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Morphological and Immunological Studies on the Blood Sources of Black Flies in Hokkaido, Japan (Diptera: Simuliidae)

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Contents

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INTRODUCTION

The members of the black fly form a small family of Diptera (Simuliidae) and have a world-wide distribution. More than 1,000 species have been described in the world 62), of which approximately 70 species are found in Japan 64, 77). The females of certain species are notorious blood-suckers, and in some countries they are veritable scourges for both human beings and domestic animals 1, 30, 43, 60). In view of their past and present infamy, black flies are familiar to many people living under a wide range of climatic conditions, from tropical to temperate, and even subarctic.

Black flies are small, usually dark-coloured and stoutly built with short legs and elongate mandibles. Their wings are broad with thick anterior veins and faint veins. Antennae are usually 11-segmented and scarcely longer than the head. The most characteristic feature is the humped thorax and resulting lowered head; this accounts for one of the black fly's alternate common names, buffalo gnat, in North America.

Adult flies occur near streams and rivers, and their eggs are laid either on herbage or stones, above or beneath the surface of the water. The larvae are aquatic and require swiftly flowing water for their environment, and for this reason they are often found congregated in the vicinity of rapids and waterfalls. The larvae form a pocket-like cocoon before pupation on stones, twig-like substances and submerged vegetations in streams.

Black flies are important to both human and domestic animals for their blood-feeding behaviour. Their effects examined from both medico-veterinary and economic view points are described by many authors 19, 25, 26, 32, 48, 72, 88). Many of these effects, such as decrease in milk and egg production, dermatitis and skin lesions, death due to toxemia and systemic shock, bovine onchocerciasis and avian leucocytozoonosis, are known in animals. Human onchocerciasis and dermatitis and systemic reactions to bites are the main effects on human health. In addition, as a public nuisance, black flies contribute to tourist revenue loss and decrease in work efficiency at recreational and agricultural sites.

The main effects caused by them is transmission of various parasites from host to host via the blood-meal. The most important of these is *Onchocerca volvulus*, a filarial nematode parasite of humans in Central

America and West Africa. In Africa, about 20,000,000 people carry this parasite 74). Black flies also transmit *Leucocytozoon smithi*, a haemosporidial blood-parasite of wild and domestic turkeys which occurs also in the U. S. A. as well as in Europe, Asia and South Africa 13, 31, 38, 86).

Many of the effects of black flies have been known and studied by researchers from many countries; however, the specification of vector species is still incomplete on many diseases. For example, one disease, the onchocerciasis, which seems to be transmitted only by black flies, has been shown to occur in Asir region of Saudi Arabia in human beings 11). However, *Simulium damnosum* s. l., the main vector of this disease in Africa and probably the vector in Yemen, is not present in that region, and this, together with the fact that there are no records of black flies biting man in Saudi Arabia, leaves the existence of human onchocerciasis in Asir epidemiologically unexplained 17).

The immunological technique for blood-meal identification is one of the useful methods to specify the vector species in many diseases transmitted by blood-sucking insects 87).

In the present study, the author examined immunologically the blood source of black flies in Hokkaido, Japan, and studied morphologically female adults, especially their blood-sucking behaviour in relation to the medico-veterinary view point.

Historical review of blood source determination studies

Since numerous diseases were determined to be transmitted by several species of blood-sucking insects, many procedures have been used to determine the host range of blood-sucking insects in order to specify the vector species 5, 21, 87).

Early studies depended principally on field observations such as by bait traps, but this method proved to be disadvantageous for some purposes. The immunological methods were recommended to determine the blood-meal of insects 91). As far as the author is aware, immunological studies on blood-meal identification were started following the suggestion of Uhlenhuth, P. *et al.* (1908) 85). They mentioned that the precipitin test may be useful to identify the blood-meal of blood-sucking insects such as bed bugs, fleas, lice and mosquitoes 49) and a large number of reports have been published 4, 24, 89).

Early immunological studies were undertaken usually by means of the precipitin test, and studies of the following insects were made: anopheline mosquitoes in relation to malaria epidemiology 7, 8, 12, 29, 37), other species of mosquitoes 45, 59, 79), sand flies 39, 42, 81), biting midges 6), tsetse flies 33, 75), ticks 28, 90) and black flies 20, 22).

Recently some other immunological methods have been adapted for insect blood-meal identification. These include the fluorescent antibody technique (FA) in mosquitoes 44), the haemagglutination inhibition test (HI) in biting midges 47), the passive hemagglutination inhibition technique (PHI) in tsetse flies 92), tabanid flies 41) and mosquitoes 80), the latex agglutination test in *Culicoides* 3) and the enzyme-linked immunosorbent assay (ELISA) in mosquitoes 9, 10, 23) and black flies 65~68).

In Japan, immunological studies on insect blood-meal identification was started by Morishita and Katagai (1933) 46). They first determined the blood-meal source of Formosan *Anopheles* species determined by the precipitin test. Subsequent studies using this method were conducted on Korean *Anopheles* species 93), on mosquitoes in the Tokyo district 82), on Heleid biting midges 50) and on mosquitoes 35, 36). The ELISA technique, the most recent labeled-reagent and the most sensitive method, was applied firstly to black flies to identify the blood-meals of blood-sucking insects in 1986 by the author and his co-workers 65).

CLASSIFICATION AND TERMINOLOGY

The classification of the family Simuliidae has been subject to controversy among workers. There are two main systems: one follows Enderlein's opinion, which accepts the binominal system devised by Stone (1964) 73), Rubtsov (1974) 61) and Ono (1982) 57); and the other is adopted for Edward's proposal of the trinominal system made by Crosskey (1969) 15), Uemoto & Okazawa (1980) 84) and Takaoka (1977) 76). Recently almost all Japanese workers accept subgenera and are using the trinominal system of nomenclature. The author recognizes certain subgenera, but refrains from their use because of the uncertain subgeneric assignment of a number of species, and is using the binominal system of nomenclature as described by Ono (1982) 57).

The morphological terminology adopted in this study follows that of Crosskey (1967, 1969) 14, 15) and Snodgrass (1944) 71).

PART 1. Morphological character of the female black fly and blood-sucking behaviour

Chapter 1 Morphology of the mouth parts

Introduction

It is well known that the mouth parts of blood-sucking insects are well developed for feeding. In black flies, the mouth parts consist of labrum, labium, jagged mandibles, maxillae and broad hypopharynx, and the mandibles and the galeae of maxillae are specially developed to wound the skin of the blood source 52, 71).

Up to the present, many detailed studies have appeared on the shape of the mouth parts of female black flies (e. g. 27, 40, 56, 70, 76, 83), and all of them were based on observations using the optical microscope.

In the present chapter, the author describes the results of examinations of the shape of the mandible and the galeae of the maxillae of female black flies by means of the scanning electron microscope (SEM).

Materials and methods

The mouth parts of 27 species belonging to 13 genera of female black flies inhabiting Hokkaido were examined (Table 1). These specimens were collected and identified by the author and Dr. H. Ono during the past ten years.

The specimens preserved in 70 percent ethanol were refixed with 2-ethoxyethanol for 2 days, then with xylene for 3 hr individually as described by Sabrosky (1966) 63).

Refixed specimens were dried on a sheet of filter paper in an open space for 2 hr. The heads of black flies were removed from the body individually, then they were put into a small Petri dish and additionally dried and degassed with diphosphorus pentaoxide and activated carbon in a desiccator for one week. Dried specimens (removed heads) were mounted on specimen stubs with a conductive adhesive and incubated in a high vacuum chamber (ic. 10^{-6} torr.) for 6 hr. Specimens were then spatter-coated with gold for 20 min (4 times of 5 min each side). After coating, they were observed and photographed with a SEM (JEOL, JSM-T200), using an accelerating voltage of 10 or 15 kV.

The number of tricuspid teeth of the outer side of the upper mandible per 10 μm, the depth of the notch of the top tricuspid tooth of the upper mandible and the shapes were counted, measured and observed. In addition, in the galeae of the maxillae, the number of teeth per 20 μm from the top was counted and the length and shape of the tooth situated at the top of the galeae were observed.

Results

1 General morphology

A pair of large toothed mandibles exists between the labrum and labium. The tricuspid teeth at the outer margin of the mandibles make a line along the insertion course, but those at the inner margin make a line counter to the insertion course.

A pair of the galeae of the maxillae with strong teeth is observed on both sides of the mandibles. The length and shapes differ among each species examined.

2 *Twinnia cannibora* (Plate I)

The teeth form a closed line at the top half and a sparse one at the end half of the mandibles. The left mandible is above the right one. The number of tricuspid teeth at the top of the outer side of the upper mandible is about 3 per 10 μm and the notch of the top tooth is 5 μm. The galeae of maxillae are strongly built and the teeth on the galeae are somewhat long and slender. Three to four teeth per 20 μm are situated at the top half of the galeae. The length of the top tooth is 8 μm.

3 *Helodon multicaulis* (Plate I)

The tricuspid teeth of the mandibles are blunt and the number of tricuspid teeth at the outer side of the top end of the upper mandible is about 6 per 10 μm. The depth of the notch of the top tooth at the top end of the mandible is about 3 μm. In this species the left mandible is above the right one. The teeth of the galeae of the maxillae are short and blunt and the number of the teeth per 20 μm is 7 to 8 at the top end. The length of the tooth at the top of the galeae is 5 μm.

4 *Distosimulium daisetsense* (Plate II)

The right mandible is above the left one. The tricuspid teeth are somewhat larger, and those at the top end of the mandible are close and at the basal half are sparse. The number of teeth per 10 μm at the top end is 2 to 3 and the depth of the notch of the top top tooth is 6 μm. The galeae of the maxillae has slender and sharp top teeth and the number of the teeth per 20 μm is 3. The length of the tooth at the top end of the galeae is 12 μm.

5 Genus *Prosimulium* (Plates II~IV)

Five species of this genus were examined in this study. Of these species, *P. sarurense* has a different mouth part from the other 4 *Prosimulium* species.

P. jezonicum has mandibles with large tricuspid teeth and the left mandible is above the right one. There are 2 to 3 teeth per 10 μm situated at the top end of the mandible. The depth of the notch of the top tooth of the upper mandible is 5 μm. The galeae of the maxillae has sharp and somewhat thick teeth, that is 2 to 3 teeth per 20 μm at the top end of the galeae. The length of the tooth at the top end of the galeae is 12 μm.

The mandible of *P. yezoense* have slightly enlarged teeth, and 4 to 5 teeth per 10 μm are counted at the top end of the upper mandible. The depth of the notch of the top tooth of the upper mandible is 5 μm. The teeth of the galeae of maxillae are slightly elongated and sharp ; these are 3 to 4 teeth per 20 μm at the top end of the galeae, and the depth of the tooth at the top is 8 μm.

P. karibaense has mandibles with enlarged tricuspid teeth, and there are 3 to 4 teeth per 10 μm at the top end of the outer side of the upper mandible. The depth of the notch of the outer side of the upper mandible. The depth of the notch of the top tooth is 4 μm. The teeth on the galeae of the maxillae are very sharp and slender, with 3 to 4 per 20 μm at the top end on the galeae. The length of the tooth at the top of the galeae is 8 μm.

P. sarurense has unusually shaped mandibles and galeae of the maxillae. The left mandible is above the right one. The teeth of the mandibles are dull and are situated 3 per 10 μm at the top end of the outer side of the upper mandible. The depth of the notch of the top tooth is 3 μm. The galeae have weak and flabby teeth, of which the number is 4 to 5 per 20 μm at the top end. The length of the top tooth of the galeae is 25 μm.

The mandibles of *P. apoinum* have somewhat large tricuspid teeth, which form a line of 3 teeth per 10 μm at the top end of the outer side of the upper mandible. The depth of the notch of the top tooth is 4 μm. The left mandible is above the right one. The teeth of the galeae of the maxillae are slender and sharp shaped and projected. Four teeth exist per 20 μm at the top end of the galeae and the length of the top tooth is 8 μm.

6 *Stegopterna nukabirana* (Plate V)

The tricuspid teeth of the mandibles are slightly enlarged and 3 teeth are counted at 10 μm of the top end of the mandible. The depth of the notch of the top tooth is 3 μm. The left mandible lies above the right one. The galeae of the maxillae have slender and sharp teeth and 3 to 4 teeth per 20 μm are counted at the top end of the galeae. The top tooth is 8 μm in length.

7 *Eusimulium erimoense* (Plate V)

The tricuspid teeth of the mandibles are slightly enlarged and the left mandible is above the right one. Four teeth per 10 μm exist at the top end of the outer side of the upper mandible, and the depth of the notch of the top tooth is 3 μm. The teeth of the galeae of the maxillae are sharp and slender and projected downward, with 4 to 5 teeth per 20 μm situated at the top end of the galeae. The length of the top tooth is 7 μm.

8 *Montisimulium sakhalinum* (Plate VI)

The right mandible is above the left one. Mandibles have somewhat dull tricuspid teeth which are situated 3 to 4 per 10 μm at the top end of the outer side of the upper mandible. The depth of the notch of the top tooth is 3 μm. The teeth of the galeae of the maxillae are sharp but stunted. There are 7 to 8 teeth per 20 μm at the top end of the galeae and the length of the top tooth is 4 μm.

9 *Gomphostilbia shogakii* (Plate VI)

The right mandible is above the left one. Mandibles have small tricuspid teeth and 8 teeth per 10 μm exist at the top end of the outer side of the upper mandible, and the depth of the notch of the top tooth is 1 μm. The teeth of the galeae of the maxillae are sharp and slender and 8 teeth per 20 μm are counted at the top end of the galeae. The length of the top tooth is 3 μm.

10 Genus *Cnetha* (Plates VII~IX)

Five species belonging to the genus *Cnetha* were examined and in all species the left mandible is above the right one. The tricuspid teeth of the mandibles of the five species are somewhat dull shaped.

There are 4 tricuspid teeth per 10 μm at the top end of the outer side of the upper mandible in *C. subcostatum* and 3 in *C. boldstemtum*, *C. uchidai*, *C. acmerium* and *C. konoi*. The depth of the notch of the top tooth of the mandibles is 4 μm in *C. subcostatum*, 3 μm in *C. boldstemtum* and *C. acmerium* and 2 to 3 μm in *C. uchidai* and *C. konoi*.

The galeae of the maxillae of *C. subcostatum* have sharp, short and slender teeth, 5 per 20 μm arranged at the top end. The length of the top tooth is 6 μm.

The galeae of *C. boldstemtum* have dull, short and slender teeth and 6 to 7 teeth per 20 μm exist at the top end. The length of the top tooth is 5 μm.

In *C. uchidai*, the teeth are thick and short but point and there are 5 teeth per 20 μm at the top end of the galeae. The length of the top tooth is 4 μm.

The teeth of *C. acmerium* are somewhat short and pointed. The numbers and length of the teeth of this species could not be calculated.

The teeth of *C. konoi* are dull as in *P. sarurense* and there are 8 to 9 teeth per 20 μm at the top end of the galeae. The length of the top tooth is 5 μm.

11 *Boophthora yonagoense* (Plate IX)

This species has deep notched tricuspid teeth on the mandibles and there are 3 teeth per 10 μm at the top end of the outer side of the upper mandible. The depth of the notch of the top tooth is 4 μm. The left mandible is above the right one. The teeth of the galeae of the maxillae are long and sharp. There are 5 teeth per 20 μm at the top end and the length of the top tooth is 7 μm.

12 *Gnus daisense* (Plate X)

The tricuspid teeth of the mandibles of this species are somewhat blunt and there are 3 to 4 teeth per 10 μm at the top of the outer side of the upper mandible. The depth of the notch of the top tooth is 4 μm. The left mandible is above the right one. The galeae of the maxillae have slightly elongated and sharp teeth and 4 to 5 teeth per 20 μm exist at the top end of the galeae. The length of the top tooth is 7 μm.

13 Genus *Odagmia* (Plates X～XI)

O. aokii and *O. nishijimai* were examined. Both species have deep notched tricuspid teeth on the mandibles and 3 teeth exist at the top 10 μm of the outer margin of the upper mandible. The depth of the top tooth is 4 to 5 μm in both species. The left mandible is over the right one. The teeth on the galeae are long and slender and sharp topped and 4 teeth per 20 μm exist at the top end of the galeae. The length of the top tooth is 7 μm in both species.

14 Genus *Simulium* (Plates XI～XIV)

Six species of this genus, namely *S. japonicum*, *S. horokaense*, *S. nikkoense*, *S. rufibasis*, *S. suzukii* and *S. tobetsuense* were examined. The right mandible is above the left one in *S. nikkoense*, and in the other 5 species, the left mandible is above the right.

The teeth are sharp and deep notched and 3 teeth per 10 μm are arranged at the top end of the outer margin of the upper mandible in *S. japonicum*. In *S. horokaense*, the teeth are somewhat blunt, with 3 to 4 teeth per 10 μm showing at the top end. The teeth of *S. nikkoense* are blunt and 2 to 3 teeth per 10 μm are observed at the top end. The depth of the notch of the top tooth is 4 μm. *S. rufibasis* has sharp and deep notched tricuspid teeth and 3 teeth per 10 μm are aligned at the top end. The depth of the notch of the top tooth is 4 μm. Somewhat blunt and small teeth are observed in *S. suzukii* and 5 teeth are counted at the top 10 μm. The depth of the notch of the top tooth is 4 μm. The tricuspid teeth in *S. tobetsuense* are slightly sharp with deep notches, and 4 teeth per 10 μm exist at the top end of the mandible. The depth of the notch of the top tooth is 5 μm.

The galeae of the maxillae of *S. japonicum* have 5 short and thick teeth arranged at the top 20 μm. The length of the top tooth is 5 μm. The galeae of *S. horokaense* have somewhat blunt teeth with 7 teeth per 20 μm at the top end. The top tooth is 6 μm long. The teeth of *S. nikkoense*

Table 1. The states of mandibles of Japanese black flies

Species	Upper mandible	Species	Upper mandible
Twinnia cannibora	Left	*Cnetha uchidai*	Left
Helodon multicaulis	Left	*C. acmerium*	Left
Distosimulium daisetsense	Right	*C. konoi*	Left
Prosimulium jezonicum	Left	*Boophthora yonagoense*	Left
P. karibaense	Left	*Gnus daisense*	Left
P. yezoense	Left	*Odagmia aokii*	Left
P. sarurene	Left	*O. nishijimai*	Left
P. apoinum	Left	*Simulium japonicum*	Left
Stegopterna nukabirana	Left	*S. horokaense*	Left
Eusimulium erimoense	Left	*S. nikkoense*	Right
Montisimulium sakhalinum	Right	*S. rufibasis*	Left
Gomphostilbia shogakii	Right	*S. suzukii*	Left
Cnetha subcostatum	Left	*S. tobetsuense*	Left
C. boldstemtum	Left		

are sharp, but their details could not be observed. In *S. rufibasis* and *S. suzukii*, the teeth of the galeae of the maxillae are slender, with 4 teeth aligned at the top 20 μm. The length of the top tooth is 7 μm in both species. *S. tobetsuense* has 4 to 5 sharp and somewhat thick teeth at the top 20 μm. The top tooth is 7 μm long.

Discussion

The mouth parts of 27 species belonging to 13 genera of black flies in Hokkaido were examined by the scanning electron microscope, and the shapes and numbers of the tricuspid teeth of the mandibles and the teeth of the galeae of the maxillae were described.

In 4 species, namely *D. daisetsense*, *M. sakhalinum*, *G. shogakii* and *S. nikkoense*, the right mandibles were arranged above the left ones (Table 1). Concerning the mandibles of *Simulium damnosum*, an important vector of human onchocerciasis, Gibbins (1938) 27) reported that the mandibles are arranged "right over left". Snodgrass (1944) 71), however, concluded that the left mandible is over the right one in all black fly species, and Gibbins's figure shows them in the reverse position.

Though the author could not examine the mandibles of *S. damnosum*, he observed the right mandible lying over the left one in 4 species inhabiting Hokkaido, Japan. Though the reasons and the functional merits are still unknown, it was confirmed that the tricuspid teeth at the inner margin make a line along the insertion course and that those at the outer margin make a line counter to the insertion course both in "right over left" and "left over right" species.

In two species, *H. multicaulis* and *P. sarurense*, mandibles with blunt tricuspid teeth and galeae of the maxillae with short and weak teeth were observed. Up to now the biting habits of these species have been unknown 54, 55). The author concluded that these two species may not have biting habits according to the results of the observation of the shapes of their mounth parts.

The number of the tricuspid teeth of the mandibles and the teeth of the galeae of the maxillae are believed to be constant according to the species 52); however, there are a few differences in the numbers in many of the species examined here.

The length of the top tooth of the galeae is varied in each species, but whether the differences of length originate in the species of blood source animals could not be confirmed.

Chapter 2 Morphology of the tarsal claws

Introduction

The shape of the tarsal claws of black flies is considered as one of the useful characteristics to identify the species 52), and the function of tarsal claws is considered to be that of gripping and pushing the hairs and feathers of host animals. Therefore, the shape is closely related to the blood-sucking behaviour, especially the blood source animal selection 16).

Up to the present, all the descriptions of the shape of the tarsal claws were based on examinations performed by the use of optical microscopes. The tarsal claws are well chitinized, so these studies describe only their outlines.

It is well known that not only the shape but also the surface structure of specimens can be observed with the scanning electron microscope (SEM). In the present chapter, the author describes the results of examinations of the shape and surface structureof the tarsal claws of black flies by means of SEM.

Materials and methods

The tarsal claws of 27 species of 13 genera of female black flies inhabiting Hokkaido, Japan, were examined in this study (Tables 2∼4). These specimens were collected and identified by the author and Dr. H. Ono during the past ten years.

The specimens, preserved in 70% ethanol, were refixed with 2-ethoxyethanol for 2 days and then with xylene for 3 hr individually as described by Sabrosky (1966) 63).

Refixed specimens were dried on a sheet of filter paper in an open space for 2 hr. The legs were removed from the body individually. Then they were put into a small Petri dish and additionally dried and degassed with diphosphorus pentaoxide and activated carbon in a desiccator for one week. Dried specimens (removed legs) were mounted on specimen stubs with a conductive adhesive and incubated in a high vacuum chamber (ic. 10^{-6} torr.) for 6 hr. Specimens were then spatter-coated with gold for 20 min (4 times of 5 min each side). After coating, they were observed and photographed with SEM (JEOL, JSM-T200), using an accelerating voltage of 10 or 15 kV.

Results

1 Fore-tarsal claws

The fore-tarsal claws of 13 genera and 27 species of female black flies were examined by means of SEM and classified into 3 types according to their shape and the presence of the basal tooth and its shape (Fig. 1). Of these, the S-type claw is simple and has no basal tooth on the base of the claw, and the carinulae of the claw run along the longitudinal axis of the claw (Fig. 1, upper). The T-type claw has a strong and well developed basal tooth on its base, and the pattern of the carinulae on the inner surface of the claw and the tooth is V-shaped (Fig. 1, lower). The P-type claw is

Table 2. The shape types of fore-tarsal claws of Japanese black flies

Species	Type	Species	Type
Twinnia cannibora	P	*Cnetha uchidai*	T
Helodon multicaulis	T	*C. acmerium*	T
Distosimulium daisetsense	P	*C. konoi*	T
Prosimulium jezonicum	S	*Boopthora yonagoense*	S
P. karibaense	S	*Gnus daisense*	P
P. yezoense	S	*Odagmia aokii*	P
P. sarurense	T	*O. nishijimai*	P
P. apoinum	S	*Simulium japonicum*	S
Stegopterna nukabirana	S	*S. horokaense*	S
Eusimulium erimoense	T	*S. nikkoense*	S
Montisimulium sakhalinum	T	*S. rufiasis*	S
Gomphostilbia shogakii	T	*S. suzukii*	S
Cnetha subcostatum	P	*S. tobetsuense*	P
C. boldstemtum	T		

S : simple untoothed
T : with strong tooth
P : with peg-like small tooth

simple but contains a small peg-like basal tooth on the base of the claw, and the carinulae of the claw and the tooth run along the longitudinal axis of the claw and tooth (Fig. 1, middle).

Of these species, 11 species belonging to the genera *Prosimulium, Stegopterna, Boophthora* and *Simulium* have the S-type fore-tarsal claws (Plates II-h, III-c, III-h, IV-h, V-c, IX-h, XI-h, XII-c, XII-h, XIII-c, XIII-h). All *Cnetha* species except *C. subcostatum, Helodon, Prosimulium sarurense, Eusimulium* and *Montisimulium* have the T-type fore-tarsal claws (Plates I1-h, IV-c, V-h, VI-c, VI-h, VII-h, VIII-c, VIII-h, IX-c). On the

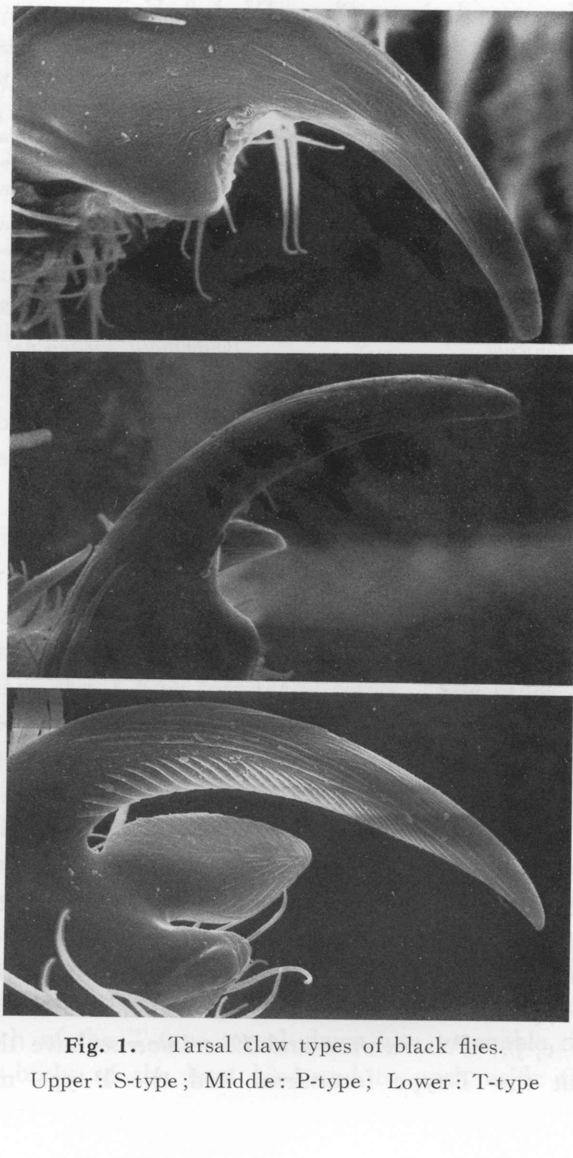

Fig. 1. Tarsal claw types of black flies.
Upper : S-type ; Middle : P-type ; Lower : T-type

other hand, *Twinnia*, *Distosimulium*, *Gnus*, *Odagmia* and *Simulium tobetsuense* have the P-type fore-tarsal claws (Table 2 and Plates I-c, II-c, VII-c, X-c, X-h, XI-c).

2 Mid-tarsal claws

The mid-tarsal claws examined were classified into 3 types according to the manner described in the previous section.

In all species except *Prosimulium sarurense* and *Eusimulium erimoense*, the type of mid-tarsal and fore-tarsal claws is the same (Table 3 ; Plates I to XIV-d, i). *P. sarurense* and *E. erimoense* have the P-type mid-tarsal claws by the T-type fore-tarsal claws (Plates IV-d & V-i).

Carinulae of both the mid-tarsal claws and their teeth show the same patterns as those of the fore-tarsal claws (Plates, I to XIV).

Table 3. The shape types of mid-tarsal clawsof Japanese black flies

Species	Type	Species	Type
Twinnia cannibora	P	*Cnetha uchidai*	T
Helodon multiczauli	T	*C. acmerium*	T
Distosimulium daisetsense	P	*C. konoi*	T
Prosimulium jezonicum	S	*Boophthora yonagoense*	S
P. karibaense	S	*Gnus daisense*	P
P. yezoense	S	*Odagmia aokii*	P
P. sarurense	P	*O. nishijimai*	P
P. apoinum	S	*Simulium japonicum*	S
Stegopterna nukabirana	S	*S. horokaense*	S
Eusimulium erimoense	P	*S. nikkoense*	S
Montisimulium sakhalinum	T	*S. rufibasis*	S
Gomphostilbia shogakii	T	*S. suzukii*	S
Cnetha subcostatum	P	*S. tobetsuense*	P
C. boldstemtum	T		

S : simple untoothed
T : with strong tooth
P : with peg-like small tooth

3 Hind-tarsal claws

The hind-tarsal claws examined were classified into 3 types according to the procedure described in the previous sections.

All species except *Prosimulium sarurense* and *Eusimulium erimoense* have the same type of hind-tarsal and fore- and mid-tarsal claws (Table 4 ; Plates I to XIV-e, j). *P. sarurense* and *E. erimoense* have the P-type hind-tarsal claws but the T-type fore-tarsal and the P-type mid-tarsal claws

Table 4. The shape types of hind-tarsal claws of Japanese black flies

Species	Type	Species	Type
Twinnia cannibora	P	*Cnetha uchidai*	T
Helodon multicaulis	T	*C. acmerium*	T
Distosimulium daisetsense	P	*C. konoi*	T
Prosimulium jezonium	S	*Boophthora yonagoense*	S
P. karibaense	S	*Gnus daisense*	P
P. yezoense	S	*Odagmia aokii*	P
P. sarurense	P	*O. nishijimai*	P
P. apoinum	S	*Simulium japonicum*	S
Stegopterna nukabirana	S	*S. horokaense*	S
Eusimulium erimoense	P	*S. nikkoense*	S
Montisimulium sakhalinum	T	*S. rufibasis*	S
Gomphostilbia shogakii	T	*S. suzukii*	S
Cnetha subcostatum	P	*S. tobetsuense*	P
C. boldstemtum	T		

S : simple untoothed
T : with strong tooth
P : with peg-like small tooth

(Plates IV-e & V-j).

Carinulae on both the hind-tarsal claw and its tooth show the same patterns as those of the fore- and mid-tarsal claws (Plates I to XIV-e, j).

Discussion

1 Fore-tarsal claws

The shape types of the fore-tarsal claws of female black flies varied even in the same genus. For example, *Prosimulium sarurense* is the only species which have the T-type fore-tarsal claws. The other 4 *Prosimulium* species all possess the S-type fore-tarsal claws.

In Crosskey's trinominal taxonomic system 18), all *Prosimulium* species except *P. jezonicum* are included in the subgenus *Helodon* with *Helodon multicaulis*. Therefore, *P. sarurense* may be more related to the genus *Helodon* than to the genus *Prosimulium*.

In both taxonomical arrangements, the shape type of the fore-tarsal claws may be a useless taxa to identify individuals into species, or even into genus.

The differences shown in the shape type of fore-tarsal claws may have behavioural significances. Crosskey (1973) 16) concluded that the function of the basal teeth of the T-type tarsal claws was to enable the black fly to grip the feather-barbs of the host bird and to push aside the overlapping feathers to reach its skin. The V-shaped carinulae on the inner surface of both the clwas and teeth of the T-type tarsal claws observed in this study provide good support for Crosskey's conclusion.

2 Mid-tarsal claw

The difference of claw type between fore-tarsi and mid-tarsi was observed in *Prosimulium sarurense* and *Eusimulium erimoense*, which is the first observation in Palaearctic black flies. This difference is rarely observed in black flies and has been observed only in South American species (Sherry, 1986) 69).

The reason for the difference is unknown, but there may be some evolutionary factors involved in the black flies' blood-sucking behaviour.

The carinulae of the 3 types of mid-tarsal claws observed had the same patterns as those of the homologous types of fore-tarsal claws in all species examined. Therefore, the pattern of carinulae on claws and teeth may have developed with the shape of tarsal claw.

3 Hind-tarsal claws

The difference of claw type between fore-tarsi and other tarsus was observed in *Prosimulium sarurense* and *Eusimulium erimoense*. Though the

Table 5. The shape types of tarsal claws of Japanese black flies

Species	Claw types			Species	Claw types		
	fore	mid	hind		fore	mid	hind
Twinnia cannibora	P	P	P	*Cnetha uchidai*	T	T	T
Helodon mulicaulis	T	T	T	*C. acmerium*	T	T	T
Distosimulium daisetsense	P	P	P	*C. konoi*	T	T	T
Prosimulium jezonicum	S	S	S	*Boophthora yonagoense*	S	S	S
P. karibaense	S	S	S	*Gnus daisense*	P	P	P
P. yezoense	S	S	S	*Odagmia aokii*	P	P	P
P. sarurense	T	P	P	*O. nishijimai*	P	P	P
P. apoinum	S	S	S	*Simulium japonicum*	S	S	S
Stegopterna nukabirana	S	S	S	*S. horokaense*	S	S	S
Eusimulium erimoense	T	P	P	*S. nikkoense*	S	S	S
Montisimulium sakhalinum	T	T	T	*S. rufibasis*	S	S	S
Gomphostilbia shogakii	T	T	T	*S. suzukii*	S	S	S
Cnetha subcostatum	P	P	P	*S. tobetsuense*	P	P	P
C. boldstemtum	T	T	T				

S : simple untoothed
T : with strong tooth
P : with small peg-like tooth

reason for the difference is unknown, the change of the tarsal claw type seems to be related to the change of the black fly's blood-sucking behaviour. The line of the evolution of the tarsal claw may go from the hind-tarsi to the fore-one corresponding with changes in the blood-sucking behaviour of the black fly.

The carinulae in 3 types of hind-tarsal claws showed the same patterns as those in each homologous type of fore- and hind-tarsal claws in all species examined. Therefore, the pattern of carinulae on claws and teeth may develop with the shape of tarsal claws.

Chapter 3 Blood sucking behaviour and its mechanism

Introduction

Black flies are well known for their blood-sucking behaviour, however, few detailed observations have been made on the behaviour on their host animals' skin. One of the reasons for the differing views among workers is that the black fly's body is too small to observe the sucking behaviour with the naked eye. Moreover, many of the reports on the blood-sucking mechanism of black flies are based primarily on morphological observations 40, 71).

In this chapter, the author describes the blood-sucking behaviour of *Prosimulium jezonicum*, observed on the arm of the author himself in Nopporo, Hokkaido, in 1986.

Methods

The observations on the blood-sucking behaviour of *Prosimulium jezonicum* were made in the forest in Nipporo, Hokkaido, in the spring of 1986. The decoy (the author himself) sat with his sleeves rolled up near a stream where adult black flies were encountered.

Black fly were lured and allowed to feed on the author's arm and their feeding and blood-sucking behaviour were examined.

Results

Descended individual walked on the decoy's arm for a few seconds, stopped walking and then bent the fore-legs. While bending, the flies inflicted a wound on the skin with the mandible and the galeae of maxillae. Then they stopped bending the lef and inserted the labrum into the wound in order to pump up the exuding blood for 2 or 3 minutes.

The position of the feeding was very unusual (Fig. 2) : the flies supported their body only with the mid- and hind-legs and the fore-legs were raised forward. In the latter half of feeding, some individuals spurted the blood

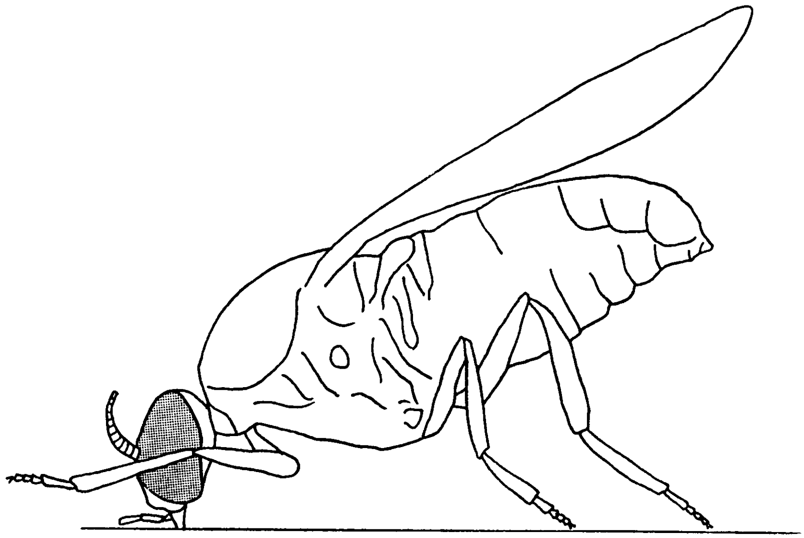

Fig. 2. Feeding position of *Prosimulium jezonicum.*

and a clear solution, which was considered to be of plant origin, from the anus, and the flies spurted the liquid more rapidly than the mosquito.

After engorging, the flies flew away from the arm of the decoy.

Discussion

The results of the observations on the blood-sucking behaviour of *P. jezonicum* described in this paper are inadequate to explain the behaviour conclusively. Furthermore, the mouth part which incises the skin at the start of feeding could not be identified clearly.

Though the exact behaviour could not be analysed, it was clarified that the black fly bends its fore-legs when it wounds the skin and supports its body only with the mid- and hind-legs when it feeds on blood. This finding may be useful to elucidate the evolutionary development of the shape type of the tarsal claws in 2 black fly species, namely *Prosimulium sarurense* and *Eusimulium erimoense,* which have T-type fore- and P-type mid- and hind-tarsal claws.

Conclusion

Twenty-seven species of 13 genera of black flies inhabiting Hokkaido were examined, their mouth parts and tarsal claws were photographed by the use of SEM and the feeding behaviour of *P. jezonicum* was observed.

Up to the present, in all the species of black flies examined, it has been reported that the left mandible is situated above the right one. But in the present investigation, the position of the mandible in 4 species, namely *D. daisetsense*, *M. sakhalinum*, *G. shogakii* and *S. nikkoense*, was right mandible over left mandible (Table 1). However, it is still unknown why this reversed position is observed only in limited populations or is found invariably observed in the above species. In species having "left over right" or "right over left" mandibles, the tricuspid teeth at the inner margin of the mandible make a line along the insertion course and those at the outer margin make a line counter to the course.

The 2 species, *H. multicaulis* and *P. sarurense*, were observed to have mandibles with blunt tricuspid teeth and galeae of maxillae with short and weak teeth (Plates I & IV). It was concluded that they have no biting habits on the basis of the shapes of the teeth of the mandibles and galeae.

The tarsal claws were classified into 3 types, namely S-, P- and T-types. S-type claw is simple and has no basal tooth at the base of the claw, and the carinulae of the claw run along the longitudinal axis of the claw. The T-type claw has a strong and well developed basal tooth at the base of the claw, and the pattern of carinulae on the inner surface of the claw and tooth is V-shaped.

There are some species in which the tarsal claw type is different from the species of the same genus or subgenus. Therefore, it is useless to type the shape of the tarsal claw to classify the black fly into species, or even genera.

On the other hand, carinulae on both the claw and tooth show the same patterns according to the shape types of the claw in every species examined. It is considered that the pattern of carinulae changed according to the development of the tarsal claws. Therefore, the shape type of the tarsal claws of black flies should be considered from the ecological view point, namely the tarsal claws develop according to the change of blood source animals. All species except *Prosimulium sarurense* and *Eusimulium erimoense* have the same type tarsal claws for their whole tarsi. The 2 species have T-type fore- and P-type mid- and hind-tarsal claws. Black flies support their body by their mid- and hind-lengs at the blood-sucking time (Fig. 1). Therefore, in the 2 species, the T-type tarsal claws were considered to be present originally and the mid- and hind-traral claws evolved into the P-type with the development of the animal range of blood source. However, whether the P-type species evolved in the same way as the above 2 species is still unknown.

The function of the legs at feeding time was clarified in this study. This finding may help to explain the evolutionary development of the tarsal claw in the 2 black fly species having T-type fore- and P-type mid- and hind-tarsal claws examined.

PART 2 Determination of the blood sources of black flies identified by means of the immunological techniques

Introduction

In spite of the many adverse effects caused by black flies in human beings and domestic animals, the knowledge of their blood source animals is still incomplete 65).

There are some reports of biting behaviour in Japan, but most of them are based on the data from human attractant or various animal-baited traps 51, 52, 57). These methods are very useful to know the bionomics of biting behaviours but they are unsuitable to determine the blood source of blood-sucking insects. Blood-sucking insects are usually attracted to carbon dioxide whatever the source is their true blood source animal or not. Therefore, all such species are not blood-suckers of the source of the carbon dioxide.

The use of immunological techniques to identify blood-meals is a valuable aid to determine the blood source animal.

In this section, the author describes the results of identification of the blood sources of 12 black fly species belonging to 8 genera collected at 12 localities in Hokkaido by the use of enzyme-linked immunosorbent assay (ELISA).

Materials and methods

1 Preparation of the blood-meal specimens

Black flies were collected by means of Co_2-lured mosquito-net trap (Fig. 3), animal trap (Fig. 4), human attractant (Fig. 5) and sweeping methods at some localities in Hokkaido (Fig. 6). The black fly specimens collected were put in an ice box and transported to the laboratory for the identification of blood sucked vertebrate species. The identified specimens were pressed individually onto a filter paper (Toyo No. 1). The smeared filter papers were dried rapidly with diphosphorus pentaoxide in a desiccator, then the smeared parts were cut off with scissors and stored individually in microvials until the assay procedure.

2 Preparation of antisera

The antisera used in this study were anti-wild brown bear (*Ursus arctos yesoensis*) whole serum rabbit serum, anti-sika deer (*Cervus nippon yesoensis*) whole serum rabbit serum, anti-Bedford's red-backed vole (*Clethrionomys rufocanus bedfordiae*) IgG rabbit serum, anti-bovine (*Bos taurus*) whole serum rabbit serum, anti-horse (*Equus caballus*) whole serum rabbit serum,

Fig. 3. CO_2-lured mosquito-net trap.

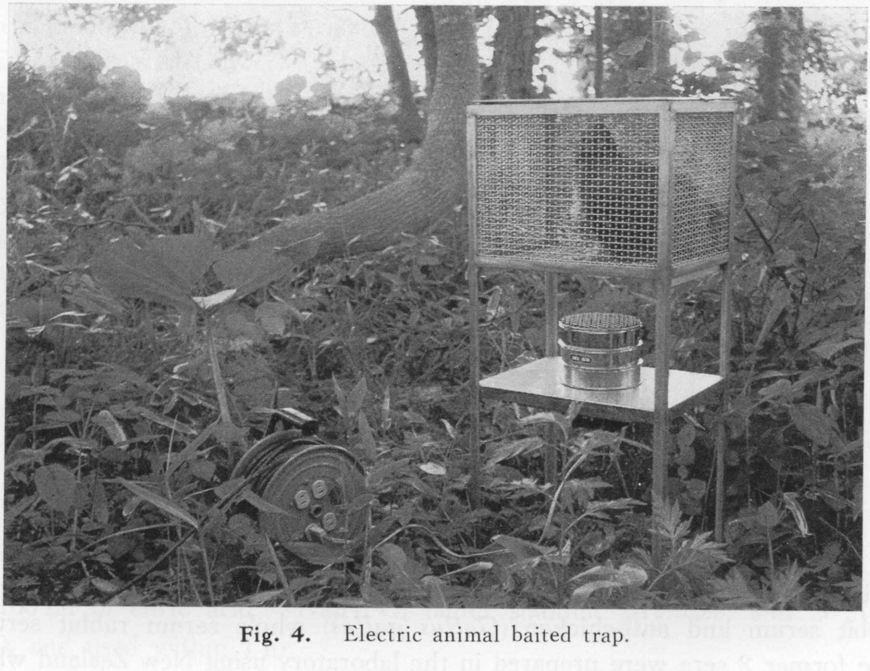

Fig. 4. Electric animal baited trap.

Fig. 5. Human attractant method.

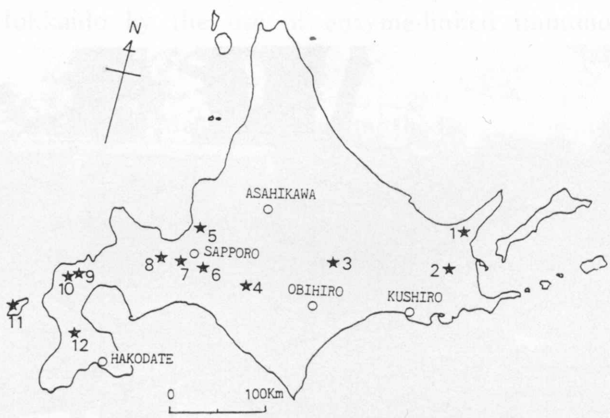

Fig. 6. Map of Hokkaido, Japan ★ show collecting localities.

1: Mt. Onnebetsu-dake	2: Shibecha	3: Nukabira
4: Hobetsu	5: Tohbetsu	6: Nopporo
7: Hitsujigaoka	8: Johzankei	9: Shimamaki
10: Mt. Oohirayama	11: Okushiri Isl.	12: Mt. Otobedake

anti-sheep (*Ovis aries*) whole serum rabbit serum, anti-human whole serum rabbit serum and anti-chicken (*Gallus gallus*) whole serum rabbit serum. The former 2 sera were prepared in the laboratory using New Zealand white rabbits: 0.5 ml of animal serum and 0.5 ml of Freund's complete adjuvant were emulsified and injected into the foot pads, followed by subcutaneous injections at two-week intervals until the rabbits were sensitized enough for assay. Two weeks after the last injection, blood was taken from the carotids. The anti-Bedford's red-backed vole serum prepared in rabbits was obtained from Dr. K. Takahashi of the Hokkaido Institute of Public Health. The other sera were commercial sera prepared in rabbits (Miles-Yeda Ltd.).

The prepared antisera were checked for their titers and cross-reactivities against heterologous species by the micro-Ouchterlony method. A 64-fold diluted antiserum formed the precipitin line against homologous antigen diluted 1 : 160 or more. No cross-reactions occurred among all of the antisera and antigens prepared (Fig. 7). These antisera were stored at $-80°C$. The anti-chicken serum prepared in rabbits was confirmed to react similarly to the serum proteins of a dewi range of bird species (Tempelis and Reeves, 1962), so the author regarded it as anti-bird serum.

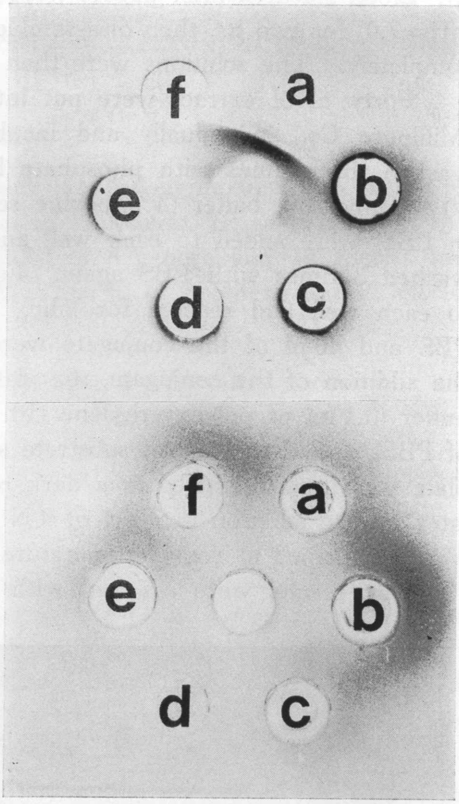

Fig. 7. The micro-Ouchterlony test on anti-serum (Upper) and absorbed anti-serum (Lower) of anti-sika deer serum.

Center well contained 6 μl of anti-sika deer serum Peripheral wells: a: sika deer serum, b: wild brown bear serum, c: Bedford's red backed vole serum, d: human serum, e: bovine serum, f: horse serum

3 Conjugate

Commercial horse radish peroxidase conjugated to the IgG fraction of goat anti-rabbit serum (Miles-Yeda Ltd.) was used.

4 Substrate

The substrate was prepared by dissolving 40 mg of *o*-phenylene diamine in 100 ml of citric acid $-Na_2HPO_4$ buffer solution (pH=5.0) and 20 μl of H_2O_2 and used within 1 hr.

5 ELISA procedure

Blood smeared filter papers were dipped in 400 μl of physiological saline (pH=7.0) for two hr, then disassembled with an insect pin to extract smears completely. The solutions were then centrifuged at 5,000 rpm for 5 min.

Forty μl of extract were put into each well of a Millititer HA plate (Millipore Co.) individually and incubated for 2 hr. The plate was then washed three times with phosphate buffered saline (PBS), pH=7.2. Then 80 μl of coating buffer (4% bovine serum albumin or 1% gelatin dissolved in PBS) were added to each well and incubated for 1 hr. The plate was washed 3 times with PBS again. Forty μl of antiserum were then added to each well and reacted for 2 hr. The plate was washed 3 times with PBS and 40 μl of the conjugate were added to each well. Two hr after the addition of the conjugate, the plate was rewashed 6 times with washing buffer (0.1 ml of polyoxyethylene (10) octylphenyl ether dissolved in 100 ml of PBS), and then 80 μl of substrate solution were added to each well. The plate was then incubated in a dark box. The reaction was stopped 45 min later by the addition of 40 μl of 4 N sulfuric acid. All procedures of assay were performed at room temperature.

The results were assessed with the eyes and the yellow colour pro-

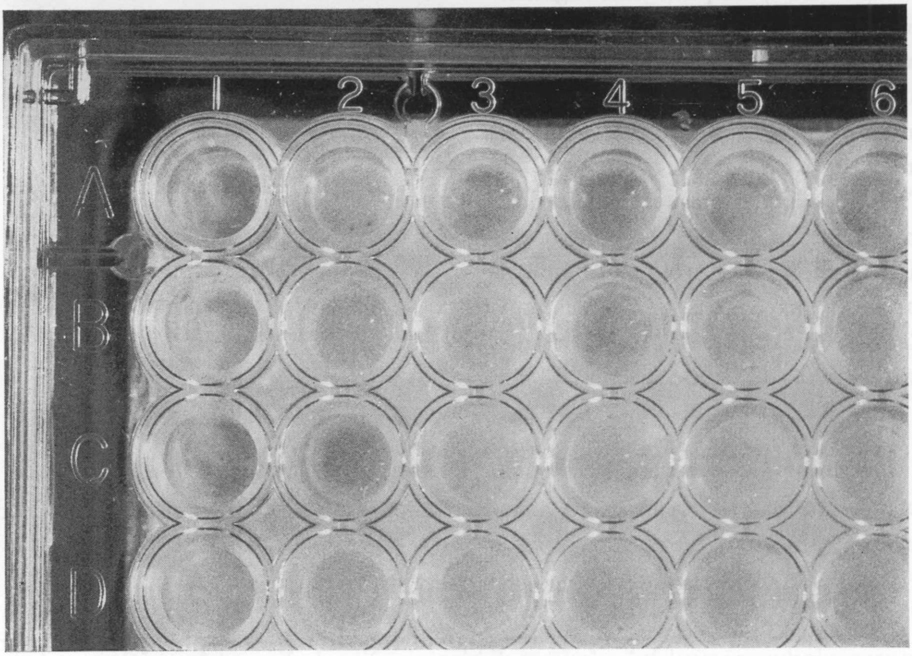

Fig. 8. ELISA reactions.

In the plate, wild brown bear serum (A-1) and the blood-meals of *Stegopterna nukabirana* were tested against anti-wild brown bear serum A-1 (Control), A-3, A-4, B-4, C-1 and C-2 show positive reactions duced in the wells of test extracts was compared with the colour of the blank wells. The homologous sera were put in several wells and tested as positive controls (Fig. 8).

Results

A total of 20,556 individuals of black flies belonging to 9 genera and 15 species were collected from 12 localities in Hokkaido, Japan (Fig. 6) in this study. Of these specimens, 1,786 (8.69%) of 8 genera and 14 species had blood-meal sources from 403 individuals (22.56%) of 8 genera and 12 species were determined in this study.

1 On the blood-meals of black flies with S-type tarsal claws

1) Genus *Prosimulium*

Three *Prosimulium* species, *P. jezonicum*, *P. karibaense* and *P. yezoense*, were collected and their blood source animals were determined in this study.

The number of *P. jezonicum* collected was 18,649 from 5 localities in Hokkaido, and 813 (4.36%) had blood-meals (Table 6). In 108 individuals (13.28%) the blood-meal sources were derermined as cattle, horse, human, Bedford's red-backed vole and sika deer (Table 7).

The number of *P. karibaense* collected was 9 from 2 localities in Hokkaido, and 1 (11.11%) had a blood-meal (Table 8), and the source was determined as human blood (Table 9).

The number of *P. yezoense* collected was 206 from 7 localities in Hokkaido, and 120 (58.25%) had blood-meals (Table 10). The blood-meals were determined as those coming from cattle, horse, sheep, human and Bedford's red-backed vole (Table 11).

Table 6. Number of *P. jezonicum* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	12	8	66.67
Nukabira	1984. 5. 18.	248	245	66.67
Hobetsu	1984. 5. 20.	15	7	98.79
Tohbetsu	1985. 5. 23.	11223	303	2.70
Nopporo	1985. 6. 4.	15	2	13.33
Tohbetsu	1985. 6. 6.	5934	228	3.84
	1985. 6. 20.	1187	15	1.26
	1985. 7. 4.	11	5	45.45
Total		18649	813	4.36

Table 7. Number and percent of *P. jezonicum* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	8	0(0.0)	0(0.0)	0(0.0)	2(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nukabira	1984. 5. 18.	245	0(0.0)	0(0.0)	0(0.0)	4(1.6)	2(0.8)	10(4.1)	0(0.0)	0(0.0)
Hobetsu	1984. 5. 20.	7	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1985. 5. 23.	303	19(6.3)	21(6.9)	0(0.0)	17(5.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nopporo	1985. 6. 4.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1985. 6. 6.	228	19(8.3)	6(2.6)	0(0.0)	3(1.3)	4(1.8)	0(0.0)	0(0.0)	0(0.0)
	1985. 6. 20.	15	0(0.0)	1(6.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 7. 4.	5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		813	38(4.7)	28(3.4)	0(0.0)	26(3.2)	6(0.7)	10(1.2)	0(0.0)	0(0.0)

Table 8. Number of *P. karibaense* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Otobedake	1985. 7. 12.	1	1	100.00
Mt. Oohirayama	1986. 8. 1.	8	0	0.00
Total		9	0	11.11

Table 9. Number and ratio (%) of *P. karibaense* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Otobedake	1985. 7. 12.	1	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 8. 3.	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		1	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 10. Number of *P. yezoense* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	2	1	50.00
Tohbetsu	1985. 6. 6.	2	1	50.00
	1985. 6. 20.	9	4	44.44
	1985. 7. 4.	59	17	28.81
Johzankei	1985. 7. 17.	7	3	42.86
Hitsujigaoka	1986. 6. 8.	1	1	100.00
	1986. 6. 24.	10	10	100.00
Okushiri Isl.	1986. 6. 27.	7	5	71.43
	1986. 6. 28.	46	40	86.96
Hitsujigaoka	1986. 7. 8.	8	8	100.00
Tohbetsu	1986. 7. 17.	11	2	18.18
Okushiri Isl.	1986. 7. 18.	30	24	80.00
	1986. 7. 19.	2	0	0.00
Shibecha	1986. 7. 26.	1	0	0.00
Mt. Oohirayama	1986. 8. 1.	4	2	50.00
	1986. 8. 3.	7	2	28.57
Total		206	120	58.25

Table 11. Number and percent of *P. yezoense* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1985. 6. 6.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 6. 20.	4	2(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 7. 4.	17	1(5.9)	1(5.9)	0(0.0)	0(0.0)	2(11.8)	0(0.0)	0(0.0)	0(0.0)
Johzankei	1985. 7. 17.	1	1(33.3)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Hitsujigaoka	1986. 6. 8.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 6. 24.	10	1(10.0)	1(10.0)	1(10.0)	3(30.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 6. 27.	5	1(20.0)	3(60.0)	0(0.0)	0(0.0)	0 (0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 6. 28.	40	3(7.5)	8(20.0)	0(0.0)	10(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Hitsujigaoka	1986. 7. 8.	8	1(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1986. 7. 17.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 7. 18.	24	0(0.0)	2(8.3)	0(0.0)	(0 0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Mt. Oohirayama	1986. 8. 1.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 8. 3.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		120	10(8.3)	15(12.5)	1(0.8)	14(15.8)	2(3.9)	0(0.0)	0(0.0)	0(0.0)

2) *Stegopterna nukabirana*

This species was the most dominant species in the survey of the Onnebetsu-dake wilderness area in Shiretoko National Park, Hokkaido, and 302 individuals were collected. Of these specimens, 106 (35.10%) had blood-meals (Table 12). The blood-meals were determined as those of human and wild brown bear (*Ursus arctos yesoensis*) (Table 13).

Table 12. Number of *S. nukabirana* collected in this investigation

locality	date	collected No.	engored No.	(%)
Mt. Onnebetsu	1984. 8. 1.	302	106	35.10
Total		302	106	35.10

Table 13. Number and ratio (%) of *S. nukrbirana* racted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt Onnebetsu	1981. 8. 1.	106	0(0.0)	0(0.0)	0(0.0)	15(14.2)	0(0.0)	0(0.0)	19(17.9)	0(0.0)
Total		106	0(0.0)	0(0.0)	0(0.0)	15(14.2)	0(0.0)	0(0.0)	19(17.9)	0(0.0)

3) Genus *Simulium*

Four *Simulium* species, namely *S. japonicum*, *S. nikkoense*, *S. rufibasis* and *S. suzukii*, were collected (Tables 14, 16, 18, 20), and in 2 species, namely *S. japonicum* and *S. rufibasis*, the blood source animals were determined.

The number of *S. japonicum* collected was 740 from 8 localities in Hokkaido, and 336 (45.41%) had blood-melas (Table 14). The blood-meal sources of 68 (20.24%) were determined as originating from cattle, horse, sheep, human, Bedord's red-backed vole and sika deer (Table 15).

The number of *S. rufibasis* collected was 161 from 4 localities in Hokkaido, and 131 (81.37%) had blood-meals (Table 18). Of these, 47 (35.88%) were determined as originating from cattle, horse, human and Bedford's red-backed vole (Table 19).

None of the blood-meals of *S. nikkoense* and *S. suzukii* reacted against the anti-sera examined (Tables 17 and 21).

Table 14. Number of *S. japonicum* cellected in this investigation

locality	date	collected No.	engorgen No.	(%)
Mt. Onnebetsu	1984. 8. 1.	161	71	44.10
Tohbetsu	1985. 5. 23.	1	1	0.00
	1985. 6. 6.	25	1	4.00
	1985. 6. 20.	115	5	4.35
	1985. 7. 4.	18	4	22.22
Johzankei	1985. 7. 17.	53	7	13.21
Tohbetsu	1985. 7. 19.	1	0	0.00
Mt. Otobedake	1985. 7. 21.	63	2	3.17
	1985. 7. 29.	3	1	33.33
Tohbetsu	1985. 9. 12.	2	0	0.00
Hitsujigaoka	1986. 6. 8.	152	129	84.87
	1986. 6. 24.	104	101	97.12
Okushiri Isl.	1986. 6. 28.	14	13	92.86
Hitsujigaoka	1986. 7. 8.	3	0	0.00
Tohbetsu	1986. 7. 17.	19	1	5.26
Mt. Oohirayama	1986. 8. 1.	4	0	0.00
Shimamaki	1986. 8. 4.	3	1	33.33
Total		740	336	45.41

Table 15. Number and ratio (%) of *S. japonicum* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	71	0(0.0)	0(0.0)	0(0.0)	1(1.4)	0(0.0)	2(2.8)	0(0.0)	0(0.0)
Tohbetsu	1985. 6. 6.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 6. 20.	5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 7. 4.	4	1(25.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Johzankei	1985. 7. 17.	7	0(0.0)	0 (0.0)	0(0.0)	1(14.3)	1(14.3)	0(0.0)	0(0.0)	0(0.0)
Mt. Otobedake	1985. 7. 21.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 7. 29.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)
Hitsujigaoka	1986. 6. 8.	129	12(9.3)	6(4.7)	14(10.9)	8(6.2)	10(7.8)	0(0.0)	0(0.0)	0(0.0)
	1986. 6. 24.	101	2(2.0)	2(2.0)	3(3.0)	3(3.0)	6(6.0)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 6. 28.	13	1(7.7)	0(0.0)	0(0.0)	2(15.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbtsu	1986. 7. 17.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Shimamaki	1986. 8. 4.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		336	16(4.8)	9(2.7)	17(5.1)	15(4.5)	19(5.7)	2(0.6)	0(0.0)	0(0.0)

Table 16. Number of *S. nikeoense* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Shimamaki	1986. 8. 4.	1	1	100.00
Total		1	1	100.00

Table 17. Number and ratio (%) of *S. nikkoense* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Shimamaki	1986. 8. 4.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 18. Number of *S. rufibasis* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	21	18	85.71
Tohbetsu	1986. 6. 6.	5	1	20.00
	1986. 6. 17.	9	3	33.33
Okushiri Isl.	1986. 6. 27.	3	2	66.67
	1986. 6. 28.	40	38	95.00
Shibecha	1986. 7. 12.	2	0	0.00
Okushiri Isl.	1986. 7. 18.	2	1	50.00
	1986. 7. 19.	12	10	83.33
Shibecha	1986. 7. 26.	12	8	66.67
Okushiri Isl.	1986. 8. 2.	55	50	90.91
Total		161	131	81.37

Table 19. Number and ratio (%) of *S. rufibasis* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	18	0(0.0)	0(0.0)	0(0.0)	2(11.1)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1986. 6. 6.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 6. 17.	3	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 6. 27.	2	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0 0)	0(0.0)	0(0.0)
	1986. 6. 28.	38	7(18.4)	2(5.3)	0(0.0)	23(60.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 7. 18.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 7. 19.	10	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Shibecha	1986. 7. 26.	8	0(0.0)	2(25.0)	0(0.0)	1(12.5)	1(12.5)	0(0.0)	0(0.0)	0(0.0)
Okushiri Isl.	1986. 8. 2.	50	1(2.0)	0(0.0)	0(0.0)	4(8.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		151	8(6.1)	5(3.8)	0(0.0)	31(23.7)	3(2.3)	0(0.0)	0(0.0)	0(0.0)

Table 20. Number of *S. suzukii* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Okushiri Isl.	1986. 6. 27.	1	1	100.00
	1986. 7. 18.	1	1	100.00
Total		2	2	100.00

Table 21. Number and ratio (%) of *S. suzukii* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Okushiri Isl.	1986. 6. 27.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 7. 18.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

2 On the blood-meals of black flies with T-type trsal claws

1) *Gomphostilbia shogakii*

Only 6 *Gomphostilbia shogakii* were collected from Okushiri Island, Hokkaido, and 5 (83.33%) had blood-meals (Table 22). In 1 of the 5 the blood-meal was determined as bird blood (Table 23).

Table 22. Number of *G. shogakii* collected in this investigation

locality	date	collected No.	engored No.	(%)
Okushiri Isl.	1986. 6. 27.	2	2	100.00
	1986. 6. 28.	3	2	66.67
	1986. 7. 18.	1	1	100.00
Total		6	5	83.33

Table 23. Number and ratio (%) of *G. shogakii* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Okushiri Isl.	1986. 6. 28.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)
	1986. 7. 18.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1986. 7. 19.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

2) *Cnetha uchidai*

Thiry *Cnetha uchidai* were collected in the Onnebetsu-dake wilderness area of Shiretoko National Park, Hokkaido, and 16 (53.33%) had blood-meals (Table 24). Of these, 2 (12.50%) showed a blood-meal of sika deer blood (Table 25).

Table 24. Number of *C. uchidai* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	30	16	53.33
Total		30	16	53.33

Table 25. Number and ratio (%) of *C. uchidai* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	16	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(12.5)	0(0.0)	0(0.0)
Total		16	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(12.5)	0(0.0)	0(0.0)

3 On the blood-meals of black flies with P-type tarsal claws

1) *Twinnia cannibora*

The number of *Twinnia cannibora* collected was 324 from 7 localities in Hokkaido and 169 (52.16%) had blood-meals (Table 26). These blood-meals were determined as originating from cattle, horse, sheep, human, Bedford's red-backed vole, sika deer and bird (Table 27).

Table 26. Number of *T. cannibora* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	87	29	33.33
Tohbetsu	1985. 6. 6.	14	4	28.57
Nopporo	1985. 6. 13.	7	0	0.00
Tohbetsu	1985. 6. 20.	2	0	0.00
	1985. 7. 4.	1	1	100.00
Mt. Otobedake	1985. 7. 12.	1	0	0.00
Nopporo	1986. 5. 30.	12	2	16.67
Hitsujigaoka	1986. 7. 8.	198	132	66.67
Mt. Oohirayama	1986. 8. 1.	2	1	50.00
Total		324	169	52.16

Table 27. Number and ratio (%) of *T. cannibora* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1.	29	0(0.0)	0(0.0)	0(0.0)	4(13.8)	0(0.0)	3(10.3)	0(0.0)	0(0.0)
Tohbetsu	1985. 6. 6.	4	0(0.0)	2(50.0)	0(0.0)	0(0.0)	0(0.0)	0 (0.0)	0(0.0)	0(0.0)
Nopporo	1985. 6. 13.	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Tohbetsu	1985. 6. 20.	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	1985. 7. 4.	1	0(0.0)	0(0.0)	0(0.0)	1(100.0)	2(11.8)	0(0.0)	0(0.0)	0(0.0)
Mt. Otobedake	1985. 7. 12.	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nopporo	1986. 5. 30.	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Hitsujigaoka	1986. 7. 8.	132	5(3.8)	7(5.3)	11(8.3)	17(12.9)	12(9.1)	0(0.0)	0(0.0)	0(0.0)
Mt. Oohirayama	1986. 8. 1.	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		169	5(4.2)	9(7.5)	11(9.2)	22(18.3)	14(11.7)	3(2.5)	0(0.0)	(8.67)

2) *Distosimulium daisetsense*

The number of *Distosimulium daisetsense* collected was 91 from the Onnebetsu-dake wilderness area of Shiretoko National Park, Hokkaido, and 55 (60.44%) were observed to have blood-meals (Table 28). The blood-meal sources of 17 (30.91%) were determined as human, sika deer and bird (Table 29).

Table 28. Number of *D. daisetsense* collected in this in vestigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	91	55	60.44
Total		91	55	60.44

Table 29. Number and ratio (%) of *D. daisetsense* reacted their blood-meals with anti-human and animal sera

locality	date	examined number	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt. Onnebetsu	1984. 8. 1	55	0(0.0)	0(0.0)	0(0.0)	6(10.9)	0(0.0)	8(14.5)	0(0.0)	(35.5)
Total		55	0(0.0)	0(0.0)	(00.0)	0(0.0)	0(0.0)	8(14.5)	0(0.0)	3(5.5)

3) *Cnetha subcostatum*

Cnetha subcostatum is the only species of the genus *Cnetha* in Hokkaido which has P-type tarsal claws, and only 2 individuals were collected in the Onnebetsu-dake wilderness area of Shiretoko National Park, Hokkaido, and all of them had blood-meals (Table 30). Only one blood-meal (50.00%) was determined as human blood (Table 31).

Table 30. Number of *C. subcostatum* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Mt. Onnebetsu	1984. 8. 1.	2	2	100.00
Total		2	2	100.00

Table 31. Number and ratio (%) of *C. subcostatum* reacted their blood-meals with anti-human and animal sera

locality	date	exami-ned num-ber	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Mt Onnebetsu	1984. 8. 1.	2	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total		2	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

4) *Gnus daisense*

The number of *Gnus daisense* collected was 31 at Hitsujigaoka, Sapporo, Hokkaido, and 28 (90.32%) had blood-meals (Table 32). Of these blood-meals, 9 (32.14%) were determined as coming from cattle, horse, sheep, human, Bedford's red-backed vole and bird blood (Table 33).

Table 32. Number of *G. daisense* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Hitsujigaoka	1986. 7. 8.	31	28	90.32
Total		31	28	90.32

Table 33. Number and ratio (%) of *G. daisense* reacted their blood-meals with anti-human and animal sera

locality	date	exami-ned num-ber	number and percent of black flies reacted against							
			anti-bovine	anti-horse	anti-sheep	anti-human	anti-vole	anti-sika deer	anti-brown bear	anti-birds
Hitsujigaoka	1986. 7. 8.	28	10(35.7)	3(10.7)	1(3.6)	2(7.1)	2(7.1)	0(0.0)	0(0.0)	1(3.6)
Total		28	10(35.7)	3(10.7)	1(3.6)	2(7.1)	2(7.1)	0(0.0)	0(0.0)	1(3.6)

5) *Odagmia aokii*

Only 2 individuals of *Odagmia aokii* were collected at Hitsujigaoka, Sapporo, Hokkaido, and none of them had blood-meals (Table 34).

Table 34. Number of *O. aokii* collected in this investigation

locality	date	collected No.	engorged No.	(%)
Hitsujigaoka	1986. 7. 21.	2	0	0.00
Total		2	0	0.00

Discussion

1 On the sources of blood-meals of black flires with S-type tarsal claws

1) Genus *Prosimulium*

Bedford's red-backed vole and sika deer were determined as the blood-source animals of *P. jezonicum* for the first time. In other species human, cattle and horse were reported to be the blood sources 51), but the reports were based on the results of human and animal attractant methods. Therefore, some doubts have remained concerning these 3 mammalian species being the blood source animals of *P. jezonicum*, as described in the introduction of this part. Moreover, this species has been confounded with *P. hirtipes* for a long time 56, 83), so the species which Ogata (1955) 51) reported cannot be distinguished clearly as *P. jezonicum* 53). In consequence, the 5 mammalian species noted above were determined as the blood source animals of *P. jezonicum*. As *P. jezonicum* was determined to have a wide range of blood sources in mammalian species, this species is considered to be an important medico-veterinary harmful species not only from the standpoint of its blood-sucking behaiour but also for its role as a possible vector and mechanical carrier of various pathogenic micro-organisms.

Ono (1982) 57) reported that *P. karibaense* attacks humans, which was supported by the findings of this study. Sheep and Bedford's red-backed vole were determined as the blood source animals of *P. yezoense* for the first time. Ogata (1955) 51) and Ono (1980) 56) reported that human, cattle and horse are the blood source animals for this species; the results obtained in this study confirmed their report immunologically. This species has a wide range of blood sources in mammal species; therefore, there is a very strong possibility that this species is a potentially harmful species to humans and animals, like *P. jezonicum*.

2) *Stegopterna nukabirana*

The blood-meals of this species were determined to be human and wild brown bear blood, and this species is considered to be only known blood-sucker of the wild brown bear by the use of the immunological technique in Japan 65). As the known habitat of this species is mountainous area 57), there is little possibility that it is a harmful species.

3) Genus *Simulium*

Of the blood source animals of *S. japonicum* determined in this study, Bedford's red-backed vole and sheep are newly known and the other 3 mammal species were confirmed by the immunological method for the first time. Takaoka (1977) 76) reported that this species ataacked some domestic animals and chickens in the Nansei Islands, but none of the blood-meals reacted against the anti-bird serum used in this study. This species is common in rural areas, and as it demonstrates aggressive blood-sucking behaviour to a wide range of vertebrates, it may be a very harmful species.

S. rufibasis has been known to attack cattle, horse, human and Bedford's red-backed vole 52, 65) and has a wide distribution in Japan; therefore, there is a strong possibility that this species, like *S. japonicum*, is hazardous to humans and domestic animals.

The black flies with S-type tarsal claws have been considered to be mammalophilic blood-suckers 16, 65) and the results of this study are good supports of these conclusions.

2 On the sources of blood-meals of black flies with T-type tarsal claws

1) *Gomphostilbia shogakii*

Only one blood-meal of this species was determined to be bird blood, and this is the first indication that the blood-meal of the black fly with T-type tarsal claws is of bird blood origin in Japan. As this species was determined to bite birds, there is a very strong possibility that it is a vector of various pathogenic micro-organisms such as leucocytozoonosis of birds, which was observed in 17 species of Japanese wild birds 34).

2) *Cnetha uchidai*

Crosskey (1973) 16) and Sasaki *et al.* (1986) 65) concluded that black flies with T-type tarsal claws are ornithophilic blood-suckers. However, the blood-meals of 2 individuals collected in the Onnebetsu-dake wilderness area of Shiretoko National Park, Hokkaido, reacted against anti-sika deer serum. The anti-sera used in this study showed no cross-reaction between sika deer and bird. Therefore, the 2 individuals of this species were considered to bite sika deer, but the feedings were concluded to be unusual phenomena.

In this study, only 2 species of black flies with T-type tarsal claws were examined and 1 individual of *G. shogakii* was determined to bite birds. The author concluded that the black flies with T-type tarsal claws are ornithophilic blood-suckers because of the shape and surface structure, especially carinulae, of the tarsal claws and the results of the blood-meal identifications of black flies with other types of tarsal claws.

3 On the sources of blood-meals of black flies with P-type tarsal claws

1) *Twinnia cannibora*

Six mammalian species and birds are determined to be the blood source for this species. Among them, human and sika deer were cited as blood sources in a former study by the author and his co-workers 65), and the other animal species are newly determined as blood sources. As this species inhabits mountains and rural areas, it was speculated to be a possible vector or carrier of various pathogenic micro-organisms. In addition, the host range of this species is not only in mammals but also in birds, and thus it may be more harmful than other species to humans and animals.

2) *Distosimulium daisetsense*

This species was collected only from the Onnebetsu-dake wilderness area in Shiretoko National Park, Hokkaido, and was derermined to show a wide host range in both mammals and birds. The known habitats of this species are mountainous areas, so it has little medico-veterinary importance.

3) *Cnetha subcostatum*

This species is reportedly the most common and widely distributed *Cnetha* species in Hokkaido 57). Only 2 individuals were collected and the blood-meal of only 1 individual was determined. More specimens of this species should be examined to clarify the blood source as either mammals or birds.

4) *Gnus daisense*

This species is known to attack goats but not humans, cattle or horses 55). However, the author determined 5 mammal species, including human, cattle, horse and birds as the optimal hosts from the blood-meals of this species. As this species has a wide distribution in Japan and a wide host range of both mammals and birds, it has more medico-veterinary significance than the other species. The black flies with P-type tarsal claws were concluded to have both mammalophilic and ornithophilic bloodlsucking behaviour 16, 65), and the results obtained immunologically concerning the host ranges for this species agree with this conclusion.

GENERAL DISCUSSION

There are many reports concerning the effects of black flies e. g. 19, 25, 26, 32, 48, 72, 88). Almost all females of the black fly species bite warm-blooded animals, and many of them are well-known as vectors and mechanical transmitters of numerous parasites and pathogenic micro-organisms, causing diseases such as human onchocerciasis.

The main effect of the black flies is not the blood loss of the host animals but the transmission of various parasites and pathogens from host to host via the blood-meal. However, the specification of vector species is still incomplete concerning many diseases which are estimated to be transmitted by black flies.

In the present study, the detailed structures of the mouth parts of black flies, especially those of the mandibles and the galeae of the maxillae observed by the use of a scanning electron microscope (SEM), were described firstly. Up to the present, the mandibles have been believed to lie "left over right" 71); however, the "right over left" species, namely *D. daisetsense*, *M. sakhalinum*, *G. shogakii* and *S. nikkoense*, were observed here. The reasons and functional merits of this arrangement are still unknown. However, the tricuspid teeth at the inner margin making a line along the insertion course and those at the outer margin making a counter line against it were observed in both "left over right" and "right over left" species. In 2 species, *H. multicaulis* and *P. sarurense*, blunt tricuspid teeth on the mandibles and short and weak teeth on the galeae of the maxillae were observed. The biting habits of the 2 species are unknown 54, 55) up to the present, but the author speculates the 2 species to be non-biting species from his observations.

The shapes and the structure of the tarsal claws of black flies were also examined by the use of SEM. Up to the present, almost all studies pertaining to the claws have been based on optical microscopic examinations and only the outlines of the claws have been described. In the present study, the author classified the claws into 3 types, namely S-, P- and T-types, according to their shapes and the presence of the basal tooth and its shape and described the carinulae on both the claw and tooth. The S-type claw is simple, having no basal tooth, and the carinulae on it run along the longitudinal axis of the claw. The P-type tarsal claw is simple but has a small peg-like basal tooth, and the carinulae on both the claw and tooth run along the longitudinal axis of the claw. On the other hand, the T-type tarsal claw has a strong and well-developed basal tooth, and the pattern of carinulae on the inner surface of the claw and tooth is V-letter shaped. Some researchers mentioned that the shape of the tarsal claw is one of the useful characters to classify the genera and even the species 52), as shown in the family Cecidomyiidae (Diptera) 58). However, there are some species which have different type tarsal claws even in the same genera or subgenera. Therefore, the author concluded that the morphology of tarsal claws is not always a useful character of the genera or subgenera for classification. It was observed that *P. sarurense* and *E. erimoense* possess T-type fore- and P-type mid- and hind-tarsal claws, a fact which may help to explain the evolution of the shapes of claw in black flies. The carinulae on both the claw and tooth show the same patterns as the shape type of claws in all the species examined. Therefore, the author concluded that in black flies the pattern of carinulae changes according to the development of the claw and the claw develops according to the change of blood source animals.

The results of the observation on the blood-sucking behaviour led to the following conclusions ; namely, that the 2 species with T-type fore- and P-type mid- and hind-tarsal claws originally had T-type claws and their mid- and hind-claws evolved into the P-type with the development of their blood source ranges.

Since the suggestion of Uhlenhuth *et al.*, (1908) 85), many researchers have searched for reliable methods to determine the blood-meal source of

Table 35. Number of collected and ratio (%) of engorged black flies examined in this investigation

Species	Number of collected	Number of engorged	(%)
Twinnia cannibora	324	169	52.16
Distosimulium daisetsense	91	55	60.44
Prosimulium jezonicum	18649	813	4.36
P. karibaense	9	1	11.11
P. yezoense	206	120	58.25
Stegopterna nukabirana	302	106	35.10
Gomphostilbia shogakii	6	5	83.33
Cnetha subcostatum	2	2	100.00
C. uchidai	30	16	53.33
Gnus daisense	31	28	90.32
Odagmia aokii	2	0	0.00
Simulium japonicum	740	336	45.41
S. nikkoense	1	1	100.00
S. rufibasis	161	131	81.37
S. suzukii	2	2	100.00
Total	20556	1785	8.68

Table 36. Number of black flies reacted their blood-meals with anti-human and animal sera

Species	Number of		reacted with anti-sera against:								total of positive reactors
	collected	engorged	bovine	horse	sheep	human	vole	deer	bear	birds	
Twinnia cannibora	324	169	5	9	11	22	11	3	0	8	70
Distosimulium daisetsense	91	55	0	0	0	6	0	8	0	3	17
Prosimulium jezonicum	18649	813	38	28	0	26	6	10	0	0	108
P. karibaense	9	1	0	0	0	1	0	0	0	0	1
P. yezoense	206	120	10	15	1	19	2	0	0	0	47
Stegopterna nukabirana	302	106	0	0	0	15	0	0	19	0	34
Gomphostilbia shogakii	6	5	0	0	0	0	0	0	0	1	1
Cnetha subcostatum	2	2	0	0	0	1	0	0	0	0	1
C. uchidai	30	16	0	0	0	0	0	2	0	1	3
Gnus daisense	31	28	10	3	1	2	2	0	0	0	18
Odagmia aokii	2	0	0	0	0	0	0	0	0	0	0
Simulium japonicum	740	336	13	6	14	11	10	2	0	0	56
S. nikkoense	1	1	0	0	0	0	0	0	0	0	0
S. rufibasis	161	131	8	5	0	31	3	0	0	0	47
S. suzukii	2	2	0	0	0	0	0	0	0	0	0
Total	20556	1785	84	66	27	134	35	25	19	13	403

blood-sucking insects. Of these, enzyme-linked immunosorbent assay (ELISA) is known to be the most sensitive method. In the present study, the author examined the blood-meals of 1,785 out of 20,556 black flies, which comprised 15 species and 9 genera by the use of ELISA. The specimens were collected at 12 localities in Hokkaido, Japan (Table 35). Among these, the blood sources were determined in 403 individuals of 12 species and 9 genera (Table 36). Despite some exceptions, the results of the blood-meal identification confirmed the estimations by Crosskey (1973) 16) and Sasaki *et al.* (1976) 65).

The species with the S-type tarsal claw, *P. jezonicum, P. karibaebsem*

Table 37. Blood sources of Japanese black flies determined by the use of ELISA and other methods

Species	blood source*	reference
Twinnia cannibora	human, cattle, horse, sheep, Bedford's red-backed vole, birds	
Distosimulium daisetsense	human, sika deer, birds	
Prosimulium jezonicum	human, horse, cattle, sika deer Bedford's red-backed vole	
P. karibaense	human	
P. yezoense	human, horse, cattle, sheep Bedford's red-backed vole	
P. apoinum		human2)
Stegopterna nukabirana	human, wild brown bear	
Gomphostilbia shogakii	birds	
Cnetha subcostatum	human	
C. uchidai	sika deer	
Gnus daisense	cattle, horse, sheep, human, birds	goat3)
Odagmia aokii		human,1) cattle1), horsel1) chicken8), pig8)
O. nishijimai		human4)
Simulium japonicum	human, horse, cattle sika deer, sheep, Bedford's red-backed vole	chicken5)
S. horokaense		human6)
S. nikkoense		horse1), cattle1)
S. rufibasis	human, horse, cattle Bedford's red-backed vole, sheep	goat1)
S. suzukii		human7), horse7), cattle7)

*: determined by the use of ELISA method
1): Ogata *et al*, 1956 2): Ono, 1977 3): Ono, 1976 4): Ono, 1978
5): Takaoka, 1977 6): Ono, 1980 7): Bentink, 1955 8): Ogata, 1955

P. yezoense, *S. nukabirana*, *S. japonicum* and *S. rufibasis*, revealed blood-meals consisting of mammalian blood, which did not react against the anti-bird serum employed. Therefore, the author concluded these S-type species to be mammalophilic type blood-suckers. Among these species, *P. jezonicum*, *P. yezoense*, *S. japonicum* and *S. rufibasis* were considered to be potentially harmful species to humans and animals because of their aggressive blood-sucking behaviour in a wide range of mammalian species (Table 37) and their wide range of distribution areas and habitats.

Only one blood-meal of T-type tarsal claw species, namely *G. shogakii* reacted against anti-bird serum, which is the first evidence showing the T-type species to bite birds. Two blood-meals of *C. uchidai* reacted against anti-sika deer serum, which the author concluded to due to a result of an unusual biting. The author concluded the black flies with T-type tarsal claws to be ornithophilic blood-suckers because of the blood-meal determination of *G. shogakii* and other claw type species, and the shape and surface structure of the tarsal claws, especially carinulae. There is a very strong possibility that some Japanese T-type species are the vectors of various avian parasites and pathogenic micro-organisms. *Leucocytozoon* has been discovered from 17 species of wild birds in Japan 34). However, the knowledge

Table 38. The feeding types of Japanese black flies

Species	group	Species	group
Twinnia cannibora	B	*Cnetha uchidai*	O
Helodon multicaulis	N	*C. acmerium*	O
Distosimulium daisetsense	B	*C. konoi*	O
Prosimulium jezonicum	M	*Boophthora yonagoense*	M
P. karibaense	M	*Gnus daisense*	B
P. yezoense	M	*Odagmia aokii*	B
P. sarurense	N	*O. nishijimai*	B
P. apoinum	M	*Simulium japonicum*	M
Stegopterna nukabirana	M	*S. horokaense*	M
Eusimulium erimoense	B	*S. nikkoense*	M
Montisimulium sakhalinum	O	*S. rufibasis*	M
Gomphostilbia shogakii	O	*S. suzukii*	M
Cnetha subcostatum	B	*S. tobetsuense*	B
C. boldstemtum	O		

M: mammalophilic type
B: both of mammalophilic and ornithophilic type
O: ornithophilic type
N: probably non biting habits

of T-type species is still incomplete to conclude them to be hazardous to human and domestic animals.

Four P-type tarsal claw species, *T. cannibora, D. daisetsense, G. daisense* and *C. subcostatum*, were determined to be blood source animals, the their blood-meals, except for those of *C. subcostatum*, reacted against anti-bird serum together with some anti-sera of mammalian species (Table 37). The author concluded that the P-type species have both mammalophilic and ornithophilic blood-sucking behaviours according to the results of the identification of the blood-meals and the shape and surface structures of tarsal claws and basal teeth. There is a great possibility that the 2 species, *T. cannibora* and *G. daisense*, are important harmful species to humans and animals because of their wide host range in mammals and birds.

The blood sources of the black flies in Hokkaido, determined by the use of ELISA and other methods, are summarized in Table 37. The blood sources of black flies described by previous researchers were confirmed immunologically in some species, and the blood source animals of many species, including the species regarded as non biting species, were determined for the first time.

Based on the results of the present study, the feeding types of the black flies in Hokkaido are discussed (Table 38). The 2 species, *H. multicaulis* and *P. sarurense*, were considered to have no biting habits, judging from the shapes of their mouth parts. *E. erimoense*, which has T-type fore- and P-type mid- and hind-tarsal claws, is included in the group of black flies with both mammalophilic and ornithophilic type blood-sucking behaviours because of the position of feeding and the function of the legs at feeding. The classification of the other black fly species into 3 feeding type groups was coincident with the shape type of their tarsal claws : S-type species is the mammalophilic type ; T-type species is the ornithophilic type and P-type species is both the mammalophilic and ornithophilic type. The blood sources of some species remain unknown and should be examined and determined in further studies.

SUMMARY

The mouth parts, especially the mandibles and the galeae of the maxillae, of 27 species belonging to 13 genera of female black flies in Hokkaido were examined with a scanning electron microscope (SEM). In 4 species, *D. daisetsense, M. sakhalinum, G. shogakii* and *S. nikkoense*, the right mandible lies over the left one, and in the other species, the arrangement is left over right. The tricuspid teeth at the inner margin make a line along the insertion course and those at the outer margin make a line counter to the insertion course in all the species examined. The tricuspid teeth on the

mandibles and the teeth on the galeae of the maxillae of *H. multicaulis* and *P. sarurense* are blunt, short and weak.

The tarsal claws of the above-noted 27 species were examined with a SEM and classified into 3 types, namely S-, P- and T-types, according to the shape of the claw and the presence and shape of the basal tooth. The S-type tarsal claw is simple without any basal tooth and the P-type one is simple with a small peg-like basal tooth. The T-type one has a strong and well-developed basal tooth. The carinulae on both the claw and tooth of S-type and P-type tarsal claws run along the longitudinal axis of the claw, and those at the inner surfaces of the T-type claws make a V-letter shaped pattern. Eleven species belonging to the genera *Prosimulium*, *Stegopterna*, *Boophthora* and *Simulium* have the S-type tarsal claws. Nine species belonging to the genera *Helodon*, *Prosimulium*, *Eusimulium*, *Montisimulium*, *Gomphostilbia* and *Cnetha* have the T-type tarsal claws. And 7 species belonging to the genera *Twinnia*, *Distosimulium*, *Cnetha*, *Gnus*, *Odagmia* and *Simulium* have the P-type tarsal claws. The 2 species, *P. sarurense* and *E. erimoense*, have T-type fore- and P-type mid- and hind-tarsal claws, and the other species have the same type of tarsal claws on each tarsus.

The blood-sucking behaviour of *P. jezonicum* was observed. After bending its fore-legs, the fly punctures the skin and inserts the labrum into the wound. While feeding, the fly supports its body only with the mid- and hind-legs and its fore-legs are raised forward.

A total of 20,556 individuals of black flies comprising 9 genera and 15 species were collected from 12 localities in Hokkaido, Japan. Of these, 1,786 individuals (8.69%) of 8 genera and 14 species had blood-meals in their alimentary canals. In 403 individuals (22.56%) of 8 genera and 12 species, the blood-meal sources were determined by means of enzyme-linked immunosorbent assay (ELISA). Despite some exceptions, the estimations stated by Crosskey (1973) 16) and Sasaki *et al.* (1976) 65) were confirmed by the results obtained in this study. The blood-meals of the species with S-type tarsal claw were determined to be of mammalian blood origin, and none of them reacted against anti-bird serum. Therefore, the S-type species were concluded to be mammalophilic type blood-suckers. Of these species, *P. jezonicum*, *P. yezoense*, *S. japonicum* and *S. rufibasis* were considered to have medico-veterinary significance as harmful species because of their aggressive blood-sucking behaviour demonstrated in a wide range of mammalian species and their wide range of habitats and distribution areas. The blood-meals of P-type tarsal claw species reacted against both anti-mammalian species and -bird sera. Therefore, the P-type species were concluded to have both mammalophilic and ornithophilic blood-sucking behaviour. Of these species, *T. cannibora* and *G. diasense* are believed to be potentially harmful to humans and domestic animals because of their wide range of hosts in both mammals and birds. T-type tarsal claw species were concluded to be ornithophilic type blood-suckers on the basis of the data of *G. shogakii*, and from a comparison of their claw shape and surface structure, especially the carinulae of tarsal claws, with other claw type species.

The black flies inhabiting Hokkaido were classified into 3 feeding types according to the results obtained in this study. *H. multicaulis* and *P. sarurense* were classified into the non-biting type group. The other species, except *E. erimoense*, were classified into groups according to the shape type of their tarsal claws. Finally, *E. erimoense* was placed in the group having both mammalophilic and ornithophilic type blood-sucking behaviour, judging from the P-type mid- and hind-tarsal claws.

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摘　　要

1. 北海道に生息する13属27種のブユ科雌成虫の口器，特に大腮 (mandible) と小腮 (maxilla) の外葉 (galea) の形態上の特徴を走査型電子顕微鏡を用いて観察した。ムカシオオブユ (*D. daisetsense*), カラフトミヤマブユ (*M. sakhalinum*), クロアシマダラブユ (*S. nikkoense*), クジツノマユブユ (*G. shogakii*) の4種で，右の大腮が左の上に位置したが，他の種ではすべて左が上であった。

大腮にある微鋸歯状突起 (tricuspid tooth) は，すべての種で外側は挿入が容易になるよう大腮の挿入方向に沿う向きに並び，内側は皮膚を傷つけやすいように大腮の挿入方向とは逆向きに並んでいた。

ハイイロオオブユ (*H. multicaulis*) と，サルルキアシオオブユ (*P. sarurense*) の2種の大腮の微鋸歯状突起と小腮の外葉の鋸歯状突起 (tooth) は鈍く短かくかつ弱々しいことが

観察された。

2. 北海道に産する13属27種のブユ科雌成虫の跗節の爪(claw)の形態上の特徴特にその形状，基部の歯(basal tooth)の有無とその形状および爪と歯の表面の隆起線(carinulae)の状態を走査型電子顕微鏡を用いて観察した。

すなわち，これらのブユは跗節の爪の歯の有無，およびその歯の形状によって，S, PおよびTの3つの型に大別された。S型には，*Prosimulium* 属，*Stegopterna* 属，*Boophthora* 属，そして *Simulium* 属の11種が含まれ，その跗節の爪は単純でその基部に歯を持たず，隆起線は爪の長辺に沿って走っていた。P型には *Twinnia* 属，*Distosimulium* 属，*Cnetha* 属，*Gnus* 属，*Odagmia* 属，および *Simulium* 属の7種が含まれ，その跗節の爪は単純だがその基部に杭状の短い歯を持ち，長辺に沿う隆起線が観察された。他方，T型は *Helodon* 属，*Prosimulium* 属，*Eusimulium* 属，*Montisimulium* 属，*Gomphostilbia* 属および *Cnetha* 属の9種を含み，その跗節の爪は複雑でその基部に強大な歯を所有し，爪と歯の内側の隆起線はV字型を呈した。

サルルキアシオオブユとエリモツノマユブユ(*E. erimoense*)の2種は，前跗節にはT型の，中・後跗節にはP型の爪を有したが，他の全ての種では前，中，後跗節とも同じ型の爪を備えていた。

3. オオブユ(*P. jezonicum*)の吸血行動を，札幌市郊外の野幌原始林内で，ヒトおとり法によって観察した。

吸血源の皮膚に降りたオオブユは，歩き回った後，一地点に定位し，前肢の屈伸を繰り返し，同時に小腮の外葉と大腮によって皮膚に傷をつける行動を行った。その後，前肢を前方に持ち上げ，中・後肢の4本のみで体を支えるという特異な姿勢で吸血することが観察された。

4. 北海道内12地点で9属15種，合計20,556個体のブユ科雌成虫を採集した。そのうちの8.69%にあたる1,786個体がその消化管内にblood-mealを持つことを観認した。そこで，そのblood-mealの同定をそれぞれウサギで調製した抗ウシ，抗ウマ，抗ヒツジ，抗ヒト，抗エゾヤチネズミ，抗シカ，抗ヒグマ，抗鳥類の8種抗血清を第一抗体として，西洋ワサビ・ペルオキシターゼ標識抗ウサギIgGヤギ血清を第二抗体として用いた酵素抗体法(ELISA)で行い8属12種合計403個体(blood-mealが認められた22.5%)について吸血源となった動物種(blood source)を明らかにした。

S型の種のblood-mealは各種の抗ほ乳類血清に対して陽性反応を示したが，抗鳥類血清に対して陽性反応を示さなかった。このことから，S型の種はほ乳類吸血性と考えられた。S型の種のうちキアシオオブユ(*P. yezoense*)，オオブユ，アシマダラブユ(*S. japoni-

cum), および，アカクラアシマダラブユ (*S. rufibasis*) の 4 種は，強烈な吸血行動，広範な吸血源動物，および，広い生息分布域などを有することから医学獣医学分野における最重要種と判断された。P 型の種より得られた blood-meal はほ乳類および鳥類双方の抗血清に対して陽性反応を示した。このことから，P 型の種は広く，ほ乳類と鳥類を吸血する習性を持つものと考えられる。これらのうち，キタクロオオブユ (*T. cannibora*) とダイセンヤマブユ (*G. daisense*) の 2 種は，ほ乳類および鳥類双方に広範な吸血源を持つことから，両種も医学獣医学分野における重要種と考えられた。T 型の種はクジツノマユブユの blood-meal が抗鳥類血清に対して陽性反応を示し，また爪や歯の形態並びにその表面構造なども羽毛や羽根をかき分けたり掴んだりするのに適した形態をしていることから鳥類吸血性と考えられた。

5. 以上のことから，北海道に生息するブユを，吸血習性を基に無吸血性，ほ乳類吸血性，鳥類吸血性および，ほ乳類一鳥類吸血性の 4 つの群に大別した。そのうちハイイロオオブユ，サルルキアシオオブユの 2 種を，大腮や小腮の外葉の歯の形態から無吸血群に編入した。また，エリモツノマユブユを中・後跗節の形状からほ乳類一鳥類吸血群に入れた。他の 24 種は，前跗節の爪が S 型の種はほ乳類吸血群に，また，P 型の種は，ほ乳類一鳥類吸血群に，T 型の種は鳥類吸血群に区分された。

Explanation of Plates

Plate I *Twinnia cannibora* (a–e) and *Helodon multicaulis* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate II *Distosimulium daisetsense* (a–e) and *Prosimulium jezonicum* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: a: 100 μm; b–j: 10 μm.

Plate III *Prosimulium yezoense* (a–e) and *Prosimulium karibaense* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate IV *Prosimulium sarurense* (a–e) and *Prosimulium apoinum* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate V *Stegopterna nukabirana* (a–e) and *Eusimulium erimoense* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate VI *Montisimulium sakhalinum* (a–e) and *Gomphostilbia shogakii* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate VII *Cnetha subcostatum* (a–e) and *Cnetha uchidai* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate VIII *Cnetha konoi* (a–e) and *Cnetha boldstemtum* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate IX *Cnetha acmerium* (a–e) and *Boophthora yonagoense* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: min-; e, j: hind-tarsal claws Scale bar: 10 μm.

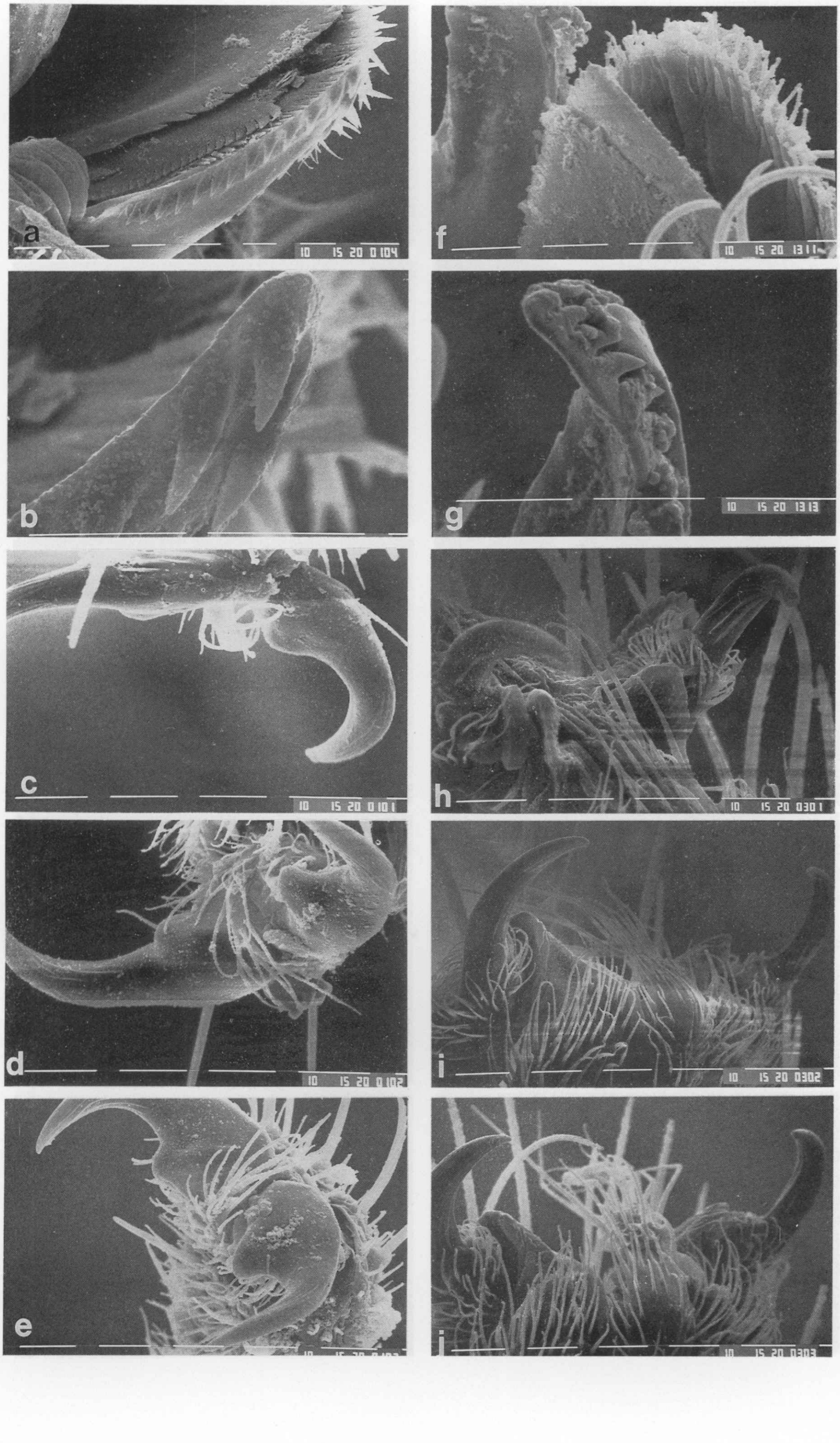
Plate X *Gnus daisense* (a–e) and *Odagmia aokii* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate XI *Odagmia nishijimai* (a–e) and *Simulium japonicum* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate XII *Simulium horokaense* (a–e) and *Simuliom nikkoense* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.

Plate XIII *Simulium rufibasis* (a–e) and *Simulium suzukii* (f–j)
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: a–h: 10 μm; i–j: 100 μm.

Plate XIV *Simulium tobetsuense*
a, f: mandibles; b, g: galea of maxilla; c, h: fore-; d, i: mid-; e, j: hind-tarsal claws Scale bar: 10 μm.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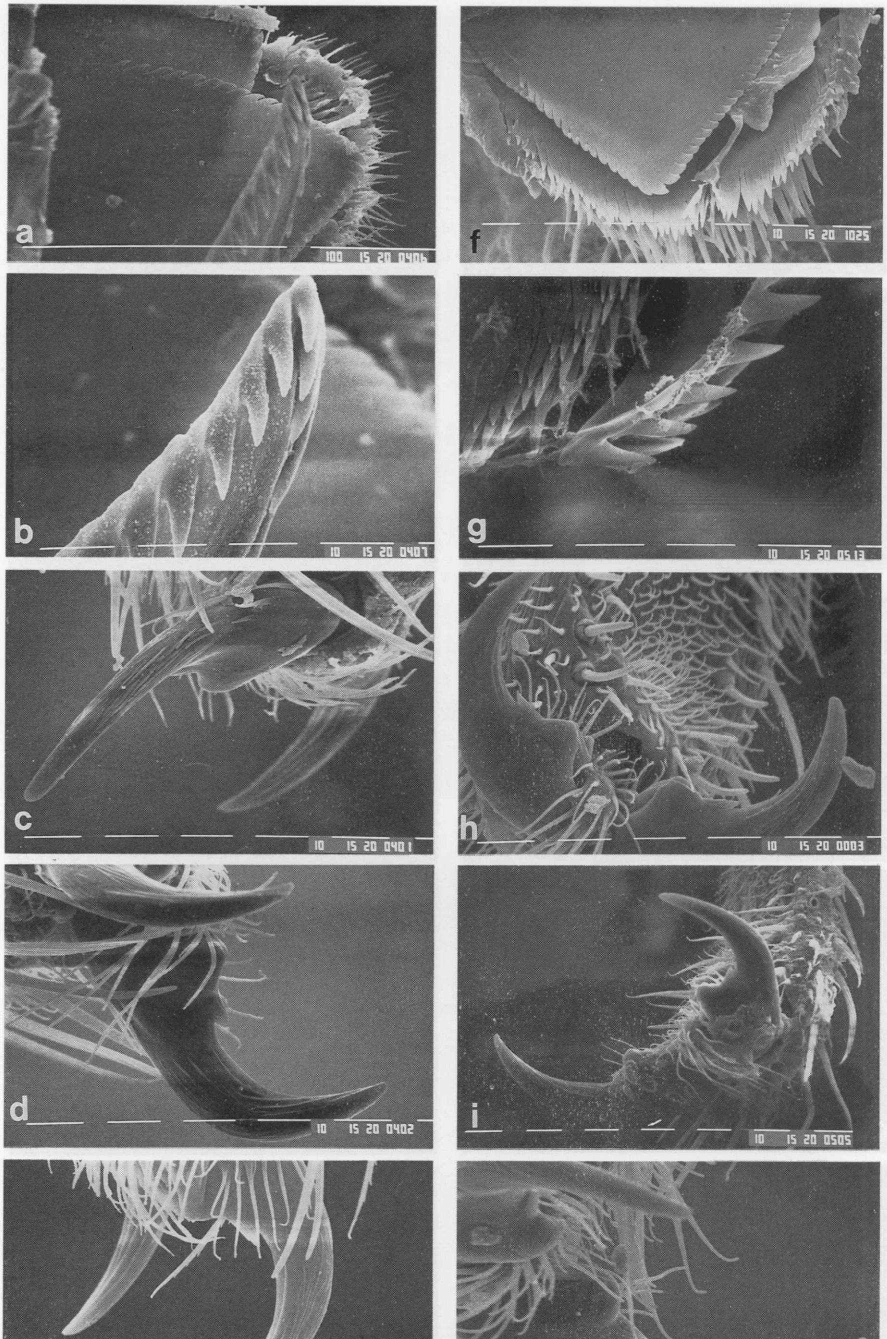
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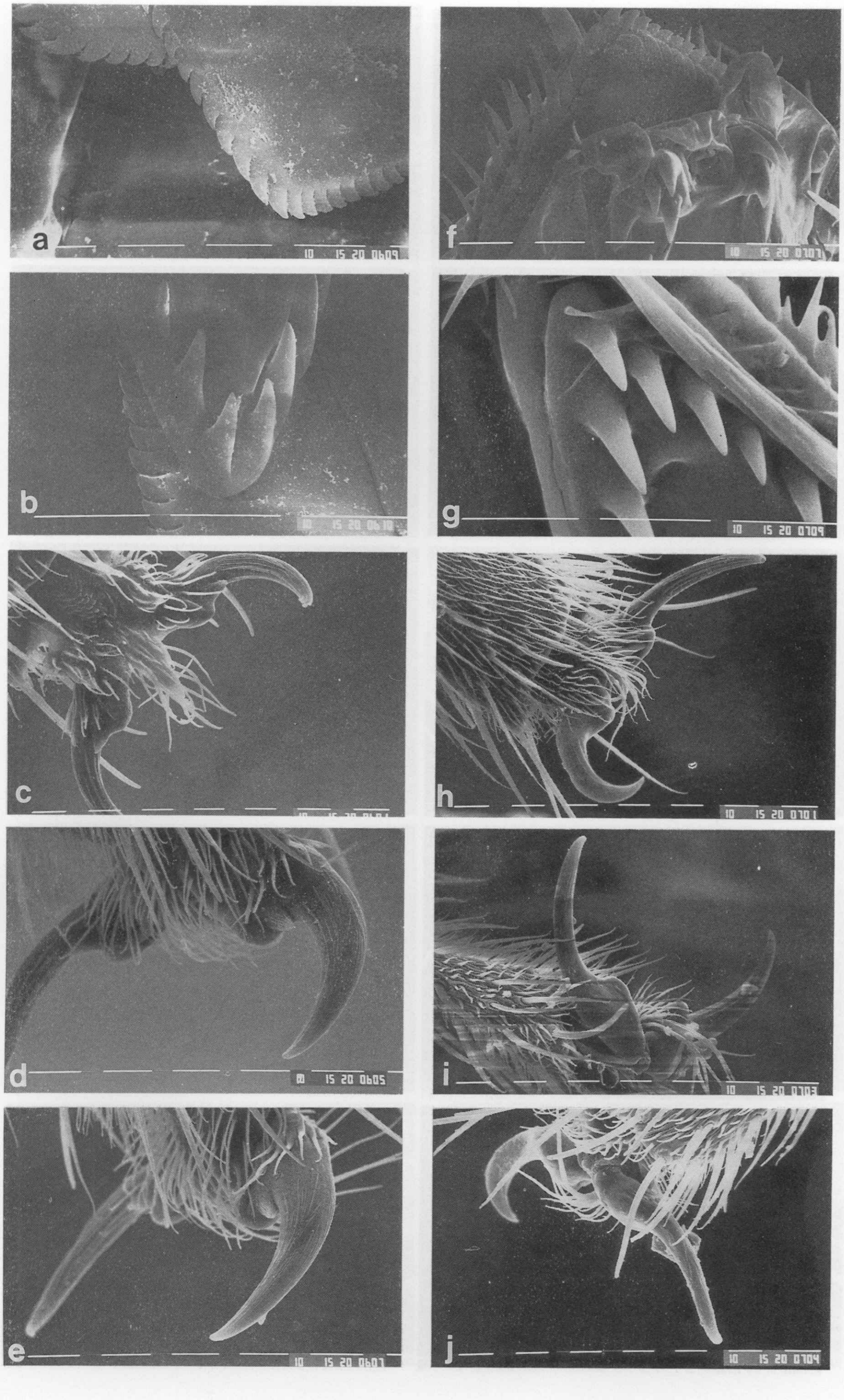
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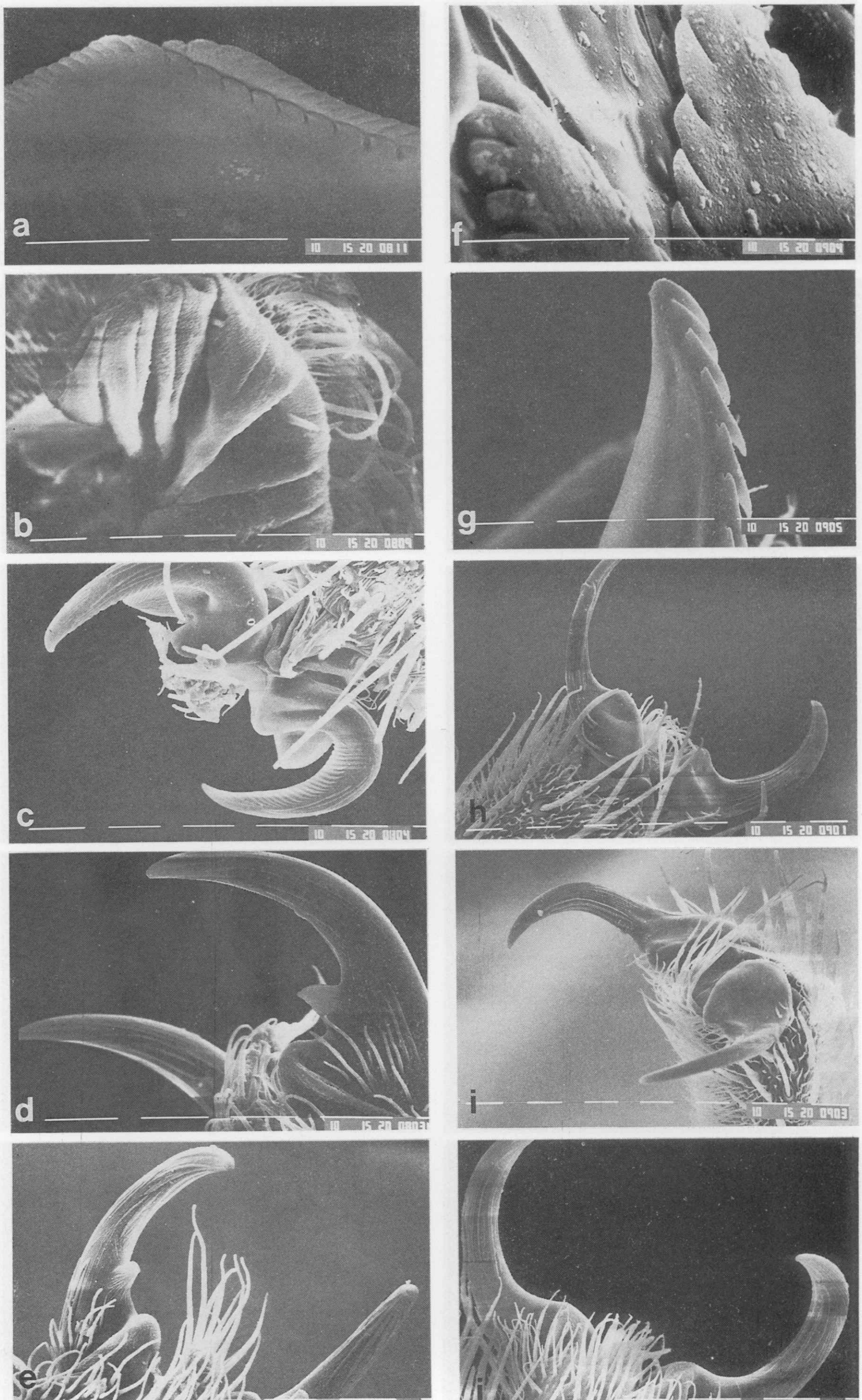
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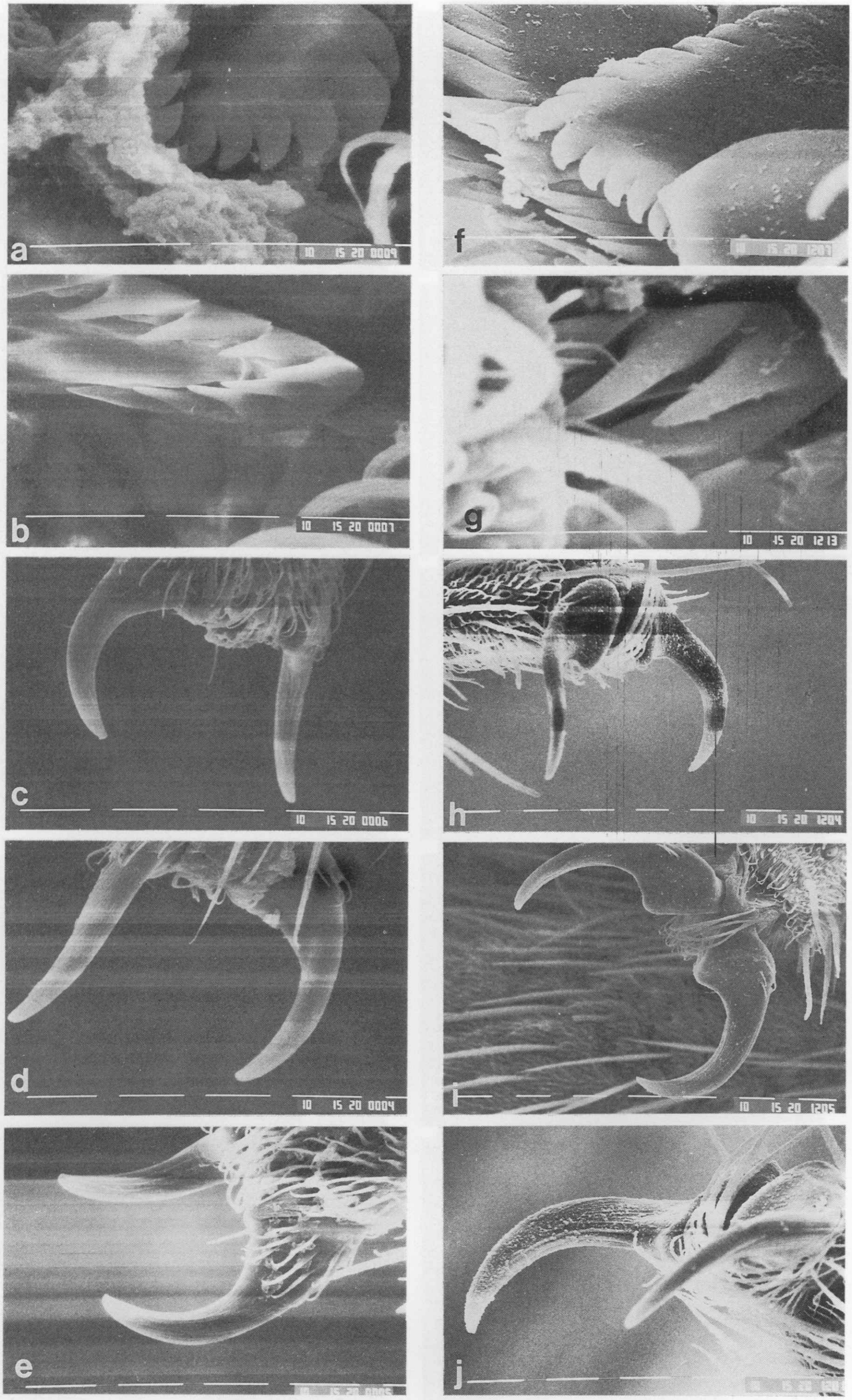
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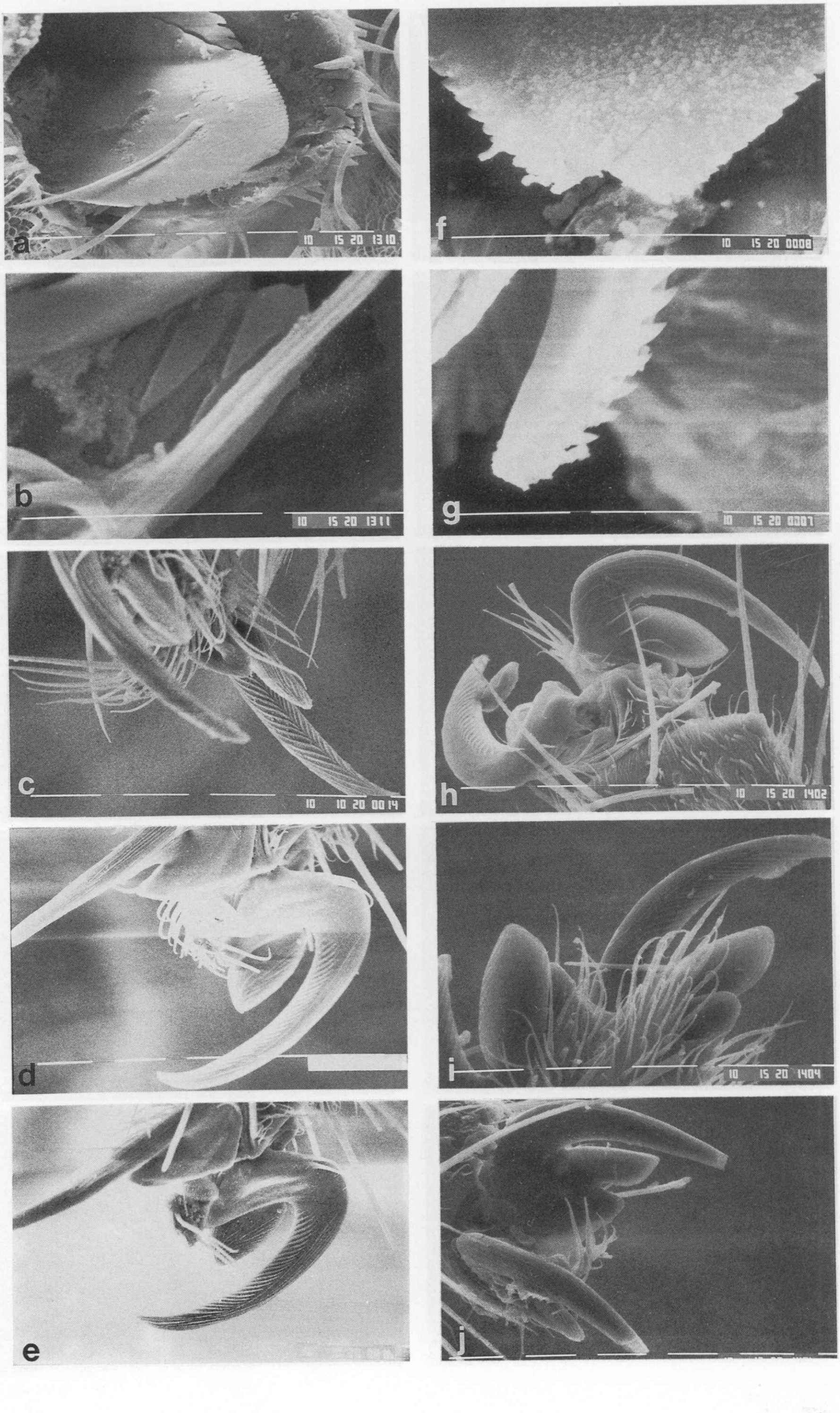
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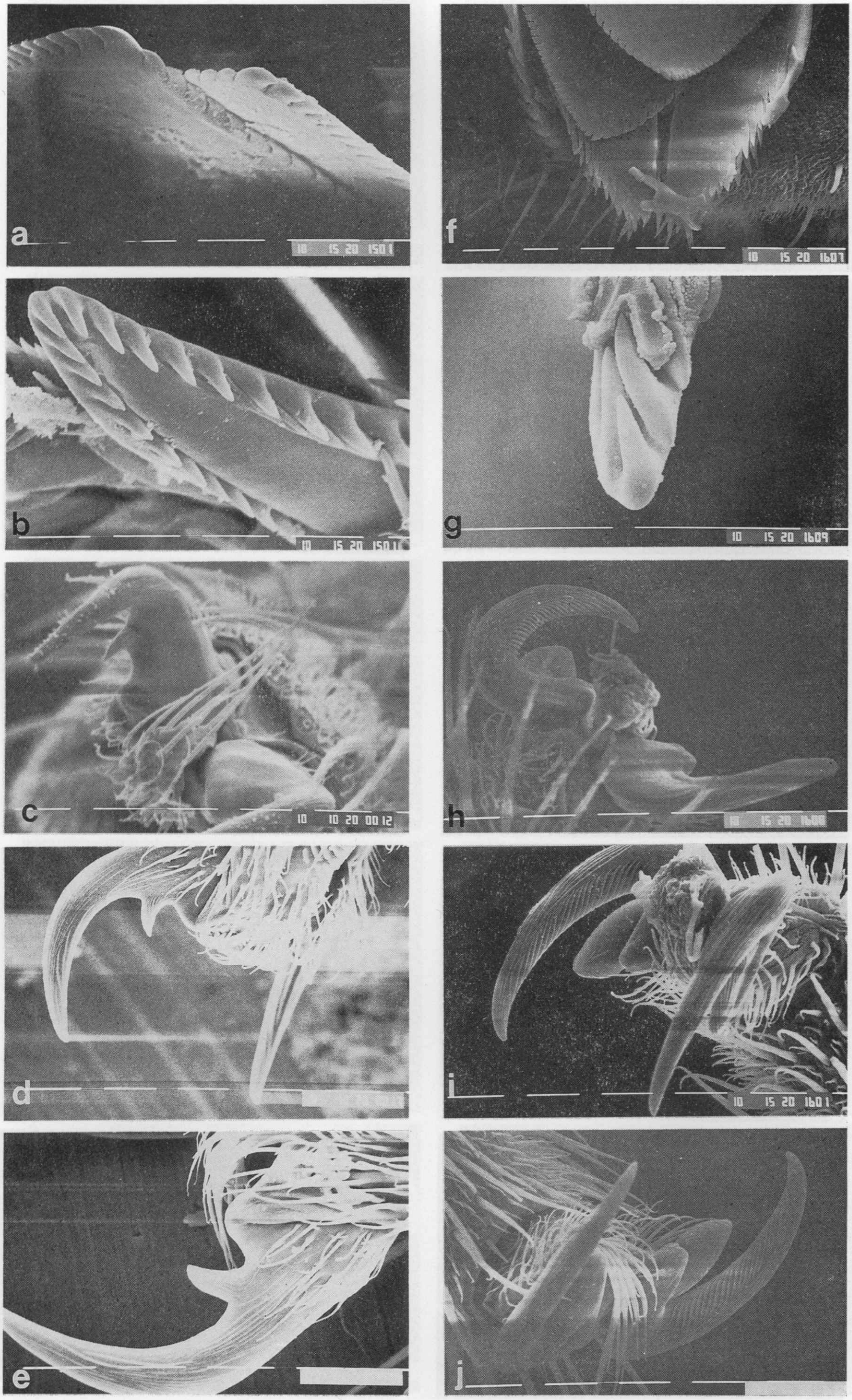
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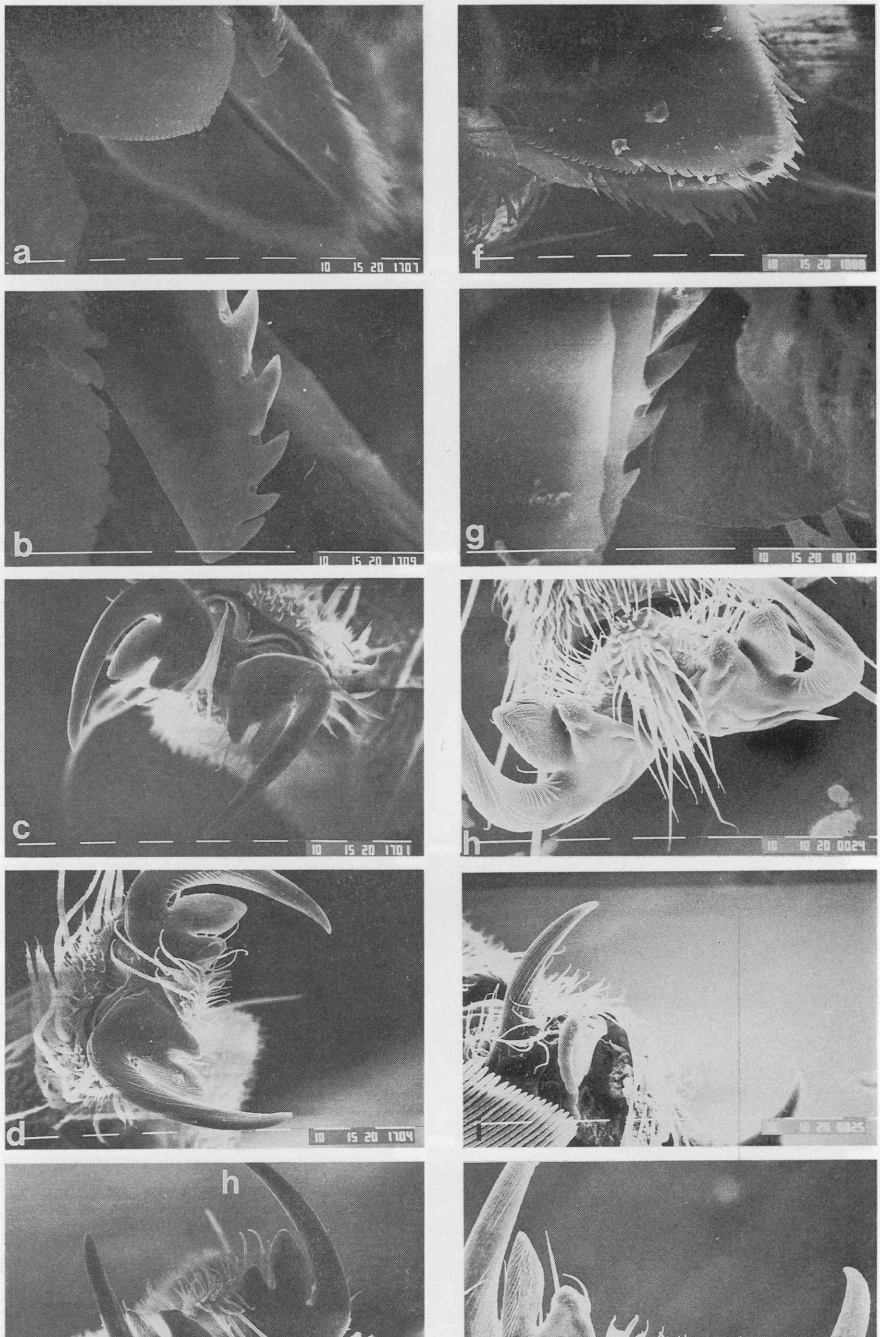
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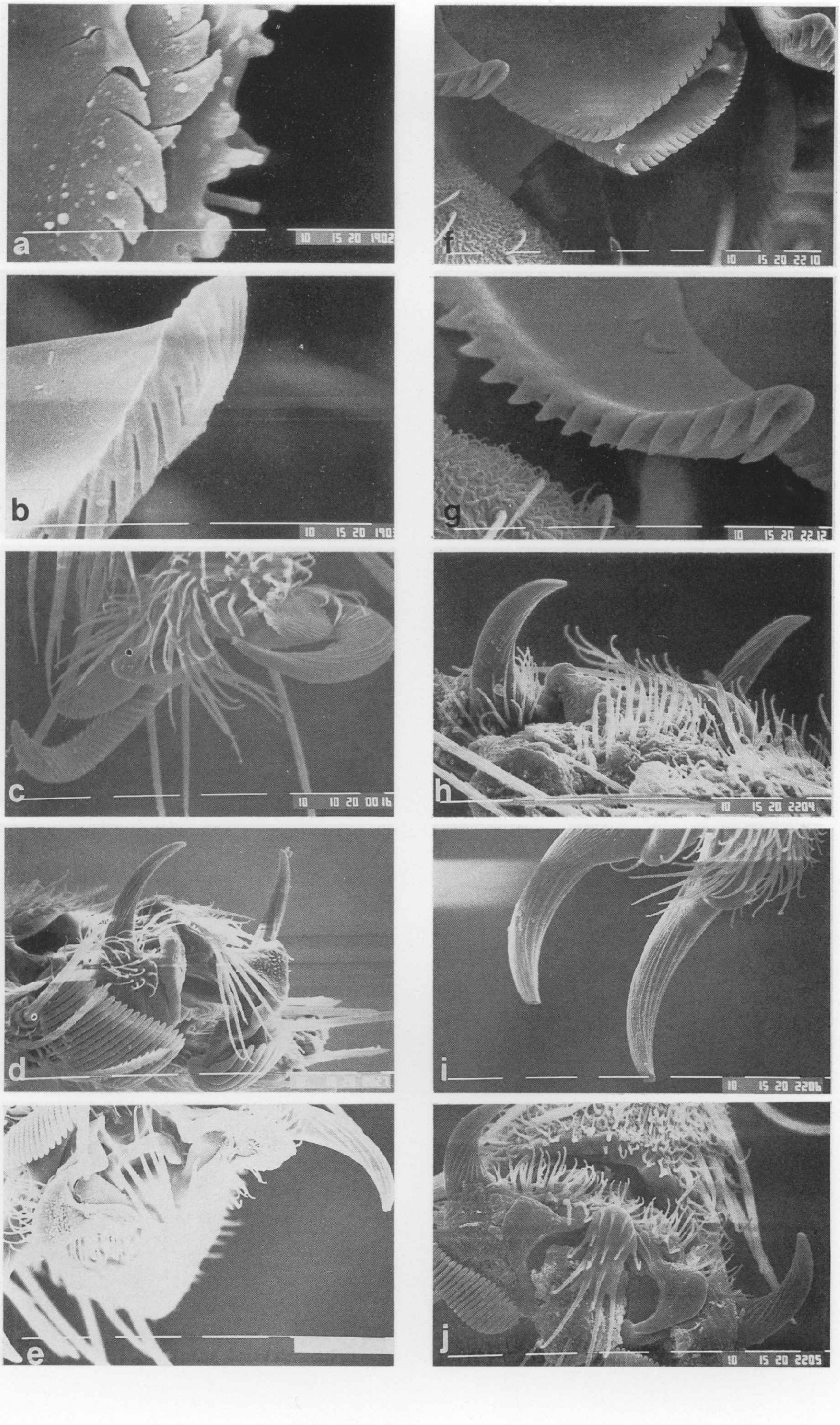
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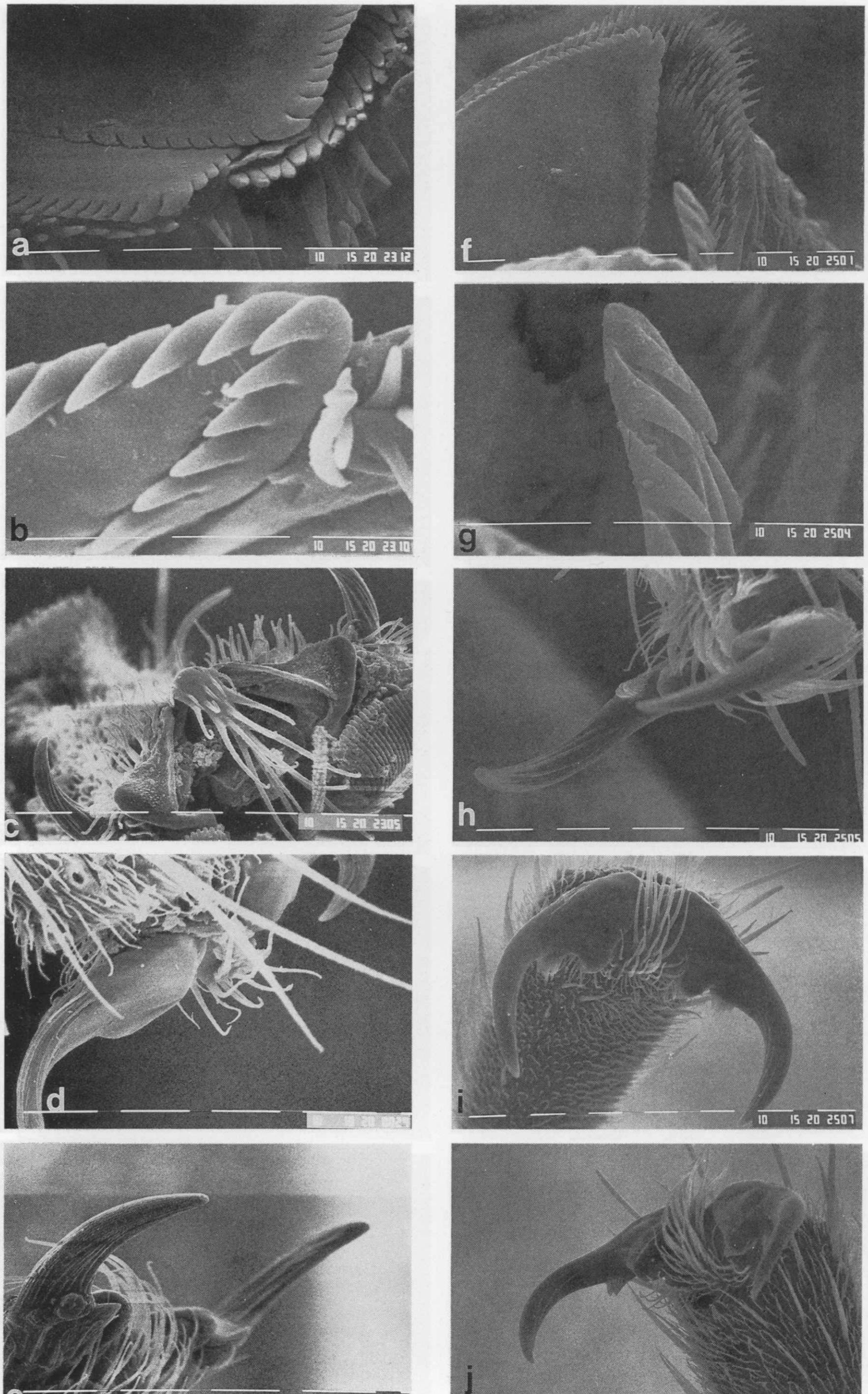
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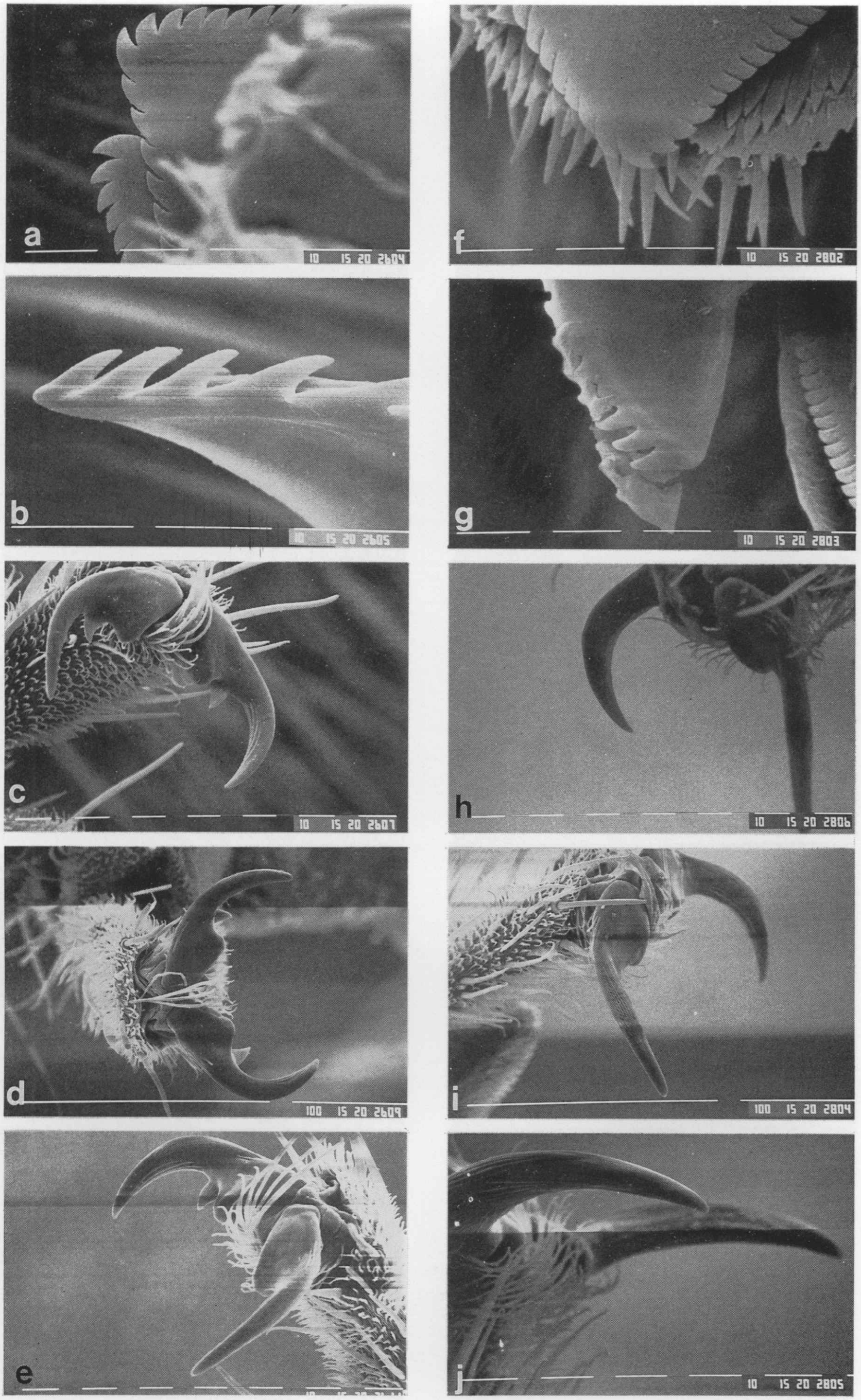
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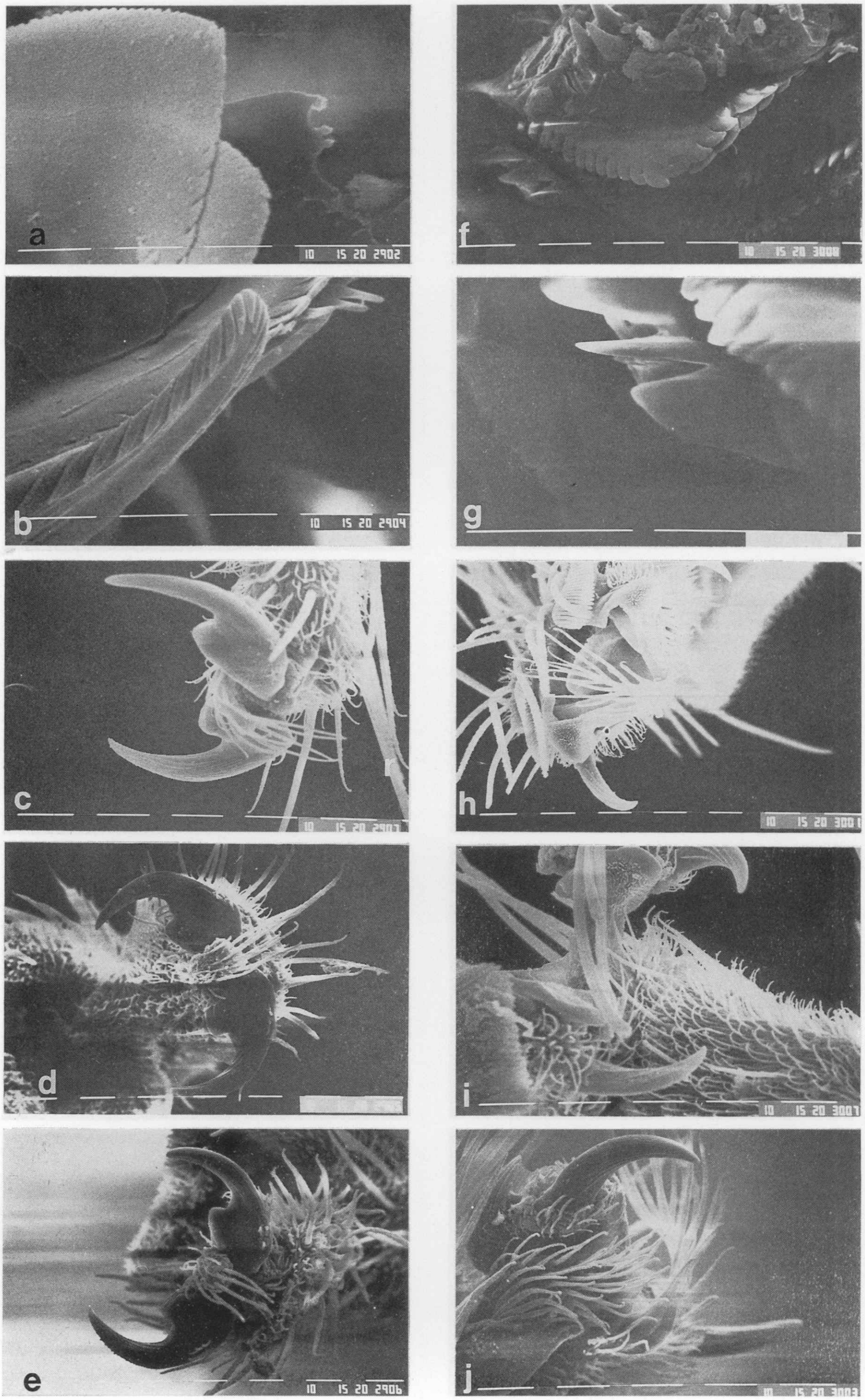
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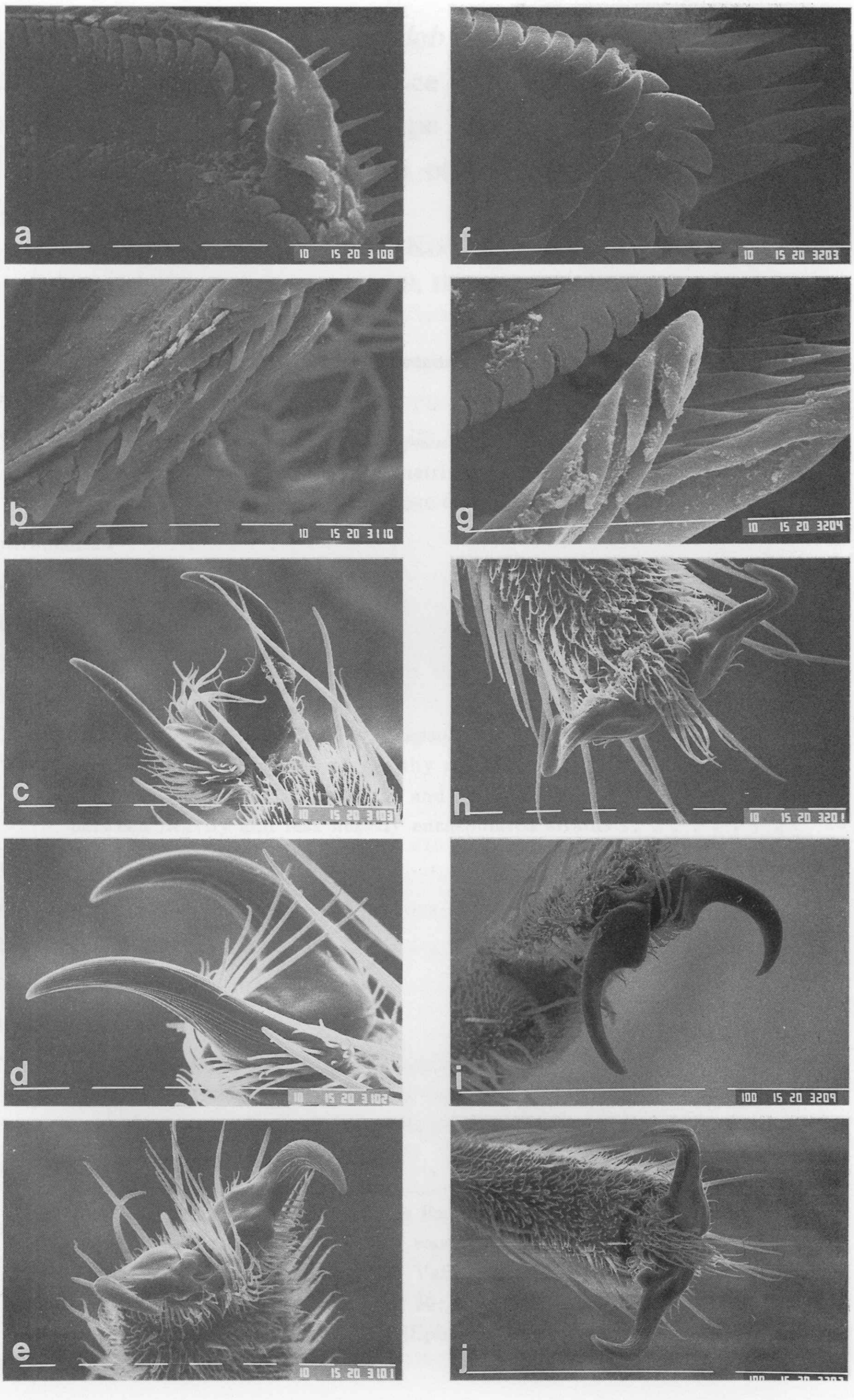
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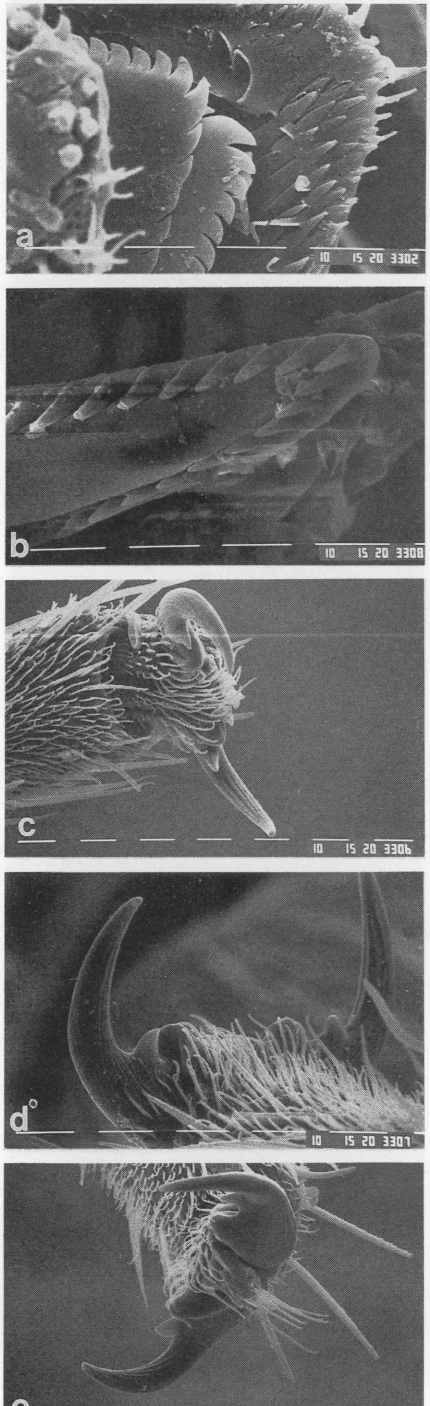
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