip of the Andean Cordillera in Colombia. The result is *R. pallescens*, which is found in northwestern Colombia extending into Panamá and southern Costa Rica¹. This species has been found invading domestic and peridomestic habitats in parts of Colombia, and in Panamá (where it is considered the primary vector of Chagas disease), but it seems particularly associated with the wine palms (*Attalea butyracea*) that extend down the Magdalena valley of central Colombia into eastern Ecuador and Perú. As it has spread southwards however, it has given rise to a slightly different form (currently known as the Tolima form, after the locality of first capture (López and Moreno, 1995), and subsequently to *R. ecuadoriensis* in Ecuador and northern Perú. *R. pallescens*, the Tolima form, and *R. ecuadoriensis* are morphologically very similar, with an apparent decline in average size from north

to south. The final separation of *ecuadoriensis* may relate to its adaptation to tagua palms (*Phytolophas aequatorialis*) along the Pacific coast of Ecuador.

In summary, our biogeographical overview indicates the genus *Rhodnius* to conform broadly to a model of adaptive radiation from a discrete ancestral form in the Amazon-Orinoco region –represented today by *R. pictipes*. Three main adaptive lines are proposed: (1) within the forest itself, to give rise to specialised forms such as *brethesi* and *paraensis*, (2) through the Sierra Nevada bottleneck in northern Colombia to give the *pallescens*-Tolima-*ecuadoriensis* cline west of the Andean Cordillera, and (3) to the widespread *R. robustus* whose 'northern form' subsequently gives rise to the domesticated *prolixus*, and whose 'southern form' gives rise to *neglectus* and *nasutus*, and also *domesticus* as a secondary re-adaptation to the Atlantic forest.

GENUS *Psammolestes*

The three species of the genus *Psammolestes* do not fit readily to a model of adaptive radiation from a discrete ancestral form, because of the geographical divergence between *Ps. arthuri* in the llanos of Venezuela and Colombia, and *Ps. coreodes* and *Ps. tertius* in the chaco-cerrado-caatinga corridor of Argentina, Paraguay and Brazil. In essence, these three species form two geographical groups completely separated from each other by the expanse of the Amazon-Orinoco forest. They are morphologically very similar, suggestive of a common ancestor, and yet their geographical distribution is difficult to rationalise with the idea of a single ancestral form. All three are intimately associated with the woven stick nests of furnariids and similar birds, and eggs of *Psammolestes* have been encountered adhering to the feathers of such birds (J.M.S. Barata, personal communication –see figure 38 in Schofield, 1994). This at least suggests the possibility that contact between the two groups may have been provided through association with

¹ An alternative possibility is that *pallescens* may represent a vicariant population separated from the ancestral Amazonian form by the emergence of the Andean mountains during the Iaramide orogeny of the late tertiary. At present however, we regard this idea as less likely because of the clear morphological cline southwards from the Sierra Nevada.

migrating birds, but an alternative is raised by the proposal of independent evolution of *Ps. arthuri* from the 'northern form' of *R. robustus*, and of *Ps. tertius* from the 'southern form', with *Ps. coreodes* representing a subsequent and contiguous derivative from *tertius*. Such an idea provides for a similar ancestral form in spite of the wide geographic separation, and also predicts that *coreodes* and *tertius* would be genetically more similar to each other than either would be to *arthuri*. In addition, arguing that the step from southern *R. robustus* to *Ps. tertius* would be most likely to have occurred in the central cerrado of Brazil, we can additionally predict that populations of *tertius* from the arid northeastern caatinga of Brazil may also present differences – mirroring the differences between *R. neglectus* of the cerrado and *R. nasutus* of the caatinga. Recent morphometric studies indeed show a clear difference between *Ps. tertius* of the central Brazilian cerrado (Minas Gerais) and those of the northeastern caatinga (Ceará), although intermediate forms have yet to be examined (L. Diotaiuti and J.P. Dujardin, unpublished).

Our biogeographical hypothesis is summarised in figure 1, which attempts to show the predicted evolutionary lines within the Rhodniini in accordance with their geographical distribution. This hypothesis was originally sketched out through discussions during the early 1990s, but can now be examined in the light of more detailed genetic and morphometric studies.

BIOCHEMICAL AND GENETIC STUDIES

A wide range of techniques has been applied to the study of relationships within the Rhodniini. However, all have faced the similar problem of obtaining authentic reference specimens to represent each species. A particular problem has been the use of laboratory colonies which have sometimes been mislabelled, and sometimes appear to have included more than one species (cf. Dujardin *et al.*, 1991). Some techniques can make use of preserved specimens, but for those such as cytogenetics or isoenzyme analysis an additional problem is posed by the need for living material. Because of these problems, published analyses of the Rhodniini have

of necessity been partial, often lacking key populations, and we attempt here to synthesise the various results to see how far they support the biogeographical hypothesis advanced above.

Results from quantitative morphometry (Dujardin *et al.*, 1999) and isoenzyme analysis (Chávez *et al.*, 1999) consistently reveal the existence of three main groupings within the genus *Rhodnius*, corresponding to (1) *pictipes*, *stali*, and *brethesi*, (2) *neglectus*, *nasutus* and domestic *prolixus*, and (3) *pallescens*, *ecuadoriensis*, and the Tolima form (also described as 'sylvatic *prolixus*' by Chávez *et al.*, 1999). By morphometry (Dujardin *et al.*, 1999) and also by isoenzymes (J.P. Dujardin and T. Chávez, unpublished), *R. domesticus* and *R. neivai* are also clustered with *neglectus*, *nasutus*, and *prolixus*. Quantitative analysis of antennal sensilla patterns of six *Rhodnius* species also suggests similarities between *pictipes* and *ecuadoriensis*, and a 'clade' formed by the two pairs *neglectus* + *nasutus*, and *robustus* + *prolixus* (Catalá and Schofield, 1994). As yet however, none of these techniques has fully resolved the relationship between *Rhodnius* and *Psammolestes*, since cladistic analysis based on isoenzymes or morphometry gives a variable position for *Psammolestes* either within or external to the other Rhodniini.

Mitochondrial DNA sequences have provided enhanced resolution of the relationships of those species so far studied. Sequencing of the 16S small subunit rRNA gene of four *Rhodnius* species showed three groups: (1) *pictipes*, (2) *ecuadoriensis*, and (3) *prolixus* and *nasutus* (Stothard *et al.*, 1998). These three groupings were also revealed by analysis based on the large subunit rRNA gene and the cytochrome-B gene fragment of seven *Rhodnius* species: (1) *pictipes* + *brethesi*, (2) *pallescens* + *ecuadoriensis*, (3) *prolixus* + *neglectus* + *robustus* (Lyman *et al.*, 1999). This latter work has since been confirmed with a larger number of populations and adding D2 nuclear DNA sequence information (F. Monteiro and C.B. Beard, personal communication), showing that the Tolima form from Colombia clusters with *pallescens* and *ecuadoriensis*, and additionally placing *R. nasutus* close to *R. neglectus* and adding *R. domesticus* and *R. neivai* to the *robustus/prolixus*/

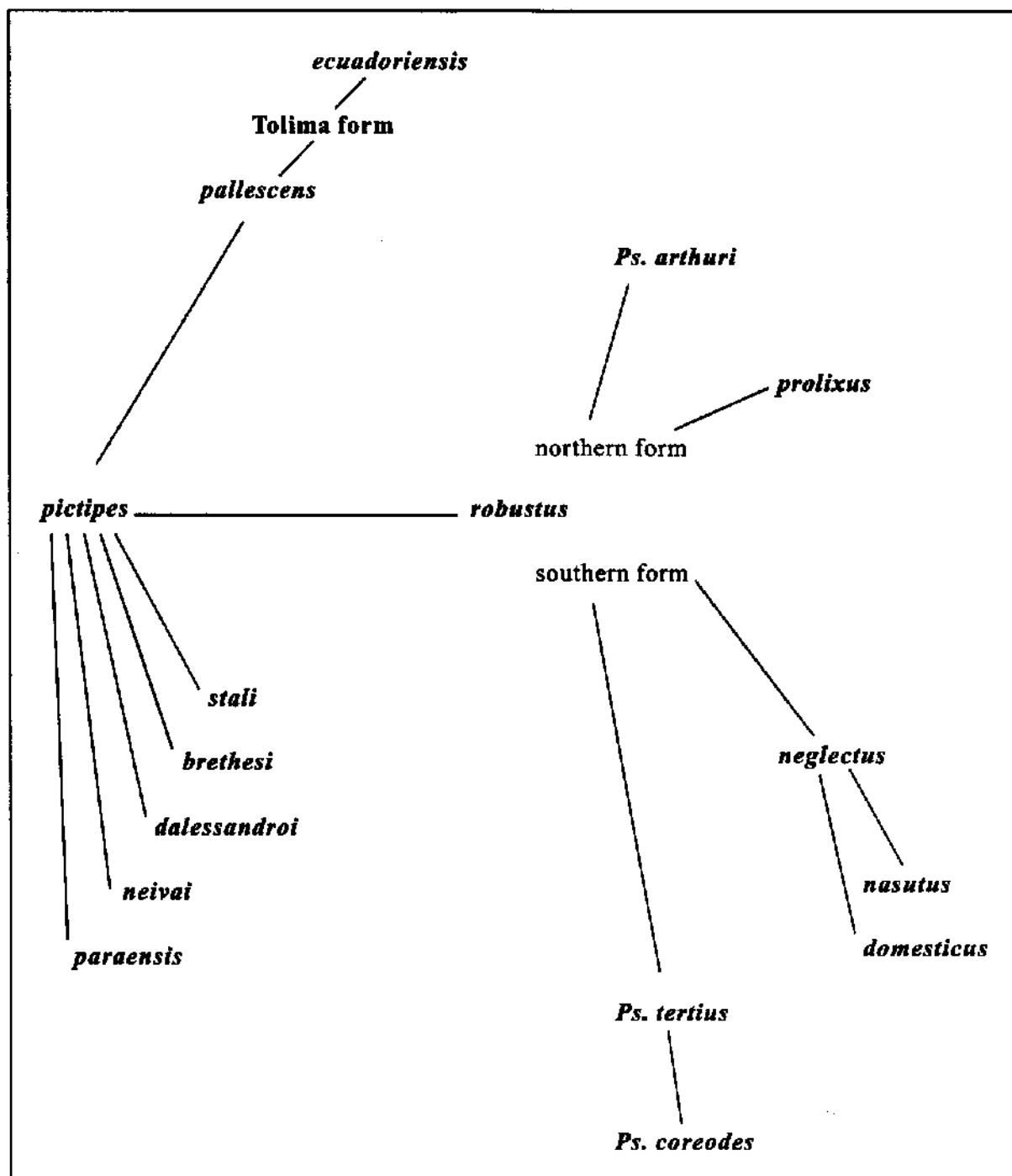


Figure 1. Diagrammatic summary of species relationships within the Rhodniini (including the three species of *Psammolestes*)

neglectus/nasutus clade (although bootstrap support for the placement of *R. neivai* was poor). The large subunit rRNA and cytochrome-B sequence analysis also placed *Psammolestes tertius* and *Ps. coreodes* at the base of the *robustus* clade (*Ps. arthuri* was not

yet sequenced) (Lyman *et al.*, 1999; F. Monteiro and C.B. Beard, personal communication).

As summarised in figure 2, these various studies lend unequivocal support to the idea of three main

evolutionary lineages within the Rhodniini, although none is as yet able to confirm the basal position of *R. pictipes* as suggested by its distribution and characteristics of the male genitalia (Jurberg, 1996). Full resolution of the robustus clade also awaits analysis of further populations, although recent RAPD analysis using populations of *R. robustus* from palm trees in Pará, Brazil, and also from palm trees in Cojedes, Venezuela, shows a clear distinction between the two, with a calculated genetic distance similar to that between domestic *R. prolixus* from Venezuela and silvatic *R. neglectus* from Brazil (M. Muñoz and J.P. Dujardin, unpublished). These results lend support to the idea that *R. robustus* populations show considerable variability that is particularly marked by comparison of the northern and southern forms. Analysis of salivary nitrophenols also indicates a clear distinction between the northern forms of *R. robustus* from Venezuela, and the southern forms from the state of Tocantins, Brazil, although this comparison unexpectedly places the southern forms closer to domestic *R. prolixus* (Soares *et al.*, 1998). Cytochrome-B gene sequence data indicate clusters of *robustus* in accordance with their geographic origin, although analysis of further populations is required to clarify these relationships (F. Monteiro and C.B. Beard, personal communication)

ORIGIN AND DISTRIBUTION OF *R. Prolixus*

The consistency of agreement between phylogenetic analyses based on several different techniques so far encourages the hypothesis that the genus *Rhodnius* originally evolved through radiative adaptation from a discrete Amazonian population. However, the process has clearly been modified in recent years through human influence, especially in the case of domestic *R. prolixus*.

R. prolixus has a wide but highly discontinuous distribution. It is common in rural houses in the llanos of Venezuela and eastern Colombia, and also along the central Magdalena valley of Colombia and adjoining valley spurs up to about 2,000 masl. It has never been recorded from northwestern Colombia, nor from Panamá or central Costa Rica. During the

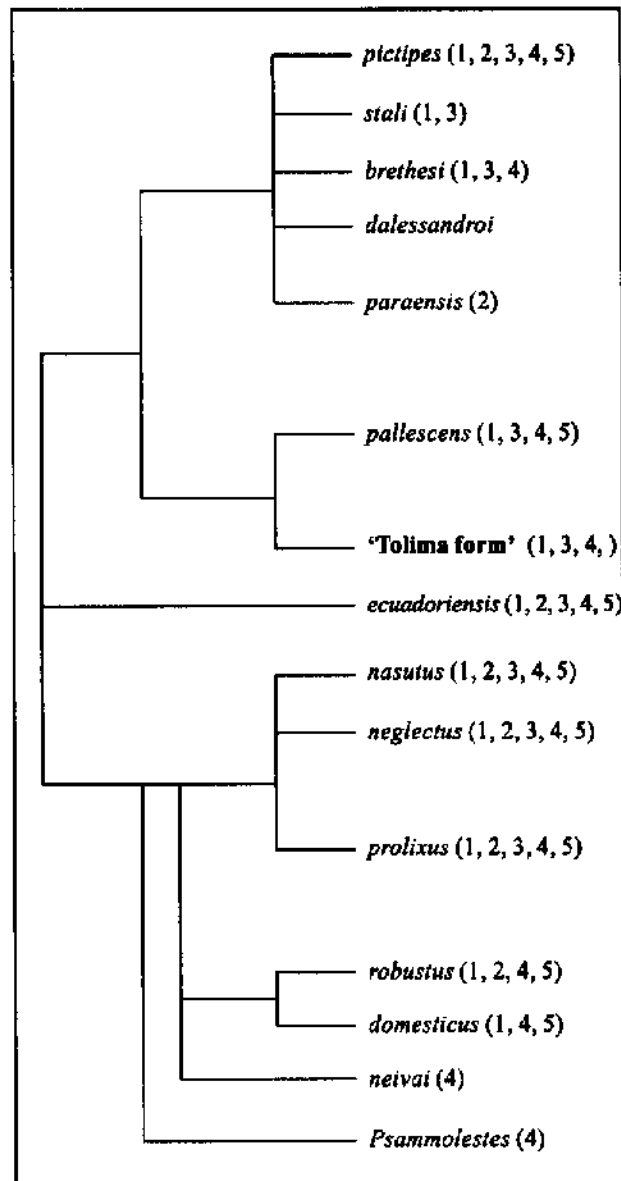


Figure 2. Clades within the Rhodniini, with relative positions supported by: (1) Morphometry (Dujardin *et al.*, 1999), (2) antennal sensilla patterns (Catalá and Schofield, 1994; S. Catalá, personal communication), (3) isoenzymes (Chávez *et al.*, 1999), (4) mtDNA sequence analysis (Stoithard *et al.*, 1998; Lyman *et al.*, 1999; F. Monteiro and B. Beard, personal communication), (5) RAPD analysis (García *et al.*, 1998; M. Muñoz, personal communication). Note that *R. dalessandroi* has not yet been analysed by any of these techniques, and its relative position is indicated only by morphology (Martínez, 1984)

1950s it was well known from southern México to northern Costa Rica (Dias, 1952) but has since been eliminated from Costa Rica (Ruiz, 1953) and from most of southern México (J. Ramsey, personal

communication) supposedly as a result of residual insecticide spraying during the antimalarial campaigns. It is still common in parts of Guatemala and Honduras, but now seems restricted in El Salvador and Nicaragua to a few localities along the Honduran border. Recently, *R. prolixus* has also been identified in the region of Teresópolis in the state of Rio de Janeiro, Brazil –possibly resulting from an accidental laboratory escape (Pinho *et al.*, 1998).

In spite of its wide and discontinuous distribution, *R. prolixus* populations from different localities are extremely homogenous-by isoenzymes (Harry *et al.*, 1992; Chávez *et al.*, 1999), by morphometry (Dujardin *et al.*, 1998, 1999), and by mtDNA sequences (Stothard *et al.*, 1998; F. Monteiro and C.B. Beard, personal communication) –which is suggestive of a common and fairly recent origin. Current theory would suggest that its domestication was a discrete event in Venezuela at some time after the establishment of European settlements in the 16th century, originating from a *robustus*-like ancestral form in palm tree crowns. Its subsequent dispersal as a domestic entity would then have been mediated primarily by passive transport in association with humans, such that its entry into the Magdalena valley of Colombia may have been originally due to colonising expeditions from Venezuela².

The origin of *R. prolixus* in Central America also seems related to human intervention, with historical

reconstruction (Zeledón, 1972) and morphometric and RAPD comparisons (Dujardin *et al.*, 1998) supporting the idea that central american *prolixus* originated from the northern part of South America. Collections of *R. prolixus* from houses in La Guayra on the northern coast of Venezuela were taken to the Institut Pasteur in Paris in 1912, from where a sample was taken to El Salvador in 1913. It is deduced that by 1915 some of these had subsequently escaped into houses in San Salvador (Neiva, 1915) from where they were gradually transported to neighbouring countries over the following decades (Zeledón, 1972). Central American specimens of *R. prolixus* are identical to those of Venezuelan and Colombia in terms of morphology and isoenzymes (Dujardin *et al.*, 1998) and mtDNA sequences (F. Monteiro and C.B. Beard, personal communication), but are generally of smaller size and with greatly reduced band numbers revealed by RAPD analysis (Dujardin *et al.*, 1998) –suggestive of substantial genomic reduction through the various ‘bottlenecks’ involved in their passage to Central America.

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² A possible early route may have been the expedition led by Ambrosio Alfinger from the northwestern Venezuelan province of Coro (now Falcon) which reached Bucaramanga, Colombia, in 1532. Triatomine bugs (of various domestic species) have been encountered amongst the saddle material of rural horseriders in Latin America, so it is at least conceivable that this was an early form of passive transport for *R. prolixus*.

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