THREE NEW BLACK-FLY SPECIES AND ECOLOGICAL NOTES

THREE NEW ONTARIO BLACK FLIES OF THE GENUS PROSIMULIUM (DIPTERA:SIMULIIDAE) AND OBSERVATIONS ON THEIR ECOLOGY

By

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SCOPE AND CONTENTS:

Cytological studies have exposed new species complexes in the dipterous family Simuliidae.

A study of one of these in the genus *Prosimulium* has established morphological, distributional and ecological characteristics of three of its members and they have been described as new species. A comparison has been made between these and other members of the complex throughout the world.

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INTRODUCTION

Systematic Methods

Taxonomy has long been based on comparative morphology and little else. In recent years however, other fields of biology such as cytology, ecology, physiology, and population genetics have become woven into the mesh of diverse approaches to this fundamental problem of taxonomy: to separate the complex of animal forms into natural and easily recognised groups.

Especially is this true of insect taxonomy, of which morphology has long been the backbone. Recent studies have proven the value of other features in recognising or distinguishing between species, and differences in growth (Smith, 1955), behaviour, and even in the ability to transmit diseases have been used (Mayr et al. 1953).

Two other approaches are coming increasingly to the front, the biochemical and the cytological. With rapid improvement in paper chromatography, it has become possible to separate mosquito species by the kinds and relative amounts of free amino acids present in their bodies (Hicks, 1956).

Chromosomal studies have resulted in an elucidation of phylogenetic relations in Drosophila (White, 1954), in mosquitoes (Succi, 1953) and in the Chironomidae (Bauer, 1936, 1945;

Rothfels and Fairlie, 1957). Since it appeared promising to apply this approach to the Simuliidae, Rothfels and Dunbar (1953) began a cytological survey of the black flies of eastern Canada during the summer of 1951. They found that in this group the giant salivary gland chromosomes provided "a wealth of descriptive morphological detail in their number and gross morphology, in the characteristics of expanded centromere regions, in the location of specific nucleolar sites, in the degree of pairing of constituents, and in the ultimate discernable banding pattern.... Since these features are not functionally related to the external environment, the confusing effects of convergence are minimized, resemblances may be taken to indicate relation, and grouping is possible according to natural affinities" (Rothfels and Dunbar, 1953).

In distinguishing between some animal species, the haploid number of chromosomes along with the relative lengths of chromosomes and their arm ratios, as governed by the position of the expanded region or centromere on each chromosome offers some help, but in most black fly species, unfortunately, these arm ratios appear to be quite constant as is the haploid number, n=3.

A more useful method is the construction of idiograms, employing besides the usual arm ratios, the distance between easily recognized "landmarks" such as the nucleolar organizer and certain conspicuous bands, and expressing those lengths as a percentage of the total complement (, 1951 of Rothfels and

Dunbar, 1953).

Easily recognized "landmarks" also make it simpler to examine two chromosomes in different species and to compare the finer detail in a particular spot. Some such "landmarks" have proven to be useful because their characteristics or position are often species-specific. These "landmarks" include the expanded region, the main nucleolus, the ends of the individual chromosomes, and the so-called Ring of Balbiani.

Two other characteristics of salivary gland chromosomes that may prove to be diagnostically useful are the degree of pairing between the two homologous constituents, and the presence or absence of a chromocentre.

Of course, the comparison of the ultimate resolvable banding patterns would provide the final criteria of species identity, but it is hoped, and seems likely, that complete maps of banding patterns will not be needed to separate many species.

A more detailed account of cytological methods, interpretations and conclusions pertaining to black flies is presented by Rothfels and Dunbar (1953).

The Taxonomic Situation of Prosimulium hirtipes (Fries)

Malloch (1914) in describing hirtipes from North America, noted variations in colour, but failed to find any significant differences between the North American and European adult

specimens, although he mentioned that the gonal respiratory organ may have a total of 60 filaments in some specimens. These he lumped with the 16-filamented type.

Dyar and Shannon (1927) also failed to find any constant differences between American and European specimens, or within the American fauna.

Later workers (Twinn, 1936; Stone and Jamback, 1955) also included the North American specimens under the name P. hirtipes (Fries).

The taxonomic confusion in Prosimulium hirtipes probably would have been less if the typical specimen of Fries had not been lost. As it is now, with only the description and no type specimen, and with all the allied forms being closely similar in morphological characters, it is only natural for workers to consider that they are dealing with a single, widely distributed species.

It was not until just recently that workers began to seriously question the validity of the name hirtipes in America. L. Davies (1957) in England remarked on the morphological variability of North American material, and the possibility that it was not conspecific with hirtipes (Fries). Rothfels (1956) in his cytological studies has discovered that the material until recently called Prosimulium hirtipes (Fries) in Ontario by taxonomists, is really a complex of species, none of which are cytologically the same as the

north European Prosimulium hirtipes (Fries). Just recently in Britain, P. inflatum Davies was distinguished on morphological grounds (L. Davies, 1957). This, too, proved to be cytologically distinct from the Ontario members of the complex (Rothfels, 1957, pers. comm.). In fact, even the north European hirtipes, according to cytological studies, consists of at least two forms which may or may not be distinct (Rothfels, 1957, pers. comm.).

Three of the four Ontario members of the complex were studied in this project. Two of these, "hirtipes 1" and "2" of Rothfels (1956), differ cytologically in a simple inversion in each arm of the third chromosome. The inversion in the long arm is always found in a homozygous condition although the inversion in the short arm is found in a heterozygous condition in the males of "hirtipes 1" and was shown to be a sex-determining mechanism (Rothfels, 1956). The two homozygous types (i.e. "hirtipes 1" and "2") were found together in the same populations at the same time over a wide area and in hundreds of larvae, indicating that the two forms of animals are at least biologically distinct species. The third species of the complex, "hirtipes 5" of Rothfels (1956, pers. comm.), differs cytologically from the other two mainly by the possession of a chromocentre and also by the cytology of sex determination and by the

possession of several characteristic inversions, both inter- and intraspecific.

The discovery that certain simuliid species, including Prosimulium hirtipes , are really species complexes, has made necessary a revaluation of morphological and ecological characteristics, and the purpose of this study was to discover those characteristics that could distinguish the three Ontario species.

Actually, this revaluation had commenced before the cytological evidence had become common knowledge. Thus, Rubtzov (1940) recognized two subspecies of hirtipes. One was P. h. hirtipes (Fries) which he described as having light yellow or yellowish brown legs, and is therefore probably not the true hirtipes as re-defined by Edwards (1915) and Puri (1925), and followed by subsequent writers such as Smart (1945) and L. Davies (1957), although Rubtzov said that it occurs in Scandinavia and northern Europe. The other was P. hirtipes tridentatum Rubtz., occurring in east Siberia and the Kamtchatka peninsula, and differing from hirtipes hirtipes in the number of teeth on the maxilla of the adult female and in other characters of colour and pilosity. Subsequently, Rubtzov (1956) raised hirtipes var. tridentatum to specific rank and described two new varieties of hirtipes. One of these, P. hirtipes var. diminutum Rubtz. differs from P. hirtipes (Fries) in size, colour of pilosity, and other characters. The other, P. hirtipes

var. lugranicum Rubts. according to Rubtzov, differs from P. h. hirtipes in size, and structure of the head and genitalia.

Examination of Rubtzov's descriptions of other Prosimulium species indicated that none of these are the species described in this paper.

Rubtzov (1956) also mentioned that close examination of a series of North American material deposited in the collection of the Zoological Institute of the Academy of Science of the U.S.S.R. showed that these individuals belong to at least three different species, all of which are distinct from the European form. He concluded that a whole revision of the hirtipes group was necessary.

Cronier (1947) realized the need for a revaluation of morphological characters and the re-examination of Edward's types of P. galli Edw., P. rufipes Meigen and P. hirtipes (Fries) to clear up a puzzle in the identity of the so-called three species. The specific name galli has subsequently been sunk as a synonym of rufipes, which also is held to be synonomous with hirtipes by some. He recognized also what he termed a subspecies of hirtipes, isolated geographically from the "typical form", and differing from it only in the greater number of respiratory filaments in the pupa. This he called P. hirtipes var. arvernense.

Novak (1956) described variations in the subcentral teeth of the larvae of "hirtipes" from various localities in Czechoslovakia, and it seems likely that he also was dealing

with a complex of species. Since he described only the larvae, and not the adults, it is difficult to tell if any of his forms were the true hirtipes.

As mentioned earlier, Dr. Lewis Davies of the University of Durham recently separated out Prosimulium inflatum in England, a species closely related to P. hirtipes (Fries). Also, in a comparative study of Holarctic forms related to hirtipes, he concluded that the dark-legged northern European Prosimulium hirtipes is distinct from the light-legged P. rufipes. He also concluded that P. hirtipes var. arvernense Grenier is most likely a distinct species, separate from both hirtipes and rufipes, and believed that all the North American material he has examined is specifically distinct from the northern European P. hirtipes (Fries). This latter conclusion, of course, was substantiated by the cytological evidence of Rothfels.

Distribution of the Complex

The genus Prosimulium is Holarctic in distribution (Smart, 1945) and confined usually to rapid, cool streams, either snow-fed or spring-fed.

Within the genus, the species complex referred to as Prosimulium hirtipes, its subspecies and recently separated relatives, is also fairly well distributed throughout the Holarctic.

In Eurasia the complex is widely distributed. The P. hirtipes (Fries) referred to by L. Davies (1957) occurs in

Great Britain and northern Europe. P. hirtipes (Fries) is reported also from Czechoslovakia (Novak, 1956) and from Japan (Bonnick, 1955; Ogata & Sasa, 1955) but whether these are the same species as the northern European one remains to be seen. P. h. dirimatum Rubtz. occurs in east Siberia and P. h. lugubricum Rubtz. occurs in the Leningrad district and in Germany (Rubtzov, 1956). P. tridentatum Rubtz. is found across Siberia into the Kamtchatka peninsula (Rubtzov, 1940, 1956), while hirtipes var. arvernense Cronier occurs in France (Cronier, 1947), Spain (Doby and Doblock, 1955), and Yugoslavia (Zivkovic and Filipovic, 1956). A related species, P. rufipes Weigen, is also found in south Europe (Zivkovic and Filipovic, 1956; L. Davies, 1957).

In North America the so-called P. hirtipes occurs in the Hudsonian, Canadian and Transition zones, from Alaska and Labrador to California and Georgia (Stone and Jamback, 1955). In Canada we are concerned with four species of the complex, of which three are treated in detail in this paper. A detailed account of their distribution in eastern Canada will be given later under Ecological Observations.

METHODS

Collecting

From April 1 to August in 1956, and from April 1 to June 18 in 1957, collections were made from various streams in Ontario to obtain larval, pupal and adult material of the various species in the "*hirtipes*" complex. Streams were usually selected that had been sampled previously by either Dr. Rothfels from the University of Toronto or Dr. Davies from McMaster University, and that were known to have produced populations of "*hirtipes*" and sometimes a relatively pure population of a particular member of the species complex being studied. They included various streams running off the Niagara escarpment near Hamilton, Ontario, similar streams north of Toronto near Caledon, Ontario, and one just south of Owen Sound, Ontario. Other streams visited were those draining or emptying into Lake Sasajewun and Lake Opeongo, Algonquin Park; the Mahsho River where it crosses 11 Highway south of Cravenhurst, Ontario; streams near Uphill, Victoria Co., Dalton Twp., Ontario and various streams draining into the Ottawa River near Chalk River, Ontario.

These streams were visited at appropriate times during the spring and summer, and the rocks, debris and vegetation were searched for mature larvae and pupae. These were trans-

forced with forceps to syracuse watch glasses containing a little water. Samples of larvae from each of these streams were examined cytologically to determine whether one or more members of the "hirtipes" complex were present.

The water temperature at each station was noted on each collecting day, as were the general features of the stream itself.

Rearing

From the collection of larvae and pupae, the pupae were sorted and reared in a manner slightly modified from that described by Twinn (1936). A small wad of wet cotton batting was placed at the closed end of glass vials 14 x 48 mm. lying on their sides. A strip of moist paper toweling was inserted lengthwise along the glass, and pupae laid on it individually or in groups of up to six per vial. Similar vials containing strips of dry toweling were placed with their mouths against those containing the pupae. The pairs of vials were wrapped in paper in this position, the paper covering the junction and the vial containing the pupae, but leaving exposed about half of the emergence tube. When the flies emerged, they were attracted to the brighter, dry end of the tube.

In some cases, and especially if insufficient pupae were collected from a locality, mature larvae were reared by placing them in battery jars of distilled water to which had

been added clean aquarium sand and some green moss (e.g. Sphagnum and Fontinalis), grass or reeds, and also a small amount of yeast or algae. Air was bubbled into these jars which were kept cool by placing them in cold running tap water. The formed pupae were allowed a few days to harden in situ before being transferred to emergence vials. This method of rearing larvae proved fairly satisfactory, providing roughly 25% emergence of adults, compared to approximately 50% emergence from stream-collected pupae.

Preserving and Pinning

In each case some larvae and pupae were preserved for morphological study by placing them in vials of 70% ethanol and 10% glycerine. Also, masses of exuviae, if present, were collected and preserved in the same manner.

Adults, when they emerged, were affixed to the side of an insect pin by means of shellac gel (Heinze,1935) and then placed immediately in a glass container with a loose fitting lid that was put into a deep freeze at -10°C. They were left in this condition for at least a week, until they had dried out. Drying while frozen prevents specimens from collapsing and shrinking, and specimens treated in this way prove much superior to those dried at room temperature. This method was developed by [illegible] who suggested its use to the author early in 1966.

A simple apparatus for applying the shellac gel to the

pin was used, and is described briefly here. Two injector-type razor blades are driven sharp side down into a sheet of ¼ in. balsa wood, parallel to each other and about ½ in. apart (the distance the specimen is to be from the head of the pin). For a right-handed worker, the upper edge of the right-hand blade is covered with shellac gel by means of a dissecting needle. The insect pin is held so that the head butts against the left-hand blade, and the shank of the pin lies across the top of the right-hand blade. A twist of the pin in this position puts a neat ring of shellac gel around the pin, precisely at ½ in. below the head. The pin is then applied to the insect in the normal fashion. In this way, the process of pinning numerous specimens can be speeded immensely.

In most cases, adult specimens were also placed in alcohol and glycerine. If the adults were raised individually, each specimen was given a number, and the same number applied to its exuvia in alcohol and glycerine. Otherwise a single number was given to each lot of specimens.

Preparation of Sensory Vesicle for Study

For the study of the sensory vesicle in the maxillary palp, dry-pinned and fresh material was prepared for sectioning by a modification of Stiles' modification of the method proposed by Mlle. Lerbaud for the dehydration and infiltration of insect material (Catenby and Beams, 1950).

Whole adult black flies were first placed in vials

containing 10 cc. of 95% ethanol for 1 hr. They were then passed through the following series of alcohols and left in each solution for the indicated times.

9 parts 45% ethanol : 1 part n butanol for 2 hr.

8 parts 68% ethanol : 2 parts n butanol for 2 hr.

6.5 parts 77% ethanol : 3.5 parts n butanol for 4 hr.

4.5 parts 90% ethanol : 5.5 parts n butanol for 12 hr.

2.5 parts 95% ethanol : 7.5 parts n butanol for 24 hr.

The specimens were then transferred to 100% n butanol where they were stored until used. Infiltration was carried out by placing the dehydrated specimens in vials containing equal parts of n butanol and paraffin (56-58° C.) and then covering them in an oven at 60° C. After 24 hours, the stoppers were removed, and the alcohol was allowed to evaporate for two days.

At this stage, the maxillary palp was removed from the specimen and imbedded in fresh paraffin (56-58° C.).

Longitudinal sections at 9μ, and transverse sections at 10μ were cut on a rotary microtome.

The serial sections were then affixed to slides with Mayer's albumin fixative, and hydrated in the following series of toluene and ethanol.

Toluene 100%

Toluene and 95% ethanol 50:50

Toluene and 95% ethanol 25:75

95% ethanol

70% ethanol

40% ethanol

distilled water

The slides were then placed in carbol-fuchsin stain (Deirne, 1955) for 1 - 2 hr., washed in tap water three times, and finally in distilled water. Excess stain was removed by dipping the slides into acid ethanol (5% HCl by volume) and quickly passing them into 95% ethanol.

A second change of 95% ethanol was followed by dupara1 mounting medium and coverglass.

Preparation of Material for Cytological Study

Fixing

Mature larvae intended for cytological work were fixed while still fresh in the field. This was done by opening the body cavity from the dorsal surface, about one-third the distance from the posterior end, with dissecting needles or fine forceps in a small drop of water in the collecting dish. When this is done, the salivary glands, which lie beside the gut in this area, pop out of the body cavity and are thus well exposed for fixing and later dissection. The entire specimen was immediately placed in a vial containing either Carnoy's fixative (1 part glacial acetic to 2 parts ethanol) or Newcomer's fixative which consists of (by volume):

6 parts isopropyl alcohol

3 parts propionic acid

1 part petroleum ether

1 part acetone

1 part dioxane

When fixed in Carnoy's fixative, the specimens must be used within a week to 10 days, but when Newcomer's fixative is used they are good for several weeks to a year. Fixed material was stored in the refrigerator until used.

Staining:

The Carnoy's- or Newcomer's-fixed mature larvae were subsequently treated to provide slides of salivary gland chromosomes by which the specimens could be identified as "hirtipes 1", "2", or "3".

The staining reaction used was of two different types:

a) (i) The fixed specimens were hydrolised for 8 min. at 60°C. in N HCl.

(ii) They were then placed in a vial of Feulgen stain for 1 hr.

(iii) The specimens were washed for 5 min. each in three changes of SO_2 water,

(iv) and washed finally in three changes of tap water.

The specimens were then placed individually under a dissecting microscope and the salivary glands were dissected out under 50% acetic acid. These were placed on a clean slide in a drop of 50% acetic acid, and covered with a [illegible]1

coverslip. The preparation was then squashed under filter paper to rupture the nuclear membranes and separate the individual chromosomes so that the banding pattern could be observed.

b) By the second method the fixed specimens were dissected in N HCl under a microscope to remove the glands. The excess HCl was removed, and orcein stain was added to the glands.

The slide was then heated slightly for a few moments, the excess orcein removed and 50 acetic acid added. The preparation was then squashed as before.

Mounting

Each squashed preparation was examined under high dry objective to determine if a satisfactory mount had been made. If so, the slide was placed coverslip up in a large petri dish of 95% ethanol overnight. The next day the coverslip was removed, a drop of Euparal placed on the specimen and the coverslip replaced. After allowing sufficient time for the Euparal to harden, the slides were identified under an oil immersion objective.

Morphological Examination

Larvae, pupae and adults from streams producing populations of a single member of the hirtipes complex were examined for morphological differences.

Since the most useful taxonomic characters are to be

found in the parts of the larval head capsule, slides were prepared by dissecting out the mandibles, maxillae, labium, mouth fans, antennae, and splitting the remaining capsule so that the submental teeth and the dorsal head pattern could be seen. This dissection was done directly in a drop of Euparal, the mounting medium, and covered with a cover-glass immediately after dissection and positioning. The components parts could then be studied at leisure under a microscope and measurements and ratios could be taken, or drawings made by camera lucida. This latter method proved quite useful in recording data that could later be compared directly. The size and colour of the whole larva were also taken into account in the examination.

In the morphological study of the pupae the respiratory filaments were cut off and spread to facilitate drawing and examination. It was found that if the respiratory filaments of the pupae, or those dissected from the histoblasts of mature larvae were placed in 50% acetic acid, or stronger, they tended to stretch and spread, making the task of counting them much easier. This procedure works best with fresh material and not so well with alcohol-hardened material. The pupal chaetotaxy was studied from whole pupae and whole exuviae.

The adults, with more taxonomic characters to examine, were treated in the same way as the larvae, except that they were first placed in hot 10% KOH in an oven at 60° C. for

20 to 60 min., usually 20 to 30 min. Specimens treated this way were washed and placed in 95% alcohol. The head capsule was removed and dissected in a drop of Euparal on a slide, as were also the genitalia except for the adminiculum of the males which was stored in glycerine in a microvial, and examined in a drop of glycerine on a slide. The legs and wings of each specimen were also mounted on a slide. Every specimen treated in this way received a slide number, and all slides of appendages belonging to that specimen received the same number. From these slides, measurements and ratios were taken of the various appendages and drawings were made of components that appeared to possess likely diagnostic characters.

Specimens were also examined dry and whole in the hope of finding characters that would not show in a wet specimen, or would be apparent only in the entire specimen.

Ecological Methods

With the idea that ecological factors might assist in separating the species, it was decided to study the emergence of a mixed population of "hirtipes 1" and "2". Thus, a stream, Hopkins Ck. crossing the York Rd. near Dundas, Ontario and known to have contained both "hirtipes 1" and "2" in 1956, was visited in 1957 and was again found to have a mixed population. A 24 mesh brass screen, cubic yard cage (Ide, 1940) was placed over the rapids harbouring "hirtipes"

larvae to try and determine if there was a significant difference in the emergence times of the two species. There was a door in one side of the cage by which the operator entered to collect adults that had emerged into the cage. This door was placed so that it was directed away from the sun when the cage was visited - i.e. early afternoon.

Trips were made daily, or every other day, to collect material that had emerged and to record the maximum and minimum temperature from a thermometer left submerged at the site. The specimens were stored in vials of alcohol and glycerine and sorted as to species and sex on the return to the laboratory.

The number of males and females was tabulated for each day to see if any difference between the emergence times existed in the two species. Also examined was material collected over a season in Algonquin Park by Dr. F.P. Ide and his students in 1940 and by Dr. D.J. Davies in 1946 and 1947. In these collections a great deal of material that had been lumped as P. hirtipes was amassed (Davies, 1950).

THE DETERMINATION OF THE PURITY OF A POPULATION

In collecting material for this project, populations of mature black fly larvae were sampled and tested for species identity by the cytological technique previously outlined. If it was found that all the mature larvae of 16-filamented Prosimulium sampled in a particular locality on a particular date were of the same species, then it was assumed that this was the only 16-filamented species of Prosimulium present in the form of mature larvae. It was then assumed that any associated pupae were also of the same species, and therefore all specimens of 16-filamented pupae and mature larvae collected from that locality at that date were of the same species as defined by the cytological testing of the sample. Adult flies reared from pupae known with this degree of certainty to be of the types "hirtipes 1", "2", or "3" became the material on which the morphological and ecological studies were based.

It was gratifying to find, and it certainly gave support to the initial assumption, that the adults reared from these collections from numerous populations fell nicely into three distinct groups based on morphological characters corresponding to "hirtipes 1", "2" and "3".

OBSERVATIONS AND RESULTS

Sensory Vesicle of the Maxilla

A brief description of earlier work on the sensory vesicle in the maxillary palp is presented along with the results of the present investigation in order to provide a better understanding of the morphology of this organ, which proved to be a key character used in the following descriptions.

According to Imms (1944), a specialized sensory organ is present on the third segment of the maxillary palp of some dipterous and necopterous families, but not in others. Thus, it occurs in the genus Edwardsina (Blephariceridae), is general in the Simuliidae, Bibionidae, and Anisopodidae, and also occurs in certain species of Culicoides among the Ceratopogonidae and is present in Asyndulum and many other genera of the Mycetophilidae. The palpal segment bearing this organ is usually dilated. It occurs also in the third palpal segment of the necopterous family Nannochoristidae.

In Culicoides pulicaris it is located on the middle of the inner aspect of its segment; as described by Jobling (1928) it comprises a group of spatulated sensoria arising from small pits. In Edwardsina the sensory organ is margined by a cuticular flange, while in the Simuliidae and Anisopodidae it is sunken within a cavity that communicates with the exterior

by means of a duct-like passage. In the two last named families, and to a lesser degree in the Bibionidae, the sensory organ is larger in the female than in the male. Its significance is unknown, but it is apparently of considerable phylogenetic importance. Davies and Peterson (1956) mentioned that the maxillary palp of a female black fly was held appressed to the skin of the host while feeding. Thus the vesicle may serve a purpose in the act of blood-sucking.

Since the morphology of the vesicle is difficult to interpret from the gross specimen and since the vesicle showed promise of providing a diagnostic character, serial sections were made of the third palpal segment.

From the slides of serial sections of the vesicles of "hirtipes 1", "2" and "3" (= Prosimulium fuscum, P. mixtum and P. fontanum respectively, new species described in this paper) it was observed that the vesicle consisted of a hollow ellipsoidal capsule within the third maxillary palpal segment, connected to the exterior at the anterior surface by means of a short tube in both "hirtipes 1" and "2" (Figs. 2 & 3), while in "hirtipes 3" this capsule opens directly to the exterior with no connecting tube, or a very short one (Fig. 4).

Also in "hirtipes 3", the vesicle is relatively larger and smoother than in "hirtipes 1" and "2" and has a consistently wider opening (Fig. 7).

The interior of the vesicle in all three is covered with stiff hairs and so-called spatulate sensoria, the latter

arising from pits in the wall of the vesicle (Fig. 1). These pits give rise to the rough or bumpy outer surface, as seen in the gross specimen. Nicholson (1945) reports that histological sections of Cnephia dacotensis (D. & S.) showed a large flask-shaped cell at the base of each of the spatulate sensillae. He also mentions that Jobling (1928) described an apparently homologous organ in the palp of Culicoides pulicaris L., and was able to show that the sensillae are connected with the palpal nerve.

The floor of the tube flattens exteriorly to merge almost imperceptibly with the outer surface of the segment, whereas the roof of the tube forms a well-defined lip at the surface, visible in the gross specimen (Figs. 5 & 6).

Descriptions of the New Species

Prosimulium fuscum sp. n.

Adult Female

Prosimulium fuscum, to the unaided eye, appears as a large, dark species. Body length 3.0 - 3.5 mm. Wing length 3.5 - 4.0 mm. Head is black with appressed yellow pilosity. Frons at antennae 0.21 width of head, and at vertex about 0.37 width of head. Clypeus black with yellow hairs laterally and ventrally, thickest at the latero-ventral corners. These hairs point medio-ventrally. Occiput with a fringe of yellow hairs, with a few black ones posterior to eyes. Antenna 11-segmented, tapering slightly from third segment; black with the first or first two basal segments brownish

in colour, mainly with pale yellow to white pilosity, larger hairs black. Maxillary palp black with black hairs. The third dilated palpal segment contains a sensory vesicle that is about 0.35 times as long as the segment and opens anteriorly to the exterior by means of a tube that arises near the distal end of the vesicle. The vesicle itself is situated at the proximal end of the segment (Fig. 5).

Thorax black with yellow pilosity. Pronotum sand-coloured to grey. Mesonotum uniformly black, except for humeral angles which are red-brown on anterior surface and which have a grey sheen on the black dorsal surface. Pile is short, yellow and appressed. Scutellum sand-coloured with erect yellow hairs and a few darker hairs. Postnotum black. Pleuron dark brown to black. Membrane light brown mottled with darker brown, occasionally sand-coloured. Metapleural tuft yellow. Haltere grey-brown with yellow hairs. Hairs on most wing veins black, becoming yellower towards base of costa and subcosta, and those on the stem vein golden. Second basal cell present. Coxae and trochanters brown with yellow hairs. Femora and tibiae yellow to sand-coloured, except for their brown extremities. Hairs yellow but brown portions of segments with black hairs. Tarsi grey to dark brown with black hairs. No pedisulcus or calcipala. Claws simple.

Abdomen with brown dorsal surface. Ventral surface grey near thorax, mottled with dark brown, and shading to

solid dark brown towards genitalia. Pile pale yellow but dark on genitalia. Basal fringe pale yellow. Scutum quadrate a little more than 3.5 times as wide as long. Anal lobe reaching posterior edge of scutum. Ovipositor lobe reaching tip of anal lobe. The pattern of dark brown pigmentation along the medial margin of the ovipositor lobe is not sigmoid in shape and possesses more or less of a shoulder at the proximal end of the lobe (Fig. 11). Genital rod with little or no infilling at point of bifurcation. Arms ending in a triangular plate.

The name *fuscum* was applied to this species because of its general dark colouration (from fuscus (Latin) = dark-coloured, dusky).

Adult Male

Body length 3.0 mm. Wing length 3.0 - 3.5 mm. Antenna 11-segmented, tapering gradually from third segment; all segments black with black hairs. Clypeus black with black erect hairs. Maxillary palp as in female except the vesicle is smaller in relation to the size of the third segment: 0.33 times the length of the segment in material from the type locality and as small as 0.22 times the length of the segment in material from some other localities. Vesicle opens to exterior by a connecting tube, as in female. Occipital fringe black.

Pronotum and mesonotum black with pale yellow appressed pile. Scutellum dark brown with erect pale yellow hairs. Pleuron and membrane both dark brown; membrane sometimes

mottled as in P. mixtum sp. n. and occasionally light as in P. fontanum sp. n. Pleural tuft pale yellow or black with pale yellow tips. Halteres black with black hairs. Wing with hairs black except for those on base of costa, subcosta and stem vein, which may be yellowish. Subcosta with less than 50 hairs on it (Fig. 29). Legs all grey to black with black hairs and some yellow hairs on femora and tibiae.

Abdomen black with black sternites. Abdominal hair pale yellow, becoming black towards genitalia. Basal fringe long and with black bases to the hairs, but the tips tend to grey or yellow. Coxite about as broad as long; style conical, about 2/5 as long as coxite, incurved; the apex compressed and bearing two teeth or occasionally three. Sometimes one style bears three teeth, while the other bears only two.[1] Ventral plate (Fig. 14) broad with a short, blunt and relatively thick ventral crest. As seen from the lateral view, the loop at the base of the median sclerite of the aedeagus usually extends farther dorsally than in P. mixtum sp. n. and P. fontanum sp. n.

Pupa

Length of body 4.5 - 5.5 mm.; of respiratory organ 2.0 - 2.5 mm. This organ consists of 16 filaments arising

[1]Davies (1949) discusses variability in this character in a population that he called P. hirtipes and that later proved to consist of P. funcum and P. mixtum.

from three main trunks. The two ventral trunks each divide twice in a dichotomy to give rise to four filaments each. The median dorsal trunk divides into three branches near its base, the two outside ones dividing again to form three filaments each, and the median branch divides once to form two filaments. This is in perfect agreement with Puri's (1925) description of Norwegian material. There is considerable variation as to the actual points of bifurcation in this organ, so that individual filaments sometimes appear to arise separately from one of the main trunks A, B or C (Fig. 20). All the 14-filamented individuals seem to arrive at this condition by the failure of (10 + 11) and (12 + 13) to branch. Pupal chaetotaxy is illustrated in Fig. 28. Caudal hooks strong. Cocoon an irregular mass of silk covering most of the body.

Mature Larva

A large, medium grey to grey-green species with medium brown head capsule. The dorsal head pattern is not distinct from either P. mixtum or P. fontanum, nor is the occipital cleft of the head capsule (Fig. 25). Outermost submental teeth higher than the remaining lateral teeth which become progressively shorter towards the median line, giving a "dished" appearance to the submentum. The median trifid tooth usually as high as the outermost teeth but not exceeding them (Fig. 21). Subapical ridge of mandible with 12 - 20 forward-pointing teeth (Fig. 21).

Holotype, female: Kahshe River where it crosses #11 Highway south of Cravenhurst, Ontario. Pupa collected on April 27, 1956. Adult emerged on May 5, 1956. P.D. Syme.

Allotype, male: Adult emerged on May 7, 1956. Otherwise same data.

Paratypes, males and females pinned, dissected and in alcohol, many pupal skins, a few pupae and some larvae, all collected on April 27, 1956.

Other Material: males and females, larvae and pupae from North Madawaska River just below Lake Kasajewan Dam, Algonquin Park, Ontario. Males and females from streams near Caledon, Caledon Twp., Peel Co., Ontario, April 1 - 8, 1956; males and females from Hopkins Ck. at Dundas, Ontario, May 7, 1956 and May 1 - 7, 1957.

The type locality of this species is a large stream with a rather turbulent flow in April and May. It is about 25 ft. wide and has a rocky bottom where it emerges from under the highway bridge.

Prosimulium mixtum sp. n.

Adult Female

A medium-sized species. Of the three species described as new in this paper, *P. mixtum* is the smallest. Body length 2.5 - 3.0 mm; wing length 3.0 - 3.5 mm. Head black with appressed golden pilosity. Frons at antennae about 0.2 width of head and at vertex about 0.4 width of head. Clypeus brown,

covered sparsely with short yellow hairs medially, but more thickly and with longer hairs laterally and on the latero-ventral corners. These hairs converge ventrally towards the median line as they do in P. fontanum, but are not as coarse and black as in this species. Occiput with a fringe of yellow hairs with a few coarser, black ones arising posterior to the eyes. Antenna 11-segmented, tapering slightly from third segment and more abruptly from ninth, dark brown with yellow pile, but the coarser hairs are black in colour. Basal two segments slightly more yellow in colour, especially their distal edges, but not orange as in P. fontanum; 1 specimen in 10 had these segments orange. The two distal segments of the maxillary palp lighter brown than the third, dilated segment which contains the sensory vesicle. This vesicle is from 0.33 to 0.50 times the length of the segment, and opens on the anterior surface by means of a tube as in P. funcum (Fig. 6a).

Thorax mainly dark brown. Pronotum dark brown. Mesonotum uniformly dark brown except for humoral angles which are slightly lighter brown with a grey shade - hardly contrasting. Pile is short, golden and appressed on the central part of the dorsal surface, but longer on the lateral edges, and longer and erect posteriorly. The pile tends to curl rather than lie straight. Scutellum sand-coloured to brown with long, erect golden hair. Postnotum dark brown. Pleuron dark brown with a light and dark mottled brown to solid brown membrane. Metapleural tuft yellow. Haltere light brown with yellow hairs.

Hairs on most wing veins black, becoming yellower towards base of costa and subcosta, and those on stem vein golden. Second basal cell present. Legs brown except for mid portions of the femora and tibiae, which are more yellow-brown, especially on the ventral surface. The tarsi sometimes tend towards yellow. Hairs on the coxae, trochanters, femora and tibiae yellow; those on the tarsi brown. No pediculeus or calcipala. Claws simple or rarely with a minute basal tooth.

Abdomen uniformly dark brown except for some grey shading on the first segment dorsally, and a grey area on the ventral surface towards the proximal end. The ventral shading is more or less mottled with dark brown. Pile light yellow, darkening to brown on the genitalia. Basal fringe yellow. Cercus quadrate, slightly less than three times as wide as long. Anal lobe reaching the posterior edge of cercus. Ovipositor lobe almost reaching tip of anal lobe and generally smaller than that of P. fuscum (Fig. 50). The pattern of dark brown sclerotisation along the medial margin of the ovipositor lobe is rather sigmoid in shape, tending sometimes to be diffuse towards the outer edge (Fig. 12). Genital rod not at all to only slightly infilled at the point of bifurcation, and with each arm ending in a triangular plate.

The Inglis Falls population near Owen Sound, Ontario differs in having a larger (extends farther towards the distal end of abdomen) and less mottled grey area on the ventral surface of the abdomen. The pleural membrane is often, though

not always, lighter in colour yet still mottled with dark brown. The humeral angles of the mesonotum are often more shaded with grey, thus resembling P. fontanum in this respect. The hairs on the clypeus are somewhat coarser in this population, but not as coarse as the corresponding hairs on P. fontanum. The Inglis Falls individuals also are more rotund than the typical specimens.

The name mixtum was applied to this species because of its similarity to P. fuscum, resulting in confusion in identification. Also the species is often found developing in the same stream with P. fuscum (from mixtus (Latin) = intermingled, confused).

Adult Male

Body length 2.0 - 3.0 mm. Wing length 2.5 - 3.0 mm. Clypeus black with black, erect hairs. Antenna 11-segmented, tapering from third segment and more abruptly from ninth; all segments black with black hairs. Maxillary palp essentially as in the female, but more black than brown, and the size of the sensory vesicle in the third segment is smaller, 0.28 (0.26 - 0.33) times the length of the segment (Fig. 6b). Occipital fringe black.

Pronotum dark brown. Mesonotum uniformly dark brown with light golden pile. Scutellum brown (to sand-coloured in the Inglis Falls population) with erect, golden hair. Pleuron dark brown with slightly lighter membrane, mottled as in female. Pleural tuft pale yellow. Haltere dark brown

with dark hairs. Wing with all hairs black except those on the stem vein and on the squama, which are sometimes pale yellow to white. Subcosta with from 19 - 99 hairs depending on the population, but usually with more than 50 hairs (Fig. 29). Legs black to grey with black hairs except on the femora and tibiae, where they sometimes tend towards yellow.

Abdomen black, shading to grey on ventral surface with black sternites. Abdominal hairs yellow to black. Basal fringe long and black to grey. One specimen had yellow hairs on the abdomen, base of costa, and trochanters. Coxite about as broad as long; style conical, about 2/3 as long as coxite, incurved, the apex compressed, and bearing two teeth or occasionally three (See footnote page 27). Ventral plate (Fig. 15) broad with a longer and thinner ventral crest than that in P. fuscum. The loop at the base of the median sclerite of the aedeagus usually not as pronounced dorsally as in P. fuscum.

Pupa

Length of body 3.0 to 4.0 mm.; of respiratory organ 2.0 to 2.5 mm. This organ consists of 16 filaments arising from three main trunks with the same branching pattern as in P. fuscum. Pupal chaetotaxy essentially the same as that of P. fuscum (Fig. 28). Caudal hooks strong. Cocoon weak and loose with no definite shape.

Mature larva

By far the smallest of the three members of the

"hirtipes" complex treated in this paper. Body colour an even medium brown; colour of head capsule dark brown. Head pattern similar to that of P. fuscum, as is the occipital cleft (Fig. 20). All submental teeth usually of the same height (Fig. 22), but sometimes the lateral teeth become progressively higher towards the outside, giving a "dished" appearance as in P. fuscum (Fig. 23). The submentum shown in Fig. 22 is extreme and shows the greatest departure from the P. fuscum type. The median trifid tooth is usually of the same height as the two outermost teeth. The subapical ridge of the mandible resembling P. fuscum with 18 - 20 forward-pointing teeth (Fig. 22).

Holotype, female: Stream crossing 15th Sideroad at Con. 6, Chinguacousy Twp., Peel Co., Ontario; 2 miles N.W. of Terra Cotta. Pupa collected on May 1, 1956. Adult emerged on May 6, 1956. P.D. Syme.

Allotype, male: Adult emerged on May 4, 1956. Otherwise same data.

Paratypes, males and females pinned, dissected and in alcohol, many pupal skins, a few pupae and some larvae, collected May 1 and May 5, 1956.

Other material: adults, pupae and larvae from about 5 mi. west of Uphill, Ontario on Highway 1505, May 13, 1956. Adults, larvae, exuviae and a few pupae from an inlet to the Sydenham R. below Inglis Falls at the 16 Sideroad, Con. 6, Derby Twp., Grey Co., Ontario (Inglis Falls inlet), 1000 yd.

S. W. of Harrison Park, just south of Owen Sound, Ontario, June 23, 1956. Males and females from Hopkins Ck., where it crosses the York Rd. near Dundas, Ontario, May 7, 1956 and May 1 - 7, 1957.

The type locality of this species is a wide and extremely shallow stream with a slow velocity. Bottom of rocks covered with moss and water cress (Nasturtium).

Prosimulium fontanum sp. n.

Adult Female

Prosimulium fontanum to the unaided eye appears as a large yellow-orange species. Body length 2.5 - 3.5 mm. Wing length 3.0 - 3.2 mm. Head black with appressed golden pilosity. Frons at antennae about 0.35 times width of head, and at vertex about 0.38 times width of head. Clypeus brown with short yellow hairs dorsally and medially, and longer black hairs arising from the latero-ventral corners, their tips curving ventro-posteriorly and converging towards the median line. Occipital fringe golden. Antenna 11-segmented, tapering slightly from third segment and more abruptly from ninth; dark brown with black hairs except basal two segments which are orange-brown with black hairs. The first three segments of the maxillary palp are dark brown with black hairs; the distal two segments lighter brown with black hairs. Third palpal segment possessing a sensory vesicle about 0.38 times as long as the segment, and opening anteriorly to the exterior directly

by means of a wide mouth, with little or no connecting tube (Fig. 7).

Thorax mostly dark brown. Pronotum light grey-brown. Mesonotum uniformly dark brown except for anterior margin and humeral angles which are usually light grey-brown and contrasting. Pile is short, golden and appressed on the dorsal surface, becoming slightly matted posteriorly, and longer on the humeral angles and laterally. Scutellum light sand-coloured with long, erect, golden pile mixed with a few darker hairs. Postnotum dark brown. Pleuron dark brown with a light, sand-coloured membrane, occasionally darkened. Pleural tuft golden. Halteres yellowish sand-coloured with golden hairs. Hairs on most wing veins black, becoming yellower towards base of the costa and subcosta, and those on the stem vein golden. Second basal cell present. Coxae and trochanters brown and sand-coloured with golden hairs. Femora sand-coloured to yellow with yellow hairs, as are the tibiae except for their distal ends which are brown with darker hairs. Tarsi dark brown to grey with black hairs and occasionally with some yellow hairs on the basitarsi. No pedisulcus or calcipala. Claws simple.

Dorsal surface of abdomen brown, shading into lighter brown on the pleura, and into grey on the ventral surface. Pile golden, darkening towards the genitalia. Basal fringe long and golden. First abdominal segment grey dorsally, occasionally dark brown, and with light grey anterior and

posterior edges. Cercus quadrate, about three times as wide as long. Anal lobe reaching the posterior edge of cercus. Ovipositor lobe almost reaching tip of anal lobe. The pattern of dark brown sclerotization along the medial margin of the ovipositor lobe is similar in shape to that of P. nixtum (Fig. 15). Genital rod partially infilled at point of bifurcation, and with each arm ending in a triangular plate. The infilling at the point of bifurcation is not always as extreme as indicated in Fig. 10.

The name fontanum is applied to this species because the larvae are often found in small spring-fed streams (from fontanus (Latin) = pertaining to a spring).

Adult Male

Body length 2.5 - 3.5 mm. Wing length 2.5 - 3.0 mm. Clypeus black with black, erect hairs. Antenna 11-segmented, tapering gradually from third segment; all segments black with black hairs. Maxillary palp essentially as in female, except the sensory vesicle in the third segment tends to be smaller (0.25 times the length of the segment) with a smaller opening (Fig. 8b), and this vesicle occasionally possesses a neck connecting it to the exterior rather than opening directly (Fig. 8a). Occipital fringe black.

Pronotum dark brown. Mesonotum dark brown except for lighter anterior margin as in the female. This margin is browner and not as grey as in the female. Pile darker golden, slightly longer and sparser on the mesonotum than it is in

the female. Scutellum sand-coloured. Pleuron essentially as in the female with a darker membrane. Dorsal tuft of golden hairs with a few black; rarely entirely black. Halteres black with black hairs. Wing with all hairs black. Legs all grey to black with black hairs, except on ventral surface of fore-coxae, sometimes the mid-coxae, and all the femora, on which the hairs are yellow.

Abdomen black dorsally shading to grey ventrally with black stemites. Abdominal hair black. Anal fringe long and black. Gonite about as broad as long; style conical, about 2/3 as long as gonite, incurved, the apex compressed and bearing two teeth on all specimens seen (Fig. 19). Ventral plate broad with a long thin ventral crest as in P. mixtum. The loop at the base of the median sclerite of the aedeagus usually not as pronounced dorsally as in P. fuscum (Fig. 18).

Pupa

Length of body 5.5 - 6.0 mm.; of respiratory organ 2.0 - 2.5 mm. This organ consists of 16 filaments arising from three main trunks with the same branching pattern as in P. fuscum and P. mixtum. Pupal chaetotaxy is the same as that of P. fuscum (Fig. 28). Caudal hooks strong. Cocoon very weak, consisting of only a few threads.

Mature larva

A large light brown species with medium brown head capsule. Dorsal head pattern similar to that in P. fuscum

and P. mixtum. Occipital cleft as shown in Fig. 27. Outermost submontal teeth enlarged, higher than the remaining two lateral teeth which are usually of equal height. The median trifid tooth usually as high as, or higher than , the outermost lateral teeth (Fig. 24). Subapical ridge of mandible with 18 - 20 upright teeth (Fig. 24).

Holotype, female: First small stream on the Tote Road on the east side of Lake Sasajewun, a mile from the Wildlife Research Station, Algonquin Park, Ontario. Pupa collected on June 22, 1956. Adult emerged on June 26, 1956. P.D. Syme and D.M. Davies.

Allotype, male: Pupa collected on June 20, 1956 and adult emerged June 29, 1956. Otherwise same data.

Paratypes, males and females pinned, dissected and in alcohol, many pupal skins, a few pupae and larvae, all collected June 20 and 22, 1956.

Other Material: males and females from second stream on the Tote Road 1/2 mi. north of the type locality. Males and females, larvae and exuviae from streams entering Ottawa River near Chalk River, Ontario, (at North Star Lodge, and crossing Laurentian Pt. road). Males and females, larvae and pupae from stream opposite Bates' Island on west shore of south arm of L. Opeongo 1½ mi. from Ontario Fisheries Research Laboratory, Algonquin Park, Ontario. Males and females from a bog-fed stream on the north shore of Sproule Bay, L. Opeongo, opposite Ontario Fisheries Research Labor-

atory . . Males and females from Smith's L. inlet in Algonquin Park.

The type locality of this species is a small bog-fed stream, which is reduced to a trickle in June, but still remains cool. The pupae were found usually embedded in the moss that covered the rocks in the stream.

Keys to P. fuscum, P. minimum and P. fontanum

Adult Females

A Ovipositor lobes with the median sclerotization pattern expanding laterally into a shoulder at the proximal end of the lobes (Fig. 11). Sensory vesicle in the third segment of the maxillary palp with a tube connecting it to the exterior (Fig. 5). Emerges in the early spring.---------- P. fuscum

A Ovipositor lobes with the sclerotization pattern sigmoid in shape and no shoulder at the proximal end of the lobe (Figs. 12 and 13). Sensory vesicle with or without a tube connecting it to the exterior. Emergence from early spring to mid summer. ---------- B

B Sensory vesicle with a tube connecting it to the exterior (Fig. 6a). Basal two segments of the antennae only slightly lighter in colour than remaining segments. Humeral angles of thorax not usually contrasting. Pleural membrane light brown mottled with dark brown, to

solid dark brown. Abdomen dark brown and thoracic pilosity yellow. Emerges from early spring on. ---- P. mixtum

B Sensory vesicle with little or no tube connecting it to the exterior; external opening wider in either case (Fig. 7). Basal two segments of antennae orange-brown and contrasting. Humeral angles of thorax lighter and usually contrasting. Pleural membrane usually sand-coloured. Abdomen brown to light brown. Thoracic pilosity golden. Emerging from late May to August. ---------- P. fontanum

Adult Males

A Sensory vesicle usually without a tube connecting it to the exterior (Fig. 8b). Pleural membrane usually sand-coloured. Ventral plate with thin ventral crest (Fig. 16). ---------- P. fontanum

A Sensory vesicle with a tube connecting it to the exterior. Pleural membrane light brown to dark brown. Ventral plate with either a thick or thin ventral crest. ----- B

B Ventral plate with a shorter thick ventral crest (Fig. 14). Hairs on subcosta usually less than 50.--- P. fuscum

B Ventral plate with a longer thin ventral crest (Fig. 15). Hairs on subcosta usually more than 50. ------- P. mixtum

Mature Larvae

A The two innermost lateral teeth of submentum distinctly lower than the outermost tooth, and of equal height (Fig. 24). Subapical ridge of mandible with upright teeth (Fig. 24). A large light brown species with medium brown head capsule. ---------- P. fontanum

A The three lateral teeth of equal height or grading down from the outermost tooth. Subapical ridge of mandible with forward-pointing teeth. ---------- B

B The three lateral submental teeth often of equal height (Fig. 22); occasionally grading down from the outermost tooth. A small brown species with dark brown head capsule. ---------- P. mixtum

B The three lateral submental teeth always grading down from the outermost tooth (Fig. 21). A large grey species with light brown head capsule. ---------- P. fuscum

Comparison of Diagnostic Characters and Their Variations

The three Ontario members of the hirtipes complex are quite similar in appearance and it seems worthwhile to summarize the points of distinction and to mention others that were not brought out in the description.

Of the three, P. fontanum is the most distinct species in both the larval and adult stages. Thus, the toothing of both the larval mandibles (Fig. 24) and the submentum (Fig. 24) is distinct from either P. fuscum or P. mixtum. The colour and size of fontanum larvae also aid in distinguishing them. The adult female of fontanum, being large and tawny, and possessing a darker golden pilosity, light humeral angles, sand-coloured pleural membranes and orange basal segments of the antennae, stands out from the females of the other two species. The shape of the sensory vesicle in the maxillary palp is the most definite diagnostic character in both sexes of fontanum. This vesicle was as indicated in Fig. 7 in more than 20 female specimens and in 19 male specimens, but in one male specimen it was flask-shaped (Fig. 8a). A few P. mixtum specimens also possessed flask-shaped vesicles (Fig. 6c), rather than the usual type (Fig. 6b). Aside from the vesicle, the only other really diagnostic character in male fontanum specimens is the light-coloured pleural membrane. Even this was occasionally dark in a few specimens of both sexes that resembled P. mixtum in all characters except the sensory vesicle.

The remaining two species, P. mixtum and P. fuscum, are much more similar in all stages. As larvae, P. fuscum is generally larger and greyer with a lighter brown head capsule than P. mixtum. The toothing of the mandibles is of no help in distinguishing between the two species, and the

submental tooth, although differing in shape, vary enough to make this means of identification unreliable in many cases. The ratio of the height of the median trifid tooth (distance "a" in Fig. 21) to the width at the base of the submental teeth (distance "b" in Fig. 21) was calculated, but the difference was slight . For twelve specimens each the average ratio arrived at was 0.249 (0.209 - 0.287) for P. fuscum and 0.281 (0.219 - 0.333) for P. mixtum. Thus the submental teeth in P. fuscum are generally wider and lower and usually have a "dished" appearance whereas P. mixtum tends to have these teeth higher and of an even height. Also a slight difference in the mouth fans was found in the ratio of the primary filament length to the stalk length. Histograms of the frequencies of these ratios are plotted in Fig. 31. It can be seen that the filaments of the mouth fan are longer in proportion to the stalk length in P. fuscum than in P. mixtum. No differences were found in the larval antennae or maxillae.

The adults of these two species are even more similar than the larvae. Thus of the two, the female fuscum specimens have a paler yellow pilosity on the thorax and a generally darker body colouration, but females of both are darker in body colour and somewhat lighter in pilosity colour than P. fontanum. There is a more pronounced reddish shade on the anterior surface of the humeral angles of the thorax in P. fuscum than on that in P. mixtum. The antennae

of P. fuscum females were found to be slightly longer than those of P. mixtum females. The average length of the female antenna in nine specimens examined was 0.73 mm. (0.66 - 0.80 mm.) for P. fuscum, and 0.56 mm. (0.45 - 0.68 mm.) for P. mixtum. Also the ratio of the third antennal segment to the tenth was less for P. fuscum females, averaging 1.42 (1.20 - 1.67) as compared to 1.81 (1.50 - 2.00) for P. mixtum. A slight difference was also noticed in the dimensions of the L-shaped anal lobe. The average length of the postero-ventral arm of the anal lobe in six specimens of each species was 0.16 mm. (0.14 - 0.17 mm.) for P. fuscum and 0.13 mm. (0.12 - 0.14 mm.) for P. mixtum. There was no real difference in the length of the dorsal arm of the anal lobe because although measurements of the dorsal arm averaged longer in P. fuscum, those in P. mixtum fell entirely within the range of variation for P. fuscum. There is, however, a difference in the size of the ovipositor lobes, those of P. fuscum being longer and wider (Fig. 30). The only reliable diagnostic character found was the shape of the sclerotization pattern on the ovipositor lobes as indicated in Figs. 11 and 12.

In the males of P. fuscum and P. mixtum the number of hairs on the subcosta provides a diagnostic character that is useful in most cases (Fig. 29), while the ventral plate of the male genitalia is stouter and has a thicker, shorter ventral crest in P. fuscum as compared to the same organ in P. mixtum (Figs. 14 and 15).

No differences could be found between the pupae of P. nixtum and P. fontanum, but the respiratory filaments of P. fuscum are shorter in proportion to the length of the pupal body than in P. nixtum or P. fontanum.

The New Species Compared With Related Members of the Complex

The three new species of the "hirtipes" complex in Ontario having been described, it seems worthwhile to compare them with their closest allies in both North America and Europe, and to point out distinguishing features that have come to the notice of the writer.

The larval individuals in the National Collection at Ottawa, collected in June and September 1949 near Whitehorse, Yukon Territory and previously identified as P. hirtipes, were found by the writer in 1957 to most resemble P. fontanum in the form of the submental teeth. Also the drawing of the Alaska hirtipes by Sommerman (1953) shows a similar resemblance. However, a collection of larvae from Alaska sent recently to Dr. Rothfels by Dr. Sommerman contained specimens that were cytologically "hirtipes 2". It has not yet been established whether the "hirtipes 2" larvae from Alaska are of the same species that possesses the P. fontanum type submental teeth. Specimens sent from Fort Richardson, Alaska to the writer by Dr. Sommerman also have the P. fontanum type submental teeth, but otherwise do not

resemble either P. fontanum or P. mixtum as larvae. There is even more contrast between the outermost and the remaining lateral teeth in these specimens than in P. fontanum. In the adult stage they agree with P. fontanum in having a light pleural membrane and a relatively light abdomen, but differ in the colour of the thorax and the shape of the sensory vesicle which possesses a tube to the exterior.

Procladius hirtipes (Fries), as defined by L. Davies (1951), and which is most likely the true hirtipes, is in the female at least according to the specimens examined in this study, a large grey dark-legged species looking superficially most like P. fuscus but differing from it in the pattern of sclerotization of the ovipositor lobes (Fig. 11), in which it most resembles P. mixtus (Fig. 12). The colour of this sclerotization is distinctly grey in hirtipes, whereas in P. mixtus and P. fontanum it is brown, and in P. fuscus it possesses at least a brown shade. The sensory vesicle in the third segment of the maxillary palp is essentially as in P. fuscus and P. mixtus, i.e., it possesses a collar, but it again is grey in colour whereas the same organ in the three North American species is brown. It appears from specimens examined by the writer and from the drawings of L. Davies (1957), that the cercus of P. hirtipes (Fries) most resembles that of P. mixtus, but there is too much variation of this character, at least in the North American material, to make it useful.

P. inflatum Davies differs from all our species in the presence of yellow hairs on most of the wing veins, the shape of the clypeus, which is inflated, and the greater infilling at the point of bifurcation of the genital rod. In the larva, the submental teeth resemble those of P. fontanum (Fig. 24), but the outermost teeth are even larger in P. inflatum. Also, in the larva, the mandibular phragma of P. inflatum extends ventrally farther than in any of our species, resembling that in P. ureinum (Edw.). The entire head capsule of mature inflatum larvae is jet black to dark brown, whereas in the Ontario complex that of P. mixtum is by far the darkest but is never black.

The female of P. tridentatum Rubtz. (Rubtzov, 1956), differs from that of P. fontanum by the colour of the antennae which are all black. It differs from our three species in that the maxilla has only three strong teeth on each side. The larvae differ from our species in the shape of the submental teeth which are of the P. fuscum type (Fig. 21), but more markedly "dished". P. hirtipes luganicum Rubt. differs from all our species in being as large as P. fuscum but possessing dull golden pilosity on the thorax rather like P. mixtum or fontanum. The thorax is greyish-black, resembling P. fuscum in this respect, but the haltores are yellow. The ovipositor lobes are smaller than those of P. fuscum and possess a pattern of sclerotization similar to that of P. mixtum. Females of P. h. diminutum Rubtz. compare in size to

those of mixtum and fontanum but the colour of the integument is black and the pilosity is silvery. The female genital rod, according to Rubtzov's figure, differs from our three species in the shape of the end plate. The larval submental teeth of P. h. diminutum most resemble those of P. fuscum (Fig. 21), but are more extreme in their "dished" appearance.

The North American P.rhizophorum Stone differs from all our species in the form of the pupal respiratory filaments, which can also be seen and recognised in mature larvae. The pupa of rhizophorum differs, in the three specimens examined, from P. fuscum, mixtum and fontanum also in the absence, or great reduction, of spines 1 and 2 on the ventral surface (Fig. 23).

P. ursinum (Edwards), a 14-filamented species, was also examined. The claspers of the male possessed two teeth, as described by Stone (1952), but the adminiculum was much stouter (Fig. 17) than that of P. fuscum. The sensory vesicle in the maxillary palp was also much smaller than any of the new species at least in the female, about 0.25 times the length of the segment in the male (Stone, 1952), and about 0.29 times the length of the segment in the female, and possessed a wide opening somewhat similar to that in P. fontanum (Fig. 10). The ovipositor lobes were similar to those of P. mixtum, but the pigmentation was more diffuse, and the anal lobes were as in Twinn's (1936) drawing (under

the name P. browni Twinn, a synonym), and this certainly distinguishes it from any of P. fontanum, fuscum or mixtum. The pupal chaetotaxy was the same as in P. fuscum, P. mixtum and P. fontanum. The respiratory filaments exhibit the same type of branching as those in the latter three species, except that (10 + 11) and (12 + 13) fail to branch, giving 14 filaments. This is the same situation that exists in P. fuscum individuals that have only 14 filaments. In three specimens examined, one had 16 filaments and the others had 14. In the larva, the mandibular phragma extends almost to the mentum, and the pigment pattern on the head is as pictured by Sommerman (1953). The median submental tooth is not always small, as described by Sommerman (1953) however.

Examination of paratypic pupal material of P. travisi Stone disclosed that the respiratory filaments exhibit essentially the same branching as in fontanum, fuscum and mixtum. However, the stalks seem to be shorter, and therefore the filaments longer. Pupal chaetotaxy shows no significant differences. In an adult female dissected from a pupa, the ovipositor lobes were short, with a pattern of sclerotization similar to that in P. mixtum. The sensory vesicle (Fig. 9) was similar to that in fuscum or mixtum. The anal lobes were short and barely exceeded the base of the stubby, rounded cercus.

The bitribes females reported from Minnesota (Nicholson and Mickel, 1950) appear from the description to be

either P. fuscum or P. mixtum, but closer to the former. The hirtipes reported from Utah (Peterson, 1955) is now considered to be something other than hirtipes (Peterson, 1957, pers. comm.).

Distribution of the Three New Pressinaldium Species

P. fuscum, P. mixtum and P. fontanum all occur in Central Ontario and Southern Quebec (Figs. 32, 33 and 34). From here P. fuscum and P. mixtum extend farther north and east to Labrador and the Maritime Provinces, west to the Bruce Peninsula and south into New York State, and possibly into Minnesota (Nicholson and Nickel, 1950). P. mixtum extends even farther north in the north-east to Fort Chimo, Quebec, and what corresponds to P. mixtum cytologically (i.e. "hirtipes 2" of Rothfels 1956) occurs in Alaska in the north-west (Rothfels, 1957, pers. comm.).

P. fontanum, according to present knowledge, is fairly well restricted to the granite portions of Ontario and Quebec, mainly the Precambrian Shield, except for one collection at Knowlton, Quebec, about 55 miles E.S.E. of Montreal. However, there are Precambrian rocks scattered in that area, and it is possible that the particular stream in question passes through granite.

"Hirtipes 4" of Rothfels until now has been found only near Uphill, Ontario and Trenton, Ontario (Rothfels, 1956, pers. comm.).

Of the four species, P. fuscum, P. mixtum, P. fontanum and P. "hirtipes 4", P. mixtum appears to be the most widely distributed and the most common and P. fuscum comes a close second.

Lack of material west of Georgian Bay, Ontario is possibly due to insufficient collecting in this area since the known distribution of these species centres around the areas of active black-fly research, namely the Toronto-Hamilton, Algonquin Park and Ottawa districts.

Ecological Observations

Feeding and Mouthparts of Adult Females

The females of the three new Ontario species possess typical biting mouthparts, averaging about 28 (24 - 31) teeth on the maxillae, and 47 (43 - 54) on the mandibles. There appeared to be no marked differences in the number of teeth on the mouth appendages between the species, nor in the form of the appendages themselves. Both P. fuscum and P. mixtum were found biting humans, and P. fontanum crawling on them. That these three species have piercing and sucking mouthparts and that all probably feed on mammals is consistent with the newly emerged females of all having little stored nutrient and eggs little developed.

Seasonal Emergence

A study was made of the seasonal emergence of the three species from several streams in Ontario to show any

characteristic differences in their emergence patterns.

Collections from Hopkin's creek in 1957 and from two streams in Algonquin Park in 1940 and 1947 showed a mixed population of P. fuscum and P. mixtum. A comparison of the seasonal emergence of each sex of these two species together with the maximum and minimum water temperatures between each collection is shown in Table I.

In Hopkin's Creek the population of Prosimulium was low, and the repeated disturbance of the cage by vandals decreased markedly the value of the results. The fact that only fuscum females were collected suggests that the cage was set up too late for the males, although earlier the stream had been searched unsuccessfully for pupae. That none were found was probably because of the low density of the population.

Larger collections of P. fuscum and P. mixtum were obtained in 1940 and 1947 in Algonquin Park. These two seasonal collections gave a good picture of what had already been suspected from earlier field work by the writer and others. Thus, P. fuscum emerged a few days earlier than P. mixtum, and the males of both species preceded the females by a few days.

A third collection made by Dr. Davies in 1946 from a bog-fed stream, Smith's Lake inlet in Algonquin Park proved to have all three species of the complex present, and this collection was analyzed to determine the relation between

emergence times of P. fontanum and the other two species. The results of this analysis are presented in Table 1.

P. fontanum emerged later than either P. fuscum or P. mixtum, and with little overlapping in the spring generation at least. This picture would be different if there were a second generation of P. mixtum present. The male emergence of P. fontanum preceded that of the females by a few days as in P. fuscum and P. mixtum.

The data obtained from the various emergence collections is summarized in Table 2. In each instance, the date of first emergence, the date of last emergence, and the date when 50% of the emergence was completed (midpoint) is recorded, along with the average water temperature during the emergence span, and the relative abundance of each species of the Prosimulium population. It is evident that where the two species exist together, fuscum usually begins to emerge a day or two ahead of mixtum, and where fontanum is present in the same stream it is by far the latest to begin emergence.

In each instance, except the Hopkin's Ck. collection in southern Ontario, the average water temperature during the emergence span is about 52° F. When this temperature is greater (Costello Ck. 1940) there is not such a marked difference between the first emergence of fuscum and mixtum. Both species develop faster at higher temperature as indicated by the Hopkin's Ck. collection, and consequently in southern Ontario where the streams warm up more quickly both fuscum

and mixtum emerged half a month earlier than in Algonquin Park.

Fontanum obviously develops more slowly than fuscum or mixtum because when fontanum occurred in the same stream as the others and therefore was subject to the same conditions of temperature, it emerged considerably later than the latter two species. Since there is no evidence to indicate that fontanum will tolerate higher temperatures than the other two, it follows that fontanum requires a stream that retains an average daily temperature of about 55°F. or less during its development.

It can be seen from Tables 3 and 4 that both P. fuscum and P. mixtum are typical Prosimulium species, emerging as adults during the early spring months, at least in the southern and central portions of Ontario, northern New York State, and southern Quebec. P. fontanum, on the other hand, which has so far only been found in central Ontario and southern Quebec, emerges from late May to August (Table 5).

It is felt that both P. fuscum and P. fontanum are single-brooded, while P. mixtum has been collected as mature larvae by Dr. Rothfels in March and April, and again in July and August of 1956 in the streams near Terra Cotta, Peel Co., Ontario, indicating that there sometimes exists a complete or partial second generation, or an extremely attenuated first generation. Davies (1950) found no such situation in P. mixtum in Algonquin Park.

P. fontanum, although not possessing a second generation, appeared sometimes to have a very drawn out emergence span. The pupation period of this species in the Tote Road stream at Lake Nasajewan occurred from mid-June to August in 1956. At Smith's Lake Inlet, however, its emergence was completed in June, 1946.

Habitat Preference

An examination of Ontario streams, other than those used for emergence studies provides a basis for understanding the habitat preference of each species.

The three species inhabit different types of streams, although P. fuscum and P. mixtum appear to overlap broadly in their requirements of habitat since they are often found together in the same streams. P. fuscum will inhabit larger, more rapid streams, such as those that drain medium-sized lakes on the Precambrian Shield, whereas P. mixtum will usually not. On the other hand, P. mixtum will inhabit streams of much slower current where P. fuscum would not be found. Both species, however, will inhabit smaller streams or creeks of moderate current, and in such streams are usually found together.

P. fontanum differs markedly in its preference of habitat by inhabiting small streams that originate from a bog or spring, usually in wooded country. These streams tend to keep a relatively constant cool temperature averaging about 55°F. during the pupation period. Actually, this

temperature differs little from those found in the streams inhabited by P. fuscum and P. mixtum during their pupation periods. Over a two year period, 1956 - 57, the average water temperature of several streams across Ontario (other than those used for emergence study) inhabited by P. fuscum was 53.7°F (46.5 - 60.0°F) from April 1 to May 8, the pupation period of this species. For a similar interval from April 26 to May 18, the average water temperature of streams inhabited by P. mixtum was 56.5°F (50 - 67°F) and from June 17 to June 22, the average water temperature of streams inhabited by P. fontanum was 54.1°F (47 - 60°F).

Thus, during the pupal period of the black flies the average water temperature did not differ significantly for streams inhabited by each of the three species, but the actual time that the black flies inhabit the streams as pupae did differ markedly in the case of P. fontanum. This emphasizes that streams that have an average water temperature of about 50°F during April and May, but become much warmer during June and July are not suitable for the slowly developing P. fontanum. Only streams that retain a temperature of about 55 - 60°F until July or August appear to provide a suitable habitat for this species. This may be related to the distribution of P. fontanum, since cool bog-fed streams are characteristic of the granite portion of Ontario where P. fontanum is found.

It does appear that P. mixtum will tolerate streams

of not only slower velocity, but also of slightly higher temperature than those inhabited by P. fuscum, as evidenced by the higher average temperature, 56.3°F, of those streams, compared to 53.7°F for P. fuscum. Also, the maximum temperature from the same data is higher for P. mixtum streams (67°F) than for P. fuscum streams (60°F). Where mixtum was found to breed in the same stream with fuscum, it was only half as abundant (Table 2).

All three species have been found in the larval stage on rocks and vegetation, although P. fuscum occurs in higher numbers and more often on rocks in larger streams of swifter current than do the other two. This species pupates in masses on rocks, the rather extensive cocoons often adhering to form a silken mat that binds the pupae together.

P. mixtum larvae, on the other hand, are usually more scattered and seem to prefer vegetation. They pupate singly, although sometimes quite thickly in vegetation, and never in large groups on rocks.

P. fontanum larvae are also usually quite scattered and pupate singly in the vegetation of the stream. Their cocoons consist of only a few strands of silk and sometimes the pupae are found lying naked in the sand at the bottom of a small pool.

It appears as if the amount of cocoon built by each species is related to the type of stream inhabited. Thus, P. fuscum with the heaviest cocoon of the three is more exposed

to abrasive action of particles, and to being torn off its support by water current in the larger, faster-flowing streams it inhabits. P. mixtum, which inhabits streams of slower velocity, also constructs weaker cocoons while P. fontanum, which usually inhabits streams of even slower current, constructs the weakest cocoon of all. This relation does not hold in every case because of the variety of stream types inhabited by each species, but in general, there does appear to be a relationship between type of stream inhabited and amount of cocoon built.

DISCUSSION

Earlier descriptions (Malloch 1914; Dyar and Shannon 1927; Nicholson and Mickel 1950; Stone and Jamnback 1955; Twinn 1956; D. Davies 1949) of *P. hirtipes* from North America indicated a considerable variation in the morphology and colouration of this species, as summarized by L. Davies (1957). It can be seen that much of this variation is caused by "*Prosimulium hirtipes*" in North America being in reality a complex of very closely related species. Another reason for the discrepancies between descriptions is that even species other than members of the complex may have been included. Malloch (1914) mentioned that the pupal respiratory organ of *hirtipes* may have 60 branches and his illustration of the submental teeth corresponded to those of *Prosimulium multidentatum* Twinn, a species not unlike *P. hirtipes* in the adult stage. Of course, a third reason for the discrepancies between descriptions is that the species do vary in colour and morphology.

Of the three species described in this paper, *P. mixtum* appears to vary the most. This species exhibits considerable variation in number of hairs on the male subcosta, in colour of the pleural membrane and of the abdominal integument, in the size of the body as indicated mostly by the

fatness rather than length, and in the larval submental teeth. The Inglis Falls population near Owen Sound, Ontario was strikingly different from all other P. mixtum examined and corresponded to P. fontanum in characters such as size, general body colouration and colour of the pleural membrane. This was so marked that it is felt that this population may prove to be a new sibling. It is, however, cytologically a "hirtipes 2" (= P. mixtum) type (Rothfels 1956, pers. comm.), and corresponds to P. mixtum in that the pattern of sclerotization on the ovipositor lobes and the shape of the sensory vesicle in the maxillary palp is definitely of the P. mixtum type. Although differences do exist it was felt that the evidence was insufficient to separate it from P. mixtum at this time. Although the differences in certain characteristics between the Inglis Falls population and the type population of P. mixtum are in some specimens greater than those between P. mixtum and P. fuscum, there is no other evidence that the Inglis Falls population is specifically distinct from the type population of mixtum.

It must be remembered here that the three species, P. fuscum, P. mixtum and P. fontanum, were not erected as species initially on morphological grounds, but on cytological evidence and on indirect evidence of their inability to interbreed. That is, the cytological studies presented strong evidence that these species did not interbreed, or at least that if they did, the resulting zygotes were inviable.

Actually "hirtipes 2" (= P. mixtum) varies cytologically more than the other two species (Rothfels, 1956, pers. comm.) and further work is needed to establish the full limits of this species.

The other two species, P. fuscum and P. fontanum, appear to be more stable in morphological characteristics. It was this stability that enabled these species to be distinguished on morphological grounds.

It was known of course at the beginning of this study that three species existed, and it was known also how pure cultures of each could be obtained and identified. Now came the search for morphological differences that would stand up to comparison over a reasonable series of individuals. This was successful, and the fact that the specimens grouped according to their morphological characteristics corresponded to the cytological grouping, confirmed the validity of the species.

As mentioned in the introduction, a fourth member of the complex exists in Ontario, and it may be necessary when describing this to resort to finer details of morphology in order to distinguish it from the three described species.

Further support for the distinctness of the three species is provided by the ecological evidence gathered during the study. P. fontanum stands out uniquely in this respect since its habitat and span of emergence differ markedly from those of the other two species. The cool, small

spring- or bog-fed streams inhabited by this species differ in one respect from most of the streams harbouring P. fuscum or P. mixtum in that they remain cool for most of the summer. It is possible that any of these streams could harbour P. mixtum and P. fuscum in the early spring, as indicated by the Smith's Lake inlet collection in 1946 (Table 1).

Even the distribution of P. fontanum, its restriction to the granite portions of Ontario and Quebec, distinguishes it somewhat from the more widely distributed P. fuscum and P. mixtum. The latter two even in their habitat preference are similar and, as in most of their morphological characters, overlap broadly in their ecological and phonological peculiarities. But the fact that these two species overlap rather than coincide in these peculiarities again suggests, rather than refutes their distinctness.

CONCLUSIONS

From the inquisitiveness of a cytologist in a purely cytological search has arisen a new problem in the identity of species. In this case, however, cytology not only posed the problem, but also aided in solving it. That is, cytology provided the means of distinguishing the species before the other characteristics were known. After this it was a relatively simple step to the morphological description of the new species. This led by a chain of events to the fulfilment of the need of an adequate description of the morphology of the sensory vesicle in the maxillary palp of simuliids, the structure of the vesicle being a useful key character.

The initiation, development and solution of this problem indicates a new approach to taxonomy where the initial groundwork is provided by cytology, the descriptive work by morphology and further substantiation by ecology. Thus once the Ontario species of the "*hirtipes*" complex were clearly separated on chromosomal characteristics, it was possible to characterize them more readily on morphological grounds as well as on differences in distribution, habitat preference, and growth as expressed by the seasonal emergence patterns of the adult flies. This work suggests that further research to clarify the classification of the

family Simuliidae could well follow the same procedure, and Dr. Rothfels has already discovered that other so-called species of black flies are really species-complexes.

The above emphasizes the close inter-relationship that must exist between the taxonomist and his fellow workers in other fields of Biology if a modern approach and a complete understanding of the species problem is to be achieved.

Fig. 1 Section of wall of sensory vesicle.

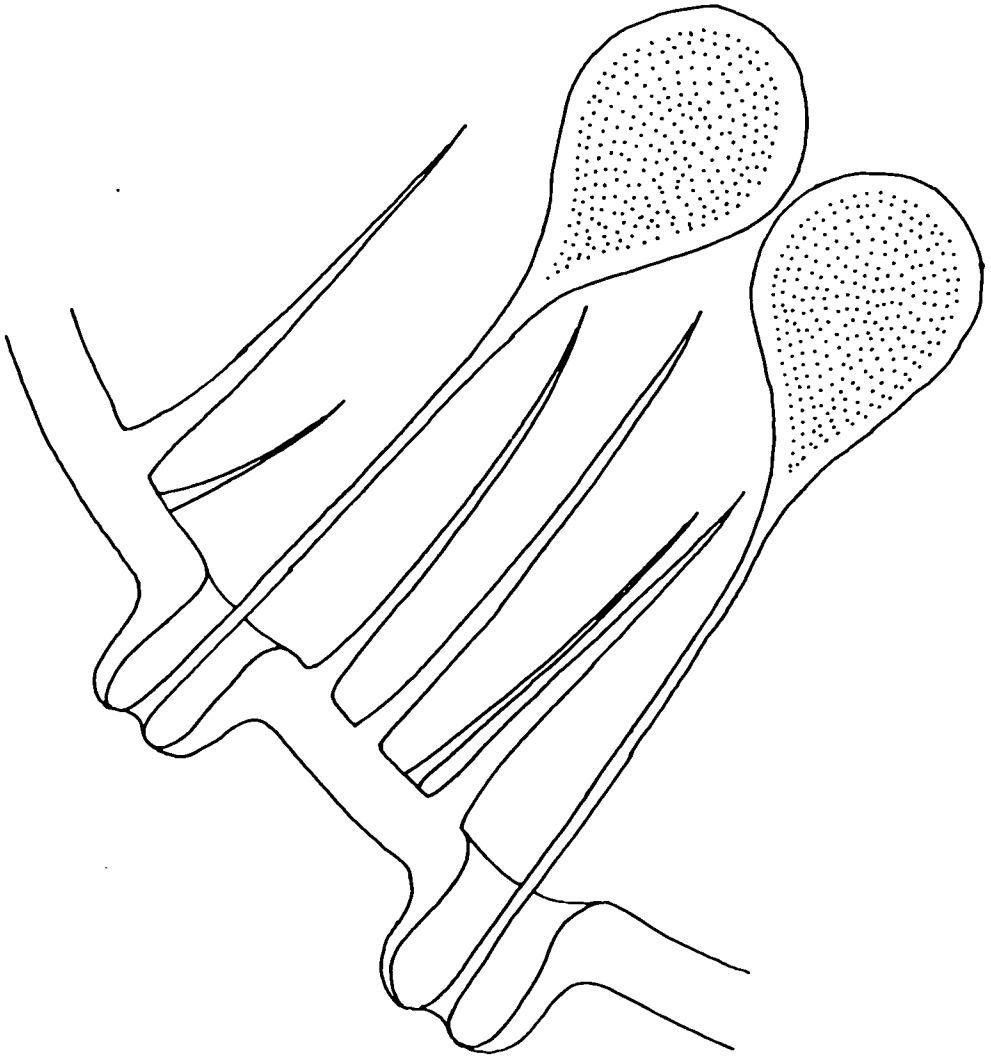

FIG. 1

Fig. 2 Serial longitudinal sections of third segment of maxillary palp showing structure of sensory vesicle in P. fuscum.

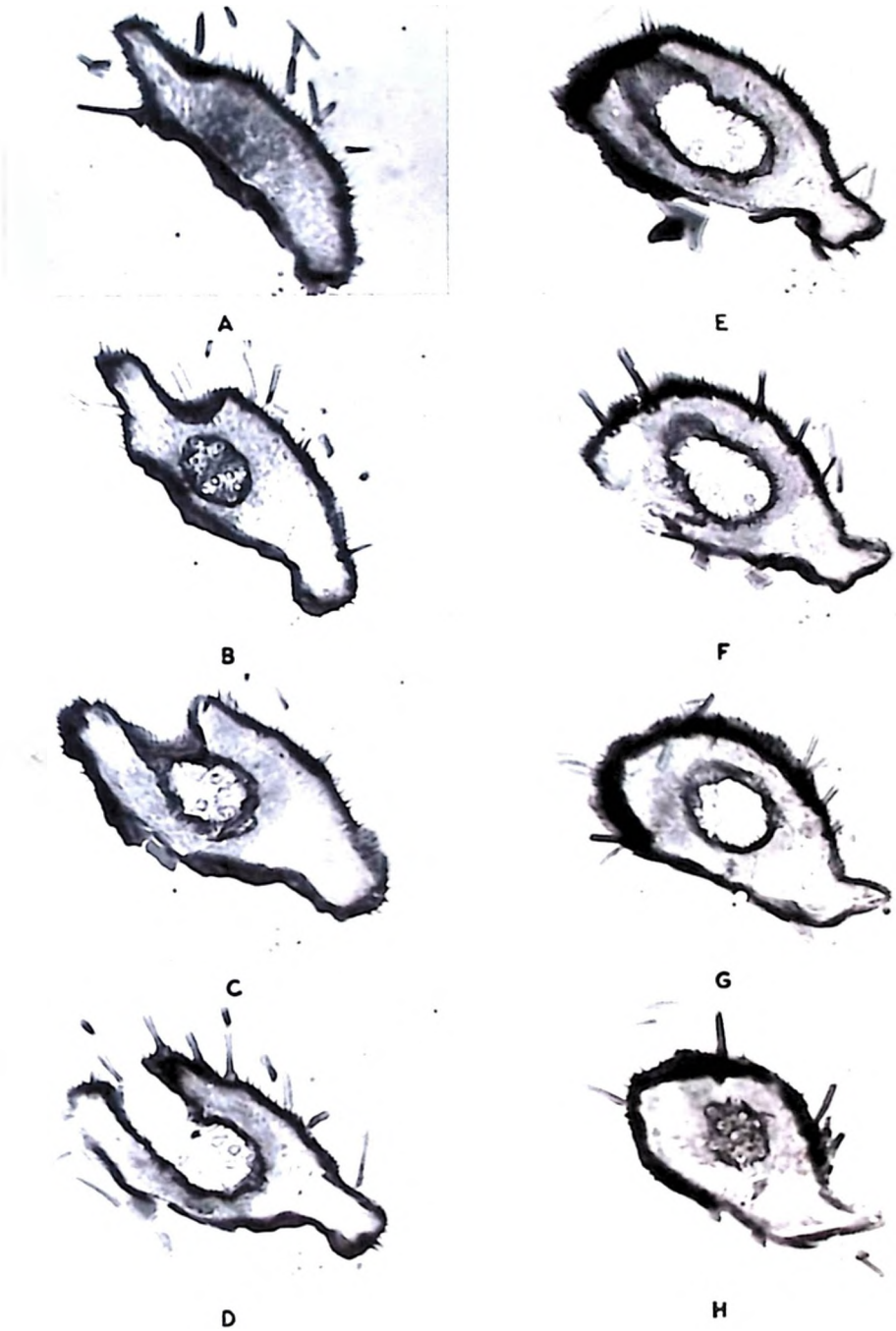

FIG. 2

Fig. 3 Longitudinal section of third segment of maxillary palp showing structure of sensory vesicle in P. mixtum.

Fig. 4 Serial transverse sections of third segment of maxillary palp showing structure of sensory vesicle in P. fontanum.

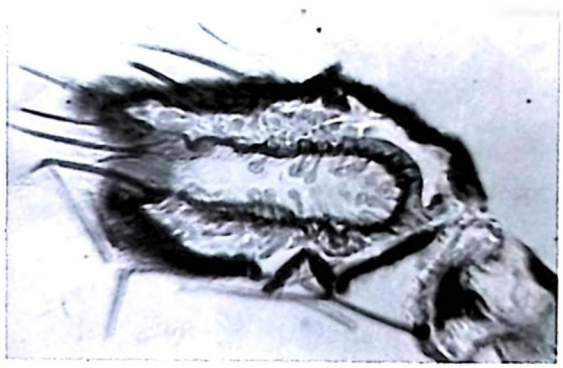

FIG. 3

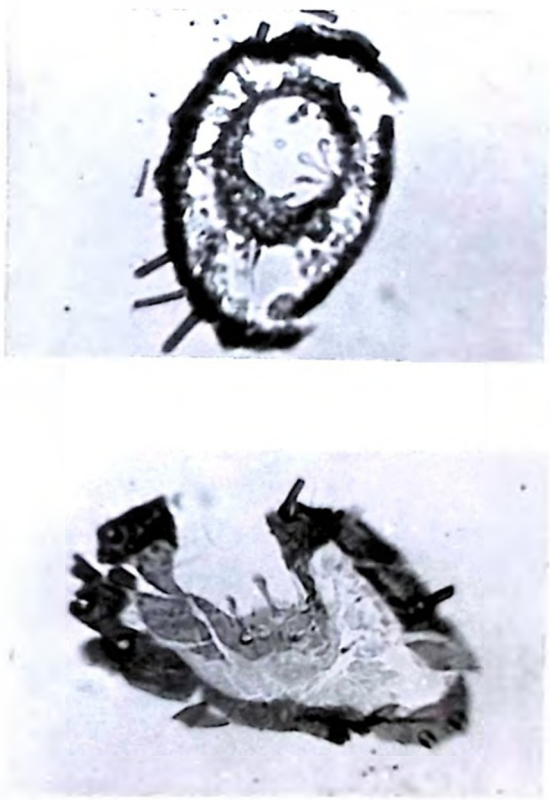

FIG. 4

Fig. 5 Sensory vesicle in third segment of maxillary palp of three P. fuscum females.

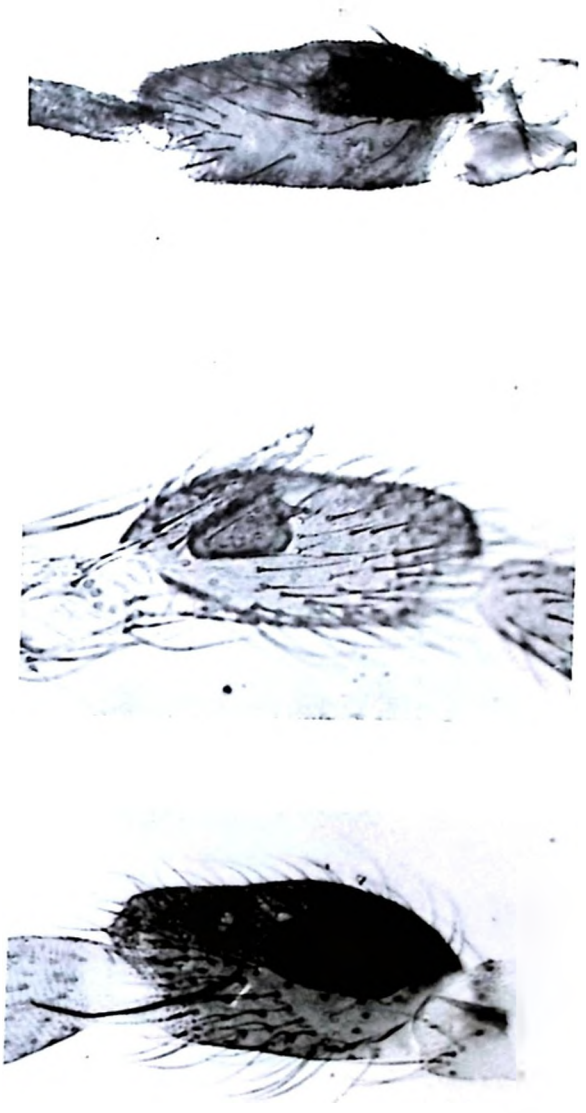

FIG. 5

Fig. 6A Sensory vesicle in third segment of maxillary palp of P. mixtum female.

B Same of P. mixtum male.

C Same of P. mixtum male (abnormal).

A

(FEMALE)

B

C

(MALES)

FIG. 6

Fig. 7 Sensory vesicle in third segment of maxillary palp of three P. fontanum females.

FIG. 7

Fig. 8A Sensory vesicle in third segment of maxillary palp of P. fontanum male (abnormal).

B Same of normal fontanum male.

FIG. 8 A

FIG. 8 B

Fig. 9 Sensory vesicle in third segment of maxillary palp of P. brevisi Stone female, (paratype).

Fig. 10 Sensory vesicle in third segment of maxillary palp of P. ursinum (Edwards) female.

FIG. 9

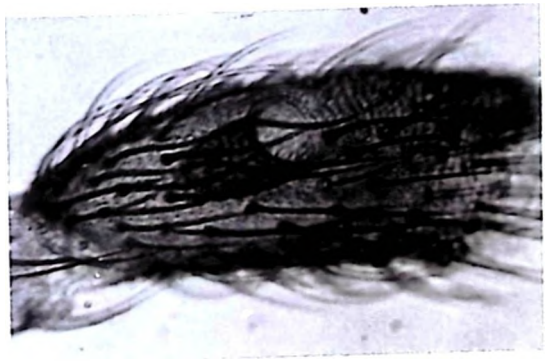

FIG.10

Fig. 11 Ovipositor lobes of P. fuscum.

Fig. 12 Ovipositor lobes of P. mixtum.

Fig. 13 Ovipositor lobes of P. fontanum.

FIG. 11

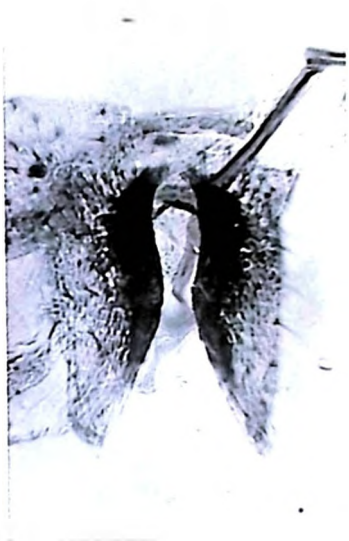

FIG. 12

FIG. 13

Fig. 14 Ventral plate of male P. fuscum.

Fig. 15 Ventral plate of male P. mixtum.

Fig. 16 Ventral plate of male P. fontanum.

Fig. 17 Ventral plate of male P. ursinum.

Fig. 18 Genital pod of female P. fontenum.

Fig. 19 Clasper of male P. fontanum.

Fig. 20 Pupal respiratory organ of P. fuscum. A and B are ventral trunks, C is dorsal trunk.

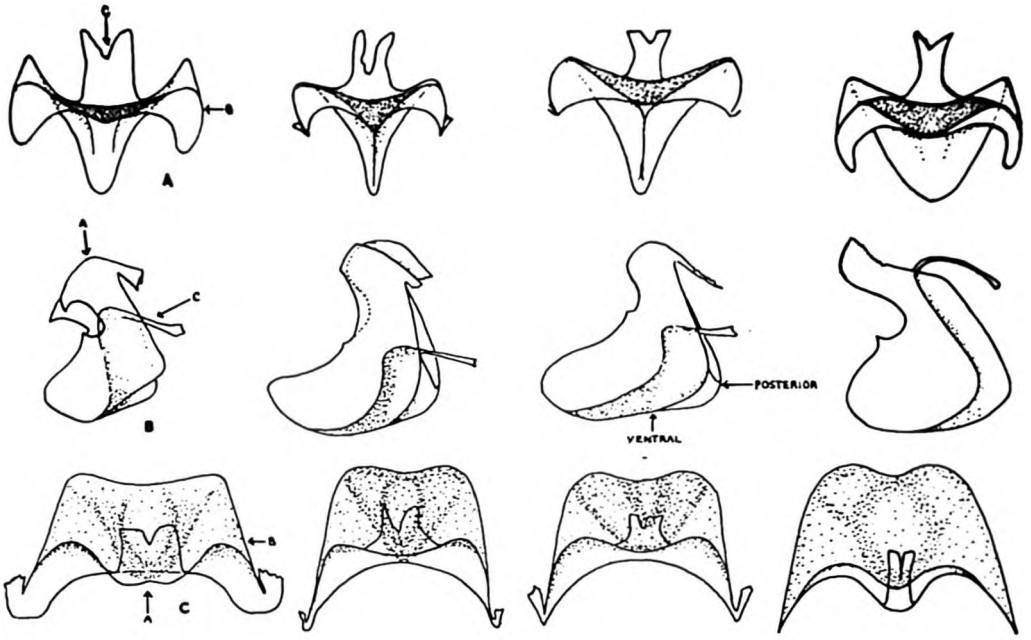

FIG.14 FIG.15 FIG.16 FIG.17

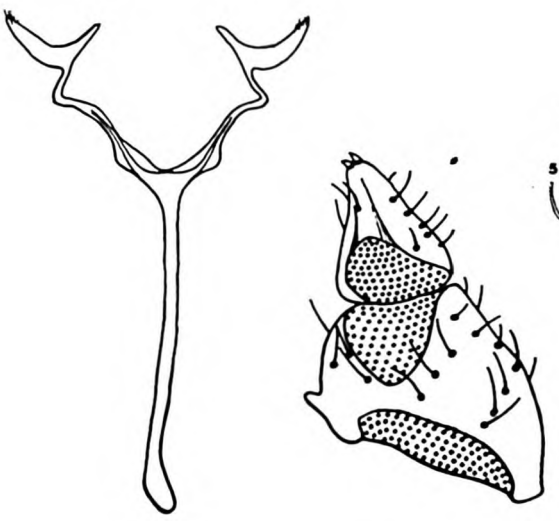

FIG.18

FIG.19

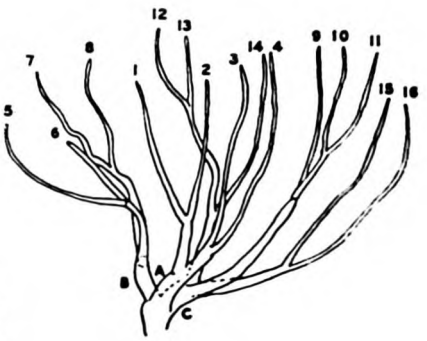

FIG.20

Fig. 21 Submental teeth and subapical mandibular ridge of *P. fuscum* larva.

Fig. 22 Submental teeth and subapical mandibular ridge of *P. mixtum* larva.

Fig. 23 Submental teeth of *P. mixtum* larva.

Fig. 24 Submental teeth and subapical mandibular ridge of *P. fontanum* larva.

Fig. 25 Occipital cleft of *P. fuscum* larva.

Fig. 26 Occipital cleft of *P. mixtum* larva.

Fig. 27 Occipital cleft of *P. fontanum* larva.

Fig. 28 Pupal chactotaxy of *P. fuscum*.

FIG. 21

FIG. 22

FIG. 23

FIG. 24

FIG. 25

FIG. 26

FIG. 27

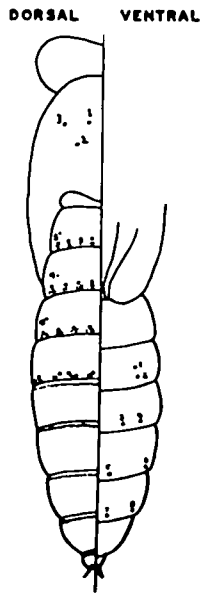

FIG. 28

Fig. 29 Histograms of the number of hairs on the male subcosta of *P. fuscum* and *P. mixtum* from various localities.

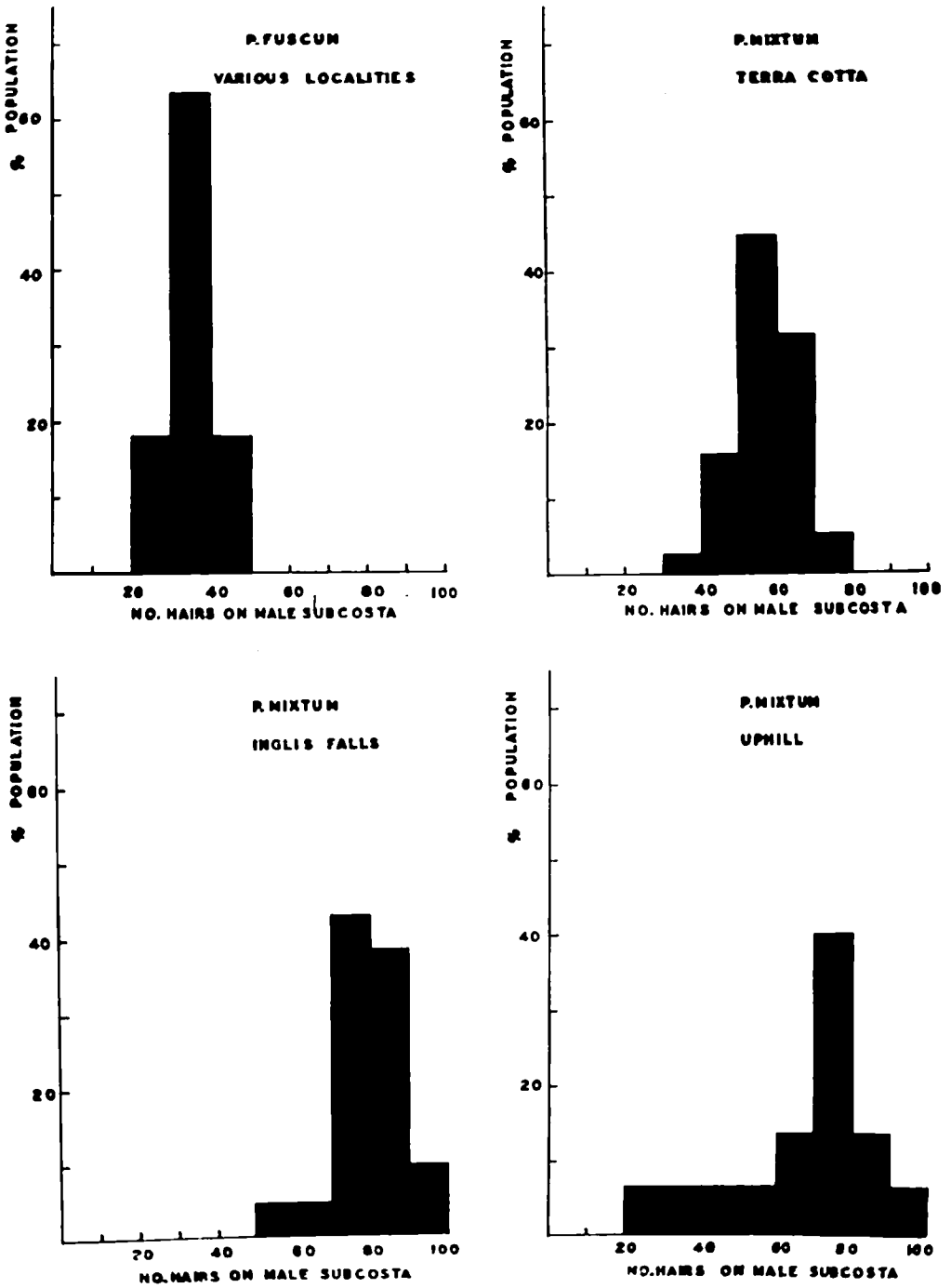
P. FUSCUM
VARIOUS LOCALITIES
% POPULATION
NO. HAIRS ON MALE SUBCOSTA
P. MIXTUM
TERRA COTTA
% POPULATION
NO. HAIRS ON MALE SUBCOSTA
P. MIXTUM
INGLIS FALLS
% POPULATION
NO. HAIRS ON MALE SUBCOSTA
P. MIXTUM
UPHILL
% POPULATION
NO. HAIRS ON MALE SUBCOSTA

FIG. 29

Fig. 30 Histograms of the lengths and widths of *P. fuscum* and *P. nigrum* ovipositor lobes.

Fig. 31 Histograms of the ratios of the filament lengths to the stalk lengths of larval mouth fans of *P. fuscum* and *P. nigrum*.

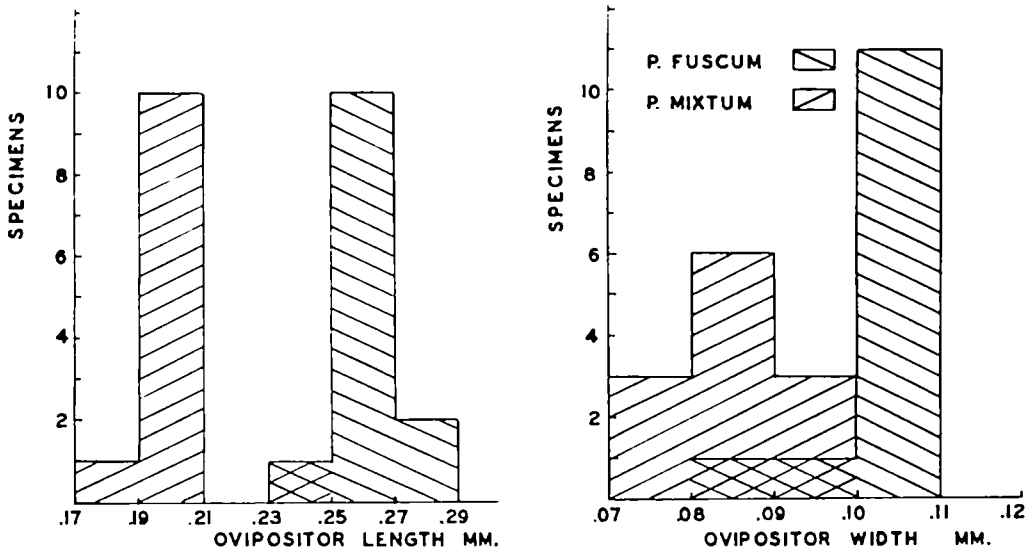
SPECIMENS
10
8
6
4
2
.17
.19
.21
.23
.25
.27
.29
OVIPOSITOR LENGTH MM.
P. FUSCUM
P. MIXTUM
SPECIMENS
10
8
6
4
2
.07
.08
.09
.10
.11
.12
OVIPOSITOR WIDTH MM.

FIG.30

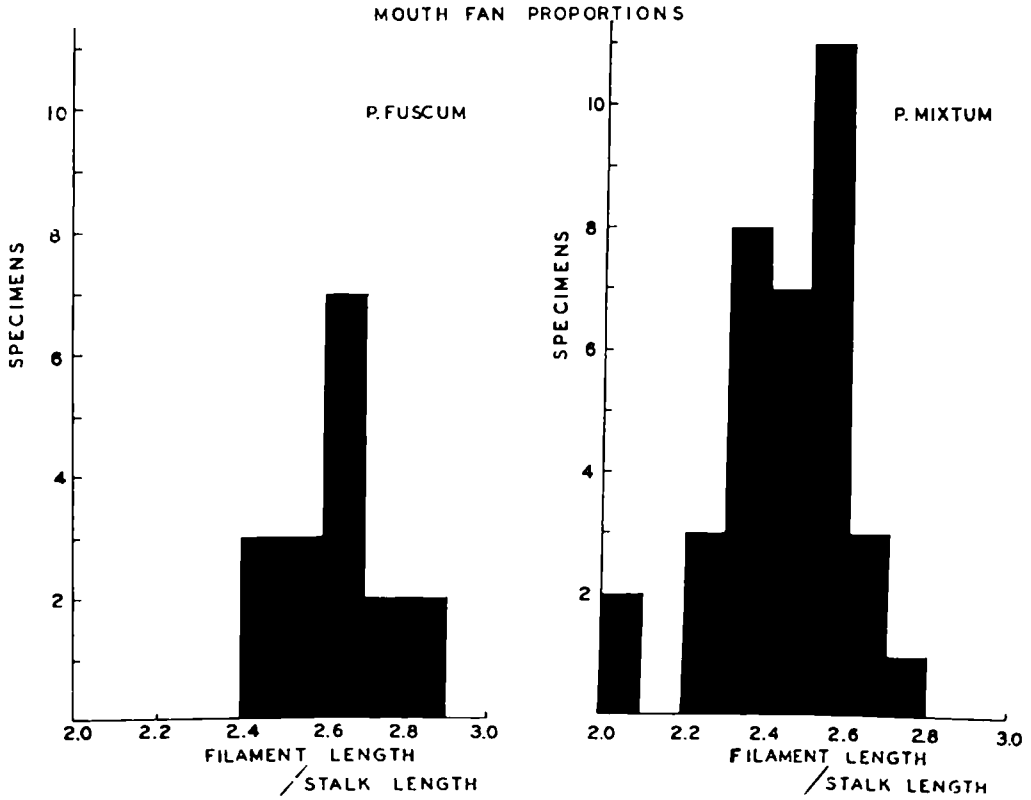
MOUTH FAN PROPORTIONS
P. FUSCUM
SPECIMENS
10
8
6
4
2
2.0
2.2
2.4
2.6
2.8
3.0
FILAMENT LENGTH
/STALK LENGTH
P. MIXTUM
SPECIMENS
10
8
6
4
2
2.0
2.2
2.4
2.6
2.8
3.0
FILAMENT LENGTH
/STALK LENGTH

FIG.31

Fig. 52 Distribution of P. fuscum in north eastern North America.

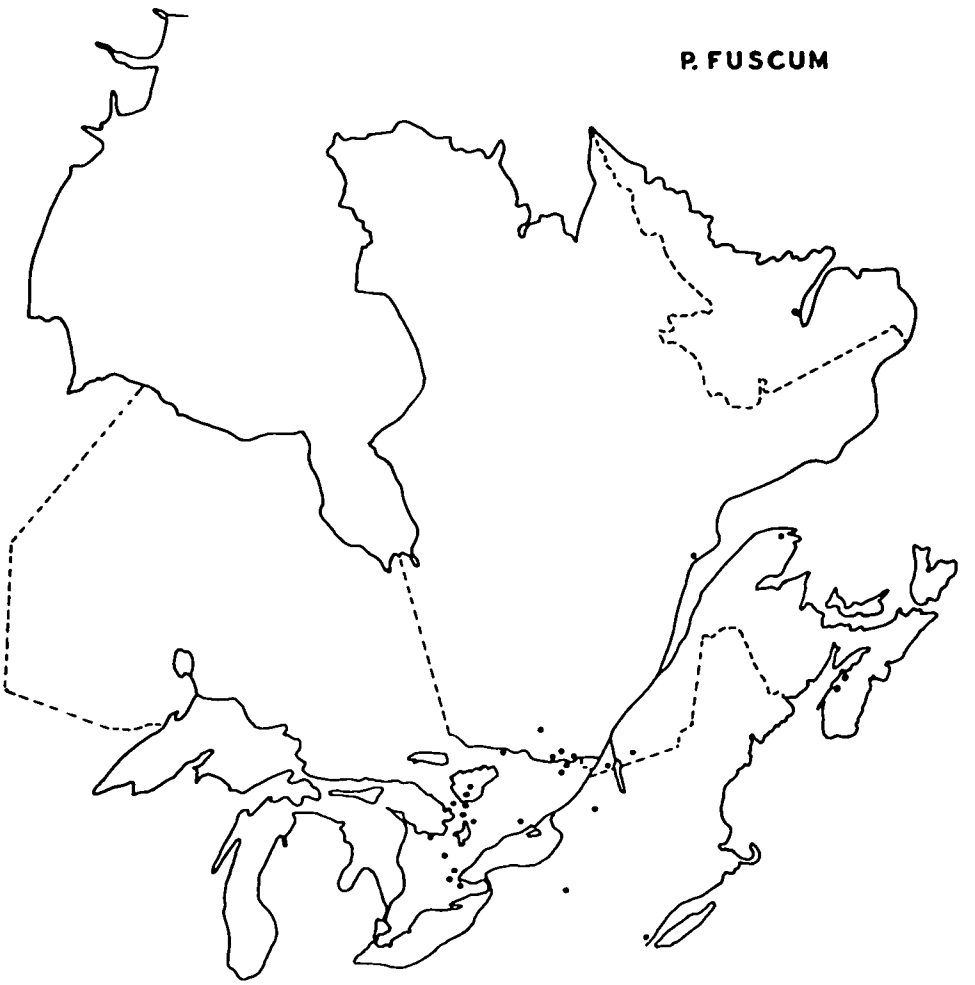

FIG. 32

Fig. 33 Distribution of *P. mixtum* in north eastern North America.

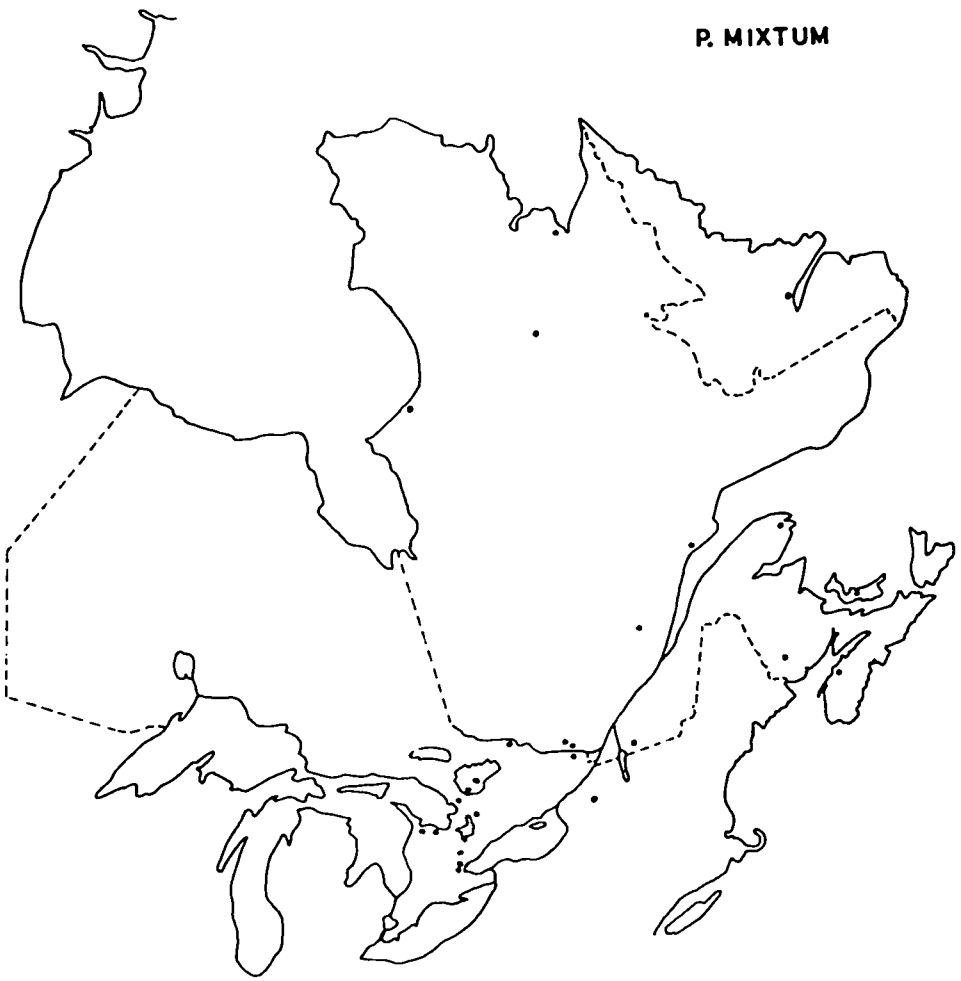

FIG. 33

Fig. 34 Distribution of *P. fontanum* in north eastern North America.

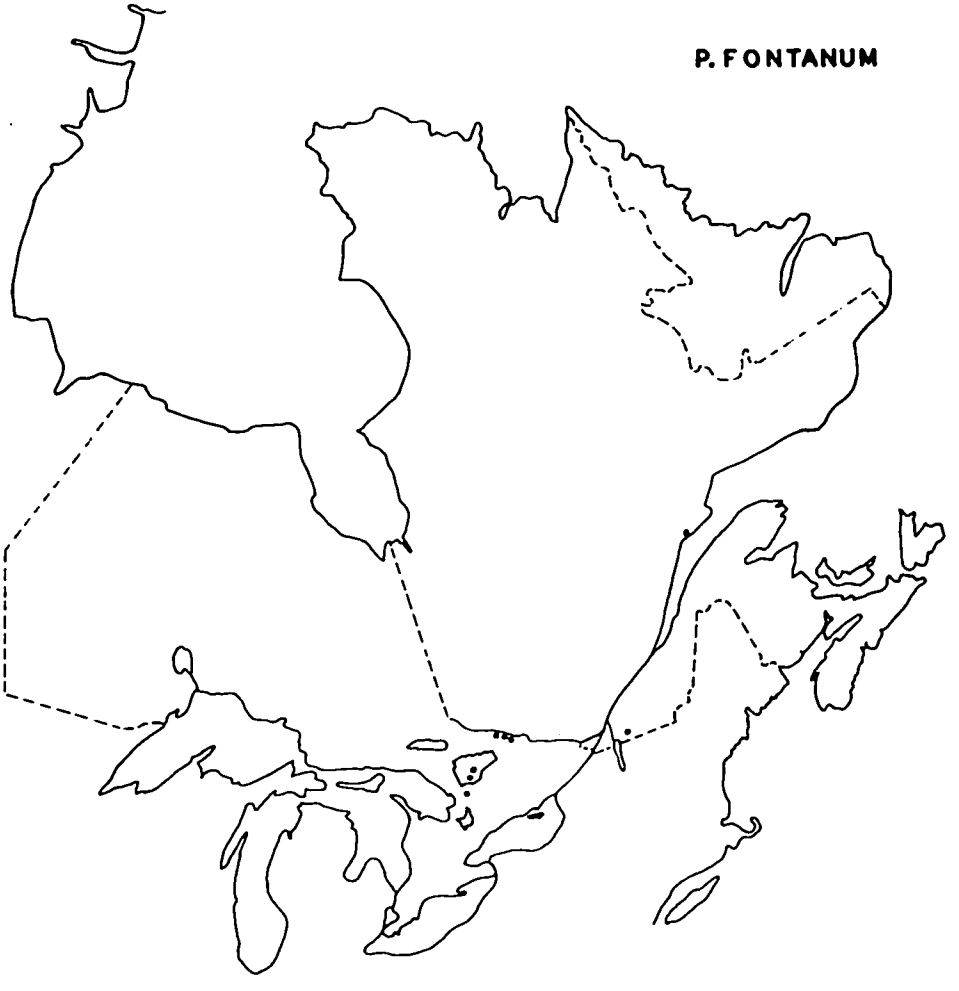

FIG. 34

TABLE I EMERGENCE COLLECTIONS

DATE	HOPKINS CK. 1957						SMITH CK. 1947					
	P.fuscum		P.mixtum		W.T.*		P.fuscum		P.mixtum		W.T.*	
	♂	♀	♂	♀	Max.	Min.	♂	♀	♂	♀	Max.	Min.
IV-23	cage in											
24												
25			1		60	55						
26			3		66	53						
27												
28												
29	cage upset				68	52						
30												
V-1	7			2	66	51						
2	8	1		2	66	48						
3					63	45						
4							cage in					
5	1			1	63	45					44.0	41.0
6	cage upset				63	45					47.5	43.5
7	1				65	48					52	43
8	cage upset				68	48					46	39.5
9											44	36.2
10											48	37.5
11											52	42.0
12							2				51	44.8
13	cage upset				68	50					53.5	46
14							2				45	42.2
15							1	5			54	44.3
16							1	2			54.8	49
17	cage upset				63	48	2	3	3		55	47.7
18							1		2	1	58	51.1
19							6	2	8	3	57.3	51.7
20							6	3		13	62.5	55.0
21											59.1	50.4
22							8	1		2	51	48.8
23								4	3	6	61.4	53.2
24							6	12	3	3	55.6	53.1
25								1		1	63.9	54.2
26								1		2		
27								1		4	56.5	50.5
28							1	1	1		58.1	52.5
29								1		1	57	52
30											54	47.7
31							1	2		1	53.1	47.9
VI-1								cage out				

* Water Temperature in °F.

TABLE I (continued) EMERGENCE COLLECTIONS

DATE	COSTELLO CK. 1940						SMITH INLET 1946							
	P. fuscum		P. mixtum		W.T.*		P. fuscum		P. mixtum		P. fontanum		W.T.*	
	♂	♀	♂	♀	Max.	Min.	♂	♀	♂	♀	♂	♀	Max.	Min.
V-11		cage in			48.0	42.8								
12	17				48.9	42.1								
13	9	1			51.2	46.1								
14	32				52.8	45.9	cage in							
15	25	17	2		57.0	49.0			6	3			52.7	46.5
16	25	37	6		52.7	52.1	3	12	12				56.5	49.5
17	6	9	6		50.9	49.8			1				53.3	42.1
18	1	29	8	1	50.0	47.6	1		6				53.8	48.0
19	1	45	11	5	52.0	47.0			3				51.0	46.7
20		6	3	5	54.9	50.2			1				52.7	46.5
21		7	2	19	60.1	50.9			2				54.0	52.5
22		11	5	12	63.5	56.0	2		1				54.5	47.5
23		5		13	61.0	56.9			1				58.5	48.7
24		1		6	58.9	58.0	1		1				61.5	53.0
25				1	58.9	58.4			1			1	61.5	54.0
26					61.3	58.0							62.0	53.5
27					62.4	60.5					2		63.0	54.0
28		7		1	60.5	57.1					1		60.2	52.0
29		2			65.9	60.0			1		1		59.5	50.7
30		4	1		67.0	61.9					1		60.2	52.2
31		3	1	3	65.8	63.1							60.0	51.0
VI- 1					68.2	63.2					2		53.5	45.5
2												1	54.5	45.5
3											9	9	55.0	48.5
4										1	7	15	54.5	48.0
5												2	55.0	47.8
6												1	55.0	48.0
7												2	52.5	48.0
8												1	53.0	49.0
9												1	56.0	50.2
10														
11							cage out					1	56.0	48.5

*Water Temperature in °F.

TABLE 2 SUMMARY OF DATA FROM EMERGENCE COLLECTIONS

	EMERGENCE	P. fuscum	P. mixtum	P. fontanum	Average water temperature
HOPKINS CK. 1957	FIRST DATE		April 25 ?		
	MIDPOINT 50% emergence	April 29 ?	April 26 ?		57°F.
	LAST DATE	May 7 ?	May 5 ?	No last stage fuscum and mixtum larvae May 3.	
	% of population	63	37		
SMITH CK. 1947	FIRST DATE	May 12	May 17		
	MIDPOINT 50% emergence	May 21	May 20		52°F.
	LAST DATE	May 31	May 31		
	% of population	57	43		
COSTELLO CK. 1940	FIRST DATE	May 12	May 13		
	MIDPOINT 50% emergence	May 16	May 21		55°F.
	LAST DATE	May 31	May 31		
	% of population	73	27		
SMITH'S L. INLET 1946	FIRST DATE			May 25	
	MIDPOINT 50% emergence		May 16 ?	June 3	52°F.
	LAST DATE	May 24	May 29*	June 11	(fontanum emergence 54°F.)
	% of population	33 ?	33 ?	33 ?	
L. SASAJEWUN TOTE RD. STREAM 1955	FIRST DATE			June 20 ?	
	MIDPOINT 50% emergence				52°F.
	LAST DATE			August 8	
	% of population			100 ?	

* One female June 4.

TABLE 3 DISTRIBUTION OF *P. FUSCUM*

DATE	LOCALITY	FORM COLLECTED	DATA FROM
III- 6-57	Caledon Trout Club	Last instar larvae	PDS
III-10-57	Caledon Trout Club	Mature larvae	PDS
III-21-57	Caledon Trout Club	Mature larvae	PDS
III-24-55	Rattlesnake Pt., Nelson Twp., Halton Co., Ont.	Mature larvae	KR
III-24-57	Kahshe R. at #11 Hwy., Ont.	Mature larvae	PDS
III-26-57	Kahshe R. at #11 Hwy., Ont.	Mature larvae	PDS
III-31-57	Kahshe R. at #11 Hwy., Ont.	Mature larvae	PDS
III-31-57	Marsh Falls, Algonquin Pk., Ont.	Mature larvae	KR
III-31-57	Park Headquarters, Algonquin Pk., Ont.	Mature larvae	KR
III-IV-56	Collingwood, Ont. (Ide's Stream)	Mature larvae	KR
III-IV-56	Stream crossing #17 Hwy. 4 mi. w. of Deep R. Ont.	Mature larvae	KR
IV- 1-56	Caledon Trout Club	Pupae	PDS
IV- 8-56	Caledon Trout Club	Pupae	PDS
IV-10-36	Yonkers, N.Y.	Adult	CNC
IV-10-49	Lake Sasajewun dam, Algonquin Pk., Ont.	Mature larvae	DMD
IV-17-53	Bell's Corners, Ottawa, Ont.	Mature larvae	CNC
IV-21-24-57	Long Lake (Eaton Pond Outlet), Adirondacks	Mature larvae	KR
IV-27-57	Kahshe R. at #11 Hwy., Ont.	Pupae	PDS
IV-29-52	Marmora, Ont.	Adult	CNC
IV-30-56	Lake Sasajewun Outlet, Algonquin Pk., Ont.	Pupae	PDS
V- 1-7-57	Hopkins Ck., Dundas, Ont.	Adult ♀	PDS
V- 3-49	Lake Sasajewun dam, Algonquin Pk., Ont.	Adult	DMD
V- 3-44	Kingston, N.S.	Adult	DMD
V- 5-53	Knowlton, P.Q.	Adult	CNC
V- 5-57	Lyons Inlet, Ancaster, Ont.	Adult ♂	PDS
V- 6-51	Gatineau Pk., Kardley, P.Q.	Adult	CNC
V- 6-51	Beech Grove, P.Q.	Adult	CNC
V- 7-56	Hopkins Ck., Dundas, Ont.	Pupae	PDS

TABLE 3 (continued) DISTRIBUTION OF P. FUSCUM

DATE	LOCALITY	FORM COLLECTED	DATA FROM
V- 8-56	Lower Buttermilk Falls, Hamilton, Ont.	Mature larvae	PDS
V- 8-56	Lyon's Inlet, Ancaster, Ont.	Pupae	PDS
V-11-57	Bala, Ont.	Adult	DMD
V-12-56	Kahshe R. at #11 Hwy., Ont.	Exuviae	PDS
V-13-50	Blanch R. Powerhouse, P.Q.	Adult	CNC
V-15-28	Hemmingford, P.Q.	Adult	CNC
V-16-50	Ashton, Ont.	Adult	CNC
V-17-26	Ithaca, N.Y.	Adult	CNC
V-18-56	Walker's Line, Halton Co., Ont.	Mature larva	PDS
V-20-23	Tetreauval, P.Q.	Adult	CNC
V-23-33	Wakefield, P.Q.	Adult	CNC
V-24-23	Kentville, N.S.	Adult	CNC
V-24-32	Meach Lake, P.Q.	Adult	CNC
V-26-27	Miner's Bay, Ont.	Adult	CNC
VI- 3-37	Lac Cayament, P.Q.	Adult	CNC
VI- 5-55	Stn. N25, Buisson, Baie Comeau, P.Q.	Adult	CNC
VI-12-55	Dry Creek, Baie Comeau, P.Q.	Adult	CNC
VI-16-48	Goose Bay, Labrador	Adult	CNC
VII-28-50	Goose Bay, Labrador	Larvae	CNC
VIII- 2-34	Gaspe Co., P.Q.	Adult	CNC
IX- 6-50	Goose Bay, Labrador	Larvae	CNC

CNC = Canadian National Collection, Ottawa.
KR = Dr. K. Rothfels, University of Toronto.
DMD = Dr. D.M. Davies, McMaster University.
PDS = P.D. Syme.

TABLE 4 DISTRIBUTION OF P. MIXTUM

DATE	LOCALITY	FORM COLLECTED	DATA FROM
III-21-57	Terra Cotta, Ont. (Credit System)	Mature larvae	KR
III-24-55	Rattlesnake Pt., Nelson Twp., Halton Co., Ont.	Mature larvae	KR
III-27-46	Palgrave, Ont.	Pupae	DMD
III-IV-56	Collingwood, Ont. (Ide's Stream)	Mature larvae	KR
III-IV-57	Stream crossing #17 Hwy. 4 mi. w. of Deep R. Ont.	Mature larvae	KR
IV- 6-57	Inglewood, Ont. (Credit System)	Mature larvae	KR
IV- 7-57	Rattlesnake Pt., Nelson Twp., Halton Co., Ont.	Mature larvae	KR
IV-15-57	Collingwood, Ont. (Ide's Stream)	Mature larvae	KR
IV-17-53	Bell's Corners, Ottawa, Ont.	Mature larvae	KR
IV-21-24-56 and 57	Long Lake (Baton Pond Outlet), Adirondacks	Mature larvae	KR
IV-25-V- 5-57	Hopkin's Ck., Dundas, Ont.	Adult	FDS
IV-26-56	Terra Cotta, Ont. (Credit System)	Mature larvae	FDS
V- 1-56	Terra Cotta, Ont. (Credit System)	Pupae	FDS
V- 3-53	Site F, Knowlton, P.Q.	Larvae	CNC
V- 3-44	Kingston, N.S.	Adult	DMD
V- 3-4-53	Knowlton, P.Q.	Larvae	CNC
V- 4-53	Site A, Knowlton, P.Q.	Adult	CNC
V- 5-56	Terra Cotta, Ont. (Credit System)	Pupae	FDS
V- 7-53	Stn. L, Missisquoi R., Bolton, P.Q.	Larvae, pupae, adult	CNC
V- 7-56	Hopkin's Ck., Dundas, Ont.	Pupae	FDS
V- 8-35	Cayuta L., N.Y.	Adult	CNC
V- 8-56	Mineral Springs, Ancaster, Ont.	Pupae, exuviae	FDS
V- 8-56	Lyon's Inlet, Ancaster, Ont.	Pupae	FDS
V- 8-43	Pagotville, P.Q.	Larvae	DMD
V-11-57	Pela, Ont.	Adult	DMD
V-12-56	3* mi. w. of Uphill, Victoria Co., Ont.	Pupae, adults	FDS
V-14-22	Kingsmere, P.Q.	Adult	CNC
V-14-44	Portugal Cove Rd., St. Johns, Nfld.	Larvae	CNC
V-15-56	Rattlesnake Pt., Nelson Twp., Halton Co., Ont.	Pupae	FDS

TABLE 4 (continued) DISTRIBUTION OF *P. LIXTUS*

DATE	LOCALITY	FORM COLLECTED	DATA FROM
V-20-35	Hull, P.Q.	Adult	CNC
V-20-27-54	Corbett Brook, Fredericton, N.B.	Larvae	CNC
V-22-49	Black Ash Ck., Collingwood Twp., Ont.	Adult	CNC
V-23-35	Hull, P.Q.	Adult	CNC
V-28-56	Rattlesnake Pt., Nelson Twp., Halton Co., Ont.	Pupae, exuviae	PDS
VI-56 and 57	Bear Ck	Mature larvae	MR
VI-14-55	Smith Lake, Baie Comeau, P.Q.	Adult	CNC
VI-18-52	Stanhope, P.E.I.	Adult	CNC
VI-21-31	Catineau Hills, P.Q.	Adult	CNC
VI-23-56	Inglis Falls Inlet (Owen Sound, Ont.)	Pupae, adults	PDS
VI-25-49	Goose Bay, Labrador	Adult	CNC
VII-6-55	Manic Depot, Baie Comeau, P.Q.	Adult	CNC
VII-10-42	Ungava Barrens, P.Q. (Lat. 56°57.3', Long.71°0.4')	Adult	CNC
VII-14-48	Knob Lake, P.Q. (Lat. 54°47', Long. 66°47')	Adult	CNC
VII-19-55	Manicouagan R., Baie Comeau, P.Q.	Adult	CNC
VII-24-49	Great Whale R., P.Q.	Adult	CNC
VII-26-33	Gaspe, P.Q.	Adult	CNC
VII-29-48	Ft. Chimo, P.Q.	Adult	CNC
VII-VIII-56	Terra Cotta and district, Ont.	Mature larvae	MR
IX-16-32	Grand Cascapedia, P.Q.	Adult	CNC

CNC = Canadian National Collection, Ottawa.
MR = Dr. R. Rothfels, University of Toronto.
DMD = Dr. D.M. Davies, McMaster University.
PDS = P.D. Syme.

TABLE 5 DISTRIBUTION OF *P. FONTANUM*

DATE	LOCALITY	FORM COLLECTED	DATA FROM
V-18-53	Site L, Knowlton, P.Q.	Larvae	CNC
V-21-53	Sulton Mts., East Hill, P.Q.	Larvae	CNC
V-22-53	Site O, Knowlton, P.Q.	Larvae	CNC
V-25-46	Smith's L. Inlet, Algonquin Pk., Ont.	Adults	DMD
VI- 1-57	Fox Point Inn, Lake of Bays, Ont.	Mature larvae	KR
VI- 2-57	N. Star Lodge, Chalk R., Ont.	Mature larvae	KR
VI- 2-57	Fox Point P.O., Lake of Bays, Ont.	Mature larvae	KR
VI- 2-57	Streams in Petawawa Forest Reserve, Ont.	Mature larvae	KR
VI-17-57	L. Sasajewun Tote Rd.,Stream #1, Algonquin Pk.Ont.	Larvae	PDS
VI-17-57	L. Sasajewun Tote Rd.,Stream #2, Algonquin Pk.Ont.	Larvae	PDS
VI-18-57	Bate's Ck., L. Opeongo, Algonquin Pk., Ont.	Mature larvae	PDS
VI-18-57	Ck. opp. Fish Lab, L. Opeongo, Algonquin Pk.,Ont.	Mature larvae	PDS
VI-20-56	L. Sasajewun Tote Rd.,Stream #1, Algonquin Pk.Ont.	Pupae	PDS
VI-21-56	L. Sasajewun Tote Rd., Stream #2, Algonquin Pk.Ont.	Pupae	PDS
VI-21-56	Bate's Ck., L. Opeongo, Algonquin Pk., Ont.	Pupae	PDS
VI-21-56	Ck. opp. Fish Lab., L.Opeongo, Algonquin Pk.,Ont.	Pupae	PDS
VI-21-56	Brewer L. S. Inlet, Algonquin Pk., Ont.	Mature larvae	PDS
VI-22-56	N. Star Lodge, Chalk R., Ont.	Pupae	PDS
VI-22-56	Laurentian Pt., Chalk R., Ont.	Mature larvae	PDS
VI-22-56	L. Sasajewun Tote Rd.,Stream #1, Algonquin Pk.Ont.	Pupae	PDS
VII-25-55	Manicouagan R., Baie Comeau, P.Q.	Adult	CNC
VIII- 8-56	L. Sasajewun Tote Rd.,Stream #1, Algonquin Pk.Ont.	Pupae, exuviae, adults	PDS

CNC = Canadian National Collection, Ottawa.
KR = Dr. K. Rothfels, University of Toronto.
DMD = Dr. D.M. Davies, McMaster University.
PDS = P.D. Syme.

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