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Genetic diversity of *Angiopolybia pallens* (Lepeletier) (Hymenoptera, Vespidae, Polistinae) explained by the disjunction of South American rainforests

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ABSTRACT. *Angiopolybia pallens* is a swarm-founding wasp that occurs in Atlantic and Amazonian rainforests, which have been isolated from each other by a large arid corridor in central Brazil since the Tertiary. In this study, we used data from polymerase chain reaction-restriction fragment length polymorphism of 16S rDNA to infer some aspects of the genetic diversity and evolutionary history of this social wasp population. Samples collected across the species range in both rainforests showed remarkable differences that separated them into two distinct haplogroups. However, some samples from isolated areas in northeastern Bahia were more related to Amazonian lineages than to southern Bahia populations. Vicariant divergenc is discussed under South American rainforests biogeographical proposals.

Key words: 16S rDNA; South American rainforests; Biogeography; Epiponini; PCR-RFLP; Social wasps

INTRODUCTION

The Atlantic and Amazonian rainforests have already been connected (Costa, 2003) but are currently disrupted by an arid corridor, the Brazilian savanna. Such disruption seems to have been caused by cyclic climatic-vegetational changes in the tropical forests during glacial events in the Cenozoic era (Ab'Saber, 1990; Muller and MacDonald, 1995; Mayle et al., 2000; Silva and Bates, 2002; Roig-Juñent et al., 2006). According to Roig-Juñent et al. (2006), the period of higher expansion of the savannas occurred in the upper Cenozoic era, which agrees with a gradual establishment of an arid corridor between Brazilian rainforests during the Tertiary and Quaternary (Costa, 2003).

Several studies using DNA markers have provided information on the lineage diversification during the Quaternary climatic oscillations (Hewitt, 2000, 2004). This approach allowed the identification of genetic profiles and their geographical distribution and has been widely applied for phylogeographical analyses (Carnaval et al., 2009; Resende et al., 2010; Thomé et al., 2010). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) has successfully provided information on the genetic diversity of bees (Bouga et al., 2005; Brito and Arias, 2005; Collet et al., 2007). However, there is no information on the geographical distribution of mitochondrial lineages of Neotropical social wasps. These analyses could be useful for understanding the historical events affecting the genetic diversity, population structure, and species conservation status.

Social Polistinae is a highly diverse wasp group in the Neotropical region. Studies carried out by Richards (1978) provided initial insights into the phylogeny and geographical distribution of this group. Moreover, Carpenter (1991, 1993) reported accurate resolution of the generic relationships within the tribes Polistini, Mischocyttarini, Ropalidiini, and Epiponini. Most of these results were corroborated by Zhu et al. (2000) and Arévalo et al. (2004) using morphological and molecular data. Pickett and Carpenter (2010), however, reviewed several molecular and morphological features of Vespidae and proposed Ropalidiini as the most basal group, followed by Mischocyttarini and the sister tribes Polistini and Epiponini.

Angiopolybia pallens (Lepeletier) is a basal swarm-founding wasp within the tribe Epiponini, restricted to South American rainforests and the Pantanal (Richards, 1978; Silveira, 2002; Silveira et al., 2005; Cruz et al., 2006a,b), with a few populations occurring in fragmented forests in dry biomes. Disjunct Amazonian and Atlantic rainforest populations of these wasps are isolated by thousands of kilometers, and gene flow among them is unlikely to occur.

In this study, we evaluated the genetic divergence among samples of *A. pallens* collected across the species range by using PCR-RFLP in a 16S rDNA fragment.

MATERIAL AND METHODS

We analyzed specimens of *A. pallens* collected in the Atlantic (N = 19) and Amazonian (N = 16) rainforests (Table 1). Two individuals of the closely related *Angiopolybia paraensis* (Spinola) (Andena et al., 2007), whose distribution is restricted to the Amazonian rainforest (Richards, 1978), were included as outgroup.

Total DNA was extracted from the thorax of one individual per nest following the method described in Han and McPheron (1997). 16S rDNA amplifications were conducted using the primers designed by Svenson and Whiting (2004). The PCR consisted of 0.3 µL 5

U/µL *Taq* polymerase (Promega), 5 µL 5X reaction buffer provided by the manufacturer (Promega), 2.5 µL 10 mM dNTPs mix, 3 µL 25 mM $MgCl_2$, 2 µL DNA, 1.25 µL 20 µM of each primer, and sterile water. PCR amplification cycles consisted of an initial heating step for 5 min at 94°C, followed by 40 cycles of 94°C for 1 min, 42°C for 1.5 min, and 64°C for 1.5 min, and an additional 5 min extension step at 64°C.

Table 1. Sampled localities (L), number of nests (N), haplogroups (H), and geographical designations.

Locality (L)	N	H	Geographical designation
1 Santa Terezinha	2	B	Atlantic rainforest - Northeastern Bahia, Brazil
2 Vera Cruz - Itaparica Island	2	B	Atlantic rainforest - Northeastern Bahia, Brazil
3 Ituberá	2	C; G	Atlantic rainforest - Northeastern Bahia, Brazil
4 Ilhéus	6	A	Atlantic rainforest - Southern Bahia, Brazil
5 Itabuna	1	A	Atlantic rainforest - Southern Bahia, Brazil
6 Santa Luzia	1	A	Atlantic rainforest - Southern Bahia, Brazil
7 Camacan	3	A	Atlantic rainforest - Southern Bahia, Brazil
8 Arataca	1	A	Atlantic rainforest - Southern Bahia, Brazil
9 Belmonte	1	E	Atlantic rainforest - Southern Bahia, Brazil
10 Altamira	5	2C; F; 2H	Amazon rainforest - Pará, Brazil
11 Presidente Figueiredo	5	4C; D	Amazon rainforest - Amazonas, Brazil
12 Iquitos	1	C	Amazon rainforest - Maynas, Loreto, Peru
13 Napo	1	C	Amazon rainforest - Ecuador
14 Paracou	1	C	Amazon rainforest - French Guiana
15 Petit Saut	2	C	Amazon rainforest - French Guiana
16 Maripasoula	1	C	Amazon rainforest - French Guiana

The amplified 16S rDNA fragment was digested with 14 restriction endonucleases (*Alu*I, *Bam*HI, *Bgl*II, *Cla*I, *Dra*I, *Eco*RV, *Hae*III, *Hinc*II, *Hph*I, *Mbo*I, *Pst*I, *Ssp*I, *Taq*I, and *Vsp*I), one at a time. The digested fragments were separated by electrophoresis on 2.0% agarose gels in 0.5X TBE buffer, stained with ethidium bromide, and visualized and photographed under UV light. We used 0.5 µg/µL ΦX174 DNA/*Bsu*RI (*Hae*III) as a size marker (Fermentas).

RESULTS AND DISCUSSION

A. pallens showed restriction fragment length polymorphism for the four enzymes, *Dra*I, *Pst*I, *Ssp*I, and *Vsp*I, in the 16S rDNA fragment (~580 bp). The restriction fragment estimated sizes are shown in Table 2. The PCR-RFLP patterns allowed us to separate the *A. pallens* samples into eight distinct haplogroups (Tables 1 and 3; Figure 1).

Table 2. Restriction fragment size estimates (in base pairs) of all fragment patterns for the16S rDNA gene fragment.

16S rDNA																	
		*Ssp*I				*Pst*I				*Vsp*I					*Dra*I		
580	—				580	—		580	—				260			—	—
300			—		490		—	400			—		220				—
250		—*						300				—	210		—*		
280			—	—*				200		—*			200	—*			
80		—			90		—	180		—	—	—	180	—			
20				—				100				—	160		—	—*	
													100				—

*Two overlapping bands.

Table 3. Designation of the haplotypes found after digestion with enzymes *Ssp*I, *Pst*I, *Vsp*I, and *Dra*I and distribution of such as haplotypes in the eight haplogroups of *A. pallens*.

Haplogroup/enzyme	A	B	C	D	E	F	G	H
*Ssp*I	250				250	580	280	580
	250	300	300	580	250		280	
	80	280	280		80		20	
*Pst*I	490	490	490	580	490	490	490	490
	90	90	90		90	90	90	90
*Vsp*I	200	400	200	300	200	200	200	200
	200	180	200	190	200	200	200	200
	180		180	90	180	180	180	180
*Dra*I	210	210	210	260	200	260	210	210
	210	210	210	160	200	220	210	210
	160	160	160	160	180	100	160	160

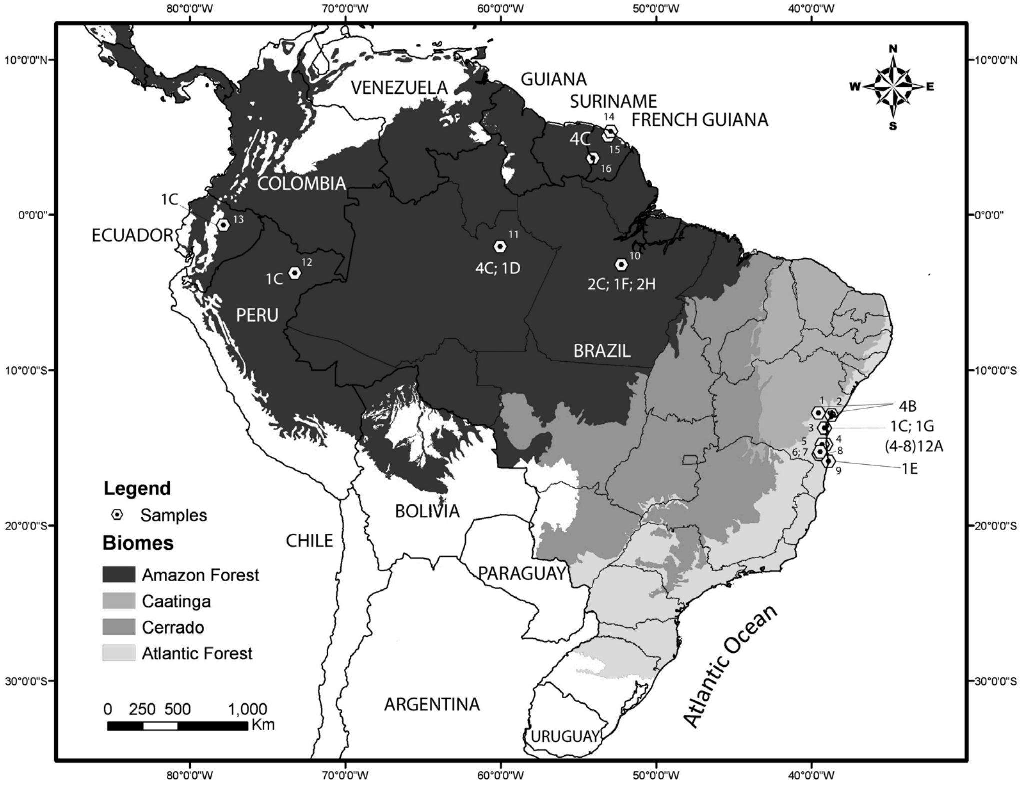

Figure 1. Haplogroup distribution of populations of *Angiopolybia pallens* in South America. Hexagons refer to the sampled sites. The numbers refer to the sampling locality codes shown in Table 1. Numbers followed by a letter represent the number of nests sampled and the haplogroup code (A - H), respectively.

Samples from southern Bahia were placed in haplogroup A. Samples from Santa Terezinha (L1) and Vera Cruz (L2), both in northeastern Bahia, were combined into haplogroup B, and samples from French Guiana, Ecuador, and Peru were grouped into haplogroup C. In the latter were also included two samples from the state of Pará (L10), four from the state of Amazonas (L11), and one from northeastern Bahia (L3, in Ituberá, Bahia). In the other five haplogroups, samples with rare haplotype combinations were included. *A. paraensis* had a haplotype combination similar to those in haplogroup C, and therefore this species was included in this group. Haplogroup C was the most frequent and widely distributed in the Amazonian region, whereas the Atlantic rainforest was mostly represented by haplogroup A.

The presence of *A. pallens* in both the Amazonian and Atlantic forests suggests that this species had an ample and continuous range in the past, which is also consistent with the hypothesis of the existence of a Cenozoic connection between these forests before glacial events (Costa, 2003). The great expansion of the Brazilian savannas on the upper Cenozoic (Roig-Juñent et al., 2006) could have helped to establish the current disjunct distribution. A similar disjunct distribution has been observed for other Polistinae species such as *Epipona media* Cooper (Menezes et al., 2010) and *Synoeca septentrionalis* (Menezes et al., 2011), but so far, there are no published studies that specifically address this issue.

The ample occurrence of haplogroup C in the Amazonian region and in northeastern Brazil (L3 in Ituberá) suggests that this haplogroup could be related to the putative ancestral lineage of *A. pallens* and that the colonization might have followed a north to northeast route. Under these assumptions, the center of origin of *A. pallens* would probably be in the Amazonian region.

A more extended analysis within a phylogeographical framework is under development in order to test if Polistinae species ecologically dependent on humid environments, such as *A. pallens*, might have had their populations disrupted after the expansion of the savannas across rainforests in the Cenozoic era, and if forest refuges have represented places of species diversification. Such investigations, allied to inferences on the biogeographical factors causing variation on the Neotropical Polistinae, will provide the required information to answer some of the questions raised here.

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