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Abundance patterns of *Anopheles pseudopunctipennis* and *Anopheles argyritarsis* in northwestern Argentina☆

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ABSTRACT

Anopheles pseudopunctipennis is an important malaria vector in Argentina but the role of *Anopheles argyritarsis* in the transmission of the parasite is still unknown. Abundance patterns of both species and their relationship to climatic variables were studied in the subtropical mountainous forest in northwestern Argentina. Adults were collected with CDC light traps from September 2002 to November 2005 in Salta (northern area) and Tucumán (southern area) provinces, from 3 localities in each province. The abundance of both species in localities was compared using the Kruskal–Wallis test, and their changes in abundance in relation to climatic variables were analyzed by Multilevel Poisson Regression. *Anopheles argyritarsis* was more abundant than *A. pseudopunctipennis*, and both reached a peak during the spring. There were significant differences in abundance in the northern localities for *A. pseudopunctipennis*, and between northern and southern localities for *A. argyritarsis*. Temperature, rainfall and relative humidity were significant predictors of the abundance of these two species.

1. Introduction

Anopheles (*Anopheles*) *pseudopunctipennis* Theobald is broadly distributed from the United States (40° N) to central Argentina (30° S), and its behavior varies throughout this distribution (Manguin et al., 1996). High densities, anthropophily, endophily and endophagy and a tendency to rest on the inner walls of human dwellings after blood-feeding are some of the reasons why it is considered an important regional malaria vector in Argentina, Bolivia, Ecuador, Guatemala, Mexico, Nicaragua and Peru (Pan American Health Organization, 1994; Manguin et al., 1996).

Prior to its redescription by Rueda et al. (2004), there were many taxonomic and molecular papers that sought to evaluate the status of *A. pseudopunctipennis* as a putative species complex (Aitken, 1945; Knight and Stone, 1977; Estrada-Franco et al., 1992, 1993a,b; Manguin et al., 1995). At least three subspecies have been recognized, namely: one from the southern USA, Mexico and Guatemala; another in Central America, Belize and South America; and the third from Grenada Island. The most recent study to address *A. pseudopunctipennis* as a complex was conducted by Coetzee et al. (1999), with specimens from Mexico and Grenada being used in cross-mating experiments. The Grenada population of *A. pseudopunctipennis* was found to be genetically different and was called *A. pseudopunctipennis* species C.

In northwestern Argentina, *A. pseudopunctipennis* has been implicated as a malaria vector since the beginning of the 20th century (e.g., Edwards, 1928). Malaria was described as endemic with epidemic outbreaks from September to October and May to June, and the abundance of *A. pseudopunctipennis* changed throughout the year and was related to variations in ecologic conditions. More recent research has documented that populations of this vector species displayed two abundance peaks during the year, one in autumn and the other during the spring, with maximum mean humidity being identified as the significant climatic variable (Dantur Juri et al., 2003, 2005).

In general, *A. pseudopunctipennis* breeds in sun-exposed clean freshwater in association with floating plants and filamentous algae (Hoffmann and Samano, 1938; Savage et al., 1990; Rejmankova et al., 1991; Manguin et al., 1996). Its population density changes seasonally and seems to increase during the dry season

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(December–April) (Fernández-Salas et al., 1994). Larval abundance was negatively associated with rainfall, which increases during the wet season. Immature stages were found in temporary habitats that disappear at the beginning of the dry season and are replaced by riverine habitats and small pools with filamentous algae (Fernández-Salas et al., 1994). Altitude and the presence of *Heteranthera* sp. are two additional environmental variables of riverine habitats positively associated with *A. pseudopunctipennis* larvae in the dry season (Savage et al., 1990).

Much less is known about the biology and ecology of *Anopheles* (*Nyssorhynchus*) *argyritarsis* Robineau-Desvoidy, which is also broadly distributed in the Neotropical region from Mexico to Argentina (Rubio-Palis, 1993). Immature stages were found in stagnant pools, animals tracks, springs, artificial containers, rock holes, and the margins of river and stream (Faran and Linthicum, 1981). Clear or turbid water exposed totally or partially to sun and the presence of grassy vegetation and green algae characterize this species' breeding sites (Faran and Linthicum, 1981). In Argentina, this species is relatively well known, since Mühlens et al. (1925) reported the presence of *A. argyritarsis* immature stages in breeding sites with clear water associated with vegetation in both anthropogenic and natural environments in Tucumán province.

Both Salta and Jujuy provinces reported *A. argyritarsis*, *A. pseudopunctipennis* and *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga immatures in the margins of rivers, which create the best breeding sites with vegetation and algae species. Bejarano (1951) characterized the breeding sites (northwestern Argentina) as mountainous rivers with green algae of *Spirogyra* sp. Human activity seems to favor breeding places, through the development of spring wells, poorly maintained irrigation ditches, dams and rice paddies.

In terms of involvement in malaria transmission, Vidal (1930) reported that this species plays an important role in the transmission of the disease in the west of Honduras, where in some localities it was the only species found. Boyd (1926) reported finding a low percentage (8%) of *A. argyritarsis* infected with malaria parasites. In Argentina, Paterson (1911) and Neiva and Bárbara (1916) reported the transmission of *P. vivax* by *A. argyritarsis*. It is still considered a primary vector in Central America by Wilkerson and Strickman (1990), and a secondary or local malaria vector in the Americas by the Organización Mundial de la Salud (1990) and Rubio-Palis (1993).

To understand the dynamics of these two species, and prior to studying their vectorial capacity and epidemiologic implications in malaria transmission, a longitudinal entomological study was conducted on *A. pseudopunctipennis* and *A. argyritarsis* in relation to climatic variables in northwestern Argentina.

2. Materials and methods

2.1. Study sites

Anopheles pseudopunctipennis and *A. argyritarsis* were collected in two subtropical mountainous areas in Argentina stretching from the Bolivian border (22° S) to northern Catamarca Province in the northwest of the country (29° S). Three sites were selected in both northern and southern areas, namely, Aguas Blancas (22°43′8″ S; 64°22′26″ W; 405 m), El Oculto (23°06′57″ S; 64°30′4″ W; 508 m) and San Ramón de la Nueva Orán (23°08′49″ S; 64°20′06″ W; 362 m) in the north (Salta province) and Sargento Moya (27°13′31″ S; 65°39′14″ W; 468 m), La Florida (27°13′45″ S; 65°37′25″ W; 452 m) and Capitán Cáceres (27°13′58″ S; 65°37′31″ W; 414 m) in the south (Tucumán province) (Fig. 1).

The subtropical mountainous forest, criss-crossed by rivers, is located on the eastern slopes of the mountain ranges of the Sub-Andean *Sierras*, *Sierras Pampeanas* and Eastern Pre-Andean range, intercepting the moisture-laden winds and rains from the Atlantic. Four layers of vegetation exist within the forest (piedmont forest, mountain forest, mountain wood and altitude grasses). This study was undertaken in the piedmont forest (northern area), which

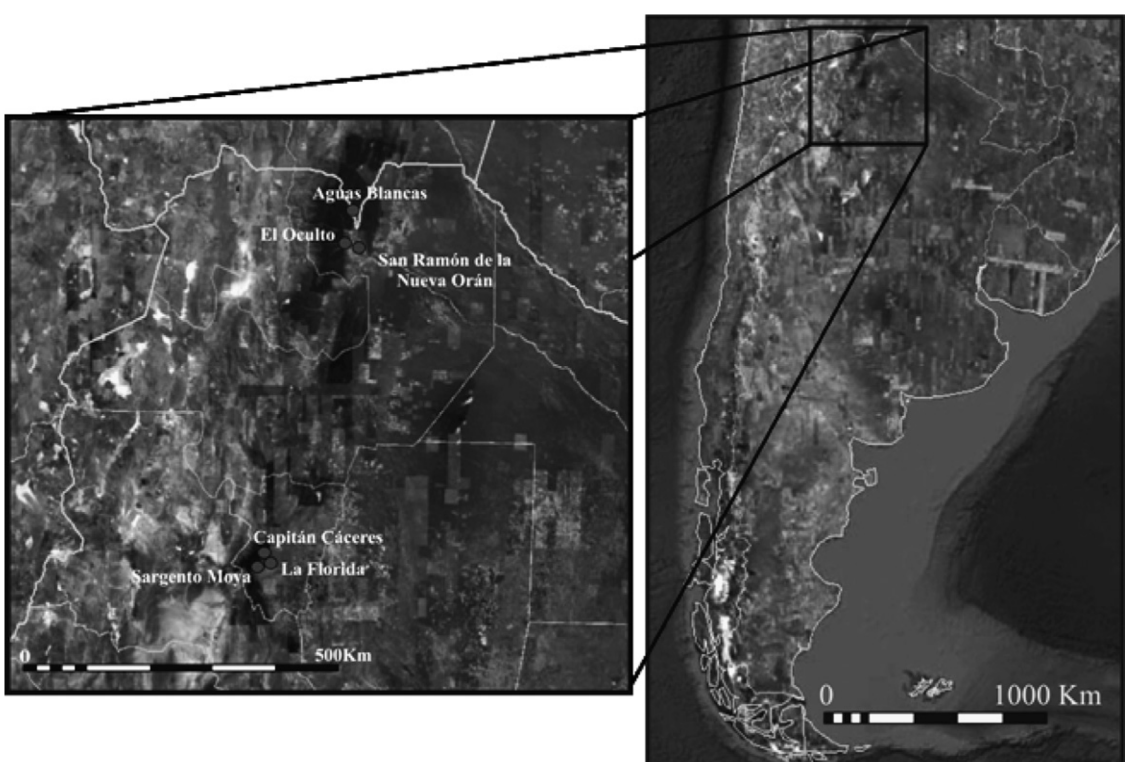

Fig. 1. Location of sampling sites, northwest of Argentina, Argentina.

corresponds to a region radically altered by human activity (deforestation and agriculture), though some patches or remains of native vegetation still exist. The canopy is approximately 30 m high, with a predominance of "*palo blanco*" (*Calycophyllum multiflorum* Griseb. (Castelo). There is also a dense shrub stratum, up to 2 m high, of lianas, climbers and epiphytes (Prado, 1995). The forest of "*tipa y pacará*" (*Tipuana tipu* (Benth.) Kuntze) and (*Enterolobium contortisiliquum* (Vell.) Morong., respectively), located in the south, was extensively modified and replaced by sugar cane, soybean and citrus plantations (Brown, 1995; Prado, 1995).

2.2. Mosquito collection

At each site, four CDC light traps supplemented with carbon dioxide were placed monthly from tree branches at an average height of 1.5 m, from September 2002 to November 2005. Traps were run for 8 h (16:00–24:00 h) from early dusk until midnight. Collection of samples could not be performed much later than midnight owing to technical difficulties affecting the field work (drug trafficking and robbery).

Mosquitoes were immobilized with dry ice and transported to the laboratory for identification, based on keys by Faran (1980) and Wilkerson and Strickman (1990). Voucher specimens were deposited in the collection of the Miguel Lillo Foundation Institute (*Instituto-Fundación Miguel Lillo—IMLA*).

Monthly measures of local weather – comprising maximum and minimum mean temperatures, maximum and minimum mean humidity, mean relative humidity and accumulated rainfall – were drawn from readings recorded at the Aguas Blancas (22°43′ S, 64°22′ W) and San Ramón de la Nueva Orán Weather Stations (23°07′ S, 64°19′ W) in the northern area, and from the Pueblo Viejo Weather Station (27°12′ S, 65°37′ W) in the southern area.

2.3. Data analysis

Monthly abundance of *Anopheles* females was expressed as the mean of individuals captured with 12 light traps for each of the northern and southern areas.

Differences in the abundance of each species as between localities in the northern and southern areas were analyzed by Kruskal–Wallis test (K–W). Monthly abundance of mosquitoes for each locality was calculated as the mean of adult females captured with the four traps. When significant differences were detected by K–W, multiple comparisons of means were conducted using the Student–Newman–Keuls test (SNK) (Zar, 1996).

Fluctuations in relation to climate variables for the two mosquito species were analyzed with Multilevel Poisson Regression (Statacorp., 2005), i.e., with Hierarchical Model for count data (HLM6), which uses the restricted Penalized Quasi Likelihood (PQL) method of estimation. This analysis is used for longitudinal studies, where the outcome variable is measured several times in the same individual. Consequently, observations are not independent (Twisk, 2004). Here, it was used to model the effects of climatic variables on anopheline abundance for the four-year sampling period.

Two levels were considered, i.e., level-1 where the unit was the month, and the level-2 where the unit was the year. The level-1 predictor was centered on the annual mean, with the level-1 linear structural model in this case being:

$$\log(\mu_{ij}) = B_0 + B_1(X_{ij1} - \overline{X}_{ij1}) + B_2(X_{ij2} - \overline{X}_{ij2}) + \cdots = B_0 + \sum_{q=1}^{Q} B_q(X_{ijq} - \overline{X}_{ijq})$$

where μ_{ij} is the event rate per time period, X_{ijq} is a level-1 predictor (climatic variables), B_0 is the intercept, B_q represents the level-1 slope for the independent predictor variable X_{ijq}, and Q is the number of level-1 predictor variables.

The level-2 model takes the following form:

$$B_0 = \alpha + u_0$$

$$B_q = \beta_q + u_q; \quad \text{with } q = 1, \ldots, Q$$

where α and β_q are the fixed effects, and u_0 and u_q are the random effects, which indicate the variability of the coefficients B_q across level-2.

In this case, the best-fit model, using the reliability estimate, was achieved with only the random intercept. Thus the model corresponding to the level-2 was:

$$B_0 = \alpha + u_0$$

$$B_q = \beta_q; \quad \text{with } q = 1, \ldots Q$$

The first of the level-2 equations indicates that, regardless of the values of the climatic variables, the incidence rate changes according to the year. The second of the level-2 equations indicates that the relationship between incidence rate and climatic variables does not change according to the year.

The Incidence Rate Ratio (IRR) of each variable was obtained for the number of specimens collected, along with standard error, *p*-values ($p < 0.001$) and confidence intervals.

3. Results

3.1. Seasonal variation and abundance of *A. pseudopunctipennis* and *A. argyritarsis*

A total of 13764 *Anopheles* females were sampled, 4012 (29.2%) of which were *A. pseudopunctipennis*, 4205 (30.5%) were *A. argyritarsis*, and 5547 (40.3%) were other species, such as *Anopheles* (*Nyssorhynchus*) *strodei* Root, *Anopheles* (*Nyssorhynchus*) *evansae* (Brèthes), *Anopheles* (*Nyssorhynchus*) *rondoni* (Neiva and Pinto), and *Anopheles* (*Nyssorhynchus*) *rangeli* Gabaldon, Cova García and López. The total number of females of *A. pseudopunctipennis* and *A. argyritarsis* differed between the two areas, with 3918 *A. pseudopunctipennis* females and 1922 *A. argyritarsis* females from the north, and 94 and 2283 from the south being collected, respectively.

Considering each locality separately, *A. pseudopunctipennis* was more abundant in El Oculto (3367 specimens), followed by Aguas Blancas ($n = 447$) and San Ramón de la Nueva Orán ($n = 104$). All the localities sampled in the southern area reported a similar amount of specimens, ranging from 22 to 45 samples (in Sargento Moya and Capitán Cáceres). *Anopheles argyritarsis* was more abundant in Sargento Moya ($n = 1117$) and Aguas Blancas ($n = 1054$). A relatively high quantity was observed in all the other localities, i.e., El Oculto ($n = 720$), La Florida ($n = 677$) and Capitán Cáceres ($n = 489$). In San Ramón de la Nueva Orán only 148 specimens were collected.

The seasonal distribution of *A. pseudopunctipennis* and *A. argyritarsis* showed that the greatest abundance of both species occurred during the spring in both areas. The second highest abundance of *A. pseudopunctipennis* and *A. argyritarsis* occurred during the winter in the north and during the summer in the south (Table 1).

Differences among abundances were significant for *A. pseudopunctipennis* from northern localities ($p < 0.001$), as well as for *A. argyritarsis* from the northern ($p < 0.001$) and southern ($p < 0.001$) localities (Table 2). In the north, *A. pseudopunctipennis* abundance was significantly different among the three localities ($p < 0.05$), with it being higher in El Oculto than in Aguas Blancas and San Ramón de la Nueva Orán. For *A. argyritarsis*, differences between localities were also significant ($p < 0.05$) but less noteworthy (Fig. 2).

Table 1
Seasonal abundances of *Anopheles pseudopunctipennis* and *Anopheles argyritarsis* in northern and southern areas of subtropical mountainous forest, in northwestern Argentina.

Years/seasons		*A. pseudopunctipennis*		*A. argyritarsis*	
		Northern	Southern	Northern	Southern
2002	Winter	696	0	1	0
	Spring	975	11	173	9
2003	Summer	28	5	2	4
	Autumn	270	6	48	5
	Winter	59	2	38	3
	Spring	220	18	134	55
2004	Summer	96	7	6	55
	Autumn	130	0	2	29
	Winter	87	0	82	58
	Spring	540	31	635	822
2005	Summer	384	6	33	180
	Autumn	188	1	75	53
	Winter	65	0	118	31
	Spring	180	7	575	979
Total		3918	94	1922	2283

Table 2
Kruskal–Wallis test results for females of *Anopheles pseudopunctipennis* and *Anopheles argyritarsis* collected from 2002 to 2005 at north and south areas of subtropical mountainous forest, in northwestern Argentina.

Area	Specie	*H*	df	*p*
Northern	*A. pseudopunctipennis*	142.501	2	<0.001
	A. argyritarsis	48.525	2	<0.001
Southern	*A. pseudopunctipennis*	10.655	2	0.005
	A. argyritarsis	14.211	2	<0.001

H: Statistic; df: degrees of freedom; *p*: *p*-value.

Whereas no significant differences were observed for *A. pseudopunctipennis* in southern localities, significant differences ($p < 0.05$) were observed between Sargento Moya and the other two localities for *A. argyritarsis* (Fig. 3).

Both species showed wide seasonal fluctuations. While abundance of *A. pseudopunctipennis* varied gradually across the entire collection period, it seemed to be lower during 2004 and 2005 in the north, where peaks occurred in October (Fig. 4A). No seasonal pattern was observed in the south, where fewer *A. pseudopunctipennis* were collected (Fig. 4B). In the north, the abundance of *A. argyritarsis* was greater in 2004 and 2005, with peaks being in evidence from September to November 2002, in November 2004 and in January 2005 (Fig. 4A). In the south, in contrast, abundance of *A. argyritarsis* was low in 2002 and 2003 but peaked in November 2004 and 2005 (Fig. 4B).

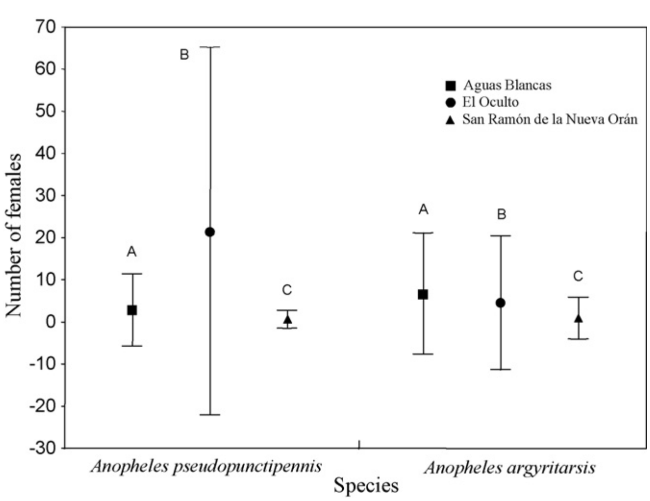

Fig. 2. Mean number (±SD) of females of *Anopheles pseudopunctipennis* and *A. argyritarsis* collected with CDC light traps between September 2002 and November 2005 at 3 localities of Salta Province (Argentine subtropical mountainous forest). Different letters means significant differences detected by SNK test ($p < 0.05$).

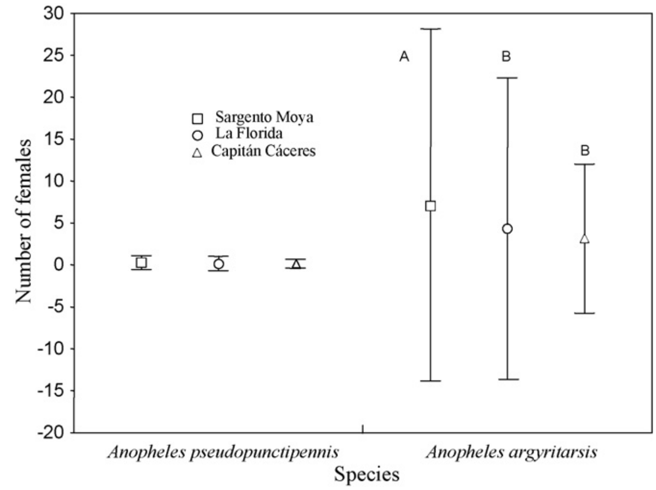

Fig. 3. Mean number (±SD) of female of *Anopheles pseudopunctipennis* and *A. argyritarsis* collected with CDC light traps between September 2002 and November 2005 at 3 localities of Tucumán Province (Argentine subtropical mountainous forest). Different letters means significant differences detected by SNK test ($p < 0.05$).

3.2. Effects of climatic variables

Analysis of the effect of climate variables on *A. pseudopunctipennis* and *A. argyritarsis* in the north showed a characteristic behavior for each species. Hence, for *A. pseudopunctipennis*, temperature, humidity and rainfall all proved highly significant ($p < 0.001$ in each case). According to the Incidence Rate Ratio (IRR): species density increased by 7% for each rise in minimum mean temperature of one degree centigrade; mosquito density decreased by 1% for each increase in rainfall of 1 mm; and mosquito density decreased by 1% for each rise in minimum mean humidity of 1% (Table 3).

An identical analysis was performed in the southern area. Here, both rainfall and mean relative humidity were significant ($p < 0.001$ and $p < 0.002$, respectively). The *IRR* was used to show that: species density increased by 2% for each increase in rainfall of 1 mm; and mosquito density decreased by 1% for each rise in mean relative humidity of 1% (Table 3).

Analysis of the effect of climate variables on *A. argyritarsis* in the northern area showed that the maximum and minimum mean temperatures, as well as minimum mean humidity, were highly significant ($p < 0.001$). The *IRR* showed that species density decreased by 1% when the maximum mean temperature fell by 1 °C, and increased by 21% when the minimum mean temperature rose by 1 °C. Insofar as the minimum mean humidity was concerned, each 1% increase led to a 1% reduction in mosquito density (Table 4).

According to the same analysis carried out in the southern area, the maximum and minimum mean temperature, mean relative humidity and accumulated rainfall were all highly significant ($p < 0.001$). Mosquitoes density increased by 14% and 4%, when the maximum and minimum temperatures increased by 1 °C, respectively. A 1-mm increase in accumulated rainfall led to a 5% increase in mosquito density. In the case of mean relative humidity, when this rose by 1%, mosquito density decreased by 0.8% (Table 4).

Table 3
Multilevel Poisson Regression results for *Anopheles pseudopunctipennis* females collected with CDC light traps from September 2002 to November 2005 at northern and southern areas of the Argentine subtropical mountainous forest.

	Northern				Southern			
	IRR	SE	*p*	95% CI	IRR	SE	*p*	95% CI
Minimum mean temperature	1.07	0.01	<0.001	1.06–1.09				
Maximum mean temperature	0.96	0.01	<0.001	0.95–0.97				
Accumulated rainfall (per month)	0.99	0.01	<0.001	0.99–0.99	1.02	0.01	<0.001	1.01–1.03
Minimum mean humidity	0.98	0.01	<0.001	0.98–0.99				
Mean relative humidity					0.92	0.02	<0.002	0.88–0.97

IRR: Incidence Rate Ratio; SE: Standard Error; CI: Confidence Interval.

4. Discussion

Anopheles pseudopunctipennis is considered a malaria vector throughout its wide geographical distribution. This species' vectorial capacity in the different countries across the Americas tends to be discussed when it is found to be sharing the same habitat with other regional malaria vectors. As stated by Gonzalez-Ceron et al. (2007), it is known that not all mosquito species are able to transmit malaria, with this situation being linked to the disruption of *Plasmodium* parasite development inside them. In this respect, these authors found that, in Mexico, the density of *P. vivax* circumsporozoite protein Vk210 phenotype had a different development in *A. pseudopunctipennis* and *Anopheles* (*Nyssorhynchus*) *albimanus* Weidemann, displaying a low susceptibility in the former species. Nevertheless, ookinete development was similar in both mosquitoes, with differences in migrations out of the blood meal bolus determining differences in infection. These results indicate that, in comparison with *A. pseudopunctipennis*, *A. albimanus* showed a greater capacity to transmit human malaria.

Another point of view is afforded by Raccurt's paper (2004), which reported the presence of *A. albimanus* in Haiti, considering it to be the main malaria vector, closely related to *P. falciparum* transmission. Yet the appearance of *A. pseudopunctipennis* assumes importance, since this species, which appears as a malaria vector in Central America, was recently introduced into the island. Its endophylic behavior was reported in Bellevue city in 1986, with *A. pseudopunctipennis* being deemed to play an important role in malaria transmission.

Brochero et al. (2006) reported that only *Anopheles* (*Nyssorhynchus*) *darlingi* Root and *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldon were the primary malaria vectors in Cimitarra, Santander, Colombia. *A. pseudopunctipennis*, in contrast, was found to be a secondary malaria vector, collected in low percentages outside homes.

Lardeux et al. (2007), who addressed host choice and human blood index of *A. pseudopunctipennis* in Bolivia, considered that this species displayed an opportunistic behavior with a feeding preference for sheep, goats, donkeys and humans, in descending order. The authors concluded that *A. pseudopunctipennis* would not seem to be strongly attracted to humans, and the low anthropophily exhibited by this species in relationship with changing environmental conditions leads to unstable malaria transmission in Bolivia. Furthermore, they note that the proximity of goats and sheep to homes at night may act as a zooprophylactic barrier to malaria transmission.

In Argentina, *A. pseudopunctipennis* did not appear in shared habitats with other malaria regional vectors, nor did it display low anthropophily, since this species is the only known malaria vector and is found close to human dwellings. Although the first reports of malaria in the northwest of the country refer to the disease as the "*chucho*" (Cantón, 1891, 1893), it was not until Paterson's (1911) study that *A. pseudopunctipennis* was implicated with malaria cases, with the internal presence of *Plasmodium* parasites being confirmed by Mühlens et al. (1925). Since 1959, when the malaria eradication program was first implemented, the parasite implicated in transmission was *P. vivax*, though mention was also made of the presence of *P. falciparum* and mixed cases (Carcavallo and Martínez, 1968). From 1989 to 1997 there were two main peaks of malaria cases, with the number rising to 1660 in 1990, subsequently declining, and then climbing as high as 2048 in 1996. Recently, there have been decreasing numbers of malaria cases, i.e., 592 in 1997, 440 in 2000, 115 in 2004, and 209 in 2006 and 2007, most of which were reported as having been imported from Bolivia, with *P. vivax* identified as the common parasite (Boletín Epidemiológico, 1997; World Health Organization, 2008).

Abundance, seasonality, behavior and effects of environmental variables have been mostly studied using immature specimens of *Anopheles*, as in the case of *A. pseudopunctipennis*, *A. albimanus*, *Anopheles* (*Nyssorhynchus*) *aquasalis* Curry and *Anopheles* (*Nyssorhynchus*) *triannulatus* (Neiva and Pinto) (Galvão et al., 1942; Savage et al., 1990; Rejmankova et al., 1991; Berti et al., 1993; Fernández-Salas et al., 1994; Flores-Mendoza and de Oliveira, 1996; Fernandes Silva-do-Nascimento and de Oliveira, 2007). The differences in population dynamics exhibited by immatures may result in characteristic adult population fluctuations related to environmental conditions.

In this study, the most abundant species was *A. argyritarsis*, which was collected from all sites and in both areas (though mainly in the south). In our sampling, this species was associated with *A. pseudopunctipennis* and *A. strodei*, among others. The main abundance of *A. pseudopunctipennis* seems to be confined to the northern area, where malaria is still an important disease.

Table 4
Multilevel Poisson Regression results for *Anopheles argyritarsis* females collected with CDC light traps from September 2002 to November 2005 at northern and southern areas of the Argentine subtropical mountainous forest.

	Northern				Southern			
	IRR	SE	*p*	95% CI	IRR	SE	*p*	95% CI
Minimum mean temperature	1.21	0.01	<0.001	1.18–1.24	1.04	0.01	<0.001	1.03–1.05
Maximum mean temperature	0.96	0.01	<0.001	0.95–0.98	1.14	0.01	<0.001	1.11–1.17
Accumulated rainfall (per month)					1.05	0.01	<0.001	1.04–1.05
Minimum mean humidity	0.98	0.01	<0.001	0.97–0.98				
Mean relative humidity					0.80	0.01	<0.001	0.78–0.82

IRR: Incidence Rate Ratio; SE: Standard Error; CI: Confidence Interval.

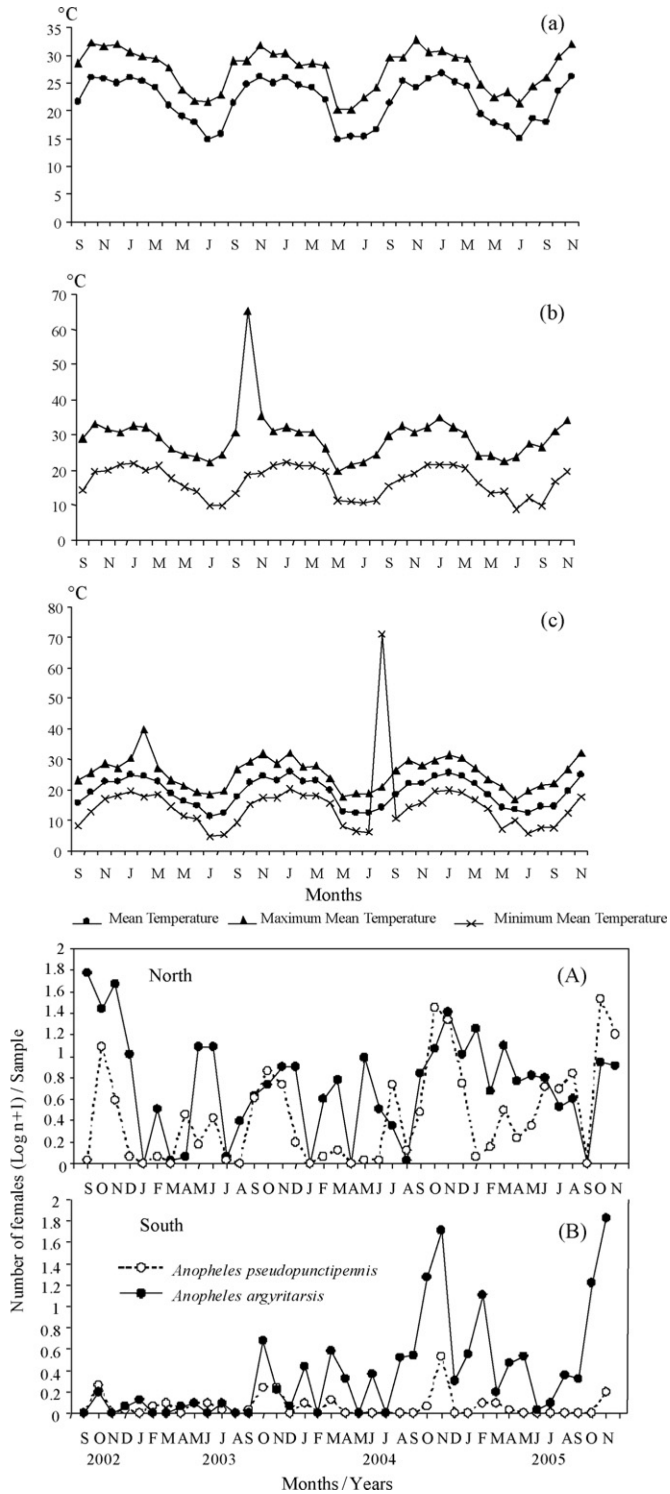

Fig. 4. (a) Monthly variation of temperature (mean, maximum and minimum) in Aguas Blancas, (b) in San Ramón de la Nueva Orán (northern area), (c) in Pueblo Viejo (southern area). (A and B) Number of females (log $n+1$) of *Anopheles pseudopunctipennis* and *A. argyritarsis* collected with 12 CDC light traps operated monthly at northern and southern areas of the Argentine subtropical mountainous forest, respectively.

In contrast to our findings, previous results from Argentina reported numerous habitats positive for *A. pseudopunctipennis* (Paterson, 1911; Petrocchi, 1924; Brèthes, 1926; Shannon and Del Ponte, 1928; Bejarano, 1951, 1956; Carcavallo and Martínez, 1968). While studying malaria cases and malaria vectors in northwestern Argentina, Mühlens et al. (1925) found that *A. pseudopunctipennis* was the most abundant species inside dwellings and around anthropogenic environments. However, *A. argyritarsis* was also abundant, and its presence was associated with environments close to bodies of water such as ponds and streams.

Shannon and Davis (1927), Rozeboom (1941), Hackett (1945) and Manguin et al. (1996) reported that *A. pseudopunctipennis* larvae were collected in fresh, clean, sun-exposed bodies of water. As reported by Mühlens et al. (1925) and by Manguin et al. (1996) vis-à-vis Argentina, immature *A. pseudopunctipennis* stages were collected in association with *A. argyritarsis*. In the same paper, Manguin et al. (1996) observed that in all American localities sampled, *A. pseudopunctipennis* larvae appeared in association with other species of Anophelinae: in Grenada, for instance, *A. pseudopunctipennis* was collected in association with *A. aquasalis* at sea level and with *A. argyritarsis* at a wide range of altitudes; in the USA, *A. pseudopunctipennis* larvae were collected in association with *Anopheles* (*Anopheles*) *punctipennis* (Say); and lastly, *A. pseudopunctipennis* larvae were found in Guatemalan high-altitude habitats along with *Anopheles* (*Anopheles*) *hectoris* Giaquinto-Mira, and in Belize in association with three species, including *A. albimanus*, *A. argyritarsis* and *Anopheles* (*Nyssorhynchus*) *darlingi* Root.

When compared against old reports, the changes observed in the abundance of *A. pseudopunctipennis* and *A. argyritarsis* could be linked to changes in the ecology of both species, as stated by Manguin et al. (1993) in the case of Grenada. They reported that *A. pseudopunctipennis* registered a smaller abundance and distribution than did *A. argyritarsis*, with the possible reason for this situation being a decline in genetic variability, which may have led to a diminished ability to adapt to environmental changes and, in turn, to a reduction in the abundance of *A. pseudopunctipennis*.

The four seasons characteristic of subtropical mountainous forests are: a hot, rainy summer from January to March; a mild, dry fall from April to June; a cold, misty winter from July to September; and a temperate, dry spring from October to December. Both species were more abundant during the spring, when moderate temperatures and low rainfall favor the existence of breeding sites and, by extension, adult abundance. Considering each species separately, whereas *A. pseudopunctipennis* was found to be the most abundant species in the northern area, reaching its highest abundance during October (spring), collections in the southern area yielded a low number of specimens. In the northern area, *A. argyritarsis* displayed greatest abundance at the beginning of and during spring. A similar situation was recorded in the southern area for the latter species, with peaks of abundance in the same season.

The dynamics of both species showed that *A. pseudopunctipennis* only displayed a well-marked fluctuation in the northern area, since its scant presence in the south rendered it impossible for a seasonal pattern to be determined. In the north, *A. argyritarsis* registered continuous activity throughout the year, with the main peaks occurring in spring and, to a lesser extent, fall: in the south, in contrast, spring was a time of very marked growth, starting from a small population that had remained in evidence during the previous months.

Similar results were obtained by Dantur Juri et al. (2003) for the northern area of the subtropical mountainous forest, where *A. pseudopunctipennis* was more abundant in the spring (September–December). In a later paper, Dantur Juri et al. (2009) reported that in El Oculto *A. pseudopunctipennis* did not exhibit marked seasonality, unlike Aguas Blancas, where high abundance was detected at the end of spring and the beginning of summer (September–January).

Flores-Mendoza and de Oliveira (1996) reported similar findings when studying bionomic aspects of *A. aquasalis* in Guraí, Brazil, where the highest abundance of this species occurred after the rainy season or during the moderate rainy period. Galvão et al. (1942) and Berti et al. (1993) found that in Pará (Brazil) and Guyana

(Venezuela) *A. aquasalis* was more abundant when the rainfall was moderate rather than during the heaviest rains.

Fernandes Silva-do-Nascimento and de Oliveira (2007), working with population dynamics of three species of the Triannulatus Complex, found that in Salobra, Brazil, the populations of *A. triannulatus* s.s. reached a peak immediately after the long rainy season. They mentioned the work of Souza-Santos (2002), who reported that *A. triannulatus* s.l. immature stages were more abundant during the rainy season or during the transition to the dry season, and that *A. halophylus* and *A. triannulatus* C peaked in the middle of the dry season.

Savage et al. (1990) and Fernández-Salas et al. (1994), studying *A. pseudopunctipennis* immatures from Mexico, found that this species was more abundant during the dry season (December–May). The rivers tended to dry up and stop flowing, and in the river beds and streams, ponds and pools appear where larval development occurred.

As reported by Rejmankova et al. (1993), *A. pseudopunctipennis* was also found to be more abundant during the dry season in Belize. Moreover, the appearance of both species was positively associated with filamentous algae, and that of *A. argyritarsis* was also positively associated with rock-pool type habitats. The authors remarked that there were problems involved in comparing their results with others because malaria vector larval ecology studies were infrequent and sporadic.

This study, which was conducted over different years, obtained similar results with respect to immatures, with the higher abundance of the species in the dry season being linked to mild climatic conditions. Whereas Rejmankova et al. (1993) reported that neither *A. argyritarsis* nor *A. pseudopunctipennis* was found during the wet season, in our study these species were seen to diminish in abundance but not disappear altogether. The authors questioned how changes in land use would affect the distribution and density of mosquito larval populations, a question that was similarly posed by us, since influence on immature habitats will inevitably affect the abundance and seasonality of the adult forms.

Insofar as the effect of climatic factors on the abundance of *A. pseudopunctipennis* was concerned, maximum and minimum mean temperatures, accumulated rainfall and minimum mean humidity were shown to be the significant variables in the northern area of the subtropical mountainous forest. In the southern area, however, the only variables that affected fluctuations in the abundance of this species were accumulated rainfall and mean relative humidity. Similar findings were reported by Dantur Juri et al. (2003, 2009), who showed that maximum mean humidity and maximum mean temperature significantly affected the fluctuation of *A. pseudopunctipennis* in different sites in the north of Argentina. Taken together, these data suggest that relative humidity could be considered a key factor in determining species abundance.

Different results were reported by Fernández-Salas et al. (1994) when working with *A. pseudopunctipennis* larvae in Mexico. Rainfall was the climatic variable most closely related to the fluctuation of this species, causing flooding of the breeding sites, washing away the immatures, and so reducing adult abundance.

While abundance of *A. argyritarsis* in the northern rainforest was influenced by maximum and minimum mean temperature and relative humidity, in the southern area the climatic variables proved to be the same plus accumulated rainfall. There are no prior records pertaining to the environmental variables that affect the presence and abundance of this species, which could be compared against the current results.

In brief, this study shows that the high abundance and wide geographical distribution of *A. pseudopunctipennis* would appear to be restricted to the northern area of Argentina. From an epidemiologic standpoint, the observed seasonality of *A. pseudopunctipennis*, marked by its high abundance in spring, is important in the northern area (where malaria cases are reported). Although the role of *A. argyritarsis* in malaria transmission is still unknown, its greater abundance in both areas in spring should be noted. Conditioning climatic factors were important, e.g., accumulated rainfall for *A. pseudopuntipennis*, and maximum and minimum mean temperatures for *A. argyritarsis* populations in both areas. Future studies on the vectorial capacity of both species will help confirm and clarify the status of *A. pseudopunctipennis* and *A. argyritarsis* as malaria vectors in Argentina.

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