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STUDIES ON "KASEN" OF HORSES IN HOKKAIDO

IV. RESEARCHES ON THE PUNKIES IN HOKKAIDO WITH DESCRIPTION OF A NEW SPECIES

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As the cause of the chronic and stubborn skin disease of horses called "kasen", there has been a theory of microfilaria, larva of *Setaria equina* or *Onchocerca cervicalis*. However, there are many unknown phases of the behavior of the microfilaria which need to be investigated in connection with the relationship between the microfilaria and "kasen".

Many cases of the disagreement between the occurrence of microfilaria and this disease have been known recently. It does not always follow that the horses which suffered from "kasen" harbored the microfilaria, and the converse holds good. RIEK (1954) has reported that the disease of horses known as Queensland-itch or allergic dermatitis, in Australia, is caused by the development of a hypersensitivity to the bites of *Culicoides robertsi* and that the lesions are confined to the dorsal parts of the horse body. In accordance with the present authors' finding that the anti-histamine preparation was beneficial to treat "kasen" as shown previously in the 2nd report of this series, it seems to be similar to Queensland-itch which occurred under allergic conditions. The punkies suck the blood from the same region as the lesion of "kasen", and also the occurring period of punkies well agrees with that of "kasen" in Hokkaido.

It is known that punkies directly cause dermatitis of men and animals by their bites, and moreover it is well known that they are also intermediate hosts of parasites or the transmitters of the virus diseases. Reports have been published of human dermatitis caused by the bites of *Culicoides miharai* by KINOSHITA (1918) in Korea and the case of a mass outbreak of human dermatitis due to *C. nubeculosus* by TAKEDA and MUKAI (1952) in Japan. YOSHIDA and GOTO (1949) have reported the disease caused by bites of *Leptoconops nipponensis*. TOKUNAGA

(1937) has pointed out the possibility of the transmission of fowl pox-virus by *C. arakawai*, and UENO et al. (1956) have found a parasite which probably the larva of *Onchocerca cervicalis*.

In other countries, the punkies are reported as the important intermediate hosts of the nematode parasites, *Onchocerca* sp. and *Dipetalonema* sp. (*Acanthocheilonema* sp.), by many investigators. The punkies as the transmitters of the incomprehensible fever, horse-sickness (virus disease) and blue-tongue of sheep in America have been reported by various investigators. There is RIEK's report, as above mentioned, of Queensland-itch in Australia caused directly by the bites of punkies. The present authors take a great interest in punkies in such various meanings. Although they have examined numerous such creatures, they have never found any endoparasite from the punkies in Hokkaido. The authors felt keenly the need of research in both fields of microfilaria and of punkies in order to make clear the cause of "kasen". As a feature of their research on the cause of "kasen", here they wish to describe their first observations concerning punkies obtained mainly from the manes of horses in Hokkaido which suffered from "kasen".

COLLECTING METHOD AND LOCALITIES

This research was carried out during 2 seasons, from July to October in 1955 and from April to October in 1956, which just agree with the season of "kasen" occurrence. The collection of the punkies was done mainly from the bodies of pasturing horses which suffered from "kasen" by use of an aspirator in the early morning and evening. The other part of collection was done by use of the light trap. Many punkies were found concealing themselves in the long hair part of horses, especially in the mane. The punkies were biting tightly on the horse skin and were sucking the blood. They were obtained in Futomi of Tobetsu and Kakuyama of Ebetsu mainly. The other areas include 4 villages; Onbetsu, Memanbetsu, Otoe and Tsukisappu. Futomi and Kakuyama have flat topography, and their climates are oceanic. The high wind blows in the spring, and the amounts of rainfall are large in summer and autumn. Geologically these areas are peaty, and there is much wet land; consequently they are best suited for the development of the blood-sucking insects.

RESULT

Species of Punkies

According to TOKUNAGA (1937), KONO and TAKAHASI (1940) and ARNAUD (1956), there have been found 12 species of punkies in Hokkaido. However, these species have been collected by the use of the light trap or the insect net without regard to the horses. The present authors' collection of the punkies was done directly from the horse body by use of an aspirator, as mentioned above. They placed their hope on their method not

only in the investigation of the relationship between punkies and "kasen", but also in the finding of unknown species of punkies which perhaps could not be collected by use of the light trap or the insect net in Hokkaido; they expected also to make clear the specific preference of punkies to horses from these observations.

The authors collected 5 species of punkies: *Culicoides peregrinus* KIEFFER, *C. obsoletus* MEIGEN, *C. pulicaris* LINNAEUS, *C. pulicaris* var. *ocellaris* KIEFFER, *Forcipomyia ishikariensis* n. sp. from the body of the horses which suffered from "kasen". But the punkies belonging to the genus *Forcipomyia* have been considered as non-blood-sucking species. So this species collected by the authors might be merely resting on the horse body. On the other hand they have collected another species, *Culicoides pictimargo* TOKUNAGA and SHOGAKI, by the light trap in the open field. A damaged specimen which is probably *Atrichopogon parviforceps* TOKUNAGA has been obtained also in Otoe in this research. The relationships between species and localities in this research are shown in table 1.

TABLE 1. *Occurrence of Punkies in Each Area*

LOCALITY	SPECIES OF PUNKIES				
	C. peregrinus	*C. obsoletus*	*C. pulicaris*	*C. p.* var. *ocellaris*	*F. ishikariensis* n. sp.
Futomi	+	+	+	+	·
Kakuyama	+	+	+	·	+
Otoe	+	+	·	·	·
Tsukisappu	+	+	·	·	·
Memanbetsu	·	+	+	+	·
Onbetsu	·	+	+	+	·

Among these species, *C. peregrinus* occurred in the greatest abundance and was widely distributed. *C. obsoletus* was far fewer than *C. peregrinus*, while its distribution was the most wide. New species were found only in Kakuyama and were remarkably few.

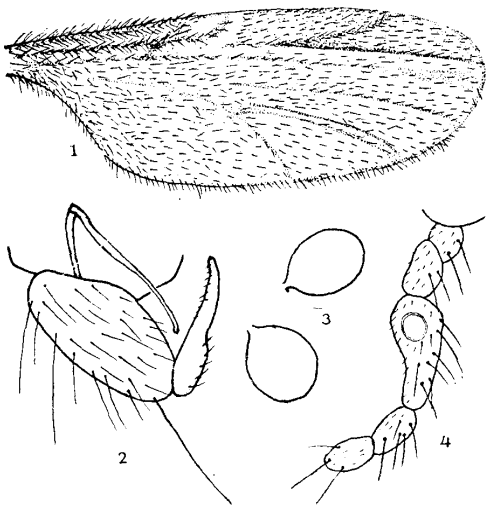
The authors tried the artificial rearing of *C. peregrinus* collected from horses by providing them with 5% grape juice and other fruit juice in the cage at room temperature 26° to 28°C; the adult punkies have survived for 4 to 7 days. About 50% of the adults laid eggs on the moist filter paper or mud. A mass of eggs contains 32 to 40 individuals. The eggs hatched after 10 to 15 days, but the rearing of the larvae failed.

Morphological Aspect of a New Species, *Forcipomyia ishikariensis* sp. nov.

Male: Body length 2.2 mm. Head, thorax, abdominal tergites black. Antennae brown, proportional lengths of distal 6 segments as follows: 4 : 6 : 6 : 10 : 9 : 12. Antennal ratio about 0.5. Maxillary palpus brown, with tuber-like form in 3rd segment. Sensory pore of 3rd segment situated upper part of swelling. Proboscis shorter than the half length of head. Scutum shining black, with sparse blackish hairs. Pleura brownish black. Fore legs brown. Middle and hind femur blackish except for brownish basal and apical

parts. Middle tibiae black. Hind tibiae black except brownish basal 2/3. Proportional lengths of 1st and 2nd tarsal segments of fore, middle and hind legs as follows: 16 : 15; 14 : 20; 17 : 23. Claws very long and curved rectangularly. Halters brown. Wings hyalin with dense black hairs, a pale yellew spot just beyond tip of radial vein. Hypopygium: coxites stout and oval. Styles slender and shorter than the coxites with a short bristle on the inner side of distal 1/4. Gonapophyses with a narrow stripe at base.

FIG. 1. *Forcipomyia ishikariensis sp. nov.*



1. Wing. 2. Male genitalia.
3. Spermatheae. 4. Female palpus.

Female: Body length 2.7 mm. Head, thorax and sternal tergites black. Antennae brown with dark brown scapes. Basal short segments of antenna conical. Proportional lengths of distal 5 segments as follows: 13 : 12 : 10 : 10 : 17. Antennal ratio about 0.65. Maxillary palpus dark brown, with tuber-like form in 3rd segment. Sensory pore circular, situated on the middle of swelling. Scutum black with sparse black hairs. Pleurae dark brown. Fore and middle femur yellow. Hind femur black, apical half of hind femur black. Basal 2/3 of the middle tibiae, and basal 3/4 of the hind tibiae black. Knee joints yellow. All tarsi yellow. Middle and hind tibiae with many lancet-like scales. Halters white. Abdomen with black setae. All tergites of abdomen black. Cerci yellow. Spermathecae 2, one is pear-like and the other is egg-plant-like shaped, each spermatheca with very short neck part. Wing membrane hyalin, with a pale yellow spot just beyond tip of radial vein. R_{4+5} ending just middle of costal margin. First radial cell absent. Second radial cell small elongated, as long as its stem. Wings covered with coarse black hairs entirely.

Holotype ♀, Allotype ♂ and paratypes 3 ♀ collected at Kakuyama, Ishikari, Hokkaido by Y. KITAMURA on July 25, 1956. The types are preserved in the Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University.

The present species is closely related to *F. brevipedicellata* KIEFFER but differs in the yellow fore legs and hind tarsal ratio.

Annual Activity of Punkies

In 1955, the authors could not make observations of the beginning of the occurrence of punkies, for this investigation was started from July. Temperature averages 22°C from early in July to late in August, and over 70% in humidity; consequently the occurrence of the punkies was remarkable. After that, there was a long spell of wet weather, and the occurrence of punkies decreased. In September when the temperature was 10° to 12°C, the number of punkies decreased, and by the 20th of September the punkies were

found to have disappeared. In 1956, the authors collected only 2 specimens of *C. peregrinus* on the 22nd of May; the punkies were gradually increased in number. September of that year was warmer than the same month of the preceding year, and the period of occurrence of punkies was prolonged. As examples of annual activities of punkies, the observations in Futomi and Kakuyama are shown in Tables 2 and 3.

TABLE 2. *Seasonal Fluctuation of Punkies in Futomi in 1955*

DATE	SPECIES OF PUNKIES			
	C. peregrinus	*C. obsoletus*	*C. pulicaris*	*C. p.* var. *ocellaris*
18/VII	130	0	0	0
21/VII	220	0	0	0
24/VII	360	0	0	0
26/VII	280	0	0	0
6/VIII	{230 (morning) {500 (evening)	3	0	0
16/VIII	400	0	0	0
3/IX	20	7	4	2
12/IX	8	9	3	0
20/IX	0	0	0	0
2/X	0	0	0	0

TABLE 3. *Seasonal Fluctuation of Punkies in Kakuyama in 1956*

DATE	SPECIES OF PUNKIES			
	C. peregrinus	*C. obsoletus*	*C. pulicaris*	*C. ishikariensis* n. sp.
22/V	2	0	0	0
27/V	28	11	0	0
2/VI	25	17	0	0
8/VI	70	28	0	0
16/VI	240	17	0	0
25/VI	420	32	8	5
10/VII	200	9	5	0
24/VII	400	2	13	0
2/VIII	600	4	7	0
12/VIII	400	25	4	0
29/VIII	130	28	2	0
18/IX	80	17	0	0

It has been known that the flying of the punkies is influenced by the meteorological factors; temperature, humidity, illumination and wind velocity, etc. According to FUJITO

(1939), many individuals of *C. miharai* occur at temperature 20°C and over 80% in humidity. TAKEDA and MUKAI (1954) have stated that the flying of *C. nubeculosis* was prosperous when temperature was about 20°C, humidity 100% and atmospheric pressure about 1010 mb. TRAVIS (1949) has reported that the wind is the most important factor for the flying of the punkies; he states that their activities disappear when wind velocity is 3 5 MPH and they stop suddenly at 55°F temperature. DOVE et al. (1932) have observed that the punkies have sensitive thermotaxis and their females, in the light breeze, swarm on the animals whose body temperatures are high. TAKAHASI (1941) has collected *C. buhetoensis* at 5°C temperature in Manchuria.

According to the authors' findings, the punkies collected in Hokkaido were sucking the blood of animals and flying in the field very actively at about 20°C temperture, while their flying disappeared when temperature was less than 10°C. Their blood-sucking and flying were observed at over 60% humidity and were the most active at over 80% humidity. Their activities were influenced remarkably by the wind.

Diurnal Activity of Punkies

The punkies began to make their appearence in the field at daybreak; they attained the greatest number just before and just after sunrise. Thereafter the number decreased gradually, and they could not be recognized at all in the open field under the direct rays of the sun.

In the daytime, the authors collected only a few specimens from the horse tethered in the forest. However, they began to fly suddenly around the bodies of horses again at about sunset; they gradually decreased in number towards the early night when they disappeared entirely. In short, the diurnal activity of punkies in Hokkaido is great during the period from 4 to 7 a.m. and from 4 to 9 p.m. in summer.

Blood-Sucking Region on Animals

The observations on *C. miharai* and *C. nubeculosus* have been done by FUJITO (1939) and TAKEDA and MUKAI (1954) respectively. These species were found mainly from the long-hair region, clothed femoral and brachial regions when they attacked women. FOOTE and PRATT (1954) have reported that the punkies creep about the long-hair region and the clothed part in order to suck the blood.

The authors observed that in horses the punkies creep about the inside of the thigh, mammary region, inside of auricle, depilated region around the lesion of "kasen" and long-hair part of the neck and root of the tail; they reach to the cutaneous surface, and then they suck the blood standing obliquely on their head, and stinging with their proboscides. There were a few cases of punkies sucking the blood at the back or ventor of horses where the hairs are short but grow in high density. It seems that they are unable to suck the blood directly through the hairs by their minute proboscides, 0.5 to 0.2 mm in length, in such regions. So it seems that the punkies attack by preference the thinly haired regions. But also it seems that the punkies attack willingly even the long-hair regions, if they are able to get into such a region as easily as in the mane of horses.

According to the authors' observation, the time required for blood-sucking of the punkies is 1 to 3 minutes in general.

Color Preference of Punkies

HILL (1947) has compared the number of punkies which rested on various colored cloths, 3 inches square in size, which were spread at a height of 4 to 5 inches from the earth. The punkies were the greatest in number on the black cloth.

According to the present authors' observation, *C. peregrinus* attacked more willingly the black horse than the chestnut horse, while the preference of *C. obsoletus* were not clear. About this subject, the authors wish to investigate further in future.

TABLE 4. *Frequency of the Occurrence of Punkies by Coat Colors of Horses.*

COAT COLORS OF HORSE	SPECIES OF PUNKIES		
	C. peregrinus	*C. obsoletus*	Total
Black	152	18	170
Chestnut	86	17	103

NAKAMURA et al. (1955) have previously reported that the rate of contraction of "kasen" is 54% in the black horses while 1% in the chestnut horses in Hokkaido. There is an agreement in respect to the color of horses between incidence of "kasen" and attacks by punkies, especially *C. peregrinus*. However, ISHIHARA and UENO (1957) have reported that the sex and coat color of horses have no relationship with the morbidity.

CONCLUSION

According to the present research, the period of the occurrence, color preference and favorite regions of attack of punkies well agree with the incidence of "kasen" in Hokkaido. *C. peregrinus* and *C. obsoletus* are widely distributed and abound; they were found more often on the horses which suffered from "kasen" than were the other species of punkies in Hokkaido. So the authors suppose that the species of *Culicoides*, especially *C. peregrinus*, may have the most important connection with "kasen" in Hokkaido, if the bites of punkies are the direct cause of this disease. The punkies adhere to the skin of horses very tightly by the sting of their proboscides and suck the blood.

The need of more detailed research about these species of punkies is keenly felt. The authors are attempting the artificial rearing of the punkies on a large scale. They believe that the success of this attempt will surely further the investigation of the cause of "kasen" and other infectious diseases.

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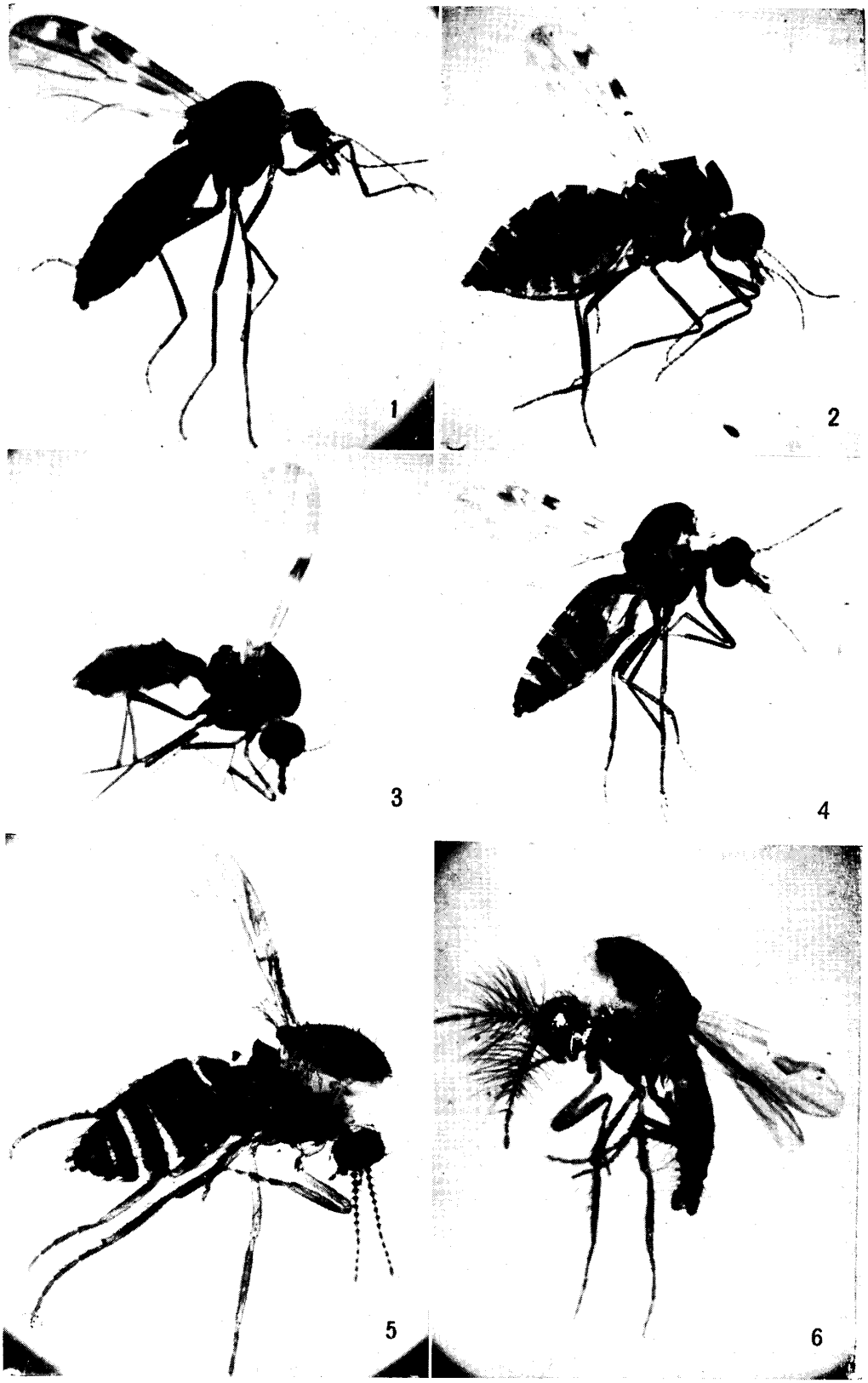
EXPLANATION OF PLATE

Fig. 1. *Culicoides peregrinus*, ♀.
Fig. 2. *C. pulicaris*, ♀.
Fig. 3. *C. obsoletus*, ♀.
Fig. 4. *C. pulicaris* var. *ocellaris*, ♀.
Fig. 5. *Forcipomyia ishikariensis* n. sp., ♀.
Fig. 6. Ditto, ♂.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