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SHORT REVIEW

The biogeography and population genetics of neotropical vector species

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Phylogenetic and population genetic data support the Pliocene or Pleistocene divergences of the co-distributed hematophagous insect vectors, the sand fly *Lutzomyia longipalpis* s.l., the mosquitoes *Anopheles darlingi* and *A. albitarsis* s.l., and the triatomines *Rhodnius prolixus* and *R. robustus*. We examined patterns of divergence and distribution in relation to three hypotheses of neotropical diversification: Miocene/Pliocene marine incursion, Pliocene/Pleistocene riverine barriers and Pleistocene refugia. Only *R. prolixus* has a pattern concordant with the refugia hypothesis, and *R. robustus* conforms to the marine incursion predictions. *A. darlingi* partially fits the refugia hypothesis. For *L. longipalpis* s.l. and *A. albitarsis* s.l., elements of both incursion and refugia hypotheses seem to fit, suggesting perhaps an interaction of factors determining their distribution patterns.
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Introduction

Neotropical diversity is near-legendary, and many hypotheses have been proposed in an effort to understand current distributions of flora and fauna. Three of these, the Miocene/Pliocene marine incursion hypothesis (Räsänen *et al*., 1995; Webb, 1995), the Pliocene/Pleistocene riverine barrier hypothesis (Wallace, 1852) and the Pleistocene refugia hypothesis (Haffer, 1969), are the focus of this review because they can be examined in the light of phylogenetics and population genetics, and because of their general applicability for vertebrates (Moritz *et al*., 2000; Bates, 2001; Aleixo, 2004) and several insects, including butterflies (Brown *et al*., 1974; Brower, 1994; Hall and Harvey, 2002), beetles (Erwin and Pogue, 1988; Erwin, 1998; Scataglini *et al*., 2006) and bees (Dick *et al*., 2004).

The earliest of the three hypotheses, the marine incursion hypothesis, includes several aspects of the previous paleogeography hypothesis. The latter focused on early tectonic movements and sea-level changes (Chapman, 1917; Frailey *et al*., 1988), while the former proposes that sea-level changes during the Tertiary caused Amazonian diversification (Räsänen *et al*., 1995; Webb, 1995). The marine incursions resulted in extensive flooding, mainly in the Amazonian lowlands, leaving three large land blocks relatively isolated: the Brazilian Shield, the Guiana Shield and the eastern Andean slopes (Figure 1). For Amazonian birds (Bates, 2001), it has been proposed that basal populations in these land blocks began to diverge and eventually speciated, resulting in major areas of endemism (Cracraft, 1985; Hall and Harvey, 2002). Many centers of endemism have been identified by mapping a wide range of Amazonian biota (for example, Prance, 1982; Patton *et al*., 2000; summarized in Hall and Harvey, 2002).

Wallace (1852) proposed the riverine barrier hypothesis while he was living and working in the Amazon in the 19th century. He postulated that the rivers would act as barriers to gene flow between populations on opposite banks, eventually resulting in speciation. The time frame for this hypothesis is around the Pliocene/Pleistocene boundary when the major Amazon river developed (Gascon *et al*., 2000; Campbell *et al*., 2006). In phylogeographic terms, sister intraspecific clades and species should exist across neotropical rivers, rather than within the areas between rivers (Aleixo, 2004). It should also be possible, using phylogenetic and population genetics data, to discriminate between primary divergence across rivers and more recent secondary contact along rivers between nonsister taxa that diverged in another geographic area (Moritz *et al*., 2000). The geology of neotropical rivers (generally narrower at the source and wider at the mouth) suggests another prediction: differentiation should be lowest among populations on either side near the headwaters and gradually increase to become highest among populations on either side at or near the mouth (Haffer, 1993; Gascon *et al*., 2000).

The Pleistocene refugia hypothesis posits the most recent time frame, and it was originally promoted for the Neotropics by Haffer (1969) to explain avian diversification. The basic model is that during Pleistocene climatic shifts associated with glaciation, areas of moister vegetation types (such as rainforest) and their populations contracted and became isolated, surrounded by drier vegetation such as savannah. During warmer,

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Figure 1 Dotted lines represent geological features and regions that were not flooded during the marine incursion.

wetter periods these vegetation islands (refugia) and their populations expanded. The contractions and expansions occurred cyclically, and are thought to have resulted in cladogenesis. Although significant changes in forest cover during Pleistocene glaciation periods have not been found (Colinvaux *et al*., 2000), and it is problematic to derive phylogenetic predictions (Patton and da Silva, 1998; Moritz *et al*., 2000), two population genetics patterns have been shown to be strongly associated with Pleistocene refugia (in North America, Zink, 1997; in Europe, Hewitt, 1999; in the Neotropics, Ruegg and Smith, 2002; Aleixo, 2004): population bottlenecks associated with refugia contraction and isolation and demographic expansions concordant with refugia expansions. Additional expectations include low genetic variability, shallow levels of mtDNA divergence and little phylogeographic structure (Avise *et al*., 1987). Allopatric speciation is also thought to be a signature of the refugia hypothesis, because small populations could have been confined in pockets of rain forest separated by savannah; then diverged by genetic drift (Monteiro *et al*., 2003).

In this review, we ask when major divergences occurred, and which of these three hypotheses seems most likely to be supported, for each of several insect vectors of human pathogens with broad neotropical distributions for which sufficient prior phylogenetic, population genetic or biogeographic data exist: sandflies (leishmaniasis), triatomines (Chagas disease) and mosquitoes (malaria). Several insect vectors of neotropical human pathogens are notorious for cryptic speciation and geographic patterning and thus could be useful in a better understanding of neotropical insect diversification.

Neotropical biota have been significantly influenced by a long and complex interplay of climatic, tectonic and paleoenvironmental changes (Vrba, 1992; de Fátima Rossetti *et al*., 2005). Since the late Oligocene (33.7–23.8 MYBP), the major geological events that have been hypothesized to play an influential role in diversification include sea-level changes congruent with a marine transgression during the late Oligocene/early Miocene, the uplifts of the Colombian eastern Cordillera, the Andean Cordillera and Central American mountain ranges, and the emergence of the Isthmus of Panama early in the Pliocene (de Fátima Rossetti, 2001). The prevailing climate was initially tropical (wet) but became dry following the emergence of the Panama Isthmus, reflecting a worldwide trend of drier, more severe climates and expansion of savannahs in place of forests during the Pleistocene–Holocene glaciations (Crowley and North, 1991; Hallam, 1994; Hooghiemstra and Van de Hammen, 1998; de Fátima Rossetti, 2001). In eastern and central Amazonia, paleoecological evidence indicates wet conditions associated with rainforest expansion since the last glacial maximum about 20 000 years ago (Burnham and Graham, 1999).

A current controversy is whether relatively recent climatic instability during the Pleistocene is the primary force driving speciation, or whether diversification followed earlier tectonic events coupled with the marine transgressions. These two scenarios need not be mutually exclusive. Highland and lowland neotropical birds were differentially affected by recent climatic changes, with highland fauna showing a late Pleistocene increase in diversification rates most likely due to habitat fragmentation by Andean glacial cycles; by contrast, diversification rates in lowland fauna were highest during the late Miocene, and decreased toward the present (Weir, 2006). Several geologically based studies suggest that species differentiation can be attributed to environmental stresses (Sheldon, 1996; Renaud and Dam, 2002) resulting from a combination of climate, sea level, sedimentary processes and tectonics (de Fátima Rossetti *et al*., 2005). This pattern was supported in a study of an Amazonian forest bird superspecies *Xiphorhynchus spixii/elegans* whose diversification is hypothesized to be the result of an interaction among geology, hydrography and sea level changes (Aleixo, 2004).

One of the main reasons we opted to begin this comparison with mtDNA data is because it is frequently used to infer biogeographical history using the genealogies of organisms that are co-distributed (Avise *et al*., 1987, 2000; Soltis *et al*., 2006), and there exist comparable mtDNA data sets among the insect species we chose. We recognize that mtDNA tracks exclusively the maternal history, and that it is prone to introgression (Besansky *et al*., 2003; Rubinoff and Holland, 2005). We also briefly compare several other markers (allozymes, pheromones, microsatellites) and, in many cases, these data add very relevant components and perspectives, but were not common to all the species of interest or else the sampling was not sufficiently broad geographically across the Neotropics to enable the comparisons we sought.

Materials and methods

In considering our choices of insect vectors we offer several caveats: first, sandflies have very limited dispersal capabilities (usually no more than 1 km, Dye *et al*., 1991; Morrison *et al*., 1993) are often abundant in peridomestic environments of rural communities, and are therefore patchily distributed (Soto *et al*., 2001); second, triatomine bugs are generally either domestic or

sylvatic, a characteristic that influences both dispersal and geographic distribution (Forattini, 1980; Abad-Franch and Monteiro, 2005); and third, the number of generations per year for each insect group is not identical which may influence the estimated dates of divergence (Monteiro *et al*., 2003). *Anopheles* are estimated to have ten generations per year (Walton *et al*., 2000); *Lutzomyia*, 5–7 (Cárdenas *et al*., 1999), and triatomines range from 3 or 4 per year to one generation every 2 years (Krinsky, 2002). Anopheline mosquitoes are likely to be more broadly naturally dispersed compared with sandflies or triatomines because their life stages are first aquatic and then terrestrial, and flight ranges have been recorded at >7.2 km (Charlwood and Alecrim, 1989 for *A. darlingi*) and >12 km (Correa *et al*., 1950 for *A. albitarsis* s.l.). We also expect differences between *A. darlingi* and *A. albitarsis* s.l. based on their preferred aquatic habitat: *A. darlingi* tends to be found along warm lowland rivers edges (Charlwood, 1996; Roberts *et al*., 2002); in contrast, *A. albitarsis* s.l. prefers shallow, sunlit pools (Wilkerson *et al*., 1995a; Conn *et al*., 2002; Brochero *et al*., 2005). We would thus expect these two species could have responded differently to the cyclical contraction and expansion of refugia.

The predictions for each of the three hypotheses in Table 1 are modified from Avise *et al*. (1987), Hewitt (1999), Gascon *et al*. (2000) and Aleixo (2004). We examined in some detail three major mtDNA sequence data sets: for *Rhodnius prolixus* and *R. robustus*, Monteiro *et al*. (2003); for *Anopheles albitarsis* s.l., Lehr *et al*. (2005); and for *A. darlingi*, Mirabello and Conn (2006a). The sequences from Arrivillaga *et al*. (2002) for *L. longipalpis* were not in GenBank or EMBO and therefore the divergence estimates are not included in Table 2. *A. albitarsis* s.l. cytochrome oxidase I (*COI*) mtDNA sequences were obtained from GenBank, accession numbers DQ076204–DQ076238 (Lehr *et al*., 2005). The *A. darlingi COI* mtDNA sequence data were from GenBank, accession numbers DQ298209–DQ298244 (Mirabello and Conn, 2006a). The *R. prolixus* and *robustus* cytochrome *b* (cyt *b*) mtDNA data were from Monteiro *et al*. (2003), and the divergence time was recalculated to an extra decimal place. The percent sequence divergence estimates were calculated using a Kimura 2-parameter model (Kimura, 1980) with MEGA version 3.1 (Kumar *et al*., 2004). The divergence time estimates were calculated assuming a mutation rate of 2.3% sequence divergence per million years as in Monteiro *et al*. (2003), based on the estimates for the complete mitochondrial genome for recently diverged arthropods (2.3% per MY; Brower, 1994).

Sandflies (Diptera: Psychodidae)

Lutzomyia longipalpis s. l. is the main vector of neotropical visceral leishmaniasis, with a discontinuous distribution from southern Mexico to northern Argentina (Young and Duncan, 1994). It is generally found in dry tropical forest or semiarid habitats, including open savannah; but also in the lower Amazon basin associated with well-drained soils subjected to periodic flooding (Lainson *et al*., 1983). Habitats within regions are often discontinuous and isolated by both geographic and climatic barriers (Soto *et al*., 2001). Multiple markers indicate that *L. longipalpis* is a species complex (reviewed in Lainson and Rangel, 2005), although the exact number of species is still under investigation. Morphological differences were the first clue to potential cryptic species in *L. longipalpis* within Brazil (Mangabeira, 1969), with two forms from the states of Ceará and Pará hypothesized to be reproductively isolated (Ward *et al*., 1983), and several forms or chemotypes subsequently proposed to be sympatrically differentiated in Brazil by sex pheromones (Ward *et al*., 1988; Hamilton *et al*., 1996a–c, 2005). There is now considerable corroborative evidence suggestive of incipient speciation in Brazil, including sequences of the *period* gene (Bauzer *et al*., 2002a, b), significant sequence divergence in the *cacophony* IVS6 intron (Bottecchia *et al*., 2004), microsatellite loci (Maingon *et al*., 2003) and courtship song differentiation (Souza *et al*., 2004). A study that combined morphology and allozymes to examine several Brazilian populations that represent three of the morphological patterns in *L. longipalpis* s.l., however, did not support the hypothesis of multiple species (de Azevedo *et al*., 2000), but allozymes evolve relatively slowly and are not the most informative markers for detecting recently diverged species; and Dipteran species complexes are rife with morphological 'look-alikes'.

In studies that focused on a broader geographical range of *L. longipalpis* s.l., crossing experiments detected male hybrid sterility among populations from Costa Rica, Colombia and Brazil (Lanzaro *et al*., 1993). Several allozyme studies (Lanzaro *et al*., 1993; Dujardin *et al*., 1997; Mukhopadhyay *et al*., 1998; Mutebi *et al*., 2002), a chromosome study (Yin *et al*., 1998), and mitochondrial DNA sequences (Soto *et al*., 2001; Arrivillaga *et al*., 2002, 2003; Hodgkinson *et al*., 2003) provide evidence for cryptic species except in Brazil, and thus are in disagreement with the pheromone, courtship song, *period* and *cacophony* sequence and microsatellite data discussed above.

Sympatric speciation in Lara state, western Venezuela, using allozymes (Lampo *et al*., 1999), parapatric and allopatric speciation followed by secondary contact in Ceará state, northwestern Brazil, based on crossing experiments, pheromones, copulation songs, *period* gene data and microsatellites (reviewed in Watts *et al*., 2005), and allopatric speciation (throughout its neotropical distribution, Soto *et al*., 2001; Arrivillaga *et al*., 2002) have been invoked, and each mode of speciation could play a significant local or regional role across the broad geographic range of this species complex.

Testing the marine incursion hypothesis is complicated because *L. longipalpis* s.l. appears to be absent from the eastern part of the Guiana Shield (no records from Guyana, Suriname or French Guiana; Arrivillaga *et al*., 2003). The Brazilian Shield populations form a monophyletic clade in both the NADH dehydrogenase subunit 4 (*ND4*) and *COI* studies, following prediction 1. No samples from the Guiana Shield were tested in the *ND4* study, and the single sample from the Guiana Shield in the *COI* study belongs to a clade with three localities in the eastern Andean Cordillera (*cis*-Andean; Figure 2). Both studies show the Brazilian as the most derived of the four clades, but disagree on which clade is basal. In the *ND4* study, the Central American localities are basal and strongly supported (95%, six synapomorphies; Soto *et al*., 2001); in the *COI* study these populations are included with northern Colombian and western Vene-

Table 1 Predictions derived from three common hypotheses of neotropical diversification for three co-distributed groups of insect vectors, *Lutzomyia longipalpis* s.l., *Anopheles albitarsis* s.l., *A. darlingi*, *Rhodnius prolixus* and *R. robustus*

Hypothesis	*Prediction*
Marine incursion[a]	(1) Brazilian shield, Guianan Shield and eastern Andean slope lineages should each be monophyletic; (2) ancestral or basal populations are found on the Brazilian Shield, Guianan Shield and along the eastern Andean slope; (3) more recently derived populations should be found in the western Amazonian lowlands
Riverine barrier[b]	(1) Sister lineages should be detected across major rivers, not within major areas between rivers; (2) rivers represent areas of primary differentiation versus secondary contact among lineages; (3) genetic similarity between populations divided by a river should be higher near the headwaters compared with near the mouth
Refugia[c]	(1) Signal of population bottleneck associated with refugia contractions and isolation; demographic expansion associated with refugia expansion; (2) low genetic variability; (3) little phylogeographic structure; (4) shallow mtDNA divergence

[a]Began in Miocene (23.8–5.3 MYBP) and continued into Pliocene (5.3–1.8 MYBP).
[b]Pliocene–Pleistocene.
[c]Pleistocene is 1.8–10 000 YBP.

Table 2 Prediction results for *L. longipalpis* s.l., *A. albitarsis* s.l., *A. darlingi*, *R. prolixus* and *R. robustus*. based on mtDNA *COI*[a] data

Insect vector	*Hypothesis support*	*Result*
	Marine	
L. longipalpis	Partial	(1) Partial support; only Brazilian shield lineages monophyletic; (2) no support; Brazilian most derived; (3) could not evaluate
R. prolixus	Untestable	
R. robustus	Yes	(1) Three monophyletic lineages; (2) ancestral lineages are Brazilian and Guianan; (3) central + western Amazonian lineages mos derived
A. albitarsis s.l.	Partial	(1) Two monophyletic lineages restricted +/− to Brazilian Shield; (2) basal clade support weak; (3) could not evaluate
A. darlingi	No	(1) Lineages not monophyletic in these areas; (2) basal lineage includes nearly all South America; (3) western Amazonian samples basal
	Riverine	
L. longipalpis	NB	
R. prolixus	Untestable	
R. robustus	NB	
A. albitarsis s.l.	No	(1) Sister lineages not detected across Amazon; (2) rivers not representing areas of primary differentiation; (3) no difference in genetic similarity at headwaters vs mouth of Amazon
A. darlingi	No	(1) Sister lineages not detected across Amazon; (2) rivers not representing areas of primary differentiation; (3) not tested
	Refugia	
L. longipalpis	Partial	(1) Bottlenecks not tested for; *ND4* data negative for expansion; (2) low genetic variability in *COI* but high in *ND4*; (3) significant phylogeographic structure; (4) deep divergence
R. prolixus	Yes	(1) Not tested; (2) low genetic variability; (3) little phylogeographic structure; (4) shallow divergence
R. robustus	No	(1) Not tested; (2) high genetic variability; (3) significant phylogeographic structure; (4) deep divergence
A. albitarsis s.l.		
A. marajoara	Partial	(1) Evidence for late-Pleistocene expansion; (2) low genetic variability; (3) some phylogeographic structure; (4) moderate divergence
A. albitarsis E	No	(1) No evidence for expansion; (2) moderate genetic variability; (3) moderate phylogeographic structure; (4) moderate divergence
A. darlingi	Partial	(1) Evidence for late-Pleistocene expansion; (2) moderate-large genetic diversity; (3) significant phylogeographic structure; (4) deep divergence

[a]For *L. longipalpis* only, the mtDNA *ND4* data set of Soto *et al*. (2001) was also evaluated.NB: Study not designed to test hypothesis.

zuelan samples in the *trans*-Andean clade (moderately well supported – 74%; Arrivillaga *et al*., 2002). No western Amazonian lowlands samples were included in either study to enable us to test whether these populations would be more derived. In summary, prediction 1 of the marine incursion hypothesis is

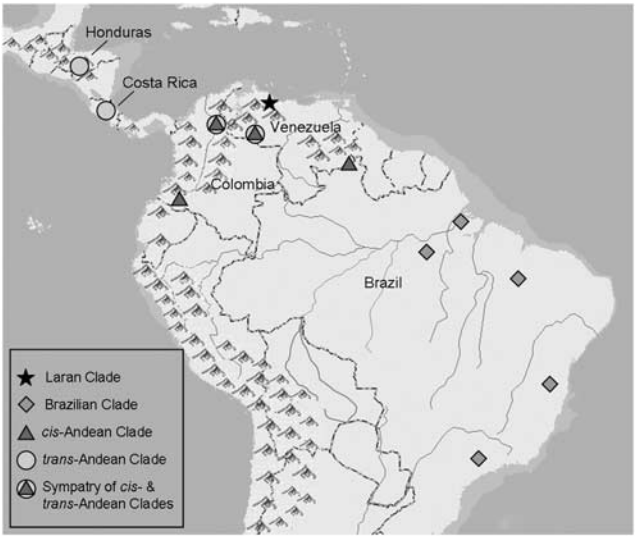

Figure 2 *Lutzomyia longipalpis* geographic locations of the populations and clades (specified in legend) based on the mtDNA *COI* phylogenetic analysis of Arrivillaga *et al*. (2002).

partially supported, prediction 2 is not supported and prediction 3 could not be evaluated (Table 2).

Limited geographic sampling in the Amazon Basin prohibits discussing the riverine hypothesis. Soto *et al*. (2001) detected four clades across the distribution of *L. longipalpis* s.l. using sequences of the mtDNA *ND4* gene: northern South America, Brazil, Central America and an isolated population in Colombia. Using additional samples from Brazil and Venezuela, and sequences of the mtDNA *COI* gene, Arrivillaga *et al*. (2002) also detected four clades (A–D): Laran (Venezuela); *cis*-Andean (Venezuela; Colombia; northern Brazil); *trans*-Andean (Venezuela; Colombia; Central America); and Brazilian (Figure 2). Although high within population genetic diversity was found using the *ND4* gene (Soto *et al*., 2001) and low variation using *COI* (Arrivillaga *et al*., 2002), both studies demonstrated deep levels of mtDNA divergence (and cladogenesis), and substantial phylogeographic structure. Additionally, no sign of a demographic expansion was detected using *ND4* sequences (this was not tested using the *COI* sequence data). These results together suggest there is very little support for the Pleistocene refugia hypothesis for *L. longipalpis* s.l. based on mtDNA (Table 2). Furthermore, the moderate genetic variability, substantial phylogeographic structure and considerable divergence among populations from Venezuela and Brazil detected using microsatellite markers (Watts *et al*., 2005) do not seem to support the refugia hypothesis either, but tests were not conducted to detect bottlenecks or expansions. Estimations of divergence time in this species complex, especially among sympatric Brazilian clades, would be a valuable contribution.

The *COI* sequence data support speciation events that began during the Pliocene, and the large geographic gap separating the Brazilian and Laran clades (Figure 2) suggests their most recent ancestor was part of a widespread sub-Andean/Amazonian gene pool (Arrivillaga *et al*., 2002). Populations are hypothesized to have subsequently dispersed across Brazil, then to the Andes, and lastly into Central America, after the final East Andean uplift in the early Pleistocene. The large genetic distances and the predominantly allopatric ranges among the Laran, Andean and Brazilian clades are attributed to vicariant geologic events as the most probable significant evolutionary force (Arrivillaga *et al*., 2002); however, this is disputed by the microsatellite data provided in Watts *et al*. (2005). Interestingly, the samples from Curarigua, Venezuela, which together comprise the Laran clade, show quite low nucleotide divergence and are considered to be relictual (Arrivillaga *et al*., 2002). The sympatric Andean clades could be the result of initial allopatric speciation followed by a more recent secondary contact via dispersal. This could be tested by assessing populations in this region for demographic expansions, frequently associated with recent range expansions resulting in secondary contact.

Our interpretation of the *COI* data differs slightly in the most probable order of dispersal, from a possible Laran ancestor (basal in the Maximum Parsimony analysis and supported by 100%; Arrivillaga *et al*., 2002, 2003), to the Andes, then into Central America, and lastly across Brazil. Based on the *ND4* study, the order of dispersal would differ, beginning with a likely Central American ancestor, and dispersing through the Andes and lastly across Brazil (Soto *et al*., 2001). Overall, the timing for *L. longipalpis* s.l. divergence is Pliocene and Pleistocene, and the primary mode of divergence could be an interaction between the marine incursion and refuge hypotheses, although neither one is well supported (Table 2).

Triatomines (Hemiptera; Reduviidae)

Triatomines are a group of hematophagous insects that transmit *Trypanosoma cruzi*, the causative agent of Chagas disease, one of the most serious parasitic diseases in the neotropics (Miles *et al*., 2003). *Rhodnius prolixus* is predominantly domestic, and is considered the primary Chagas disease vector in Central America, Colombia and Venezuela (Lent and Wygodzinsky, 1979). *R. robustus* is sylvatic, although its taxonomic status has been uncertain (Schofield, 2000). The two species overlap broadly in South America, and the distribution of *R. prolixus* extends as far north as southern Mexico (Lent and Wygodzinsky, 1979). No diagnostic allozyme loci have been detected in these species (Harry *et al*., 1992), and overlapping morphological characters make field identification problematic (Harry, 1993). They were found to be distinctive evolutionary lineages using two mtDNA fragments, cytochrome *b* and large subunit ribosomal RNA (Lyman *et al*., 1999). *R. robustus* was determined to be heterogeneous based on mtDNA and nuclear fragments combined, and hypothesized to consist of multiple cryptic species (Monteiro *et al*., 2000).

The two Orinoco region *Rhodnius* (*R. prolixus* and *R. robustus* clade I) are estimated to have diverged from the three *R. robustus* Amazonian clades (II–IV; Figure 3) during the Pliocene (Monteiro *et al*., 2003; Table 3). Amazonian clade III consists of localities from the Brazilian Shield and clade IV comprises four localities from the Guiana Shield plus one from the Brazilian Shield, and these clades are reciprocally monophyletic (*R. robustus* II, the most derived of the three *R. robustus* Amazonian clades, is primarily from the western

Amazonian lowlands (Figure 3)). Thus prediction 1 of the marine incursion hypothesis is strongly supported (Table 2). The Brazilian and Guiana Shields are ancestral for *R. robustus*, because the two most basal *R. robustus* clades, IV and III, are both strongly supported (80 and 99%, respectively) in the MP tree (Monteiro *et al*., 2003). Together these data provide some support for prediction 2 of the marine incursion hypothesis (Table 2). Furthermore, the most derived of the Amazon region *R. robustus* clades (II; Figure 3) spans the central and western Amazonian lowlands, giving some support to prediction 3 of the incursion hypothesis (Table 2). The geographic sampling was not designed to examine the riverine hypothesis.

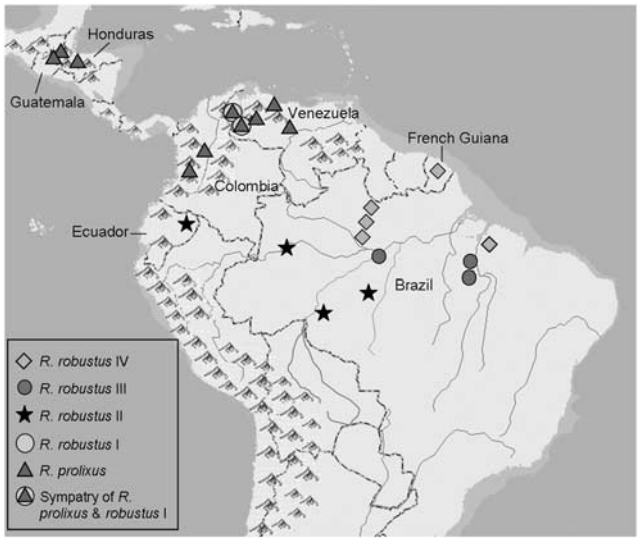

Figure 3 *Rhodnius robustus* I–IV and *R. prolixus* geographic locations of the five clades (specified in legend) based on the mtDNA *cyt b* phylogenetic analysis of Monteiro *et al*. (2003).

Monteiro *et al*. (2003) detected a molecular signature consistent with the refugia hypothesis for *R. prolixus* that included an extremely low level of nucleotide diversity, shallow levels of mtDNA divergence and little phylogeographic structure (Table 2). A recent population bottleneck with subsequent human-aided dispersal was hypothesized, which would fit prediction 1 (Table 1). Tests were not conducted for an expansion. The estimated divergence time between *R. prolixus* and *R. robustus* I (in the Orinoco region), and among *R. robustus* II, III and IV within both the Amazon and Orinoco regions is less than two MYA (Table 3), providing support for diversification during the Pleistocene (Monteiro *et al*., 2003), despite deep levels of divergence, and considerable phylogeographic structure. Monteiro *et al*. (2003) suggest that *R. prolixus* originated 1.4 MYA in the Orinoco lowland forests from an ancestral *prolixus*/*robustus* I stock that was separated in distinctive refugia, consistent with our interpretation (Table 2). In summary, *R. prolixus* was both monophyletic and homogeneous, and strongly supported the refuge hypothesis, in striking contrast to *R. robustus*, which consists of four monophyletic allopatric clades and supported the marine incursion hypothesis (Table 2).

Anopheline mosquitoes (Diptera: Culicidae)

Anopheles darlingi is a primary malaria vector responsible for transmission of *Plasmodium falciparum*, *P. vivax* and *P. malariae* in several endemic regions throughout much

Table 3 Mean mitochondrial sequence divergence levels (Kimura 2-parameter model) and the estimated time of divergence between the clades of *R. prolixus* and *R. robustus*, *A. albitarsis* s.l., and *A. darlingi* genotypes

Between clades	*Sequence divergence*[a] *(range)*	*Divergence time*[b]
Rhodnius prolixus and robustus[c]		
Orinoco region		
R. prolixus vs *R. robustus* I	3.3 (3.0–3.3)	1.43
Amazon region		
R. robustus II vs *R. robustus* III	4.0 (3.6–4.4)	1.74
R. robustus II vs *R. robustus* IV	3.4 (3.0–3.9)	1.48
R. robustus III vs *R. robustus* IV	2.3 (2.0–2.8)	1.00
Orinoco clades vs Amazon clades	7.2 (5.6–8.5)	3.13
Anopheles albitarsis s.l.[d]		
A. albitarsis s.s. vs *A. albitarsis* B	3.1 (2.6–3.6)	1.35
A. albitarsis s.s. vs *A. marajoara*	3.9 (2.7–4.9)	1.69
A. albitarsis s.s. vs *A. deaneorum*	3.4 (3.0–3.9)	1.48
A. albitarsis s.s. vs *A. albitarsis* E	4.2 (3.8–4.7)	1.83
A. marajoara vs *A. albitarsis* B	4.2 (3.5–5.1)	1.83
A. marajoara vs *A. deaneorum*	2.7 (1.7–3.6)	1.17
A. marajoara vs *A. albitarsis* E	4.9 (4.3–5.6)	2.13
A. albitarsis B vs *A. deaneorum*	3.7 (3.4–4.1)	1.61
A. albitarsis B vs *A. albitarsis* E	4.2 (3.9–4.5)	1.83
A. albitarsis E vs *A. deaneorum*	4.4 (4.1–4.7)	1.91
Anopheles darlingi[e]		
Genotype 1 vs genotype 2	1.3 (0.9–2.0)	0.57

[a]Divergence estimates are given in percents.
[b]The divergence time estimates are given in million years.
[c]Rhodnius cyt b sequence data from Monteiro *et al*. (2003).
[d]*Anopheles albitarsis* s.l. *COI* sequence data from Lehr *et al*. (2005).
[e]*Anopheles darlingi COI* sequence data from Mirabello and Conn (2006a).

of its extensive range from southern Mexico to southern Brazil (Deane, 1947, 1988; Forattini, 1962; Roberts *et al*., 1997). Its distribution is discontinuous, with no records from Panama, Costa Rica or Nicaragua (Faran, 1980). *A. darlingi* has been considered a single species, although there has been evidence of heterogeneity in polytene chromosomes (Kreutzer *et al*., 1972; Tadei *et al*., 1982), allozymes (Steiner *et al*., 1982; Rosa-Freitas *et al*., 1992), mtDNA (Freitas-Sibajev *et al*., 1995; Conn *et al*., 1999; Mirabello and Conn, 2006a), rDNA ITS sequences (Malafronte *et al*., 1999; Mirabello and Conn, unpublished data), nuclear *white* gene (Mirabello and Conn, unpublished data) and biological variation (Charlwood, 1996; Voorham, 2002).

The geographic sampling for the mtDNA *COI* gene fragment study of *A. darlingi* (Mirabello and Conn, 2006a) was not designed specifically to examine the marine incursion hypothesis. However, none of the populations within the Brazilian or Guianan Shields are in distinctive clusters or monophyletic clades (Mirabello and Conn, 2006a), falsifying prediction 1 (Table 2). In addition, Manguin *et al*. (1999) hypothesized that *A. darlingi* originated in South America and then established populations in Central America, and since the largest isozyme mean heterozygosity occurred in southern Brazil and Bolivia these populations may be more ancestral. In support of Manguin *et al*. (1999), the mtDNA data found the southern Brazil populations (located on the Brazilian Shield) to be more ancestral than those on the Guiana Shield, and elsewhere (Figure 4), which is inconsistent with predictions 2 and 3 of the incursion hypothesis (Table 2).

The riverine barrier hypothesis was not specifically tested, but populations of *A. darlingi* north of the Amazon River (Amapá State) were differentiated from those south of the Amazon River (Pará State) using mtDNA sequence data, and this was not due to isolation by distance (Mirabello and Conn, 2006a). Perhaps not surprisingly, microsatellite markers also detected significant divergence between populations of *A. darlingi* on either side of the mouth of the Amazon (approximately 270 km wide; Conn *et al*., 2006) compared with very limited divergence for *A. darlingi* on either side of the Negro River, a major tributary of the Amazon in north central Brazil (Scarpassa and Conn, 2007). Because *A. darlingi* is most common along warm lowland river edges (Charlwood, 1996; Roberts *et al*., 2002) and rare in rain forest, rivers *per se* are unlikely to represent areas of primary differentiation.

A significant division was detected between (1) Central America and Colombia and (2) Amazonia and southern Brazil populations of *A. darlingi* using sequences of the *COI* gene (Mirabello and Conn, 2006a), and confirmed by nuclear gene sequences (Mirabello and Conn, unpublished data) that also detected three localities in Venezuela, Peru and Bolivia (Figure 4) where genotypes 1 and 2 were sympatric. However, this division does not specifically correspond to a prediction of any of the main hypotheses proposed to explain neotropical diversity. A demographic expansion was found in Amazonian and southern South America using mtDNA, and the time to expansion was estimated to be 16 237 years ago (95% confidence interval 5488–25 814; Mirabello and Conn, 2006b). The exactness of this estimation is questionable because it was calculated

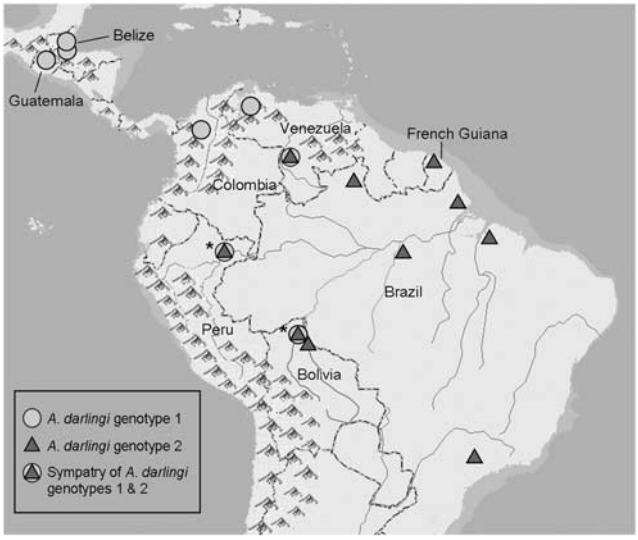

Figure 4 *Anopheles darlingi* geographic locations of the two clades (specified in legend) based on *white* gene phylogenetic analysis of Mirabello and Conn, unpublished data. *Only 1 of 28 individuals in Iquitos Peru and 1 of 14 individuals in Guayaramerín, Bolivia was genotype 1, as compared to half of the individuals in Fortuna de Albarico, Venezuela. The distribution of *A. darlingi* based on the *COI* data set is virtually identical to this except that sympatric sequences were not detected (Mirabello and Conn, 2006a).

based on a *Drosophila* mutation rate, although the confidence interval range includes a large margin of error. The timing of this expansion is consistent with the premise of rainforest expansion in eastern and central Amazonia about 20 000 years ago (Burnham and Graham, 1999), and a main prediction of the refugia hypothesis (Table 1). *A. darlingi* populations in Amazonia have demonstrated a moderate to large amount of genetic variability and considerable phylogeographic structure (mtDNA; Freitas-Sibajev *et al*., 1995; Conn *et al*., 1999; Mirabello and Conn, 2006a; microsatellites; Conn *et al*., 2006), which contradicts the prediction of low genetic diversity and shallow phylogeographic structure of the refugia hypothesis. The amount of *COI* sequence divergence between *A. darlingi* genotypes 1 and 2 is 1.3% and the estimated divergence time is 0.57 MYA (Table 3). In summary, *A. darlingi* diverged into two genotypes during the Pleistocene, and a portion of the divergence may be accounted for by the refuge hypothesis (Table 2). Although we recommend the collection of additional data to formally test the riverine hypothesis, the habitat preference of *A. darlingi* makes it unlikely that this hypothesis will provide a satisfactory explanation.

Anopheles albitarsis s.l. is a neotropical species complex distributed throughout Latin America and on some Caribbean islands (Linthicum, 1988) that currently consists of five species (*A. albitarsis* s.s., *A. albitarsis* B, *A. albitarsis* F, *A. deaneorum* and *A. marajoara*; Wilkerson *et al*., 1995a, b; Brochero *et al*., 2007) and one putative species (*A. albitarsis E*; Lehr *et al*., 2005). *A. marajoara* has been implicated as a regional malaria vector in savannah habitats (Rubio-Palis and Zimmerman, 1997) and in lowland rainforest in eastern Amazonian Brazil (Conn *et al*., 2002; Galardo *et al*., 2007), and *A. albitarsis* E is important in malaria transmission in savannah habitats in northern Roraima state, Brazil (Póvoa *et al*., 2006).

A. deaneorum is quite competent in laboratory studies (Klein *et al*., 1991a, b) and *A. albitarsis* F is suspected as a local or regional vector in Colombia (Brochero *et al*., 2007).

The species determinations for members of this complex are based on several markers, and include crossing experiments that support the specific status of *A. albitarsis* B and *A. deaneorum* (Klein *et al*., 1991c), and *A. albitarsis* s.s. and *A. deaneorum* (Lima *et al*., 2004). Studies of *A. marajoara* using chromosome inversions (Kreutzer *et al*., 1976) and allozymes and mtDNA restriction fragment length polymorphisms (Narang *et al*., 1993), both provide data consistent with multiple species. More recently, neither random amplified polymorphic DNA banding patterns nor ITS2 could discriminate between *A. marajoara* and *A. albitarsis* E (Wilkerson *et al*., 1995b; Li and Wilkerson, 2005), but sequences of the complete *COI* gene (Lehr *et al*., 2005) and a combination of 767 bp of the *COI* gene plus 909 bp fragment of the mtDNA NADH dehydrogenase subunit 5 (*ND5*) gene (Shaw *et al*., unpublished data) both determine *A. marajoara* and *A. albitarsis* E to be distinctive monophyletic clades. Based on Bayesian analysis, microsatellites also discriminate between the two (Conn *et al*., unpublished data).

The *A. deaneorum* and *A. albitarsis* B clades, seemingly restricted to the Brazilian Shield or to a transition area between the Shield and the lowlands (Figure 5), do conform to prediction 1 of the marine incursion hypothesis (Table 2). However, the *A. albitarsis* s.s. clade is completely to the south of the Brazilian Shield, the *A. marajoara* clade stretches across the Guiana and Brazilian Shields, and the *A. albitarsis* E clade appears to be restricted to savannah and Andean foothills habitat in Venezuela and Trinidad. The position of each of the five clades (species), excluding *An. albitarsis* F (not discovered until after the publication of Lehr *et al*. (2005) and only identified from one (Colombian) locality to date) is fairly stable among the three analyses undertaken (MP, maximum likelihood (ML), and Bayesian; Lehr *et al*., 2005) except for the basal clade. The second prediction of the marine incursion hypothesis suggests, for the *A. albitarsis* complex, that *A. deaneorum* and *A. albitarsis* B should be basal, and *A. deaneorum* is basal in the ML analysis, but *A. albitarsis* E is basal in the MP and Bayesian analyses. There were no samples collected from the western Amazonian lowlands so the third prediction could not be tested. Based on these data, ancestry for *A. albitarsis* s.l. is most likely in Venezuela, followed by dispersal south into the Amazon Basin and then onto the Brazilian Shield. Not all the MP, ML and Bayesian analyses (Lehr *et al*., 2005) agree on this dispersal order, but all found the most southern species, *A. albitarsis* s.s., to be the most derived clade, and sister to it is *A. albitarsis* B, the species found on the eastern side of the Brazilian Shield (Figure 5).

The only comment relative to the riverine hypothesis is the observation that samples of *A. marajoara* from Salvaterra on eastern Marajó Island and those from Macapá in Amapá state across the northern arm of the Amazon at its mouth were not significantly differentiated with mtDNA sequences (Lehr *et al*., 2005) and, using a Bayesian analysis with microsatellite markers, the probability was extremely high that they were in the same population (Conn *et al*., unpublished data).

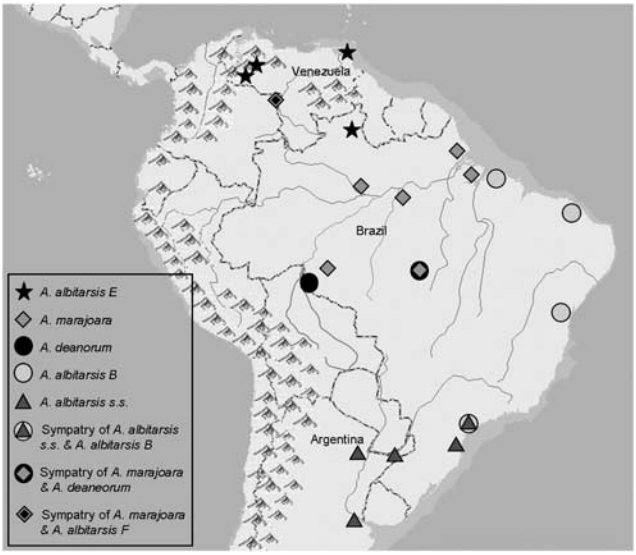

Figure 5 Geographic locations of the five species of the *A. albitarsis* complex based on the mtDNA *COI* gene of Lehr *et al*. (2005) plus *A. albitarsis* F (Brochero *et al*., 2007) and additional mtDNA data on localities of *A. albitarsis* E (Shaw *et al*., in manuscript).

The refugia hypothesis can only be discussed in terms of *A. marajoara* and *A. albitarsis* E, for which sufficient data have been collected and analyzed. Both *COI* (Lehr, 2003) and *ND5* (Shaw *et al*., unpublished data) sequences show strong support for a demographic expansion during the Pleistocene only in five localities in eastern Amazonian Brazil (north of the Amazon River), and low genetic variability for *A. marajoara*, supporting predictions 1 and 2 (Table 2). However, for samples of *A. marajoara* overall (including those from three Central Amazonian localities) there is considerable phylogeographic structure and deeper than expected mtDNA divergence. *A. albitarsis* E, in contrast, has the signature of an old stable population in equilibrium, with no compelling evidence for any of the four refugia hypothesis predictions (Shaw *et al*., unpublished data). The sequence divergence between *A. marajoara* and *A. albitarsis* E is also the highest, the only one estimated to be during the Pliocene, compared with the other members of this complex (Table 3). Taken together, data for *A. albitarsis* s.l. suggest an interaction between the marine incursion and refugia hypotheses, congruent with the estimated divergence time frame of Pliocene/Pleistocene.

Common patterns?

The similarities among *L. longipalpis* s.l., *R. prolixus*/*R. robustus* and *A. albitarsis* s.l. include ancestral divergence during the Plicoene (despite distinctive generation times), and predominantly reciprocal allopatric clades. The congruent sympatric clades in the east Andean Cordillera (Figures 2 and 3) within each of *L. longipalpis* s.l. and *R. prolixus*/*R. robustus* are possibly the result of a combination of continuous tectonic activity over a long period of geological history and limited dispersal. However, the triatomines strongly support marine incursion and refuge hypotheses (Table 2), whereas support for each of these hypotheses for *L. longipalpis*

s.l. is only partial. The triatomines and sand flies also differ in ancestry: South American or Amazonian for *R. robustus/R. prolixus* vs western Venezuela for *L. longipalpis* s.l. *A. albitarsis* s.l. also consists of several reciprocal allopatric clades, shares likely ancestry in Venezuela with *L. longipalpis* s.l. and there are two regions of relative geographical congruence with *L. longipalpis* s.l. One is the Brazilian Shield for *A. albitarsis* B (Figure 5) and the *L. longipalpis* Brazilian clade (Figure 2) and the other is northwestern South America: the basal *A. albitarsis* E clade and the nearly-basal *cis*-Andean *L. longipalpis* clade. Despite very distinctive habitat preferences, *A. darlingi* genotype 2 shares evidence for a Pleistocene expansion in the Amazon with *A. marajoara*, but other aspects of its phylogeography differ considerably (Table 2). Perhaps in part because of its high gene flow, its divergence is not easily explained by the available hypotheses.

Conclusion

Because samples of vector insects tend to be collected in or near disease foci, a concerted effort needs to be made to collect more broadly in order to rigorously examine the three testable divergence hypotheses discussed, particularly within each of the three insect vector groups. Further investigations into the role of ancestral Venezuelan localities for each of the three insect groups may lay the foundation for new hypotheses, and could be useful in a better understanding of locations of disease foci and their potential expansions. Molecular dating techniques for insect vector groups could provide more insight into the influence of historical events on the phylogeographic patterns of variation.

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References

Abad-Franch F, Monteiro FA (2005). Molecular research and the control of chagas disease vectors. *An Acad Bras Cienc* **77**: 437–454.

Aleixo A (2004). Historical diversification of a *Terra-Firme* forest bird superspeices: a phylogeographic perspective on the role of different hypothesis of Amazonian diversification. *Evolution* **58**: 1303–1317.

Arrivillaga J, Mutebi JP, Pinango H, Norris D, Alexander B, Feliciangeli MD *et al.* (2003). The taxonomic status of genetically divergent populations of *Lutzomyia longipalpis* (Diptera: Psychodidae) based on the distribution of mitochondrial and isozyme variation. *J Med Entomol* **40**: 615–627.

Arrivillaga JC, Norris DE, Feliciangeli MD, Lanzaro GC (2002). Phylogeography of the neotropical sand fly *Lutzomyia longipalpis* inferred from mitochondrial DNA sequences. *Infect Genet Evol* **2**: 83–95.

Avise JC (2000). *Phylogeography. The History and Formation of Species*. University Press: Massachusetts.

Avise JC, Arnold J, Ball Jr RM, Bermingham E, lamb T, Neigel JE *et al.* (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* **18**: 489–522.

Bates JM (2001). Avian diversification in Amazonia: evidence for historical complexity and a vicariance model for a basic pattern of diversification. In: Viera I, D'Incão MA, Silva JMC, Oren E (eds). *Diversidade Biológica e Cultural da Amazônia. Museu Paraense Emilio Goeldi*. Belém: Brazil. pp 119–138.

Bauzer LG, Gesto JS, Souza NA, Hamilton JG, Kyriacou CP, Peixoto AA (2002b). Molecular divergence in the period gene between two putative sympatric species of the *Lutzomyia longipalpis* complex. *Mol Biol Evol* **19**: 1624–1627.

Bauzer LG, Souza NA, Ward RD, Kyriacou CP, Peixoto AA (2002a). The period gene and genetic differentiation between three Brazilian populations of *Lutzomyia longipalpis*. *Insect Mol Biol* **11**: 315–323.

Besansky NJ, Krzywinski J, Lehmann T, Simard F, Kern M, Mukabayire O *et al.* (2003). Semipermeable species boundaries between *Anopheles gambiae* and *Anopheles arabiensis*: evidence from multilocus DNA sequence variation. *PNAS* **100**: 10818–10823.

Bottecchia M, Oliveira SG, Bauzer LG, Souza NA, Ward RD, Garner KJ *et al.* (2004). Genetic divergence in the cacophony IVS6 intron among five Brazilian populations of *Lutzomyia longipalpis*. *J Mol Evol* **58**: 754–761.

Brochero HL, Li C, Wilkerson RC (2007). A newly recognized species in the *Anopheles (Nyssorhynchus) Albitarsis* complex (Diptera: Culicidae) from Puerto Carreño, Colombia. *Am J Trop Med Hyg* (in press).

Brochero HL, Rey G, Buitrago LS, Olano VA (2005). Biting activity and breeding sites of *Anopheles* species in the municipality Villavicencio, Meta, Colombia. *J Am Mosq Control Assoc* **21**: 182–186.

Brower AVZ (1994). Rapid morphological radiation and convergence in the butterfly, *Heliconius erato*, inferred from patterns of mitochondrial DNA evolution. *Proc Nat Acad Sci USA* **91**: 6491–6495.

Brown Jr KR, Sheppard PM, Turner JRG (1974). Quaternary refugia in tropical America: evidence from race formation in *Heliconius* butterflies. *Proc R Soc Lond B Biol Sci* **187**: 369–378.

Burnham RJ, Graham A (1999). The history of the neotropical vegetation: new developments and status. *Ann Mo Bot Gar* **86**: 546–589.

Campbell Jr KE, Frailey CD, Romero-Pittman L (2006). The Pan-Amazonian Ucayali Peneplain, late Neogene sedimentation in Amazonia, and the birth of the modern Amazon River system. *Palaeogeogr Palaeoclimatol Palaeoecol* **239**: 166–219.

Cárdenas E, Ferro C, Corredor D, Martínez O, Munstermann LE (1999). Reproductive biology of *Lutzomyia shannoni* (Dyar) (Diptera; Psychodidae) under experimental conditions. *J Vector Ecol* **24**: 158–170.

Chapman FM (1917). The distribution of bird-life in Colombia: a contribution to a biological survey of South America. *Bull Am Mus Nat Hist* **36**: 1–729.

Charlwood JD (1996). Biological variation in *Anopheles darlingi* root. *Mem Inst Oswaldo Cruz* **91**: 391–398.

Charlwood JD, Alecrim WA (1989). Capture-recapture studies with the South American malaria vector *Anopheles darlingi*, root. *Ann Trop Med Parasitol* **83**: 569–576.

Colinvaux P, de Olivera PE, Bush MB (2000). Amazonian and neotropical plant communities on glacial time-scales: the failure of the aridity and refuge hypotheses. *Quat Sci Rev* **19**: 141–169.

Conn JE, Rosa-Freitas MG, Luz SLB, Momen H (1999). Molecular population genetics of the primary neotropical malaria vector *Anopheles darlingi* using mtDNA. *J Am Mosq Control Assoc* **15**: 468–474.

Conn JE, Vineis JH, Bollback JP, Onyabe DY, Wilkerson RC, Póvoa MM (2006). Population structure of the malaria vector *Anopheles darlingi* in a malaria-endemic region of eastern Amazonian Brazil. *Am J Trop Med Hyg* **74**: 798–806.

Conn JE, Wilkerson RC, Segura MN, de Souza RT, Schlichting CD, Wirtz RA *et al.* (2002). Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use. *Am J Trop Med Hyg* **66**: 18–22.

Correa RR, Lima FO, Coda D (1950). Observations on the flight and longevity in nature of *Anopheles albitarsis domesticus*. *J Nat Mal Soc* **9**: 280–284.

Cracraft J (1985). Historical biogeography and patterns of differentiation within the South American areas of endemism. *Ornithol Monogr* **36**: 49–84.

Crowley TJ, North GR (1991). *Paleoclimatology (Oxford Monographs on Geology and Geophysics 16)*. Oxford University Press: New York. pp 339.

de Azevedo AC, Monteiro FA, Cabello PH, Souza NA, Rosa-Freitas MG, Rangel EF (2000). Studies on populations of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) in Brazil. *Mem Inst Oswaldo Cruz* **95**: 305–322.

de Fátima Rossetti D (2001). Late Cenozoic sedimentary evolution in northeastern Pará, Brazil, within the context of sea level changes. *J S Am Earth Sci* **14**: 77–89.

de Fátima Rossetti D, Mann de Toledo P, Góes AM (2005). New geological framework for western Amazonian (Brazil) and implications for biogeography and evolution. *Quat Res* **63**: 78–89.

Deane LM (1947). Observacoes sobre a malaria na Amazonia brasileira. *Rev Soc Bras Med Trop* **24**: 13–20.

Deane LM (1988). Malaria studies and control in Brazil. *Am J Trop Med Hyg* **33**: 223–230.

Dick CW, Roubik DW, Gruber KF, Bermingham E (2004). Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography. *Mol Ecol* **13**: 3775–3785.

Dujardin JP, Torrez EM, Le Pont F, Hervas D, Sossa D (1997). Isozymic and metric variation in the *Lutzomyia longipalpis* complex. *Med Vet Entomol* **11**: 394–400.

Dye C, Davies CR, Lainson R (1991). Communication among phlebotomine sandflies: a field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Anim Behav* **42**: 183–192.

Erwin T, Pogue M (1988). *Agra*, arboreal beetles of neotropical forest. Biogeography and forest refugium hypothesis (Carabidae). In: Vanzolini P, Heyer W (eds). *Proceedings of Workshop on Neotropical Distribution Patterns*. Academia Brasileira de Ciencias: Rio de Janeiro.

Erwin TL (1998). Forests and insects. In: Watt AD, Stork NE, Hunter MD (eds). *A Review in Biodiversity and Conservation*. Chapman & Hall: London. pp 1–406.

Faran ME (1980). Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus section of the subgenus Nyssorhynchus of Anopheles. *Contrib Am Entomol Inst* **15**: 1–215.

Forattini OP (1962). Parte Geral, Diptera, Anophelini Faculdade de Higiene e Saúde Pública, Universidade de São Paulo, São Paulo, Brasil. *Entomologica Medica* **1**: 622.

Forattini OP (1980). Biogeografia, origem e distrbuição da domiciliação de triatomíneos no Brasil. *Rev Saúde publ São Paulo* **14**: 265–299.

Frailey CD, Lavina EL, Rancy A, de Souza JP (1988). A proposed Pleistocene/Holocene lake in the Amazon basin and its significance to Amazonian geology and biogeography. *Acta Amaz* **18**: 119–143.

Freitas-Sibajev MG, Conn J, Mitchell SE, Cockburn AF, Seawright JA, Momen H (1995). Mitochondrial DNA and morphological analyses of *Anopheles darlingi* populations from Brazil (Diptera: Culicidae). *Mosq Syst* **27**: 79–99.

Galardo AK, Arruda M, D'Almeida Couto AA, Wirtz R, Lounibos LP, Zimmerman RH (2007). Malaria vector incrimination in three rural riverine villages in the Brazilian Amazon. *Am J Trop Med Hyg* **76**: 461–469.

Gascon C, Malcolm JR, Patton JL, da Silva MNF, Bogart JP, Lougheed SC *et al.* (2000). Riverine barriers and the geographical distribution of Amazonian species. *Proc Nat Acad Sci* **97**: 13672–13677.

Haffer J (1969). Speciation in Amazonian forest birds. *Science* **165**: 131–137.

Haffer J (1993). On the 'river effect' in some forest birds of southern Amazonia. *Bol Mus Para Emilio Goeldi, Sér Zool* **8**: 217–245.

Hall JPW, Harvey DJ (2002). The phylogeography of Amazonia revisited: new evidence from Riodinid butterflies. *Evolution* **56**: 1489–1497.

Hallam A (1994). *An Outline in Phanerozoic Biogeography*. Oxford Univ Press: New York. pp 246.

Hamilton JG, Dawson W, Pickett JA (1996a). 9-Methylgermacrene-B, proposed structure for novel homosesquiterpene from the sex pheromone glands of *Lutzomyia longipalpis* (Diptera:Psychodidae) from Lapinha, Brazil. *J Chem Ecol* **22**: 1477–1491.

Hamilton JG, Dawson W, Pickett JA (1996b). 3-Methyl-α-himachalene, proposed structure for novel homosesquiterpene from the sex pheromone glands of *Lutzomyia longipalpis* (Diptera:Psychodidae) from Jacobina, Brazil. *J Chem Ecol* **22**: 2331–2334.

Hamilton JG, Maingon RD, Alexander B, Ward RD, Brazil RP (2005). Analysis of the sex pheromone extract of individual male *Lutzomyia longipalpis* sandflies from six regions in Brazil. *Med Vet Entomol* **19**: 480–488.

Hamilton JG, Ward RD, Dougherty MJ, Maignon R, Ponce C, Ponce E *et al.* (1996c). Comparison of the sex-pheromone components of *Lutzomyia longipalpis* (Diptera:Psychodidae) from areas of visceral and atypical cutaneous leishmaniasis in Honduras and Cost Rica. *Ann Trop Med Parasitol* **90**: 533–541.

Harry M (1993). Isozymic data question the specific status of some blood-sucking bugs of the genus *Rhodnius*, vectors of Chagas disease. *Trans R Soc Trop Med Hyg* **87**: 492–493.

Harry M, Galindez I, Cariou ML (1992). Isozyme variability and differentiation between *Rhodnius prolixus, R. robustus* and *R. pictipes*, vectors of Chagas disease in Venezuela. *Med Vet Entomol* **6**: 37–43.

Hewitt GM (1999). Post-glacial re-colonization of European biota. *Biol J Linn Soc* **68**: 87–112.

Hodgkinson VH, Birungi J, Quintana M, Dietze R, Munstermann LE (2003). Mitochondrial cytochrome b variation in populations of the visceral leishmaniasis vector *Lutzomyia longipalpis* across eastern Brazil. *Am J Trop Med Hyg* **69**: 386–392.

Hooghiemstra H, Van de hammen T (1998). Neogene and quaternary development of the neotropical rainforest. *Earth Sci Rev* **44**: 147–183.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**: 111–120.

Klein TA, Lima JBP, Tada MS (1991a). Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondonia Brazil. *Am J Trop Med Hyg* **44**: 598–603.

Klein TA, Lima JBP, Tada MS, Miller R (1991b). Comparative susceptibility of anopheline mosquitoes in Rondonia Brazil to infection by *Plasmodium vivax*. *Am J Trop Med Hyg* **45**: 463–470.

Klein TA, Lima JBP, Toda Tang A (1991c). Hybridization evidence supporting separate species status for *Anopheles albitarsis* and *Anopheles deaneorum* (Diptera: Culicidae) in Brazil. *J Am Mosq Contrl Assoc* **7**: 301–303.

Kreutzer RD, Kitzmiller JB, Ferreira E (1972). Inversion polymorphism in the salivary gland chromosomes of *Anopheles darlingi* root. *Mosq News* **32**: 555–565.

Kreutzer RD, Kitzmiller JB, Rabbani MG (1976). Cytogenetically distinguishable populations of the mosquito *Anopheles albitarsis*. *Acta Amaz* **6**: 473–481.

Krinsky WL (2002). True bugs (Hemiptera). In: Mullen G, Durden L (eds). *Medical and Veterinary Entomology*. Academic Press: Orlando, Florida. pp 69–85.

Kumar S, Tamura K, Nei M (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**: 150–163.

Lainson R, Rangel EF (2005). *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. *Mem Inst Oswaldo Cruz* **100**: 811–827.

Lainson R, Shaw JJ, Silveira FT, Fraiha H (1983). Leishmaniasis in Brazil. XIX: visceral leishmaniasis in the Amazon region, and the presence of Lutzomyia longipalpis on the island of Marajó, Pará state. *Trans R Soc Trop Med Hyg* **7**: 323–330.

Lampo M, Torgenson D, Marquez LM, Rinaldi M, Garcia CZ, Arab A (1999). Occurrence of sibling species of Luyzomyia longipalpis (Diptera: Psychodidae) in Venezuela. First evidence from reproductively isolated sympatric populations. *Am J Trop Med Hyg* **61**: 1004–1009.

Lanzaro GC, Ostrovska K, Herrero MV, Lawyer PG, Warburg A (1993). *Lutzomyia longipalpis* is a species complex: genetic divergence and interspecific hybrid sterility among three populations. *Am J Trop Med Hyg* **48**: 839–847.

Lehr MA (2003). *MS Thesis*. University of Vermont, Burlington, Vermont.

Lehr MA, Kilpatrick CW, Wilkerson RC, Conn JE (2005). Cryptic species in the *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) complex: incongruence between random amplified polymorphic DNA-polymerase chain reaction identification and analysis of mitochondrial DNA *COI* gene sequences. *Ann Entomol Soc Am* **98**: 908–917.

Lent H, Wygodzinsky PW (1979). Revision of the triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas disease. *Bull Am Mus Nat Hist* **163**: 123–520.

Li C, Wilkerson RC (2005). Identification of *Anopheles* (*Nyssorhynchus*) *albitarsis* complex species (Diptera: Culicidae) using rDNA internal transcribed spacer 2-based polymerase chain reaction primes. *Mem Inst Oswaldo Cruz* **100**: 495–500.

Lima JBP, Valle D, Peixoto AA (2004). Analysis of reproductive isolation between sibling species *Anopheles albitarsis* sensu stricto and *Anopheles deaneorum*, two malaria vectors belonging to the Albitarsis complex (Diptera: Culicidae). *J Med Entomol* **41**: 888–893.

Linthicum KJ (1988). A revision of the *Argyritarsis* section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst* **20**: 98–271.

Lyman DE, Monteiro FA, Escalante AA, Cordon-Rosales C, Wesson DM, Dujardin JP *et al*. (1999). Mitochondrial DNA sequence variation among triatomine vectors of Chagas disease. *Am J Trop Med Hyg* **60**: 377–386.

Maingon RD, Ward RD, Hamilton JG, Noyes HA, Souza N, Kemp SJ *et al*. (2003). Genetic identification of two sibling species of *Lutzomyia longipalpis* (Diptera: Psychodidae) that produce distinct male sex pheromones in Sobral, Ceara State, Brazil. *Mol Ecol* **12**: 1879–1894.

Malafronte RS, Marrelli MT, Marinotti O (1999). Analysis of ITS2 DNA sequences from Brazilian *Anopheles darlingi* (Diptera: Culicidae). *J Med Entomol* **36**: 631–634.

Mangabeira O (1969). Sôbre a sistematica e biologia dos *Phlebotomus* do Ceará. *Rev Bras Malariol D Trop* **21**: 3–25.

Manguin S, Wilkerson RC, Conn JE, Rubio-Palis Y, Danoff-Burg JA, Roberts DR (1999). Population structure of the primary malaria vector in South America, *Anopheles darlingi*, using isozyme, random amplified polymorphic DNA, internal transcribed spacer 2, and morphologic markers. *Am J Trop Med Hyg* **60**: 364–376.

Miles MA, Feliciangeli MD, Arias AR (2003). American trypanosomiasis (Chagas' disease) and the role of molecular epidemiology in guiding control strategies. *Br Med J* **28**: 1444–1448.

Mirabello L, Conn JE (2006a). Molecular population genetics of the malaria vector *Anopheles darlingi* in central and South America. *Heredity* **96**: 311–321.

Mirabello L, Conn JE (2006b). Molecular population genetics of the malaria vector *Anopheles darlingi* in central and South America. *Heredity* **97**: 438.

Monteiro FA, Barrett TV, Fitzpatrick S, Cordon-Rosales C, Feliciangeli D, Beard CB (2003). Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. *Mol Ecol* **12**: 997–1006.

Monteiro FA, Wesson DM, Dotson EM, Schofield CJ, beard CB (2000). Phylogeny and molecular taxonomy of the Rhodniini derived from mitochondrial and nuclear DNA sequences. *Am J Trop Med Hyg* **62**: 460–465.

Moritz C, Patton JL, Schneider CJ, Smith TB (2000). Diversification of rainforest faunas: an integrated molecular approach. *Annu Rev Ecol Syst* **31**: 533–563.

Morrison AC, Ferro C, Morales A, Tesh RB, Wilson ML (1993). Dispersal of the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. *J Med Entomol* **30**: 427–435.

Mukhopadhyay J, Ghosh K, Rangel EF, Munstermann LE (1998). Genetic variability in biochemical characters of Brazilian field populations of the Leishmania vector, *Lutzomyia longipalpis* (Diptera: Psychodidae). *Am J Trop Med Hyg* **59**: 893–901.

Mutebi JP, Tripet F, Alexander JB, Lanzaro GC (2002). Genetic differentiation among populations of *Lutzomyia longipalpis* (Diptera: Psychodidae) in central and South America. *Ann Entomol Soc Am* **95**: 740–752.

Narang SK, Klein TA, Perera OP, Lima JB, Tang AT (1993). Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis* complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphisms. *Biochem Genet* **31**: 97–112.

Patton JL, da Silva MNF (1998). Rivers, refuges, and ridges: the geography of speciation of Amazonian mammals. In: Howard DJ, Berlocher SH (eds). *Endless Forms: Species and Speciation*. Oxford University Press: Oxford, UK. pp 202–213.

Patton JL, da Silva MNF, Malcom JR (2000). Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. *Bull Am Mus Nat Hist* **224**: 1–306.

Póvoa MM, de Souza RT, Lacerda RN, Rosa ES, Galiza D, de Souza JR *et al*. (2006). The importance of *Anopheles albitarsis* E and *An. darlingi* in human malaria transmission in Boa Vista, state of Roraima, Brazil. *Mem Inst Oswaldo Cruz* **101**: 163–168.

Prance GT (1982). *Biological Diversification in the Tropics*, vol. 32. Colombia University Press: New York.

Räsänen M, Linna AM, Santos JCR, Negri FR (1995). Late Miocene tidal deposits in the Amazonian foreland basin. *Science* **69**: 386–390.

Renaud S, Dam JV (2002). Influence of biotic and abiotic environment on dental size and shape evolution in a Late Miocene lineage of murine rodents (Teruel basin Spain). *Palaeogeogr Palaeoclimatol Palaeoecol* **184**: 163–175.

Roberts DR, Laughlin LL, Hsheih P, Legters LJ (1997). DDT, global strategies, and a malaria control crisis in South America. *Emerg Inf Dis* **3**: 295–302.

Roberts DR, Manguin S, Rejmankova E, Andre R, Harbach RE, Vanzie E *et al*. (2002). Spatial distribution of adult *Anopheles darlingi* and *Anopheles albimanus* in relation to riparian habitats in Belize, Central America. *J Vector Ecol* **27**: 21–30.

Rosa-Freitas MG, Broomfield G, Priestman A, Milligan JJM, Momen H, Molyneaux DH (1992). Cuticular hydrocarbons, isoenzymes and behavior of three populations of *Anopheles darlingi* from Brazil. *J Am Mosq Control Assoc* **8**: 357–366.

Rubinoff D, Holland BS (2005). Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Syst Biol* **54**: 952–961.

Rubio-Palis Y, Zimmerman RH (1997). Ecoregional classification of malaria vectors in the neotropics. *J Med Entomol* **34**: 499–510.

Ruegg KC, Smith TB (2002). Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). *Proc Biol Sci* **269**: 1375–1381.

Scarpassa VM, Conn JE (2007). Population genetic structure of the major malaria vector *Anopheles darlingi* (Diptera: Culicidae) from the Brazilian Amazon, using microsatellite markers. *Mem Inst Oswaldo Cruz* (in press).

Scataglini M, Lanteri AA, Confalonieri VA (2006). Diversity of Boll Weevil populations in South America: a phylogeographic approach. *Genetica* **126**: 353.

Schofield CJ (2000). Area-wide control of chagas disease vectors in Latin America. In: Tan KH (ed). *Area-Wide Control of Fruit Flies and Other Insect Pests*. Penerbit Universiti Sains Malaysia: Penang. pp 131–133.

Sheldon PR (1996). Plus ça change – a model for stasis and evolution in different environments. *Palaeogeogr Palaeoclimatol Palaeoecol* **127**: 209–227.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006). Comparative phylogeography of unglaciated eastern North America. *Mol Ecol* **15**: 4261–4293.

Soto SI, Lehmann T, Rowton ED, Velez ID, Porter CH (2001). Speciation and population structure in the morphospecies *Lutzomyia longipalpis* (Lutz & Neiva) as derived from the mitochondrial *ND4* gene. *Mol Phylogenet Evol* **18**: 84–93.

Souza CM, Vigoder FM, Araki AS, Ward RD, Kyriacou CP, Peixoto AA (2004). Analysis of the copulatory courtship songs of *Lutzomyia longipalpis* in six populations from Brazil. *J Med Entomol* **41**: 906–913.

Steiner WWM, Narang S, Kitzmiller JB, Swofford DL (1982). Genetic divergence and evolution in neotropical *Anopheles* (*Nyssorhynchus*). In: Steiner WWM, Tabachnick WJ, Rai KS, Narang S (eds). *Recent Developments in the Genetics of Insect Vectors*. Stripes: Champaign, IL. pp 523–550.

Tadei WP, Santos JMM, Rabbani MG (1982). Biologia de anofelinos amazônicos. V. Polimorfismo cromossômico de Anopheles darlingi Root (Diptera: Culicidae). *Acta Amazonica* **12**: 353–369.

Voorham J (2002). Intra-population plasticity of *Anopheles darlingi*'s (Diptera, Culicidae) biting activity patterns in the state of Amapa, Brazil. *Rev Saude Publica* **36**: 75–80.

Vrba ES (1992). Mammals as a key to evolutionary theory. *J Mammal* **73**: 1–28.

Wallace AR (1852). On the monkeys of the Amazon. *Proc Zool Soc Lond* **20**: 107–110.

Walton C, Handley JM, Tun-Lin W, Collins FH, Harbach RE, Baimai V *et al*. (2000). Population structure and population history of *Anopheles dirus* mosquitoes in Southeast Asia. *Mol Biol Evol* **17**: 962–974.

Ward RD, Phillips A, Burnet B, Marcondes C (1988). The *Luzyomyia longipalpis* complex reproduction and distribution. In: Service MW (ed). *Biosystematics of Haematophagous Insects*, Vol. 37. Claredon: Oxford. pp 257–269.

Ward RD, Ribeiro AL, Ready PD, Murtagh A (1983). Reproductive isolation between different forms of *Lutzomyia longipalpis* (Lutz & Neiva, 1912), (Diptera: Psychodidae), the vector of *Leishmania donovani chagasi* Cunha & Chagas and its significance to kala-azar distribution in South America. *Mem Inst Oswaldo Cruz* **78**: 269–280.

Watts PC, Hamilton JG, Ward RD, Noyes HA, Souza NA, Kemp SJ *et al*. (2005). Male sex pheromones and the phylogeographic structure of the *Lutzomyia longipalpis* species complex (Diptera: Psychodidae) from Brazil and Venezuela. *Am J Trop Med Hyg* **73**: 734–743.

Webb SD (1995). Biological implications of the middle Amazon seaway. *Science* **269**: 361–362.

Weir JT (2006). Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. *Evolution* **60**: 842–855.

Wilkerson RC, Gaffigan TV, Bento Lima J (1995b). Identification of species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* by random amplified polymorphic DNA-polymerase chain reaction (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* **90**: 721–732.

Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV, Bergo E, Consolim J (1995a). Diagnosis by random amplified polymorphic DNA polymerase chain reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. *J Med Entomol* **32**: 697–704.

Yin H, Mutebi JP, Marriot S, Lanzaro G (1998). Metaphase karyotypes and G-banding in sandflies of the *Lutzomyia longipalpis* species complex. *Med Vet Entomol* **13**: 72–77.

Young DM, Duncan MA (1994). Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Mem Am Entomol Inst*, No. 54. Assoc. Pub.: Gainesville, FL.

Zink RM (1997). Phylogeographic studies of North American birds. In: Mindel DP (ed). *Avian Molecular Evolution and Systematics*. Academic: San Diego, CA. pp 301–324.