

CHINESE PHLEBOTOMINE SANDFLIES OF SUBGENUS *ADLERIUS* NITZULESCU, 1931 (DIPTERA: PSYCHODIDAE) AND THE IDENTITY OF *PHLEBOTOMUS SICHUANENSIS* LENG & YIN, 1983 Part I – Taxonomical study and geographical distribution

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Summary :

Four species of *Adlerius* phlebotomine sandflies have been recorded in China, namely: *P. chinensis* Newstead, 1916 (Pc); *P. fengi* Leng & Zhang, 1994; *P. longiductus* Parrot, 1928 and *P. sichuanensis* Leng & Yin, 1983 (Ps). *Adlerius* phlebotomines are the main vectors of visceral leishmaniasis (VL) in China; three of them are acknowledged as VL vectors and *P. fengi* is considered a potential VL vector for southwestern mountainous region. Different opinion has been raised to the validity of identity of Ps by some investigators from Shanghai and Shanxi who consider Ps to be a large type of Pc instead of an isolate species. The center of controversy is whether Ps is an isolate taxon or a large type of Pc. The present authors have carried out a series of comparative studies for these two flies on: 1 quantitative and qualitative morphological characters of four Chinese *Adlerius* phlebotomines; and 2. differences in geographical distribution. All specimens of Pc and Ps used in the present study are collected where their holotypes-paratypes were produced – West Mountain, West Suburb, Beijing and Lixian County, Sichuan Province. The results have forcefully proved that Ps is an isolate species instead of a so-called large type Pc according to the concept of species. The clarification of their taxonomical identities is meaningful because both of them are VL vectors in different epidemic areas in China; especially Ps is an important VL vector in high mountainous regions of southwestern China and some extend to the Loess Plateau of northwestern China, where VL still exists and it is also the first Phlebotomine sandfly discovered in Tibet, the locality being near Assam in India (Leng *et al.* 1990).

KEY WORDS : taxonomy, Diptera, *Phlebotomus*, *Adlerius*, *P. sichuanensis*.

MOTS CLÉS : taxonomie, Diptera, *Phlebotomus*, *Adlerius*, *P. sichuanensis*.

Résumé : LES PLÉBOTOMES DE CHINE DU SOUS-GENRE *ADLERIUS* NITZULESCU, 1931 (DIPTERA : PSYCHODIDAE) ET IDENTITÉ DE *PHLEBOTOMUS SICHUANENSIS* LENG & YIN, 1983. I : TAXONOMIE ET DISTRIBUTION GÉOGRAPHIQUE

Quatre espèces de phlébotomes du sous-genre *Adlerius* sont connues de Chine. Elles sont respectivement nommées : *P. chinensis* Newstead, 1916 (Pc), *P. fengi* Leng & Zhang, 1994; *P. longiductus* Parrot, 1928 et *P. sichuanensis* Leng & Yin, 1983 (Ps). Trois d'entre elles sont incriminées dans la transmission de la leishmaniose viscérale en Chine et *P. fengi* est considéré comme vecteur potentiel dans le sud-ouest montagneux.

La validité du taxon *P. sichuanensis* a été remise en question par les chercheurs de Shanghai et Shanxi (ISS). Ils considèrent Ps comme un variant de grande taille de *P. chinensis* plutôt qu'une espèce à part entière. Cette différence d'identification est devenue le centre d'une controverse. Le présent travail est basé sur une série d'études comparatives de ces deux insectes.

Les études morphologiques, anatomiques et morphométriques des quatre espèces du sous-genre *Adlerius* de Chine sont rapportées ainsi que les différences de distribution géographique. Tous les échantillons de Pc et Ps utilisés dans cette étude ont été collectés dans les localités des holotypes et paratypes, région montagneuse de l'ouest, région sud-ouest (Shanghai), région de Pékin et Lixian et la province de Sichuan.

Les résultats montrent clairement que Ps est une espèce en soi et non un représentant de Pc de grande taille. La clarification de la position taxonomique des deux espèces est fondamentale en raison de leur responsabilité dans la transmission de la leishmaniose viscérale épidémique dans les différentes localités chinoises.

Ps est un vecteur important de VL dans les régions de haute montagne du sud-ouest de la Chine et quelques foyers du plateau de Loess du nord-ouest où la VL existe encore. Par ailleurs c'est dans cette région que le premier phlébotome tibétain a été découvert localisé près d'Assam en Inde (Leng *et al.*, 1990).

ABBREVIATIONS:

Pc: *Phlebotomus chinensis*, **Ps:** *Phlebotomus sichuanensis*,
PS: phlebotomine sandfly, VL: visceral leishmaniasis,
ISS: investigators from Shanghai Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai and Shanxi Medical University, Taiyuan, Shanxi Province.

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INTRODUCTION

Leng & Yin (1983) reported 10 species of PS from northwestern Sichuan, including two new species among which *Phlebotomus sichuanensis* (Ps) was described for the first time. Since then Ps has been incriminated as a vector of VL in Shanxi, Gansu, Qinghai and Sichuan Provinces (Yin & Leng, 1985). This sandfly is also distributed in Yunnan Province and Tibet Autonomous Region. (Leng, Zhang & Zhang, 1991; Leng, 1997). Xiong, Jin, Hong *et al.* (1990) synonymized Ps to *Phlebotomus chinensis* (Pc) and consi-

dered **Ps** to be a large type of **Pc**. But they have not given any description to define a measurable distinction between so-called small and large type **Pc** except they stressed on the altitude where the specimens are collected, at above 2,000 m or below 1,500 m of sea level because they considered that increasing altitude made usual **Pc** changing into a large type **Pc** and this changing might reverse (Xiong, Jin, Hong *et al.*, 1990). To go further, ISS carried out a series of study concerning this item, they confirmed that **Ps** is a large type of **Pc** instead of an isolate taxon based on the following reasons: 1. large and small type imagoes of **Pc** were collected from different heights (980-2,370 m altitude) and the fourth instar hatched from them in laboratory are identical in morphology and measurement ratios. 2. The male ascoid formula of **Pc** has two patterns, 2/3-15 and 2/3-8, 1/9-15; following the increasing altitude "the higher the altitude the more the latter pattern ratio", up to 96.6 % at above 2,000 m sea level. Large type is often distributed at above 1,500 m sea level (Xiong & Jin *et al.*, 1990). 3. Zhao, Li *et al.* (1987) and Zhao, Qiao *et al.* (1998) compared the SEM imagines of female pharyngeal armature – a subgenus character for *Adlerius* instead of inter-species characters – of large and small type **Pc** and according to its similarity concluded that **Ps** is a large type **Pc**.

MATERIALS AND METHODS

Origin of specimens and materials: for the present research **Ps** specimens were collected by aspirator from above 900 m altitude, Lixian County, northwestern Sichuan, where its holotype-paratypes were collected for the first time. **Pc** specimens were collected from: 1. Temples in West Mountain, West Suburb, Beijing where its holotype was collected in 1915; 2. Lixian County and Nanping County, northwestern Sichuan, in the latter where both **Ps** and **Pc** are present. *P. fengi* specimens measured are their holotype and paratypes preserved in Jinan University (Leng & Zhang, 1994) and the measurement materials of *P. longiductus* are quoted from original descriptions and well known papers (Parrot, 1928;

Wang *et al.*, 1966; Artemiev, 1980; Lewis, 1982; Feng, 1983). The taxonomical systems of Artemiev (1980), Lewis (1977, 1982, 1987) and Theodor (1958) are adopted.

Quantitative and qualitative morphological study: comparative morphological structures are studied under microscope. Its corresponding imagines are drawn under camera lucida and measured in μm scale for quantitative analysis.

Zoogeographical distribution: according to the data studied by the present author (Leng, 1997).

RESULTS

MORPHOLOGICAL CHARACTERS

By comparing of ten morphometric and two morphostructural characters of males and four morphometric and two morphostructural characters of females for the four *Adlerius* PS in China (Tables I, II, III and Fig. 2 for comparison between **Ps** and **Pc**), the following facts are found:

Morphometrically: 1. **Ps** is one of the largest PS in China and over the world. 2. A3, A3/L, ascoid on A4, style and aedeagus tubercle distance of **Ps** is the largest but its c/b is the smallest among the species in subgenus *Adlerius* (Tables I, II). 3. A4 in **Ps** is much longer than that of **Pc** [male = 7.6:1 (257:34), female = 2.9:1 (148:51)]. 4. The male wing length is much longer in **Ps** [**Ps/Pc** = 1.5:1 (3,180:2,104), Table III]. 5. A3/L: δ **Ps** > δ **Pc**, η **Ps** < η **Pc** (Tables I, II). 6. Aedeagus and genital filament: **Ps** > **Pc**. 7. The aedeagus tubercle distance of **Ps** is the longest of the four (**Ps**: **Pc** = 1.4; **Ps/P. longiductus** = 2.6; **Ps/P. fengi** = 4.2). 8. Genital filament/Genital pump = **Ps** > **Pc** (7.1:5.9). 9. The following characters of **Ps** are opposite to the other three Chinese *Adlerius* phlebotomines: 1. Male A4 in **Ps** and *P. fengi* are much longer (δ/η = 1:0.58 and 1:0.88, Table III) instead of shorter than that of females; 2. Wing of male **Ps** is longer instead of shorter than that of females (δ/η = 1:0.58, Table III).

Morphostructurally: **Ps** differs from **Pc** in 1. **Ps** has no common spermathecal duct. 2. Male ascoid formula

δ	A3	A3/L	AF	HC	FHC	ST	GF	Style	Aed	F/P
Pc	419	1.59	2/3-15	24	0.66	31	679	202	175	5.9
Pf	460	1.47	2/3-8,15	1/9-57	0.59	10	1,333	206	233	10.5
Pl	417	1.52	2/3-8,15	1/9-64	0.57	16	1,400	204	195	10.1
Ps	550	1.67	2/3-8,15	1/9-39	0.71	42	1,130	250	190	7.1

Pc: *P. chinensis*; Pf: *P. fengi*; Pl: *P. longiductus*; **Ps**: *P. sichuanensis*.

A3: antenna 3; A3/L: antenna 3/labrum; AF: ascoid formula; HC: hairs on inner surface of coxite; FHC: farthest hair from base of coxite; ST: subapical tubercle of aedeagus; GF: genital filament; Aed: aedeagus; F/P: genital filament/genital pump.

All measures in μm .

Table I. – Some morphological characters of four Chinese *Adlerius* phlebotomines (δ).

♀	A3	A3/L	CDS	SS
<i>P. chinensis</i>	325	1.27	+	13-14
<i>P. fengi</i>	455	1.38	-	-
<i>P. longiductus</i>	388	1.06	+	11-13
<i>P. sichuanensis</i>	370	1.02	-	15-18

CDS: common duct of spermatheca; SS: segments of spermatheca.

Table II. – Some morphological characters of four Chinese *Adlerius* phlebotomines (♀).

Species	Antenna 4			Wing length		
	♂	♀	♂/♀	♂	♀	♂/♀
<i>P. chinensis</i>	34	51	1:1.50	2,104	2,320	1:1.10
<i>P. fengi</i>	203	178	1:0.88	2,587	3,116	1:1.20
<i>P. longiductus</i>	56	66	1:1.18	2,363	2,707	1:1.15
<i>P. sichuanensis</i>	257	148	1:0.58	3,180	2,750	1:0.86

Table III. – Length of antenna 4 and wings of four Chinese *Adlerius*.

(Table III) in **Ps** is 2/3-8, 1/9-15 which differs from that of **Pc** 2/3-15. Hundreds of **Ps** male specimens collected from Yunnan, Tibet and Sichuan are repeatedly examined: it shows that all male **Ps** have only one pattern of ascoid formula without variations. 3. Hairs on the inner surface of coxite in **Ps** covered a wider area and are more in number (**Ps:Pc** = 39:24). The above morphological characters make up sharp species distinction between **Ps** and **Pc** (Tables I, II, III).

ZOOGEOGRAPHICAL DISTRIBUTION

The topography of China by according to height is clearly divided into three platforms of steps. The first platform covers a huge area from the coast of Pacific Ocean where it is lower than 500 m altitude. The second platform covers western loess plateau and western mountainous area where it is lower than 1,000 m altitude. The third platform covers southwestern mountainous region higher than 1,000 m altitude including Tibet, the roof of the Earth. *P. chinensis* is mainly distributed in the first platform up to certain depth into the border of second platform. *P. sichuanensis* is distributed in second and third platforms from



Fig.1. – Map of the zoogeographical distribution of four Chinese *Adlerius* phlebotomine sandflies.

900 m to 2,800 m altitude with some overlaps with *P. chinensis* in the border of second platform. This has been well studied for years (Leng, 1997; Zhang & Leng, 1997; Fig. 1).

DISCUSSION

SPECIMENS USED IN THE STUDY

In the studies of ISS, all phlebotomines used were said to be: “The locality of specimens collected” that is in a limited area in northwestern Sichuan, southern Shaanxi and Shanxi (Fig. 1), just at/near to the places in a parapatric zone for **Pc** and **Ps**. But, from the contradictory descriptions given by them (Table IV), people can easily find that the origin of specimens used in their works are mixed and blurred. ISS have never collected specimens from the place where original holotype or paratype of **Pc** and **Ps** were formally collected.

The standard considered by ISS to differentiate **Ps** and **Pc** are: 1. The Size on which depends identification

Same photos	Zhao, Li, Qiao & Yin (1997)#	Zhao, Qiao, Yin, Yin, Li (1998)#
1 (1997) = D (1998)	Large type Pc from Nanping, Sichuan Province (2,400 ×)	Pc from Yichuan 2, Shaanxi Province
2 (1997) = A (1998)	Small type Pc from Nanping, Sichuan Province (1,600 ×)	Large type Pc from Nanping, Sichuan Province
3 (1997- = C (1998)	Pc from Jixian, Shaanxi Province (2,000 ×)	Pc from Yichuan, Shaanxi Province
4 (1997) = E (1998)	Pc from Yichuan, Shaanxi Province (1,300 ×)	Pc from Jixian, Shanxi Province

The names underlined are the same writer's in both papers.

Table IV. – The same SEM imagine illustrated in different origin of places and species.

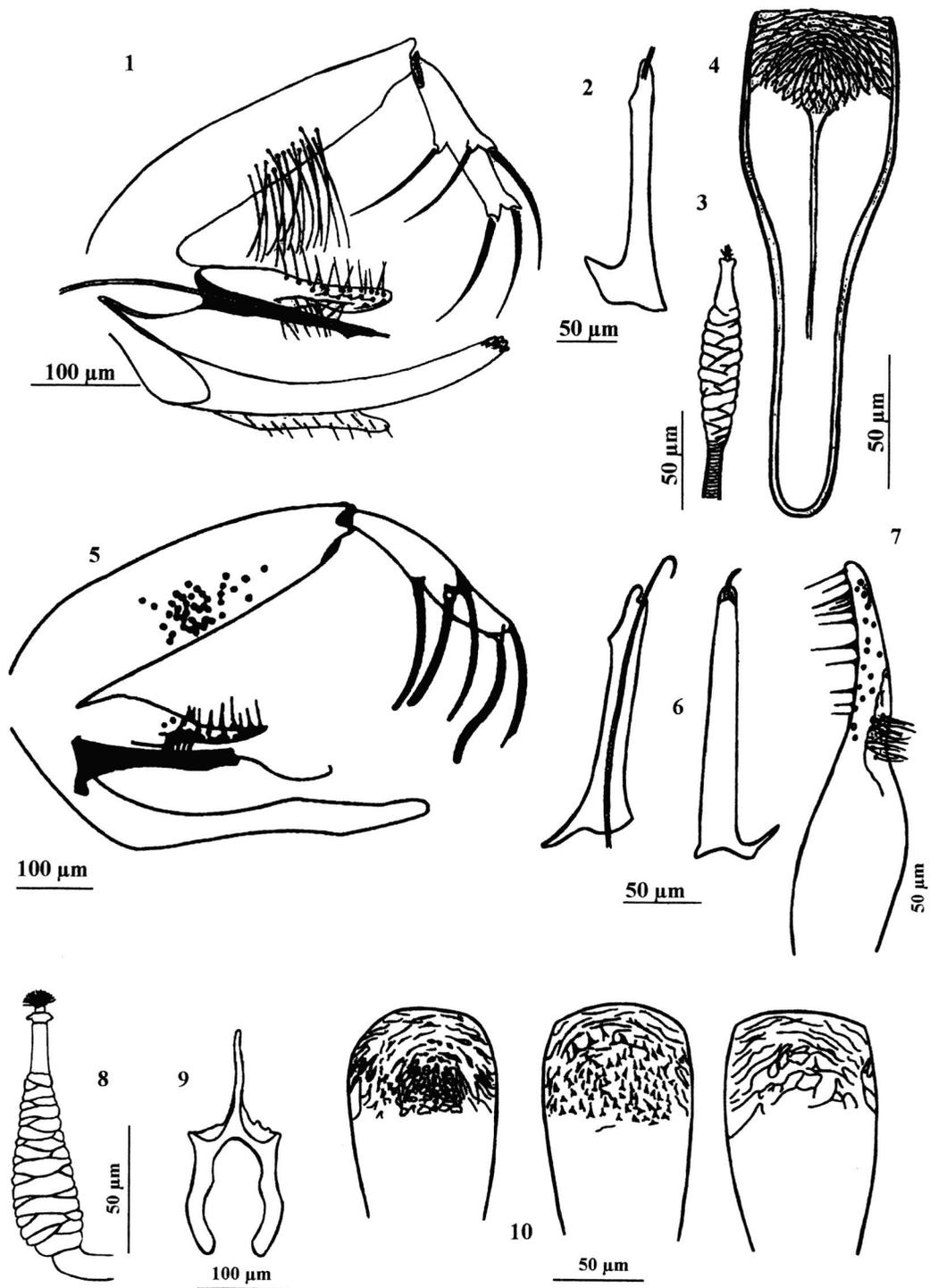


Fig. 2. – *P. chinensis*. 1, genitalia; 2, aedeagus lateral view; 3, spermatheca; 4, pharynx (after Theodor, 1958); *P. sicbanensis*. 5, genitalia; 6, aedeagus, lateral view and ventral view; 7, paramere showing the short hairs in the middle of lateral side; 8, spermatheca; 9, furca; 10, pharynx viewed at three different focal depths for the same specimen (after Leng & Yin, 1983).

of large and small type **Pc** by these investigators is probably corresponding to a microscopical aspect without distinctive data for size distinction. 2. The height is considered to be an important factor for dividing **Ps** and **Pc** because Investigators of Shanghai Institut consider, that following the increasing altitude, the size of phlebotomine sandflies is enlarging. When reached 1,500-2,000 m in altitude large type **Pc** appeared. So the specimen collected above or lower 1,500 m were treated as large and small **Pc** respectively for their studies. But we have found **Ps** appeared in the height of 900 m above sea level in northwestern Sichuan, 2,800 m in Yunnan and 2,500 m in Tibet.

Most proper morphological differences useful for identifying these two flies described in the world literature under the light of contemporary taxonomy do not seem to have been considered by ISS.

The specimens used for their studies came from a similar origin (Xiong & Lu's old dry preserved specimens). Upon the facts mentioned above it is hard to avoid wrong identification and confusions for the original specimens used in their work.

Xiong, Jin, Hong *et al.* (1990) stated in their paper that "We checked the specimens sent to us as a gift by Dr Yin Zhi-cheng of Sichuan Institute in Parasitic Diseases collected from Huan (Lixian?) County at 2,400 m and 2,800 m altitude. Then these very large specimens were renamed by Leng *et al.* (It is really Leng Y.J. & Yin Z.C., 1983) as *P. sichuanensis*, which is actually what we have repeatedly explained the large type *P. chinensis* distributed 2,000 m above sea level. So that *P. sichuanensis* should be a synonym of *P. chinensis*." Dr Yin Zhi-cheng collected a large number of PS in an area which covers 29-33° N and 102-106° E in northwestern Sichuan in 1976-1980. These specimens were studied by Leng & Yin (1983) and ten species of phlebotomine sandflies have been identified including two new species – *P. sichuanensis* and *S. zhengjiani*. At that time there was no publication nor personal communication stating that these specimens had been authorized by Xiong as large type **Pc** which can not be legally studied, named or renamed by others.

COMPARABILITY AND RELIABILITY

Following the updated method of phlebotomine taxonomy, *P. sichuanensis* was carefully described and named following the International Code of Zoological Nomenclature (Leng & Yin, 1983). The morphometric and morphostructural comparison of twelve male characters and six female characters for four species recorded in Chinese *Adlerius* has shown obvious specific differences between **Pc** and **Ps**. (Tables I, III)

In the papers of ISS, there were lack of morphological descriptions and standards for supporting the differentiation between **Ps** and **Pc**. This will undoubtedly

make obscure their species identification between **Pc** and **Ps**. Furthermore, many contradictory facts and data have been given in the SEM studies carried out by investigators from Shanxi (Zhao, Li, Qiao & Yin, 1997). They published four SEM imagines of **Pc** female pharyngeal armatures which were re-used and re-illustrated differently as to the site of collection and the species naming without explanation by Zhao, Qiao, Yin, Yin & Li (1998), of whom three were the 1997 writers.

The obscure ness of ISS works is also denoted in the magnification labeled in SEM imagines and their ratios to real size of **Pc** and **Ps**. In the specimens collected from Nanping, northwestern Schuan (Zhao, Li, Qiao & Yin, 1997), the large type **Pc** (PS) in photo 1 (2,400×) is almost the size of small type **Pc** (**Pc**) in photo 2 (1,600×), which means that the real size ratio is about large type: small type = 2:3, **Ps**<**Pc**. The real size of these two flies calculated by female wing length (Table III) is **Ps** (2750): **Pc** (2320) = 1.19:1.00, **Ps**>**Pc**. The above confusions are clear just with a glance to Table IV. It is impossible to evaluate the accuracy of ISS's work and it would be strange to treat **Ps** as a large lype of **Pc** upon such obscure bases.

MALE ASCOID FORMULA

Xiong *et al.* (1990) have reported that they dissected 53 males from 1,500 m altitude and six males above 2,000 m altitude (for observing ascoïd dissection is not needed, it is hard to understand why they have dissected the flies to observe ascoïd), and discovered that the ascoïd formula of large type **Pc** (**Ps**) has two patterns, 2/3–15 and 2/3–8, 1/9–15. But on the basis of examining hundreds of specimens from Sichuan, Yunnan and Tibet the authors claim that the ascoïd formula of male **Ps** is constantly 2/3–8, 1/9–15, in one pattern. In the view of ascoïd formula patterns among the members of subgenus *Adlerius* over the world as shown in Table V, it is clear that they are different species with their own ascoïd formula pattern. According to the obscure original specimens from a parapatric zone observed by Xiong *et al.* it is obvious that their wrong conclusion has come from mixed original materials of **Ps** and **Pc** observed.

Ascoïd formula	Species
2/3–15	<i>chinensis, simici</i>
2/3–5, 1/6–15	<i>angustus, balcanicus, comatus, hyreniae, salangensis, zulfagarensis</i>
2/3–7, 1/8–15	<i>davidi, hindustanicus</i>
2/3–8, 1/9–15	<i>arabicus brevis, fengi, halpensis, kabulensis, longiductus, rupester, sichuanensis, turanicus</i>

Table V. – Ascoïd formula of male subgenus *Adlerius* phlebotomine sandflies.

Neglecting most papers meticulously worked under the light of updated taxonomy on this item and published in world literature including Leng & colleagues studies, and hiding the characters of identification described by these investigators, ISS repeatedly sophisticated that Leng has only taken the difference of male ascoid formula to name a normal (?) **Pc** as **Ps** (Xiong *et al.*, 1990; Zhao *et al.*, 1997; 1998). It is really a kind of distort quotations commonly used which would not even alter facts. Scientific conclusion can only be made on the basis of facts and scientific data instead of a hasty announcement.

ZOOGEOGRAPHICAL DISTRIBUTION

It seems that the Chinese *Adlerius* phlebotomines may be divided into two groups according to morphological approximation and geographical distribution. High Altitude Group: *P. fengi*, and *P. sichuanensis*. Low Altitude Group: *P. chinensis* and *P. longiductus* stands in the middle (Table VI).

Species	♂ AF	♂ A4	♀ A4	♀ CSD
<i>P. chinensis</i>	2/3–15	< 100 (34)	< 100 (51)	+
<i>P. longiductus</i>	2/3–8, 1/9–15	< 100 (56)	< 100 (66)	+
<i>P. sichuanensis</i>	2/3–8, 1/9–15	> 100 (203)	> 100 (148)	–
<i>P. fengi</i>	2/3–8, 1/9–15	> 100 (257)	> 100 (178)	–

AF = ascoid formula, CSD = common spermathecal duct.

Table VI. – Some morphological differences in Chinese *Adlerius*.

TAXONOMIC PRINCIPLE AND RULE

Every contemporary phlebotomine taxonomist knows that the morphology of female pharyngeal armature and spermatheca are subgenus characters for *Adlerius*, which are usually similar in morphology with uncertain minute difference between species within the subgenus and are usually not used for identifying species. Male characters are mainly used for identifying species in *Adlerius*, and of course, in zoological taxonomy, different morphological characters are used for identification in different level of taxa and for different objects. Xiong, Jin, Hong *et al.* (1990) denied the classification system of Theodor (1958) and Artemiev (1980) on subgenus *Adlerius* without dealing with which characters should be needed for this purpose; they affirmed that the main differential characters for **Pc** to other members in subgenus *Adlerius* are 1. spermatheca, 2. female pharyngeal armature and 3. male genitalia especially the ratio of aedeagus tubercle distance to aedeagus length, and said that these useful characters are not different between **Pc** and **Ps**. Updated phlebotomine taxonomists should be shocked by the above unusual words. Many incomplete and over-simplified description and inaccurate or inadequate comparison for establishing

new phlebotomine species have also been done by some investigators causing much chaos. A new taxon has to be created with adequate descriptions, as stipulated in the International Code of Zoological Nomenclature: “to be available every new scientific name... must be accompanied by a description of definition that states in words characters that are purposed to differentiate the taxon...” [ICZN (1985) Article 13(i)]. For the brightness, the International Code had lead to the 1st International Symposium for Phlebotomine Sandflies (ISOPS I), 1991, Rome where a standardization was finalized for describing phlebotomines and the Chinese version has been published in China (Leng, 1991). Unfortunately ISS neglected the principle and rule for taxonomy and the standard of ISOPS, they have laid undue emphasis on the importance of female pharyngeal armature and spermatheca for identifying phlebotomine species in common. Zhao, Qiao *et al.* (1998) quoted such idea of Xiong, Jin *et al.* (1987): “Female pharyngeal armature is one of most important morphological accordance for identifying a species in subgenus *Larrousius*. A species can be correctly identified if combined with the morphology of spermatheca”. Then this idea was grafted on **Pc** and **Ps** and according to the similarity of pharyngeal armature under SEM imagines, Zhao, Qiao *et al.* (1997) repeatedly concluded that **Ps** is nothing more than a large type **Pc**. But the truth is that, under an inconceivable idea, SEM imagines could not prevent them to avoid inevitable mistakes.

It is strange, furthermore, that Hsiung (Xiong), Guan & Jin from Shanghai (1981) simply compared two phlebotomines sandflies in different subgenera, *S. sintoni* Pringle, 1953 of subgenus *Sergentomyia s. str.* Franca & Parrot, 1920 and a newly found fly of subgenus *Parrotomyia* Theodor, 1958 from southern Xinjiang, instead of comparing members within the same subgenus, to create the latter as a new taxon, “*Sergentomyia turfanensis* sp. nov.” *Sergentomyia turfanensis* is considered being *S. (Parrotomyia) palestaniensis* Adler & Theodor, 1924 in morphology and geographical distribution (Leng, 1997). Disobeying the well-established taxonomic rules and law made the proposed name invalid.

General rule and principle for taxonomy have been established by generations of taxonomists (Zhen, 1987). Present taxonomic system for phlebotomine sandflies has been mainly formed within this century and is constantly improving. At present new techniques of molecular biology are often used for understanding evolutionary tendency of a population and for probing sibling species. General principle and rule, knowledge and method of zoological taxonomy should always be strictly followed. It has been proved that the contemporary system for phlebotomine taxo-

onomy is effective and reflective to natural history and must not be neglected.

CONCLUSION

According to the above mentioned facts, it is taxonomically clear that *P. sichuanensis* is an isolate species instead of a large type of *P. chinensis*.

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