EIGHTY-YEAR RESEARCH OF PHLEBOTOMINE SANDFLIES (DIPTERA: PSYCHODIDAE) IN CHINA (1915-1995).

II. PHLEBOTOMINE VECTORS OF LEISHMANIASIS IN CHINA

ZHANG L.M. & LENG Y.J.*

Summary :

The human leishmaniasis vector – phlebotomine sandfly – was proven in China, i.e. *P. alexandri*, *P. chinensis*, *P. longiductus*, *P. sichuanensis* and *P. smirnovi*. The infectious rate, biology and distribution of these vectors are shown in this review. Two disputed questions about *P. chinensis* and *P. sichuanensis*, *P. smirnovi* and *P. wui* are discussed. At last, the vectors of animal leishmania are provided, i.e. the vectors of great gerbil leishmania: *P. alexandri*, *P. andrejjevi*, *P. caucasicus*, *P. mongolensis* and *P. smirnovi*, and the vector of lizards leishmania: *S. sinkiangensis*.

KEY WORDS : phlebotomine sandflies, vectors, leishmaniasis, China.

Résumé : QUATRE-VINGT ANS DE RECHERCHE SUR LES PHLÉBOTOMES (DIPTERA : PSYCHODIDAE) EN CHINE (1915-1995). II. LES PHLÉBOTOMES VECTEURS DE LEISHMANIOSE EN CHINE

Les phlébotomes dont on a démontré qu'ils sont vecteurs de la leishmaniose en Chine sont *P. alexandri*, *P. chinensis*, *P. longiductus*, *P. sichuanensis* et *P. smirnovi*. Les taux de contamination, la biologie et la distribution géographique de ces vecteurs sont précisés. Deux controverses concernant *P. chinensis* et *P. sichuanensis* d'une part, *P. smirnovi* et *P. wui* d'autre part, sont abordées. Enfin, les vecteurs de la leishmaniose animale sont répertoriés : *P. alexandri*, *P. andrejjevi*, *P. caucasicus*, *P. mongolensis* et *P. smirnovi* pour la grande gerbille, *S. sinkiangensis* pour le lézard.

MOTS CLÉS : phlébotome, vecteur, leishmaniose, Chine.

The presence of visceral leishmaniasis (VL) in China was first confirmed after an autopsy of a German soldier in 1909. VL used to be one of the most serious parasitic diseases in China. The vector, phlebotomine sandfly, was proven in China as early as in the twenties. In this paper, a brief review of phlebotomine vectors in China is given.

HUMAN LEISHMANIASIS VECTORS

THE VECTOR SPECIES IN CHINA

In China, five species of phlebotomine sandflies are incriminated to be the vectors of VL, i.e. *Phlebotomus* (*A.*) *chinensis*, *P.* (*A.*) *longiductus*, *P.* (*A.*) *sichuanensis*, *P.* (*P.*) *alexandri* and *P.* (*L.*) *smirnovi* (Leng, 1988a).

P. chinensis was proven a vector of VL in the early twenties according to epidemiological study, coincidental distribution with VL, artificial infection, presence of natural infection and successful experimental transmission. After the fifties, four more flies were incriminated as vectors/suspected vectors (Tables I, II). After having been fed on cotton rats or hamsters infected with *Leishmania donovani*, 85.1 to 93.3 % of *P. alexandri* were infected, the promastigotes not only developing in the stomach but also invading the pharynx, buccal cavity and proboscis (Guan, 1986a, b). The natural infection of *P. alexandri* is about 2.0 %. In addition, golden hamsters could also experimentally be infected by the bite of *P. alexandri* infected with promastigotes of *L. donovani* (Guan *et al.*, 1986b).

P. smirnovi: during the sandfly season, the natural infection rate of promastigotes was 5.7 %, and experimental infection rate is about 35.3 to 85.1 % after feeding, in which case the promastigotes invade into the pharynx (Xiong *et al.*, 1974; Wang, 1983; Guan *et al.*, 1986a, 1992, 1994a). The experimental infection rate of *P. longiductus* and *P. sichuanensis* is 54.2 to 62.2 % and 60.8 % respectively (Xiong *et al.*, 1974; Yin, 1985; Wang, 1983; Guan, 1986a).

* Department of Medical Parasitology, Faculty of Medicine, Jinan University, Guangzhou, 510632, China PR.

Correspondence: Y.J. Leng.

Tel: 86-20-8551-6511 ext 4653/4629.

e-mail: oxyx@maina.jnu.edu.cn.

Distribution of promastigotes in sandflies

Species*	Infected rate (%)	Proboscis	Buccal cavity	Pharynx	CEsophagus	Pro-gut	Mid-gut	Hind-gut	Reference
P. al	48.9	1.5	—	10.6	30.3	63.6	100.0	12.1	Xiong, 1963
P. al	85.1	2.5	2.5	2.0	85.0	100.0	100.0	5.0	Guan, 1986a
P. al	93.3	5.2	1.3	29.1	78.9	99.6	100.0	5.7	Guan, 1986b
P. ch	69.2	2.2	—	17.5	45.4	84.3	100.0	19.7	Xiong, 1988
P. ch	65.0	3.4	—	27.1	53.6	85.4	100.0	19.3	Xiong, 1992
P. lo	54.2	—	—	8.7	70.2	88.1	97.7	20.2	Xiong, 1974
P. lo	62.2	7.1	0	25.0	75.0	100.0	100.0	3.6	Guan, 1986a
P. si	60.8	—	—	6.7	48.9	55.6	100.0	8.9	Yin, 1985
P. sm	85.1	—	—	6.6	35.0	64.9	100.0	39.3	Xiong, 1974
P. sm	35.3	—	—	33.3	83.3	100.0	100.0	33.3	Guan, 1986a

* P. al: *P. alexandri*; P. ch: *P. chinensis*; P. lo: *P. longiductus*; P. si: *P. sichuanensis*; P. sm: *P. smirnovi*.

Table I. — Artificial infection of five vectors with *L. donovani*.

Distribution of promastigotes in sandflies

Species*	Infected rate (%)	Proboscis	Buccal cavity	Pharynx	CEsophagus	Pro-gut	Mid-gut	Hind-gut	Reference
P. al	2.0	7.7	7.7	46.2	61.5	92.3	100.0	—	Guan, 1986b
P. ch	0.23	—	—	+++	+++	++++	++++	—	Xiong, 1988
P. ch	0.31	++	—	+++	+++	++++	++++	—	Xiong, 1992
P. sm	5.7	—	—	23.5	70.6	88.2	94.1	29.4	Xiong, 1974

* See table I.

Table II. — Natural infection of vectors with *L. donovani*.

BIOLOGY AND DISTRIBUTION OF VECTORS

The vectors could be grouped into two categories: endophilic (*P. alexandri*, *P. chinensis* and *P. longiductus*) and exophilic vectors (*P. sichuanensis* and *P. smirnovi*). Their zoogeographical distribution are showed in figure 1.

P. chinensis, one of the three endophilic vectors, was the main and most important vector widely distributed over Northeast, North, East and eastern part of Northwest China. It is an anthropophilic and a predominant species in the old VL epidemic areas. The other two endophilic vectors are: *P. longiductus* distributed in old residential quarters of western Xinjiang; *P. alexandri* distributed in western Xinjiang, western Inner Mongolia and western Gansu as high as 3,200 m altitude, Qilian-shan Mt. (Leng & Zhang, 1993). The experimental results indicate that *P. alexandri* is also an anthropophilic species of epidemiological significance in the transmission of VL in the studied area (Guan *et al.*, 1986b).

P. sichuanensis is found to be an exophilic vector in the mountainous region of northwestern Sichuan, southern Gansu, Qinghai and Tibet (above 1,000 m altitude). *P. smirnovi* is another exophilic vector in the desert and savannah area of Xinjiang, western Inner Mongolia and Gansu. These two species prefer sucking blood of wild animals, and also bite man. They have an important role in transmission of zoonotic leishmaniasis. Studies indicate that sandflies of the different species act as vectors of VL transmission in different regions.

SEVERAL OBJECTS IN DISPUTATION

1/ *P. chinensis* and *P. sichuanensis*
(Leng & Zhang, 1992)

P. sichuanensis is considered by some authors as a large type of *P. chinensis*. The authors carried out a series of comparative studies of these two flies according to the following facts, and proved that *P. sichuanensis* is a separate species instead of a « large type of *P. chinensis* ».

Fig. 1. — Diagrammatic distribution of phlebotomine vectors of VL in China.

Species*	Male							Female				
	Wing	A3	A4	AF	HC	GF	F/P	Wing	A3	A4	CDS	SS
<i>P. ch</i>	2,104	419	34	2/3-15	24	679	5.9	2,320	325	51	+	13-14
<i>P. si</i>	3,180	550	257	2/3-8, 1/9-15	39	1,130	7.1	2,750	370	148	-	15-18

* See table I. A3: antenna 3; A4: antenna 4; AF: ascoid formula; CDS: comduct of spermatheca; GF: genital filament; F/P: genital filament/genital pump; HC: hairs on inner surface of coxite; SS: segments of spermatheca.

Table III. — Some morphological characters of *P. chinensis* and *P. sichuanensis*.

Fig. 2. — Developmental comparison of *P. sichuanensis* and *P. chinensis* (from Leng & Zhang, 1992).

Pc: *P. chinensis*; Ps: *P. sichuanensis*; O: oval stage; I: first larval stage; II: second larval stage; III: third larval stage; IV: fourth larval stage; P: pupal stage.

- Morphological Characters (Leng & Yin, 1983) ((Table III))
- Development (Fig. 2)

The time from oviposition to emergence of *P. sichuanensis* (83 days) is much longer than that of *P. chinensis* (55 days).

Especially the third larval stage is much longer in *P. sichuanensis* (22 days) than that of *P. chinensis* (5 days) (Zhang, 1990).

- Characteristics at molecular level (Zhang & Leng, 1991)

The allozyme phenotypes at different loci of phosphoglucumutase (PGM) and malate dehydrogenase (MDH-2) showed differences between *P. sichuanensis* from Sichuan and *P. chinensis* from Sichuan and Beijing. The MDH2 revealed a relative moving distance (RMD) 80 of alleles in *P. chinensis* from Beijing, but absent from Sichuan; and RMD 84 of alleles in *P. sichuanensis*. The PGM locus for genotype AA was over 77 % in *P. chinensis* and 8.3 % in *P. sichuanensis*; for genotype DD they were negative and 75 % respectively (Fig. 3).

The molecular message showed evidences that *P. sichuanensis* and *P. chinensis* are two closely related but different species.

* Diagrammatic from of PGM electrophoretic bands

Fig. 3. — A comparison of the phosphoglucumutase enzyme genotype of *P. chinensis* and *P. sichuanensis* from Beijing and Sichuan (from Zhang & Leng, 1991).

A: *P. chinensis* from Beijing; B: *P. chinensis* from Sichuan; C: *P. sichuanensis* from Sichuan.

	<i>P. smirnovi</i>					« <i>P. wui</i> »		
	Perfiliew (1941)	Perfiliew (1948)	A. & N. (1984)	L. L. & L. (1987)	Leng (1991)	Y. & X. (1965)	A. & N. (1984)	Remarks
Male								
Aedeagus	0.096-0.115	0.12	0.143 (0.120-0.160)	0.15 (0.12-0.16)	0.147 (0.130-0.160)	—	0.138 (0.128-0.144)	No difference
Coxite	0.27-0.29	0.27-0.35	0.301 (0.284-0.320)	0.31-0.37	0.300 (0.272-0.314)	0.30	0.291 (0.264-0.316)	No difference
H/C	23+	18+	28 (25-36)	20-25	26 (21-29)	—	25 (15-23)	Overlapping
Farmost hair	—	—	0.55 (0.51-0.57)	—	0.52 (0.41-0.61)	—	0.54 (0.51-0.57)	No difference
Style	0.14-0.15	0.15-0.18	0.166 (0.152-0.180)	0.17 (0.16-0.18)	0.163 (0.154-0.178)	0.15	0.148 (0.134-0.160)	No difference
C/S	1.92-1.93	1.80-1.94	1.67-1.95	1.94-2.05	1.63-2.01	2	1.85-2.03	No difference
GF/GP	—	3	3.27	3	3.56	—	3	No difference
A. F.	2/3-7, 1/8-15	same	same	same	same	same	same	Identical
Asc4/A4	—	1/5	0.21	1/5	0.23	—	0.25	No difference
W. L.	1.90	1.92-2.16	1.81 (1.64-2.14)	1.93 (1.83-2.10)	1.73 (1.62-1.84)	1.83	1.66 (1.52-1.80)	Overlapping
Female								
A3	—	0.23-0.28	0.231 (0.200-0.248)	0.227 (0.225-0.230)	0.223 (0.206-0.238)	0.21	0.236 (0.208-0.260)	No difference
A3/L	—	0.88-0.96	0.86 (0.81-0.94)	0.82 (0.77-0.87)	0.91 (0.79-0.99)	—	0.93 (0.87-1.07)	Overlapping
Asc4/A4	—	—	0.31 (0.28-0.35)	> 1/5	0.32 (0.22-0.42)	—	0.35 (0.30-0.40)	Overlapping
P. B.	—	—	0.056 (0.048-0.064)	0.063 (0.055-0.070)	0.065 (0.058-0.070)	0.058	0.060 (0.052-0.064)	Overlapping
S. S.	15	14-15	16 (14-18)	12-16	15 (14-17)	14-16 (12-19)	16 (14-19)	Overlapping
W. L.	2.28	2.28	2.05 (1.96-2.16)	2.08	1.95△ (1.80-2.08)	2.24	2.12 (1.98-2.46)	Overlapping
Locality	USSR	USSR	USSR	USSR & China (Inner Mongolia)	China	China	Outer Mongolia	

A3: antenna 3; A3/L: antenna 3/labrum; A. F.: antennal formula; A. & N.: Artemiev & Neronov; Asc4/A4: ascoid (antenna 4) length/antenna 4 length; C/S: coxite/style; GF/GP: genital filament/genital pump; H/C: hairs/coxite; L. L. & L.: Leng, Lane & Lewis; P. B.: pharynx breadth; S. S.: spermatheca segments; Y. & X.: Yang & Xiong; △: measured from the alulet; □: Artemiev & Neronov (1984) stressed main differential points.

Table IV. — Comparison of *Phlebotomus (Larrousius) smirnovi* from USSR, China and Mongolia.

• Different geographical distribution (Fig. 1)

P. chinensis is distributed up to 44°N in Jilin and Changchun, Jilin Province and southwards down to Danxian County, Hainan Island around 19.5°N; in the east from the sea coast westward to a zigzag line running north to south along the eastern parts of Inner Mongolia, Shaanxi, Gansu, Qinghai, northern Sichuan, Guizhou, Guangxi and Hainan Island.

P. sichuanensis is distributed mainly in the south-western mountainous areas of China. There is only a very small area of vertical and horizontal overlapping in geographical distribution between the two species.

2/ *P. smirnovi* and *P. wui*

(Leng, Lane & Lewis, 1987; Leng, 1988a)

Ting & Ho (1962) reported a new species named as *P. major* collected from Xinjiang Uyghur Autonomous Region. Yang & Xiong (1965) described a new subspecies of *Larrousius*, *P. major wui* and concluded that Ting & Ho's *P. major* was *P. major wui*. Artemiev & Neronov (1984), Xiong & Jing (1987) treated this subspecies as *P. wui*.

Leng *et al.* (1987) compared the paratype of Ting & Ho's *P. major* and Yang & Xiong's *P. major wui* with *P. smirnovi* (included syntype and paratype) from pro-USSR, and considered that *P. wui* is really a synonym of *P. smirnovi*.

• Morphological Characters (Table IV) have not obvious proper differences from the species mentioned above

- Geographical distribution (Fig. 4) is in continuation from Central Asia to the northwest of China

- Ecological similarity (Table V)

Phlebotomine season of these « species » is similar.

- Evolutionary difference

Artemiev & Neronov (1984) suggested that, during the historical migration, *P. smirnovi* spread eastward from Central Asia to the East of Junggar and Tarim Basins and evolved to a new species *P. wui* in these two basins. In fact, the first discovery and main distribution of *P. major wui*/*P. wui* are the west of Junggar and Tarim Basins. It is difficult to imagine that Artemiev & Neronov's suggestion is true.

Fig. 4. — Zoogeographical distribution of *Phlebotomus smirnovi* (from Leng, 1988b).

THE VECTORS OF ANIMAL LEISHMANIA

WILD MAMMALS

The natural infection by leishmanial parasites is quite common in great gerbil, *Rhombomys optimus*, in the desert area in north Xinjiang Uygur Autonomous Region and Ejin Banner, Inner Mongolia Autonomous Region (Guan *et al.*, 1982,

1994b). These parasites can cause ear-lesion in great gerbil. Two species of *Leishmania*, *L. turanica* and *L. gerbilli* have been found in great gerbil's ear lesion. The main vectors of these two leishmanial parasites are *P. mongolensis* and *P. andrejevi* in Ejin Banner, Inner Mongolia Autonomous Region (Guan *et al.*, 1982); *P. smirnovi*, *P. andrejevi*, *P. mongolensis* and *P. caucasicus* in Xinjiang Uygur Autonomous Region (Xiong *et al.*, 1964; Guan *et al.*, 1994b) and *P. alexandri*, *P. mongolensis* in Gansu Province (Wang *et al.*, 1964). In gerbil burrows, these sandflies are predominant and their natural infection rates by *Leishmania* are shown in table VI. Infected case of *P. smirnovi* with gerbil *Leishmania* is different in some places. It is considered being the main vector in Karamay, Xinjiang, but the possibility to be vectors in Ejin Banner, Inner Mongolia and Gansu is very little (Guan *et al.*, 1982, 1986a, 1994a; Liu *et al.*, 1982; Xiong *et al.*, 1964). Other experiments suggest that *P. alexandri* might be a favourable host for *L. gerbilli* (experimental infection rate is about 67.6 %, Guan *et al.*, 1986a) (Table VII). Other facts have shown that after inoculating the *Leishmania* isolated from Karamay big gerbils, a monkey showed an ulceration of dermis and one of two human volunteers developed cutaneous leishmaniasis. *Leishmania* parasites were found in macrophages of the dermis and in the epithelial cells of hair follicles. This indicates that *Leishmania* parasite of the Karamay great gerbils is pathogenic to monkey and man, and that great gerbil is a potential reservoir of human cutaneous leishmaniasis in this area (Guan *et al.*, 1992).

WILD LIZARD

The natural infection of *Leishmania* in lizards is found in the Ejin Banner, Inner Mongolia Autonomous Region. The main vector of lizards leishmania is considered to be *S. (S.) sinkiangensis* with a natural infected rate of 13.0 % and promastigotes filled up fore-, mid-, hind- gut (Guan *et al.*, 1982) (Table VI). This sandfly prefers the blood of lizard and is tightly associated with the lizard in nature (Guan *et al.*, 1991).

Species	Distribution	Season				Reference
		Start	Peak	End	Period	
<i>P. wui</i>	Xinjiang	E/May	① M/June ② M/August	L/September	4.5-5 m	Xiong, 1979
	Inner Mongolia	E/June	L/June to E/July	L/August	3 m	Xiong, 1976, 1979
<i>P. smirnovi</i>	Central Asia	M/May	① L/June ② L/July	L/September	4.5-5 m	Artemiev, 1984

Table V. — Ecology of *P. smirnovi* and *P. wui*.

Distribution of promastigotes in sandflies

Species*	Infected rate (%)	Proboscis	Buccal cavity	Pharynx	Esophagus	Pro-gut	Mid-gut	Hind-gut	Reference
P. an	12.8	—	—	—	8.3	41.7	100.0	8.3	Guan, 1982
P. an	13.0	6.3	—	0	12.5	18.8	100.0	25.0	Guan, 1994b
P. al	4.6	11.1	—	77.8	88.9	88.9	100.0	11.1	Wang, 1964
P. ca	22.8	—	—	9.5	19.0	52.4	95.2	28.6	Xiong, 1964
P. mo	0.8	—	—	+	+	++	+	++	Wang, 1964
P. mo	17.2	0.7	—	9.3	17.9	43.0	100.0	8.6	Xiong, 1964
P. mo	10.0	2.9	—	—	11.4	54.3	94.3	17.1	Guan, 1982
P. mo	8.1	0	—	0	13.3	33.3	100.0	0	Guan, 1994b
S. si	1.6	+	—	+	+	+	+	+	Xiong, 1965
S. si	13.0	0	—	10.0	30.0	60.0	100.0	40.0	Guan, 1982
P. sm	13.0	23.8	—	38.1	66.6	85.7	100.0	23.8	Xiong, 1964
S. sm	1.1	—	—	—	—	33.3	100.0	33.3	Guan, 1982

* P. an: *P. andrejevi*; P. ca: *P. caucasicus*; P. mo: *P. mongolensis*; S. si: *S. sinkiangensis*.

Table VI. — Natural infection of several species of sandfly with animal *Leishmania*.

Distribution of promastigotes in sandflies

Species*	Infected rate (%)	Proboscis	Buccal cavity	Pharynx	Esophagus	Pro-gut	Mid-gut	Hind-gut	Reference
P. al	67.6	8.7	—	13.0	47.8	91.3	100.0	8.7	Guan, 1986a
P. mo	33.3	0	—	0	15.0	45.0	100.0	0	Xiong, 1964
P. lo	32.8	0	—	10.5	73.7	100.0	100.0	10.5	Guan, 1986a
S. si	16.2	—	—	—	+	+	+	+	Xiong, 1965
P. sm	20.6	0	0	0	0	71.4	100.0	14.3	Guan, 1986a

* See table I and table VI.

Table VII. — Artificial infection of sandflies with animal *Leishmania*.

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