https://doi.org/10.25100/socolen.v26i2.9727

The salivary gland chromosomes of the black fly *Simulium (Pternaspatha) limay* (Diptera: Simuliidae) from Argentina

Los cromosomas de las glándulas salivares de *Simulium (Pternaspatha) limay* (Diptera: Simuliidae) de Argentina

CECILIA L. COSCARÓN ARIAS[1]

Revista Colombiana de Entomología 26(3-4): 137-143 (2000)

Resumen. Larvas de *Simulium (Pternaspatha) limay* Wygodzinsky del Parque Nacional Lanín, Neuquén, en el Sudoeste de Argentina se analizaron citogenéticamente. No se observaron citoformas diferentes. Se presentan mapas de cromosomas politénicos, idiogramas y una tabla comparativa de caracteres de la especie standard y las otras especies de la región Neotropical conocidas citogenéticamente. *S. limay* se puede diferenciar de la especie estándar (*Simulium venustum/verecundum*) por las inversiones fijas que presenta, así como por el intercambio de los brazos de los cromosomas; este último carácter no se ha observado en otras especies neotropicales.

Palabras clave: *Simulium*. Jejenes. Cromosomas politénicos. Citotaxonomía. Argentina. Región neotropical.

Summary. Larvae of *Simulium (Pternaspatha) limay* Wygodzinsky from two creeks from the Lanin National Park, Neuquen in Southwestern of Argentina were cytogenetically examined. No evidence of different cytoforms were observed. Polytene chromosome maps, idiograms, table with similarities of standard and known cytogenetically neotropical species are presented. *S. limay* could be distinguished from the standard species (*Simulium venustum/verecundum)* on the basis of different fixed inversions and an interchange of the chromosome arms; the last character is not found in the other neotropical simuliid species.

Key words: *Simulium*. Black fly. Polytene chromosomes. Cytotaxonomy. Argentina. Neotropical realm.

Introduction

The cytotaxonomy of the black flies from the Neotropical region based on the analysis of chromosomal rearrangements of salivary gland chromosomes is becoming better known. However, most of the literature is restricted to species involved in the transmission of the human onchocerciasis in South as well as in Mesoamerica (Conn 1988, 1990; Conn *et al.* 1989; Charalambous *et al.* 1993a, 1993b; Charalambous *et al.* 1996; Hirai 1985, 1987a, 1987b; Hirai and Uemoto 1983,1984; Hirai *et al.* 1994; Millest 1992; Shelley *et al.* 1986). Nevertheless, there are some other chromosomal studies performed in Neotropical *Simulium* species even though the species analyzed have not been yet reported as vectors of onchocerciasis (Chubareva *et al.* 1976; Duque *et al.* 1988; Campos and Muñoz de Hoyos 1990; Campos *et al.* 1996; Coscaron Arias 1998; Muñoz de Hoyos 1990, 1995).

From the 15 neotropical subgenera that are committed to the genus *Simulium* (Crosskey y Howard 1997) only the species within to *S. (Notolepria)*, *S. (Simulium)*, *S. (Hemicnetha)*, *S. (Chirostilbia)*, *S. (Ectemnaspis)* and *S. (Trichodagmia)*= *S. (Grenierella)* have been analyzed.

The systematics of the genus *Simulium* in the Neotropical realm is extremely complex and needs to be clarified (Coscarón 1987). For example among the taxa, *S. (Pternaspatha)* is a subgenus with subantarctic lineage possessing morphological characters which clearly can be separated from the remaining Neotropical genera (Coscarón and Coscarón Arias 1996) such as male dystistyle shape, different types of pupae trichomes or the great variation on female abdomen ornamentation. Accordingly, a chromosomal analysis of *S. (Pternaspatha)* could help to clarify the systematics of the genus.

S. (Pt.) limay, from Argentina, is endemic to the Subantarctic and Patagonia areas from the Southeastern Andes (Mendoza to Santa Cruz provinces) (Coscarón and Coscarón Arias 1995, 1996). It is an hematophagous insect but not considered to be a serious pest for men. It breeds in a diversity of habitats from creeks to big rivers in crystalline hard current waters in a cold to temperate area between 400 to 700 meters of altitude. It is sometimes sympatric with *S. (P.) nigristrigatum* and *S. (P.) simile*, *Gigantodax marginalis*, *G. fulvescens*, *G. chilensis* and *Cnesia dissimilis*. Preimaginal stages are found on submergent or fallen tree branches and leaves. According to previous reports, it belongs to *nigristrigatum* species group, and morphological characteristics admit the identity as a morphoespecies well differentiated (Coscarón and Wygodzinsky 1972; Coscarón 1991; Coscarón and Coscarón Arias 1996).

In the present report *S. (Pt.) limay* is cytologenetically described. The cytological features of this species could be useful to establish similarities and differences with other Neotropical species, and to infer which are the closest taxa of this subgenus. *S. limay* is studied from two main locality during different times of the year in order to see if there was a seasonal variation.

Materials and Methods

Larvae from the two last stages were collected from natural substrate. The sampling was done monthly from 1983 to 1984 from the Quitrahue and Chapelco Grande Brooks in the Lanin National Park (Neuquen Province, Argentina) (Fig. 1), in the northern part of the patagonian Andes with elevations ranging from 650 to 2,000 m.

Collection date and number of male and female larvae analized are given in tables 1 and 2.

---

1 Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, C. C. 85, 8303 Cinco Saltos, Río Negro, Argentina

Table 1. Collection date of *Simulium limay* from Chapelco Grande brook.

Date	Females	Males	Total
4/03/83	17	9	26
10/07/83	5	6	11
12/08/83	1	0	1
11/11/83	24	13	37
12/04/84	24	10	34
6/08/84	2	1	3
18/09/84	3	8	11
14/10/84	6	5	11
16/11/84	14	12	26
17/12/84	22	19	41
TOTAL	118	83	201

Table 2. Collection date of *Simulium limay* collected in Quitrahue brook.

Date	Females	Males	Total
5/04/83	11	14	25
6/06/83	3	1	4
12/08/83	2	2	4
11/11/83	15	9	24
10/03/84	6	7	13
6/08/84	0	1	1
18/09/84	4	8	12
14/10/84	9	9	18
16/11/84	16	12	28
TOTAL	66	63	129

Taxonomic determination of larval instars was performed following Coscarón (1989).

After collection, larvae were fixed in absolute ethanol: acetic acid (3:1), and stored at 4° C until dissection under stereoscopic microscope. Chromosomal spreads were obtained using the Feulgen method described in detail elsewhere (Rothfels and Dunbar 1953; Rothfels *et al.* 1978). Slides were examined with an Olympus microscope under phase contrast. When necessary, photographs were taken by using Kodak Imagen Link black-and-white film.

From a total of 330 larvae, 184 were females and 146 males, were analyzed (Tables 1-2).

Chromosome nomenclature and mapping conventions observed followed previous recommendations (Gordon 1984; Rothfels and Dunbar 1953; Rothfels *et al.* 1978). Briefly, the chromosomes were identified using landmarks. They are numbered as I, II and III in decreasing order of size and each arm is S (short) or L (long) depending on their size. Major chromosomal features, namely Ring of Balbiani, para-Balbiani, nucleolar organiser, grey band, shield, frazzle, blister are designated as RB, pB, NO, gB, S, F, and B, respectively (Gordon 1984), are used to identify the chromosome arms. The entire complement is divided into 100 sections. The proportion of the complement assigned to an specific arm is expressed as mean percentage contribution of the arm in the total complement length after chromosomal measuring in at least 10 nuclei. The sections are numbered 1 to 100 beginning in IS and ending at IIIL. The proximal, central, and distal segments of a section are indicated by a, b, and c, respectively. Nomenclature of inversions followed previously established conventions (Basrur 1959; Bedo 1977; Gordon 1984; Rothfels *et al.* 1978). Mainly, they are indicated by brackets on illustrations or photographs and are numbered arbitrarily starting from one. The fixed (interspecific) inversions are designated by underlining the inversion number. Floating inversions are designated by an inversion number not underlining. Sex-linked polymorphisms were analyzed by comparing the frequencies of inversions in males and females.

The standard banding pattern for comparison with *S. (Pt.) limay* was *Simulium venustum/verecundum* (Rothfels *et al.* 1978). This species was chosen as it has the standard map for the subgenus *Simulium* and it is one of the species of *Simulium* genus better cytologically studied and frequently used for comparison in the New World.

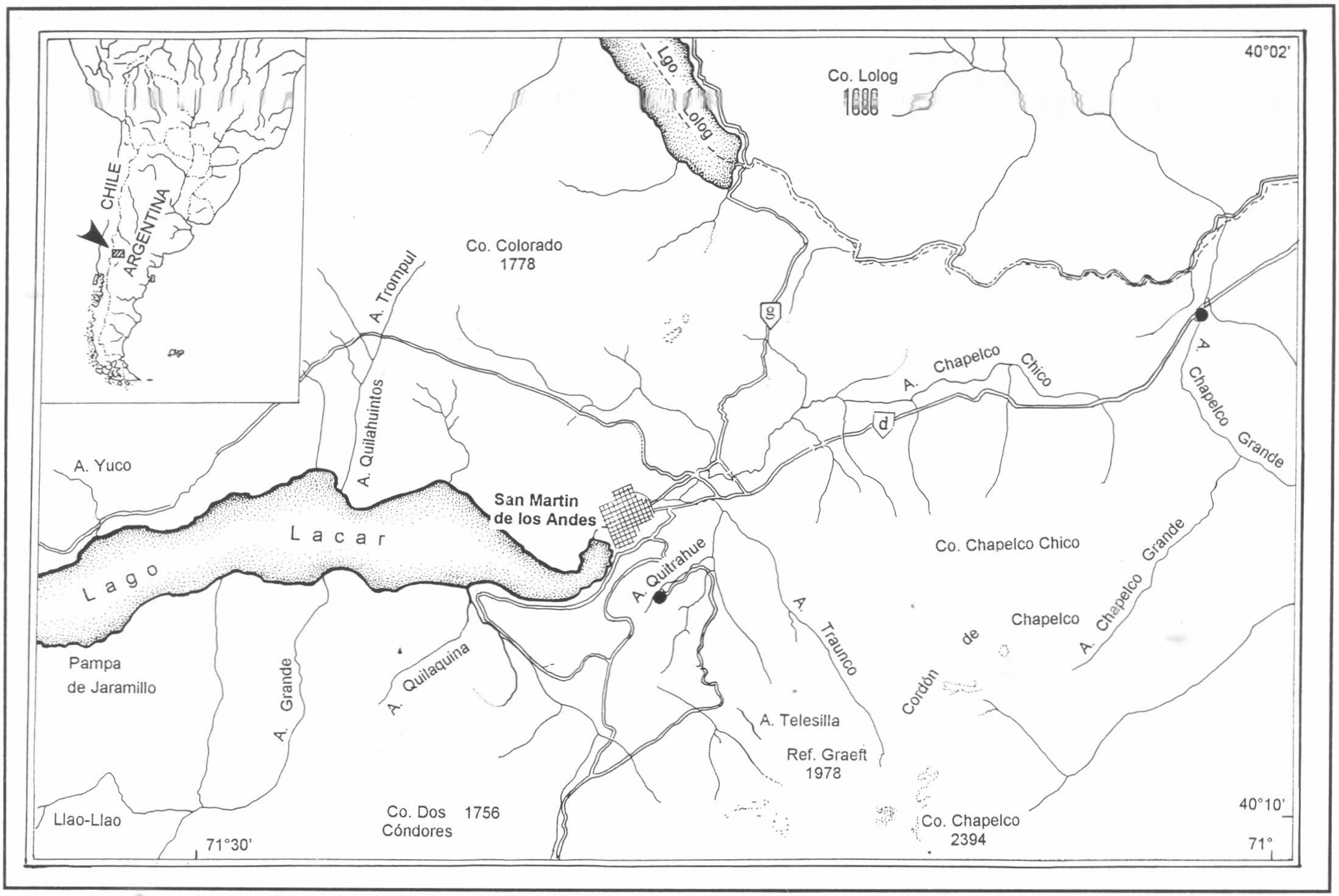

Figura 1. Quitrahue and Chapelco Grande Brooks (Lanín National Park, Neuquen, Argentina).

Results

Description of S. limay (Figs. 2-4)

The arms of the chromosomes were identified using the landmarks of *S. venustum/verecundum* (Fig. 2).

Simulium limay shows an interchange of the whole arm between chromosomes II and III. The arms are associated as shown: IIIL-IIL; IIIS-IIS. Because of this arm-association, the "chromosomes" show great difference in size with "chromosome III" being very small because it is composed of two short arms: IIS+IIIS (Fig. 3).

Chromosome I (Figs. 2; 3 A, B; 4). Section 1 has a characteristic marker as was described by Landau in 1953. This arm (IS) has only one inversion, which involves sections 7-12 (IS-1). The IL has the standard species banding pattern and the N.O. is present in this arm with no shift of other chromosome bands.

"Chromosome II" (Fig. 2). This chromosome is formed by the IIL and IIIL association due to the arm interchange between chromosomes II and III (Fig. 4). The IIL arm (Figs. 3 C, D) has a

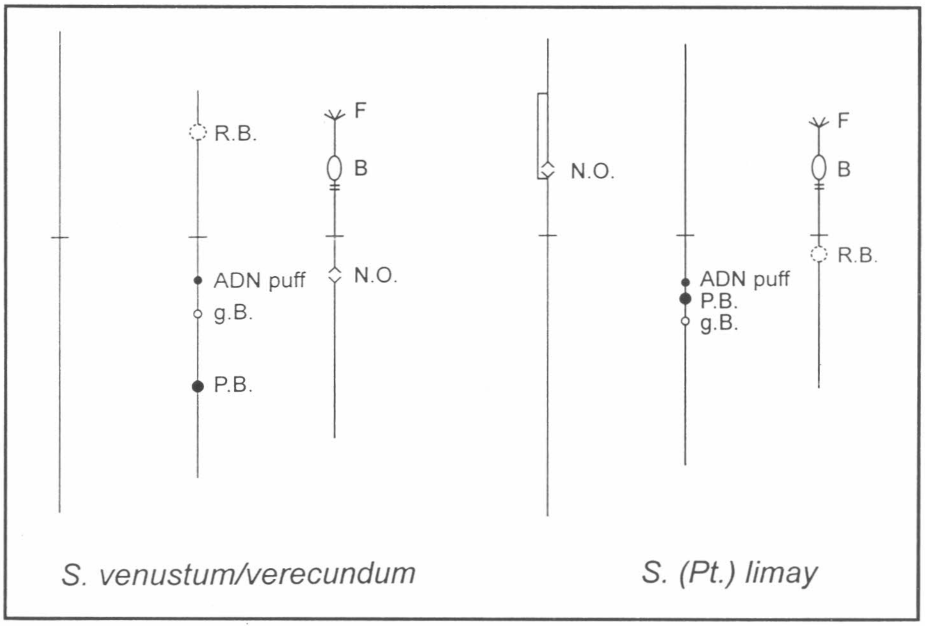

Figura 2. Idiograms of *Simulium venustum/verecundum* and *S. limay*. Interspecific inversions shown by brackets to the left of chromosome arms. Abreviations: RB, ring of Balbiani; pB, parabalbiani; NO, nucleolar organizer; gB, grey band; F, frazzled; B, blister; ADN puff; C, centromere.

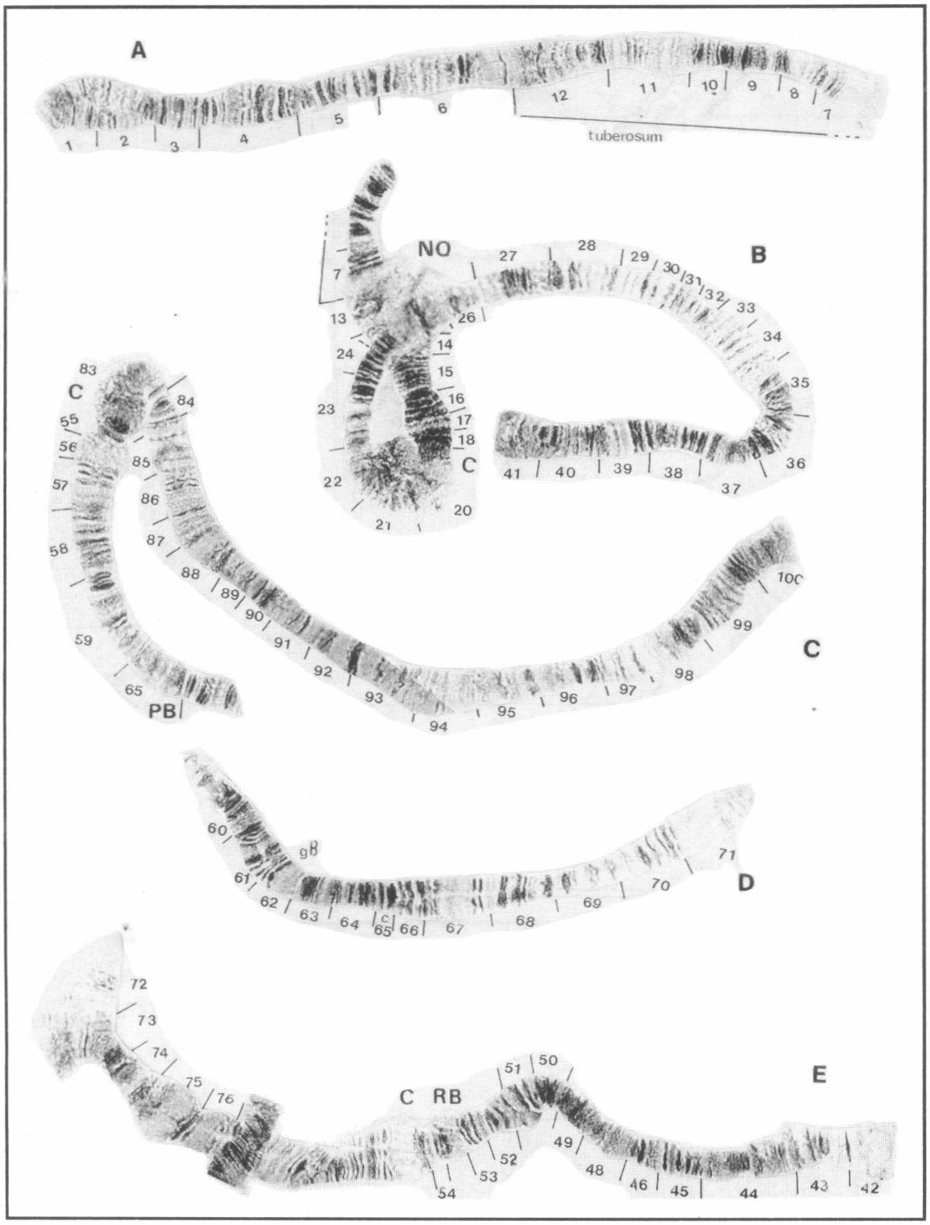

Figura 3. *S. limay* A: chromosome IS (part), B: chromosome IS (part) and chromosome IL, C: "chromosome II" (IIIL + IIL), D: "chromosome III" (part IIL), E: "chromosome III" (IIIS + IIS). Abbreviations: RB, ring of Balbiani; pB, parabalbiani; NO, nucleolar organizer; C, centromere; c, distal portion of subsection.

translocation of the section 65 a,b including the pB which is relocated between sections 59-60. The IIIL arm (Fig. 3 C) has a characteristic end (section 99) with three groups of dark bands. The landmark denominated "cup and saucer" is present in *S. limay* in section 92. This arm matches with the banding pattern of the standard species.

"Chromosome III" (Figs. 3 E, 4). It is very small, formed by the association of IIS with IIIS. The IIS has a similar banding pattern as the IIS of *S. venustum/verecundum*. The only difference is that the Ring of Balbiani (section 47) (Fig. 3 E) is between section 53/54. The IIIS which is usually constant within species has a characteristic fan tailed end (frazzled) (section 72). The best arm marker is the heavy group (section 75), which separates two lightly banded and blister-prone regions. In *S. limay* this section is inverted. As mentioned before, the N.O. has moved from chromosome III to I.

No inversions or polymorphisms differentiating the sexes were observed. No cytological differences were observed during the different seasons of the year.

Discussion

The results demonstrate that the appearance of the centromere, the beginning of IS, IL and IIIL, the frazzled end of IIIS of *S. limay* are chromosome features shared with *S. venustum/verecundum*. As it is the "A" ending for IS stated by Landau (1953) for some species of *Simulium* like *Simulium damnossum* and *Eusimulium* and also present in *Gymnopais, Twinnia, Prosimulium* spp., etc (Rothfels *et al.* 1978).

The unique inversion observed (IS-1) is also described in *S. tuberosum* by Landau (IS-*tuberosum*), being this species later renumbered by Rothfels *et al.* (1978).

On the other hand, several characters of *S. limay* have been observed to differ from *S. venustum/verecundum*. Among them the arm interchange between chromosome II and III, producing a small chromosome, the presence of N.O. in chromosome I (the change of position of the N.O. is common to other members of the family (Procunier *et al.* 1985), the interspecific inversion in IS, the translocation of the paraBalbiani within IIL and the inversion involving a landmark of IIIS.

Comparison of one of the most obvious landmark from the Neotropical *Simulium* species reveals the existence of some similarities among several species (Table 3). In *S. limay* the N.O. occupies a basal position in IS, resembling the localization of this chromosome marker as in *S. exiguum* (Procunier *et al.* 1985; Charalambous *et al* 1993 a, b), *S. furcillatum* (Campos and Muñoz de Hoyos 1990), *Simulium* "C" (Duque *et al.* 1988); *S. ochraceum* (Hirai and Uemoto 1983; Hirai *et al.* 1994; Millest 1992); *S. pertinax* (Campos Gaona *et al.* 1996); *S. bicornutum* and *tunja* as well as *S. lewisi lutzianum* (Miranda and Muñoz de Hoyos 1995). It should also be mentioned that the R.B. is located in *S. limay* in IIS occupying a proximal position to the centromere as it has been also observed in *S. furcillatum* (Campos and Muñoz de Hoyos 1990), *S. metallicum* "H" and "I" (Conn *et al.* 1989), *S. pertinax* (Campos Gaona *et al.* 1996) *S. bicornutum* and *S. tunja* (Miranda and Muñoz de Hoyos 1995).

In *S. limay* the pB and the gB usually are two characteristic landmarks for IIL. The pB is proximal to the centromere as it has been previously reported for *S. metallicum* "A", "C", "D", "E", "F" , "G" and "K" (Conn *et al.* 1989) and "L" (Arteaga and Muñoz de Hoyos 1999).

The presence of the IIIS frazzled end in *S. limay* is a common character within the *Simulium* species cytogenetically analysed. It has been observed in *S. furcillatum* (Campos Gaona y Muñoz de Hoyos 1990), *S. metallicum* "A" to "K" (Conn *et al.* 1989), *S. muiscorum* (Muñoz de Hoyos 1990) and *S. ochraceum* (Hirai *et al.* 1994; Millest 1992). Accordingly to it, it could be suggested that almost all known Neotropical *Simulium* species share several landmarks, and that chromosome III is the most homosequential with the standard pattern, especially IIIL.

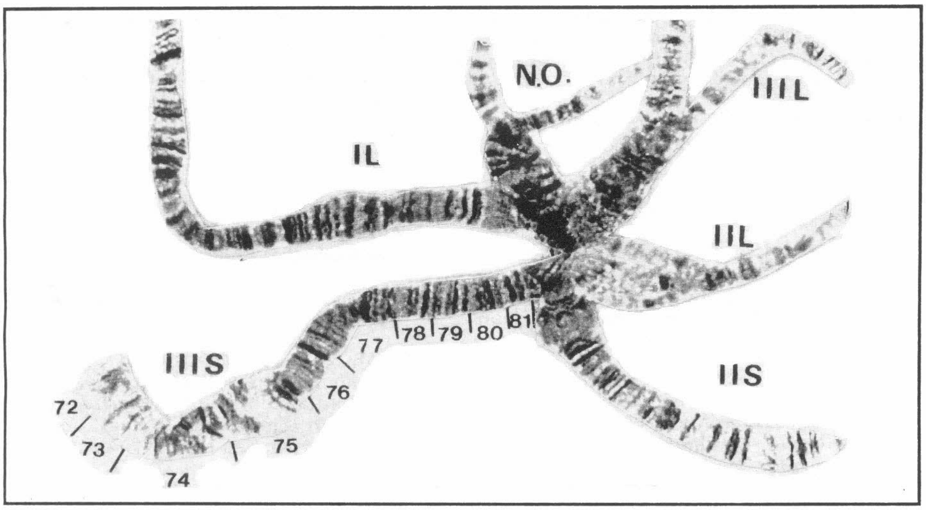

Figura 4. *S. limay* general view of the chromosomes illustrating chromosome IIIS.

In *S. limay* no inversions or polymorphisms differentiates sexes. These results are in good agreement with previous reports demonstrating that the genetic X and Y chromosomes appear alike. Procunier *et al.* (1985) have observed no chromosomal rearrangements between sexual chromosomes in specimens from *Simulium exiguum (Cayapa)*, and similar observations have been found by Duque *et al.* (1988) in *Simulium "C"*, and in *S. metallicum "E"*, *"F"* and *"K"* by Conn *et al.* (1989). *S. guianense* (Charalombous *et al.* 1996) and *S. pertinax* (Campos Gaona *et al.* 1996), suggesting that X_0Y_0 is, therefore, a primitive character of this species group (Rothfels 1987).

In *S. limay* an interchange of chromosome arm was observed so that IIIL-IIL, IIIS-IIS are associated. This interchange has not been observed in other Neotropical

Table 3. Chromosomal characters of the known neotropical species

SPECIES		AUTHOR	SEX DETERM	IS	IL	IIS	IIL	IIIS	IIIL
S.standard		40	IIL			RBd; gBd	pBd	F	NOb, "3"
S. bicornutum		33		NOb		RBb			
S. exiigum	*Cayapa*	36	no	NOb		RBd	pBd; gBd		
	Aguarico	36	IIS; IIL	NOb		RBd	pBd; gBd		
	Bucay	7; 40	IIL	NOb		RBd	pBd; gBd		
	Quevedo	7	IIS; IIL	NOb		RBd	pBd; gBd		
S. furcillatum		5; 33		NOb		RBb	pBd; gBd	F	"3"
S. guianense	*A*	9	no		NOb	RBb	pBb; gBd	B	
	B		no		NOb	RBb	pBb; gBd	B	
	C		no		NOb	RBb	pBb; gBd	B	
	D		no		NOb	RBb	pBb; gBd	B	
S. ignescens		23; 33; 34	IIS			RBd	pBd; gB	NOb	
S. lewisi-lutzianum		33		NOb		RBd	pBd; gBd	B	
S. limay		14	no	NOb		RBb	pBb	F, B	
S. metallicum	*A*	11; 12; 25; 26; 27; 30	IL			RBd, NOb	pBb; gBd	F, B	
	B		IS	NOd		RBd	pBd; gBb	F, B	
	C		IIL			RBd, NOb	pBb; gBd	F, B	
	D		IIL			RBd, NOb	pBb; gBd	F, B	
	E		no			RBd, NOb	pBb; gBd	F, B	
	F		no			RBd , NOb	pBb; gBd	F, B	
	G		IIL			RBd , NOb	pBb: gBd	F, B	
	H		IIIL			RBd , NOb	pBd; gBb	F, B	
	I		IL			RBd , NOb	pBd; gBb	F, B	
	J		IS		NOd	RBd	pBd; gBb	F, B	
	K		no		NOd	RBb	pBb; gBd	F, B	
	L	1	IIL			RBd , NOb	pBb; gBd		
S. muiscorum		34	IIIL			RB ; F	pBd; gBb; F	NOb , F, B	F
S. ochraceum	*A*	28; 29; 32; 33	IL	NOb		RBd	pBd; gBd	F, B	
	B		IS; IL	NOb		RBd	pBd; gBd	F, B	
	C		IL ; IIIL	NOb		RBd	pBd; gBb	F, B	
S. pertinax		6	no	NOb		RBb	pBd		"3"
S. romanai		33			NOb	RBd	pBb; gBd	B	
S. tunja		33		NOb		RBb	pBd; gBb		

(1) Arteaga & Muñoz de Hoyos 1999; (2) Campos Gaona & Muñoz de Hoyos 1990; (3) Campos Gaona *et al.* 1996; (4) Charalambous *et al.* 1993b; (5) Charalambous *et al.* 1996; (6) Conn 1988; (7) Conn 1990; (8) Coscarón 1989; (9) Duque *et al.* 1988; (10) Hirai 1985; (11) Hirai 1987a; (12) Hirai 1987b; (13) Hirai *et al.* 1994; (14) Hirai & Uemoto 1983; (15) Hirai & Uemoto 1984; (16) Millest 1992; (17) Miranda & Muñoz de Hoyos 1995; (18) Muñoz de Hoyos 1990; (19) Procunier *et al.* 1985; (20) Rothfels *et al.* 1978.

simuliid species but does appear in *Twinnia hirticornis* (Gymnopaidinae) (Rothfels 1980). The fact that the same interchange of chromosome arms occurs in *S. limay* and *Twinnia* spp. could reflect a close phylogenetical relationship. However, they belong to different tribes (sensu Crosskey and Howard 1997). It is more likely that, this interchange of arms is probably a homoplasic character occurring in different taxa. *S. limay* has a morphology which is different from the rest of the Neotropical species and its distribution is closed to the Subantarctic fauna (Coscarón and Coscarón Arias 1995, 1996), which has a close relationship with the Australian fauna (Crisci *et al.* 1991). Therefore, *S. limay* was compared with *Austrosimulium bancrofti* (Ballard y Bedo 1991) but no evidences was found of close phylogenetical relationship among them. Comparing *S. limay* with *S. ornatipes* and *S. neornatipes* (Bedo 1977) it was observed that they share a proximal position of the pB in IIL and the frazzled end of IIIS. However, these characters alone are not enough to suggest a close relationship between *S. limay* and the australian species.

In conclusion, the interchanges observed in the arms of the chromosomes from *S. limay* may reinforce the opinion based on morphology that *S. (Pternaspatha)* is an independent subgenus, (Coscarón y Wygodzinsky 1972; Wygodzinsky and Coscarón 1967; Coscarón 1987) and justifies *S. Pternaspatha* as a valid supraespecific taxon. With the present information (Table 3) there is not enough chromosomal information that allows to establish that any neotropical or australian species are closest to *S. (Pternaspatha)* and to infer a phylogenetic relationship.

Aknowledgements

The author would like to acknowledge the helpful suggestions of Magda Charalombous (Department of Entomology of The Natural History Museum, London, U.K.) and Paulina Muñoz de Hoyos (Universidad Nacional de Colombia, Facultad de Ciencias, Instituto de Ciencias Naturales). Also to Prof. Nelida Caligaris (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata) for her drawings.

References

ARTEAGA, L.; MUÑOZ DE HOYOS, P. 1999. New cytotype in the *Simulium metallicum* Complex (Diptera: Simuliidae) from Cundinamarca, Colombia. J. Med. Entom. 36 (2): 133-140.

BALLARD, J.; BEDO D. 1991. Population cytogenetics of *Austrosimulium bancrofti* (Diptera: Simuliidae) in eastern Australia. Genome 34: 338-353.

BASRUR, P. K. 1959. The salivary gland chromosomes of seven segregates of *Prosimulium* with a transformed centromere. Can. J. Zool. 37: 527-570.

BEDO, D. G. 1977. Cytogenetics and evolution of *Simulium ornatipes* Skuse (Diptera: Simuliidae). I. Sibling speciation. Chromosoma 64: 37-65.

CAMPOS GAONA, J.; MUÑOZ DE HOYOS, P. 1990. Los cromosomas politénicos de *Simulium furcillatum* (Diptera: Simuliidae) Chisacá, Cundinamarca, Colombia. Rev. Acad. Colomb. Cienc. 17 (67): 715-723.

CAMPOS GAONA, J.; RECCO-PIMENTEL, S. M.; ANDRADE, C. F. 1996. Polytene chromosome analysis of a population of *Simulium pertinax* (Diptera: Simuliidae). Brazilian Journal of Genetics 19 (1): 47-52.

CHARALAMBOUS, M.; SHELLEY, A.; ARZUBE, M. 1993a Distribution and taxonomic status of chromosomal forms of the onchocerciasis vector *Simulium exiguum* in Central Ecuador. Med. and Veter. Entom. 7: 299-303.

CHARALAMBOUS, M.; READY, P.; SHELLEY, A.; ARZUBE, M.; LOWRY C. 1993b Cytological and isoenzime analysis of the Bucay and Quevedo Cytotypes of the Onchocerciasis vector *Simulium exigumm* (Diptera: Simuliidae) in Ecuador. Mem. Inst. Oswald Cruz 88 (1): 39-48.

CHARALAMBOUS, M.; SHELLEY, A.; HERZOG, M.; LUNA DIAS, A.P. 1996. Four new cytotypes of the onchocerciasis vector blackfly *Simulium guianense* in Brazil. Med. and Vet. Entomol.10: 111-120.

CHUBAREVA, L.; RUBTZOV, I.; PETROVA, N. 1976. Morphological and karyological similarities and difference in Paleartic and Neotropical species of the genus *Hemicnetha* End. (Diptera, Simuliidae). Entomol. Rev. 55 (7): 137-142.

CONN, J. 1988. A cytological study of the *Simulium metallicum* complex (Diptera: Simuliidae) from Central and South America: 221-243. En M. W. Service (ed.) Biosystematics of haemotophagous insects, Clarendon, Oxford.

CONN, J. 1990. Chromosome key to the larvae of *Simulium metallicum* complex (Diptera: Simuliidae) from Latin America. J. Med. Entom. 27 (4): 459-466.

CONN, J.; ROTHFELS, K.; PROCUNIER, W.; HIRAI, H. 1989. The *Simulium metallicum* species complex (Diptera: Simuliidae) in Latin America: a cytological study. Can. J. Zool. 67: 1217-1245.

COSCARON, C. L. 1989. Estudios bioecológicos y citotaxonómicos de simúlidos de Argentina. Tesis doctoral Fac. Cs. Nat. y Museo. Univ. Nacional de La Plata. 293pp.

COSCARON ARIAS, C. 1998. The polytene chromosomes of *Cnesia dissimilis* (Edwards) and three species of *Gigantodax* Enderlein (Dop[tera: Simuliidae) from Lanin National Park (Argentina) Mem. Inst. Oswaldo Cruz 93 (4): 445-458.

COSCARON, S. 1987. El género *Simulium* Latreille en la región neotropical: análisis de los grupos supraespecíficos, especies que lo integran y distribución geográfica (Simuliidae, Diptera). Mus. Paranense Emilio Goeldi, Col. Emilie Snethlage: 112 pp.

COSCARON, S. 1991. Simuliidae, Insecta, Diptera. En Z. A. Castellanos (Dir.). Fauna de Agua Dulce de la República Argentina, 28 fasc. 2: 304 pp, 67 pl.

COSCARON, S.; COSCARON ARIAS, C. 1995. Distribution of Neotropical Simuliidae (Insecta, Diptera) and its areas of endemism. Rev. Acad. Colomb. Cienc. 1 9 (75): 717-732.

COSCARON, S.; COSCARON ARIAS, C. 1996. Análisis cladístico de *Simulium (Pternaspatha)* Enderlein con datos sobre su distribución geográfica (Diptera: Simuliidae). Acta Entom. Chilena *20*:71-82.

COSCARON, S.; WYGODZINSKY, P. 1972. Taxonomy and distribution of the black fly subgenus *Simulium (Pternaspatha)* Enderlein (Simuliidae, Diptera, Insecta). Bull. Am. Mus. Nat. Hist. 147 (4): 199-240.

CRISCI, J., CIGLIANO, M.M.; MORRONE, J.; ROIG JUÑENT, S. 1991. Historical biogeography of Southern South America. Syst. Zool. 40(2): 152-171.

CROSSKEY, R. W.; HOWARD, T. M. 1997. A New taxonomical and geographical inventory of world blackflies (Diptera: Simuliidae). Department of Entomology. The Natural History Museum. London. 144 pp.

DUQUE, S., MUÑOZ DE HOYOS, P.; ROTHFELS, K. 1988. The polytene chromosomes of *Simulium (Ectemnaspis) ignescens* Roubaud and the related species *Simulium* "C" both from Colombia. Can. J. Zool. 66: 300-309.

GORDON, A. E. 1984. The cytotaxonomy of three species in the jenningsi-group of the subgenus *Simulium* (Diptera: Simuliidae) in New York State. Can. J. Zool. 62: 347-354.

HIRAI, H. 1985. The salivary gland chromosomes of *Simulium metallicum* Bellardi from northern Venezuela: 66-73. En: Tada, I. (ed.). A comparative study on onchocerciasis between South and Central America. Shimoda, Japan.

HIRAI, H. 1987a. IV- 2. C banding patterns in polytene chromosomes of *Simulium metallicum* complexes *A* and *B*, 39-47, 4 figs., En Tada, I. (ed.). A comparative study on onchocerciasis between South and Central America. Shimoda, Japan.

HIRAI, H. 1987b. IV- 5. Cytotype of *Simulium metallicum* in Miranda, an endemic area in northern Venezuela for onchocerciasis, 66-67, 1 fig, En Tada, I. (ed.). A comparative study on onchocerciasis between South and Central America. Shimoda, Japan.

HIRAI, H.; UEMOTO, K. 1983. The analysis of salivary gland chromosmes of *Simulium ochraceum* from Guatemala and Mexico. Japan J. Sanit. Zool. 34: 120.

HIRAI, H.; UEMOTO, K. 1984. Polytene chromosome analysis in *Simulium metallicum* complex from Guatemala. Japan J. Sanit. Zool. 35: 188.

HIRAI, H.; PROCUNIER, W. S.; OCHOA, J.; UEMOTO, K. 1994. A cytogenetic analysis of the *Simulium ochraceum* species complex (Diptera: Simuliidae) in Central America. Genome 37: 36-53.

LANDAU, R. (Zimrig). 1953. A comparative study of the salivary gland chromosomes of five realted blackfly species. M. A. Thesis, Univ. of Toronto, Toronto.

MILLEST, M.1992. Identification of members of *Simulium ochraceum* species complex in the three onchocerciasis foci in Mexico. Medical and Veterinary Entomology 6: 23-28.

MIRANDA ESQUIVEL, D.; MUÑOZ DE HOYOS, P. 1995. *¿Ectemnaspis* o *Psilopelmia?* he ahí el dilema. Revista Colombiana de Entomología 21 (3): 129-144.

MUÑOZ DE HOYOS, P. 1990. La importancia de los cromosomas politécnicos en la determinación taxonómica de los simúlidos. Rev. Colomb. Cienc. 16 (66): 511-520.

MUÑOZ DE HOYOS, P. 1995. Género *Gigantodax* (Diptera: Simuliidae) en Colombia. Rev. Colomb. Cienc. 19 (74): 607-630.

PROCUNIER, W.; SHELLEY, A.; ARZUBE, M. 1985. Sibling species of *Simulium exiguum* (Diptera: Simuliidae), the primary vector of Onchocerciasis in Ecuador. Rev. Ecuat. Hig. Med. Trop. 35 (2): 49-59.

ROTHFELS, K.H. 1980. 13. Chromosomal variability and speciation in blackflies. Insect Cytogenetics, Symposia of the Royal Entomological Society of London; N° 10. Ed. Blackman, R.L.; Hewitt; G.M. y M. Ashburner.

ROTHFELS, K.H. 1987. Cytological approaches to black fly taxonomy. En: Black flies: Ecology, Population Management and Annotated World list (Kim, K. C. and Merrit, R. W., eds.). Pennsylvania State University Press, University Park and London, pp. 39-52.

ROTHFELS, K. H.; DUNBAR, R. W. 1953. The salivary gland chromosomes of the back fly *Simulium vittatum* Zett. *Canad. J. Zool. 31:* 226-241.

ROTHFELS, K. H.; FERADAY, R.; KANEPS, A. 1978. A cytological description of sibling species of *Simulium venustum* and *S. verecundum* with standard maps for the subgenus *Simulium* Davies (Diptera). Can. J. Zool. 56: 1110-1128.

SHELLEY, A.; PROCUNIER, W.; ARZUBE, M. 1986. Direct incrimination of *Simulium exiguum* Cayapa form as vector of *Onchocerca volvulus* in Ecuador. Trans. Roy. Soc. Trop. Med. Hyg. 80:845.

WYGODZINSKY, P.; COSCARON, S. 1967. A review of *Simulium (Pternaspatha)* Enderlein (Simuliidae, Diptera). Bull. Am. Mus. Nat. Hist. 136 (2): 49-116.