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POST EMBRYOLOGICAL DEVELOPMENT OF EPHEMEROPTERA (MAYFLIES). EXTERNAL CHARACTERS ONLY[1]

BY F. P. IDE[2]

Abstract

The development of external structures is followed from hatching to maturity. The life histories of two species *Stenonema canadense* Wlk. and *Ephemera simulans* Wlk. are described in detail and less complete accounts given of nine others.

Each moult results in a change in the number of segments in the caudal filaments and on this basis it has been possible to determine the number of instars. In *Stenonema canadense* the number was found to be between 40 and 45, and in *Ephemera simulans* about 30. Segments are added to the caudal filaments of the former at each moult in the following way: one in each of the first two moults, two in each of the next three, three in the next and four at each subsequent nymphal moult. In the change from nymph to subimago distal segments are dropped in *Ephemera* and probably in *Stenonema* also.

Mouth parts are very different in the newly hatched than in the full grown nymph as described for *Stenonema canadense.*

A definite case of a hypermetamorphosis of the tarsus and its claws appears in the life history of *Epeorus humeralis* and *Iron pleuralis.*

Gills are absent in the first instar in all the species studied. In some species they appear simultaneously on all the gill bearing segments at the first moult. In others they appear on segments five and six only at this moult, those of the other segments appearing only after several moults. The internal or secondary ramus of the gills appears much later in the nymphal life. The ultimate shape of the gill is influenced in some cases by the fact that the gills of segments five and six are segmented or unsegmented.

In *S. canadense* the wing pads make their appearance in about the fifteenth from the last instar and the claspers and external genitalia of the male are apparent in about the eighth from the last instar.

At each moult there is some structural change in the nymph adapting it to the environment. The environmental relation is being constantly changed by increase in size of the organism, thus necessitating these adaptations to preserve an equilibrium.

Introduction

There is relatively little known of the younger stages of Ephemerid nymphs and a study of these is important not only to a better knowledge of the insects themselves but of their distribution and ecology.

Lubbock (1864 and 1867) describes very fully the development of *Cloeon dimidiatum* but has missed at least one of the early instars and misinterpreted the way in which segments are added in the caudal filaments. Joly (1872) describes in brief some of the changes taking place in the nymphs of *Ephoron* (*Palingenia*) *virgo.* (See also Joly (1876).) Vayssiere (1882) describes the development of *Ecdyonurus* (*Heptagenia*) *venosus* (*longicauda*) describing nine stages which, however, do not correspond to instars. Lestage (1921) divides the nymphal life into three stages, stade larvulaire, stade larvaire and stade larva-nymphal. Murphy (1922) has reared *Baetis posticatus* Say and finds that there are 26 instars in this species. Gros (1923) in a study of *Ecdyonurus forcipulus* Koll. has described more stages than Vayssiere, basing the separation mainly on the growth of the gills. Wiebe (1926) describes the first three instars of *Hexagenia bilineata* Say and Neave (1932) the first instar of *Hexagenia limbata occulta* Walk.

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Contribution from the Department of Biology, University of Toronto, Canada.
[2] *Lecturer in Biology, University of Toronto.*

In the present paper nymphal stages of 11 species of mayflies commonly found in the upper reaches of trout streams of Ontario are described. The species are:

1. *Ephemera simulans* Walker;
2. *Stenonema canadense* Walker;
3. *Stenonema fuscum* Clemens;
4. *Stenonema tripunctatum* Banks;
5. *Heptagenia pulla* Clem;
6. *Heptagenia hebe* McD.;
7. *Epeorus humeralis* Morgan;
8. *Iron pleuralis* Banks;
9. *Isonychia bicolor* Wlk.;
10. *Ephemerella subvaria* McD.;
11. *Leptophlebia debilis* Wlk.

The life histories of the first two species mentioned were worked out fairly completely, but even in these all the instars were not determined. In both cases, however, the method of addition of segments in the caudal filaments and antennae was determined. It was found that each moult results in a change in the segmentation of the caudal filaments. The number of instars through which the full-grown nymph has passed may be estimated fairly closely by an examination of the caudal filaments and could be definitely determined in this way if there were not a fusion and obliteration of some of the segments basally. In *Ephemera simulans* the number of instars was estimated at about 30 and in *Stenonema canadense* at between 40 and 45.

For other species listed much less complete accounts are given, especially for *Ephemerella subvaria* and *Leptophlebia debilis*. The description is of the eggs and first instar of the former, and the gills of the latter.

The material was collected from the Mad and Pine rivers, tributaries of the Nottawasaga river, in Dufferin and Simcoe counties, Ontario, in connection with an ecological study of mayflies of these streams conducted during the summers of 1930, 1931 and 1932.

All the species herein treated have already been adequately described in the adult and full-grown nymphal state; references to these descriptions are given in the case of each species.

Method

Nymphs were collected periodically in the stream and examined under a compound microscope in the following way. A film of water was spread over a microscope slide and nymphs of one species placed dorsal side up in rows to facilitate the taking of measurements. When thus arranged the nymphs were covered with a cover slip and water was introduced, care being taken to exclude all air bubbles. The temporary mount thus made was placed on a larger piece of plate glass for convenience in handling under the objective of the microscope. Measurements were made using a squared whipple disc in a 10× Spencer ocular. For the later instars in which the mesothoracic wing pads are growing out rapidly relative to the growth of the nymph as a whole, the length of these pads was measured and also the length of the seventh abdominal tergum. The length of the wing pad was then divided by the length of the seventh tergum of the same individual to give a factor x.

By this means the differences in sizes of nymphs at the same instar were compensated for. As the wing pads grow out, not evenly, but by sudden increases in length at each moult, frequency diagrams made by plotting values of x as abscissas and the number of individuals as ordinates, gave a separation of the later stages into groups. Each group, it was shown, did not always represent one instar, since in one case it was found that there was little increase in length of the wing pads, but that in male individuals the instars could be easily separated by the change in size of the claspers which are growing out at the same time as the wing pads.

It was found that the intermediate stages could be roughly grouped by making a count of the segments in the antennae. Other structures which showed metamorphosis at each moult were the gills, and in the case of *Ephemera simulans*, the mandibular tusks. Very minute changes in morphological structure were found to be very constant and to indicate true instars.

The very early stages were never taken in collections in the stream owing to their small size (less than a millimetre). These were secured by capturing female adults on their ovipositing flight and relieving them of their egg masses which were set to incubate in fresh water in glass containers. Usually it was found that the eggs incubated under these conditions and hatched, even in the case of rapid water forms. With the rapid water forms, however, all individuals died either in the first or second instar. *Stenonema canadense*, and *Ephemera simulans* were reared further, up to the eighth or ninth instar.

Samples were taken from the rearing jars at intervals of two or three days and notes made on all the individuals contained in the sample, special attention being given to the changes in segmentation of the caudal filaments and of the antennae, the change in the number of spines on the femora and the appearance and subsequent increase in size of the gills. These structures were very sensitive indicators of change at moulting, especially the caudal filaments which, it will be shown in this paper, register every moult through which the individual passes.

Examination of the early instars was carried out under the compound microscope, using a 4 mm. Spencer objective and a 10× ocular, and examination of the older stages under a 16 mm. Spencer objective and a 10× ocular. The oil immersion was used to examine the mouth parts of the first and fifth instars of *Stenonema canadense*.

All drawings were made on cards ruled in quarter-inch squares and each is drawn to one of three scales of magnification, designated X, Y or Z, depending on whether the 16 mm., 4 mm., or oil immersion objective was used. The scales of magnification are given in Fig. 2,–13, and the magnification of each figure is mentioned in the explanation of the plate concerned. In any one figure drawings of structures having the same number were made from one individual (except in one or two instances), letters designating the separate structures.

Description of Instars

In the description of the instars of *E. simulans* and *Stenonema canadense* some characters have been tabulated to save repetition. These characters are omitted from the general accounts so that the data in the tables should be consulted as supplementary material.

Ephemera simulans Walker

The full grown nymph of *E. simulans* was described by Clemens (1915). This stream form, which may eventually be shown to be specifically different

TABLE I

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Ephemera simulans*

Instar	Length in mm.			No. of segments in antennal flagellum (including terminal piece)	Apparent no. of segments in ant.	No. of segments in caudal filament including terminal piece	Gills	Setae on posterior border of femur
	No. of individuals	Average	Lower and upper limits					
1	(25)	0.79	(0.64–0.85)	3		5	Absent	1 (and hairs)
2	(27)	0.82	(0.72–0.91)	4		5.5	On Segs. 2-7	1 (and hairs)
3	(4)	0.94		5	4	6	On Segs. 2-7	2
4	(4)	0.99	(0.87–1.12)	6	5	7	On Segs. 2-7	
5				7	6	9	On Segs. 2-7	5
6				8	7	12	Gill 7, sec. ramus begun	6
7				9	8	16		12
8								
9				11	10			
?10				12	10			13
11				13	11			
12								
13				15	13			26
14- about 23								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
?Nonult	0.29			visible
Octult				visible
Septult	0.42	0.40	1	0.05 mm.
Sextult	0.42	0.50	1	0.10
Quintult	0.64	0.50	2	0.10
Quartult	0.74	0.50	3	0.22
Tertiult	1.10		3	0.34
Penult	1.36		3	0.53
Last	2.60		3	0.85

The length of the nymphs is measured from the front of the head to the tip of the abdomen excluding the length of the caudal filaments. Where the length is the average of a number of individuals the number measured is placed in brackets and also the upper and lower limits of the lengths. In recording the segments of the antennae the two basal segments are omitted but the terminal segment is included in the count throughout. Similarly in the count of segments in the caudal filaments the terminal segment is included. The arrangement of setae on the femur refers to the series on the posterior border only.

Some segments are fused basally in the antenna and caudal filament and in these cases the true number and the apparent number are listed.

from typical *E. simulans* of lakes, was present in countless numbers in the slower flowing reaches of the Mad river at Singhampton, Ontario, where its nymphs burrow in the gravel and marl of the bottom in the channel of the stream.

Eggs oblong, about .25× .15 mm. (Fig. 1,–1). Surface of the egg reticulate as shown (Fig. 1,–1). The egg figured is one from which the nymph had hatched. Eggs extruded in two cylindrical masses, one from each oviduct.

First instar (Fig. 1,–2). Average length of 25 individuals .79 mm. with upper and lower limits of .85 and .64 mm. respectively. Head somewhat pentagonal, front margin forming two sides of pentagon; compound eyes rather less prominent than lateral ocelli and directly behind them; antennae composed of two thick basal segments and terminal flagellum in which are two segments differentiated and a terminal undifferentiated portion about as long as the second segment; hairs present subapically in these segments. Yolky enteron visible as an opaque body (stippled in figure); femora with one rather weak distal seta and two proximal hairs. Gills absent; three subequal caudal filaments, each of five segments of which the three basal are well differentiated and the others indistinct; rosette of spines present apically on Segment 1 only; Segments 2 and 3 armed apically with fine hairs.

Second instar, (Fig. 1,–3, 3a and 3b). Basal segment of antennal flagellum shorter and thicker than in first instar. Gills on Segments 2-7 inclusive as projections of the postero-lateral angles. The basal segment of caudal filament thickened, and shortened, the second segment better differentiated; a rosette of rather weak spines apically as on the basal segment; Segments 3 and 4 longer than in previous instar; Segment 5, the terminal piece more than twice as long as Segments 4 and 5 of the previous instar. The writer is inclined to think that this segment represents two undifferentiated segments; if this is so it means that the equivalent of a segment has been added in this instar to those formed in the previous instar.

Third instar, (Fig. 1,–4, 4a, 4b, 4c). The basal segment of antennal flagellum more elongate than in the previous instar, and probably the product of the two basal segments fused, making five segments in all; on the basal segment of the antenna adjacent to the flagellum a pair of very stout curved setae, which are present in this position in all subsequent stages. Femora each with a distal stout seta on posterior border and immediately proximad of it a weaker seta; gills elongate, uniramous; Gill 7 shown in Fig. 1,–4; on the lateral border of the gill in the distal third, a process, the first of the series of filaments which develop along the lateral border.

Caudal filaments (Fig. 1,–4b) have added one segment to the number already formed in the second instar, so that there are now six segments in all; basal segment much shortened and thickened.

Fourth instar, (Fig. 1,–5, 5a, 5b and 5c). Rostrum (Fig. 1,–5) developed as a shelf with anterior margin convexly curved; seventh gill of right side shown in Fig. 1,–5a. It is about twice as long as in the third instar, still

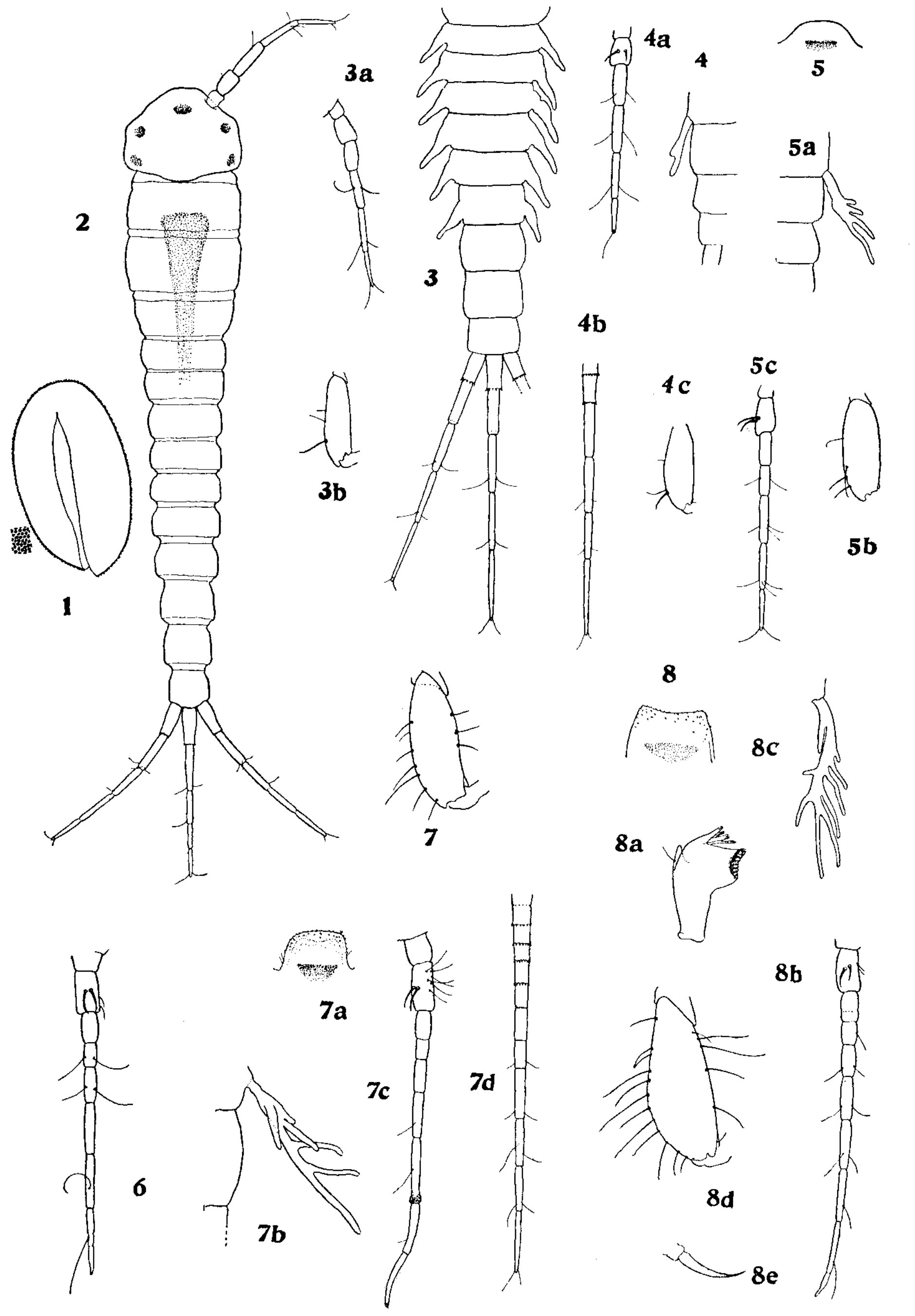

FIG. 1. *Ephemera simulans. All mag. Y. 1, Egg. 2, First instar. 3, Second instar, abdomen; 3a, antenna; 3b, femur. 4, Third instar, Gill 7; 4a, antenna; 4b, caudal filament; 4c, femur. 5, Fourth instar, rostrum; 5a, Gill 7; 5b, femur; 5c, antenna. 6, Antenna, fifth instar. 7, Sixth instar, femur; 7a, rostrum; 7b, seventh gill; 7c, antenna; 7d, lateral caudal filament. 8, Seventh instar, rostrum; 8a, mandible; 8b, antenna; 8c, seventh gill; 8d, hind femur; 8e, tarsal claw.*

uniramous, but with three lateral processes instead of one. The three basal segments of the caudal filaments now armed apically with a rosette of short spines.

Fifth instar (Fig. 1,–6). The rostrum is more truncate than in the fourth instar; caudal filaments with nine segments in flagellum, an addition of two to those present in the fourth instar. Gill 7 with five filamentous appendages but still uniramous.

Sixth instar, (Fig. 1,–7, 7a, 7b, 7c and 7d). At distal end of Segment 5 of the antenna is a thick sclerotized region, and in most individuals of this stage the antenna was broken off at this point. Usually the antennae were broken off at different points, one losing practically the whole flagellum and the other about one third of it. Rostrum (Fig. 1,–7a), more extended and truncate than in previous instar, and its surface roughened with numerous tubercles. Gill 7 more complex and with the beginning of the internal or secondary ramus, (Fig. 1,–7b). Caudal filaments show an addition of three segments in the flagellum, making 12, including the terminal piece.

Seventh instar, (Fig. 1,–8, 8a, 8b, 8c, 8d and 8e). Rostrum slightly emarginate. This condition is forecast in the previous instar figured where the new rostrum is visible through the old nymphal skin. Mandible (Fig. 1,–8a) with tusk well under way in development; tarsal claw long, scimitar-shaped and lacks pectinations.

Ninth instar, (Fig. 2,–9, 9a and 9b). Mandibular tusk shown in Fig. 2,–9; internal or secondary ramus of Gill 7 more elongate and conspicuous than in seventh instar, and the outer or primary ramus more complex.

? Tenth instar, (Fig. 2,–10, 10a, 10b, 10c and 10d). Mandibular tusk more developed, with a rather blunt apex, and a seta basally. Rostrum more concave on its anterior border than in seventh instar. Seventh gill (Fig. 2,–10b) with an elongation and basal enlargement of the internal or secondary ramus.

Eleventh instar, (Fig. 2,–11, 11a, 11b and 11c). Rostrum more emarginate and small setae appearing along anterior border; mandibular tusk longer than in previous instar and acutely instead of bluntly pointed; apically also it is much more heavily sclerotized than in the tenth instar; basally the tusk has two setae, one of which is longer and stronger than the other. Gill of seventh segment shows a change in the secondary ramus in the appearance of a bifid apex and the beginning of one medial filament.

? Thirteenth instar, (Fig. 2,–12, 12a, 12b, 12c and 12d). Rostrum has become more emarginate and six short setae are present around concavity in front of median ocellus. Mandibular tusk with much the same proportions as in previous instar, but longer, more heavily sclerotized and with four long setae proximally Setae on anterior border of femur more numerous than in tenth instar, and a row of seven short, thick setae has appeared in this position. Secondary or medial ramus of Gill 7 increased in length and with seven filaments, an addition of four to those started in the eleventh instar.

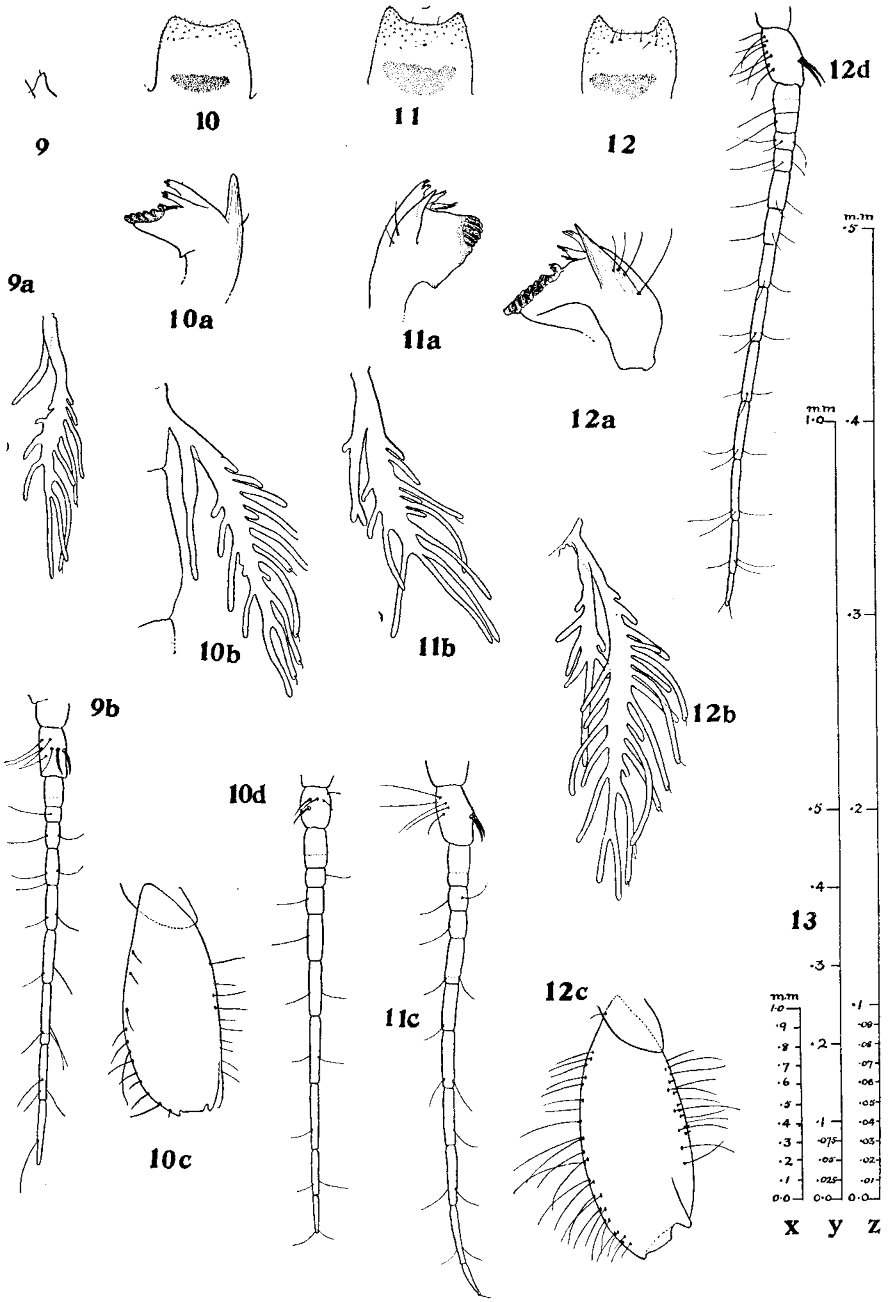

FIG. 2. *Ephemera simulans. 9-12d mag. Y. 9, Ninth instar, mandib. tusk; 9a, seventh gill; 9b, antenna. 10, Rostrum; 10a, mandible; 10b, seventh gill; 10c, hind femur; 10d, antenna. 11, Rostrum; 11a, mandible; 11b, seventh gill; 11c, antenna. 12, Rostrum; 12a, mandible; 12b, seventh gill; 12c, hind femur; 12d, antenna. 13, Scales of drawings with compound microscope using 10X ocular; X, using 16 mm. objective; Y, using 4 mm. objective; Z, using oil immersion.*

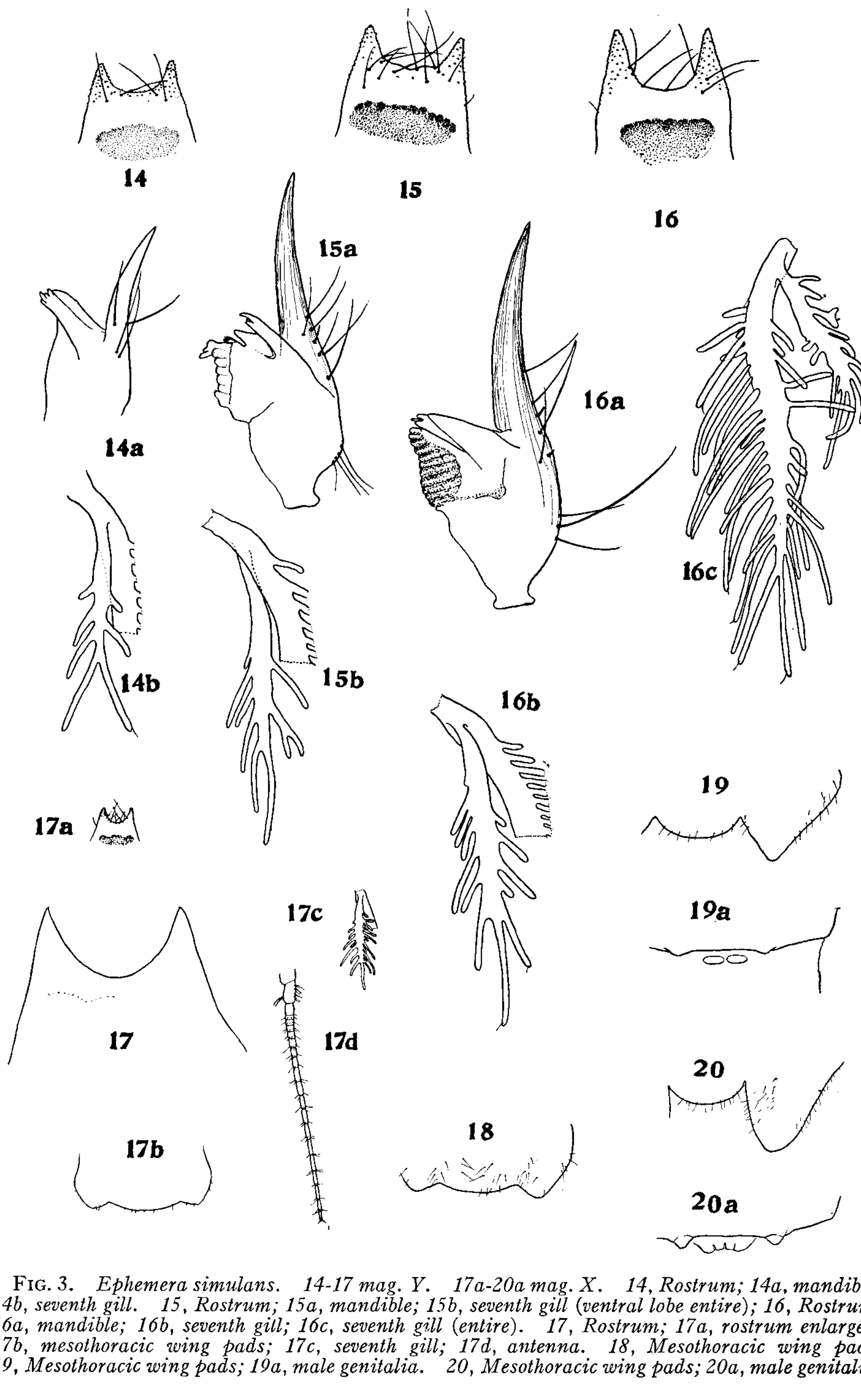

FIG. 3. *Ephemera simulans. 14-17 mag. Y. 17a-20a mag. X. 14, Rostrum; 14a, mandible; 14b, seventh gill. 15, Rostrum; 15a, mandible; 15b, seventh gill (ventral lobe entire); 16, Rostrum; 16a, mandible; 16b, seventh gill; 16c, seventh gill (entire). 17, Rostrum; 17a, rostrum enlarged; 17b, mesothoracic wing pads; 17c, seventh gill; 17d, antenna. 18, Mesothoracic wing pads. 19, Mesothoracic wing pads; 19a, male genitalia. 20, Mesothoracic wing pads; 20a, male genitalia.*

In Fig. 3,–14-16 are illustrated corresponding structures of three later instars which it will be seen at a glance cannot be consecutive instars. Fig. 3,–14, 14a, 14b, are taken from an individual which has 15 segments distinguishable in the antennal flagellum so that it is probably the 17th instar. Fig. 3,–15, 15a, 15b, are of a stage which is probably about the 18th instar, judging by the number of segments in the antennae. Similarly Fig. 3,–16, 16a, 16b, probably represent the 20th instar, since about 18 or 19 segments could be made out in the antennal flagellum. Adding to these one or two lost by fusion at the base the true number is calculated at about 20, and this, as we have seen in previous cases, gives approximately the number of the instar.

Through these stages the rostrum shows a deepening of the anterior concavity until its ultimate bifid character is developed. After the twentieth instar there is little change in its relative shape, merely an increase in size. The tusks of the mandibles also develop rapidly their final proportions during the stages immediately preceding the twentieth instar. The gills of the seventh segment show increasing complexity of structure which is easier to observe in the secondary ramus which alone is shown in full in Fig. 3,–14b, 15b, 16b. In Fig. 3,–16c is shown a complete seventh gill for a comparison of the secondary with the primary ramus. The wing pads have begun to form in this series probably in the stages immediately before the twentieth instar.

In Fig. 3,–17, 17a, 17b, 17c and 17d show structures of a later instar which is probably about the twenty-second, judging by the number of segments in the antennae, the increased size of the rostrum and the greater complexity of the secondary ramus of Gill 7. The wing pads in this instar reach back to a point in line with the posterior extremity of the mesonotum. The figures of this instar and subsequent ones are drawn at a lower magnification (Mag. X Fig. 2,–13), but Fig. 3,–17, of the rostrum is drawn to the same scale (Mag. Y, Fig. 2,–13) as that used in all previous figures as a basis of comparison.

Fig. 3,–18, shows the mesonotum of what is probably the next instar in which the wing pads project back beyond the posterior margin of the notum. There were about 23 segments discernible in the antennal flagellum. Obviously there is a gap between the instar represented in Fig. 3,–18 and that represented in Fig. 3,–19 and 19a.

During the last eight or nine instars the wing pads and male external genitalia are rapidly growing back so that the series is probably more complete, but even so it has some gaps; for instance there is apparently an instar missed between those represented in 19 and 19a, and in 20 and 20a, Fig. 3.

A system of nomenclature used by Dr. P. P. Calvert has been followed for the later instars.

? Nonult instar (Fig. 3,–19, 19a). In this instar the wing pads are triangular and measure about .29 mm. in length. The claspers in the male are present as mere rounded elevations on the posterior margin of the ninth sternum. The penes have made their appearance as elongate, slightly elevated tubercles on the intersegmental membrane behind the ninth sternum.

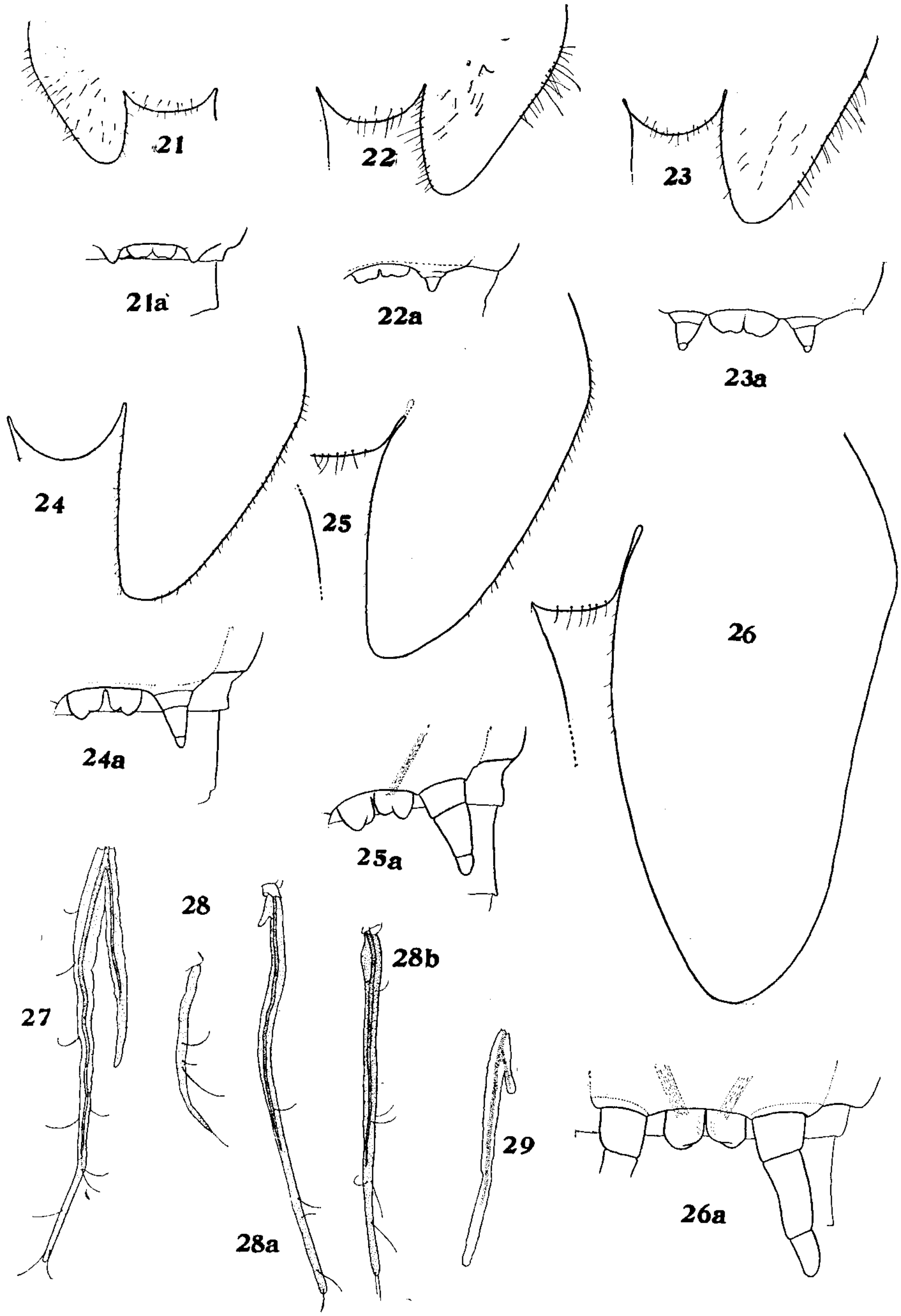

FIG. 4. *Ephemera simulans.* *21-26a mag. X.* *27-29 mag. Y.* *21, Mesothoracic wing pads; 21a, male genitalia.* *22, Mesothoracic wing pads; 22a, male genitalia.* *23, Mesothoracic wing pads; 23a, male genitalia.* *24, Mesothoracic wing pads; 24a, male genitalia.* *25, Mesothoracic wing pads; 25a, male genitalia.* *26, Mesothoracic wing pads; 26a, male genitalia.* *27, Leptophlebia debilis; fifth gill of left side.* *26, Leptophlebia debilis first gill; 28a, fourth gil; 28b, fifth or sixth gill.* *29, Leptophlebia debilis; sixth gill of left side.*

Septult instar, (Fig. 3,–20, 20a). The penes (penes + parameres) now betray their double nature in the bilobed condition of each.

Sextult instar, (Fig. 4,–21, 21a).

Quintult instar, (Fig. 4,–22, 22a). Wing pads are relatively longer than in previous instar; male claspers about .1 mm. in length, and of two segments, a short basal and a longer conical apical one.

Quartult instar, (Fig. 4,–23, 23a). Wing pads have increased to a length of .74 mm. which is not as great an increase as there was between the two previous instars; distance between wing pads about .5 mm. which is about the same as in the two former instars. The two distal segments of the claspers represent the single apical segment of the previous instar.

Tertiult instar (Fig. 4,–24, 24a).

Penult instar, (Fig. 4,–25, 25a). Wing pads have not increased very greatly in length, measuring now about 1.36 mm. Instead of being directed posteriorly as in previous instar, they converge noticeably at their apices. Male claspers have lengthened and lost some of their conical symmetry, but are still composed of three well defined segments. It is interesting to note that although there has been little increase in the length of the wing pad over that in the last instar, there has been a much greater increase in the length of the claspers.

Last instar, (Fig. 4,–26, 26a). Wing pads increased to a length of 2.6 mm. Towards the end of this instar the wing pads become first grayish and then black, prior to emergence.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to adult are illustrated in Fig. 5.

A count of the single segments in a lateral caudal filament (Fig. 5,–30, 30a) of a female nymph of the last instar gives about 94 segments formed during the thirty or more instars. The subimago (Fig. 5,–32, 32a, 32b) has fewer (about 74) and the adult female (Fig, 5,–33) and male (Fig. 5,–34) have each about 72 segments. In spite of this great reduction in the number of segments the caudal filaments of the adult are much longer than those of the subimago, and those of the subimago are in turn much longer than those of the full-grown nymph. This great increase in length is produced by a great elongation and swelling of the segments, and not by any increase in their number. Indeed, there is actually a reduction in the number of segments in the change from nymphal to subimaginal state. The twelve distal segments of the nymphal caudal filament do not represent segments of the subimago or adult, but are dropped in the manner shown in Fig. 5,–31a, where the developing subimaginal filament could be made out within.

Fig. 5,–31, 31a, of the nymphal filament, show the developing subimaginal filament within and Fig. 5,–32, 32a, show the developing imaginal filament within the subimaginal. In Fig. 5,–30, 30a, the segments formed at each ecdysis are indicated, and in the other figures an attempt has been made to designate homologous segments similarly. A more detailed description of Fig. 5 will be given later in a discussion of segmentation.

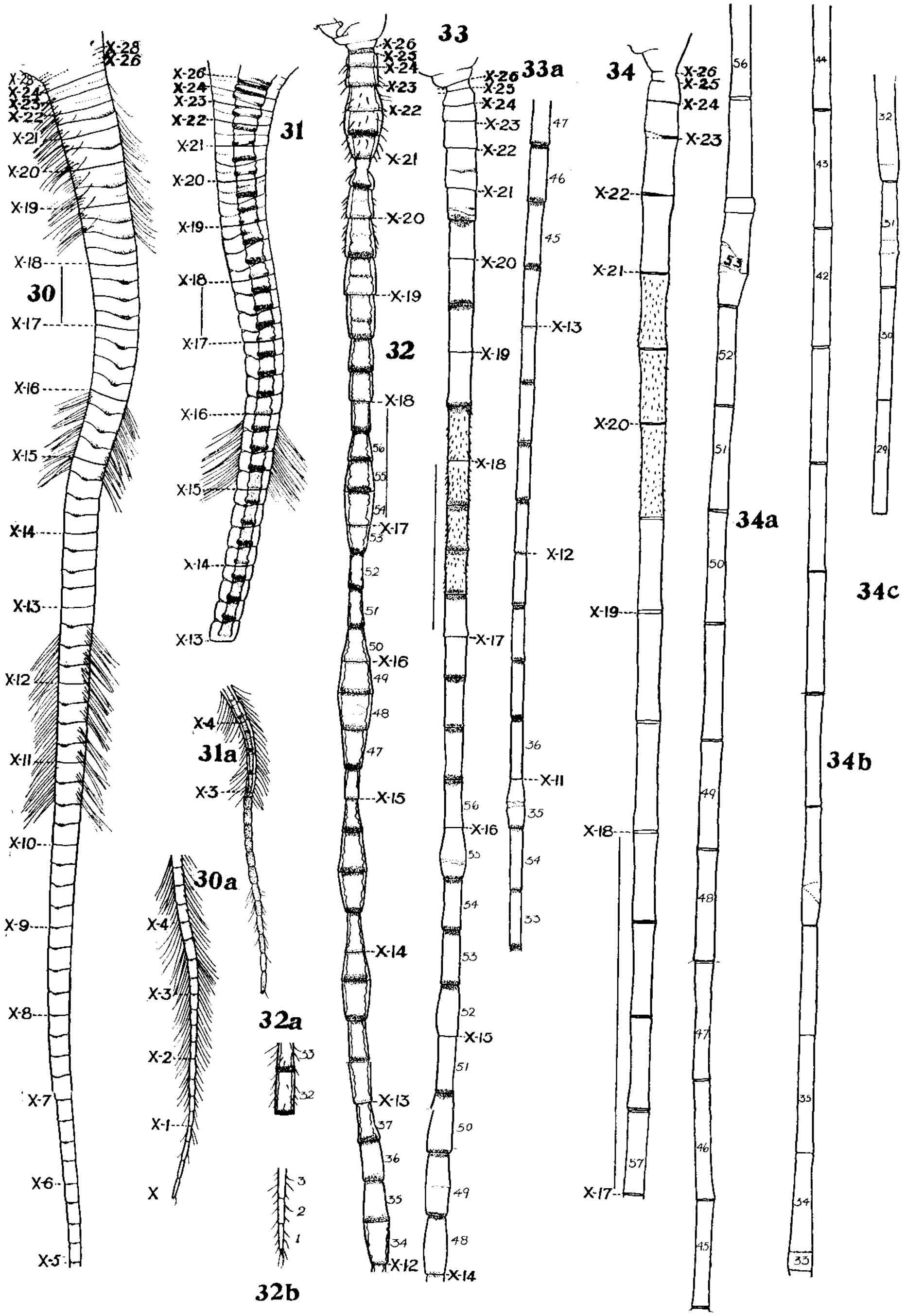

FIG. 5. *Ephemera simulans. All mag. X. 30 and 30a, Lateral caudal filament of female, last instar nymph. 31 and 31a, Lateral caudal filament of last instar female nymph, showing the subimago filament within. 32, 32a and 32b, Lateral caudal filament of female subimago showing lateral caudal filament of imago within. 33, 33a, Basal half of lateral caudal filament of adult female. 34, 34a, 34b and 34c, Basal portion of lateral caudal filament of adult male.*

In *Ephemera simulans* there is considerable metamorphosis during development, involving the antennae, caudal filaments, external genitalia, wing pads, eyes, mouth parts, gills and the number of setae and hairs.

The antennal segments are added one at each instar through most of the nymphal life and the caudal filaments add segments, in the following way,— one-half in the second instar, one in the third instar, one in the fourth instar, two in the fifth instar, three in the sixth instar, four in the seventh and in all subsequent nymphal instars. The twelve distal segments are dropped in the subimago. The mouth parts show considerable metamorphosis as shown by the appearance and rapid development of the mandibular tusks, beginning about the sixth and lasting to about the twentieth instar, after which the tusk increases at approximately the same rate as the body as a whole. The rostrum in its development keeps pace with the mandibular tusks. The gills make their appearance at the second instar as uniramous appendages, and increase in complexity and size during development. The internal or secondary ramus appears at the fifth or sixth instar, and develops rapidly, never, however, reaching the length nor the complexity of the primary ramus. The wing pads make their appearance at about the twelfth from the last instar, and grow out rapidly. The genitalia are not apparent as early as the wing pads, but are visible soon after, the claspers as rounded elevations on the posterior border of the ninth sternum, and the penes as rounded esicles on the intersegmental membrane behind the ninth sternum.

Stenonema canadense Walker

The full-grown nymph of this species is well described by Clemens (1915).

The eggs are of a reddish color, and when shed into water adhere in a mass held together by a gelatinous material, and also by threads in skeins as described by Morgan (1913).

First instar, (Fig. 6,–1, 1a, 1b, 1c). Head as long as broad, posterior angles squarish; anterior border somewhat truncate; antenna with two basal segments and a flagellum in which one basal segment is differentiated and provided with an apical crown of spines, the remainder of the flagellum tapering and faintly marked off in pseudo- or sub-segments; compound eyes about the same size as lateral ocelli. The mouth parts were dissected out and are shown in Fig. 6,–1a, 1b, 1c (mandible, maxilla and labium); mandibles with cardinal teeth directed medially; molar surface not developed and there is an indication of a suture or joint across the mandible; maxilla and labium both unmodified and very different from the same structures in the full-grown nymph (Clemens, 1915).

In the thorax is the reddish, opaque mass of the yolk-containing enteron. Legs long and spindly, terminating in a long, scimitar-shaped, non-pectinate tarsal claw more than half the length of the tarsus; on each femur in the distal third a single posterior stout seta. Three subequal caudal filaments of which the telson or median is the longest; each filament composed of three well formed basal segments, each provided apically with a crown of spines,

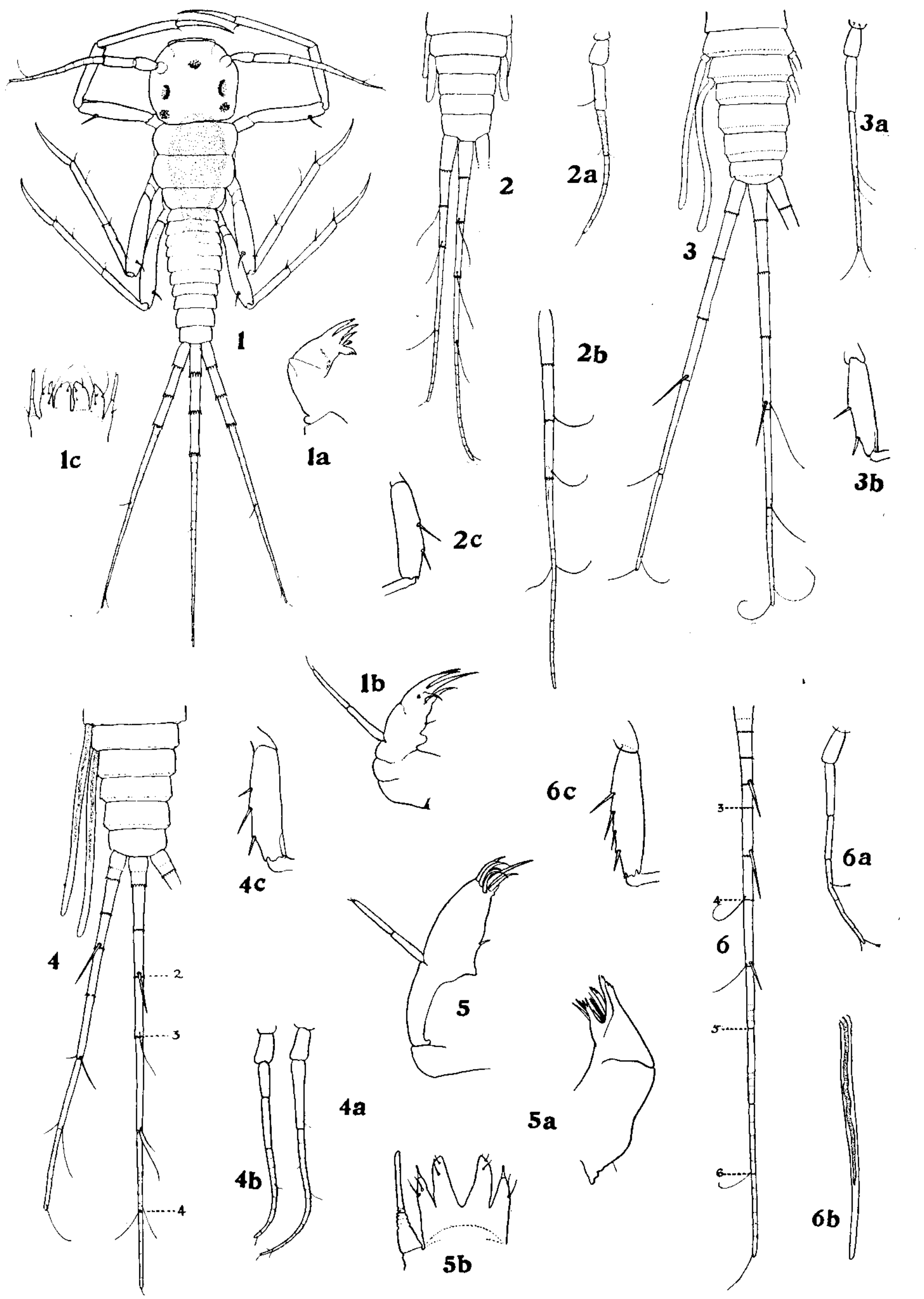

FIG. 6. *Stenonema canadense. 1a, 1b, 1c, 5, 5a, 5b, mag. Z; others mag. Y. 1, First instar; 1a, mandible; 1b, maxilla; 1c, labium. 2, Second instar; 2a, antenna; 2b, caudal filament, showing next instar within; 2c, femur. 3, Third instar, 3a, antenna; 3b, femur. 4, Fourth instar; 4a, antenna; 4b, antenna; 4c, femur. 5, Fifth instar, maxilla; 5a, mandible; 5b, labium. 6, Sixth instar, caudal filament; 6a, antenna; 6b, sixth gill; 6c, femur.*

TABLE II

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Stenonema canadense*

Instar	Length in mm.			Segments in ant. flagellum, excluding terminal piece	Apparent no. of segments in antenna	No. of segs. in caudal filament, excluding terminal piece	Setae on posterior border of femur	Gills
	No. of indivi-duals	Aver-age	Lower and upper limits					
1	(20)	0.50	(0.45–0.55)	1		3	1	Absent
2	(28)	0.54	(0.47–0.59)	1½		4	2	On Segs. 5 and 6
3	(23)	0.62	(0.57–0.68)	2		5	2	On Segs. 5 and 6
4	(5)	0.69	(0.64–0.75)	3		7	3	On Segs. 5 and 6
5	(2)	0.72		3		9	3	On Segs. 5 and 6
6	(4)	0.81	(0.76–0.85)	4	3	11	4	On Segs. 5 and 6
7						14		On Segs. 5 and 6
?8				6	5	18	7	On Segs. 1-7.
9						22		On Segs. 1-7.
10				6	5	26 (app. 24)		On Segs. 1-7.
11				7		30 (app. 28)	17	On Segs. 1-7.
12 to about 32								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
Octult	0.19			visible
Septult	0.32	0.37		visible
Sextult	0.36	0.48		visible
Quintult	0.50	0.50	1 segment	visible
Quartult	0.69	0.38	2 segments	visible
Tertiult	0.91	0.45	2 segments	0.16 mm.
Penult	1.16	0.58	2 (and indistinct basal)	0.26 mm.
Last	1.95	0.32	3 segments	0.30 mm.

The length of the nymph is taken from the front of the head to the end of the abdomen, excluding the length of the caudal filaments; the number of individuals and the upper and lower limits of the measurements for each instar appear in brackets. As with Ephemera the two basal antennal segments are omitted in the count; but in this case the terminal portion of the antennal flagellum has also been omitted. Similarly in the count of segments in the caudal filaments the terminal undifferentiated portion has been excluded from the count. The setae on the femur refer to the posterior series only. Owing to fusion of segments basally in the antennae and caudal filaments the true number cannot be made out. The apparent number in these cases is also listed.

and a tapering flagellum divided indistinctly into about twenty subsegments; about half way along this undifferentiated portion of the flagellum, but slightly proximad, a hair which probably marks the position of what will be the apex of the next segment to be formed. Gills absent, but in some individuals the postero-lateral angles of Segments 5 and 6 project more than the others owing, probably, to the formation of gills below the cuticle, which will appear at the next ecdysis.

Second instar, (Fig. 6,–2, 2a, 2b and 2c). The antennae have changed slightly; the small subsegments of the flagellum adjacent to the segment already formed in the first instar have shortened and fused, or in other words, another segment has been partially differentiated; antenna has lengthened by a corresponding amount. Gills on Segments 5 and 6, only, and measure from $1\frac{3}{4}$–$2\frac{1}{4}$ times the length of an abdominal segment. A fourth segment has been differentiated in the flagellum of each caudal filament and the filaments have been increased in length; distally in Segments 2, 3 and 4, a single long, fine hair. Fig. 6,–2b shows one caudal filament of this instar inside which the new filament of the next instar can be seen. A long stout seta is discernible on this filament, beneath the cuticle at the junction of Segments 3 and 4 of the old filament.

Third instar, (Fig. 6,–3, 3a and 3b). Antenna now with two segments basally in the flagellum, *i.e.*, the segment which was being differentiated in the last instar is now formed and has elongated, although there are still vestiges of the former subsegments of which it is composed. Gills present on Segments 5 and 6 only, but they have elongated so that they measure from five to six times the length of a typical abdominal segment. Apically in Segment 4 of the caudal filament a stout seta which was mentioned above in the description of the second instar.

Fourth instar, (Fig. 6,–4, 4a, 4b and 4c). Two conditions of the antennae were found. The commoner shown in Fig. 6,–4a had a very long basal segment in the flagellum, with a thickened base which is probably the fusion of two segments. The other condition Fig. 6,–4b, had a much shorter basal segment in the flagellum. Probably one segment has been added to those formed in the third instar.

Gills present on Segments 5 and 6 only, but longer than in the previous instar, measuring from seven to eight times the length of a typical abdominal segment; caudal filaments with seven segments differentiated basally, of which Segments 1 and 2 are rather intimately fused and shortened, so that the separation is not very easily made out in some individuals; distally in Segment 4 the stout seta mentioned above, and a somewhat weaker seta present apically in Segment 6. Two segments have been added this time in contrast to the single segment added in the previous instars.

Fifth instar, (Fig. 6,–5, 5a and 5b). Gills present on Segments 5 and 6 only, and each about seven times as long as a typical abdominal segment.

In Fig. 6,–5, 5a and 5b, the mouth parts are shown greatly enlarged. These show some change since the first instar. Terminal lobes of the labium longer; molar process of the mandibles more developed. At this instar the mouth parts are still very different from those of the last instar, as will be seen by referring to Clemens' figures of the full-grown nymph (Clemens, 1915). At the stage in which about thirteen segments are recognizable in the antennae, however, the mouth parts are very similar to those of the full-grown nymph, so that the change in these parts must be rather abrupt and occur early in the life of the nymph.

Sixth instar, (Fig. 6,–6, 6a, 6b and 6c). Caudal filaments now with eleven segments differentiated, so that two new segments have been added distal to those already formed in the previous instar.

In the sixth instar there is, as in the fifth, a stout seta apically on Segment 4 of the caudal filaments, and also one present apically on Segments 6 and 8; apically in Segment 11 a long, thin hair. In Fig. 6,–6, the portion of the filament which has been differentiated at each ecdysis is indicated.

In the seventh instar three segments have been added to those formed in the sixth instar. At every subsequent ecdysis four segments are added to the caudal filaments.

The change into the eighth instar seems to be a very critical stage in the life history of this species. This is the stage at which the gills make their appearance on Segments 1 to 4 and 7. Only one stage was taken which appears to be the eighth instar. The stages beyond the eighth were not reared from the egg, but collected in the stream.

?Eighth instar, (Fig. 7,–7, 7a, 7b). Gills now present on all segments from 1 to 7 inclusive; on Segments 1 to 4 as short, rounded appendages, on 5 and 6 linear as in the fifth instar, and on 7, linear gills in length equivalent to the eighth abdominal segment. The caudal filaments were broken off in this specimen.

Tenth instar, (Fig. 7,–8, 8a, 8b and 8c). Gills of Segments 1 and 2 shown enlarged in Fig. 7,–8a; Gill 7 (Fig. 7,–8c) about twice as long as in the preceding instar. In the caudal filaments there are about 24 segments apparent, with stout bristles present apically in Segments 6, 8, 10, 12, 16 and 20; seta of Segment 4 has been lost. The regions of the filament which were differentiated at each instar are enumerated in Fig. 7,–8.

Eleventh instar, (Fig. 7,–9, 9a. 9b). Gill 7 has increased in length so that it is nearly as long as Gills 5 and 6. The segments of the caudal filament formed at each instar are shown in the figure.

The femora are provided with about ten long setae and about seven shorter intercalary setae (Fig. 7,–9b).

?Twelfth instar, (Fig. 7,–10, 10a, 10b, 10c, 10d, 10e, 10f, 10g, and 10h). The number of this instar could not be determined with certainty. Anterior gills show an increase in length over the previous instar, and are shown under greater magnification; Gill 2 longer than others of the anterior series; Gills 5, 6 and 7 long and linear. Antennae with seven segments differentiated in the flagellum.

Fourteenth instar, (Fig. 7,–11 and 11a, 11b and 11c). At this stage the anterior gills have grown out to a fair size and are lamellate. As yet there is no appearance of the tuft of filaments at the bases of the gills. The posterior gills, 5 to 7 inclusive, are still long and linear, but their length has decreased in relation to that of the nymph.

The mesothorax is shown in Fig. 7,–11a. The postero-lateral angles, at which point the wing pads later develop, are quite evenly rounded.

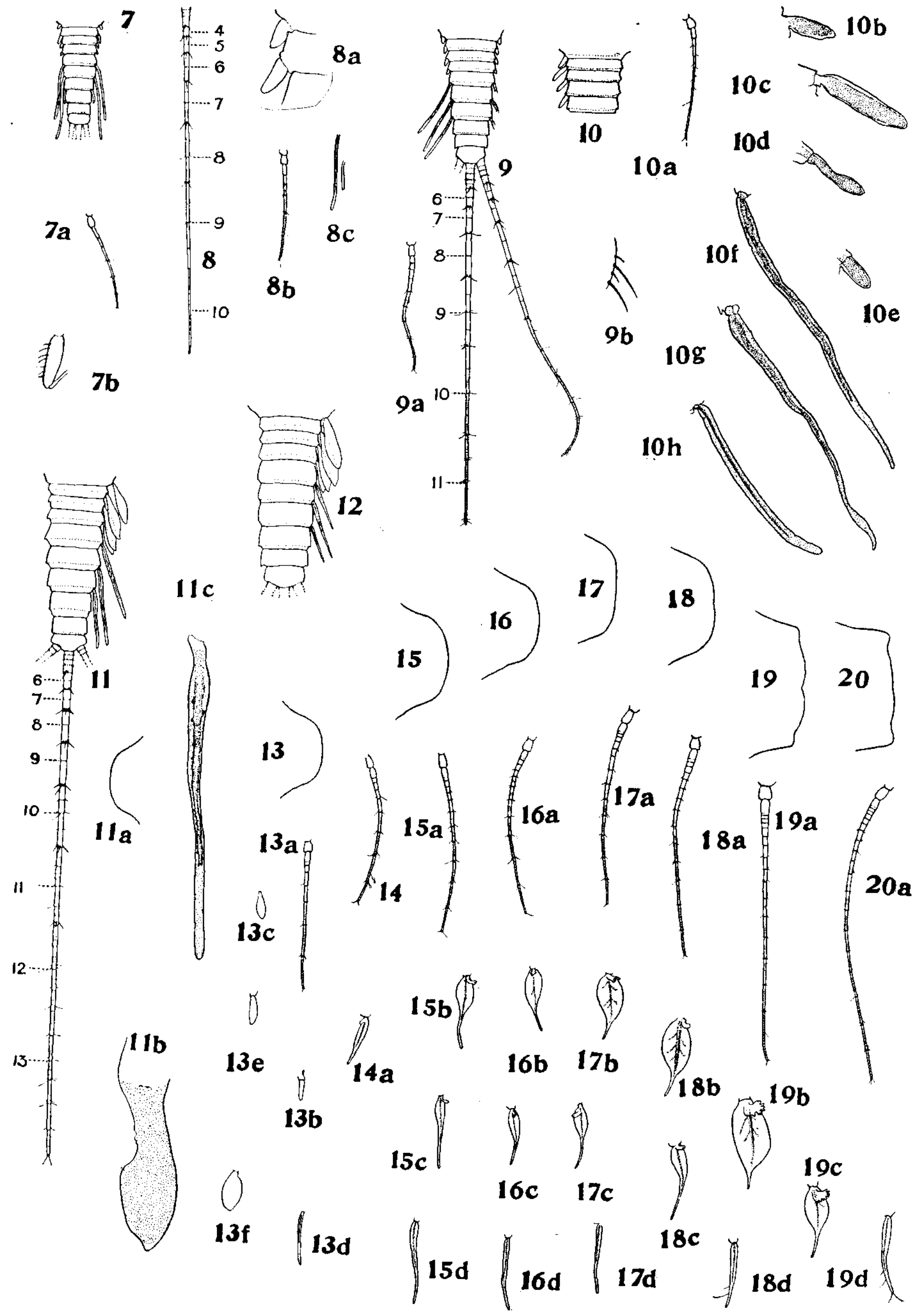

FIG. 7. *Stenonema canadense. 8a, 9b, 10b-10h, 11b, 11c, mag. Y; others mag. X. 7, ?Eighth instar, gills; 7a, antenna; 7b, femur. 8, Tenth instar, caudal filament; 8b, antenna; 8c, sixth and seventh gills; 8a, Gills 1 and 2. 9, Eleventh instar; 9a, antenna; 9b, part of femur. 10, Twelfth instar (anterior four gills); 10a, antenna; 10b–10h, Gills 1–7 under mag. Y. 11, Fourteenth instar; 11a, mesothorax; 11b, 11c, Gills 1 and 6. 12, Fifteenth instar. 13, Mesothorax; 13a, antenna; 13b, regenerating fifth gill; 13c, fourth gill; 13d, seventh gill; 13e, sixth gill; 13f, second gill. 14, Antenna; 14a, fifth gill. 15, Mesothorax; 15a, antenna; 15b, fifth gill; 15c, sixth gill; 15d, seventh gill 16, Mesothorax; 16a, antenna; 16b, fifth gill; 16c, sixth gill; 16d, seventh gill. 17, Mesothorax; 17a, antenna; 17b, fifth gill; 17c, sixth gill; 17d, seventh gill. 18, Mesothorax; 18a, antenna; 18b, fifth gill; 18c, sixth gill; 18d, seventh gill. 19, Mesothorax; 19a, antenna; 19b, fifth gill; 19c, sixth gill; 19d, seventh gill. 20, Mesothorax; 20a, antenna.*

?Fifteenth instar, Fig. 7,–12. Anterior gills larger and posterior ones have become smaller and more lanceolate; Gill 5 more affected by this process than 6, and in subsequent instars the former keeps ahead of the latter in its metamorphosis.

Beyond this point in the life history it was not possible to determine the number of the instar accurately from the material which was collected until nearer the end of nymphal life, when the last seven instars were fairly accurately determined.

Fig. 7,–13 to 20a, show the changes taking place in certain structures in an incomplete series of nymphs from a stage (13a) in which there were eight or nine segments differentiated in the antennae up to a stage (20a) in which nineteen segments were differentiated. Gradual change at each ecdysis is evident in the posterior border of the mesonotum, with the incidence of wing pad formation at about the middle of the series as a gradual prolongation of the postero-lateral angles of the notum. Gill 7 shows little change throughout the series, but Gills 5 and 7 show considerable change from the linear type found in Gills 5 to 7 in early life to the lamellate type of the anterior segments.

At the stage in which there are eight segments differentiated in the antennal flagellum, the ventral lobe of the fifth and sixth gills has made its appearance and during subsequent instars this lobe becomes more complex until finally it is a tuft of filaments. The basal part of these gills becomes more and more expanded and the terminal part shortens until finally Gills 5 and 6 from their very different appearance early in development come to resemble the anterior gills. Gill 7 does not change noticeably and never develops the tuft of filaments at the base.

Fig. 8,–21, 21a, 21b, 21c and 21d show parts of a later instar in which there are twenty segments differentiated in the antennal flagellum. The mesothoracic wing pads are more prominent than in previous instars. Gills 5, 6 and 7 are shown greatly enlarged in Fig. 8,–21b, 21c and 21d.

Fig. 8,–22, 22a, illustrate a later instar in which 21 or 22 segments are differentiated in the antennal flagellum.

Fig. 8,–23, 23a, 23b, 23c and 23d are of a later instar in which 23 or 24 segments are differentiated in the antennal flagellum. The mesothorax is shown in Fig. 8,–23. In Fig. 8,–23b and 23c are shown Gills 5 and 6 which are now much like the anterior gills and have lost the terminal filamentous portion.

Fig. 8,–24 and 24a are of a still later stage, in which about 25 segments are present in the antennal flagellum. The wing pads measure about .1 mm. in length. In this instar the developing external genitalia are not apparent, but in the next instar they can be made out so that it is possible from here on to separate male and female individuals on this character. With the wing pads and external genitalia developing through the next instars it was possible by an examination of a number of individuals to determine what are believed to be the last eight instars.

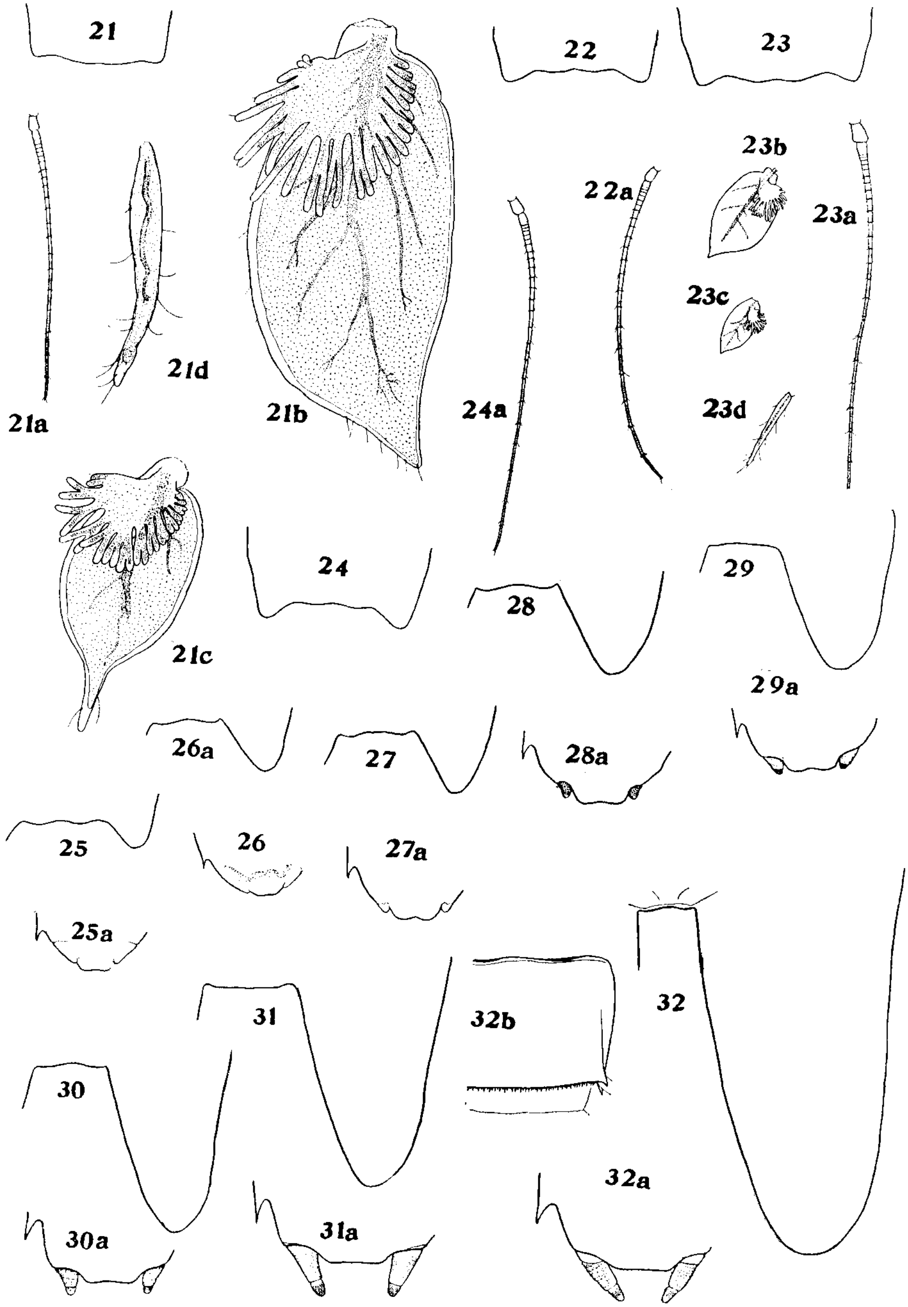

FIG. 8. *Stenonema canadense. 21b, c, d, mag. Y; others mag. X. 21, Mesothorax; 21a, antenna; 21b, fifth gill; 21c, sixth gill; 21d, seventh gill. 22, Mesothorax; 22a, antenna. 23, Mesothorax; 23a, antenna; 23b, fifth gill; 23c, sixth gill; 23d, seventh gill. 24, Mesothorax; 24a, antenna. 25, Mesothoracic wing pad; 25a, developing male genitalia. 26a, Mesothoracic wing pad; 26, male genitalia. 27, Mesothoracic wing pad; 27a, male genitalia. 28, Mesothoracic wing pad; 28a, male genitalia. 29, Mesothoracic wing pad; 29a, male genitalia. 30, Mesothoracic wing pad; 30a, male genitalia; 31, Mesothoracic wing pad; 31a, male genitalia. 32, Mesothoracic wing pad; 32a, male genitalia; 32b, seventh abdominal tergum.*

Octult instar, (Fig. 8,–25, 25a). The developing male claspers may be distinguished at this stage as small indentations on the postero-lateral margins of the sternum. There is a suture separating the posterior portion of the sternum from the anterior sternite proper, which suggests that this posterior part of the sternum may be the fused appendages of this segment, part of which will develop into the claspers and the medial part be associated with the penes.

Septult instar, (Fig. 8,–26, 26a). Male claspers have not increased much in size and are rather difficult to distinguish as in the previous instar.

Sextult instar (Fig. 8,–27, 27a). Male claspers not very distinct, but visible as rounded projections on the lateral borders of the sternum; their bases visible; they extend less than half way from their bases to the posterior border of the ninth sternum.

Quintult instar, (Fig. 8,–28, 28a). Male claspers composed of one segment only, darkly pigmented with a pale tip, extending only slightly more than half way from their bases to posterior border of ninth sternum.

Quartult instar, (Fig. 8,–29, 29a). Male claspers of two segments; small distal segment pigmented to tip; claspers reach the level of posterior border of ninth sternum or project very slightly beyond it.

Tertiult instar, (Fig. 8,–30, 30a). Apical segment of clasper pigmented in proximal half and unpigmented distally; basal segment pigmented, the pigment being distributed in annuli.

Penult instar, (Fig. 8,–31, 31a). Male claspers about .26 mm. in length, composed of two distinct segments and an indistinctly marked off basal segment; middle segment of clasper long and smooth; apical segment short and darkly pigmented distally to the apex.

Last instar, (Fig. 8,–32, 32a, 32b). In Fig. 8,–32b is shown the tergum of the seventh abdominal segment. The females are larger as a rule, having a wing pad length of about 2.2 mm.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to imago are illustrated in Fig. 9,–33 to 36. In Fig. 9,–33, 33a, 33b, of the caudal filament of penultimate nymphal instar, there are about 122 segments, formed as indicated in about 40 instars. In the subimago and imago filament there are decidedly fewer segments, about 70 in the imago. This reduction may come about as in *Ephemera*, by the dropping of distal segments and probably also by the loss of a few basal segments by fusion and obliteration of the sutures.

There is considerable metamorphosis of structure during nymphal development in *Stenonema canadense*. Some structures, such as antennae and caudal filaments, develop continuously with the growth of the nymph, maintaining their length with little increase in relation to the size of the nymph. These structures, particularly the caudal filaments, are the most sensitive indicators of change, and record every ecdysis as far as can be made out. Other structures, such as the mouth parts, wing pads and external genitalia, develop during a particular period of the nymphal life and during this time change greatly relative to the changes in the nymph as a whole.

The mouth parts change radically during the early instars, but by about the twelfth have a form similar to that of the full-grown nymph. The gills show definite metamorphosis apart from an increase in size. The gills of Segments 5 and 6 appear at the second instar and those of Segments 1 to 4 and 7 do not make their appearance until about the eighth instar. Gills 1 to 4 grow directly into flat lamellae, but Gill 7 becomes long and linear and similar to Gills 5 and 6. The latter pair gradually metamorphose until they are similar to Gills 1 to 4, but Gill 7 remains as a single lanceolate appendage known as the vestigial gill. Gills 1 to 6 develop a tuft of filaments ventrally near the base.

The wing pads make their appearance about the fifteenth from the last instar, and grow back at first rather slowly, then by gradually accelerated growth. The external male genitalia are first distinguishable in about the eighth from the last instar. The claspers in this instar are slightly rounded projections from the ninth sternum and the anlage of the penes lies between them. The caudal filaments at hatching have three segments differentiated basally and a terminal undifferentiated termina part to the flagellum. In the second instar there is one further segment differentiated in the caudal filaments, making four in addition to the terminal piece, which, however, is only about half as long as in the first instar. Apparently there has been very little growth during the first instar. In the third instar there are five segments differentiated, and the fourth bears a stout seta apically. In the fourth instar there are seven segments differentiated, show'ng that two segments are being added at each instar instead of one as in the earlier instars. In subsequent instars two segments are added up to the seventh, when three segments are added. In the eighth instar and in all subsequent instars, four segments apparently are added at each ecdysis except in the subimaginal and imaginal instars. The individual new segments added at each instar are of approximately equal length and volume in the early stages, so that change in the rate of growth is indicated by the manner in which the segments are added, the supplemented segments increasing in number in the ratio of 1 : 1 : 2 : 2 : 2 : 3 : 4 : 4, from the first to the ninth instars.

Curiously it is at the eighth instar that the gills make their appearance on Segments 1 to 4 and 7, a fact which may be more than coincidental since the increase in rate of growth probably implies a greater oxygen requirement. Also, as mentioned above, this stage is a very critical one in the life of the nymph as indicated by failure to rear them beyond this stage in conditions under which they incubated well and passed through the first stages successfully.

In Fig. 9,–33 a complete lateral caudal filament of a female individual of the next to last nymphal instar is shown. The portions of the filament laid down at each instar are indicated and a count of these regions beginning at the apex reveals that there are 39 instars recorded which, with the last,

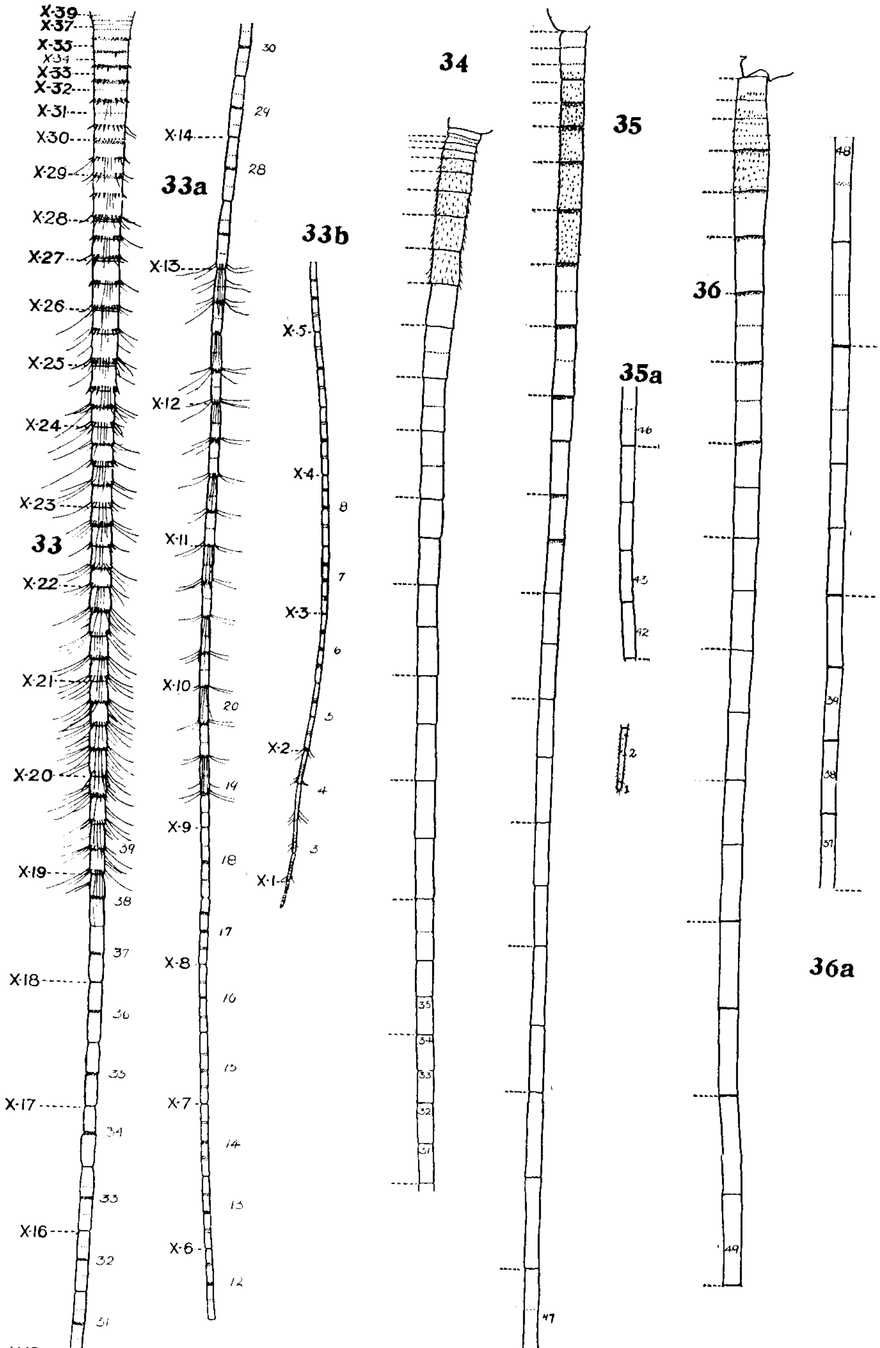

FIG. 9. *Stenonema canadense. All mag. X. 33, 33a, 33b, Complete lateral caudal filament (cercus) of a female nymph in the penultimate instar. 34, Lateral caudal filament of subimaginal female. 35, 35a, Lateral filament of adult female; 36, 36a, Lateral filament of adult male.*

make 40. Unfortunately, this is not the full number of instars represented during the nymphal period. Fusion of segments at the base and their almost entire obliteration results in a smaller number than was actually differentiated during development. There are probably between 40 and 45 instars in this species. Of these, 35 have been illustrated showing how they differ morphologically, and obvious gaps in the sequence noted.

Ephemerella subvaria McDunnough

An account of the full-grown nymph of this recently described species is given by McDunnough (1931). This was a common species in the streams studied by the author and females were frequently taken ovipositing. The eggs are extruded in a spherical mass of a brownish yellow color, which is dropped in flight into the water of the rapids.

The eggs are ovoid in shape, finely reticulate, and measure about .2 by .15 mm. They are stuck end to end by a mass of very elastic gelatinous material so that when the string of eggs is stretched this material pulls out into a thin elastic thread. Sometimes the strings branch and anastamose, three eggs instead of two being held together by one mass of the elastic material.

First instar, Fig. 12,–8. Three individuals measured on an average .495 mm. in length, exclusive of the caudal filaments. The head is somewhat triangular with the apex directed more ventrally than in *S. canadense;* antennae with two distinct basal segments, a very short proximal and a longer distal one, and a flagellum in which three segments are distinguishable including the apical portion; in front of each compound eye a curved hair.

Each of the femora bears distally a curved hair or fine seta; tarsal claws pointed and with two rows of pectinations converging on the large apical claw.

Caudal filaments relatively short, equalling about one third the length of the body; three well developed basal segments and an apical undifferentiated part which shows in some cases an indication of weak joints; median caudal filament slightly longer than the lateral ones; gills absent.

Isonychia bicolor Walker

The full-grown nymph of this species is described by Needham (1905) under the name *Chirotenetes albomanicatus* Needh. which is placed as a synonym by McDunnough.

The eggs, (Fig. 12,–2), are spherical in shape with a diameter of .25 mm. They are extruded from the female in the form of a spherical mass of an olive green color, in which form they are dropped on the surface of the water during flight, and quickly disperse.

First instar, (Fig. 12,–1). The average length of six individuals was .71 mm. with a range of from .67–.75 mm. Gills have not made their appearance and the yolk is still present as the opaque body shown in stipple. The compound eyes are less prominent than the lateral ocelli and are situated directly behind them.

Antennae with two basal segments and a tapering, flexible flagellum in which one proximal segment has been differentiated; tarsal claws pectinate; femora with only one rather stout distal seta on the posterior border and a somewhat weaker dorsal one; three caudal filaments each consisting of three well developed proximal segments, each of which bears a conspicuous crown of spines and a terminal undifferentiated part bearing a few hairs.

Second instar, (Fig. 12,–3, 3a, 3b). Gills have not appeared; antennae with no increase in number of segments; still but one segment differentiated proximally in the flagellum; the terminal portion of the flagellum is not, however, tapered as in the first instar, but more cylindrical with a slight constriction in the middle; setae on posterior border of femora increased to two on fore femur and three on hind femur, on which they are well developed, the middle one being the longest; caudal filaments show a definite change in the addition of one segment, making four well differentiated segments proximally; this change may easily be overlooked, however, since the crown of spines on the proximal segment has been so reduced that it is visible only with the closest observation. The new distal segment (Segment 4), has been added from the undifferentiated distal part of the filament of the first instar; basal Segments 1 and 2 intimately fused together, the crown on Segment 1 being almost obliterated. In the next instar it is very probable that the separation between 1 and 2 will not be apparent, the fusion being complete, and so on through the life of the nymph, segments are formed apically and proximal ones lose their identity in the process of fusing to form a strong rigid base for the filament.

One individual in this stage had a portion of the exuvia of the first instar attached, showing plainly the single spine distally in the third femur, and also the three-segmented condition of the bases of the caudal filaments. The fusion of Segments 1 and 2, which makes it impossible to distinguish them in some individuals and very difficult in others, probably led to Lubbock's conclusion that growth takes place by an elongation of the basal segment and its ultimate division into two. In describing the second instar of *Cloeon dimidiatum*, Lubbock, (1864) writes: "The two tails have increased to a length of 25/200, and consist of twenty segments." (It should be mentioned that this large number of segments is due to the fact that Lubbock includes the small subsegments of the flagellum in his count.) "Here again as in the antennae almost the whole change has taken place in one segment, which however, is in the present case the basal one. The remainder are almost exactly as they were before. As already mentioned the basal segment was in the first stage 5/800 in length; in the present it has divided into two segments which, taken together, are 9/800 and we see therefore that almost the whole increase of length is in this one part."

In another section of the present paper the addition of segments has been discussed and it is evident that segments are added from the distal end to those already present, and not by a division of segments which have been differentiated. The basal segments are not becoming shorter by being subdivided, but by thickening and fusing together.

Fig. 12,–4, 4a, 4b show a later instar whose number was not determined owing to the absence of antennae and caudal filaments which had been broken off. From its size (1.20 mm.) and the number of setae on the femur, it is probably about the fourth. At this stage the gills are making their appearance as rounded projections from the postero-lateral angles of the segments. They are slightly longer on Segments 4, 5 and 6, but probably appear simultaneously on all segments as was the case with *Ephemera simulans*. The claws are pectinate.

These early stages were reared from the egg in water from the stream standing in an open jar. Eggs from several females were set to incubate on July 6, 1931. On July 24 some had hatched although none were out on July 21. Four first instar individuals were taken in a sample from the jar on July 24. On July 28 a sample was taken which contained one individual in the first instar and twenty in the second instar. On August 11 one individual was removed from the jar and this one was in about the fourth instar. No others were seen on this date. On August 15 one first instar nymph was taken. Development in this species is apparently very rapid. The temperature of the water in the jar was of course higher than is normal for hatching of this species.

Epeorus humeralis Morgan

The full-grown nymph is described by Dr. Anna Morgan (1911).

The eggs (Fig. 10,–1a) which are of a reddish color, are extruded from the female in two ribbon-like masses but quickly disperse on touching the water. The length is about .20 mm. and the width .12 mm. They are not provided with special organs of attachment.

First instar, (Fig. 10,–1). The average length of six nymphs was .40 mm., the limits being about .33 to .42 mm. The head broader than long and convex in front; the compound eyes (the pigmented portion of them at least) smaller than the lateral ocelli; antennae with two basal segments and a flagellum in which one segment is differentiated basally; portion of flagellum distal to this segment divided into small faintly demarcated subsegments as mentioned above in the description of *S. canadense*. The opaquely reddish mass of the enteron is visible through the integument of the thorax. This is shown as a stippled area in the figure.

Abdomen without gills; lateral caudal filaments (cerci) with three segments differentiated at the base, each with a crown of spines apically; remaining portion of caudal filament tapered and divided into small subsegments; median caudal filament with one basal segment and a distal three-segmented flagellum.

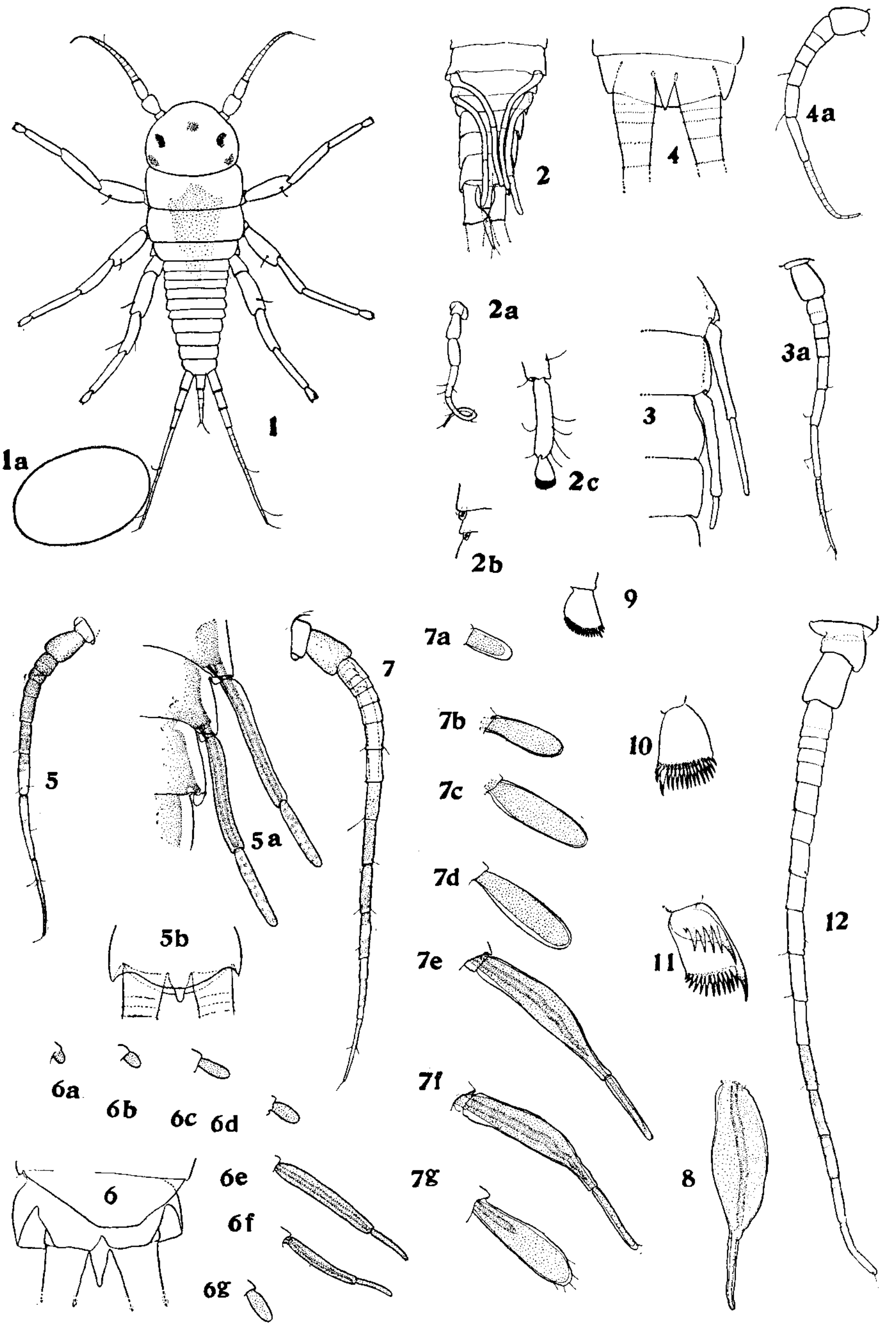

FIG. 10. *Epeorus humeralis. All mag. Y. 1, First instar; 1a, egg. 2, Ventral view of later instar; 2a, antenna; 2b, dorsal view of Gills 5 and 6; 2c, tarsus and tarsal claw. 3, Later instar, Gills 5 and 6; 3a, antenna. 4, Posterior end of earlier instar showing reduced median caudal filament; 4a, antenna. 5, Antenna; 5a, Gills 5 and 6; 5b, posterior end of abdomen. 6, Post end of abdomen, ventral view; 6a–g, Gills 1–7. 7, Antenna; 7a–g, Gills 1–7. 8, Gill 6. 9–11, Tarsal claws of 3 instars last showing new type of claw within. 12, Antenna.*

A later instar (Fig. 10,–2, 2a, 2b and 2c) shows two well developed segments in the antennal flagellum and measures .73 mm. Gills are present on Segments 5 and 6 only, long and filamentous, directed ventrally, and divided into two segments, a long basal and a shorter distal one. The fact that the gills are directed ventrally makes them very inconspicuous, and it was some time before they were noticed.

Lateral caudal filaments thick and bulbous at the base, narrowing somewhat suddenly into the main filament which had dropped off in most individuals; median caudal filament present as a short three-segmented appendage; tarsal claws are particularly interesting, consisting of a series of about 15 curved hooks of nearly equal size. One of these at one end of the series is, however, stouter. In development it is this stouter claw which will become the tarsal claw proper and those of the others which remain will become the pectinations along one side of the main claw.

In a later instar (Fig. 10,–4, 4a) in which there are six segments in the antennal flagellum, the terminal segments of the median caudal filament are dropped, leaving a short conical projection in its place. A later stage (Fig. 10,–3, 3a) is shown in which the antennal flagellum has about seven segments. The gills are present on Segments 5 and 6, but are now directed laterad rather than ventrad.

In the stage in which eight segments are formed in the antenna (Fig. 10,–5, 5a and 5b), the gills are present on 5 and 6, as two-segmented structures, and the gills of some of the other segments have appeared as mere rounded elevations on one or two of the other segments. The relative sizes of the gills (Fig. 10,–6, 6a–g) are shown on all segments in a nymph measuring 1.9 mm. The gills of 5 and 6 are still two-segmented. The gills are also present now on Segments 1, 2, 3, 4 and 7 as short, rounded appendages. The gills are shown (Fig. 10,–7, 7a–g) at the stage in which the antennae have 12 segments differentiated. Those on Segments 1-4 and 7 are becoming lamellate but are still rather thick and the tracheae are not apparent. The tracheae are showing in the basal segments of Gills 5 and 6. The latter pair are broadening at the base. Length of nymph is 2.33 mm. Gill 5 is shown (Fig. 10,–8) in a later stage in which the nymph measured 3.4 mm. Gills 5 and 6 are now very similar to the other gills, except that they are formed of two segments, the distal one of which is not becoming lamellate. The antennal flagellum at this stage is composed of 16 well developed segments.

The terminal segment of Gills 5 and 6 is dropped after the stage in which the antennae contain 22 well developed segments in the flagellum and is absent from the later instars.

Fig. 11,–13, 13a–d illustrate some of the characters of this stage which measured 4.13 mm. Gill 6 still has a vestige of the terminal part which is dropped at the next shedding. The wing pads have been developing through several ecdyses. The tarsal claw has changed, the number of hooks or claws having been reduced from about fifteen in the stage illustrated on Fig. 10,–10, to five of which one is much longer than the others and more curved. The smaller ones form now the pectinations of the larger claw.

Fig. 11,–14 and 14a–e show some structures of the last instar. Fig. 11,–14c is of the tarsal claw of the third leg, which now consists of one great, curved claw bearing four smaller straight ones as pectinations. Gills 1, 5 and 7 are shown in ventral view. Of particular interest is the chitinous thickening around the outer margin of the gills armed with numerous thickly set short stiff spines directed downward and backward, which have been described by Morgan (1913). When the nymph is facing upstream into the current, as they usually do, these spines are in contact with the stone on which it is resting, thus firmly anchoring the nymph to the rock. It should be noticed that the limpet-like adaptation is not as perfectly developed in *Epeorus* as in *Iron pleuralis*, since *Epeorus* lacks the long anterior process which is present on Gill 1, of *Iron*, and also the fold in the seventh gill of the latter, which would allow the gills of the seventh segment to fit more closely together. The appearance of the genitalia of female and male which have been developing during the later instars is shown in Fig. 11,–14d and 14e respectively.

Curiously enough the abrupt transformation of the tarsal claws illustrated in Fig. 10,–11, coincides with the point in development at which the gills have grown out far enough to come in contact with the stone on which the nymph is resting, thus aiding in anchoring it. It also coincides roughly with the time in the life of the nymphs at which there is a marked tendency to drop out of the rapids into the somewhat quieter water below. This was determined in an ecological study of this species, which is presented in another paper, (Ide, 1935).

The nymphs of this species occur commonly along with those of *Iron pleuralis*, the next species, but in the younger stages can be readily distinguished from the latter in the field by the presence of a light yellow area on the anterior part of the dorsum of the abdomen, which is contrasted with the dark greenish color of the rest of the nymph. As a young nymph *Iron pleuralis* is uniformly light brown.

Iron pleuralis Banks

The full-grown nymph of this species has already been described, with some notes on its habits (Ide, 1930).

This species follows *Epeorus humeralis* very closely in its post-embryonic development. The earliest stage collected in the stream is one (Fig. 11,–15, 15a and 15b), in which the antenna has but three well formed segments in the flagellum, and which measures about 1.10 mm. in length. The lateral caudal filaments have 19 segments and the median filament is vestigial having but two segments present. Gills are present on Segments 5 and 6 only, where they are very long and filamentous, and composed of two segments, a distal short and a basal long one.

At the stage (Fig. 11,–16, 16a–f) in which there are six segments in the antennal flagellum, the gills on Segments 1, 2, 3, 4 and 7 have appeared as minute bud-like processes. The gills on Segments 5 and 6 are composed of two segments, but have become somewhat stouter. The median caudal filament

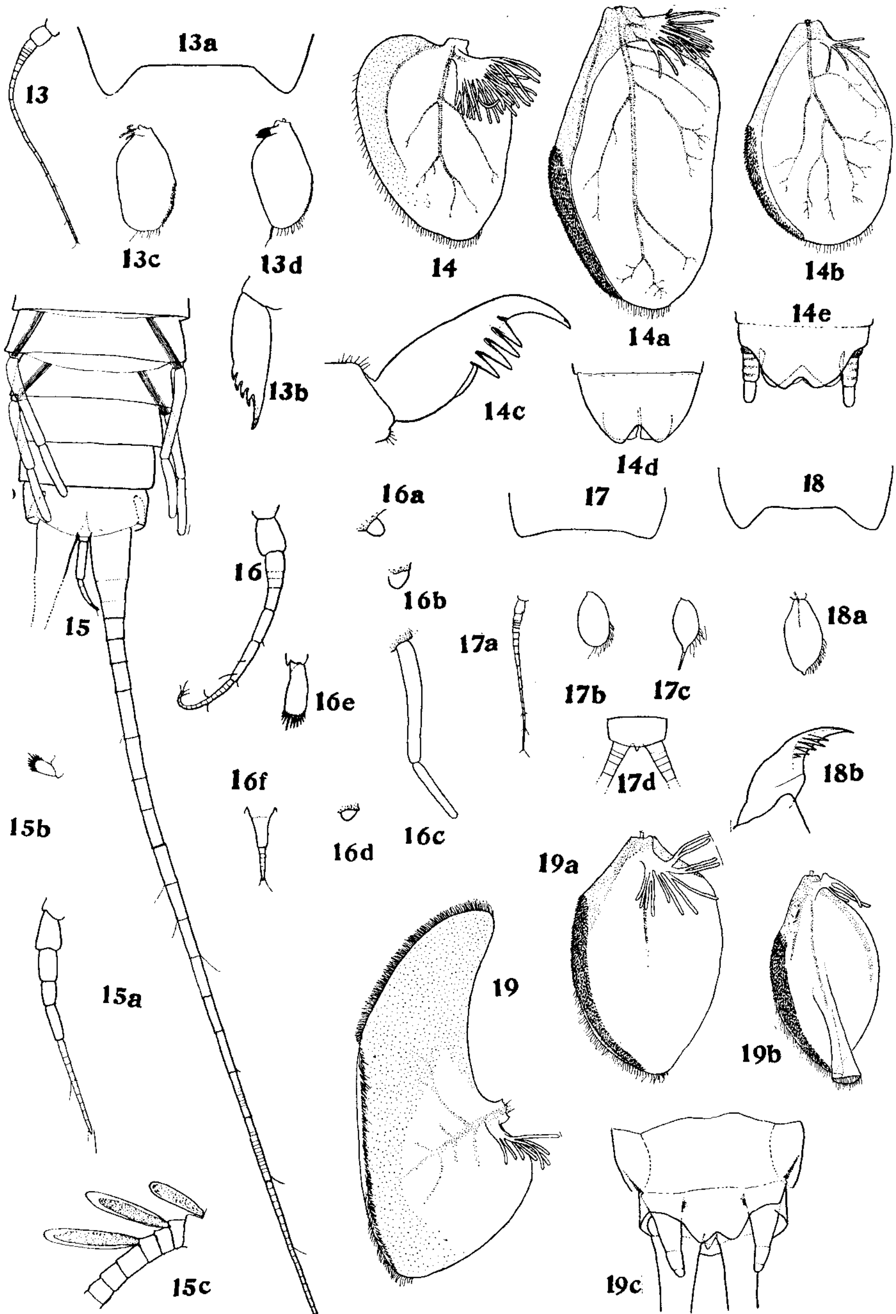

FIG. 11. *Epeorus humeralis. 13b, 14c, mag. Y. others mag. X. 13, Antenna; 13a, mesothoracic wing pads; 13b, tarsal claw; 13c, fourth gill of right side; 13d, sixth gill of right side. 14, Last nymphal instar Gill 1, ventral view; 14a, Gill 5; ventral view; 14b, Gill 7, ventral view; 14c, tarsal claw; 14d, posterior end of female, ventral view; 14e, male genitalia.*

Iron pleuralis. 15–16f, and 18b, mag. Y; 17–19c mag. X. 15, Nymph about seventh instar; 15a, antenna; 15b, tarsal claw; 15c, parasitic fungus protruding from the anus. 16, Antenna; 16a, b, c, d, Gills 1, 2, 5, 7; 16e, tarsal claw; 16f, median caudal filament. 17, Mesothorax; 17a, antenna; 17b, Gill 3; 17c, Gill 6; 17d, posterior end of abdomen, showing reduced median caudal filament. 18, Mesothorax; 18a, Gill 6; 18b, tarsal claw. 19, Last nymphal instar, Gill 1; 19a, Gill 5; 19b, Gill 7; 19c, male genitalia, ventral view.

is similar to the last described stage except that Segment 2 has become relatively shorter and more conical. The tarsal claws of *Iron pleuralis* differ from those of *Epeorus* in that the most strongly developed claw is near the middle of the series of claws rather than at one end, as was the case in the latter. Length in this stage is about 1.48 mm.

A later stage is illustrated (Fig. 11,–17, 17a–d) in which the specimen is 2.90 mm. long and the antenna contains 14 well developed segments in the flagellum, showing that the nymph has passed through several moults since the last described instar. The gills are now flat lamellae as shown in Fig. 11,–17b, 17c for gills of Segments 3 and 6 respectively. The terminal segment is present on Gills 5 and 6 as a narrow, cylindrical process. The distal segments of the median caudal filament have now been dropped (Fig. 11,–17d). About two instars later, when the antennae have about seventeen segments, the terminal segments of Gills 5 and 6 are dropped (Fig. 11,–18, 18a, 18b). The sixth gill is shown in Fig. 11,–18a, with a slight tubercle at its apex, the remnant of the former distal segment. At this stage the gills are all rather similar, although they have originated at different times in development.

From now on the metamorphosis is chiefly in the growing wing pads, the external genitalia, the antennae and caudal setae. In Fig. 11,–19, 19a–c, the detail of Gills 1, 5 and 7 of the last instar is given. The most notable feature is the anterior extension of Gill 1 which passes beneath the thorax ventrally, where it nearly meets the corresponding gill of the opposite side. In the gill of the seventh segment there is a fold medially directed in such a way that the gills of both sides nearly meet below the abdomen. All the gills, by this arrangement, form a sucking disc, the water pressure keeping the gills firmly pressed against the rock. Along the lateral borders, where the gills meet the rock, are series of short spines directed backwards, which form an efficient anchor, as Morgan has pointed out for *Iron fragilis* (1913).

In this species as in *Epeorus* there has been a considerable metamorphosis in gills and tarsal claws. In the younger stages the gills are not developed as organs of retention and so the whole load comes on the tarsal claws, which are numerous, small and arranged in a V-shaped line at right angles to the force so that they provide a maximum grip. The nymph is so small that it is only the minute irregularities of the surface of stones which are of use to it for retention, and in order to grip these irregularities there must be many, closely set, tarsal claws. As the nymph grows in size the gills become important as gripping organs and the claws change, until ultimately there is one large tarsal claw with pectinations along the inner border. These pectinations are derived from some of the smaller tarsal claws present in the early stages of development. The claws of the larger nymphs are probably used more for crawling than for clinging to the surface of the stone, and the irregularities against which they pry are not of the order which are useful earlier in the life history but much larger ones which are naturally much farther apart. A series of small tarsal claws in the form of a rake probably would not be as efficient now as the single pectinate claw. This is an interesting example

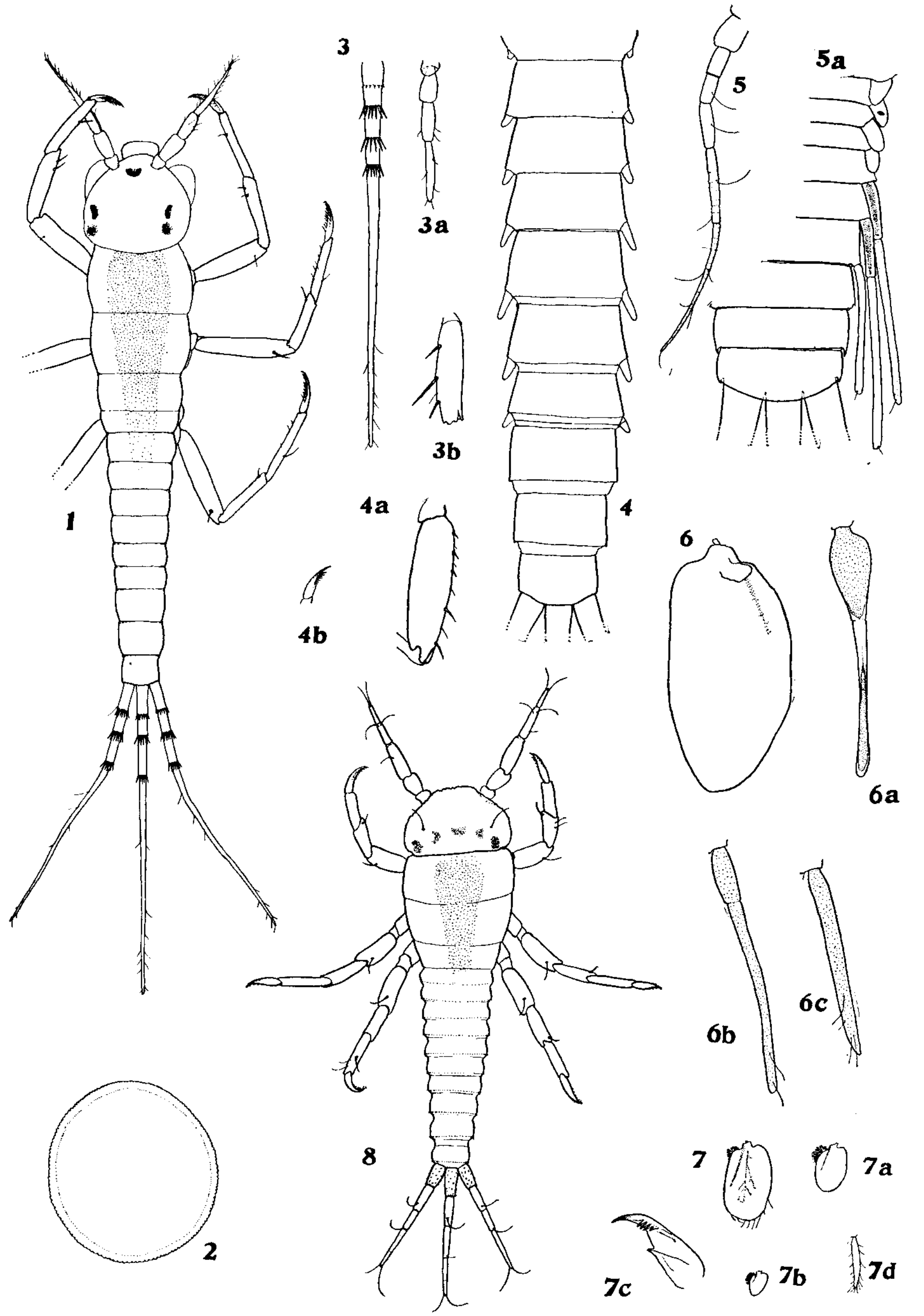

FIG. 12. *Isonychia bicolor. All mag. Y. 1, First instar. 2, Egg. 3, Second instar, lateral caudal filament; 3a, antenna; 3b, hind femur. 4, About fourth instar, showing gills; 4a, hind femur; 4b, tarsal claw.*

Stenonema fuscum. 5–6c, 7c, mag. Y; 7, 7a, b, 7d, mag. X. 5, Antenna; 5a, gills; 6, Third gill, ventral aspect; 6a, fifth gill; 6b, sixth gill; 6c, seventh gill. 7, Third gill; 7a, fifth gill; 7b, sixth gill; 7d, seventh gill; 7c, tarsal claw of foreleg.

Ephemerella subvaria. Mag. Y. 8, First instar.

of an organism radically changing its relation to its environment by growth. As it grows larger it has to adjust itself differently to the same flow of water, so that it is rather the relation of the environment to the organism which is important as a cause in metamorphosis than change in the environment itself.

This change in the tarsal claws is probably quite abrupt as was the case with *Epeorus* described above. An individual was not found which showed the change, but intermediate steps in the process were not seen in considerable material examined.

Heptagenia pulla Clemens

The last nymphal stage is described and figured by Clemens (1915). The first stage was not found in the present study.

A nymph (Fig. 13,–1, 1a, 1b) measuring 1.6 mm. in length showed the following points. The antennae have four well developed segments in the flagellum. The three caudal filaments, of which the central one is slightly longer than the others, are composed of 23 segments, the basal three of which are fused so as to be indistinguishable. Strong setae are present apically in Segments 6, 8, 10, 12 and 16. On Segment 14 there is a stiff hair only, so that probably Segments 15, 16, 17 and 18 were added at one ecdysis; this is probably the ninth or tenth instar.

Gills are present on Segments 2, 3, 4, 5, 6 and 7. On Segment 2 the gill is a mere bud; on Segments 3, 4 and 7 the gills are unsegmented filaments, a little longer than their respective segments. On Segments 5 and 6 the gills are about twice as long as on 4 and 7, and composed of two segments. By analogy with *Epeorus* and *Iron* the gills presumably appear on Segments 5 and 6 at an earlier ecdysis than on the other segments.

During subsequent development the gills assume a lamellate form and the tuft appears at the base of the gill, as shown for an intermediate stage in Fig. 13,–4, of Gill 5. All gills from 1 to 7 are now very similar except that 5 and 6 retain the terminal segment. This terminal segment is present on Gills 5 and 6 in the penultimate instar (Fig. 13,–3, 3a–c) and sometimes even in the last instar, as a mere vestige which has been generally overlooked in the published figures of this species.

Heptagenia hebe

A full-grown nymph was figured and described by Clemens (1915) under name *Ecdyonurus maculipennis*. This species was not reared in connection with the present study, but nymphal material was collected from the stones in the streams.

The earliest stage found (Fig. 13,–5 and 5a) shows the antennae with four well developed segments in the flagellum. The three caudal filaments are similar except for the slightly greater length of the median one. They consist of 16 well marked segments with strong setae distally on Segments 4, 6, 8, 10 and 12, and a terminal flagellum. It is probably about the seventh instar. Gills are present as long filaments on Segments 4, 5, 6 and 7, those on 5 and 6

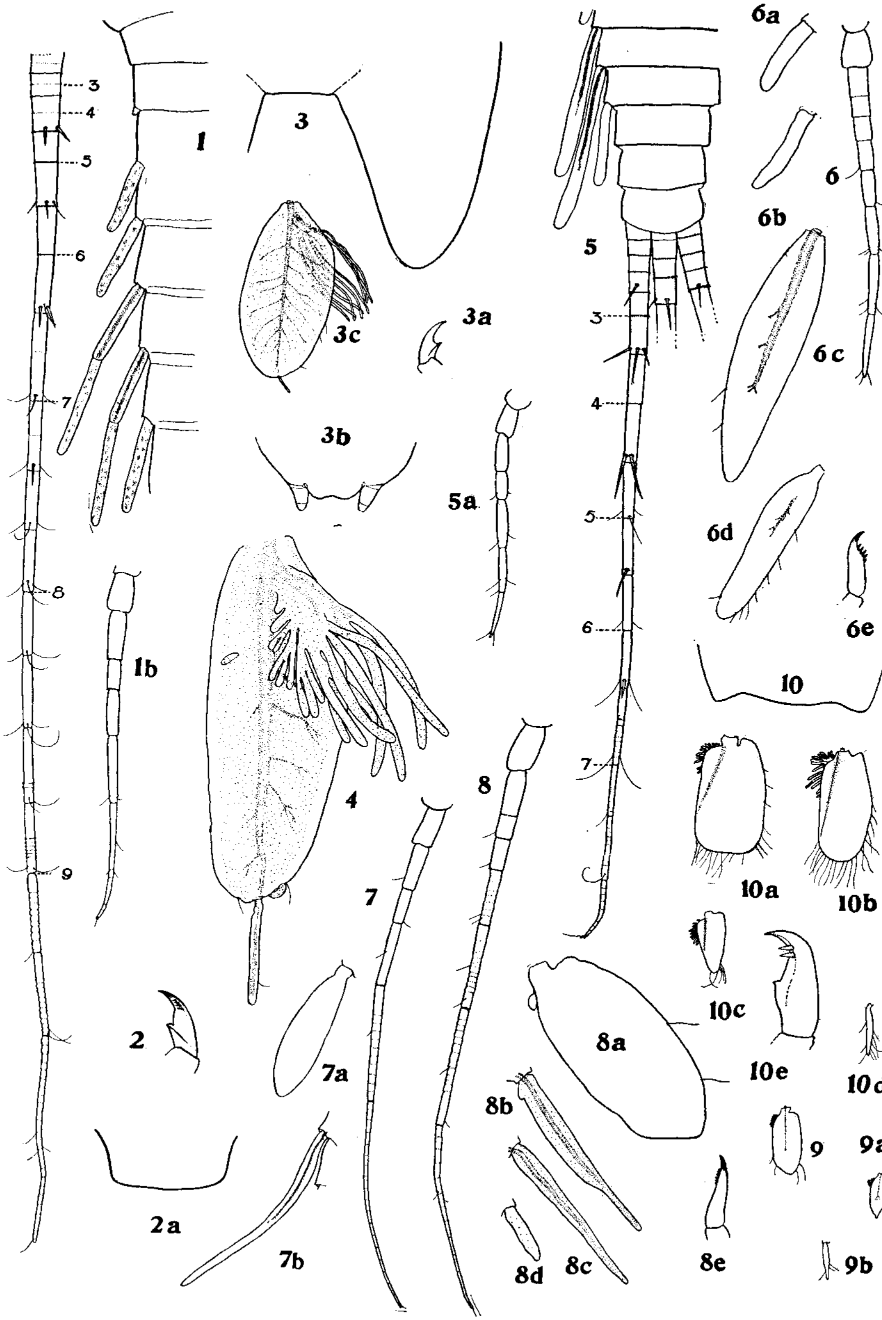

FIG. 13. *Heptagenia pulla. 1, 1a, 1b, 2 and 4, mag. Y; 2a, 3–3c, mag. X. 1, Ninth instar showing gills on Segments 2–7; 1a, lateral caudal filament; 1b, antenna; 2a, Mesothorax; 2, tarsal claw; 3, Penultimate instar, mesothoracic wing pads; 3a, tarsal claw; 3b, male genitalia; 3c, Gill 5. 4, Fifth gill.*

Heptagenia hebe. 5–6e mag. Y. 5, Seventh instar; 5a, antenna. 6, Antenna; 6a, 6b, 6c, 6d, Gills 1, 2, 5, 7 respectively; 6e, tarsal claw.

Stenonema tripunctatum. 7–8e and 10e mag. Y; 9–10d, mag. X. 7, Antenna; 7a, third gill; 7b, fifth and sixth gills. 8, Antenna; 8a, second gill; 8b, c, d, fifth, sixth, seventh gills; 8e, tarsal claw. 9, Gill 5; 9a, Gill 6; 9b, Gill 7; 10, Mesothorax; 10a, Gill 3; 10b, Gill 5; 10c, Gill 6; 10d, Gill 7; 10e, tarsal claw.

being about twice the length and of greater thickness than those on 4 and 7, but not segmented as in *Heptagenia pulla*. Here again, as in other cases referred to earlier, it seems extremely probable that there were gills present on Segments 5 and 6 before there were any on the other segments.

In a later stage (Fig. 13,–6, 6a–e) in which the antennae are composed of seven well differentiated segments, the gills are further advanced in development, and present on Segments 1 to 7. On Segments 1 and 2 they are short, on Segments 3 and 7 about twice as long as on 1 and 2, and on 4 they are slightly longer than on 3 and 7. On 5 and 6 they are longer than on any of the other segments but lack the terminal segment found in *Heptagenia pulla*. This seems to be a significant difference between the two groups in the genus *Heptagenia*, but of course may not prove to be a group character.

As development proceeds the gills grow broader, develop the tuft of filaments at the base, but otherwise do not change much and are very similar in form to one another. The gills on 5 and 6 keep ahead of the others in size, however, and the others retain their differences in relative length. It seems probable that in this form both segments of Gills 5 and 6 of *Heptagenia pulla* are represented in the corresponding unsegmented gills, and that the whole length of the gill has entered into the formation of the lamella. This would account for their relatively greater length in the ultimate nymphal instar.

In development of gills this species probably follows closely *Ecdyonurus forcipulus* Koll (see Gros, 1923).

The tarsal claws are pectinate as shown for the stage in which the antenna is of seven segments as shown in Fig. 13,–6e.

Stenonema tripunctatum Banks

The full-grown nymph is well described by Clemens (1915). In the earliest instar found the antennal flagellum contains four well differentiated segments (Fig. 13,–7). The anterior gills were lamellate and similar to the third of the left side which is figured in Fig. 13,–7a. Gills 5 and 6 (Fig. 13,–7b) are long and filamentous and similar to one another, and the gills of 7 are lacking entirely. It seems likely that in *Stenonema tripunctatum*, as in *S. canadense*, the gills of Segments 5 and 6 appear before those of any of the other segments.

The next stage found was the instar in which the antennal flagellum is composed of six well differentiated segments Fig. 13,–8. At this stage the gills on 1 to 4 are lamellate with the tuft of filaments beginning to form at the base. Gill 2 is shown in Fig. 13,–8a. The gills on 5 and 6 (Fig. 13,–8b and 8c) are long and filamentous, that of 5 being somewhat broader at the base and constricted toward the distal extremity. Gill 7 (Fig. 13,–8d) is not linear, but rather short, differing in this respect from *S. canadense*. The tarsal claw (Fig. 13,–8e) has the middle spine very much developed and the others arranged on each side of it as two rows of pectinations.

In a later stage the antennae have about 13 segments differentiated in the flagellum. The gills of this instar on Segments 5, 6 and 7 are illustrated in Fig. 13,–9, 9a and 9b. Gill 7 is lanceolate and lacks the tuft of filaments at the base. Gill 6 is lamellate and has dropped the terminal portion whose former position is marked by a small tubercle at the apex of the gill. Gill 5 is lamellate and shows no sign of the linear tip; it has metamorphosed so that it is similar to the anterior gills. Gills 5 and 6 both have the tuft of filaments at the base developed at this stage.

In a still later stage (Fig. 13,–10, 10a–e), in which the antennae have about 19 well developed segments in the flagellum the gills are very similar to those of the full-grown nymphs. The main change now takes place in the greater size of the gills and the greater complexity of the tuft of filaments at the base. In this stage the wing pads (Fig. 13,–10) have been developing through three or four instars and the claws (Fig. 13,–10e) have changed somewhat, the change being mainly in the reduction in the number of pectinations. In one series there are two, and in the series of the opposite side, but one angular projection which may be a different structure entirely from the claws which form the pectination. Gill 7 has not changed its form. Gill 6 has lost the vestige of the terminal part, is triangular in shape and shows a greater development of the tuft of filaments at the base. Gills 3 and 5 are similar to one another and have a truncate posterior border.

Stenonema fuscum Clemens

The full-grown nymph is described by Clemens (1915) and also by McDunnough (1933). This form was not studied in great detail, but three stages are figured, which throw some light on the development of gills in this group. At the stage (Fig. 12,–5, 5a) in which the antennal flagellum contains four segments proximally, and the nymph is about 1 mm. in length, gills are present on Segments 1 to 4 as short rounded projections varying slightly in length, but in general being about equal to the length of the corresponding abdominal segment. Gill 3 is slightly longer than the other gills of the anterior series; Gill 7 is long and unsegmented, similar to the gills of the same segment in *S. canadense.* The gills on 5 and 6 are similar to each other and are divided into a short basal segment and a terminal segment which is nearly three times as long as the basal one. These gills are more like those of *Iron, Epeorus* and *Heptagenia pulla* than those of *Stenonema canadense* in this respect. Their greater length and the presence of the trachea in the basal segment suggest that these gills appeared earlier than the other gills, probably at the second instar. The tarsal claws are similar to those of *S. tripunctatum* in possessing two rows of pectinations, one on either side of the central claw.

Fig. 12,–6, 6a–c, taken from an individual 1.7 mm. long in which nine segments were differentiated in the antennal flagellum, forecast the probable fate of each segment of the fifth and sixth gills. The basal portion has become expanded (6a), the content has been constricted off at the intersegmental region, so that at the next instar the distal segment will be dropped altogether and the basal segment will form the ultimate gill lamella as in *Epeorus.* Gill 7 is unsegmented and remains practically the same throughout development, and in the full-grown nymph is referred to as a vestigial gill.

Fig. 12,–6 shows the tuft of gill filaments forming at the base of the lamella of Gill 3. Gills 1–4 are all lamellate and similar to Gill 3, although varying somewhat in size. In Fig. 12,–7, 7a–d are shown Gills 3, 5, 6 and 7 at a later stage (antennae 16 segments) when Gills 5 and 6 have lost their distal segments and are similar to Gills 1 to 4. Gill 7 retains its simple and lanceolate character.

Leptophlebia debilis Walker

The full-grown nymph of this species is described by the author (Ide, 1930).

Some of the younger stages of this species were found. In the stage in which there are eleven segments in the antennal flagellum, the gills (Fig. 4,–27 to 29) are very long, slender filaments, showing the beginning of the inner or upper ramus of the gill. This ramus, which later becomes nearly as long as the primary ramus, is at this stage a mere bud at the base of the gill. A branch of the trachea runs into it. Undoubtedly in an earlier stage the gills would be simple lanceolate filaments without the side branch. A European genus, *Paraleptophlebia*, has been figured in this stage (Lestage, 1916).

Discussion and Conclusions

A study of the life histories of these nymphs has brought out some points which will be discussed under five headings:

(a) Origin of segments in the antennae and caudal filaments.

(b) Variation in the number of caudal filaments present in mayflies.

(c) Metamorphosis of claws and mouth parts.

(d) Appearance and metamorphosis of gills.

(e) Metamorphosis in general.

Origin of Segments in the Antennae and Caudal Filaments

Both antennae and caudal filaments show some points regarding the formation and addition of segments during growth, which should be noted. The antennae and filaments roughly maintain their length relative to the size of the instar, with a few exceptions (see *Baetis*).

In the very small nymphs a very flexible flagellum is satisfactory, but as the nymph grows in size the basal part of the flagellum must become more solid and rigid for some distance, the intermediate part must be flexible, accomplished by the production of segments or joints which allow of movement and yet are rigid enough to give support. Rigidity and increase in size at the base of the flagellum is accomplished by the compression of the segments until they are much thicker than long, and then by fusion to form a rigid base. This gives the appearance of a growing point at the base of the flagellum, the segments originating by a splitting of the original segments into two or four parts, as suggested by Lubbock, (1864 and 1867). The development of the flagellum of the antennae and caudal filaments in *S. canadense* and others demonstrates that the segments do not arise in this way, but rather by a differentiation of segments from the proximal end of the unsegmented flagellum, the new segments being added distal to those formed in the previous instar. The clearest evidence for this is found in an examination and comparison of the terminal unsegmented part and the adjacent segments of the antennae in early instars of *S. canadense.* The terminal part will be seen to be divided into minute subsegments of about equal volume (Fig. 6,–2a). As we pass proximad a place is found where these subsegments quite suddenly become noticeably shorter and appear to be more immovably united to one another. This point is usually roughly shown by the appearance of one or several hairs a little proximad of it. About ten of these subsegments make up one segment and what is being examined then is a segment in the process of formation. Next to this region proximally is a well formed segment in which, however, the original subsegments of which it is composed are clearly distinguishable. Examination of more proximal segments in later instars shows that the vestiges of these subsegments are lost entirely nearer the base.

The origin of segments in the caudal filaments takes place in the same way except that here the segmentation is still more complex. The small subsegments of the terminal part fuse in numbers to form a segment. Then at the base of the filaments in later stages the segments themselves become indistinguishably fused with one another to form a rigid base. Further, the segments formed are not uniformly definite, even at the time of their appearance. In *S. canadense* one segment makes its appearance at the first ecdysis. At the next ecdysis one segment is added and a stout seta appears distally in Segment 4. At the next ecdysis two segments are added, the proximal one (Segment 6) bearing apically a stout seta similar to that on Segment 4. The separation between the fifth and sixth segments is not nearly as well defined as that between 6 and 7, and thus, as development proceeds, Segments 5 and 6 will soon become fused so as to be indistinguishable and this fusion will not take place between 6 and 7 till a later period in development. Segments 13 to 16 are produced also at one shedding; now the joint between 12 and 13 and that between 16 and 17 are each much more prominent than the joint between 14 and 15, and the joint between 14 and 15 is in turn more definite than the joint between 13 and 14 and that between 15 and 16. Thus, as development goes on and these segments begin to fuse and enter into the basal part of the filament, the junctions between 13 and 14, and between 15 and 16 will disappear first; then the joint between 14 and 15, and finally the joint between 12 and 13 and that between 16 and 17.

The limit of the segment is determined in the undifferentiated terminal part of the flagellum. Usually these joints form in a definite way in regard to the position of the hairs and setae, so that these structures are in the distal part of the segment. Sometimes, however, something goes wrong and the segment limit or joint does not fall at the right place, and the hairs are not present distally in the proper segment. For example in the third instar of *S. canadense* there is, with very few exceptions, a strong seta distally in Segment 4 of the caudal filaments. In an occasional specimen, however, (and in these it is usually in but one of the filaments) the seta is absent from Segment 4 but is present distally in 5. In this case, however, Segment 4 is much shorter than the normal, and Segment 5 is also rather short, showing that the joint had occurred at the wrong place. Even so, the circlet of spines forms distally in both 4 and 5. From this it would appear that the position of the setae in the caudal filaments is more fundamental than the position of the segments. In this connection Lubbock noted that the dark bar on the caudal filaments of *Cloeon dimidiatum* retained its position in the distal third throughout the nymphal period, in spite of great changes in the position of the segments.

In the caudal filament (Fig. 5,–30, 30a) of a full-grown nymph of *Ephemera*, there are a number of intimately fused segments at the base, but many of them are still distinguishable. As the segments are examined distally they become more distinct and then show a division into two, which is at first very indistinct. The suture dividing these secondary segments forms a sinuate line rather than the straight line between the original or primary segments. Still further out on the tail the primary segments show division into four, or a subdivision of the two halves, also by sinuate lines. At first these sutures, which might be termed tertiary sutures, are indistinct, but further out they become very distinct so that each primary segment is delimited by a straight suture from adjacent primary segments and divided into two secondary segments by a sinuate line, and these secondary segments are each divided into two tertiary segments by a sinuate line or suture. In development, each primary segment represents the addition to the caudal filament at one moult. The basal primary segments are undivided; a few adjacent segments are divided into two secondary segments; those formed subsequent to the eighth instar are composed of four tertiary segments. The caudal filament (Fig. 5,–30, 30a) does not show all the segments formed owing to loss through fusion at the base. And furthermore, as pointed out above, in the process of fusion and disappearance of the intersegmental sutures, the tertiary ones disappear first, then the secondary and finally the primary. If all the segments remained separate and distinguishable it would be possible to count the number of primary segments in the filament and thus have the number of instars through which the nymph has passed. This number, together with the well known subimago and adult instars, would be the full number passed through by the individual. In the subimago instar there is apparently a freeing again of at least some of the segments fused during nymphal life. In Fig. 5,–30 to 34c, the corresponding segments in full-grown nymph, subimago, and male and female imagos are similarly designated. The determination of these homologies was made possible by the finding of nymphs about to emerge, which showed the enclosed segments of the subimaginal caudal filaments, and also by the fact that the imaginal segments were apparent within the subimaginal cuticle. The segments, it will be seen, correspond very well with those of the previous stage, the main difference being an increase in length of the segments. In the case of *Ephemera*, the pigmentation at the joints helped greatly in working out the homologies in the last three instars. Fairly well out on the tail, as mentioned above, each primary segment is composed of four tertiary segments, these being separated not by straight lines as are the joints between the primary segments, but by sinuate lines. The subimaginal tail developing within the nymphal tail near the end of the last nymphal instar fortunately shows a difference in pigmentation at the junction of the segments. This indicates that the segments correspond. Between the primary segments there is very little pigmentation, while between the secondary and tertiary segments there is very dark pigmentation, resulting in the middle third of the caudal filament in the formation of a light band followed by three dark bands, then another light and three dark bands, and so on. This plan can be followed well out on the tail, a few segments only, near the tip, not showing distinctly the grouping into four by the pigmentation. Towards the base the dark rings between the tertiary segments disappear first and then finally those between the secondary segments. There is a narrow constriction at the base of the subimago caudal filament, designated X, which can be fairly accurately placed and so serve as a starting point in a count of the segments.

In *Stenonema* also the segments are apparently added in fours at each ecdysis through most of the nymphal life. Here the segments are also nicely grouped in fours not by the difference in character of the joints themselves as was the case with *Ephemera*, but by the presence of setae at the apices of the segments and also somewhat by the pigmentation. In the basal part the primary segments from the fourth on are armed in the middle with very stout spines in a single row. Farther out the spines disappear, but they have been present far enough out on the tail to show that the method of addition of the segments is apparently the same as in *Ephemera simulans*. In the basal third of the filament the apices of the primary segments are not armed with thick, stout setae, but with longer, thinner ones and possess a band of pigment. Following out the tail to the region of the tertiary segments it is seen that the pigmented band disappears from the region between the primary segments and is now found on the line dividing the tertiary segments. After the disappearance of the stout setae in the middle of the primary segments distally in the caudal filament there is nothing to distinguish them, so that a primary segment is represented by two secondary segments which were added at one ecdysis and are similar to one another as far as could be ascertained.

Each of these secondary segments is divided by a suture into two tertiary segments with darkened bands between. Basally the segments are fused together so that first the tertiary joints disappear, leaving two secondary segments only to each primary, and then the secondary joints disappear, leaving only the primary joints, which eventually disappear also.

In *Ephemera* the change in the caudal filaments from the subimago to the imago stage was the most difficult to understand. The imaginal filament was much longer than the corresponding subimaginal filament, and it was surprising to find that in spite of this there were fewer segments present and that the lengthening had been caused by the elongation of segments. At first it was concluded that the decrease in the number of segments had been caused by fusion of segments at the base, but an individual which was about to change into the subimago showed that this was not the case. In this individual (Fig. 5,–31a) the reduction was caused by the dropping of the twelve distal segments of the nymphal filament. Other specimens of this species examined later showed the same phenomenon. The process is the same as that taking place in the antennae between the last nymphal instar and the subimago as described by Lubbock (1867) for *Cloeon dimidiatum*.

Variation in the Number of Caudal Filaments Present in Mayflies

The number of tails present in mayflies has always been an interesting question. Several different conditions seem to exist.

1. Nymphs hatch with three caudal filaments of about equal length, and retain these throughout nymphal and adult life, *e.g.*, *Ephemera*, *Ephemerella*.

2. Nymphs hatch with three caudal filaments of about equal length, retain these throughout nymphal life, and lose the middle tail in the subimaginal and adult stages, *e.g.*, *Stenonema*, *Heptagenia*.

3. At hatching, there are two well developed lateral filaments and a reduced median, one which is further reduced in early nymphal life, and remains so throughout the later stages, including the subimago and adult, *e.g.*, *Iron* and *Epeorus*.

4. Nymphs hatch with two well developed lateral filaments, but no median one. The median one grows out during nymphal life though not attaining the length of the lateral ones. In the subimago and imago the median filament is dropped and the lateral ones retained, *e.g.*, *Baetis posticatus* (Murphy 1922), *Baetis vagans* and *Cloeon dimidiatum* (Lubbock).

5. There is perhaps another condition too in which there are only two caudal filaments at hatching (the two lateral ones or cerci), the third or median filament not being present or making its appearance during subsequent stages including the subimago and adult. *Pseudocloeon* seems to illustrate this type but since first stage nymphs have not been examined it may turn out that *Pseudocloeon carolina* has a reduced median filament which is dropped early in development as was the case with *Iron* and *Epeorus*.

The two-tailed condition in nymphs seems to be associated with life in very rapid water (*Iron*, *Epeorus* and *Pseudocloeon*) where obviously the tails are not used for swimming and therefore the addition of a middle tail could be of little advantage.

The lateral caudal filaments are generally conceded to be the cerci, appendages of the eleventh segment, and the median caudal filament the greatly elongated telson.

The evidence available seems to indicate that the three-tailed condition is primitive within the order Ephemeroptera, the two-tailed condition having arisen secondarily by the dropping of the median filament. The great majority of species have three caudal filaments in the nymphal instars, some of which drop the median caudal filament in the subimago and adult, and others retain it. Some of the nymphs which have but two tails present have a vestigial median filament in the early instars and many adults which apparently have but two filaments have a rudiment of the median present. Apparently the median filament is a very variable structure, being present in the adults of some species and absent in the adults of other species, even in the same family. In order to consider the two-tailed condition primitive, the assumption would have to be made that the median tail had been independently developed in several families, which is extremely improbable. The logical conclusion is that the three-tailed condition is primitive and that mayflies had three-tailed insects as ancestors.

Metamorphosis of Claws and Mouth Parts

In *Stenonema* the molar surface of the mandible is relatively undeveloped at the time of hatching, and the other mouth parts (Fig. 6,–1a–c) also differ greatly from their final condition in the half-grown or full-grown nymphs. The canine teeth on the mandibles of each side are directed medially so that they probably function in ingestion. Later these canine teeth are directed anteriorly and probably do not take part in ingestion in the same way as formerly, that function being taken on by the molar surface. This suggests that the canine teeth function as early larval organs.

An examination of stomach content was not made, but, assuming that the young nymph eats the same organisms and materials as the older nymph, we would expect that a different mechanism would be required in a small and large nymph in order to capture effectively and take in the same organisms. The relation of food organism and nymph has altered not by any change in the former, although this may occur also, but merely by the increase in size of the nymph which necessitates morphological changes to meet the altered environmental relation thus created.

A series of tarsal claws of *Epeorus humeralis* is shown (Fig. 10,–2c, 9, 10, 11, Fig. 11,–13b and 14c), illustrating the metamorphosis which takes place in these parts. There is a gradual increase in the number of claws up to the stage illustrated by Fig. 10,–11, at which time there is a reduction in the number of claws as indicated by the new claw developing within. The new claw has changed its form very abruptly. There is from now on one great curved claw with some of the other claws present as a row of pectinations on the great claw. This abrupt change in the type of tarsal claw coincides with a probable change in function of the claw as described under *Epeorus humeralis*.

The nymph throughout its life cycle must be adapted to hold itself in position in a strong current of water. Obviously the very small nymph will hold to very minute irregularities on the surface of the stones. The many-pectinated or many-clawed last tarsal segments of the early stage nymphs are adapted to clinging to such a surface. With growth these claws increase in number up to the stage shown in Fig. 10,–11, when there is a sudden metamorphosis of the claws, to form the type shown in Fig. 11,–13b, 14c, with one claw greatly elongated and hooked and the four others present as pectinations along its lower surface. The nymph by this stage is much larger than at hatching (about 6 mm. long), so that the minute irregularities on the surface of the rock will no longer provide a suitable hold, but irregularities of a larger order, and therefore less in number relative to the nymph's immediate environment, will be used. For this purpose the new type of claw will be much more satisfactory since it will hook around these larger irregularities and projections. This sudden metamorphosis of the claws also coincides with the time at which the gills reach the substratum and develop along their lower edge combs of posteriorly directed setae, which function in holding the nymph in place in the current of water. It is just at this time in their life that the nymphs of *Epeorus humeralis* drop down-stream out of the very rapidly flowing water into the more slowly flowing water below the rapids (Ide 1935).

Appearance and Metamorphosis of the Gills

The gills appear as prolongations from the postero-lateral angles of the segments. In *Ephemera* and *Isonychia* they appear simultaneously on Segments 1 to 7 inclusive. In *Stenonema canadense, Epeorus humeralis* and *Iron pleuralis* the gills of Segments 5 and 6 appear several instars earlier than those on 1, 2, 3, 4 and 7 and this is probably true for *Heptagenia pulla, H. hebe, Stenonema tripunctatum* and *Stenonema fuscum*. The gills of 5 and 6 in these forms grow out as long filaments which later metamorphose into lamellae similar to the anterior gills which have developed into lamellae directly, never having the filamentous form. Gill 7 in some forms becomes filamentous, *e.g.*, *Stenonema canadense*, or may develop as a lamellate plate as in *Heptagenia*. The metamorphosis of Gills 5 and 6 from filaments to lamellae may take place in two ways. If these gills are composed of two segments as in *Epeorus humeralis, Iron pleuralis, Heptagenia pulla* and *Stenonema fuscum* the change involves only the basal segment which becomes expanded and lamellate and the distal segment remains unchanged and is eventually dropped. If the gills are unsegmented, as in the case of *Heptagenia hebe, Stenonema canadense* and *Stenonema tripunctatum*, the whole gill enters into the formation of the lamella. In the former process the result is a gill with a rounded or truncate posterior border while in the latter the result may be a pointed gill as in *S. canadense.* All gills appear first as uniramous structures, but early in their growth most of them develop a secondary medial ramus which may take several forms. In *Ephemera* and *Leptophlebia* it grows out and becomes similar to the primary ramus though not attaining its length or complexity. In *Epeorus, Iron, Stenonema* and *Heptagenia* the secondary ramus takes the form of a plumose tuft of filaments. The secondary ramus may be absent from all the gills as in *Baetis*, or may be absent from one or more gills, *e.g.*, Gill 7 in *Stenonema* which remains uniramous throughout development.

The gills contain tracheae and some of them at least are provided with muscles which move them to and fro in the water. Fig. 11,–15 represents a young nymph of *Iron pleuralis* in which muscles pass from a suture on the venter of the segment (line separating the zygosternum into two parts, the sternum and the limb bases) to the proximal part of the gill. This muscle does not continue into the gill even when the latter is a two-segmented structure. Durken (1907) has discussed the musculature of the tracheal gills of ephemerids and has come to the conclusion that the gills are dorsal or notal structures, although he does not homologize them with wings as some others have done. The more generally accepted view is that they represent abdominal legs (Snodgrass, 1931, and Spieth, 1933).

Metamorphosis in General

The number of instars in mayflies is very great compared with that in other insects, a phenomenon probably inherited from ancestors and retained because of the special requirements of this group. At each of the moults there is some increase in size of the nymph but the moult seems primarily for the purpose of changing the morphological structure. Although the nymph is increasing slowly in size, some structures such as mouth parts, wing pads, gills and genitalia are changing radically in size and complexity by differential growth rate of the parts. Some of this metamorphosis is merely concerned in perfecting the adjustment of nymphal structure to the changing conditions. Examples are the changes in mouth parts, eyes, gills and tarsal claws. Other metamorphosis is concerned with the development of such adult structures as the wings and external genitalia.

The cuticle of insects is very elastic so that it seems unlikely that so many moults could be concerned primarily with increase in size. Many other insects grow to much larger size with but four or five moults. The reason for the great number of moults seems to be in the constant adjustment which the nymph must make to its physical environment (*e.g.*, food, rapid flow of water). Every change in external structure, even to the addition of a seta or hair, can be accomplished only by a moulting of the old skin and the emerging of the new stage adapted to its brief duration as was each stage prior to it. A long resting period is precluded in these forms because of the necessity of their being maintained in a rapid current and actively feeding all the time. Consequently any large structure does not form completely at one moult, but requires several, as for example, in the formation of the mandibular tusks in *Ephemera*. Very abrupt changes are rather the exception than the rule in this group and most of these occur when the insect leaves the water, at which time the environment is radically changed. Examples of such changes are found in the development of turbinate eyes in some forms, in the great reduction in size of the antennae, often in the dropping or aborting of the median caudal filament or in the shortening of the filaments as was demonstrated for *Ephemera simulans*. There is a striking difference in the time required to develop a structure such as a caudal filament or an antenna and the time required to get rid of the same structure. By a process of differential growth, which probably follows closely the exponential curve, the structure is gradually developed through several instars but it may be eliminated in much less time, at one ecdysis.

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