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Taxonomy / Taxinomie

Anopheles of Bolivia: new records with an updated and annotated checklist

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Abstract

Anopheles squamifemur has been identified from CDC light trap collections carried out at Arca de Israel, a small community located in the extreme north-east of Bolivia (Pando Department) on the banks of the river Madera, on the border with Brazil. *Anopheles costai* and *An. forattinii* have been identified in place of *An. mediopunctatus* which has been removed from the Bolivian list of *Anopheles* species. The first identification of *An. trinkae* in Bolivia by Dr. J.C. Lien in 1984 is cleared. The presence of *An. deaneorum* in Bolivia has been confirmed by our mosquito captures carried out in Guayaramerín (Pando Department, north-east of Bolivia), a border city separated from the type locality of *An. deaneorum*, the Brasilian city of Guajara-Mirin, by the large Mamoré River. These new findings increase to 43 the total number of known *Anopheles* species for Bolivia for which an updated and partially annotated checklist is given. ***To cite this article: F. Lardeux et al., C. R. Biologies ••• (••••).***

Résumé

Anopheles* de Bolivie : Nouveaux signalements avec une liste d'espèces actualisée et commentée.** *Anopheles squamifemur* a été identifié à partir de collectes faites avec des pièges lumineux de type CDC à Arca de Israel, une petite communauté située à l'extrême nord-est de la Bolivie (Département de Pando) sur les rives du fleuve Madera, à la frontière avec le Brésil. *Anopheles costai* et *An. forattinii* ont été identifiés en lieu et place d'*An. mediopunctatus* qui est supprimé de la liste des *Anopheles* de Bolivie. La première identification d'*An. trinkae* en Bolivie par le Dr. J.C. Lien en 1984 est précisée. La présence d'*An. deaneorum* en Bolivie a été confirmée par nos captures effectuées à Guayaramerín (Département de Pando, nord-est de la Bolivie), une ville frontière séparée de la localité type d'*An. deaneorum* (la ville brésilienne de Guajara-Mirin) par le grand fleuve Mamoré. Ces nouveaux résultats augmentent à 43 le nombre des espèces d'*Anophèles* connus de Bolivie, pour laquelle une liste actualisée et partiellement commentée est donnée. ***Pour citer cet article : F. Lardeux et al., C. R. Biologies ••• (••••).

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Keywords: *Anopheles squamifemur*; *Anopheles costai*; *Anopheles forattinii*; *Anopheles deaneorum*; New record; Bolivia

Mots-clés : *Anopheles squamifemur* ; *Anopheles costai* ; *Anopheles forattinii* ; *Anopheles deaneorum* ; Nouvelle occurrence ; Bolivie

1. Introduction

Mosquito species that transmit *Plasmodium* parasites to humans belong to the genus *Anopheles*. In 2004, the genus *Anopheles* comprised six subgenera totaling 484 species: *Anopheles* (189 species), *Cellia* (239 species), *Kerteszia* (12 species), *Lophopodomyia* (6 species), *Nyssorhynchus* (33 species) and *Stethomyia* (239 species) [1]. Only *Cellia* is absent from South America where a provisional total of 85 *Anopheles* species were identified [2]. Taxonomical reorganizations are frequent, especially in Neotropical *Anopheles*. For example, in the *Nysshorhynchus* subgenus, intraspecific variations of morphological traits (that often exceed differences between species) have provoked in-depth taxonomical research, the constant discovery of new species and frequent modifications in the *Anopheles* classification [3]. These reorganizations are also the consequences of new molecular approaches to taxonomy that have led to the discovery of new species, mainly sibling or cryptic ones. As such, checklists are always provisional and evolve with time.

At a country level, a significant step toward the understanding of malaria transmission is to be acquainted with a catalog of local *Anopheles* species and the correct identification of vector species among the list. This local catalog may evolve with time as it is generally prepared gradually by sampling surveys, accumulated knowledge on the local mosquito fauna and the evolution of taxonomical tools.

Bolivia is characterized by the varying nature of its biogeographical units and a high level of species diversity [4,5]. Not surprisingly, the number of mosquito species recognized there so far is high (≈ 150) [2] and it is likely that new species (even for Science) will be discovered in this still poorly prospected country. Despite some founding works [4,6], the mosquito fauna of Bolivia remains partially unknown. In the present study, a new record of *Anopheles squamifemur* from Bolivia is reported; *An. costai* and *An. forattinii* are identified in place of *An. mediopunctatus*; the discovery of *An. trinkae* in 1981 is commented and the presence of *An. deaneorum* in Bolivia is specified. These new findings increase to 43 the total number of known *Anopheles* species for Bolivia for which an updated and partially annotated checklist is given.

2. New records: discovery and comments

2.1. *Anopheles (Lophopodomyia) squamifemur* Antunes, 1937

Three *An. squamifemur* specimens were collected on the 5th of September 2005 at Arca de Israel (Pando Department, Federico Roman Province, Municipality of Nuevo Manoa (Nueva Esperanza)) a small community located in the extreme north-east of Bolivia, on the banks of the river Madera, on the border with Brazil (Fig. 1). This small settlement was founded in the year 2000, but is at present the most populated of the area (≈ 250–300 inhabitants grouped in ≈ 70 houses). The area is characteristic of the humid Amazonian forest (thermicity index 710–740 °C, ombrothermic index 4.5–6.5 mm °C^{-1} and dry season ombrothermic index 0.5–2.3 mm °C^{-1}), at a mean elevation of ≈ 120 m [5]. Two CDC light-traps were placed inside and outside a house (S10°15′40″S, 65°19′11″W, altitude 117 m) located outside of the community center, on the road to Nueva Esperanza, between 18:00 h and 6:00 h next morning. The site was surrounded by the Amazonian forest. One *An. squamifemur* female was captured inside the house along with two *An. oswaldoi*, while outside, two *An. squamifemur* females were collected along with four *An. darlingi*, four *An. oswaldoi* and one *An. triannulatus*. The presence of the characteristic tuff of long, erect black scales on the distal third of hind femurs permitted the unambiguous classification of the specimens as *An. squamifemur*.

The monotypic Subgenus *Lophopodomyia* was established in 1937 by Antunes with *An. squamifemur* as the type species [7]. At present, along with *An. squamifemur*, five other species belong to this strictly Neotropical Subgenus [1]: *An. gilesi* (Peryassú 1908); *An. gomezdelatorrei* Levi-Castillo, 1955; *An. oiketorakras* Osorno-Mesa, 1947; *An. pseudotibiamaculatus* Galvão and Barreto, 1941; and *An. vargasi* Gabaldón, Cova-García and López, 1941. From the *Lophopodomyia* Subgenus, only *An. gilesi* has been previously recorded from Bolivia [8,9] (Table 1).

Adults of the six species of this Subgenus are morphologically very close to those of the *Anopheles* Subgenus and are generally best distinguished by examining the male genitalia [10,11], except for *An. squamifemur* for which a special distinguishing mark exists:

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Table 1
Checklist of the *Anopheles*[1] and their presence in Departments of Bolivia.

Subgenus		Species	Beni	Chuquisaca	Cochabamba	La Paz	Oruro	Pando	Potosi	Santa Cruz	Tarija
Anopheles	[2]	*apicimacula* Dyar & Knab, 1906	X							X	
	*	*costai* Fonseca & Ramos, 1939	X		X			X		X	X
		eiseni Coquillett, 1902	X			X					
	*	*fluminensis* Root, 1927			X					X	
	*	*forattinii* Wilkerson & Sallum, 1999	X		X			X		X	X
		intermedius (Peryassú, 1908)								X	
		maculipes (Theobald, 1903)	X							X	
	*	*mattogrossensis* Lutz & Neiva, 1911	X		X			X		X	X
		neomaculipalpus Curry, 1931				X				X	
	*	*peryassui* Dyar and Knab, 1908	X					X			
	*	*pseudopunctipennis* Theobald, 1901	?	X	X	X			X	X	X
		punctimacula Dyar and Knab, 1906								X	
	*	*shannoni* Davis, 1931	X					X			
		tibiamaculatus (Neiva, 1906)				X					
Kerteszia		*bambusicolus* Komp, 1937								X	
	*	*boliviensis* (Theobald, 1905)			X	X					
		cruzii Dyar & Knab, 1908			X	X					
	*	*homunculus* Komp, 1937			X	X					
	*	*laneanus* Corrêa & Cerqueira, 1944			X	X		X		X	
	*	*lepidotus* Zavortink, 1973			X						
		neivai Howard, Dyar & Knab, 1913				X					
Lophopodomyia		*gilesi* (Peryassú, 1908)			X	X					
	*	*squamifemur* Antunes, 1937						X			
Nyssorhynchus	[3]*	*albitarsis* s;s; Lynch Arribálzaga, 1878	X	X	X	X		X		X	X
	*	*argyritarsis* Robineau-Desvoidy, 1827	X	X	X	X				X	X
	*	*benarrochi* Gabaldón, Cova-García & López, 1941			X	X		X			X
	*	*braziliensis* (Chagas, 1907)	X	X	X		X	X		X	
	*	*darlingi* Root, 1926	X					X		X	?
	*	*deaneorum* Rosa-Freitas, 1989	X					X			
	*	*evansae* (Brethes, 1926)	X		X			X		X	X
		konderi Galvão & Damasceno, 1942	X					X			
		lutzii Cruz, 1901			X						
	*	*marajoara* Galvão & Damasceno, 1942			X						
	*	*nuneztovari* Gabaldón, 1940	X		X	X		X		X	X
	*	*oswaldoi* (Peryassú, 1922)	X		X	X		X		X	
	[4]	species "C" (*oswaldoi* complex)			X						
	*	*parvus* (Chagas, 1907)				X					
	*	*rangeli* Gabaldón, Cova-García & López, 1940	X		X	X		X		X	X
	*	*rondoni* (Neiva et Pinto, 1922)	X		X					X	X
	*	*strodei* Root, 1926	X		X			X		X	X
	[5]*	*triannulatus* (Neiva and Pinto, 1922)	X		X	X		X		X	X
	*	*trinkae* Faran, 1979	X		X	X		X		X	X
Stethomyia		*nimbus* (Theobald, 1902)	X								

* Species present at least in one of the Bolivian collections at INLASA or Escuela Técnica de Salud.
? Indicates that in the past the species has been mentioned in the Department (by [4] in particular) but that the present authors do not agree with that distribution. The species is not present anymore and therefore either it has disappeared from the region, either the species could have been misidentified at that time.

(1) *An. (Nysshorhynchus) galvaoi, An.(N.) lanei* and *An. (Kerteszia) bellator* have not been added to this list (see text).
(2) Doubtful presence; probably confused with *intermedius*.
(3) *An. allopha* Peryassú, 1921 (*nomen dubium)* as described by Faran & Linthicum (1981) is also present.
(4) Identified by molecular tools (Brelsfoard et al., 2005), not yet described, close to *oswaldoi*.
(5) Various mutant forms exist in Bolivia, at least *triannulatus davisi* (see text).

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both sexes show a characteristic tuff of long, erect black scales on slightly less than distal third of hind femurs that permits an easy and undoubted identification. In the Neotropical region, *An. squamifemur* is the only *Anopheles* species exhibiting such scale tuffs on its hindlegs. Other *Anopheles* species are known to exhibit tuffs of scales on their legs such as all the species of the *Lophoscelomyia* Series (Subgenus *Anopheles*) from the Hindu-Asiatic region and the Malaysian species *An. wellingtonianus* Alcock, 1912 (Subgenus *Anopheles*, Series *Anopheles*).

The description of *An. squamifemur* female was first published by Antunes [7], then the male was described [12] and later the larva, pupa and egg [13]. All stages of *An. squamifemur* can easily be distinguished from the other species of the *Lophopodomyia* Subgenus [13]. However, using usual identification keys [14,15], the 4th stage larvae would be misidentified as *An. (Anopheles) eiseni* which is also present in Bolivia (Table 1). However, *An. squamifemur* larvae can be easily differentiated by the presence of a palmate tuft hair on the first abdominal segment (seta 1-I) and a generally shorter size as compared to *eiseni* [13].

An. squamifemur is also known from Brazil (States of Para and Amazonas, close to the Venezuelan border [12,16] and State of Rondonia, frontier with Bolivia [17,18]), Colombia [7] from where comes the holotype, Costa Rica [19], French Guiana [20], Panama [21], Peru [22], Suriname [23] and Venezuela [24].

Lophopodomyia mosquitoes are sylvatic species, biting man and animals at twilight and their aquatic stages are passed in shaded side pools of streams rich in organic matter [25]. *An. squamifemur* follows these rules: it has always been captured in tropical humid forest areas [[18,20,21,26], among others]. It is, for example, widespread and locally common in the Atlantic coastal forests of the Isthmus of Panama [13]. Little is known of the bionomics of *An. squamifemur*. Immature stages have rarely been collected and the only types of breeding sites known at present are from Venezuela [27] and Peru [28]. They were encountered in small shaded areas in the banks of small or large rivers, where the stream is slow and permits the accumulation of natural floating debris and vegetation (*Eichhornia crassiipes* y *Pistia stratiotes*). Such an area may also be the consequence of a fallen tree trunk (for example) which enables the accumulation of organic debris and the slowing down of the stream. Few *An. squamifemur* were also collected in ponds [27]. Adults were also collected in Panama by horse-bait traps [13,29] and light traps [21,26], in a forest area of French Guiana [20] and at the borders of forests using animal baits at twilight [30]. *An. squamifemur* may bite humans [13], but nothing is known on its importance as a *Plasmodium* vector. It is probably limited, due to generally low densities of this mosquito in areas where human populations are also scarce.

In Bolivia, the surroundings of the collection site were similar to the few descriptions of other adult collecting sites elsewhere [18,20,21,26,30]. The Bolivian collecting site was situated in a characteristic Amazonian semi-evergreen seasonal forest (Fig. 1) and it is likely that *An. squamifemur* may extend its distribution range in Bolivia throughout the extension of this biogeographic region in that country.

2.2. *Anopheles (Anopheles) costai Fonseca & Ramos, 1939 and An. (An.) forattinii Wilkerson & Sallum, 1999*

These two species belong to the *Anopheles* subgenus (Series Arribálzagia) and are very closely related to *An. mediopunctatus*. *An. costai* has been recently resurrected from synonymy with *An. mediopunctatus* [31], while *An. forattinii* has been recently described [32]. These three species are morphologically similar and therefore were misidentified as *An. mediopunctatus* for several years in South America, as a consequence of identifications based only on adult female characters which could not discriminate [31]. *An. costai* and *An. forattinii* females are undistinguishable, and apparently undistinguishable from *An. mediopunctatus* except by the shape of the cercus [31,32]. Differences amongst the three species are more marked in male genitalia, pupae and larvae. In Bolivia, where the usual keys of Gorham et al. [9,15] or Stojanovich et al. [14] are routinely used to identify *Anopheles* species, *An. costai* and *An. forattinii*, absent from these keys, were *de facto* confused with *An. mediopunctatus*. The correct identification of these two species in Bolivia was recently carried out by mounting larvae collected during the "Bolivian Vectors Mapping Project" and using the differentiating characters from [31,32]. Both species were first identified in the Cochabamba Department, Chapare Province, Municipality of Villa Tunari: *An. forattini* from the Moleto locality and *An. costai* from the Villa 14 de septiembre locality (Fig. 1). However, only a few larvae from these two localities were classified undoubtedly as *costai* or *forattinii*. Other several tens of larvae examined from five other localities of the Chapare Region (Aroma, Moleto, San Mateo Bajo, San Rafael, Villa 14 de septiembre) and two localities from the Tarija Department in the south of Bolivia (Sachapera in the Municipality of Yacuiba (Gran Chaco Province), and Valle Dorado in the Municipality of Padcaya (Arce Province)) (Fig. 1) presented a mixture of characters that prohibited a clear

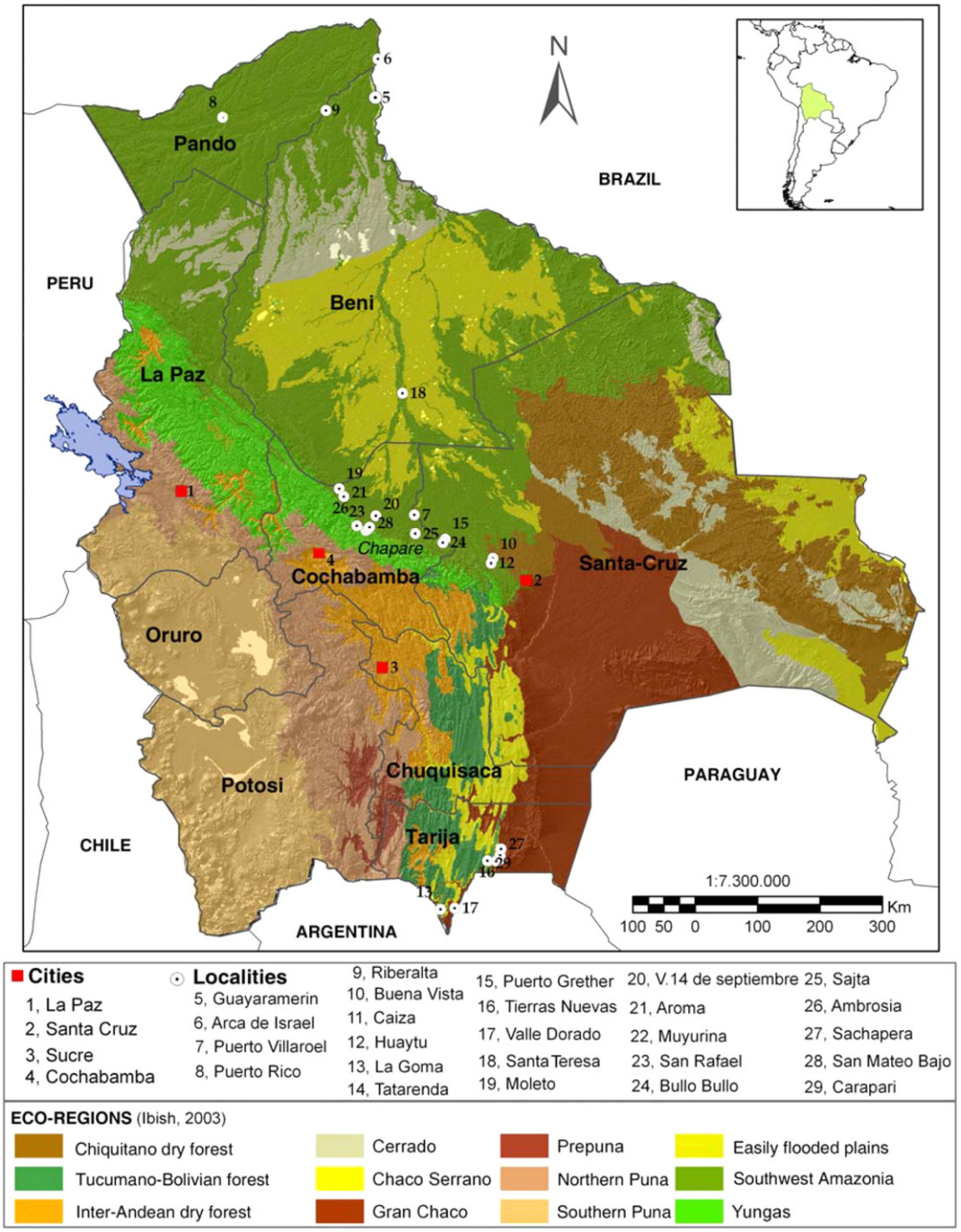

Fig. 1. The nine Departments of Bolivia, eco-regions [following [68]] and localities cited in the text.

classification. They shared characters of both species, even amongst the most discriminating ones (*i.e.*, the antenna length/width, setae 3C, 4C, 9C, 12C, 14C, 7P, 13P, 2S and 1-II) [31]. To sum up the determination results, some larvae shared more characters with *forattini* (in particular in the south of Bolivia), while others more with *costai*. However, none were very close to *mediopunctatus s.s.* although sharing some of its characters (on setae 8P for example). If *costai*, *forattini* and *mediopunctatus* are really separated species and not mutant forms, some larvae from Bolivia may represent something else characterized by a mixture of

(at least) the two former species. Genetic analysis (in prep.) of Bolivian specimens will likely give some cues to clear this situation. Larval sites were shaded pools (> 40 cm deep and < 10 m^2) in the margin of small rivers, in forest situations, at low altitude (200–300 m) with cold clear water (with or without floating debris and algae).

Adults *An. mediopunctatus s.l.* formerly identified from the Bolivian collections came from various sites of Bolivia, at low altitude: (1) in the Cochabamba Department, Chapare Province, localities of Muyurina, Ambrosia, San Mateo alto, San Mateo Bajo and Aroma; Carrasco Province, localities of Puerto Villaroel, Bulo Bulo and Sajta; (2) Santa Cruz Department, Ichilo Province, localities of Buena Vista and Huaytu, (3) Pando Department, Manuripi Province, locality of Puerto Rico; (4) Beni Department, Vaca Diez Province, localities of Riberalta and Guayaramerin, and (5) Tarija Department, Gran Chaco Province, localities of Carapari, Estacion Caiza and Sachapera; Arce Province, localities of La Goma and Valle Dorado (Fig. 1). These adults were examined using the discriminating character of the cercus (elliptical for *An. costai* and *An. forattinii*, and triangular for *An. mediopunctatus*) [31,32]. Although it was impossible to classify them as *An. costai* or *An. forattinii* because of the exact morphological similarity of the two species for this character, they were not *An. mediopunctatus s.s*. It seems that *An. mediopunctatus s.s*. is at present only known from southeastern Brazil [32], and because none of the specimen examined from Bolivia was identified as *An. mediopunctatus s.s.*, this species was removed from the list of Bolivian *Anopheles* (Table 1). However, in Bolivia, more research is needed to clarify the taxonomic status of *An. costai*, *An. forattini* and the closely related forms encountered.

An. costai is also known from Argentina, Brazil, Colombia, French Guiana, Trinidad, Tobago, Panama, Peru, Surinam [31], and Venezuela [33] and might be present in all other countries where *An. mediopunctatus* was formerly (mis)identified: British Guiana, Colombia, Ecuador, Mexico [31]. *An. forattinii* is also known from Amazonian Brazil, Colombia, French Guiana, Peru, Surinam [32] and Venezuela [33].

Little is known about the biology of the two mentioned species, but in Bolivia their distribution range is wide, from the north (Amazonian region) to the south, but always in areas of low altitude (< 600 m), in ecoregions such as Southwest Amazonia and in the lower parts of the Yungas and the Tucumano-Bolivian Forest (Fig. 1). *An. mediopunctatus s.l.* can be involved in malaria transmission [31]. However, in Bolivia, because of the rarity and low densities of *An. costai* and *An. forattinii* and the low human densities where these species have been found, their role as a malaria vector might be limited.

2.3. *Anopheles (Nysshorynchus) deaneorum* Rosa-Freitas, 1989 and the albitarsis complex

This species was captured during surveys we carried out in 2002–2003 in Guayaramerín, a city of $\approx 35\,000$ inhabitants on the banks of the Mamoré River, a river ≈ 1 km wide, on the border with Brazil (Fig. 1). Guayaramerín is facing its "sister" Brazilian city (Guajara-Mirin) of similar size, and from which *An. deaneorum* was first described and included in the *albitarsis* complex [34]. Adults *An. deaneorum* captured in Guayaramerín were distinguished from the closely related species *An. albitarsis s.s*. also present in the samples by a paler general external appearance for *An. deaneorum*, and the insertion of postlateral tufts of black scales appearing from the third tergite for *An. albitarsis* and from the fourth tergite for *An. deaneorum* [34]. The presence of *An. deaneorum* in Guayaramerín was also reported shortly before us in 2002 [35], but it has been impossible to examine specimens from these collections and therefore the presence of the species could not be confirmed at that time.

An. deaneorum is one of the five species of the *albitarsis* complex, along with *albitarsis* s.s., *albitarsis* sp. B, *albitarsis* sp. E and *An. marajoara* [36–38]. In Bolivia, it is likely that *An. deaneorum* has been confused for several years with *An. albitarsis s.s*. because of their very close morphological appearance. In other countries, species of the *albitarsis* complex are known to transmit *Plasmodium* sp., but further research is needed to ascertain the malaria vector status of each of these cryptic species and in particular the role of the three (*An. deaneorum*, *An. albitarsis* s.s. and *An. marajoara*) that have been identified in Bolivia (Table 1). In the past, *An. albitarsis* has also been divided in two closely related species: *An. albitarsis* and *An. allopha* [39]. Some specimens labelled *An. allopha* were found in the collection of pinned adults at Escuela Técnica de Salud Boliviano–Japonés de Cooperación Andina (Cochabamba). Although the two species might be morphologically distinguished [39], *An. allopha* is now in a *nomen dubium* [2,40]. However, Bolivian specimens matched the description of both "species" (*An. allopha* and *An. albitarsis*) as described by Faran and Linthicum [39].

2.4. *Anopheles (Nyssorhynchus) trinkae Faran, 1979*

The history of *An. trinkae* in Bolivia was made possible by the examination of apocrypha literature, mainly internal reports of Dr. Lien (a former collaborating scientist) to the Bolivian Ministry of Health from 1980 to 1985, and held in the Entomology Laboratory at Escuela Tecnica de Salud Boliviano–Japonés de Cooperación Andina (Cochabamba, Bolivia) [41]. *An. trinkae* has been described in 1979 and its presence in Bolivia was at that time hypothesized [42]. Its real identification in Bolivia was confirmed by Lien in 1981 [43]. Unfortunately this discovery went unnoticed because of the unavailability of the local Bolivian journal where it was published. Lien, who at that time worked in Bolivia with one of us (RR), first identified *An. trinkae* from San Mateo Bajo (S 17.0165, W 65.4378, Altitude 351 m), a small community in the Chapare Province of the Cochabamba Department, by a careful examination of larvae and adults reared in the laboratory.

An. trinkae belongs to the *Albimanus* Section of the *Nyssorhynchus* Blanchard Subgenus of *Anopheles*. In this Section, it is classified in the Oswaldoi Subgroup of the Oswaldoi Group of the Oswaldoi Series [44]. The Subgroup comprises eleven species [1] of which two: *An. rangeli* Gabaldón, Cova García and López, 1940 and *An. nuneztovari* Gabaldón, 1940 (or more precisely the *nuneztovari* complex) are morphologically very close to *An. trinkae*. As such, the adult females of *An. trinkae, An. nuneztovari* and *An. rangeli* can sometimes be confused due to intraspecific variability and the paucity of reliable differentiating characters [39]. As a consequence, before 1979, *An. trinkae* was misidentified as *An. nuneztovari* or *An. rangeli*. In Bolivia, where the three species are present (Table 1), the latter misidentification was the rule. Since the identification of *An. trinkae* in Bolivia, its presence has been confirmed by molecular tools [45,46]. In Bolivia, it has a wide distribution range from the Andes (tropical areas) in the Departments of La Paz (Yungas region), Cochabamba (Chapare region), Santa-Cruz and Tarija, to the Amazonian region in the Departments of Pando and Beni (to a lesser extent) (Fig. 1). This species is also known to occur along the eastern slope of the Andes in Colombia [42], Ecuador [42] and Peru [47]. It is known as a poor malaria (*P. vivax*) transmitter [39,44,47]. In Bolivia, it is suspected to be a malaria vector in some localities of the Chapare region of the Cochabamba Department, along with *An. species* C [48]. However, because of its feeding behavior (mostly zoophilic) and its low susceptibility to *Plasmodium* infection, its role as an efficient (and locally important) vector in Bolivia still remains to be clarified.

3. Updated checklist of *Anopheles* of Bolivia and comments on some species

The establishment of the checklist was based on (1) examination of collected material from the "Bolivian Vectors Mapping Project" carried out since 2005 at Instituto Nacional de Laboratorios de Salud (INLASA), La Paz (Laboratorio de Entomología Medica, curators TC and LT) and from previous collected material from collections at Escuela Técnica de Salud Boliviano–Japonés de Cooperación Andina, Cochabamba (Laboratorio de Entomología Medica, curator RR), and (2) on previously published but incomplete lists [[4,6,8–10,15, 41,49–55], amongst the most significant].

The *Anopheles* checklist for Bolivia is given in Table 1 with a provisional distribution of the species within the nine Departments of Bolivia. The Department of Oruro lacks the presence of any *Anopheles* species because of the ecological conditions in this Altiplanic region (> 3500 m altitude, mean annual temperature 11 °C). The Department of Potosi shares the same general ecological conditions but has some parts of its northern territory in the dry valley ecosystem of the Andes spur where only *An. pseudopunctipennis* can be found. Elsewhere, the *Anopheles* diversity is variable, but higher areas with a tropical or sub tropical climate, at altitudes 200–700 m: Amazonian region of Pando and Beni Department, Yungas region of La Paz Department, Chapare region of the Cochabamba Department, "Wet Chaco" of the Tarija Department, principally in the Carapari/Yacuiba area etc.). The five subgenera of South America are present in Bolivia. The subgenus *Anopheles* is represented by 14 species, and the subgenera *Kerteszia, Lophopodomyia, Nysshorhynchus* and *Stethomyia* by 7, 2, 19 and 1 species, respectively. A total of 43 *Anopheles* species are identified in Bolivia of which 28 are preserved in at least one of the current Bolivian collections kept at the above mentioned Institutions.

The presence in Bolivia of some of the listed species is still questionable. In Bolivia, *An. apicimacula* is probably confused with *An. intermedius* [15]. As a matter of fact, *An. apicimacula* is essentially a "northern" species of South America, with confirmed literature records from Colombia, Ecuador, Guyana, Mexico, Suriname, Trinidad and Tobago, Central America and Venezuela [2]. Likewise, *An. punctimacula* could be *An. calderoni*, described from Peru (Bolivia's neighbour country) [56] and where *An. punctimacula* was erroneously identified.

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It could also be *An. malefactor*, which was retrieved from synonymy with *An. punctimacula* [57]. Because we have not had access to Bolivian specimens of *"An. punctimacula"*, either from Bolivian collections or from our captures, these doubts could not be removed. *An. neivai* is another species never captured again in Bolivia although mentioned (with an interrogation point) in the "Catalog of the Mosquitoes of the World" [4].

An. pseudopunctipennis is known to be a species complex comprised of at least three species [58], with numerous subspecies and varieties [2]. In Bolivia a larval form ("short tail") sympatric with the standard form is under study (Lardeux, unpublished data). This form can be distinguished by a very short black tail (in opposition to the long black tail of the standard description) in each posterior spiracular plate of L4 larvae. In the past, the species has also been mentioned in the Beni Department (Moxos y Vaca Diez Provinces) [4]. However, these Provinces are far from the known geographical distribution range of that species in Bolivia (mainly in the foothills of the Andes) and therefore, the presence of *An. pseudopunctipennis* in the two above mentioned Provinces of the Beni Department is doubtful and has never been confirmed since.

An. darlingi is also known to exhibit morphological variations [59] and in Bolivia, a form sympatric with the standard one has been discovered in Guayaramerín. It exhibits a lack of prehumeral and humeral pale costal spots on its wings as compared to a standard specimen and as such, the first third of the costa is entirely black. However, the "black" form could not be separated from the standard one by molecular analysis (Lardeux, in prep.). In the past, *An. darlingi* has been identified from the Gran Chaco Province of the Tarija Department (very few individuals in one single location) [4]. However, despite numerous efforts we were unable to recapture this species again in that region and there is a reasonable assumption that this species is nowadays absent from the Gran Chaco area. In Bolivia, its distribution area is clearly the Southwest Amazonia Eco-region in the north of the country, and in the west, areas border with Brazil.

Recently in the Cochabamba Department, an apparently new to Science species, very close to *An. oswaldoi*, has been identified by molecular analysis and provisionally named "species C" [55]. It is very common in the Chapare and Carrasco Provinces (in or near the foothill regions at 200–500 m above sea level close to the locality of Chimoré) and is suspected to be a vector of *Plasmodium vivax* in that region, because of its abundance and the absence of other possible vectors (except *An. trinkae*) [48]. This species has been added to the list, though its description has not yet been published.

An. (Nysshorhynchus) triannulatus is closely related to *An. rondoni*, *An. benarrochi* and *An. strodei* also present in Bolivia (Table 1). It is known to be highly polymorphic, with numerous mutant forms [44] and even cryptic species [60,61]. However, two subspecies are generally recognized: *An. triannulatus triannulatus* and *An. triannulatus davisi* [62]. The principal characteristics used to distinguish *t. triannulatus* from *t. davisi* are: (1) larval hair 1-P filamentous and with more branches than in *davisi*, (2) females smaller than that of *davisi*, (3) adult wing with sectoral dark spot of Cu larger and reaching the furcation, and (4) IV hind tarsomer generally all black in *t. triannulatus* and white in its distal part in *t. davisi*. According to the key of Consoli and Lourenço-de-Oliveira [63], most Bolivian larvae from various geographical sites belong to the *triannulatus davisi* form (*i.e.*, 4th instar larva with seta 1-P leaflets lanceolate, and long lateral arms of the spiracular apparatus). However, variations exist and many specimens exhibit mixed characteristics of the various described forms. For example, larvae from the north of the country (Pando and Beni Departments) exhibit large lateral arms of the spiracular apparatus while in some (but not all) places in the south (for example, the locality of Tatarenda, [Tarija Department, Gran Chaco Province, Yuacuiba Municipality]), these arms are very small in comparison, and make one think of a partial description of the *t. triannulatus* form. Similarly, adults can be divided into two distinct groups by their overall size. For example, in the north of Bolivia (Localities of Guayaramerín, Puerto Rico, etc.) and in the Chapare region in the center, *An. triannulatus* is a very small species (somewhat similar to the *t. triannulatus* form) while in the south of the country, the size of the adults is much larger. Moreover, inside these groups, other differences arise, such as the color of the IV hind tarsomer whose pattern varies from one region to another (for example, the "black" tarsomer form is more abundant in the south of Bolivia and the white one in the north, although some localities may exhibit "black" and "white" forms in sympatry). Some variations have also been noticed in the sectoral dark spots of Cu reaching or not the furcation and in the dark spots of Cu1, but without any relationship with the color of the IV hind tarsomer. However, even if *davisi* is the predominant form, observed variations and mixed characters account for more complex relationships that do not match with previous descriptions. In Bolivia the clarification of the various forms is under study (small and large adults, 4th instar larvae with long or very short lateral arms, "black" and "white" IV hind tarsomer etc. and specimens which exhibit mixed characters). The mix-

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ing of characters of both *t. davisi* and *t. triannulatus* has also been noticed in Brazil [10], which might support the hypothesis that *triannulatus* is one single but variable species [44]. Recently, *An. halophylus* has been described from Brazil [64]. It is a very closely related species which differ from *An. triannulatus* by some 4th instar larvae characteristics (seta 1-P with very narrow and filiforme branches, and very small size of the lateral arms of the spiracular apparatus) and by the shape of the ventral lobe of the claspette of the male genitalia. In Bolivia, larvae and male genitalia examined from several localities indicated that *An. halophylus* is not present (Localities sampled, from north to south of the country: Puerto-Rico [Pando Department]; Guayaramerín and Santa Teresa [Beni Department]; Puerto Grether [Ichilo Province, Santa Cruz Department]; Caiza, Tatarenda and Tierras Nuevas [Gran Chaco province, Tarija Department]) (Fig. 1).

The *albitarsis* complex is represented in Bolivia by the three species *albitarsis s.s.*, *deaneorum* and *marajoara*. As stated above, some *Anopheles* specimen found in the Cochabamba collection also matched the description given by Faran and Linthicum [39] for *An. allopha*. However, *An. allopha* has been declared to be a *nomen dubium* [2,40] and since, its status has never been cleared [65]. Some recent works have dealt with the molecular identification of cryptic species in the *albitarsis* complex [38], and it would be interesting to process Bolivian "*allopha*" specimens with the published primers to clear their taxonomic status: do they belong to the *albitarsis* complex?, are they part of another cryptic species?, or are they a variety of *argyritarsis* as some authors seem to place "*allopha*" (see review in [65])? Because of the unclear taxonomic status of *An. allopha*, it was not maintained as a valid name in the Bolivian list of *Anopheles*.

Although three species have been cited for Bolivia in the past, they are not in the present list because (1) of the uniqueness of the sources that could not have been cross-verified and (2) we were unable to capture these species again in the field where they were reported or encounter them in one of the actual Bolivian collections. The first one is *An. (Nysshorhynchus) galvaoi* Causey, Deane & Deane, 1943, which has been listed only by Lien in a provisional list of Bolivian *Anopheles* [41], but we unfortunately did not find it in his collection at Escuela Técnica de Salud Boliviano–Japonés de Cooperación Andina (Cochabamba). The second one is *An. (Kerteszia) bellator* Dyar et Knab, 1906, listed again by Lien but absent from his collection. Larvae may also have been collected in the Chapare region of the Cochabamba Department [66]. However, the species has never been captured again in the mentioned Chapare spots and, moreover, the larval sites described [63] did not match the standard larval site description for that species. It is thus likely that *An. bellator* has been misidentified. The third one is *An. lanei*, identified once by Lien [41]. However, *An. lanei* is probable an endemic species of Serra da Mantiqueira, state of São Paolo, Brazil [67] and therefore, its presence in Bolivia is very doubtful.

4. Conclusion

Bolivia is still a poorly prospected country for its entomofauna and it is likely that new species (even for Science) will be discovered there. New molecular tools will help in better understanding the *Anopheles* classification, and better identifying species which differ only in subtle morphological characteristics that are not always easy to track with the current taxonomical identification keys. The actual *Anopheles* checklist for Bolivia will evolve, gaining in diversity and also in precision with the work required to remove all the above-mentioned doubts.

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