The Embryology of the Mayfly *Ephemera japonica* McLachlan (Insecta: Ephemeroptera, Ephemeridae)

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CONTENTS

INTRODUCTION

The insects account for three quarters of all animal species, and more than 99 percent of them are the wing-acquired insects or the Pterygota. For elucidating the evolution of insects or pterygotes which have attained their spectacular prosperity and radiation, the understanding of their ground plans of body and morphogenesis is the most interesting subject. In this account, the mayflies or Ephemeroptera is one of the most significant groups, because the Ephemeroptera is widely accepted as one of the representatives closest to early pterygote ancestors (cf. Hennig, 1953).

The phylogeny of insects or the basal clades of pterygotes still remains controversial. That is, three possible phylogenies are presented concerning the interrelationships of three major pterygote groups, i.e., the Ephemeroptera, the Odonata, and the Neoptera which includes all the pterygote groups other than the former two. Hennig (1953, 1969) morphologically and Rohdendorf et al. (1962) paleontologically supported the phylogeny formulated as [Paleoptera (= Ephemeroptera + Odonata) + Neoptera]. Kristensen (1975, 1991) mainly from the morphological bases and Wheeler and Carpenter (1996) from the 'total evidence' analysis supported the phylogeny formulated as [Ephemeroptera + (Odonata + Neoptera)]. Lemche (1940) and Matsuda (1981) morphologically and Boudreaux (1979) functional

morphologically and from the development of wing buds supported the phylogeny formulated as [Odonata + (Ephemeroptera + Neoptera)]. In attempting to elucidate the evolutionary transition of the basic body plan and ground plan of morphogenesis in insects or pterygotes and to reconstruct their phylogeny, the examination of Ephemeroptera may be especially significant.

Comparative embryology is one of the most promising and useful methods for understanding the ground plans of body and morphogenesis, for elucidating the evolutionary transition of ground plan and for speculating the phylogeny. Whereas for another most primitive pterygote representative the Odonata we can refer to comprehensive embryological studies (Ando, 1962; etc.), our embryological knowledge on the Ephemeroptera remains rather fragmented, although we have some studies on its embryology: Joly's (1876) brief note on the embryology of *Palingenia virgo* (Palingeniidae), Heymons' (1896a, b, c) general embryological studies on *Ephemera vulgata* (Ephemeridae), Murphy's (1922) embryological study on *Baetis* sp. (Baetidae) focusing on the external morphology, Ando and Kawana's (1956) embryological study on *Ephemera strigata* by external observation, Wolf's (1960) karyological study on the maturation and cleavage of *Cloeon dipterum* (Baetidae), Bohle's (1969) developmental physiological study on *Ephemerella ignita* (Ephemerellidae), and Tsui and Peters' (1974) note on embryonic development of *Tortopus incertus* (Polymitarcyidae).

For this reason, I began an embryological study of the Ephemeroptera, using *Ephemera japonica* McLachlan (Ephemeridae) as materials. The purposes of the present study are: 1) to describe the embryogenesis of the species, covering the whole developmental process, and, comparing the results with the previous studies, to reconstruct and revaluate the ephemeropteran body plan and embryological ground plan; 2) based on this, to discuss the basic body plan and the ground plan of embryogenesis in insects or pterygotes; 3) to illustrate the evolutionary transition of the body plan and ground plan of embryogenesis, so as to re-examine the phylogeny of insects or pterygotes in higher level.

MATERIALS AND METHODS

The mature females of *Ephemera japonica* McLachlan (family Ephemeridae) already copulated were collected at the Kakuma and the Nakanosawa Rivers, and a branch of the Karasawa River, Sanada, Nagano Prefecture, and the Uchimura River, Maruko, Nagano Prefecture, and the Hakii River, Minobu, Yamanashi Prefecture, in June to August of 1993 to 1998, and eggs were obtained in the laboratory. Unfertilized eggs were dissected out of mature, uncopulated imagos or subimagos, or ultimate instar nymphs.

The eggs were incubated in water at room temperature (20 ± 2℃). Most of eggs were fixed with alcoholic Bouin's fluid (saturated alcoholic solution of picric acid : formalin : acetic acid = 15 : 5 : 1) at room temperature for 24 hours. The fixed eggs were stored in 70% ethyl alcohol. A small opening was made by fine forceps in the chorion of some eggs prior to fixation, and these eggs were then fixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde) buffered at pH 7.2 with HCl-sodium cacodylate at 4℃ for 2 hours, and then stored in the HCl-sodium cacodylate buffer at 4℃.

The fixed eggs were processed into methacrylate resin Technovit 7100 (Kulzer, Wehrheim) : stylene = 4 : 1 or Technovit 8100 (Kulzer, Wehrheim) sections of 1-2 μm thickness, in accordance with Machida et al.(1994a, b). Some eggs were immersed in a solution of 70% ethanol : 50% ammonium

mercaptoacetate = 9 : 1 for a few hours, prior to the infiltration of resin, in order to soften the chorion.

Sections were stained with Delafield's hematoxylin and eosin (in some cases supplementarily stained with 0.1% fast green FCF), or with Azan (Domagk's modification). For tatal staining of eggs or embryos, 0.1 % thionin was used. Some newly laid eggs were stained with a DNA specific fluorescent dye Hoechst 33342 (Calbiochem, La Jolla), prior to the staining a small opening was made in the chorion.

For examination of the fertilization and blastokinesis, a time-lapse VTR system (Olympus, Tokyo: CK-2 inverted microscope; Victor, Tokyo: SR-9070 video tape recorder; and Kenis, Osaka: QB CCD camera) was employed.

For scanning electron microscopy, fixed eggs or embryos dissected out of eggs were sonicated for a few seconds with an ultrasonic cleaner, dehydrated in a graded ethyl alcohol series, and then transferred to acetone. The eggs or embryos were dried in a critical-point drier, coated with gold, and observed under a scanning electron microscope (JEOL, Tokyo: JSM-T200).

OBSERVATIONS

1. Egg structure

A female of *Ephemera japonica* deposits eggs of about 3,000 to 5,000 in a form of egg mass a time. The egg is ellipsoidal, about 200 μm in length and about 100 μm in width, colorless and translucent: the egg long axis proves to correspond to the anterior-posterior axis, from the observation of developed eggs (Fig. 1A, C). The adhesive layer or gelatinous layer on chorion is very thin, about 0.1 μm, and it has a weak rugged-mesh pattern about 1 μm in diameter on its surface: the mesh pattern of this layer derives from and corresponds to that of chorion (Fig. 1A, B, D).

Chorion is 3-4 μm in thickness, and it has a weak rugged-mesh pattern about 1 μm in diameter (Fig. 1D). One micropyle is on the equator of egg, and it proves to be located in the mid-dorsal side of egg from the observation of later stages (Fig. 1A-C). In the egg of *Ephemera japonica*, the sperm guide is not developed, which is often found to well develop in the other ephemeropteran eggs. Micropylar opening is about 1.5 μm in diameter. Micropylar canal about 20 μm in length obliquely penetrates the chorion along the equator of egg.

2. Early development

2.1. Maturation

At the time of oviposition, the egg nucleus (oocyte nucleus s. str.), which is in the polar plasm (cytoplasmic island) that proves to be located in the mid-ventral side of the egg from the observation of later eggs, is at the metaphase of the first maturation (meiotic) division, with its spindle axis being vertical to the egg surface (Figs. 3, 4A, C, 5, 15A). The first maturation division has already started at the ultimate instar nymphal stage, but it is arrested at the metaphase, so that the division is equational in phase. A male pronucleus, into which a spermatozoon converted itself, is found just beneath the micropyle which is situated at the opposite to polar plasm, on the equator of egg (Figs. 3, 4B, D, 15A).

At 0.5-1 hour after oviposition, the first maturation division finishes, and the first polar body is formed, to move just beneath the chorion in the polar plasm (Figs. 6A, 15B). The first polar body is beginning to degenerate at this point, and we could not determine its fate, i.e., whether it undergoes the following division.

At 1.5 hours after oviposition, the second maturation division occurs, producing the female pronucleus, and the second polar body is given off toward the outer periphery of the polar plasm (Figs. 6B, 15B, C). The female pronucleus soon starts to migrate into the yolk toward the center of the egg,

accompanied by a part of the polar plasm (Figs. 7, 15D). During the second maturation division, the male pronucleus starts to migrate toward the anterior pole in the periplasm (Figs. 8A, B, 9A, B, 15B-D).

2.2. Fertilization

During the second maturation division, the male pronucleus starts to migrate toward the anterior pole in the periplasm (Figs. 8A, B, 9A, B, 15B-D).

At 2-3 hours after oviposition, the female and male pronuclei arrive at the center and the anterior pole of egg, respectively (Figs. 8A, B, 9A, B, 15D). In a living egg, the male pronucleus accompanied by cytoplasm is recognized as a depression of yolk at the anterior pole of the egg (Fig. 9A). On arriving there, the male pronucleus changes its direction inwards into the yolk, and proceeds along the long axis of the egg to approach the female pronucleus, accompanied by surrounding cytoplasm (Figs. 10, 15E, F). As a result, the anterior yolk depression gradually becomes shallow, and the surrounding cytoplasm of the male pronucleus is observed to be partially left behind on the path that the male pronucleus has traced from the anterior pole to the center of the egg (Figs. 10, 15F).

Thus, the male pronucleus approaches the female pronucleus, and the female pronucleus exhibits an approaching movement, although slightly, toward the male pronucleus, just before their conjugation. At 5-6 hours after

oviposition, the male and female pronuclei conjugate with each other near the center of egg, and the fertilization is completed (Figs. 11, 15G).

The time-lapse VTR reveals not only the migration of pronuclei in the yolk but also a yolk stream which coordinates in time and direction with the migration of the male pronucleus mentioned above. During the prefertilization stage, the yolk stream first moves anteriorwards at the egg surface, and then enters and sinks inwards into the yolk from the anterior pole of the egg (Fig. 16A-C). The fertilization then occurs, and a reversal stream involving the whole yolk takes place (Fig. 16D).

In unfertilized eggs of *Ephemera japonica*, which are activated by immersing in water, a depression of yolk is also observed to be formed at the anterior pole of the egg 2-3 hours after activation (Fig. 17A, B). Time-lapse VTR shows that in unfertilized eggs, a yolk stream is generated which is similar to that observed in the prefertilization stage of fertilized eggs, although the unfertilized egg yolk stream is slightly less extensive (Fig. 18A-C).

2.3. Cleavage

The cleavage of *Ephemera japonica* is of the typical superficial type. About 10 hours after oviposition, the first cleavage takes place at the site of fertilization or at the center of the egg, with its spindle axis oblique to the long

axis of the egg (Figs. 12, 15H). The second cleavage occurs vertically to the direction of the first cleavage (Fig. 15I), and the following cleavages occur at intervals of approximately 9 hours. The first five cleavages are synchronized in phase, and the number of nuclei accords with the 2^n-rule (Fig. 15H-J). The cleavage nuclei migrate centrifugally, and some nuclei are observed to reach the egg surface or periplasm by the fifth cleavage stage.

After the sixth cleavage, the synchrony of division fades, and nuclei of various phases are encountered (Fig. 15K-M). In the sixth cleavage stage, many cleavage nuclei are found in the periplasm (Figs. 13, 15L, M), and thereafter the peripheral cleavage nuclei are observed to undergo radial divisions. As a result of eight cleavages, about 250 cleavage nuclei arrive at the periplasm to form the syncytial blastoderm, i.e., the blastema (Fig. 15N). About ten cleavage nuclei are observed to be left behind in the yolk, and they are the primary yolk cells (cf. Figs. 15O, 19).

3. External features of embryos and blastokinesis

The egg period of *Ephemera japonica* ranges from 15 to 17 days at room temperature (20±2℃). Herein, I describe its embryonic development after the fertilization, which is divided into 13 stages, and the first instar nymphal stage, with special reference to external features of embryos and blastokinesis.

Stage 1 Egg cleavage (Figs. 2A, 11-13, 15G-M)

The fertilized egg starts to divide mitotically, and undergoes the typical superficial cleavage, as described in detail in "2.3. Cleavage". The process of cleavage can be externally observed through the chorion.

Stage 2 Blastoderm formation (Figs. 2B, 14, 15N-O, 19)

Cleavage nuclei arrive at the egg periphery after eight cleavages, and the syncytial blastoderm (blastema) is formed (Fig. 15N). Cell membranes soon appear between the syncytial blastodermal nuclei, and the blastoderm s. str. is completed (Figs. 14, 15O, 19).

Stage 3 Formation of germ disc (Figs. 2C, 15P, 20A, B)

Even in the newly formed blastoderm, the thick posterior half embryonic and thin anterior half extraembryonic areas are distinguishable (Figs. 15O, 19). The posterior cells concentrate at the posterior pole of the egg, to form the germ disc of about 80 μm in diameter. The anterior cells become more flattened, and form the future serosa (Figs. 2C, 15P, 20A, B).

Stage 4 Pear-shaped embryo (Figs. 2D, D', 21A, B)

The germ band starts to elongate backwards. It assumes a pear shape, and the anterior broad protocephalon and posterior protocorm differentiate

(Figs. 2D, D', 21A, B).

Stage 5 Start of invagination of germ band (Anatrepsis I) (Figs. 2E, E', 22A, B)

The germ band further elongates, and its caudal end starts to proceed into the yolk. At the same time, the amnion starts to be produced from the embryonic margin, resulting in the start of the amnioserosal fold formation. This amnioserosal fold is derived mainly from the posterior area, the anterior amnioserosal fold being not well-developed (Figs. 2E, E', 22A, B; cf. Fig. 32A-C).

Stage 6 S-shaped embryo: completion of invagination (Anatrepsis II) (Figs. 2F, F', 23A-C)

With active cell proliferation, the embryo further elongates and deeply invaginates into the yolk. The caudal end of the embryo reaches the middle of the egg long axis, and the embryo acquires an S-shape (Figs. 2F, F', 23A-C). At this stage, the posterior amnioserosal fold extends anteriorly to the cephalic level, and anatrepsis completes (cf. Fig. 33A, B). The ventral side of the embryo is completely covered by the amnion or the amnioserosal fold.

Stage 7 Longest embryo (Figs. 2G, 24A-F)

The embryo further elongates, and it acquires its maximum length, reaching near the anterior egg pole (Figs. 2G, 24A-F; cf. Figs. 35A, 36A, B), with its posterior half bending three times. With the bendings, the posterior half of embryo or the future abdomen is folded and divided into four regions: regions I to IV from the anterior to posterior (Figs. 2G, 24E, F, 35A, 48A). In cross section, regions I and II and regions III and IV are respectively connected to each other by the amnion (Fig. 35B). The stomodaeum appears (Figs. 24E, 35A, 36A, B, 37).

As a result of completion of anatrepsis in the preceding stages, the entire egg surface is occupied by the serosa (Fig. 33A, B), and the latter secretes the serosal cuticle beneath the chorion (Figs. 43A-D, 44A, B) .

Stage 8 Segmentation of embryo (Figs. 2H, 25A-E)

The segmentation of embryo commences (Fig. 2H). It proceeds from the head to the rear, and simultaneously appendage rudiments appear in the cephalic and thoracic segments: a pair of antennal, mandibular, maxillary and labial, and three pairs of thoracic appendages. A clypeolabral rudiment, as not paired but single swelling, appears anteriorly to the stomodaeum. The intercalary segment, which appears between the antennal and mandibular segments, is much shorter than the other segments, and it is devoid of

appendage (Fig. 25A, B, E). With the progressive cephalic formation in later stages, this segment becomes obliterated, and it is finally undetectable in and after stage 12.

In regions I and II of the abdomen, which is now divided into four regions, the first to fifth and the sixth to eleventh abdominal segments develop respectively (Figs. 25D, 48A; cf. Figs. 39B, 40, 42). Late in this stage, neuroblasts and neuropiles can be clearly observed in all the first to eleventh abdominal segments (cf. Figs. 39A, B, 40, 45). In contrast, no attributes showing segmental nature can be found in regions III and IV. No appendages develop in the abdomen of *Ephemera japonica*, except for the eleventh segment, at which the cerci develop as its appendages in the next stage.

Stage 9 Start of appendicular annulation (Figs. 2I, 26A-F, 34A, 41)

The appendages of the head and thorax are enlarged and segmented. The antenna is divided into two parts, i.e., the proximal scapus and distal part which later develops into pedicellus and flagellum. The maxilla and labium are also divided into two parts, i.e., the proximal part of which medial region later develops into galeolacinia (maxillary endite) and distal maxillary palp, and the proximal part of which medial region later develops into glossa and paraglossa (labial endites) and distal labial palp, respectively. The mandibular appendage, however, never undergoes such a partition into the

proximal and distal as observed in antenna, maxilla and labium, nor develops the structures identifiable with the maxillary and labial palps (Figs. 26A-C, 34A).

Three paired bulges appear in head lobe, named the bulges 1, 2 and 3 from the lateral to medial. These correspond to the protocerebral lobi 1, 2 and 3: the mostmedial and mostlateral pairs are the preantennal ganglia (lobi 3) and optic ganglia (lobi 1), respectively (Fig. 26C). The sternal area of the mandibular to maxillary segments begins to bulge out, and it leads to the formation of hypopharynx (Figs. 26C, 41).

Each of thoracic appendages is divided into the proximal and distal parts, which are identified to be the coxopodite and telopodite, respectively (Fig. 34A).

Regions III and IV of the abdomen fuse with each other to form the proctodaeum (Fig. 26D, E; cf. Figs. 40, 42, 43A-D, 48B, B'). Then, the proctodaeum itself is soon enclosed by the definitive dorsal closure of region II (cf. Figs. 43A-D, 48B, B'). The caudal filament develops at the apex of region IV (Fig. 26E, F; cf. Figs. 39A, B, 40, 42, 43A-D, 48B, B'). A pair of cerci develop, as appendages of the eleventh abdominal segment which is the extremity of region II (Figs. 26E, F; cf. Figs. 43A-D, 46A-C, 48B, B'). With the progression of definitive dorsal closure, the cerci move from the original ventrolateral to the dorsolateral position, to reach their definitive position at the same level as

the caudal filament (Figs. 46A-C, 48B, B' vs. Figs. 26F, 41, 47, 48C).

Stage 10 Revolution (Katatrepsis) (Figs. 2J, 27A-C)

The amnioserosal fold tears near the labrum, and the embryo appears on the egg surface. The embryo, which is slightly shortened temporarily, moves along the ventral surface of the egg toward the anterior pole, with its head to the traveling direction, and the anteroposterior axis of the embryo reverses (Fig. 2J).

In this stage, the clypeolabrum enlarges and is divided into two parts, i.e., the proximal clypeus and distal labrum: this proximal-distal partition of clypeolabrum is obviously delayed, in comparison with those found in the appendicular structures such as antennal, maxillary, labial and thoracic (Fig. 27B vs. Fig. 27C). The distal part of the elongated antenna is subdivided into two parts, i.e., the proximal pedicellus and distal flagellum: the antenna acquires the composition by three parts, i.e., scapus + pedicellus + flagellum (Fig. 27C). The mandible enlarges, and the incisor and molar appear. The incisor and molar do not develop into such structures as tough teeth until the middle postembryonic stages. The maxillary endite, which now develops into the galeolacinia, and labial ones enlarge, and gather toward the ventral median line. The developing hypopharynx further swells and extends its area, with its posterior extremity reaching the labial segment (Fig. 27B, C).

Stage 11 Post-revolution I (Figs. 2K, 28A, B, 34B)

The serosal cells are condensed at the anterodorsal part of the egg, to form the secondary dorsal organ just posteriorly to the head. With the progressive condensation and withdrawal of serosal cells, the amnion replaces the serosa and finally spreads over the dorsal yolk as a provisional dorsal closure (Figs. 2K, 44A, B).

The flagellum of antenna elongates, to be the same as the scapus plus pedicellus in length. The labial appendages of both sides, of which endites enlarge and develop into the medial glossa and lateral paraglossa, move to medial line and approach each other. The rotation of labial appendages is involved in their approach, as a result, the glossa and paraglossa direct themselves anteriorwards as well as the labial palp (Fig. 28A, B).

Each of thoracic appendages, especially telopodial region, much elongates. Late in this stage, annular partitions become observable, although faint, as are the subcoxa + coxa, the trochanter, the femur, the tibia and the tarsus + pretarsus (Fig. 34B).

Stage 12 Post-revolution II (Figs. 2L, 29A-D, 34C)

The flagellum further elongates, and divides into four annuli (Fig. 29A). The mandible and maxilla elongate toward the median line, and the maxillary palp divides into two annuli (Fig. 29C, D). The labial appendages of both

sides unites with each other at their proximal regions, resulting in the formation of the prementum and postmentum (Fig. 29D). The labial palp divides into two annuli (Fig. 29C, D). The hypopharynx is divided into three parts, i.e., the medial lingua and lateral, paired superlinguae (Fig. 29B-D). The head fundamentally acquires its definitive configuration. The maxillary endite or the galeolacinia remains to be solid, and the differentiation into the galea and lacinia as is common in the other insects does not occur.

Late in this stage, the tarsus and pretarsus and the subcoxa and coxa differentiate in thoracic appendages, and the annulations become obvious and complete (Figs. 29C, 34C). The subcoxa and coxa and the femur, tibia, tarsus and pretarsus are derived from the coxopodite and the telopodite, respectively (cf. Fig. 34A-D). Because the trochanter originates from the boundary zone between the coxopodite and telopodite, it is difficult to determine whether it is coxopodial or telopodial.

The secondary dorsal organ which formed at the previous stage sinks into the yolk and disappears. The definitive dorsal closure further proceeds anteriorwards (cf. Fig. 48C). A pair of cerci and caudal filament elongate and become segmented. Late in this stage, compound eyes and three ocelli can be clearly observed (Fig. 2L).

Stage 13 Post-revolution III (Figs. 2M, 30A, B)

Early in this stage, a fine embryonic cuticle is secreted over the embryonic body. The definitive dorsal closure is completed, and the embryo acquires its definitive form. Late in this stage, the larval cuticle is secreted beneath the embryonic cuticle, and a sclerotized egg tooth which assumes a longitudinal fringed ridge is visible on the frons (Figs. 2M, 30A, B). In the inner side of mandible, two small lobes appear, which are probably identical to the structures Murphy (1922) called the galea and lacinia.

First instar nymph (Fig. 31)

In 15 to 17 days after oviposition, the first instar nymph breaks through the chorion, using the egg tooth, and hatches out. At the same time of emergence, the nymph moults the embryonic cuticle, with exuvia left on the egg.

The first instar nymph is about 500 μm in body length (caudal filament and antenna omitted). A small process "mandibular tusk" appears at the outer side of mandible. The tracheal gills on abdomen which are characteristic of ephemeropteran nymphs have not been well-developed, but remain to be filamentous processes on the third to sixth abdominal segments (Fig. 31). The rudiments of wings or the wing buds are not developed until the later nymphal stages. The midgut formation has not been completed, and the

midgut is still filled with yolk materials. The nymph of *Ephemera japonica* feeds for the first time in the second instar nymph, of which midgut becomes functional.

4. Embryonic membranes

4.1. Serosa, amnion and amnioserosal fold

In *Ephemera japonica*, the germ disc is formed by the concentration of broad and thick blastoderm area distinguished at the posterior half of egg surface (Fig. 20A). The rest of blastoderm or the thin extraembryonic area is now called the serosa (Fig. 20A). In stage 5, the embryo starts to invaginate into the yolk, accompanied by the production of a second embryonic membrane called the amnion from the embryonic margin (Figs. 2E, E', 32A-C). As a result of the invagination of embryo and the production of amnion, a folded structure "amnioserosal fold" is formed, and it starts to spread over the embryonic venter, to finally close there in stage 6 (cf. Fig. 33A, B). Thus, the entire egg surface is to be occupied by the serosa, and the embryo is doubly covered by the serosa and amnion. The space surrounded by the embryo and amnion of amnioserosal fold is the amniotic cavity (Fig. 33B), which is but too narrow to distinguish by external observation, and the amnioserosal fold-amniotic cavity system completes (cf. Machida and Ando, 1998). Soon after

the completion of anatrepsis or the completion of amnioserosal fold, the serosa secretes a cuticular layer or the serosal cuticle beneath the chorion (cf. Figs. 43A-D, 44B).

In stage 10, the amnioserosal fold which is formed in stages 5 to 6 begins to regress in the reverse way to the process of its formation, to be withdrawn, and finally the amnion and then the embryo reach the egg surface (Fig. 2J). It is not unusual, however, that the amnioserosal folds are precociously regressed earlier to stage 10, as extreme examples, in stage 8 (Fig. 49A-C).

4.2. Dorsal closure and secondary dorsal organ

The katatrepsis or the withdrawal of amnioserosal fold occurs, and the serosal cells begin to be condensed anterodorsally on the egg surface, to form the secondary dorsal organ just posteriorly to the head (Fig. 44A, B). With the progressive condensation and withdrawal of serosal cells, the amnion replaces the serosa and spreads over the dorsal yolk as a provisional dorsal closure (Fig. 44A, B). The secondary dorsal organ finally sinks into yolk and is consumed there by late stage 12.

The dorsal closure by the amnion is, however, temporal. The amnion itself is replaced by the extension of lateral plates of the embryo, which is called the definitive dorsal closure. The definitive dorsal closure first occurs

at the abdominal region in stage 9 (Figs. 43A-D, 48B, B', C), at the gnathal region in stage 11, and the dorsal closure at the rest region proceeds from the rear toward the anterior. The definitive dorsal closure finishes at the anterior thoracic area and completes just before hatching or in stage 13.

5. Formation of inner layer and mesodermal somites

5.1. Formation of inner layer

In stage 5, a thin, cellular layer is found to be segregated on the dorsal side of germ band which is initially composed only of the ectoderm. This is the commencement of inner layer formation or mesodermal segregation (cf. Figs. 33B, 35A, B, 36B). This cellular layer or inner layer extends and spreads over the ectoderm (embryo proper), keeping a step with the elongation of embryo. As a result, the entire dorsal side of elongated embryo is lined with the inner layer or mesoderm, including the non-segmental regions III and IV of abdomen.

Newly segregated inner layer is thin, but in stages 6 to 7 the regions on both sides thicken, to form a pair of thick longitudinal mesodermal bands. The region of inner layer between these thick mesodermal bands is the median mesoderm, and it remains thin, especially at the stomodaeal area and at the junction of the regions III and IV of abdomen which is the future blind

end of proctodaeum (Fig. 35A).

5.2. Segmentation of inner layer and mesodermal somites

In stage 8, in which the ectoderm (embryo proper) is segmented, the inner layer is likewise segmented. The segmentation of inner layer proceeds from the anterior to posterior, the same as the ectoderm. The segmentation of inner layer is, however, not so definite. Namely, the adjacent mesodermal segments or mesodermal somites are not demarcated clearly, but they are communicated with each other by thin layer of mesoderm. This situation is very true of in preoral area and intercalary segment, in which the mesodermal somites are in contact with the adjacent ones by not a thin but rather thick mesodermal layer, as known for the other insects, for example, the most primitive ectognathan insect, Archaeognatha (Larink, 1969; Machida, 1981b).

The cells of mesodermal somites assume a radial arrangement in section. The coelomic cavities are ill- or underdeveloped, only in thoracic segments a small lumen is discerned at the center of radial cell arrangement (Fig. 38).

The cells of somite facing the developing midgut are of splanchnic mesoderm, and later develop into alimentary musculature (Fig. 38). The rest of somite cells is the somatic mesoderm, and they later develop into the mesodermal organs or tissues other than the alimentary muscutature (Fig. 38).

In stage 9, the somatic mesoderm of somites enters the cavity of appendage, and it is called the appendicular mesoderm. Before long, the mesodermal somites of all segments begin to collapse, and the coelomic cavities are mingled with the epineural sinus, to be converted into mixocoel, which is characteristic of the arthropods.

6. Organogenesis

6.1. Nervous system

6.1.1. Central nervous system

6.1.1.1. Ventral nerve cord

Externally a pair of swellings appear on the ventral side of each segment in stages 8 to 9. It represents the first sign of differentiation of ganglion rudiments, and the midventral groove between paired swellings is the neural groove. In section, the neuroblasts, which are characterized by the large-sized nuclei and less basophilic karyoplasm, are found to appear in the ventral region of each segment (Figs. 39B, 50, 53). Differentiation of ganglion rudiment is progressive in time in the anterior segments, and a little delayed in the posterior: it occurs at the gnathal and thoracic segments in stage 8, and at the abdomen in stage 9.

The neuroblasts begin a series of divisions, with the spindle axis

approximately at right angle to the surface of embryo, to give rise to daughter cells or ganglion cells, which have smaller nuclei and more basophilic karyoplasm in comparison with those of neuroblasts (Fig. 53). With each subsequent division, the previously formed ganglion cells are pushed farther away from the neuroblast, forming a straight row above the latter (Fig. 53).

The ganglion cells thus increase in number, and gradually become concentrated into the form of ganglia which acquire segmental arrangements corresponding to the external demarcation of body segment.

Late in stage 9, when the ventral nerve cord is observed to consist of three gnathal, three thoracic and eleven abdominal ganglia, a pair of fibrous structures, i.e., neuropiles, begins to be produced at the dorsal side of ganglion of each segment (Figs. 40, 45, 56A, B, 57A-C). A pair of neuropiles of each segment fuse medially with each other, with their extension. Two thick bundles are observed between a pair of neuropiles in their communicating area (in the eleventh abdominal segment only one commissure is found) (Figs. 40, 45). These bundles are the anterior and posterior commissures, and each is observed to be composed of two subbundles. At the same time, the communication of neuropiles between the anterior and posterior segments is established with the connectives. Each ganglion is observed to be surrounded with a fine cellular membrane or the neurilemma (Fig. 39B; cf. Figs. 39A, 40, 44A, B, 50), but I could not precisely designate its origin and describe the

process of its formation.

Three gnathal or the mandibular, maxillary and labial ganglia are initially formed separately, but in stage 9 they fuse with each other, to form a single suboesophageal ganglion, which is now situated in the region surrounded anteroventrally with the developing hypopharynx and posteroventrally with the developing postmentum (Figs. 40, 55A, 57B, C). The suboesophageal ganglion becomes short and thick, and three thoracic ganglia also increase in thickness. At the same time, the eleventh abdominal ganglion is fused with the ganglion of the tenth abdominal segment.

Late in stage 12, the terminal abdominal ganglion formed by the fusion of the tenth and eleventh ganglia moves forward into the preceding segment, to fuse with the ninth ganglion. Then, the ventral nerve cord acquires its definitive construction, i.e., one suboesophageal, three thoracic and nine abdominal ganglia (Fig. 56A, B).

6.1.1.2. Brain

6.1.1.2.1. Tritocerebrum

The tritocerebrum is derived from the ganglion of intercalary segment (Fig. 44A; cf. Fig. 40). The intercalary ganglion undergoes the development similar to that in ventral nerve cord, but it is difficult to distinguish two commissures, i.e., the suboesophageal commissures, and practically only one

commissure can be observed in the intercalary ganglion.

The intercalary ganglion originates in postoral area, but with its progressive development into the tritocerebrum, it moves to the preoral position, and tightly unites with the developing deutocerebrum there, as a result, the circumoesophageal connectives are much elongated.

6.1.1.2.2. Deutocerebrum

The deutocerebrum is derived from the ganglion of antennal segment. The antennal ganglion undergoes the development very similar to those of ventral nerve cord.

The paired antennal ganglia are situated just anteriorly to the stomodaeum, and are anteriorly in contact with the protocerebral ganglia lobi 3 (Figs. 55A, 56A, 57A, C, 60).

6.1.1.2.3. Protocerebrum

In stage 7, the anterior broad region of the protocephalon is divided into three paired lobes, from the lateral to medial, the lobus 1, lobus 2 and lobus 3 (Fig. 37). These are the future three pairs of protocerebral ganglia. The lobus 1 is the optic ganglion and lobus 3 is the ganglion of preantennal segment. The structures called the bulges 1, 2 and 3 in external observations correspond to the lobi 1, 2 and 3, respectively.

Early in stage 9, the neuropiles are formed in the dorsal regions of each of the lobi 1 to 3 (Figs. 40, 55A). The neuropiles of three paired lobi extend and fuse with each other. In stage 11, the epidermis ventral to the lobus 1 becomes thick, and is the future optic plate or rudimentary compound eye, which is later innervated from the lobi 1 with the postretinal fiber (Fig. 60). Three ocelli are formed and arranged in inverted triangle on the frons in stage 12, but their innervations could not be precisely designated.

Each of the lobi 1, 2 and 3 enlarges, and their neuropiles increase in volume. These protocerebral ganglia begin to fuse with each other, to form the protocerebrum (Figs. 57A, C, 60). In parallel with the fusion of protocerebral ganglia, the developing protocerebrum fuses with the deutocerebrum. The fusion of protocerebral ganglia makes it difficult to distinguish the lobi 1, 2 and 3 from each other, but it is not impossible, because each of protocerebral lobi is separately sheathed with a neurilemma.

6.1.1.2.4. Morphogenesis of brain

As a result of dorsal-curving and extending of the head lobe (cf. Fig. 37), the head capsule is formed, and it acquires the definitive form in stage 12. With this process and the enlargement of cerebral ganglia themselves, the relative positions of each ganglion of the brain, i.e., the protocerebral lobi 1, 2 and 3, the antennal (deutocerebral) and the intercalary (tritocerebral) ganglia,

are considerably altered. The deutocerebrum mounts the tritocerebrum, and the deutocerebrum itself is mounted by the protocerebral ganglia lobi 3. The lobi 2 shift their positions posteromedially (Fig. 50), keeping the communications with the median lobi 3 and with the deutocerebrum anteroventrally. The lobi 1 hanging over the lobi 2 move posteromedially in accompany with the lobi 2, to fuse with the median lobi 3. Thus, the solid brain is completed (Fig. 60).

6.1.2. Stomatogastric nervous system

In stage 11, the frontal ganglion and recurrent nerve are recognized (Figs. 57A, C, 60). The frontal ganglion is derived from the dorsal wall of the stomodaeum and lies on the middle part of the labrum. The reccurent nerve cord originates from the frontal ganglion, to run backwards along the stomodaeum.

6.2. Apodeme

6.2.1. Tentorium

The tentorium is the endoskeletal brace of the cranium giving attachments to the antennal and gnathal muscles. The tentoria typical to pterygotes are composed of the anterior and posterior tentoria, and the central body is formed by the fusion of them.

In stage 9 just before the revolution, two pairs of ectodermal invaginations appear medially to the mandibular bases in the intersegmental groove between the intercalary and mandibular segments (Fig. 51) and between the lateral bases of the maxillary and labial appendages (Fig. 52). These are respectively the rudiments of anterior and posterior tentoria.

With the progressive cephalization, the anterior and posterior tentoria of both sides elongate and spread medially, and fuse with those of another side. Then, the anterior and posterior tentoria approach and finally unite with each other, to form the central body. In stage 12, the tentorium acquires the definitive form of H shape (cf. Fig. 54).

6.2.2. Salivary gland

In stage 11 when the labial appendages of both sides start the medial extension leading to the formation of mentum, a pair of ectodermal invaginations arise close to median side of each labial base (Fig. 52). They are the rudiments of salivary glands.

The rudiments of salivary glands elongate and develop inwards. The prementum and postmentum are formed by the fusion of labial appendages of both sides in stage 12, and the ductal regions of developing salivary glands of both sides unite with each other, to form a common salivary duct (Fig. 57B, C). The apices of invaginations of salivary glands differentiate into glandular

tissues.

6.3. Alimentary canal

6.3.1. Stomodaeum

The stomodaeal invagination, which has a uniformly thick wall, appears near the cephalic end of the germ band just before segmentation of the germ band in stage 7 (Figs. 35A, 37). For a while, the stomodaeum elongates and develops, as it is.

Shortly before katatrepsis, late in stage 9, the stomodaeum, which initially directs itself dorsally, curves posteriorly and becomes tubular, and its blind end, directly contacting the yolk, is thinned (cf. Fig. 57A, C). On the dorsal wall of the stomodaeum, the rudiment of the frontal ganglion differentiates (Fig. 55A). At the same time, a mass of ectodermal cells which later takes part in the formation of the anterior part of midgut epithelium is found to differentiate from the stomodaeal ventral wall near the blind end (Fig. 55A).

While little regional differentiation is seen in the stomodaeum in previous stages, it acquires the definitive construction as the foregut in stage 13, and in the stomodaeum the various regions such as the pharynx, oesophagus, proventriculus and cardiac valve are distinguished (Fig. 60; cf. Fig. 61A).

6.3.2. Proctodaeum

In stage 7, invaginating embryo further grows, with its posterior half bending three times. With the bendings, the posterior half of embryo or the future abdomen is folded and divided into four regions: regions I to IV from the anterior to posterior (Figs. 35A, 39B, 40, 42, 48A). In cross section, regions I and II and regions III and IV are respectively connected to each other by the amnion (Fig. 35B).

In stage 9, the proctodaeum is formed by the fusion of the regions III and IV of the abdomen. Then, the proctodaeum itself is soon enclosed by the definitive dorsal closure of region II (Figs. 43A-D, 48B, B', C).

After katatrepsis, the proctodaeum or the hindgut elongates extensively. The proctodaeal wall becomes thick, except for the blind end which is conversely thinned. The lumen of the proctodaeum which was narrow before katatrepsis is enlarged. A mass of ectodermal cells similar to that found on the stomodaeal blind end differentiates from the proctodaeal ventral wall near the blind end (Figs. 55B, 57B). These cells later forms the posterior region of midgut epithelium (cf. Fig. 63A, B).

In stage 9, the middle region of the dorsal wall of the proctodaeum begins to thicken, to form the rectal pad. The cells of rectal pad are vacuolated and have a large nucleus. In stage 11, after the katatrepsis, the rectal pad further develops, and its cells become columnar (Fig. 57A, B). In the first

instar nymphal stage, the rectal pad cells increase in volume, and their cytoplasm becomes highly vacuolated. As for the formation of rectal pad, we have only one report for a plecopteran *Kamimuria tibialis* (Kishimoto, 1987). The structure and development of *Ephemera japonica* closely resembles those in *K. tibialis*. Nothing definite is known for the function of the organ.

6.3.3. Midgut

The midgut epithelium of *Ephemera japonica* has a dual origin: the anterior and posterior parts ectodermal, and the middle part of yolk cell in origin (Fig. 63A, B).

A mass of ectodermal cells which arises from the stomodaeal ventral wall near the stomodaeal blind end spreads posteriorly and dorsally over the ental membrane as a form of flap, with the progression of definitive dorsal closure, and develops into the anterior part of midgut epithelium (Fig. 55A; cf. Fig. 63A, B).

Likewise, a mass of ectodermal cells differentiates from the proctodaeal ventral wall near the proctodaeal blind end (Figs. 55B, 57B). This mass first separates bilaterally at its proximal region, and assumes the form of a pair of ribbons. The ribbons begin to spread anteriorly and dorsally over the ental membrane, and then they widen and contact each other, to form the posterior part of midgut epithelium. The formation of midgut epithelium in the

posterior part is more progressive than in the anterior part (Fig. 63A, B).

The middle part of midgut epithelium is formed by yolk cells. First, in stage 9, the yolk mass begins to be encircled by the ental membrane (cf. Figs. 39B, 55) and segmented into yolk blocks (cf. Figs. 44A, B, 56A, B, 58A, B). Encirclement of yolk mass by ental membrane completes by the end of stage 11.

Each yolk block contains some yolk cells inside, which were differentiated in the cleavage stage and then have gradually proliferated. A part of yolk cells migrate onto the ental membrane and settle themselves there (Figs. 56A, B, 58A, B, 59). Yolk materials inside the yolk blocks are gradually consumed. As a result, the yolk blocks regress or decrease in volume, and the lumen of midgut appears (Fig. 58B). With the progressive consumption of yolk, yolk cells settled on the ental membrane accumulate cytoplasm, and develop into the midgut epithelium (Fig. 63A, B). The midgut epithelium completes also in its middle part by the end of the first instar nymphal stage.

6.3.4. Malpighian tubule

The malpighian tubules, which function as an excretory organ, originate from the evaginations appeared in stage 11 on the boundary region between the developing midgut and proctodaeum (Figs. 58A, B, 59; cf. Fig. 61B). The malpighian tubules of *Ephemera japonica* are ectodermal in origin, as in the

other insects.

In this stage, the malpighian tubules of *Ephemera japonica* are only two, with their origins on the both sides of the boundary region. Each tubule branches out during later developmental stages including the postembryonic, and the tubules are found to be composed of numerous branches in the ultimate instar nymph.

Landa and Soldán (1985) studied the number and branching pattern of malpighian tubules in the Ephemeroptera and reported their high diversity.

6.4. Derivatives of splanchnic and somatic mesoderms

6.4.1. Splanchnic mesoderm and musculature of alimentary canal

The splanchnic mesoderm is the dorsal wall of mesodermal somites (Fig. 38), that is, the mesodermal wall directly facing the developing alimentary canal.

In stage 11, the splanchnic mesoderm of gnathal to anterior abdominal segments starts to spread medially and dorsally on the (outer surface of) developing ental membrane of which formation is closely linked with the (definitive) dorsal closure. With the progressive dorsal closure and formation of ental membrane, the splanchnic mesoderm more extends on the ental membrane, and finally it encircles the developing midgut up, to form the midgut musculature, at the completion of dorsal closure and formation of

ental membrane (stage 13) (Fig. 58B). The dorsal closure and formation of ental membrane are progressive in the posterior abdominal and gnathal regions, so that the formation of midgut musculature is also progressive in these regions. It is probable that the median mesoderm also participates in the formation of midgut musculature.

The musculature of stomodaeum originates from a part of the antennal and intercalary mesoderms. The mesoderm which is initially located adjacently to the stomodaeum spreads on the developing stomodaeum, keeping a step with the elongation of the latter, to form the stomodaeal musculature.

As previously described, the proctodaeum is formed by the fusion of regions III and IV of abdomen, which are lined with the mesodermal sheets (Fig. 35A, B). The mesodermal sheets on the regions III and IV fuse with each other at the time of the fusion of these regions (stage 9), to form the mesodermal sheath of proctodaeum and later to develop into the proctodaeal musculature.

6.4.2. Derivatives of somatic mesoderm

The somatic mesoderm is the ventral wall of somites (Fig. 38), and from the somatic mesoderm, all the organs or tissues other than the musculature of alimentary canal originate, such as the somatic musculature, heart, fat body,

and so on.

Major part of somatic mesoderm differentiates into the somatic musculature and fat body which is found in the space between the definitive dorsal closure and midgut (Figs. 56A, B, 58A, B, 59).

The somatic mesodermal cells situated mostlaterally contribute to the formation of circulatory system. With the progression of dorsal closure, these mesodermal cells are carried dorsally. At the completion of dorsal closure, a pair of mesodermal cells, called the cardioblasts, contact with each other on the dorsal side of body, to form the heart (Figs. 58A, B, 59). The heart is accompanied by the cells called the pericardial cells which are also derived from the somatic mesoderm (Figs. 58A, B, 59).

In stage 11, the gonads are found to be situated lateroventrally to the developing proctodaeum as paired structures (cf. Fig. 61A, B). This is only the information obtained in the present study on the development of gonads in *Ephemera japonica*, and nothing definite is known for their origin and further development. Generally in insects, it is known that the gonad is formed by the genital ridge originated from the somatic walls.

The mesoderm of the preoral region or the antennal and preantennal segments is not well-defined segmentarily. The mesodermal cells of this region differentiate into the musculature of the antenna and labrum: a part of mesodermal cells of antennal segment takes part in the formation of

stomodaeal musculature.

In stage 11, a pair of clumped glandular cells are found to differentiate beneath the stomodaeum. These are the suboesophageal bodies, and derived from the intercalary mesoderm (Figs. 50, 57C, 60).

6.5. Other structures

6.5.1. Ental membrane and epineural sinus

In stage 9, the embryo is found to be covered dorsally with a fine layer (Figs. 39A, B, 55A, B, 58A, B, 59; cf. Fig. 56A, B). It covers the embryo throughout all its dorsal surface, and its anterior and posterior extremities end at the margin of head and tip of abdomen. The structures similar to it are reported for Archaeognatha (Machida, 1981b), Odonata (Ando, 1962), Orthoptera (Graber, 1888; Roonwal, 1937), Plecoptera (Miller, 1940), Hemiptera (Sander, 1956) and so on, and they are named the ental membrane. The structure here observed for *Ephemera japonica* may be identified as the ental membrane, but I could not refer to its origin. As for the origin of ental membrane, the argument has not been settled: for example, as possible candidates for the origin of ental membrane, for Orthoptera Graber (1888) and Roonwal (1937) referred to the lateral edges of ectoderm, for Hemiptera Sander (1956) and for Odonata Ando (1962) to the blind ends of stomodaeum and proctodaeum, and for Plecoptera Miller (1940) to the lateral ectoderm and

coelomic sacs. Miller (1940) also speculated that the ental membrane serves a "rail" for the spread of midgut epithelial rudiments.

The space between the ental membrane and ganglia is the epineural sinus, but the epineural sinus is rather narrow in *Ephemera japonica* (Fig. 39A, B). As a result of the collapse of mesodermal somites occurred about in stage 10, the epineural sinus is mingled with the coeloms, to become a mixocoel.

DISCUSSION

1. Egg structure

Koss and Edmunds (1974) generalized the features of the eggs of Ephemeridae or *Ephemera* as follows: 1) possessing a thick adhesive layer, 2) lacking well-developed attachment structures (other than the adhesive layer), and 3) possessing a sperm guide. They also pointed out that the egg of *Ephemera japonica* is exceptional within eggs of Ephemeridae or *Ephemera* in: 1) possessing a very thin adhesive layer and 2) lacking a sperm guide. Their reference to *E. japonica* is justified in the present study. However, the dissimilarities of *E. japonica* to the other ephemerids or *Ephemera* should not always be so essential, because regarding the adhesive layer, "thick" or "thin" may be a matter of degree, and the presence or absence of micropyle may correlate to the thickness of the adhesive layer. Koss and Edmunds (1974) enumerated "smooth chorion" for *E. japonica* egg as another exceptional feature within the Ephemeridae or *Ephemera.* However, SEM reveals that the chorion of *E. japonica* has a mesh pattern on its surface, although it is ambiguous (Fig. 1D).

As is clear from the above, it is of no use to regard *Ephemera japonica* as an exception within the Ephemeridae or *Ephemera.* Thus, the eggs of the genus *Ephemera* or the family Ephemeridae can be re-characterized as follows,

also with reference to previous works (Degrange, 1960; Koss, 1968; Koss and Edmunds, 1974). 1) The egg is ellipsoidal and is translucent and colorless, 2) the egg has an adhesive layer on its surface regardless of thickness, 3) the egg develops no attachment structures other than the adhesive layer, 4) the egg has a single micropyle on its equator, and 5) the chorion has a mesh pattern on its surface.

2. Early development

We have had little knowledge on the early development of Ephemeroptera. The present study is the first comprehensive contribution to this account.

2.1. Maturation

The newly laid egg of *Ephemera japonica*, of which nucleus is in the polar plasm localized at the mid-ventral side of the egg, is at the metaphase of the first maturation (meiotic) division, with its spindle axis being vertical to the egg surface, as is common in the insects (cf. Johannsen and Butt, 1941; Schwalm, 1988; Ando and Kobayashi, 1996).

The second polar body heads for degeneration, so that I can not determine its fate whether it undergoes a further division, namely, I can not

determine the number of polar bodies, whether two or three.

2.2. Fertilization

It is quite striking in the early embryonic development of *Ephemera japonica* that an extensive and circuitous migration of the male pronucleus is involved in the fertilization process: the male pronucleus goes to the female pronucleus not in a straight path, but circuitously, first, anteriorwards in the periplasm from the entrance (i.e., micropyle) on the mid-dorsal side of the egg to the anterior pole of the egg, and then, changing its direction, posteriorwards in the yolk along the egg long axis to the site of syngamy, near the center of the egg.

The time-lapse VTR reveals that in the prefertilization egg of *Ephemera japonica* there exists a yolk stream that moves in accord with the migration of the male pronucleus in time and direction (Fig. 16A-D). Hence, it may be concluded that the migration of the male pronucleus in *E. japonica* is directed by or closely related to the observed yolk stream. The yolk stream is also generated in activated unfertilized eggs, a result implying that the yolk stream occurs regardless of the entry of sperm or the presence of a male pronucleus and that it may be intrinsic to the egg (Figs. 17A, B, 18A-C). However, the mechanism generating the yolk stream is unknown, and investigations especially of the cytoskeletal system are needed to elucidate this issue.

Rempel and Church (1965) found a cytoplasmic stream in the early eggs of a meloid coleopteran *Lytta viridana* by the examination of sectioned materials, and they admitted the possibility that the yolk stream is closely related to the male pronucleus movement. The cytoplasmic stream of *L. viridana* strikingly resembles the yolk stream of *Ephemera japonica* in direction and phase. The findings in the present study may thus verify Rempel and Church's correlation of the male pronucleus movement and cytoplasmic stream.

It may be interesting, in the light of phylogeny, that a unique behavior of the male pronucleus in association with fertilization, which may be controlled by or is closely related to the extensive yolk or cytoplasmic stream, is commonly found in both a primitive mayfly *Ephemera japonica* and a remote advanced coleopteran *Lytta viridana*. The phylogenetic implication of this type of male pronucleus behavior, however, remains to be clarified, because this behavior has not been reported in any other insect groups, and our knowledge of fertilization throughout the insects itself still remains insufficient.

Sauer (1966) used time-lapse cinematography to observe the fertilization of a cricket *Gryllus domesticus*. He found that in this species the approach of male and female pronuclei, which does not involve an extensive circuitous migration of the male pronucleus as observed in *Ephemera japonica* but is fulfilled in the shortest way, is caused by contraction waves of the yolk.

2.3. Cleavage

The fertilized egg of *Ephemera japonica* undergoes a typical superficial cleavage, which is characteristic of dicondylian insects (= Zygentoma + Pterygota) (Johannsen and Butt, 1941; Sharov, 1966), and the successive eight cleavages, of which the first five are synchronized, result in the formation of the blastoderm. The number of cleavages required for the completion of blastema may be small in *E. japonica* in comparison with that in the other insects (cf. Anderson, 1972a, b). This probably results from the small size of *E. japonica* egg, because even a relatively small number of cleavage nuclei may be enough to cover the entire egg surface in such a small egg.

3. Germ disc formation and blastokinesis

The germ disc of *Ephemera japonica* is formed with a concentration of cells at the embryonic area which is broadly defined at the posterior half of the blastoderm. A similar manner of germ disc formation is observed in other ephemeropterans, i.e., *Ephemera strigata* (Ando and Kawana, 1956), *Baetis rhodani* and *B. vernus* (Bohle, 1969) and *Tortopus incertus* (Tsui and Peters, 1974), and this manner of germ disc formation may be regarded as an important characteristic of ephemeropteran embryogenesis.

The embryo of *Ephemera japonica* can be categorized into the typical

short germ type (cf. Krause, 1939), characterized by the sequential proliferation of segments from the anterior to posterior. Because the short germ type dominates in primitive pterygotes (e.g., Odonata: Ando, 1962; Plecoptera: Kishimoto and Ando, 1985) as well as in ectognathan apterygotes (cf. Sander, 1984), this type of germ band may be regarded as an ancestral feature.

As a result of anatrepsis and elongation of the germ band, the embryo of *Ephemera japonica* acquires an S-shape, as observed in other primitive pterygotes such as an odonatan *Epiophebia superstes* (Ando, 1962) and a plecopteran *Kamimuria tibialis* (Kishimoto and Ando, 1985) [and as sporadically found also in higher orders such as paraneopterans (Anderson, 1972a)], and this type of blastokinesis may be considered to be basic type in the pterygotes and apomorphic to their stock.

4. Amnioserosal fold

In the embryogenesis of the Dicondylia (= Zygentoma + Pterygota) or the Ectognatha (= Archaeognatha + Dicondylia), one of the most outstanding is the formation of amnioserosal fold in association with the blastokinesis (Johannsen and Butt, 1941; Anderson, 1973; Zeh et al., 1989; Ando and Kobayashi, 1996; Machida and Ando, 1998). First, the embryo superficially

formed starts to sink into the yolk, and completely invaginates or immerses in the yolk (anatrepsis). During this process, the amnioserosal fold is formed by the production of the amnion from the embryonic margin and by the invasion of serosa underneath the embryo, and the amnioserosal fold then fuses beneath the embryo. In the Dicondylia, the amniotic pore is completely closed, to form a amniotic cavity, and the amnioserosal fold-amniotic cavity system is established (Machida and Ando, 1998). For a time, embryogenesis continues in the same situation (diapause). Then, the amnioserosal fold is withdrawn, and the amnion and then the embryo appear at the egg surface (katatrepsis or revolution), to lead to the degeneration of serosa and formation of secondary dorsal organ. The above also occurs and is very true of in *Ephemera japonica*.

The amnioserosal fold-amniotic cavity system in the Dicondylia seems to be so firmly incorporated in the developmental process, that it should be recognized as a synapomorphy of the group (Machida et al., 1994a; Machida and Ando, 1998). As for the function of this system, some mechanical advantages, such as protection of embryo, have been assumed (Sharov, 1966; Ando, 1970, 1988; Zeh et al., 1989), but nothing definite is known about its functional role (cf. Anderson 1972a).

However, Machida et al. (1994a) and Machida and Ando (1998) recently extended an interesting discussion concerning the functional role of the

amnioserosal fold, based on the evo lutionary transition of functional specialization between the embryo proper and embryonic membranes in the Atelocerata (= Myriapoda + Insecta). They deduced that the amnioserosal fold should have been acquired during the evolution of insects in order to secrete the serosal cuticle beneath the embryo that had lost this ability in due course of atelocerate evolution.

It has been confirmed that in *Ephemera japonica*, the serosal cuticle is not secreted until the completion of anatrepsis, that is, until the embryo is ventrally covered by the amnioserosal fold and the entire egg surface is occupied by serosa. This may be very favourable to the assumption of Machida et al. (1994a) and Machida and Ando (1998) that the principal functional role of the amnioserosal fold and the amnioserosal fold-amniotic cavity system as its advanced form should lie in the secretion of serosal cuticle beneath the embryo.

Machida et al. (1994a) and Machida and Ando (1998) indeed admitted that the evolutionarily established amnioserosal fold-amniotic cavity system in dicondylians, which is temporarily maintained at a fixed embryogenetic stage for each group (as a diapause stage), should be recognized to have a special role in embryogenesis and, as has been often suggested, might be related to the protection of the embryo. They, however, further assumed that in early forms of dicondylians the amnioserosal fold-amniotic cavity system

should have been an ephemeral structure which is not so firmly fixed in duration, because the primary functional role of the amnioserosal fold or the amnioserosal fold-amniotic cavity system should only lie in the secretion of cuticular layer beneath the embryo. The present data on the amnioserosal fold in Ephemeroptera which is one of the most ancestral pterygote groups seem to be also very favorable to their assumption of this. That is, the diapause stage in *E. japonica* considerably ranges in time: the break of diapause is from the late of stage 8 to stage 10. Moreover, an example that the amnioserosal fold is withdrawn in the earliest period at the late of stage 8 does imply that the fold must have been regressed just after the secretion of serosal cuticle occurred during stage 7 (Fig. 49A-C).

5. Body plan

5.1. Cephalic construction

5.1.1. Metamerism

The head segmentation or cephalic metamerism in insects is one of the most interesting and controversial subjects in the insect morphology. Detailed reviews on this subject have been given by Matsuda (1965) and Rempel (1975). According to Rempel (1975), the total number of segments has been places as low as three and as high as seven. Regarding to the cephalic metamerism in

insects, there is no doubt for the validity of the three gnathal, or the mandibular, maxillary and labial segments, and the controversy may concern the pregnathal region. For the elucidation of metamerism and the determination of a segment, the following three criteria are generally utilized: the presence of 1) a pair of appendages, 2) a pair of ganglia and 3) a pair of coelomic sacs. Here I discuss the pregnathal metamerism, based on the present data obtained from the embryonic development of *Ephemera japonica*, according to these three criteria.

In *Ephemera japonica*, a pair of antennal ganglia is formed independently of the others, and the antennal segment possesses a pair of well-developed appendages as well as mesodermal somites, although their coelomic cavities are not well developed. These are good enough to postulate the antennal segment as a eusegment. This must be, in all possibility, true of all the other insects. The fact that the antennal ganglia are formed independently of the other ganglia deserves special emphasis. The arthropod morphlogists such as Holmgren (1916), Hanström (1927, 1928) and Snodgrass (1935, 1960) supposed that the antennal ganglion is not independently present, to be a homologue of a part of the annelid archicerebrum: that is, they did not regard the antennal ganglion or deutocerebrum as representing a neuromere of eusegment. However, the embryological data including the present works suggest that it is not necessary to correlate the antennal ganglion with the archicerebrum.

In *Ephemera japonica*, the intercalary segment fundamentally fulfills the criteria for metamerism: it possesses a pair of well-developed nuromeres differentiating into the tritocerebrum and a mesodermal sheet which may represent its segmental mesoderm, but it is devoid of appendages, as in most of insects. However, a pair of well-developed appendages are found in some insects such as an anopluran *Haematopinus suis* (Young, 1963), a hemipteran *Oncopeltus fasciatus* (Butt, 1949), a coleopteran *Lytta viridana* (Rempel and Chruch, 1971), a lepidopteran *Pieris rapae* (Eastham, 1930), and a hymenopteran *Pimpla turionellae* (Bronskill, 1959), and it is well known that crustaceans develop a pair of second antennae in the segment homologizable to the intercalary segment of insects (cf. Snodgrass, 1935). Taking these facts into account, the intercalary segment may well be regarded as a eusegment.

In *Epehemera japonica*, as in the other insects, three pairs of ganglia are recognized anteriorly to the antennal segment. These ganglia are the protocerebral lobi 1, 2 and 3, to form the protocerebrum. On the basis of the comparative neuroanatomy on the Articulata (= Annelida + Onychophora + Arthropoda + the other small groups) (cf. Hanström, 1928; Scholl, 1969; Rempel, 1975), may be settled the argument on the lobi 1 and 2 of insects, although the data obtained from the present study provide little bases for the discussion on them: that is, the lobus 1 corresponds to the optic lobe of lower articulates or annelids, the lobus 2 is characterized by the corpus

pedunculatum, and the lobi 1 and 2 constitute the neuromere homologizable to the archicerebrum of annelids, to represent the acron as its neural constituent. In *E. japonica*, a mesodermal sheet which probably represents a segmental somite is found to extend from the antennal mesoderm, it may be possible to advocate supposing an additional eusegment, of which candidate of segmental ganglion is lobus 3, anteriorly to the antennal segment, as the preantennal segment.

A difficult problem, however, remains what is the appendage of the preantennal segment. In an odonatan *Epiophlebia superstes* (Ando, 1962), an anopluran *Haematopinus suis* (Young, 1953), a hemipteran *Oncopeltus fasciatus* (Butt, 1949), a coleopteran *Lytta viridana* (Rempel and Chruch, 1971), a lepidopteran *Pieris rapae* (Eastham, 1930) and so on, the labrum is found to formed from paired rudiments, and the authours such as Snodgrass (1935, 1938), Butt (1957, 1960) and Rempel (1975) regarded the labrum as the preantennal appendage. However, it should deserve much attention that in all of the primitive, apterygote insects which have hitherto been studied embryologically such as Collembola (Jura, 1972; Uemiya and Ando, 1987), Diplura (Uzel, 1898; Silvestri, 1933; Ikeda and Machida, 1998), Archaeognatha (Larink, 1969; Machida, 1981a, b) and Zygentoma (Heymons, 1897a; Sharov, 1953; Wellhouse, 1954; Woodland, 1957; Larink, 1970), the labrum does not arise as paired, but as a single structure, and the present

study reveals that the labrum is also derived from a single labral rudiment in the Ephemeroptera which is one of the most primitive pterygote representatives. Moreover, in the myriapods Symphyla (Tiegs, 1940) and Chilopoda (Heymons, 1901), the unpaired labral rudiment forms. Furthermore, Heymons (1901) observed a pair of small structures, which may represent the preantennal segment as its appendicular constituent, to be formed independently of the labral rudiment in Chilopoda. Thus, it may be more likely to conclude that: 1) the labrum cannot be attributed appendicular nature to, in the Atelocerata (= myriapods + insects), and 2) the preantennal appendages are degenerate in insects (and vestigial in myriapods).

As the most unique of the theories for cephalic metamerism in insects (cf. Rempel, 1975), we can refer to Folsom's (1900) and Chaudonneret's (1966): both of them recognize a segment of which appendages are superlinguae. The superlinguae are found in lower insects such as apterygotes and Ephemeroptera, and they seem to function as tongues. The present study reveals that in *Ephemera japonica* the superlinguae originates from the sterna of mandibular and maxillary segment as is confirmed in apterygotes (cf. Machida, 1981a), this implying that the structures are not appendicular but sternal and that Folsom's and Chaudonneret's superlingual segment is not acceptable.

Consequently, the head of *Ephemera japonica* is concluded to be

composed of the acron plus six segments, i.e., the preantennal, intercalary, mandibular, maxillary and labial segments. This is coincident with the interpretations of insect cephalic metamerism by Tiegs (1940, 1947), Imms (1957), Weber (1952), Manton (1960, 1964), Machida (1981b), Kishimoto (1987) and Uemiya (1987).

5.1.2. Appendage

5.1.2.1. Antenna

The antennal rudiment is divided into the proximal and distal parts, and the scapus and the pedicellus plus flagellum originate from the former and the latter, respectively. This partition occurs at the same time as does in the thoracic appendages (see "5.2. Thoracic appendage" in Discussion), and in the light of serial homology, it may be safely asserted that the proximal and distal parts are regarded as the coxopodite and telopodite, respectively, that is, the scapus and the pedicellus plus flagellum are respectively coxopodial and telopodial.

This is the first, direct reference to the antennal coxopodial-telopodial partition, from the morphological basis. Niwa et al. (1997), who molecular embryologically investigated the appendage formation in a cricket *Gryllus bimaculatus*, reported that an expression of a homeobox gene *Distal-less* (*Dll*) is found only at the pedicellus and flagellum in the antenna the same as in the

telopodite of thoracic appendages.

5.1.2.2. Mandible

The mandible had been recognized as coxopodial (Börner, 1909; Crampton, 1921; Matsuda, 1965), but Manton (1977) functional morphologically and Kraus and Kraus (1996) myriapodologically claimed that the mandible of Uniramia (= Atelocerata) is of whole-limb in origin or that it contains not only coxopodite but also telopodite. The argument has not been settled.

The present observation on the mandible formation in *Ephemera japonica* may give a good basis to this argument. That is, the mandible never undergoes any partition as occurs in the antennal, maxillary, labial and thoracic appendages. It never develops its palp, and entire the mandible seems to serial-homologously correspond to the coxopodite of other segmental appendages. Machida (1996, in preparation) investigated the development of mandible and maxilla of an archaeognathan *Pedetontus unimaculatus*, to determine the serial homologies between each part of them: he came to the similar conclusion to the present study, and concluded that the mandible of insects is coxopodial in origin. Recent molecular embryological studies on insect morphogenesis using *Dll* (Carroll, 1994; Panganiban et al.,

1994; Popadić et al., 1996; Niwa et al., 1997) also reveal that the mandible is coxopodial. Thus, it is more probable that the mandible of insect is not of whole-limb, but coxopodial in origin.

From his observation on the nymphal mandible of *Ephemera vulgata*, Heymons (1896a) regarded the mandibular tusk as the palp. Similar structure is observed in the first instar nymph of *E. japonica*, but it arises much delayed to the stage the maxillary or labial palps differentiate (stage 9), so that the correlation of the mandible to the maxillary and labial palps may be misleading, as Murphy (1922) considered that the mandibular tusk should be a secondary structure.

On the other hand, Murphy (1922) recognized the structures, which he called the galea and lacinia and homologized with the structures bearing the same names in the maxilla, in the nymphal mandibles of an ephemeropteran *Polymitarcys* sp. The structures which are to be identical to Murphy's mandibular galea and lacinia are also observed to be formed at stage 13 in *Ephemera japonica*. However, it is more reasonable that these structures are understood as the specialization of mandibular incisor, because these structures and the maxillary galea and lacinia or galeolacinia, which develops in stage 10, are much different in the time of formation.

5.1.2.3. Maxilla

In *Ephemera japonica*, in stage 9 in which the thoracic appendages divide themselves into two parts or the coxopodite and telopodite (see "5.2. Thoracic appendage" of Discussion), the maxillary rudiment is also divided into two parts, later the proximal part developing into the maxilla proper, i.e., the cardo + stipes + their endite, and the distal part into the maxillary palp. The proximal and distal parts of maxilla in *E. japonica* may be respectively homologized with and regarded as the coxopodite and telopodite, in the light of serial homology with the thoracic appendage. The result of this is well supported by the recent molecular embryological investigations on insect appendage formation using the expression of a homeobox gene *Distal-less* (*Dll*), in which only the palpal region is stained as is the telopodial region of thoracic appendage (Cohen and Jürgens, 1989; Cohen et al., 1989; Carroll, 1994; Panganiban et al., 1994; Niwa et al., 1997). The similar proximal-distal partition of maxilla is also confirmed embryologically in the Collembola (Uemiya and Ando, 1987), Archaeognatha (Machida, 1981a, b, 1996) and Plecoptera (Kishimoto, 1987).

Generally in insects, the coxopodial endites, i.e., the medially protruding lobes of coxopodites, of maxilla are two in number, called the galea (outer) and lacinia (inner). It is, however, well known that the maxillary coxopodial endite in Ephemeroptera is a single lobe, and it is called the

galeolacinia.

In the Odonata, the maxillary coxopodial endite is also a single and called the galeolacinia. Ando's (1962) comparative embryological study of odonatans confirmed that the differentiation of galea and lacinia never occurs throughout the developmental processes of all the species examined. Taking importance on the single-lobed coxopodial endite of maxilla and regarding it as its autapomorphy, Hennig (1969) supported the Paleoptera (= Ephemeroptera + Odonata).

Indeed, in *Ephemera japonica*, the differentiation of galea and lacinia was not observed. However, Murphy (1922) did confirm that in a ephemeropteran *Baetis* sp. the singleness of maxillary coxopodial endite is brought about by the secondary fusion of once differentiated galea and lacinia. On the formation of maxillary coxopodial endites, further and detailed investigations are needed.

5.1.2.4. Labium

A close homology between the labium and maxilla is recognized and demonstrated in insects (Snodgrass, 1935; Matsuda, 1965). In *Ephemera japonica*, in stage 9 in which the thoracic appendages divide themselves into two parts or the coxopodite and telopodite (see "5.2. Thoracic appendage" of Discussion), the labial rudiment also divides into two parts as the maxillary rudiment does, and later the proximal part developing into the mentum (=

prementum + postmentum) + their endites or the paraglossa and glossa, and the distal part into the labial palp. The proximal and distal parts of labium in *E. japonica* may be respectively homologized with and regarded as the coxopodite and telopodite, in the light of serial homology with the thoracic appendage. The result of this is well supported by the recent molecular embryological investigations on insect appendage formation (Niwa et al., 1997; etc.), and the similar proximal-distal partition of labium is embryologically confirmed in the other insects (Machida, 1981a, b; etc.).

In *Ephemera japonica*, in stage 12, the coxopodites of labial rudiments of both sides approach and fuse with each other, to form the mentum, this implying that the mentum is coxopodial in origin. It is confirmed that the mentum derives from the coxopodial element also in the other insects, i.e., Collembola (Uemiya and Ando, 1987), Diplura (Ikeda and Machida, 1998), Archaeognatha (Machida, 1981a), Orthoptera (Roonwal, 1937).

5.2. Thoracic appendage

In stage 9, the thoracic appendage divides into the proximal and distal parts, and later in stage 12 the subcoxa and coxa, and the femur, tibia, tarsus and pretarsus differentiate from the former and the latter, respectively (Fig. 34A-D). Because the trochanter originates from the boundary zone between the proximal and distal parts, it is difficult to attribute it to which parts.

For some insects such as Archaeognatha (Machida, 1981a), Collembola (Uemiya and Ando, 1987), Plecoptera (Kishimoto, 1987), in which also the thoracic appendage is first divided into two parts, and the proximal and distal parts are respectively identified as the coxopodite and telopodite, however, it is reported that the trochanter derives from the distal part. Taking this into account, it may be assumed that the proximal-distal partition of thoracic appendage in *Ephemera japonica* can be understood to be the coxopodial-telopodial, and that the trochanter belongs to the telopodial part.

5.3. Abdominal construction

5.3.1. Formation of proctodaeum

In the longest-embryo stage (stage 7), the abdomen of *Ephemera japonica* is folded and divided into four regions I to IV. Regions III and IV fuse together to form the proctodaeum: the ventral and dorsal walls respectively originate from regions III and IV. A similar manner of proctodaeum formation is found in another paleopteran group, the Odonata (Ando, 1962), and this type of proctodaeum formation, in which the proctodaeum is formed by the fusion of two belt-like rudiments, may be regarded as the most basic in Pterygota.

The proctodaeum formations categorized into this type are sporadically found in some neopteran orders such as hemipterans *Pyrrhocoris apterus*

(Seidel, 1924), *Oncopeltus fasciatus* (Butt, 1949) and *Pyrilla perpusilla* (Sander, 1956), a mecopteran *Panorpa pryeri* (Suzuki and Ando, 1981), a trichopteran *Stenopsyche griseipennis* (Miyakawa, 1975), and lower lepidopterans *Endoclita signifer* (Kobayashi et. al., 1981) and *Neomicropteryx nipponensis* (Kobayashi and Ando, 1988). In these insects, region IV has been regarded as amniotic in origin, because of its lack of inner layer, and their proctodaeum has been recognized to be dual in origin, i.e., the dorsal wall of the amnion and the ventral wall of the embryo proper in origin (Seidel observed that both the dorsal and ventral walls of *P. apterus* proctodaeum lack the inner layer, and he concluded the whole proctodaeum of this insect to be amniotic in origin) (cf. papers mentioned above). In the light of this, it may also be said that region IV, namely the whole proctodaeum, of the Paleoptera [Ephemeroptera: herein; Odonata: Ando (1962)] is embryonic proper in origin, because the inner layer is found to be segregated also on region IV, namely on the entire proctodaeum.

Accordingly, the difference in proctodaeum formation may be recognized, concerning the differentiation of the inner layer, between the paleopterans and neopterans. However, it is suggested that the difference is not so essential as to specifically designate the partially different origin of the proctodaeum. I believe that the difference can be explained merely by the term of heterochrony concerning the timing of the segregation of the inner

layer and the morphogenesis of the proctodaeum, or by the term "substitution in the developmental process" of Matsuda (1976), namely, the evolutionary alteration of the manner of mesodermal supply to the wall of the proctodaeum. So far as any new bases for the amniotic nature of region IV in neopterans cannot be found, it is better to recognize the region to be of embryo proper in origin as well.

5.3.2. Formation of caudal filament and abdominal metamerism

Heymons (1896a, b, c) deduced that the caudal filament is the elongation of the eleventh abdominal tergum. His idea has been followed by many insect morphologists such as Crampton (1917, 1918), Snodgrass (1935), Brinck (1957), Birket-Smith (1971) and Matsuda (1976), and has remained largely unchallenged.

In the present study, it is clarified that the caudal filament of *Ephemera japonica* originates from the posterior extremity of region IV and has its origin much away from the eleventh abdominal segment, with regions III and IV or the precursor of proctodaeum interposing. This may be critical of Heymons' interpretation. That is, if the caudal filament, as Heymons deduced, is a derivative of the eleventh abdominal segment, we should regard regions III and IV or the proctodaeum as a part of the eleventh abdominal segment as well, and we should suppose an enormously long eleventh abdominal

segment. Regarding the derivation of the caudal filament, another interpretation may be more plausible. That is, the caudal filament should not be attributed to the eleventh abdominal segment, but should be correlated to the so-called telson which has been thought to be represented in insects only by the proctodaeum and anal lobes (cf. Snodgrass, 1935; Matsuda, 1976).

The abdominal formation and metamerism of *Ephemera japonica* can be summarized as follows. Its embryonic abdomen is composed of four regions I to IV. From regions I and II, the first to fifth and the sixth to eleventh abdominal segments are respectively originated. Each of these eleven segments is characterized by a pair of ganglia, and the eleventh also by a pair of cerci as appendages as well. Regions III and IV following the eleventh abdominal segment are devoid of any attributes suggestive of "segments". Regions III and IV develop into the proctodaeum. At the terminal of region IV, the caudal filament forms as a derivative of the telson which may be represented by the proctodaeum and anal lobes.

6. Formation of midgut epithelium

The midgut epithelium is exclusively derived from the yolk cells in the apterygotes other than the dicondylian apterygote Zygentoma, i.e., the Collembola (Uljanin, 1875; Claypole, 1898; Uzel, 1898; Prowazek, 1900; Jura,

1972; Jura and Krzysztofowicz, 1977), Diplura (Heymons, 1897b) and Archaeognatha (Machida and Ando, 1981). On the other hand, it is well known that in the pterygotes, more strictly the neopterans, the midgut epithelium is entirely originated from the midgut epithelial rudiments arising from the blind ends of stomodaeum and proctodaeum, and that it is ectodermal in origin (cf. Johannsen and Butt, 1941; Anderson, 1972a, b; Ando and Kobayashi, 1996).

The present study reveals that the midgut epithelium in *Ephemera japonica* has a dual origin: the anterior and posterior parts are respectively stomodaeal and proctodaeal, i.e., ectodermal in origin, and the middle part is of yolk cell in origin. We can say that the midgut epithelium in *E. japonica* is formed in the blending or intermediate way of the apterygote and pterygote types. Such a midgut epithelium formation as observed herein for Ephemeroptera has been also reported for the Zygentoma (Heymons, 1897a; Sharov, 1953) and Odonata (Ando, 1962), that is, the most ancestral dicondylian Zygentoma, and another representative of the most primitive pterygotes the Odonata.

The present results that the midgut epithelium in the Ephemeroptera is formed in the intermediate way of the apterygote and pterygote types seem to be more significant in illustrating the anagenetical changes of midgut epithelium formation in insects. That is, the Zygentoma, Odonata and

Ephemeroptera represent the basal clades of Dicondylia, and it is revealed that all of them have an intermediate type of midgut epithelium formation. The present study on the Ephemeroptera may cover a piece unknown but requisite for anagenetically elucidating the midgut epithelium formation in insects.

7. Proposed affinity of Ephemeroptera based on the embryological knowledge

In the present study, I have examined the embryonic development of an ephemeropteran, *Ephemera japonica* in detail, extended discussions on the insect or pterygote body plan and embryogenesis in the light of evolution, and referred to the ground plan of body construction and embryogenesis as well as their evolutionary transition.

In this section, based on the embryological data obtained from *Ephemera japonica*, comparing with the previous works on the other insects, I reconstruct the affinity of Ephemeroptera or the phylogeny of Pterygota in higher level which concerns the major pterygote groups, i.e., the Ephemeroptera, Odonata and Neoptera. For the interrelationships among them, there exist three possibilities, that is, 1) Paleoptera (= Ephemeroptera + Odonata) + Neoptera (cf. Hennig, 1969), 2) Ephemeroptera + (Odonata + Neoptera) (cf. Kristensen, 1975; Wheeler and Carpenter, 1996), and 3) Odonata + (Ephemeroptera +

Neoptera) (cf. Boudreaux, 1979), but the argument has not been settled.

For the basal clades of insects is adopted Hennig's "Entognatha-Ectognatha system" which may be currently the most reliable (cf. Hennig, 1969; Kristensen, 1975). Although the phylogenetical discussion here presented is aimed to examine the phylogeny of Pterygota in higher level, the Zygentoma should be also taken into account: unless be done, it would be as much as unconditionally confessing the Ephemeroptera - Odonata - Neoptera to be monophyletic: the monophyly of them or Pterygota should be primarily examined, as the prerequisite for the discussion on their affinities. For determination of character state of each character, the Archaeognatha is set as the out-group of Dicondylia (= Zygentoma + Pterygota).

Here the following twelve characters are examined and utilized for elucidating the phylogeny: 1) amnioserosal fold, 2) germ type 3) cleavage, 4) egg tooth, 5) formation of midgut epithelium, 6) invagination type of embryo, 7) formation of proctodaeum, 8) micropyle, 9) broadly-formed embryonic area, 10) clypeolabral rudiment, 11) superlingua and 12) caudal filament as an elongation of telson. Because the characters may be too short in number to illustrate the phylogeny directly and cladistically, I adopt the way to map these characters each of which character state is determined on the three phylogenies above-mentioned, to examine their validity: these phylogenies agree in supporting monophylies of Pterygota, Dicondylia and Ectognatha. In

Figure 64A to C, the solid and open circles respectively imply the apomorphic (derived) and the plesiomorphic (ancestral) character states; the double-headed and single-headed arrows respectively imply the synapomorphic (or synplesiomorphic) relationship and the transformation of character; the double-headed shaded line implies the parallel acquisition of the character by the groups pointed with the arrows. The arrowed numerals in parentheses imply that the character is transformed into another form in one or some groups of the lineage.

1) Amnioserosal fold

In the myriapods and entognathous insects, the embryonic membrane is represented only by the serosa, and the amnion and the amnioserosal fold are not developed. These structures have first appeared in the Ectognatha (= Archaeognatha + Dicondylia) as its autapomorphy (cf. Machida and Ando, 1998).

In Dicondylia (= Zygentoma + Pterygota), the amnioserosal fold is completely fused, to form a closed amniotic cavity, resulting in the formation of the amnioserosal fold-amniotic cavity, which is considered to be an autapomorphy of the group. The amnioserosal fold is inherited into the archaeognathan and dicondylian (including the Ephemeroptera) lineages as a synapomorphy, and in the latter it transforms itself into the amnioserosal fold-

amniotic cavity system.

The numerals 1 and 1' respectively imply the amnioserosal fold and the amnioserosal fold-amniotic cavity system.

2) Germ type

The germ rudiment of short germ type is first appeared in the Archaeognatha and also inherited into the dicondylian lineage, and it is an autapomorphy of Ectognatha (cf. Sander, 1984).

The numeral 2 implies the short germ type.

Appendix: In the higher neopterans, the long germ type appears, which is recognized as an apomorphy of them (shown by 2').

3) Cleavage

The cleavage is fundamentally holoblastic in the arthropods, and in primitive insects including the Archaeognatha the total cleavage is performed. The superficial cleavage in insects is recognized as a autapomorphy of Dicondylia (cf. Machida et al., 1990).

The numerals 3 and 3' respectively imply the total and superficial cleavages.

4) Egg tooth

The egg tooth is not found in the entognathous insects and archaeognathans (cf. Jura, 1972; Machida, 1981a). It is first appeared in the dicondylian lineage, which can be recognized as an autapomorphy of the lineage (cf. Sharov, 1966; Ando and Kobayashi, 1996).

The numerals 4 and 4' respectively imply the absence and the presence of egg tooth.

5) Formation of midgut epithelium

As previously mentioned (see "6. Formation of midgut epithelium" of Discussion), the anagenetical transition concerning the manner of midgut epithelium formation in insects is found: the midgut epithelium is formed exclusively by yolk cells in entognathous insects and archaeognathans, whereas it is ectodermal in origin in neopterans, and in the zygentomans, ephemeropterans and odonatans it is formed by the intermediate or blending way.

This anagenetical transition concerning the midgut epithelium formation in insects can be translated as follows, in terms of the character states. First, the midgut epithelium formation by yolk cells is recognized as a plesiomorphic to insects (cf. Machida and Ando, 1981), because the midgut epithelium formation by yolk cell is basic in the myriapods and crustaceans

which can be respectively accepted as the out-group of insects and Atelocerata (= Myriapoda + Insecta) (Johannsen and Butt, 1941; Anderson, 1973; Machida and Ando, 1981). The participation of ectoderm in the formation of midgut epithelium is apomorphic to the stem of Dicondylia. In the Neoptera the midgut epithelium is exclusively ectodermal in origin, this implying the renouncement of ability for differentiation into the midgut epithelium by the yolk cells, which can be recognized as apomorphic to the group.

The numerals 5, 5' and 5" respectively imply the midgut epithelium formation exclusively by yolk cells, the participation of ectoderm in the midgut epithelium formation, and the midgut epithelium formation exclusively by the ectoderm, i.e., the renouncement of ability by the yolk cells for the differentiation into the midgut epithelium.

6) Invagination type of embryo

The invagination of embryo into the yolk is, in all possibilities, considered to be first acquired by the stock of Dicondylia, because this phenomenon should be recognized to be closely linked to the acquisition of amnioserosal fold-amniotic cavity system (see "4. Amnioserosal fold" of Discussion), so that the invagination of embryo can be recognized as a synapomorphy of the Zygentoma and Pterygota.

The S-shaped, deep invagination of embryo as found in Ephemeroptera

also occurs in the representatives of the most primitive pterygotes (and besides in some higher pterygotes such as the paraneopteran orders), whereas in the Zygentoma the invagination is not so extensive. It may be safely asserted that the S-shaped invagination is basic in the pterygotes and apomorphic to their stem. The Archaeognatha has an amnioserosal fold, but it does not develop into a form of amnioserosal fold-amniotic cavity system, so that its embryo does not perform the invagination comparable to that discussed here.

The numerals 6, 6' and 6" respectively imply the primitive condition found in Archaeognatha, the invagination of embryo not extensive as in Zygentoma and the S-shaped invagination of embryo.

Appendix: In some higher neopterans, the type of invagination of embryo is transformed into the other forms, which are recognized as apomorphic to each of them (shown by 6"').

7) Formation of proctodaeum

In myriapods and apterygotes, i.e., the entognathans, archaeognathans and zygentomans, the proctodaeum is formed as a simple ectodermal invagination as found in myriapods and crustaceans, and this manner of proctodaeum formation is recognized as plesiomorphic to the insects.

In the Ephemeroptera, Odonata and some neopteran groups, the

proctodaeum is formed by the fusion of belt-like proctodaeal rudiments, the manner of formation can be regarded as basic in pterygotes and apomorphic to their stem (see "5.3.1. Formation of proctodaeum").

The numerals 7 and 7' respectively imply the proctodaeum formations by the simple invagination and by the fusion of belt-like rudiments.

Appendix: In some groups of neopterans, the proctodaeum is formed by the simple ectodermal invagination, which may be regarded as a new character of each group (shown by 7").

8) Micropyle

The micropyle is the structure only developed in the Pterygota within the Atelocerata (= Myriapoda + Insecta), and it is regarded as an autapomorphy of the group.

The numerals 8 and 8' respectively imply the lacking of micropyle and the acquisition of it.

9) Broadly-formed embryonic area

In the Ephemeroptera, a very broad embryonic area is formed, to form a small germ disc by its condensation. This broad embryonic area of which condensation results in the formation of germ rudiment is unique within insects, and is regarded as an autapomorphy to the Ephemeroptera.

The numerals 9 and 9' respectively imply the regular embryonic area ordinary to ectognathans and the broad embryonic area found in the Ephemeroptera.

10) Clypeolabral rudiment

In myriapods and apterygotes, the clypeolabrum arises as a single structure, the same as in the Ephemeroptera, and this is regarded as plesiomorphic to the insects. In the Odonata and Neoptera, the clypeolabrum is formed by the fusion of paired rudiments, and this is to be regarded as apomorphic to the Odonata and Neoptera.

The numerals 10 and 10' respectively imply the clypeolabral rudiment as a single structure and that as paired structure.

11) Superlingua

In myriapods, apterygotes and ephemeropterans, the hypopharynx differentiates into the superlinguae and lingua, whereas in the odonatans and neopterans it remains to be single and develops only into a lingua. The differentiation of superlinguae is to be recognized as plesiomorphic in the insects, and the loss of their differentiation as apomorphic to the odonatans and neopterans.

The numerals 11 and 11' respectively imply the differentiation of

superlinguae and the loss of their differentiation.

12) Caudal filament as an elongation of telson

The present study reveals that the caudal filament of the Ephemeroptera is an elongation of telson. Machida (1981b) alluded a similar interpretation for the caudal filament of the Archaeognatha. The structures closely resembling the caudal filament of Ephemeroptera are present in the Zygentoma and Paleozoic monuran *Dasyleptus* spp., of which origin could be similarly identified to that in the Ephemeroptera: the Monura is regarded to constitute the Dicondylia with the Zygentoma and Pterygota (Kukalová-Peck, 1987). Such structures as the caudal filaments found in these insects are not reported for any other insects or atelocerates, so that the caudal filament can be recognized as an autapomorphy of the Ectognatha.

The ectognathans other than the Archaeognatha, Monura, Zygentoma and Ephemeroptera, namely, the Odonata and Neoptera, do not possess the caudal filament, and this character condition, i.e., the loss of caudal filament, may be recognized as an apomorphy to the Odonata and Neoptera.

The numerals 12 and 12' respectively imply the acquirement of caudal filament and the loss of it.

The character states of characters 1 to 12 are determined as discussed

above. In Figure 64A to C, these characters are mapped on three phylogenies currently proposed: A) the phylogeny supported by Kristensen (1975) and Wheeler and Carpenter (1996), B) the phylogeny supported by Hennig (1969), and C) the phylogeny supported by Boudreaux (1979). The lineages leading to the Archaeognatha, Zygentoma and the stem of Pterygota are shown only in A (Fig. 64A), because these phylogenies agree in supporting the monophylies of Ectognatha, Dicondylia and Pterygota.

Although the characters here examined may be not much in number enough to positively elucidate the phylogeny, I try to read the distribution of characters. First, it seems to be well shown that both of the Dicondylia (= Zygentoma + Pterygota) and the Pterygota (Ephemeroptera + Odonata + Neoptera) should be monophyletic, which are characterized by five (characters 1, 3-6) and three (characters 6-8) autapomorphies, respectively.

Regarding the interrelationships within the Pterygota, the phylogeny A (Fig. 64A) proves to be the most parsimonious. Both of phylogenies B (Fig. 64B) and C (Fig. 64C) require the supposition of parallel acquisitions three times, concerning the characters 10-12, i.e., the clypeolabral rudiment, the superlingua and the caudal filament: any suppositions of parallel acquisition of characters are not needed in the phylogeny A (Fig. 64A).

Consequently, the comparative embryological examination presented here supports the phylogeny A (Fig. 64A) that is supported by Kristensen

(1975) and Wheeler and Carpenter (1996) and which is formulated as [Ephemeroptera + (Odonata + Neoptera)] concerning the Pterygota.

SUMMARY

1. The embryonic development of a mayfly, *Ephemera japonica* McLachlan (Insecta: Ephemeroptera, Ephemeridae) was described in detail, divided into thirteen stages, and the results obtained were discussed in comparison with the previous works, to examine the ground plans of body and morphogenesis in the Ephemeroptera as well as in the insects and pterygotes.
2. The egg is ellipsoidal about 200 μm in length and about 100 μm in width, and colorless translucent. One micropyle is on the equator of mid-ventral side of the egg.
3. The egg newly oviposited is at the metaphase of first maturation division, and the egg nucleus is situated in the polar plasm or cytoplasmic island localized at the mid-dorsal side of egg.
4. An extensive and circuitous migration of the male pronucleus is involved in the fertilization process. It is demonstrated that the movement of male pronucleus is generated by the yolk stream intrinsic to the egg itself. Similar behavior of male pronucleus to this was reported for a coleopteran *Lytta viridana* (Rempel and Church, 1971). For discussing the basic plan of fertilization in insects, it may be interesting that a unique behavior of the male pronucleus is commonly found in both a primitive mayfly and a

remote advanced coleopteran.

5. The cleavage is of the typical superficial type. The successive eight cleavages, of which the first five are synchronized, result in the formatio n of the blastoderm, and about ten primary yolk cells are left behind in the yolk.
6. Even in the newly formed blastoderm, the thick posterior half, embryonic, and the thin anterior half, extraembryonic areas are distinguished.
7. The cells of embryonic area concentrate to the posterior egg pole, to form a germ disc there, and the cells of extraembryonic area become thinned to be a serosa. This broadly-formed embryonic area is characteristic of Ephemeroptera, and is recognized as its autapomorphy.
8. The germ disc undergoes the following embryonic development of typical short-germ type, which may be recognized as an ancestral type within insects.
9. The blastokinesis is extensive. As a result of anatrepsis, the embryo deeply invaginates into the yolk, to acquire an S-shape. This S-shaped embryo is characteristic of the lower pterygotes, and it may be recognized as an ancestral feature within the pterygotes.
10. A second embryonic membrane or the amnion is produced from the margin of embryo, in association with the progressive anatrepsis, to form the amnioserosal fold. The fold is completely closed to form an amniotic

cavity, this leading to the establishment of the amnioserosal fold-amniotic cavity system.

11. As a result of the katatrepsis or the withdrawal of amnioserosal fold, the serosal cells begin to be condensed anterodorsally on the egg surface, to form the secondary dorsal organ, which is degenerate by the hatching.
12. With the progressive condensation of serosal cells, the amnion replaces the serosa, to function as a provisional dorsal closure. The provisional dorsal closure is itself replaced by the progressive definitive dorsal closure by the growth of lateral walls of embryo during the late developmental stages.
13. Just after the completion of anatrepsis, the serosa secretes a cuticular layer, i.e., the serosal cuticle, beneath the chorion. Also taking it into account that the timing of the katatrepsis is considerably deviated, it may be possibly assumed that the principal and primary function of amnioserosal fold lies in the secretion of serosal cuticle (beneath the embryo).
14. The inner layer or the mesoderm is segregated in the entire dorsal side of developing embryo.
15. The clypeolabrum arises as not paired but an unpaired, single structure, and its appendicular nature is rejected, this implying likewise the rejection of labral segment.
16. The superlinguae are derived from the mandibular and maxillary sterna, and are not appendicular but sternal in origin, this leading to the rejection

of superlingual segment.

17. On the basis of serial homology, the cephalic appendages are recognized as follows. The antenna is divided into the proximal and distal parts, which are homologized with the thoracic coxopodite and telopodite, and from each the scapus and the pedicellus + flagellum differentiate, respectively. The palpi of maxilla and labium are homologous with the telopodites of the thoracic appendages, and the other parts proximal to the palpi are homologous with the coxopodites. The mandible lacks the telopodial element or the palp, so that it is determined to be coxopodial.
18. The labial coxopodites of both sides approach each other, to form the prementum and postmentum.
19. The galeolacinia and the glossa + paraglossa develop from the medial parts of maxillary and labial coxopodites as the endites, respectively.
20. No appendages develop in any abdominal segments at least during the embryonic stages, except for the eleventh abdominal segment, of which appendages largely develop into a pair of cerci.
21. In the longest-embryo stage, the abdomen is folded and is divided into four regions (regions I-IV, from anterior to posterior). All the first to eleventh segments are derived from regions I and II. Regions III and IV, which have no attributes good enough to speculate their segmental nature, fuse together to form the proctodaeum, and they represent the telson.

22. The caudal filament which has been interpreted as the elongation of eleventh abdominal tergum is revealed to be of telson in origin.
23. The metamerism in *Ephemera japonica* is embryologically determined to be composed of the head [= acron + six segments (= preantennal + antennal + intercalary + mandibular + maxillary + labial segments)] + the thorax (= three thoracic segments) + the abdomen (= eleven segments + telson). This interpretation may be applicable to the other ectognathous insects.
24. The protocerebrum consists of three pairs of ganglia (lobi 1, 2 and 3). The deutocerebrum is derived from the antennal, and the tritocerebrum from the intercalary ganglia. The protocerebral lobi 1 and 3 are respectively regarded as the optic and preantennal ganglia.
25. The ventral nerve cord consists of seventeen pairs of ganglion from the mandibular to the eleventh abdominal ganglia. The mandibular, maxillary and labial ganglia fuse with each other, to form the suboesophageal ganglion. The ganglia of the tenth and eleventh abdominal segments are incorporated into the ganglion of the ninth.
26. The frontal ganglion is derived from the dorsal wall of the stomodaeum.
27. The anterior and posterior tentoria arise from the intersegmental groove between the intercalary and mandibular segments and that between the maxillary and labial segments, respectively, and they fuse with each other, to form the central body.

28. The salivary gland is formed from a pair of ectodermal invaginations arising close to median side of each labial base.
29. The stomodaeum arises as a simple invagination of ectoderm. The proctodaeum is formed by the fusion of regions III and IV of abdomen.
30. The midgut epithelium has a dual origin. Its anterior and posterior parts are derived from the rudiments arising from the stomodaeal and proctodaeal blind ends, and they are ectodermal in origin. The middle part of midgut epithelium is formed by the migration of yolk cells, and it is of yolk cell in origin. The manner of formation of midgut epithelium is regarded as an intermediate type between or blending type of those found in the apterygotes and pterygotes.
31. The malpighian tubules arise from the boundary between the developing midgut and proctodaeum as a pair of evaginations.
32. The musculatures of the stomodaeum and the midgut are respectively derived from the antennal and intercalary mesoderms and the splanchnic mesoderm of the gnathal to abdominal segments. The musculature of proctodaeum is developed from the inner layer appeared in the dorsal surface of regions III and IV of abdomen.
33. The development of somatic mesoderms was described, which differentiate into various organs or tissues such as: the somatic musculature, heart, pericardial cell, fat body and suboesophageal body.

34. In the embryos of later stages, the ental membrane appears, which entirely covers the dorsal surface of embryo, to form the epineural sinus.

35. From the comparison of the results obtained herein for *Ephemera japonica* with the knowledge for the other insects, the evolutionary transition of the ground plans of body and morphogenesis in the insects or pterygotes was discussed, and the interrelationships of the basal clades of pterygotes was elucidated. Using the twelve characters, i.e., 1) amnioserosal fold, 2) germ type, 3) cleavage, 4) egg tooth, 5) formation of midgut epithelium, 6) invagination type of embryo, 7) formation of proctodaeum, 8) micropyle, 9) broadly-formed embryonic area, 10) clypeolabral rudiment, 11) superlingua, and 12) caudal filament as an elongation of telson, the phylogenies of pterygote basal clades currently proposed were examined, and the phylogeny formulated as [Ephemeroptera + (Odonata + Neoptera)] was strongly supported.

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FIGURES

Fig. 1. Newly laid eggs of *Ephemera japonica*. SEM micrographs of the egg (A, B, D), and living egg (C).

1A. Dorsal view (anterior to the left). A micropyle (Mp) is located on the dorsal side of the egg. Bar = 50 μm.

1B. Enlargement of egg with adhesive layer, showing the micropyle. Bar = 5 μm.

1C. Lateral view (anterior to the left, dorsal to the top). Bar = 50 μm.

1D. Enlargement of egg with adhesive layer. Bar = 5 μm.

Ch, chorion; Mp, maicropyle; MpO, micropylar opening; Y, yolk.

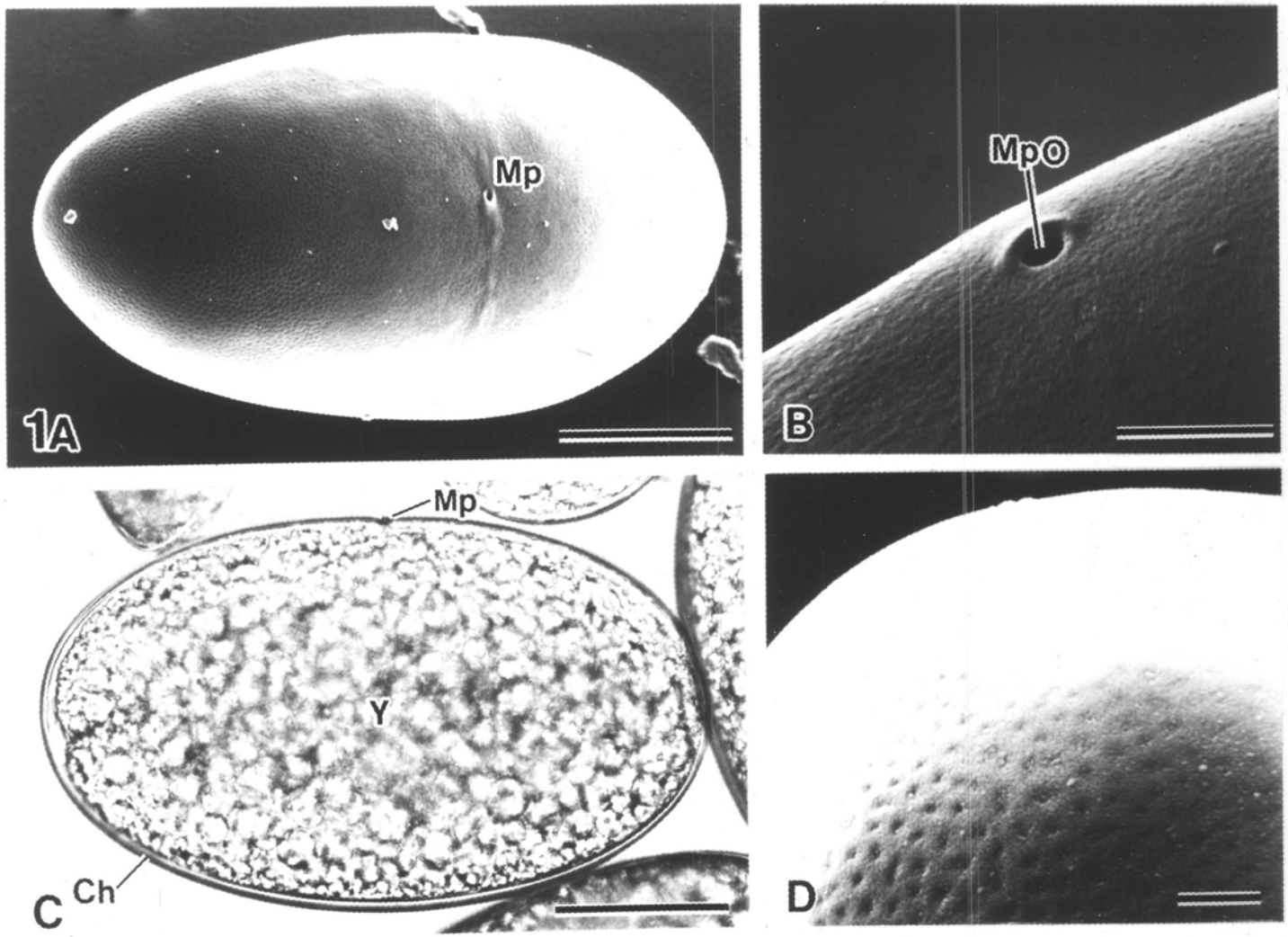

Mp
1A
MpO
B
Mp
Y
Ch
C
D

Fig. 2. Successive stages of embryonic development. Lateral views. Bar = 50 μm.

2A. Stage 1 (Egg cleavage)

2B. Stage 2 (Blastoderm formation)

2C. Stage 3 (Formation of germ disc)

2D, D'. Early (D) and late (D') stage 4 (Pear-shaped embryo)

2E, E'. Early (E) and late (E') stage 5 (Start of invagination of germ band, anatrepsis I)

2F, F'. Early (F) and late (F') stage 6 (S-shaped embryo: completion of invagination, anatrepsis II)

2G. Stage 7 (Longest embryo)

2H. Stage 8 (Segmentation of embryo)

2I. Stage 9 (Start of a appendicular annulation)

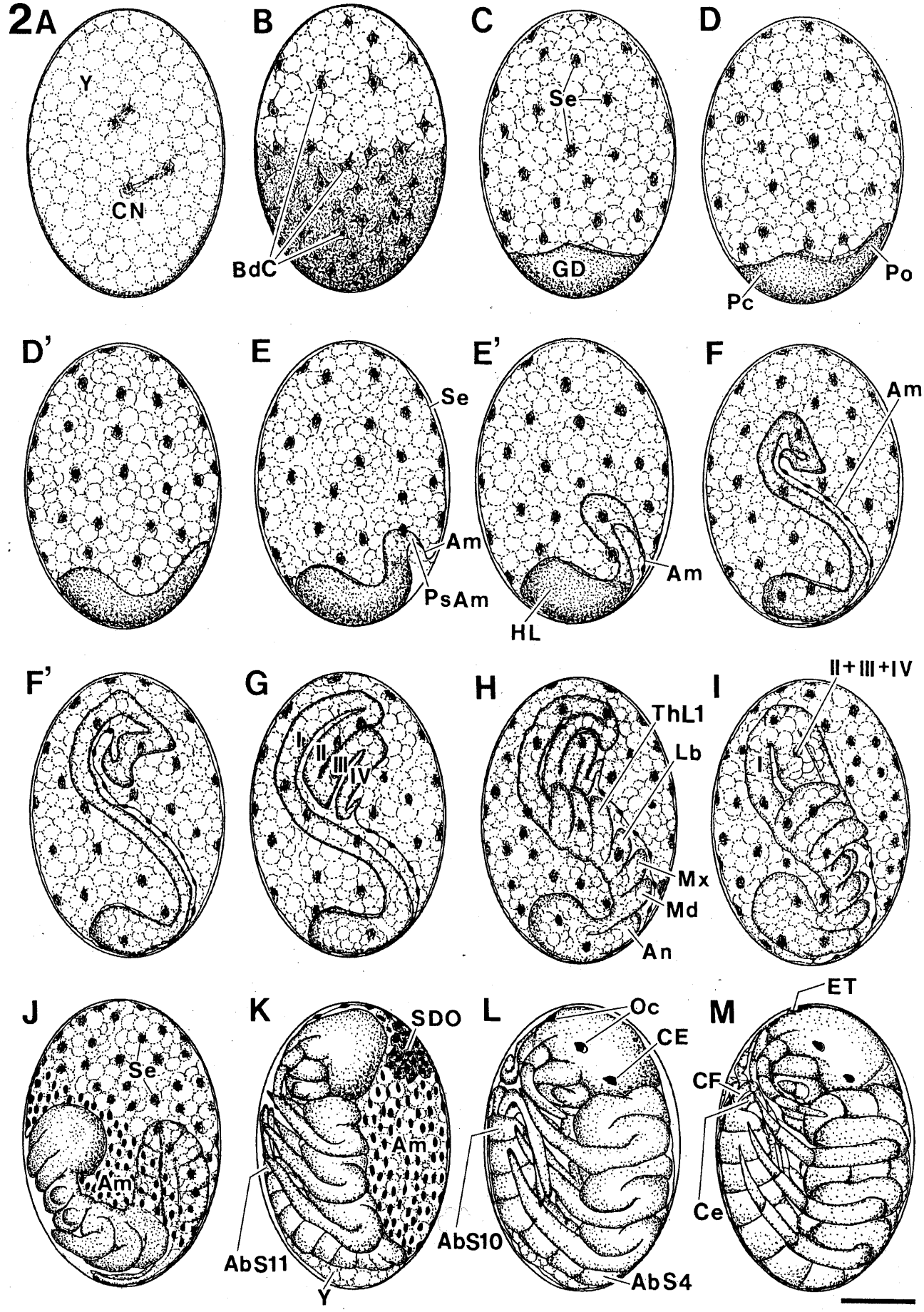
2J. Stage 10 (Revolution, katatrepsis)

2K. Stage 11 (Post-revolution I)

2L. Stage 12 (Post-revolution II)

2M. Stage 13 (Post-revolution III)

I-IV, abdominal regions I to IV; AbS4, 10, 11, 4th, 10th and 11th abdominal segments; Am, amnion; An, antenna; BdC, blastodermal cell; CE, compound eye; Ce, cercus, CF, caudal filament; CN, cleavage nucleus; ET, egg tooth; GD, germ disc; HL, head lobe; Lb, labium; Md, mandible; Mx, maxilla; Oc, ocellus; Pc, protocephalon; Po, protocorm; PsAm, presumptive amnion; SDO, secondary dorsal organ; Se, serosa; ThL1, proleg; Y, yolk.


2A
Y
CN
B
BdC
C
Se
GD
D
Po
Pc
D'
E
Se
Am
PsAm
E'
Am
HL
F
Am
F'
G
I
II
III
IV
H
ThL1
Lb
Mx
Md
An
I
II+III+IV
I
J
Se
Am
K
SDO
Am
AbS11
Y
L
Oc
CE
AbS10
AbS4
M
ET
CF
Ce

Figs. 3-7. Early embryonic development I.

Fig. 3. A newly laid egg. Lateral view (anterior to the top, ventral to the left). The egg was stained with Hoechst 33342, and observed through a green filter (UV excitation, fluorescence microscopy). The egg nucleus (oocyte nucleus s. str.) and male pronucleus can be seen at the ventral (arrowhead) and opposite or dorsal (arrow) sides of the egg, respectively. The vague fluorescences at the anterior egg pole was artificially caused by pricking the chorion. Bar = 50 μm.

Fig. 4. A longitudinal sections of the egg shown in Figure 4.

4A. A section through polar plasm. Bar = 50 μm.
4B. A section through male pronucleus. Bar = 50 μm.
4C. Enlargement of A. Bar = 10 μm.
4D. Enlargement of B. Bar = 10 μm.

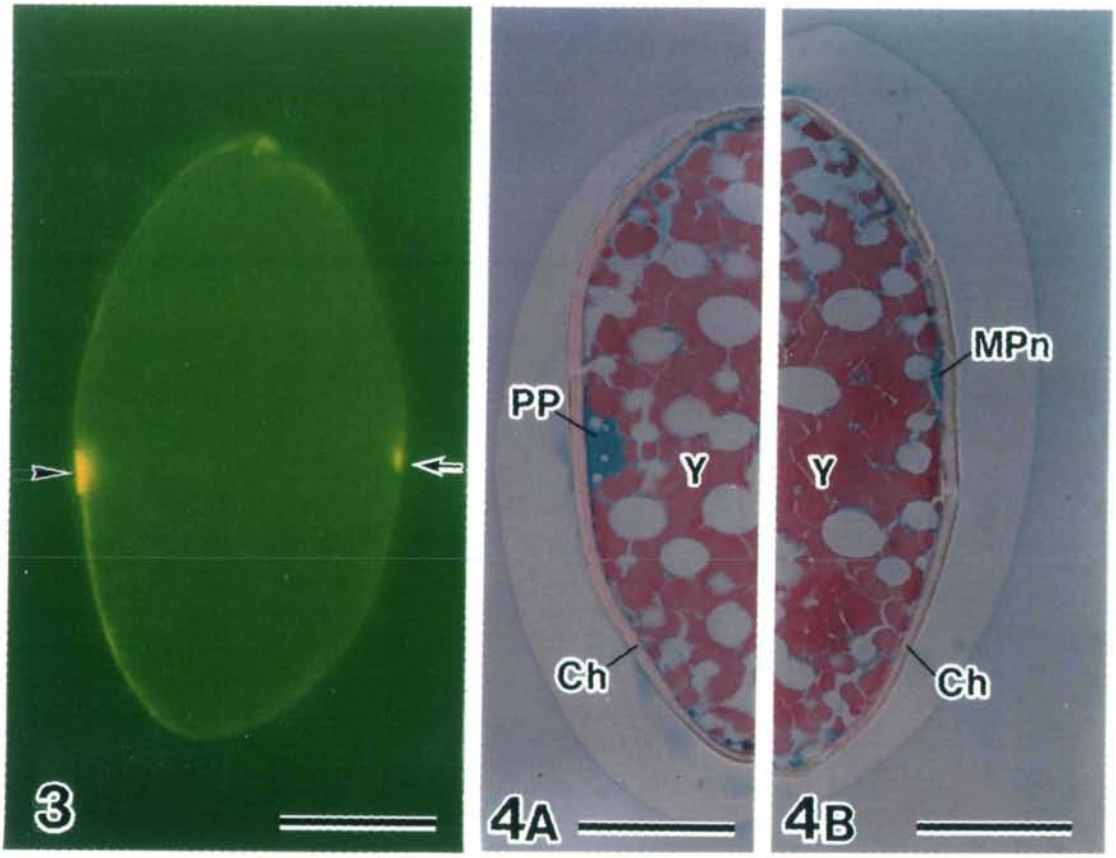
Fig. 5. Longitudinal section of a newly laid egg through the polar plasm (metaphase of the first maturation division). Bar = 10 μm.

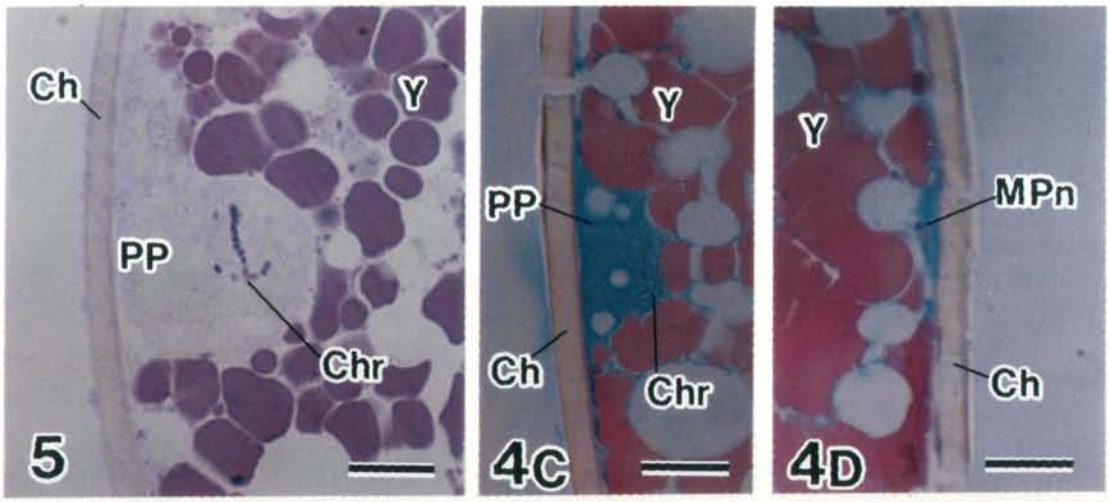
Fig. 6. A longitudinal sections of an egg, at the telophase of the second maturation division, 1.5 hours after oviposition. A and B are 4 μm apart. Bar = 10 μm.

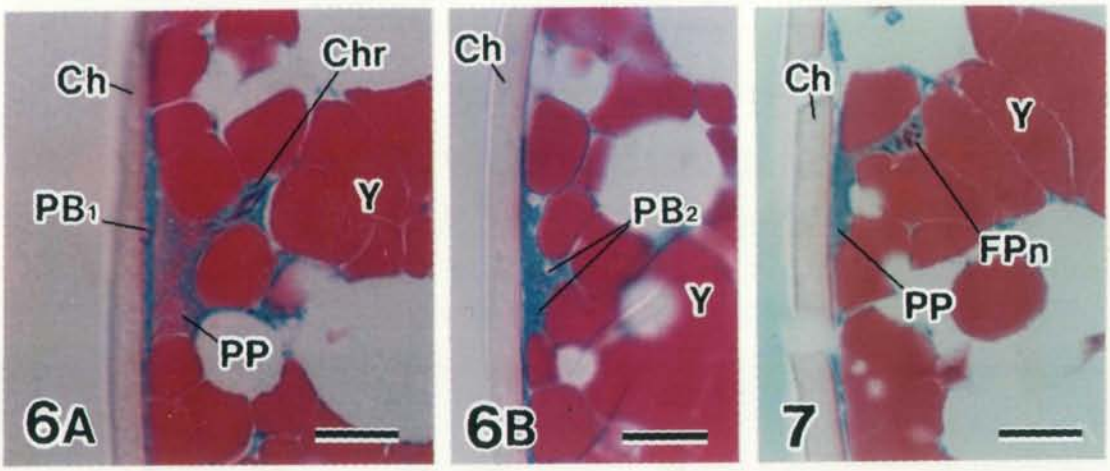
6A. A section through first polar body and egg nucleus.
6B. A section through second polar body.

Fig. 7. A longitudinal section of an egg, 1.5 hours after oviposition. The second maturation division finishes, and the female pronucleus starts its migration toward the center of the egg. Bar = 10 μm.

Ch, chorion; Chr, chromatin; FPn, female pronucleus; MPn, male pronucleus; PB_1, first polar body; PB_2, second polar body; PP, polar plasm; Y, yolk.


3
4A
PP
Y
Ch
4B
MPn
Y
Ch


5
Ch
Y
PP
Chr
4C
Y
PP
Ch
Chr
4D
Y
MPn
Ch


6A
Ch
Chr
PB1
Y
PP
6B
Ch
PB2
Y
7
Ch
Y
FPn
PP

Figs. 8-14. Early embryonic development II.

Fig. 8. A longitudinal section of an egg about 2 hours after oviposition. The male pronucleus is migrating toward the anterior pole in the periplasm, and the female pronucleus arrives at the center of the egg.

8A. Anterior region of the section. Bar = 50 μm.
8B. Enlargement, showing the male pronucleus. Bar = 10 μm.

Fig. 9. Anterior region of eggs, about 2.5 hours after oviposition. The male pronucleus arrives at the anterior egg pole. Bar = 20 μm.

9A. A living egg. The cytoplasm surrounding the male pronucleus is recognized as a depression of yolk at the anterior pole of the egg (arrow).
9B. A sectioned egg.

Fig. 10. A longitudinal section of an egg 3 hours after oviposition. The male pronucleus migrates in the yolk along the egg long axis, approaching the female pronucleus. Bar = 20 μm.

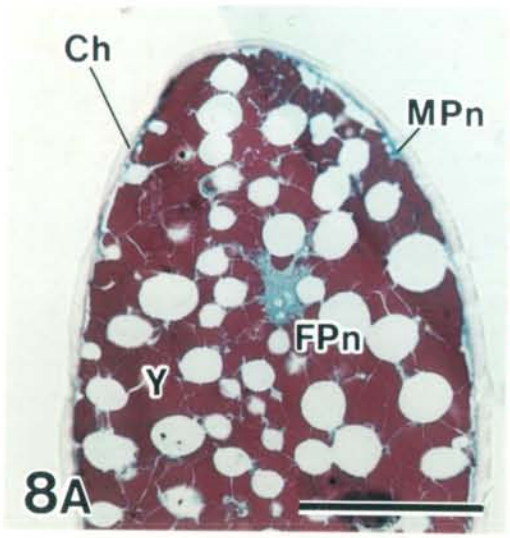
Fig. 11. A longitudinal section of an egg at fertilization, about 5.5 hours after oviposition. Bar = 20 μm.

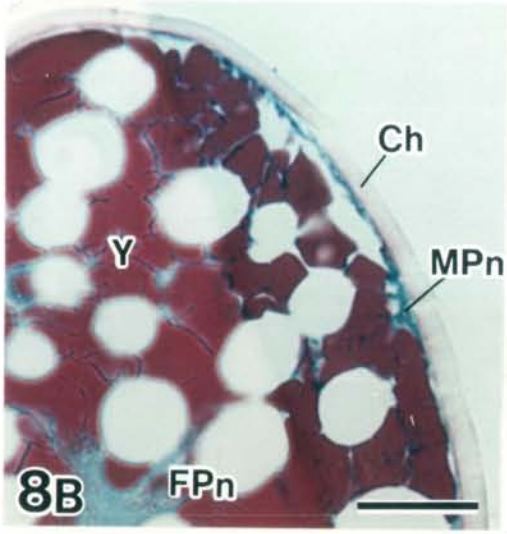
Fig. 12. A longitudinal section of an egg at first cleavage division, 7 hours after oviposition. Bar = 20 μm.

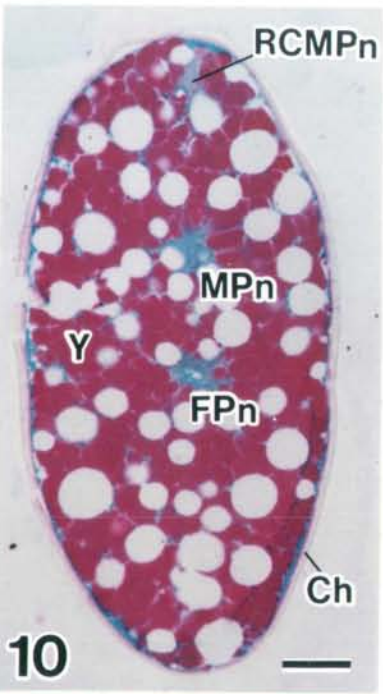
Fig. 13. A longitudinal section of an egg at the sixth cleavage stage, 2 days after oviposition. Bar = 20 μm.

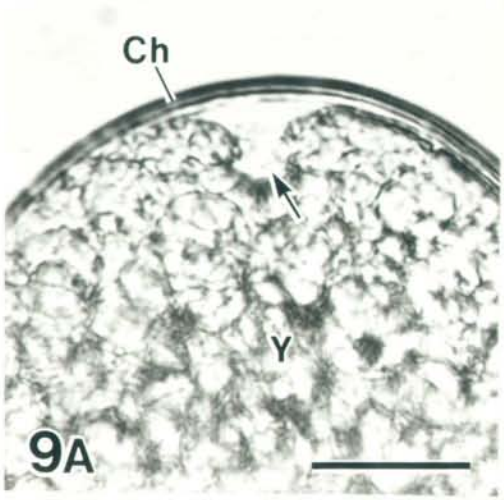
Fig. 14. A longitudinal section of an egg at the cellular blastoderm stage, about 3 days after oviposition. Bar = 20 μm.

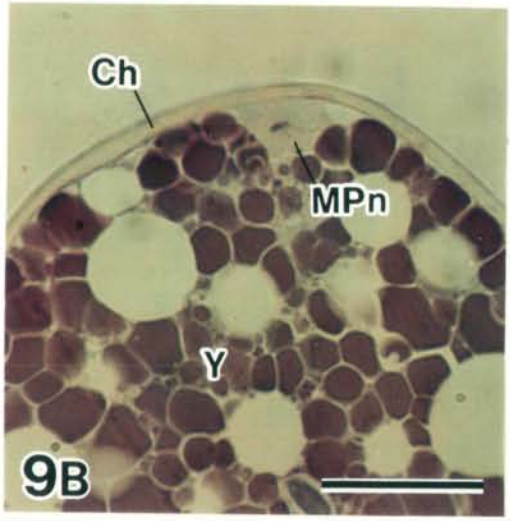
BdC, blastodermal cell; Ch, chorion; CN, cleavage nucleus; FPn, female pronucleus; MPn, male pronucleus; RCMPn, remnant of cytoplasm of male pronucleus; Sk, synkaryon; Y, yolk.

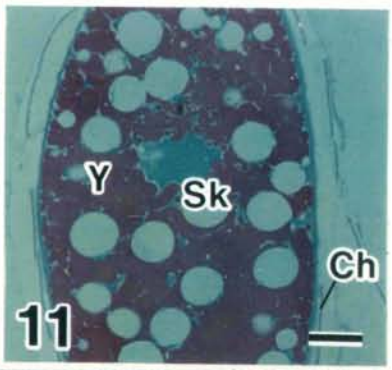

Ch
MPn
FPn
Y
8A

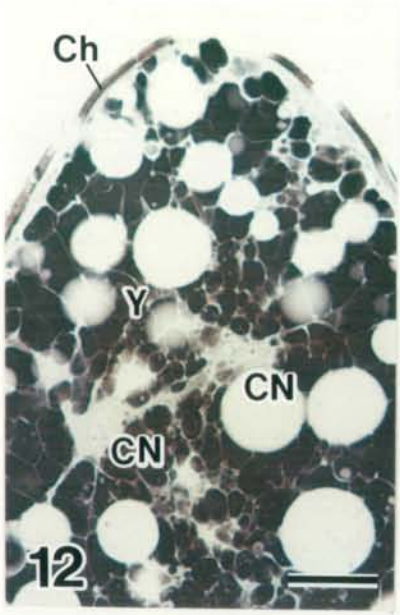

Ch
Y
MPn
FPn
8B

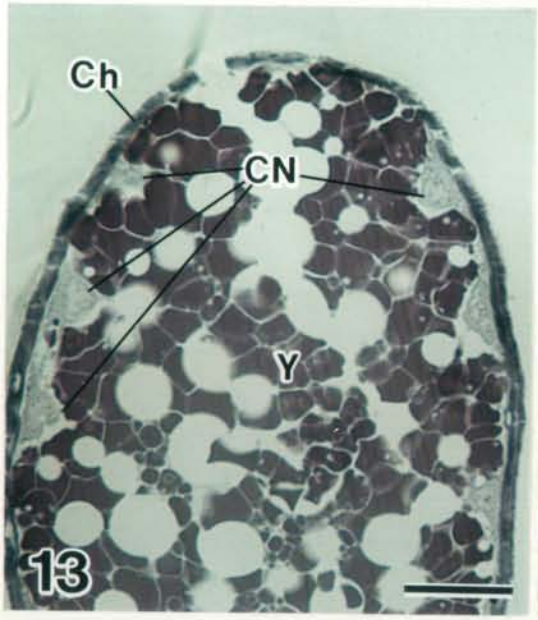

RCMPn
MPn
Y
FPn
Ch
10


Ch
Y
9A


Ch
MPn
Y
9B


Y
Sk
Ch
11


Ch
Y
CN
CN
12


Ch
CN
Y
13

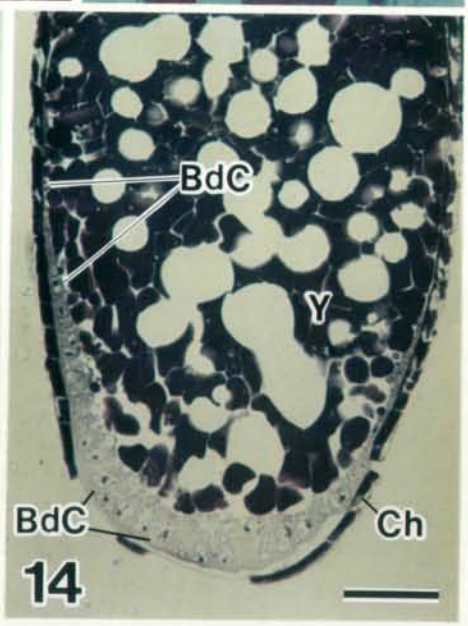

BdC
Y
BdC
Ch
14

Fig. 15. Diagrammatic representation of the developmental process from oviposition to germ disc formation (A-P). See text.

BdC, blastodermal cell; Ch, chorion; CN, cleavage nucleus; EN, egg nucleus (oocyte nucleus); FPn, female pronucleus; GD, germ disc; Mp, micropyle; MPn, male pronucleus; PB_1, first polar body; PB_2, second polar body; PP, polar plasm (cytoplasmic island); PYN, primary yolk necleus; RCFPn, remnant of cytoplasm of female pronucleus; RCMPn, remnant of cytoplasm of male pronucleus; Se, serosa; Sk, synkaryon; Y, yolk.

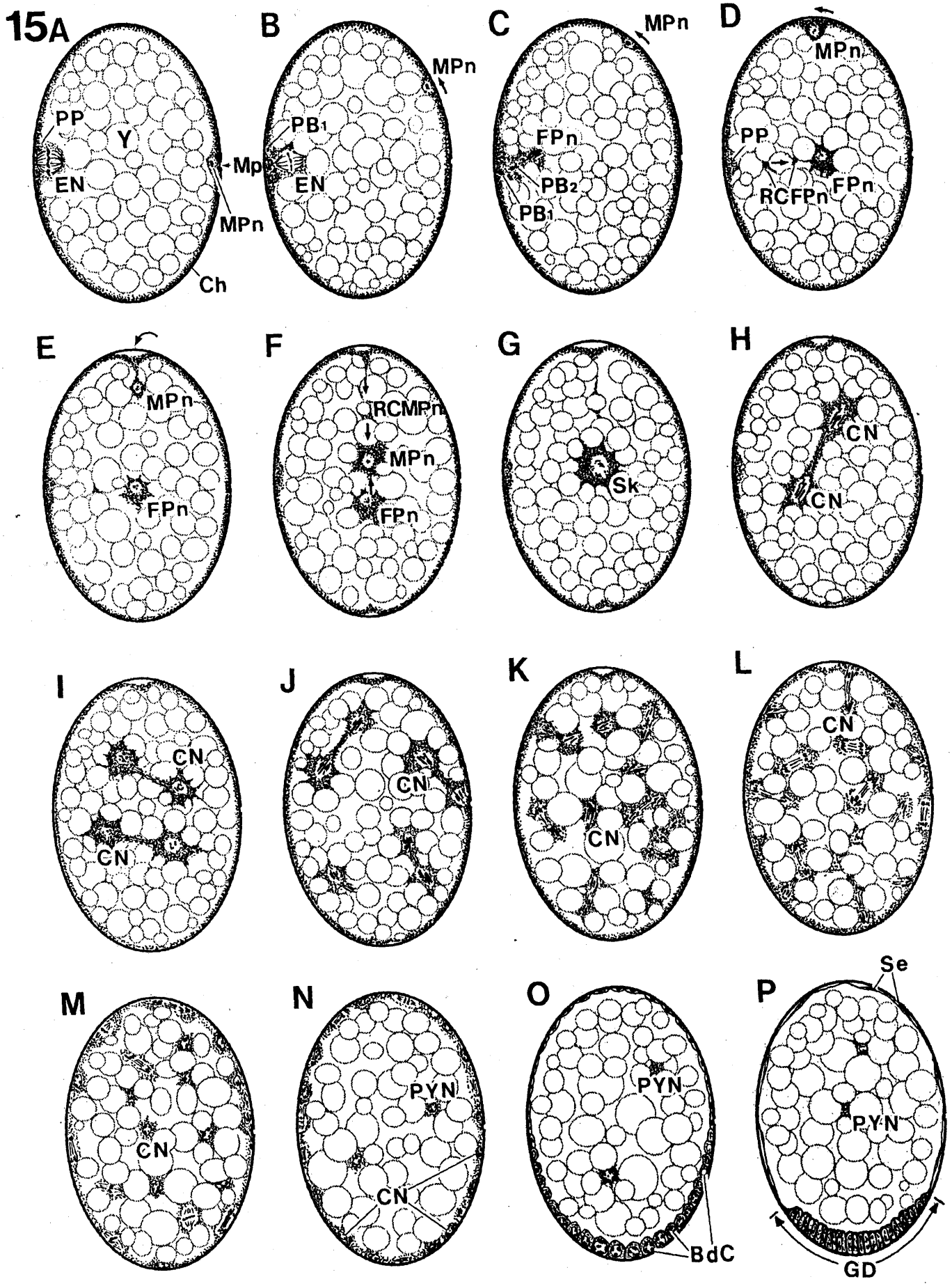
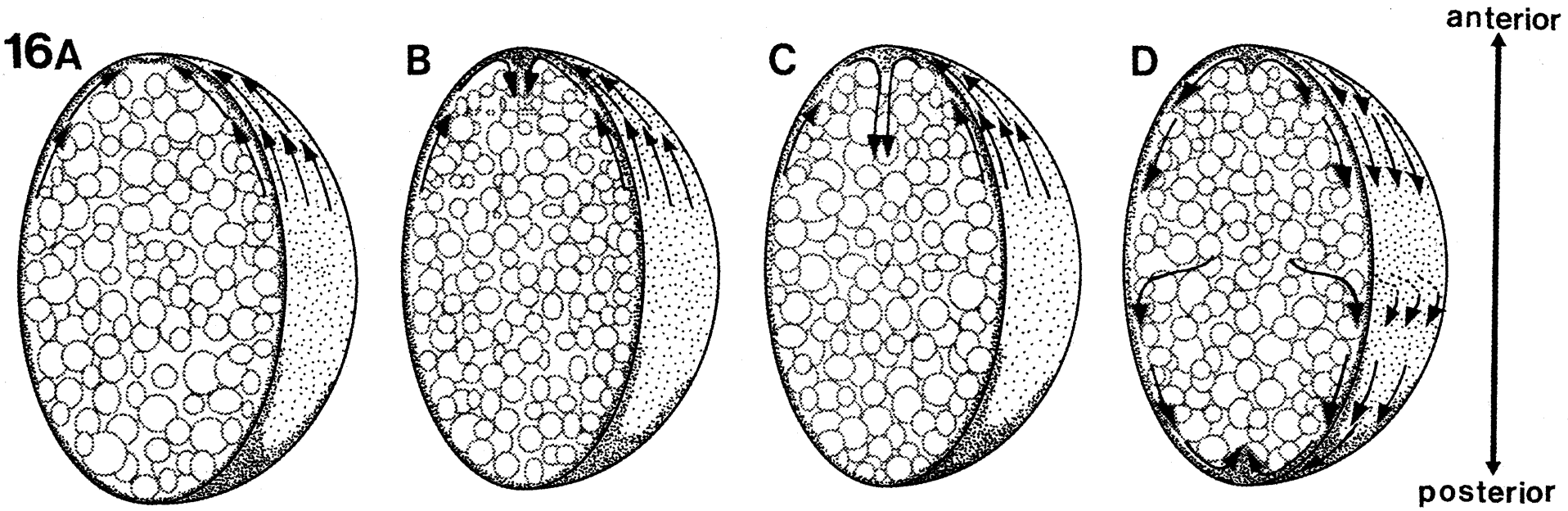

15A
PP
Y
EN
Mp
MPn
Ch
B
MPn
PB1
EN
C
MPn
FPn
PB2
PB1
D
MPn
PP
RC
FPn
FPn
E
MPn
FPn
F
RC
MPn
MPn
FPn
G
Sk
H
CN
CN
I
CN
CN
J
CN
K
CN
L
CN
M
CN
N
PYN
CN
O
PYN
BdC
P
Se
PYN
GD

Fig. 16. Diagrammatic representation of the yolk stream of the egg in association with the fertilization (A-D). Arrows represent the yolk stream. See text.


16A
B
C
D
anterior
posterior

Figs. 17, 18. Activated unfertilized eggs, which were dissected out of subimagos, 2-3 hours after activation.

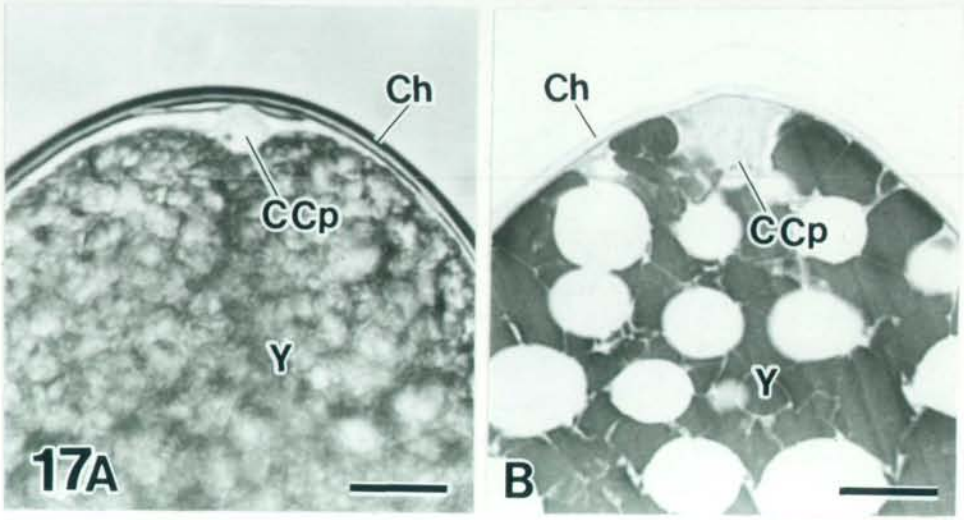
Fig. 17. Anterior region of egg. Bar = 20 μm.

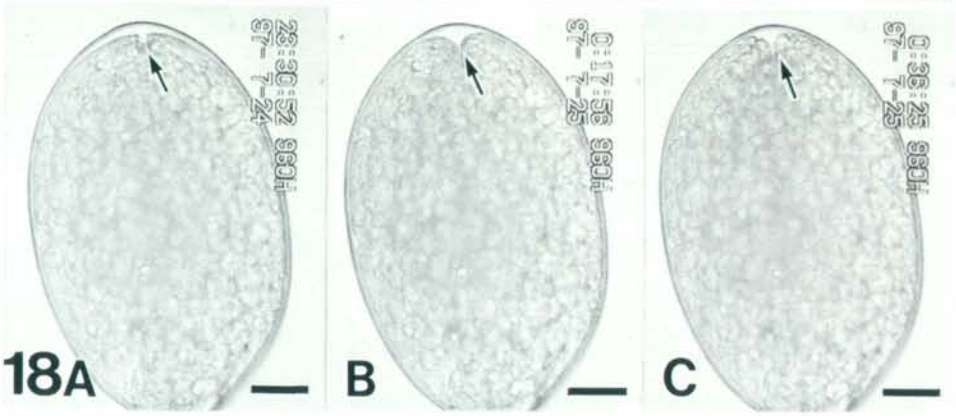
17A. A living egg. A depression of yolk is observed to be formed at the pole.

17B. A sectioned egg. Condensed cytoplasm devoid of male pronucleus can be seen at the anterior pole of the egg.

Fig. 18. Time-lapse VTR observation of an activated unfertilized egg (A-C). The egg was activated about 135 min before A (21:15, July 24, 1997). A depression of yolk appears at the anterior pole of the egg (A), deepens (B), and finally attains its maximum depth (C). Arrows show the depression of yolk or condensed cytoplasm. Bar = 20 μm.

CCp, condenced cytoplasm; Ch, chorion; Y, yolk.


Ch
CCp
Y
17A
Ch
CCp
Y
B


18A
B
C

Figs. 19-22. External features of embryos I. Bar = 50 μm.

Fig. 19. Stage 2 (blastoderm formation). A sectional diagram. In this stage, the posteior half embryonic and anterior half etraembryonic areas are distinguishable.

Fig. 20. Stage 3 (Formation of germ disc).

20A. A sectional diagram.

20B. Lozenge-shaped germ disc.

Fig. 21. Stage 4 (Pear-shaped embryo).

21A. Anterolateral view.

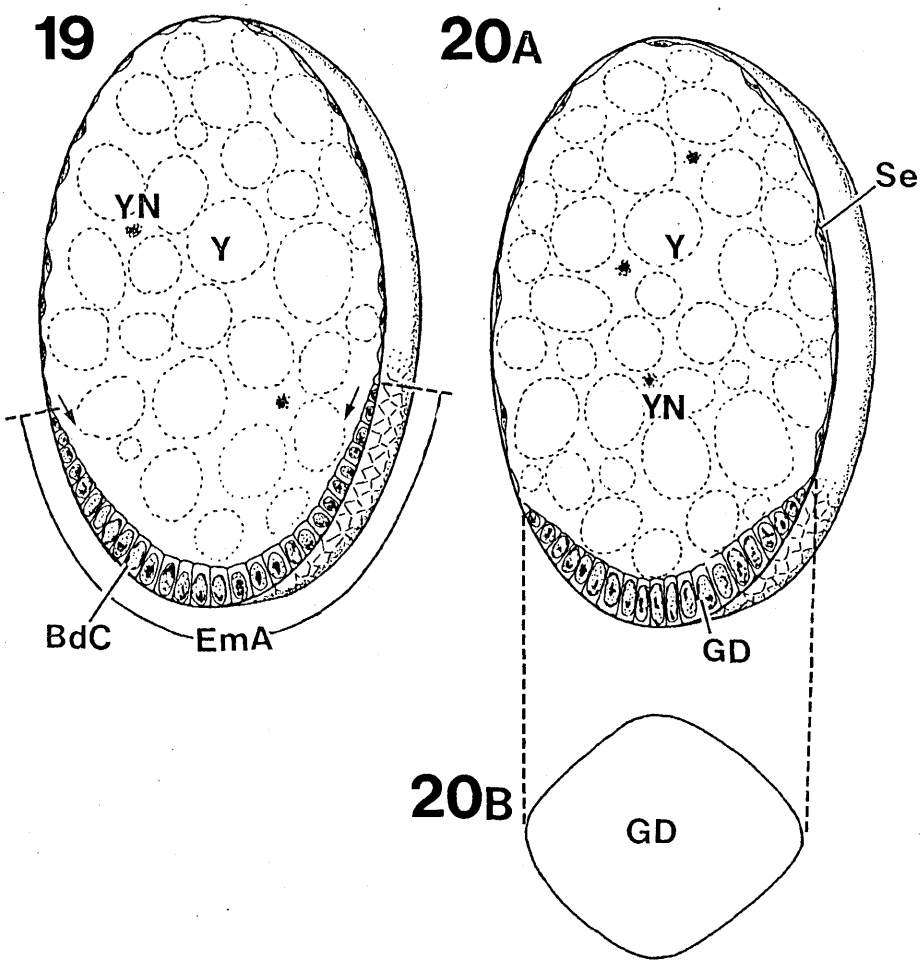
21B. Ventral view.

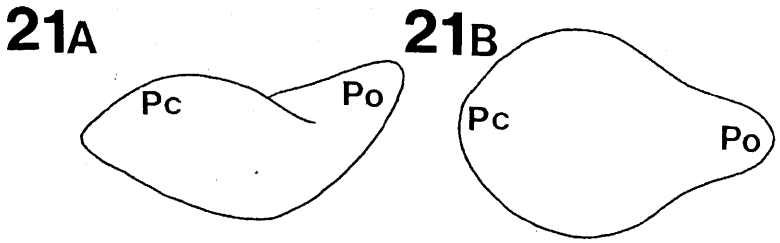
Fig. 22. Stage 5 (Start of invagination of germ band, Anatrepsis I).

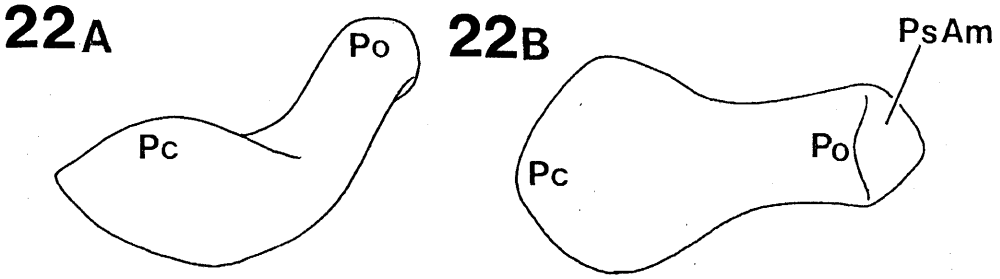
22A. Anterolateral view.

22B. Ventral view.

BdC, blastodermal cell; EmA, embryonic area; GD, germ disc; Pc, protocephalon; Po, protocorm; PsAm, presumptive amnion; Se, serosa; Y, yolk; YN, yolk necleus.


19
YN
Y
BdC
EmA
20A
Se
Y
YN
GD
20B
GD


21A
Pc
Po
21B
Pc
Po


22A
Po
Pc
22B
PsAm
Po
Pc

Figs. 23, 24. External features of embryos II. Bar = 50 μm.

Fig. 23. Stage 6 (S-shaped embryo: completion of invagination, anatrepsis II)

23A. Ventral view.

23B. Ventrolateral view.

23C. Lateral view.

Fig. 24. Stage 7 (Longest embryo I).

24A, B. Early stage 7.

24A. Ventrolateral view.

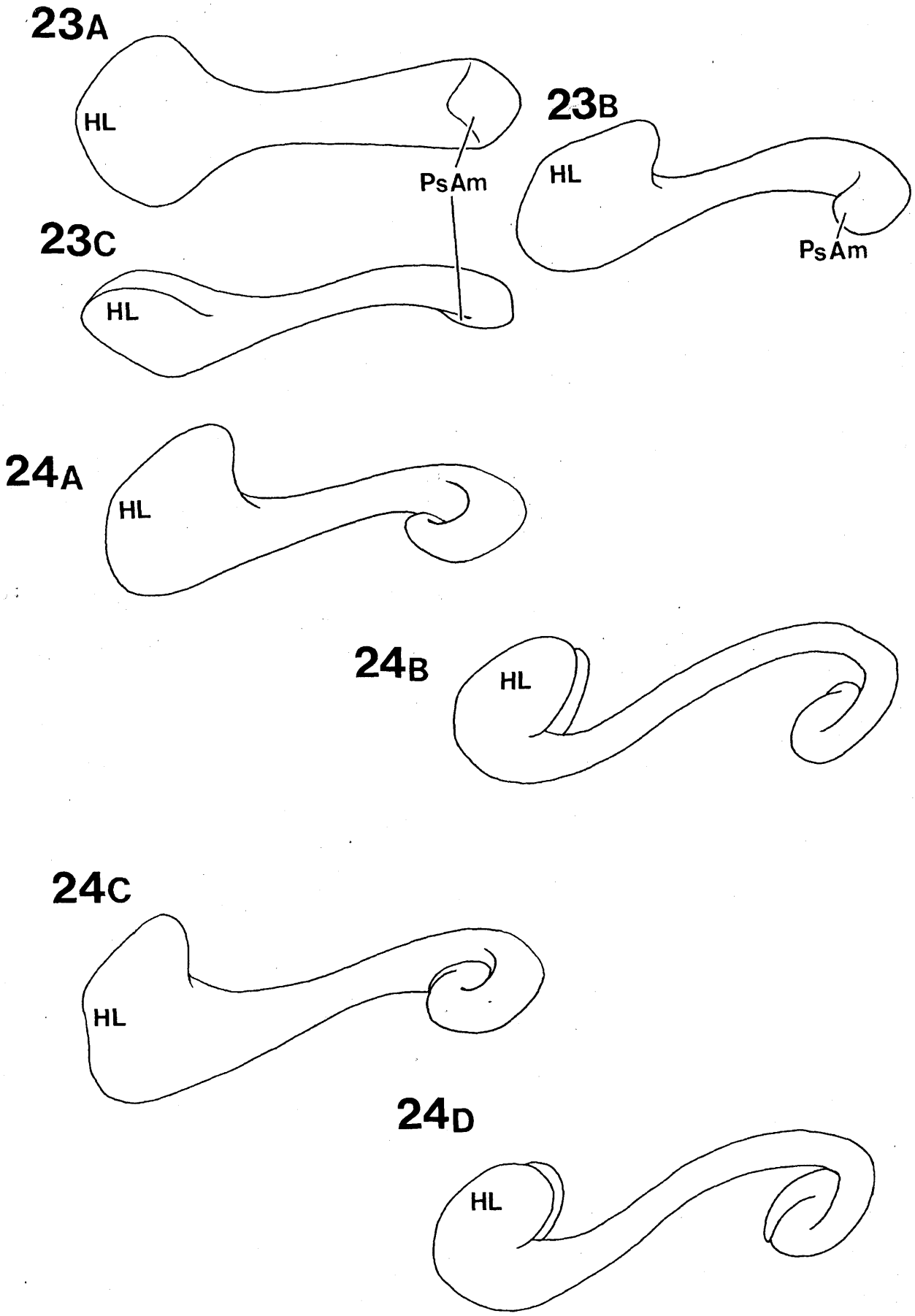
24B. Lateral view.

24C, D. Middle stage 7.

24C. Ventrolateral view.

24D. Lateral view.

HL, head lobe; PsAm, presunptive amnion.


23A
HL
PsAm
23B
HL
PsAm
23C
HL
24A
HL
24B
HL
24C
HL
24D
HL

Figs. 24, 25. External features of embryos III. Bar = 50 μm.

Fig. 24. Stage 7 (Longest embryo II).

24E, F. Late stage 7.

24E. Ventrolateral view.

24F. Lateral view.

Fig. 25. Stage 8 (Segmentation of embryo).

25A-C. Early stage 8.

25A. Venrolateral view.

25B. Ventral view.

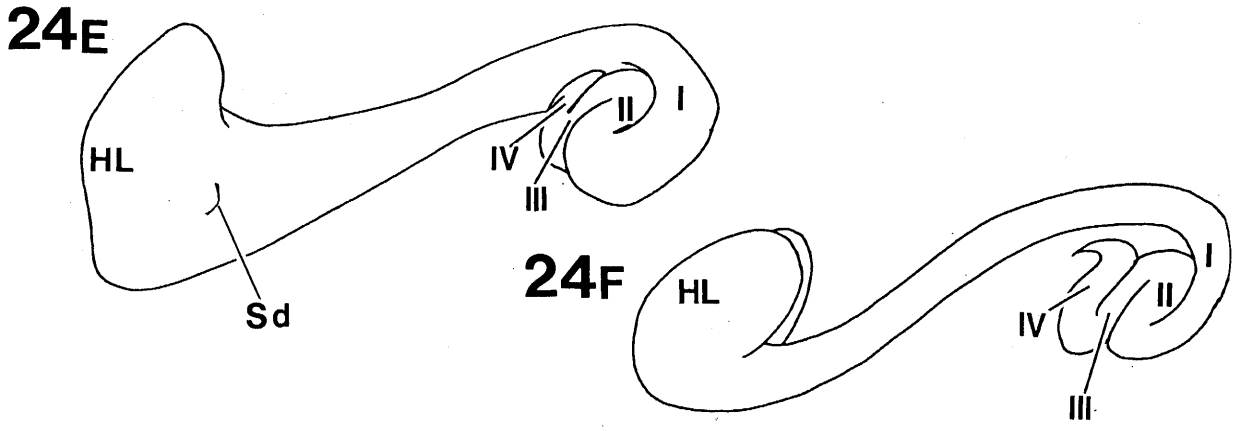
25C. Lateral view.

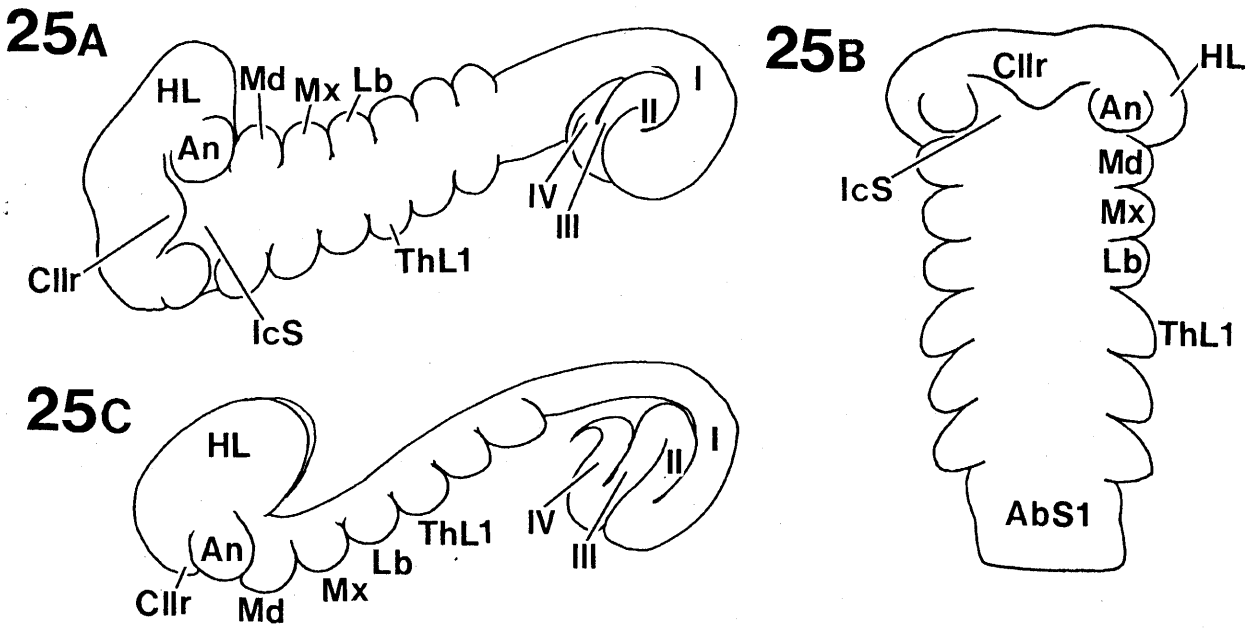
25D, E. Late stage 8.

25D. Lateral view.

25E. Ventral view.

I-IV, abdominal regions I to IV; AbS1, 6, 11, 1st, 6th and 11th abdominal segments; An, antenna; Cllr, clypeolabrum; HL, head lobe; IcS, intercalary segment; Lb, labium; Md, mandible; Mx, maxilla; Sd, stomodaeum; ThL1, proleg.


24E
HL
Sd
IV
III
II
I
24F
HL
IV
III
II
I


25A
HL
Md
Mx
Lb
An
CIIr
IcS
ThL1
IV
III
II
I
25B
CIIr
HL
An
IcS
Md
Mx
Lb
ThL1
AbS1
25C
HL
An
CIIr
Md
Mx
Lb
ThL1
IV
III
II
I

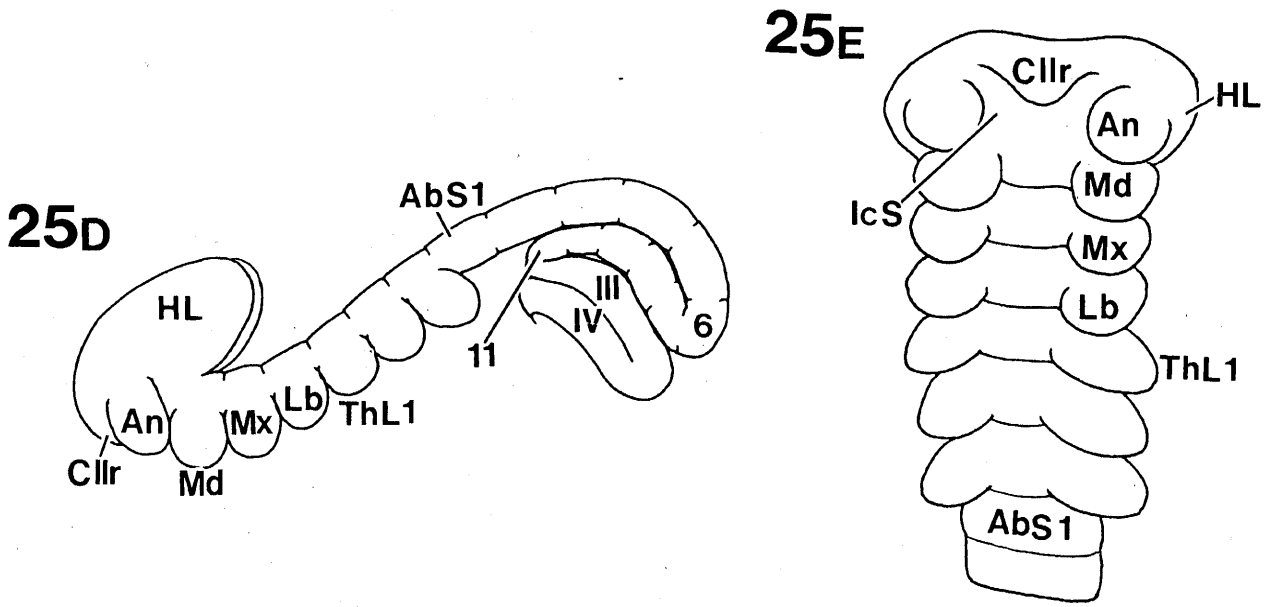

25D
AbS1
HL
An
CIIr
Md
Mx
Lb
ThL1
11
III
IV
6
25E
CIIr
HL
An
IcS
Md
Mx
Lb
ThL1
AbS1

Fig. 26. External features of embryos IV. Bar = 50 μm.

Fig. 26. Stage 9 (Start of appendicular annulation).

26A, B. Early stage 9.

26A. Ventral view of head to 2nd abdominal segment.

26B. Lateral view of head to 2nd abdominal segment.

26C-E. Middle stage 9.

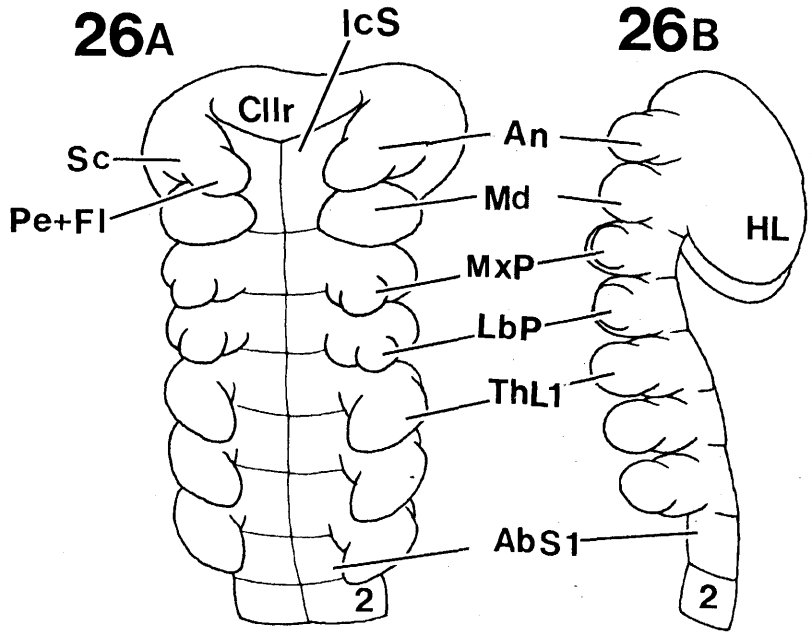
26C. Ventral view of head to 2nd abdominal segment.

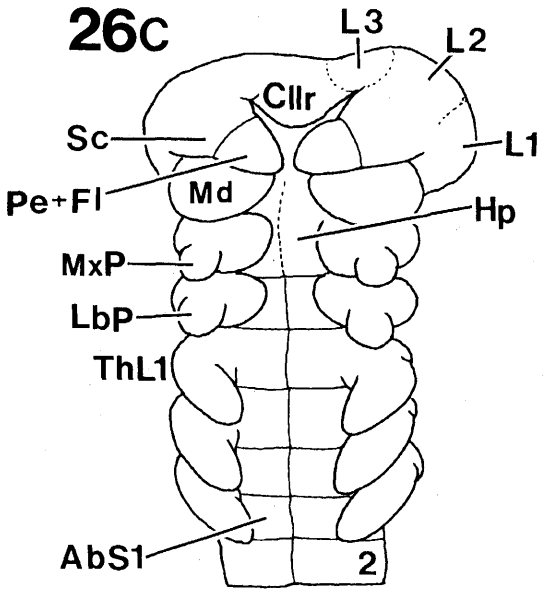
26D. Ventrolateral view of abdomen.

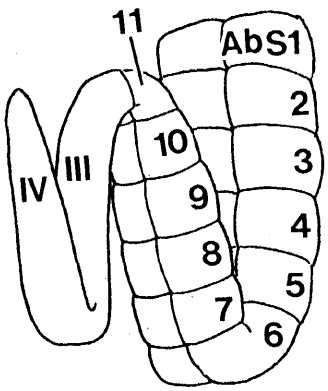
26E. Ventrolateral view of abdomen.

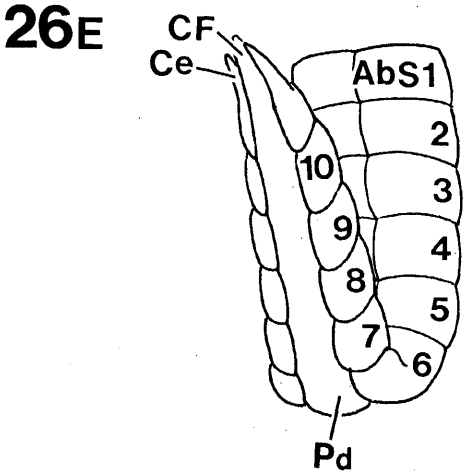
26F. Late stage 9. Ventrolateral view of abdomen.

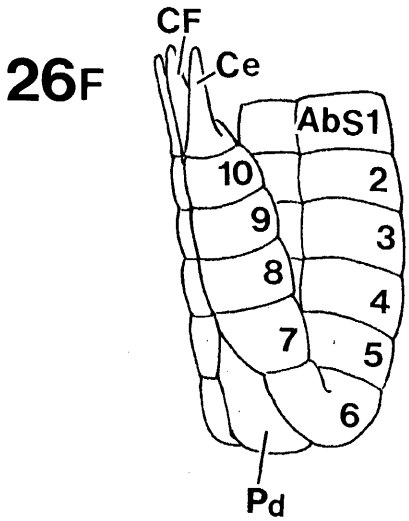
I-IV, abdominal regions I to IV; AbS1-11, 1st to 11th and 11th abdominal segments; An, antenna; Ce, cercus; CF, caudal filament; Cllr, clypeolabrum; Fl, flagellum; HL, head lobe; Hp, hypopharynx; IcS, intercalary segment; L1-3, protocerebral lobi 1 to 3; LbP, labial palp; Md, mandible; MxP, maxillary palp; Pd, proctodaeum; Pe, pedicellus; Sc, scapus; ThL1, proleg.


26A
IcS
CIIr
Sc
Pe+Fl
26B
An
Md
HL
MxP
LbP
ThL1
AbS1
2
2


26C
L3
L2
CIIr
Sc
L1
Pe+Fl
Md
Hp
MxP
LbP
ThL1
AbS1
2


26D
11
AbS1
2
3
4
5
6
7
8
9
10
III
IV


26E
CF
Ce
AbS1
2
3
4
5
6
7
8
9
10
Pd


26F
CF
Ce
AbS1
2
3
4
5
6
7
8
9
10
Pd

Figs. 27, 28. External features of embryos V. Bar = 50 µm.

Fig. 27. Stage 10 (Revolution, katatrepsis).

27A, B. Early stage 10.

27A. Lateral view.

27B. Ventral view of head.

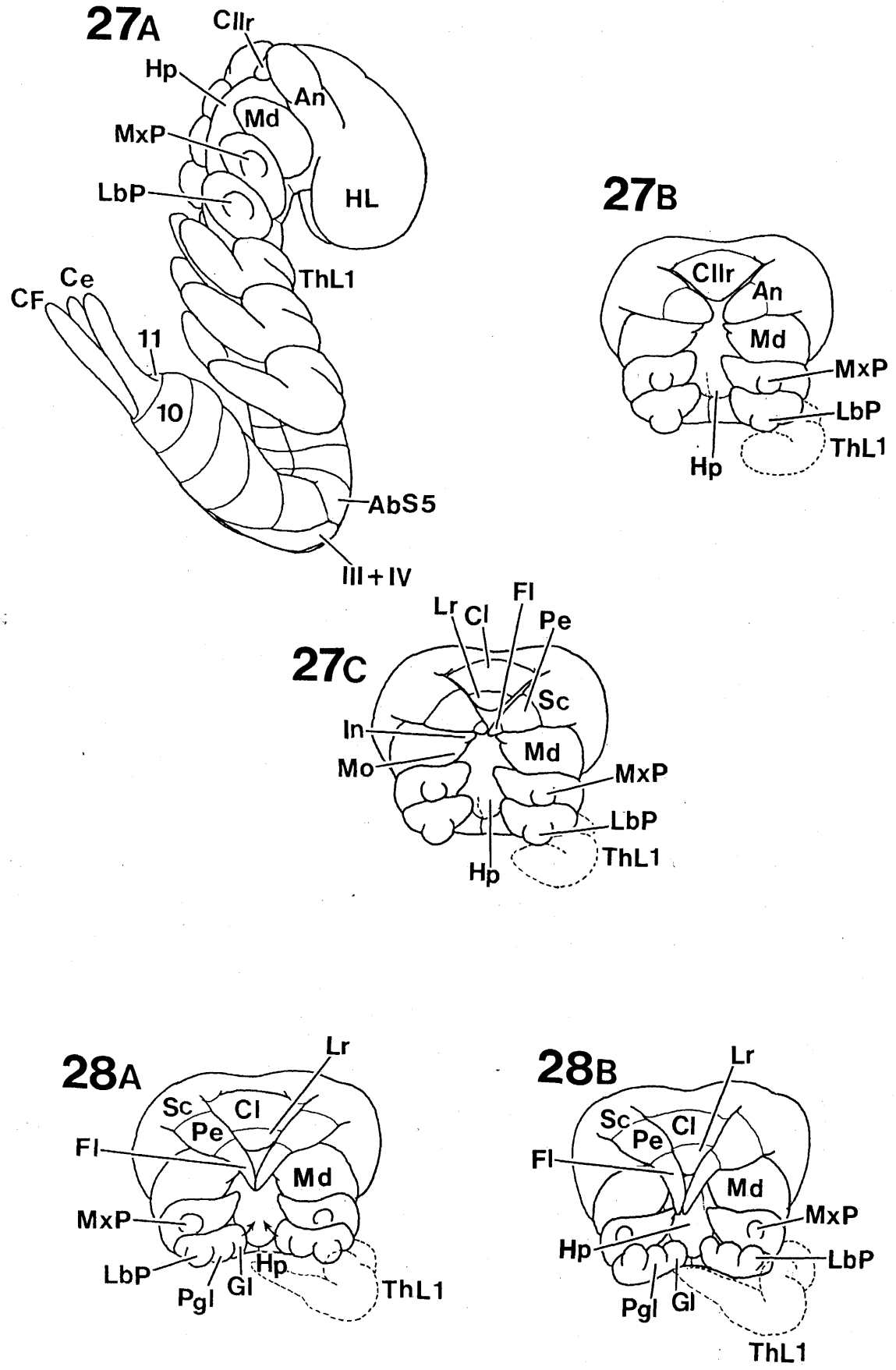
27C. Late stage 10. Ventral view of head.

Fig. 28. Stage 11 (Post-revolution I).

28A. Early stage 11. Ventral view of head.

28B. Late stage 11. Ventral view of head.

III, IV, abdominal regions III and IV; AbS5, 10, 11, 5th, 10th and 11th abdominal segments; An, antenna; Ce, cercus; CF, caudal filament; Cl, clypeus; Cllr, clypeolabrum; Gl, glossa; Fl, flagellum; HL, head lobe; Hp, hypopharynx; In, incisor; LbP, labial palp; Lr, labrum; Md, mandible; Mo, molar; MxP, maxillary palp; Pe, pedicellus; Pgl, paraglossa; Sc, scapus; ThL1, proleg.


27A
CIlr
Hp
An
Md
MxP
LbP
HL
ThL1
Ce
CF
11
10
AbS5
III+IV
27B
CIlr
An
Md
MxP
LbP
ThL1
Hp
27C
Lr
Cl
Fl
Pe
Sc
In
Md
Mo
MxP
LbP
ThL1
Hp
28A
Lr
Sc
Cl
Pe
Fl
Md
MxP
LbP
Hp
Pgl
Gl
ThL1
28B
Lr
Sc
Cl
Pe
Fl
Md
MxP
Hp
LbP
Pgl
Gl
ThL1

Figs. 29-31. External features of embryos and first instar nymph.

Bar = 10 μm.

Fig. 29. Stage 12 (Post-revolution II).

29A. Early stage 12. Ventral view of head.

29B. Early stage 12. Ventral view of hypopharynx.

29C. Middle stage 12. Lateral view of hed and thorax.

29D. Late stage 12. Vetrolateral view of head.

Fig. 30. Stage 13 (Post-revolution III)

30A. Lateral view of head and prothorax.

30B. Ventrolateral view of head.

Fig. 31. First instar nymph. Dorsal view.

AbS1, 5, 10, 1st, 5th and 10th abdominal segments; An, antenna; CE, compound eye; Ce, cercus; CF, caudal filament; Cl, clypeus; Co, coxa; ET, egg tooth; Gl, glossa; Fe, femur; Fl, flagellum; LbP, labial palp; Li, lingua; Lr, labrum; Md, mandible; MxP, maxillary palp; Oc, ocellus; Pe, pedicellus; Pgl, paraglossa; Prm, prementum; Psm, postmentum; Pta, pretarsus; Sc, scapus; Sco, subcoxa; Sli, superlingua; Ta, tarsus; ThL1, proleg; Ti, tibia; Tr, trochanter.

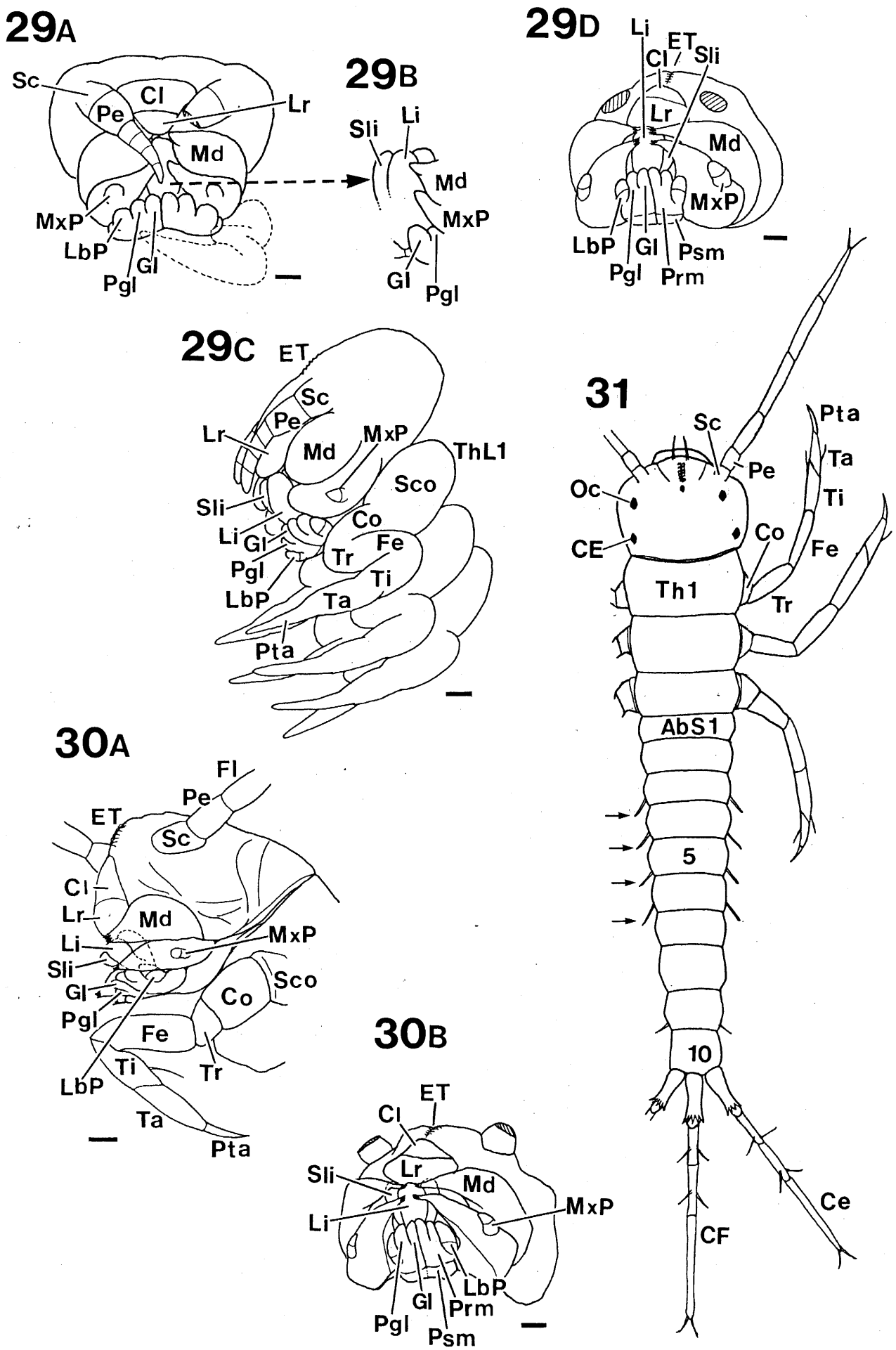

29A
Sc
Cl
Pe
Lr
Md
MxP
LbP
Gl
Pgl
29B
Sli
Li
Md
MxP
Gl
Pgl
29D
Li
ET
Cl
Sli
Lr
Md
MxP
LbP
Gl
Psm
Pgl
Prm
29C
ET
Sc
Pe
Lr
Md
MxP
ThL1
Sco
Sli
Co
Li
Gl
Tr
Fe
Pgl
Ti
LbP
Ta
Pta
31
Sc
Pta
Ta
Pe
Ti
Oc
Co
Fe
CE
Th1
Tr
AbS1
5
10
CF
Ce
30A
Fl
Pe
ET
Sc
Cl
Lr
Md
MxP
Li
Sli
Sco
Gl
Co
Pgl
Fe
Ti
Tr
LbP
Ta
Pta
30B
ET
Cl
Sli
Lr
Md
MxP
Li
LbP
Gl
Prm
Pgl
Psm

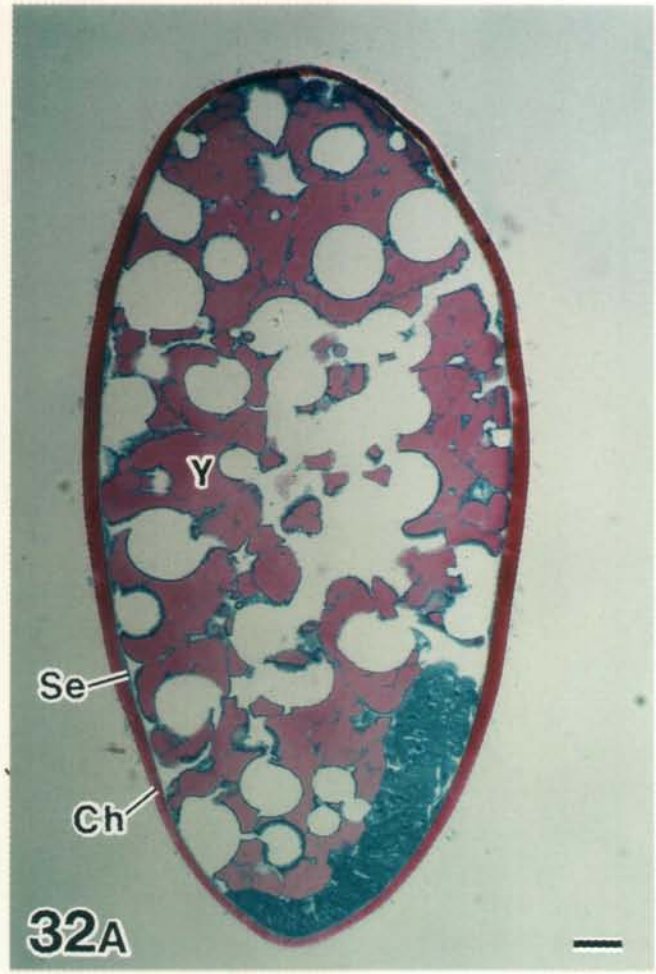
Fig. 32. Invagination of embryo and amnioserosal fold formation.

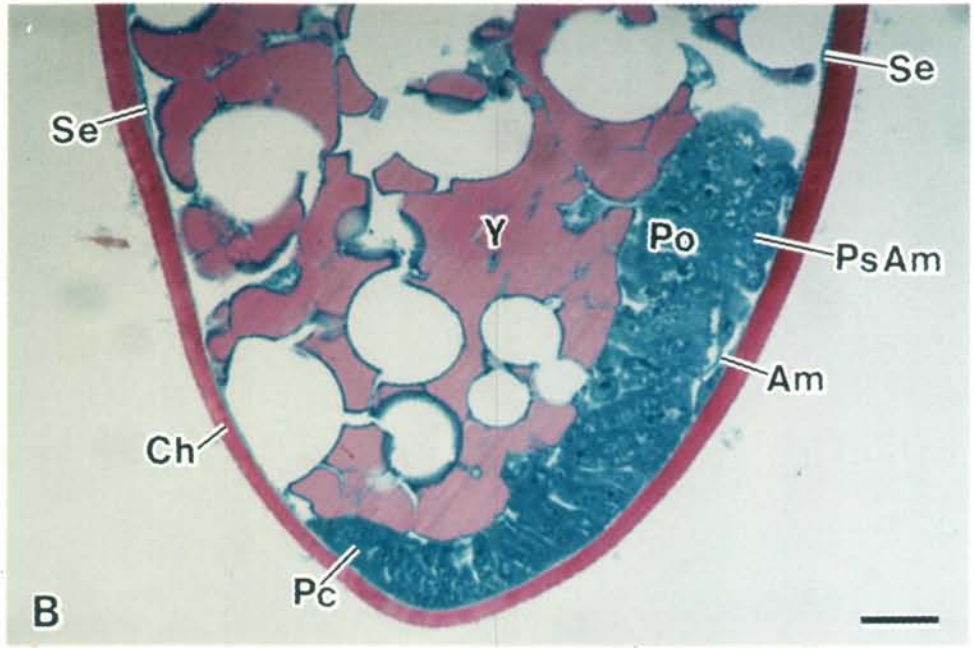
Bar = 20 μm.

32A. A sagittal section of an egg at stage 5.

32B, C. Enlargement.

Am, amnion; Ch, chorion; Pc, protcephalon; Po, protocorm; PsAm, presumptive amnion; Se, serosa; Y, yolk.


Y
Se
Ch
32A


Se
Se
Y
Po
PsAm
Am
Ch
Pc
B

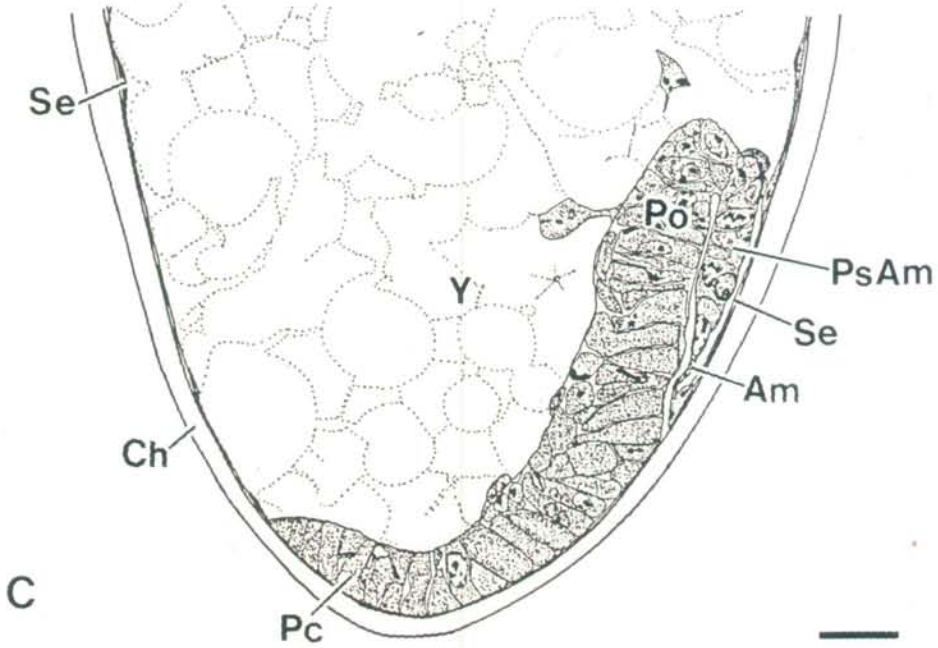

Se
Po
PsAm
Y
Se
Am
Ch
Pc
C

Fig. 33. Amnioserosal fold formation.

33A. A sagittal section of an egg, at late stage 6, in which anatrepsis is just completed. Bar = 50 μm.

33B. Enlargement. The amnioserosal fold fuse with each of those beneath the embryo at the level of labrum (arrow), and anatrepsis is completed. Bar = 10 μm.

Am, amnion; AmC, amniotic cavity; AmSeF, amnioserosal fold; An, antenna; Ch, chorion; Em, embryo; Md, mandible; Me, mesoderm; Se, serosa.

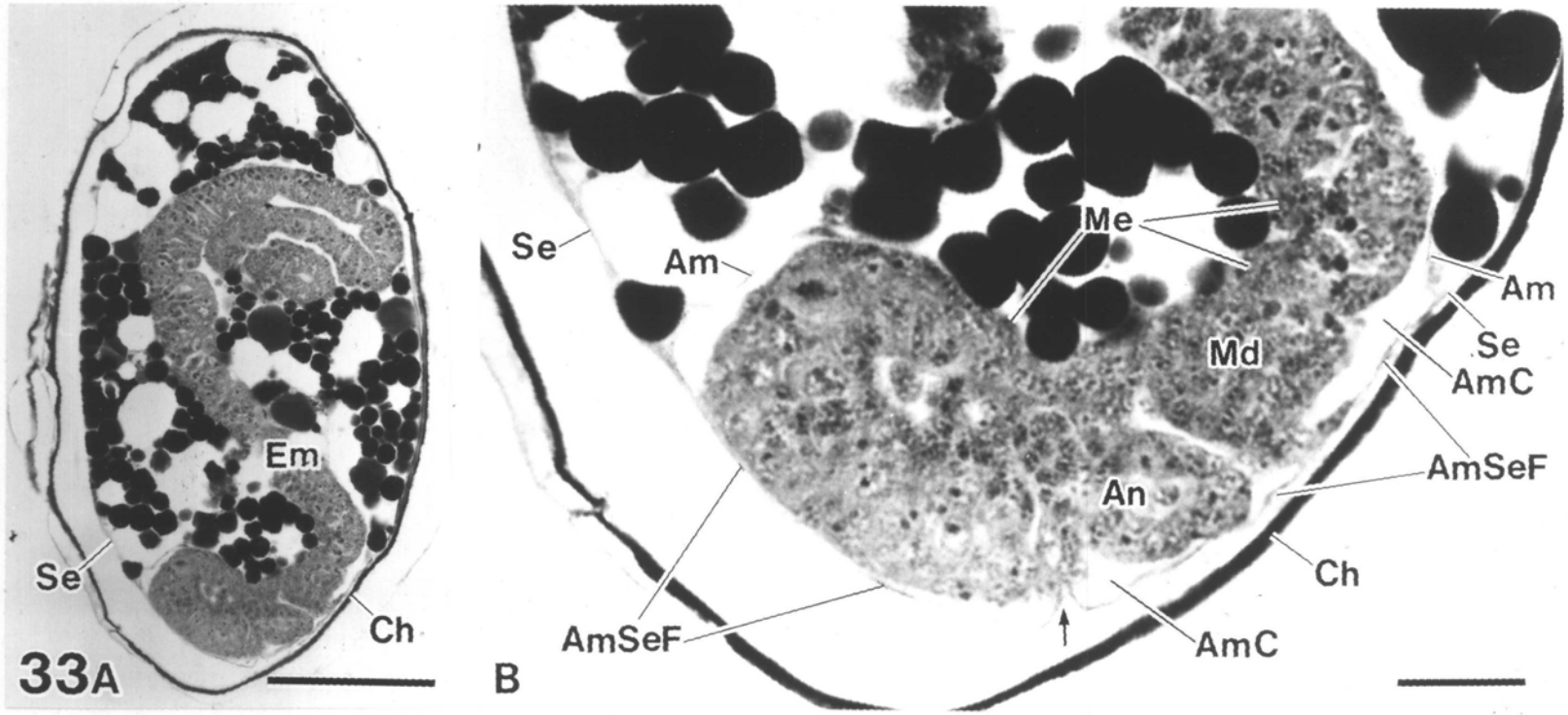

Em
Se
Ch
33A
B
Se
Am
Me
Md
Am
Se
AmC
AmSeF
An
Ch
AmSeF
AmC

Fig. 34. Successive stages of annulation of cephalic and thoracic appendages (A-D).

34A. Stage 9. Telopodites are dotted.

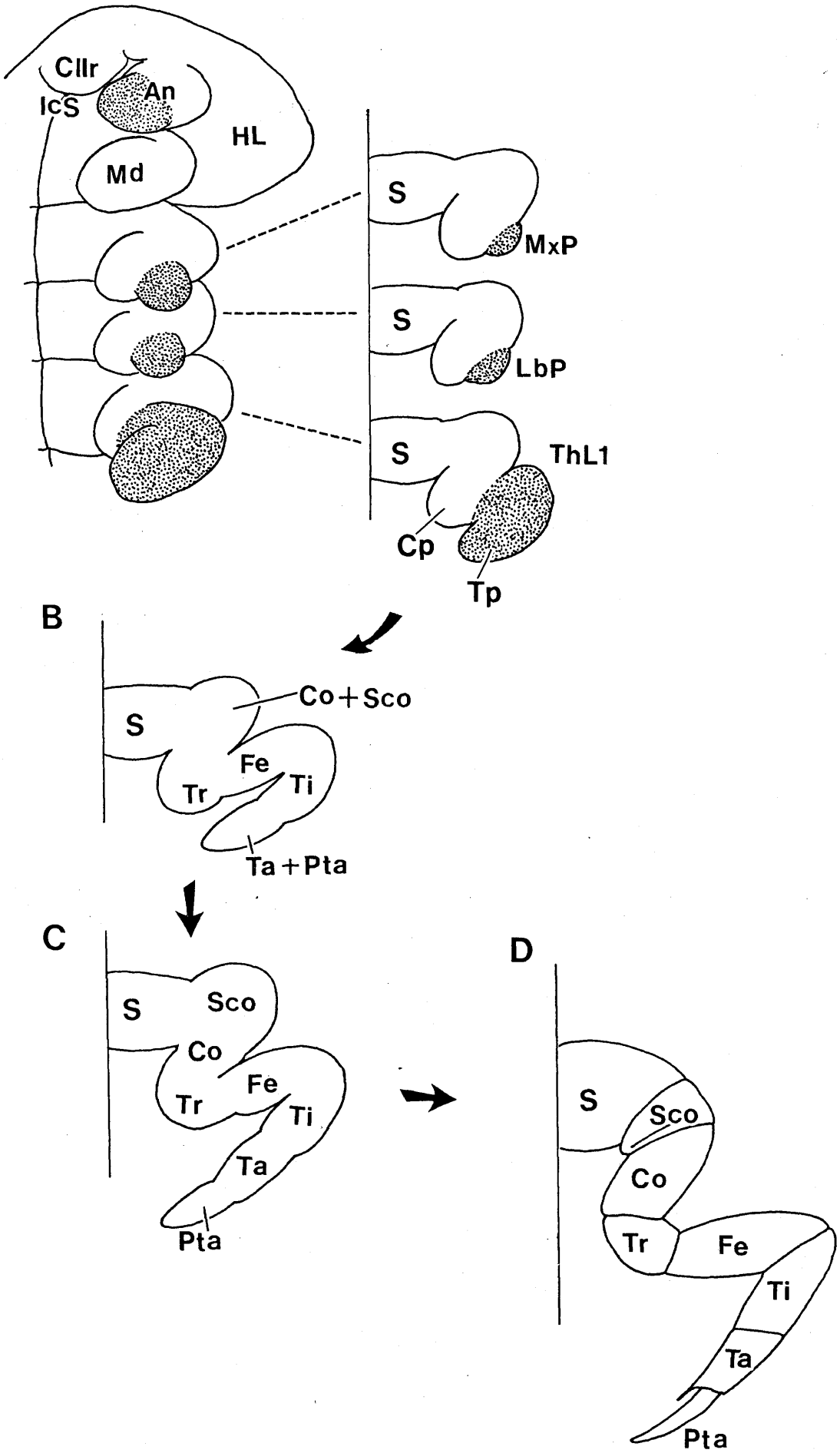
34B. Stages 10 and 11.

34C. Late stage 12 to early stage 13.

34D. Late stage 13.

An, antenna; Cllr, clypelabrum; Co, coxa; Cp, coxopodite; Fe, femur; HL, head lobe; IcS, intercalary segment; LbP, labial palp; Md, mandible; MxP, maxillary palp; Pta, pretarsus; S, sternum; Sco, subcoxa; Ta, tarsus; ThL1, proleg; Ti, tibia; Tp, telopodite; Tr, trochanter.

34A


CIIr
An
IcS
HL
Md
S
MxP
S
LbP
S
ThL1
Cp
Tp
B
Co+Sco
S
Fe
Tr
Ti
Ta+Pta
C
S
Sco
Co
Fe
Tr
Ti
Ta
Pta
D
S
Sco
Co
Tr
Fe
Ti
Ta
Pta

Figs. 35-37. Embryos of stages 7.

35A. A sagittal section of embryo. In this stage, the stomodaeum invagination starts, and the abdomen is folded and divided into four regions: regions I to IV from the anterior to posterior. Bar = 20 μm.

35B. A cross section of embryo through abdomen. Regions I and II and regions III and IV are respectively connected to each other by amnion. Bar = 20 μm.

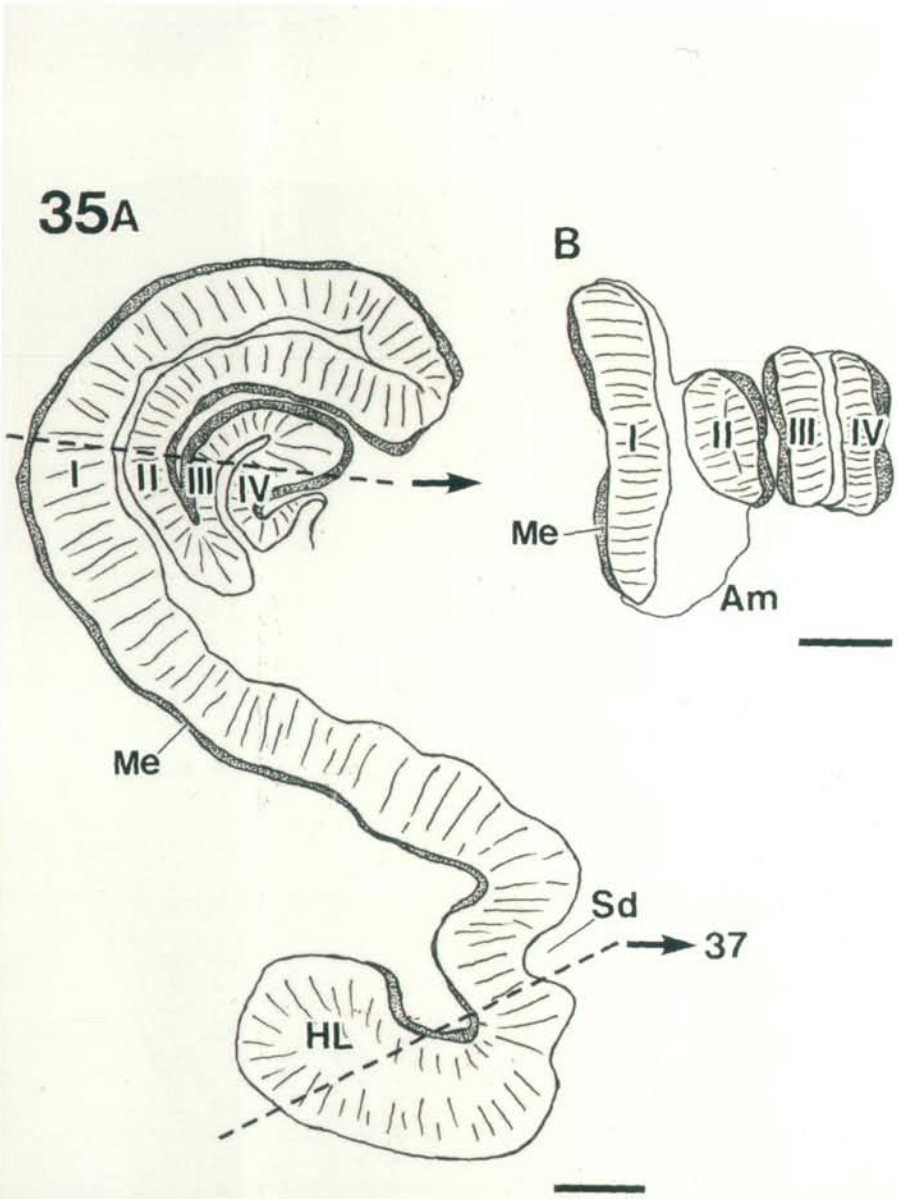
36A. A sagittal section of egg through embryo. Bar = 20 μm.

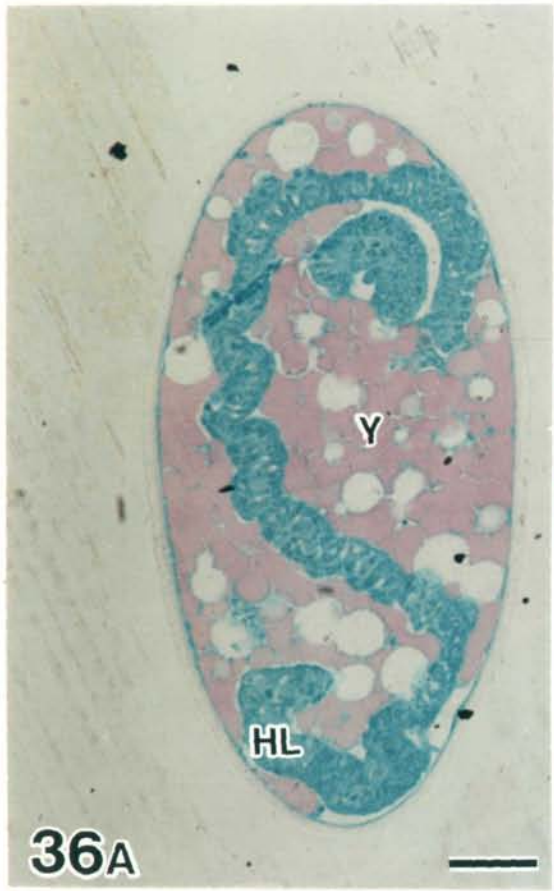
36B. Enlargement. Bar = 20 μm.

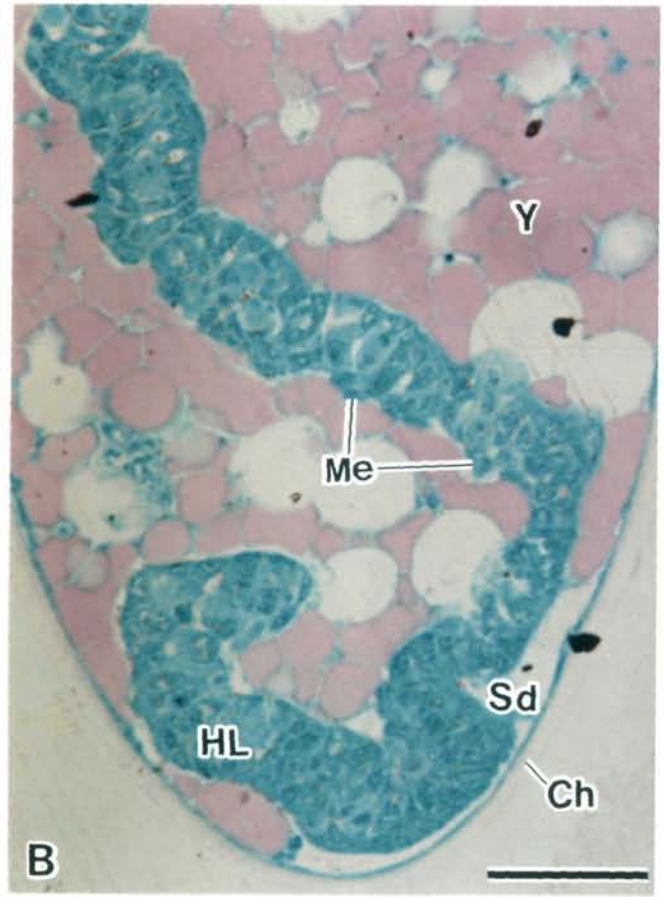
Fig. 37. A cross section of head through the plane shown with "37" in Fig. 35A.

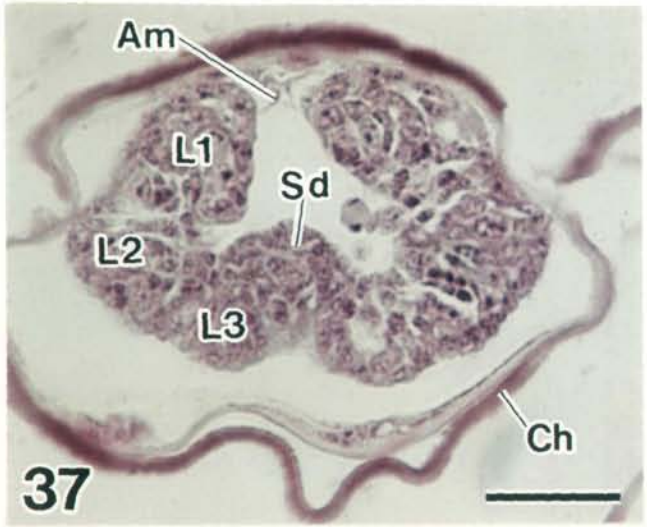
Fig. 38. A cross section through thorax, at the early stage 8.

I-IV, abdominal regions I to IV; Am, amnion; Ch, chorion; HL, head lobe; L1-3, lobi 1 to 3; Me, mesoderm; Nb, neuroblast; Sd, stomodaeum; SoMe, somatic mesoderm; SpMe, splanchnic mesoderm; Y, yolk.


35A
B
I
II
III
IV
Me
Am
Sd
37
HL


Y
HL
36A


Y
Me
HL
Sd
Ch
B


Am
L1
Sd
L2
L3
Ch
37

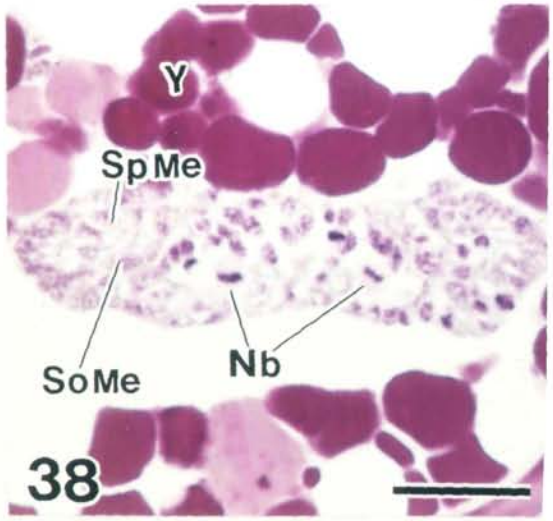

Y
Sp Me
So Me
Nb
38

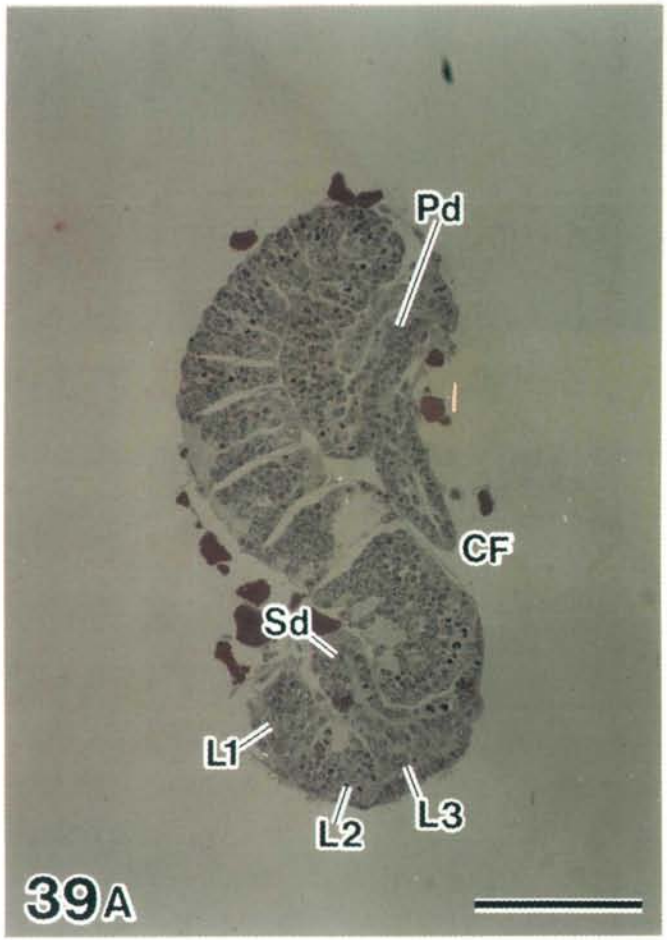
Fig. 39. Just before revolution (stage 9).

39A. A sagittal section of embryo. Bar = 50 μm.

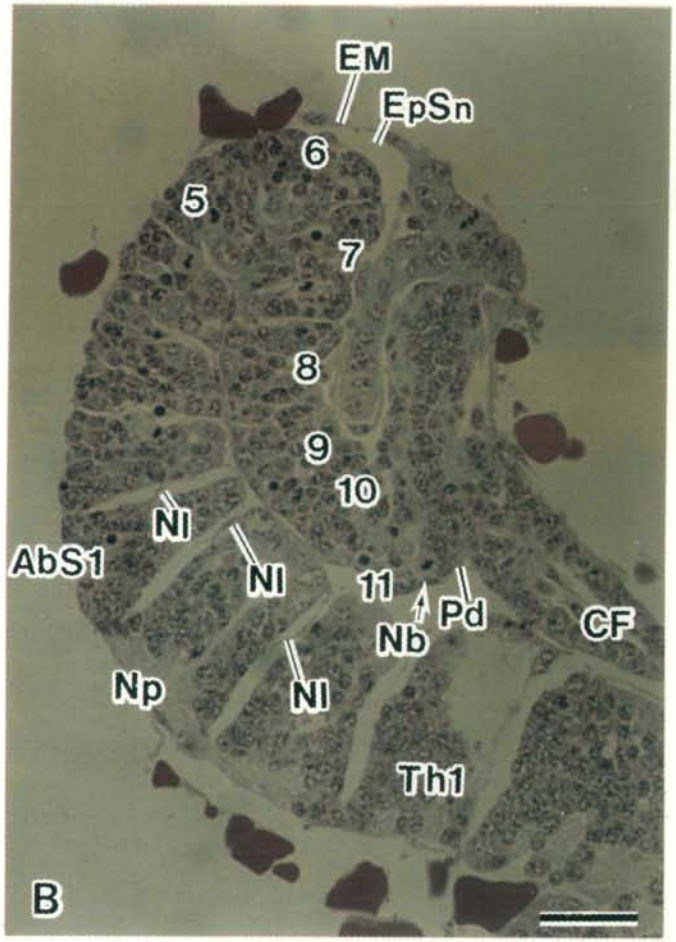
39B. Enlargement. Eleven abdominal segments and proctodaeum are visible. Caudal filament originates in abdominal region IV. Bar = 20 μm.

Fig. 40. Diagrammatic sagittal section of embryo just before revolution (stage 9).

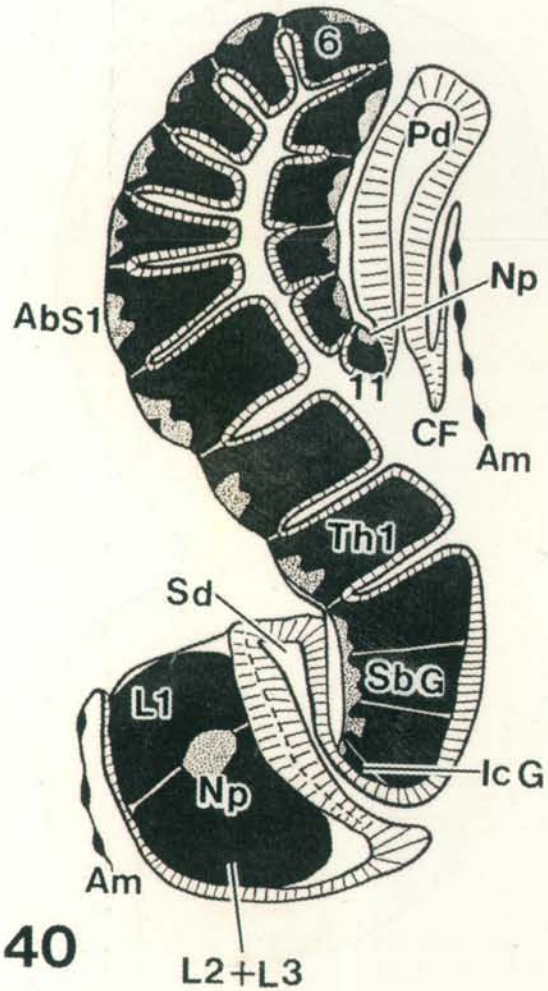
AbS1, 5-11, 1st and 5th to 11th abdominal segments; Am, amnion; CF, caudal filament; EM, ental membrane; EpSn, epineural sinus; IcG, intercalary ganglion; L1-3, lobi 1 to 3; Nb, neuroblast; Nl, neurilemma; Np, neuropile; Pd, proctodaeum; SbG, suboesophageal ganglion; Sd, stomodaeum; Thl, prothorax.


Pd
CF
Sd
L1
L2
L3

39A


EM
EpSn
6
5
7
8
9
10
Nl
AbS1
Nl
11
Pd
Nb
CF
Np
Nl
Th1

B


6
Pd
Np
AbS1
11
CF
Am
Th1
Sd
SbG
L1
Ic G
Np
Am
L2+L3

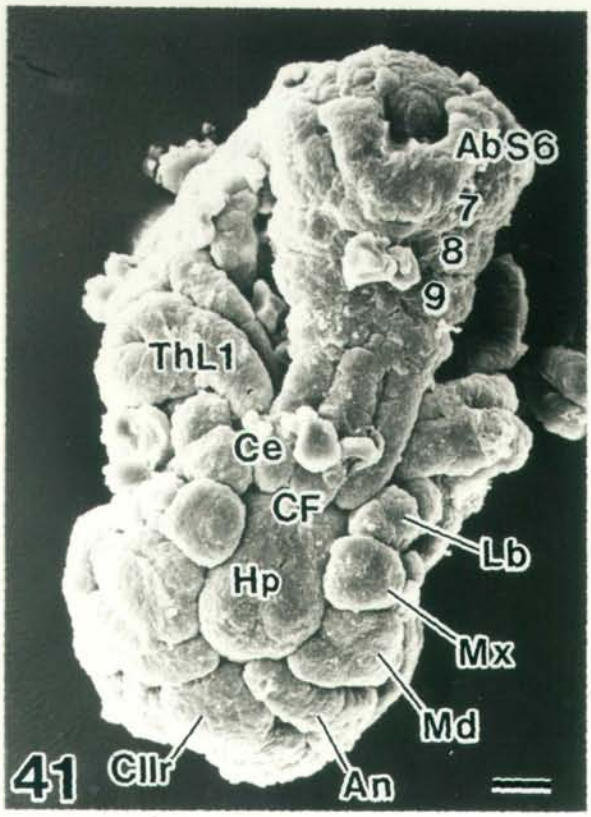
40

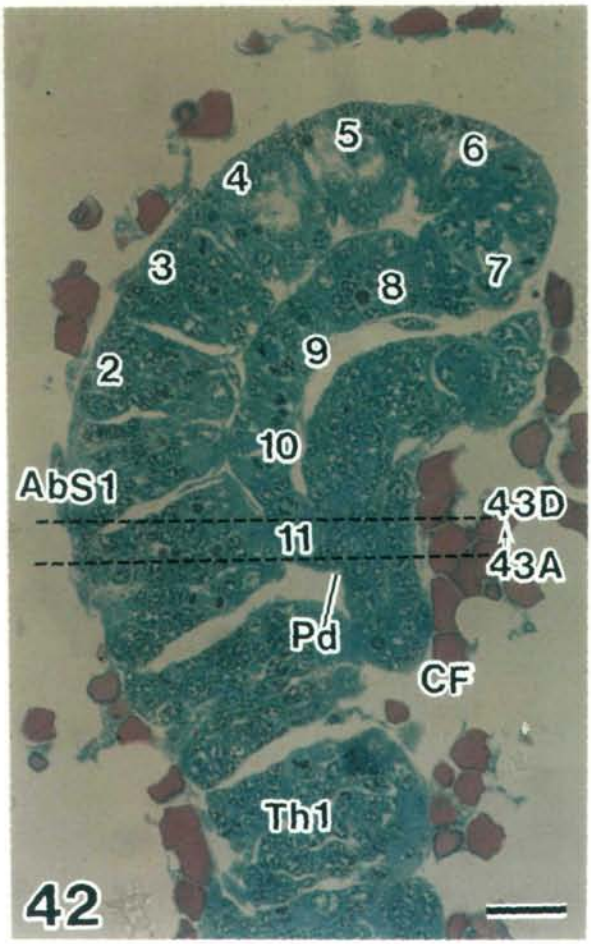
Fig. 41. An SEM micrograph of an embryo just before revolution (stage 9). Bar = 20 μm.

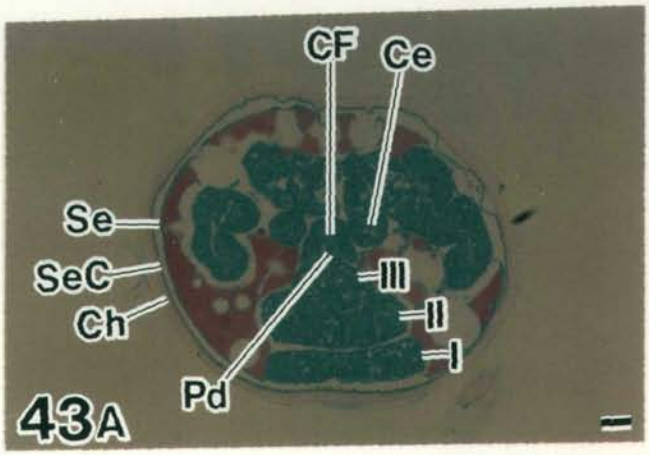
Fig. 42. A sagittal section of an embryo (posterior region) just before revolution (stage 9). Bar = 10 μm.

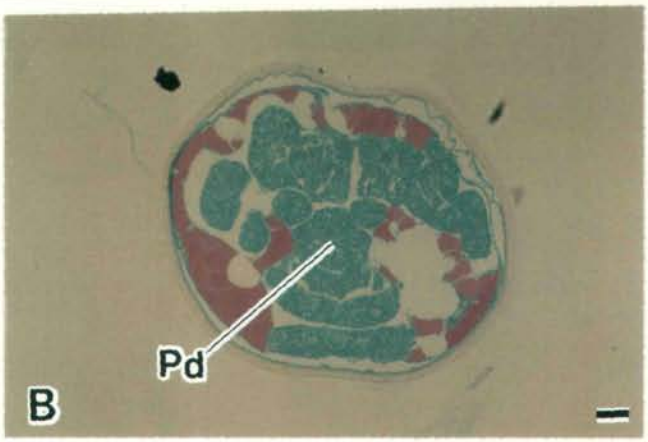
Fig. 43. Serial cross sections of an embryo at stage 9, through the planes shown with "43A-43D" in Fig. 42.

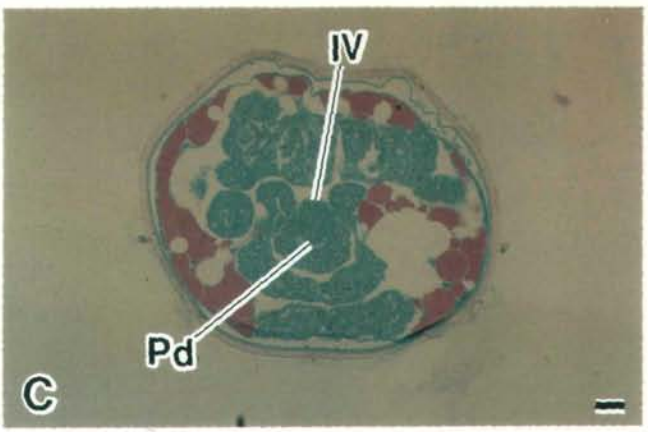
I-IV, abdominal regions I to IV; AbS1-11, 1st to 11th abdominal segments; An, antenna; Ce, cercus; CF, caudal filament; Ch, chorion; Cllr, clypeolabrum; Hp, hypopharynx; Lb, labium; Md, mandible; Mx, maxilla; Pd, proctodaeum; Se, serosa; SeC, serosal cuticle; Th1, prothorax; ThL1, proleg.


AbS6
7
8
9
ThL1
Ce
CF
Lb
Hp
Mx
Md
CIIr
An
41


5
6
4
3
8
7
9
2
10
AbS1
43D
11
43A
Pd
CF
Th1
42


CF
Ce
Se
SeC
Ch
III
II
I
Pd
43A


Pd
B


IV
Pd
C

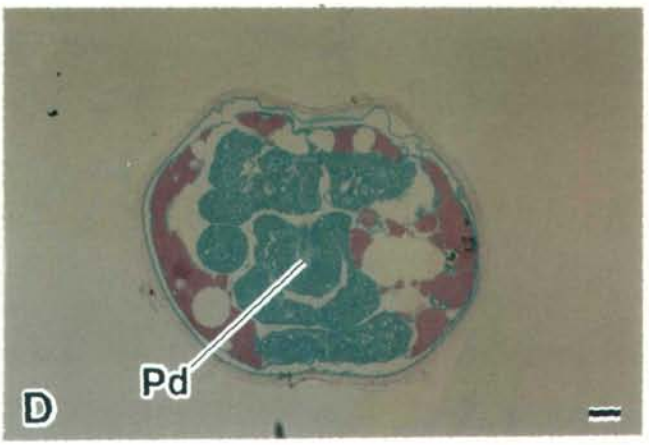

Pd
D

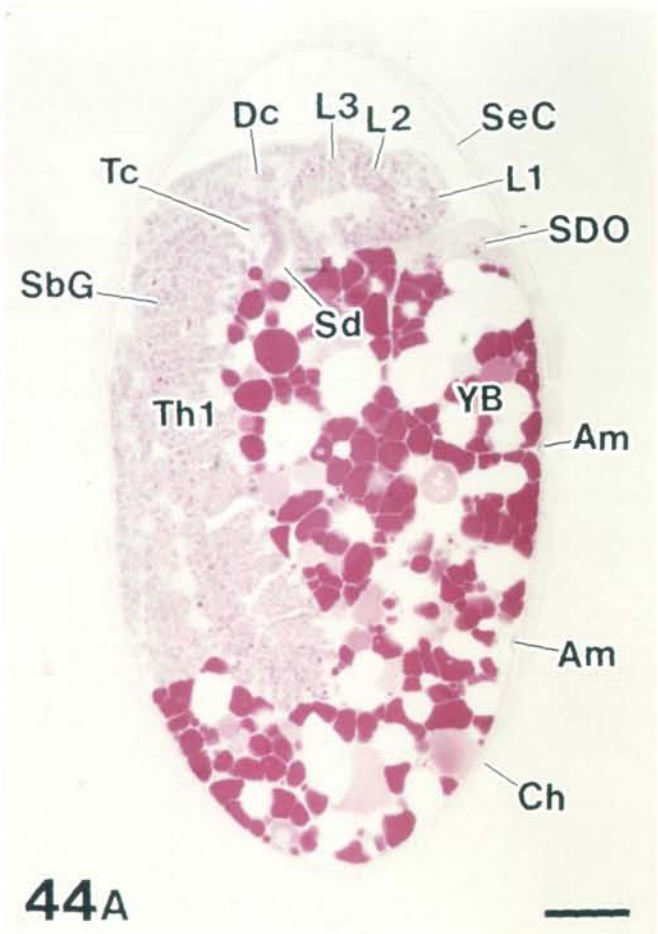
Fig. 44. Just after revolution (stage 11). Bar = 20 μm.

44A. A sagittal section of an egg.

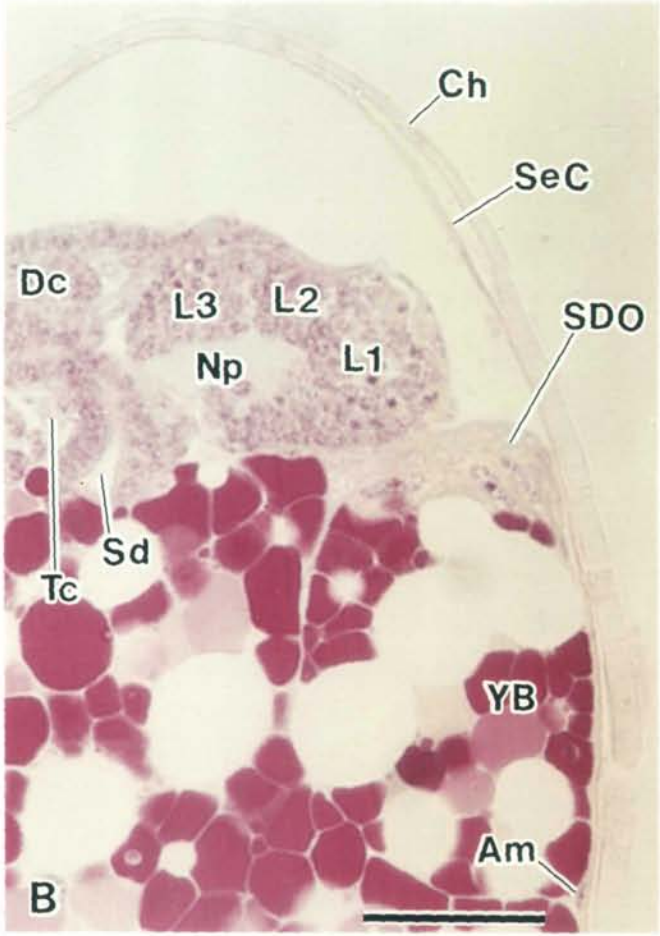
44B. Enlargement of its anterodorsal region.

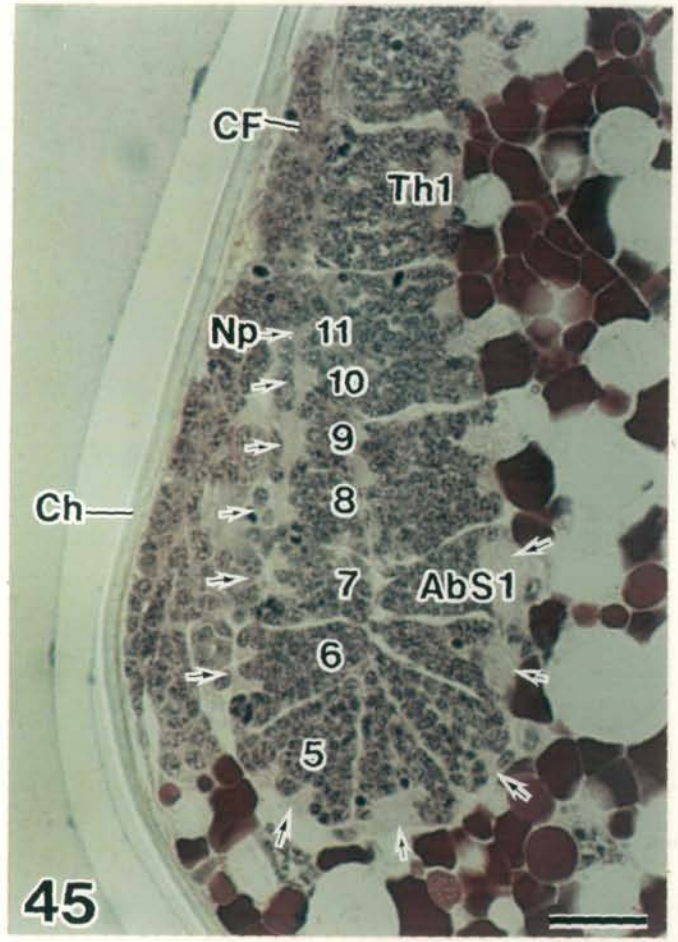
Fig. 45. A sagittal section of abdomen. Arrows show the neuropiles. Bar = 10 μm.

AbS1, 5-11, 1st, 5th to 11th abdominal segments; Am, amnion; CF, caudal filament; Ch, chorion; Dc, deutocerebrum; L1-3, lobi 1 to 3; Np, neuropile; SbG, suboesophageal ganglion; Sd, stomodaeum; SDO, secondary dorsal organ; SeC, serosal cuticle; Tc, tritocerebrum; Thl, prothorax; YB, yolk block.


Dc
L3
L2
SeC
Tc
L1
SDO
SbG
Sd
Th1
YB
Am
Am
Ch

44A


Ch
SeC
Dc
L3
L2
SDO
Np
L1
Sd
Tc
YB
Am
B


CF
Th1
Np
11
10
9
Ch
8
7
AbS1
6
5

45

Figs. 46, 47. SEM micrographs of abdomens at stage 9. Bar = 30 μm.

Fig. 46. Eary stage 9.

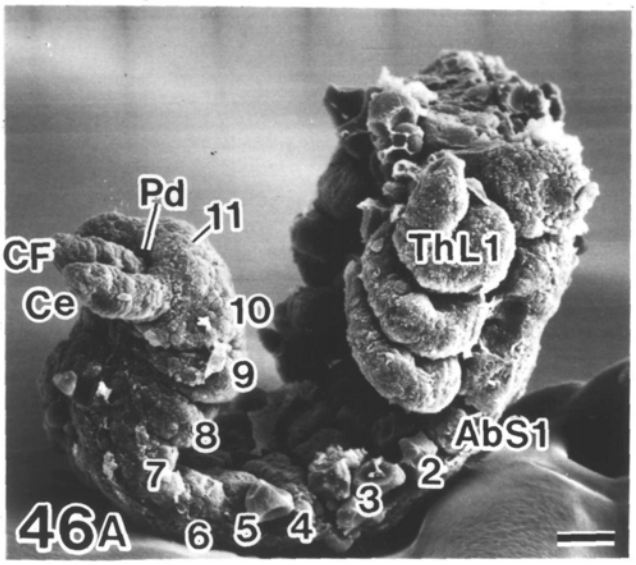
46A. Lateral view of the thorax and abdomen.

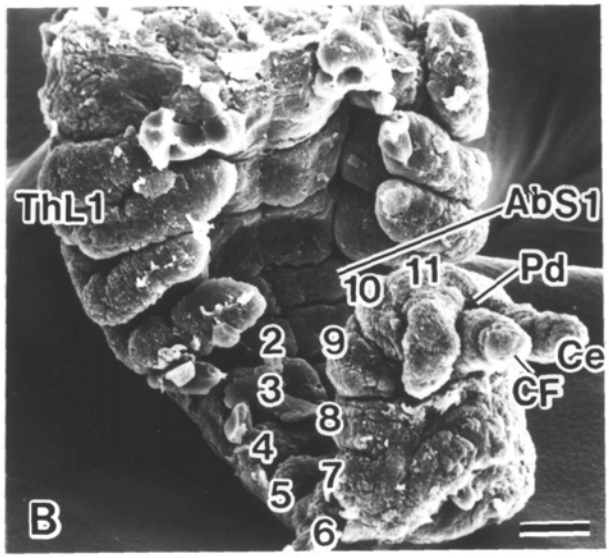
46B. Ventrolateral view of the thorax and abdomen.

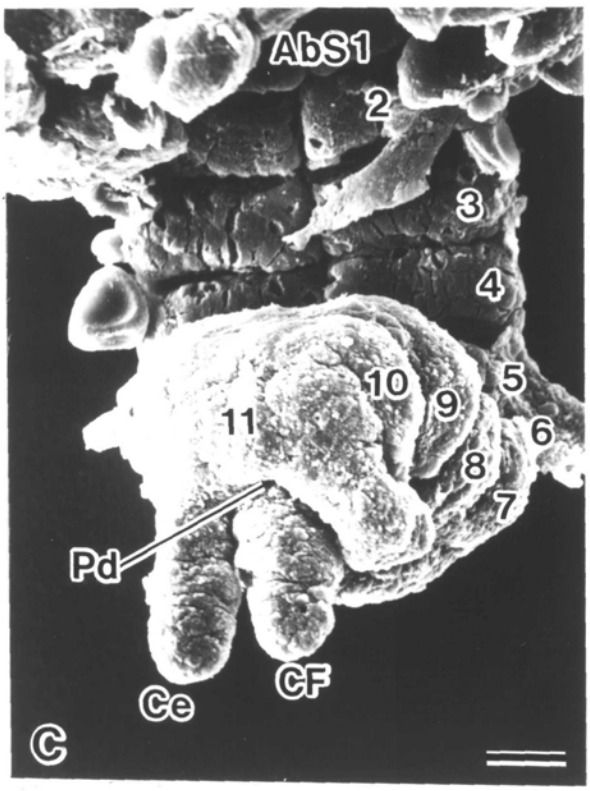
46C. Enlargement from a different angle, demonstrating the different origins of the cerci and the caudal filament.

Fig. 47. Late stage 9. Ventrolateral view of the abdomen, more developed than in Fig. 46. The cerci move from their original ventro-lateral to the dorsolateral position.

AbS1-11, 1st to 11th abdominal segments; Ce, cercus; CF, caudal filament; Pd, proctodaeum; ThL1, proleg.


Pd
11
CF
Ce
10
9
8
7
6
5
4
3
2
ThL1
AbS1
46A


ThL1
AbS1
10
11
Pd
2
9
Ce
3
CF
8
4
7
5
6
B


AbS1
2
3
4
10
9
5
11
6
8
7
Pd
Ce
CF
C

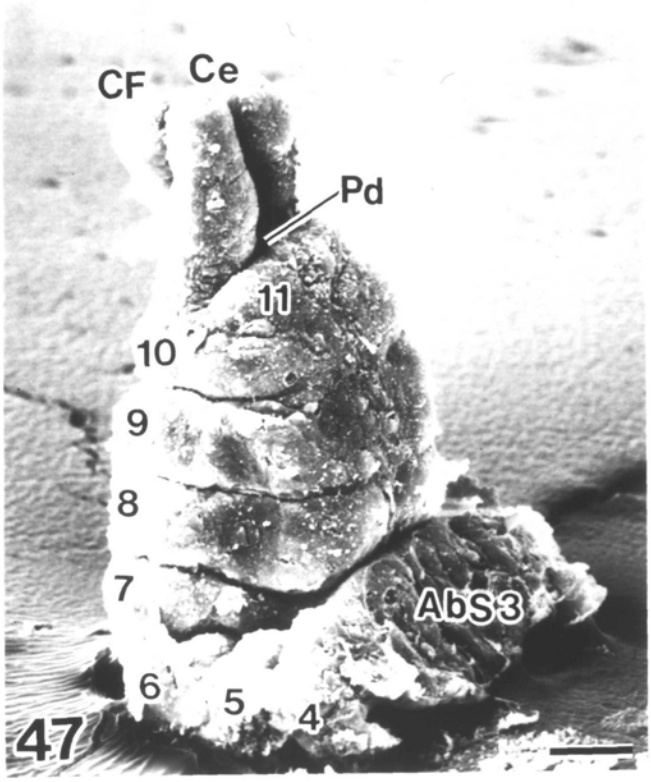

CF
Ce
Pd
11
10
9
8
7
AbS3
6
5
4
47

Fig. 48. Diagrammatic representation of abdominal development.

48A. Stage 8.

48B, B'. Early stage 9. In B', regions III and IV are pulled apart from region II to show the proctodaeum in formation.

48C. Late stage 9. The proctodaeum is enclosed by the definitive dorsal closure of region II.

I-IV, abdominal regions I to IV; AbS1-11, 1st to 11th abdominal segments; Ce, cercus; CF, caudal filament; Pd, proctodaeum.

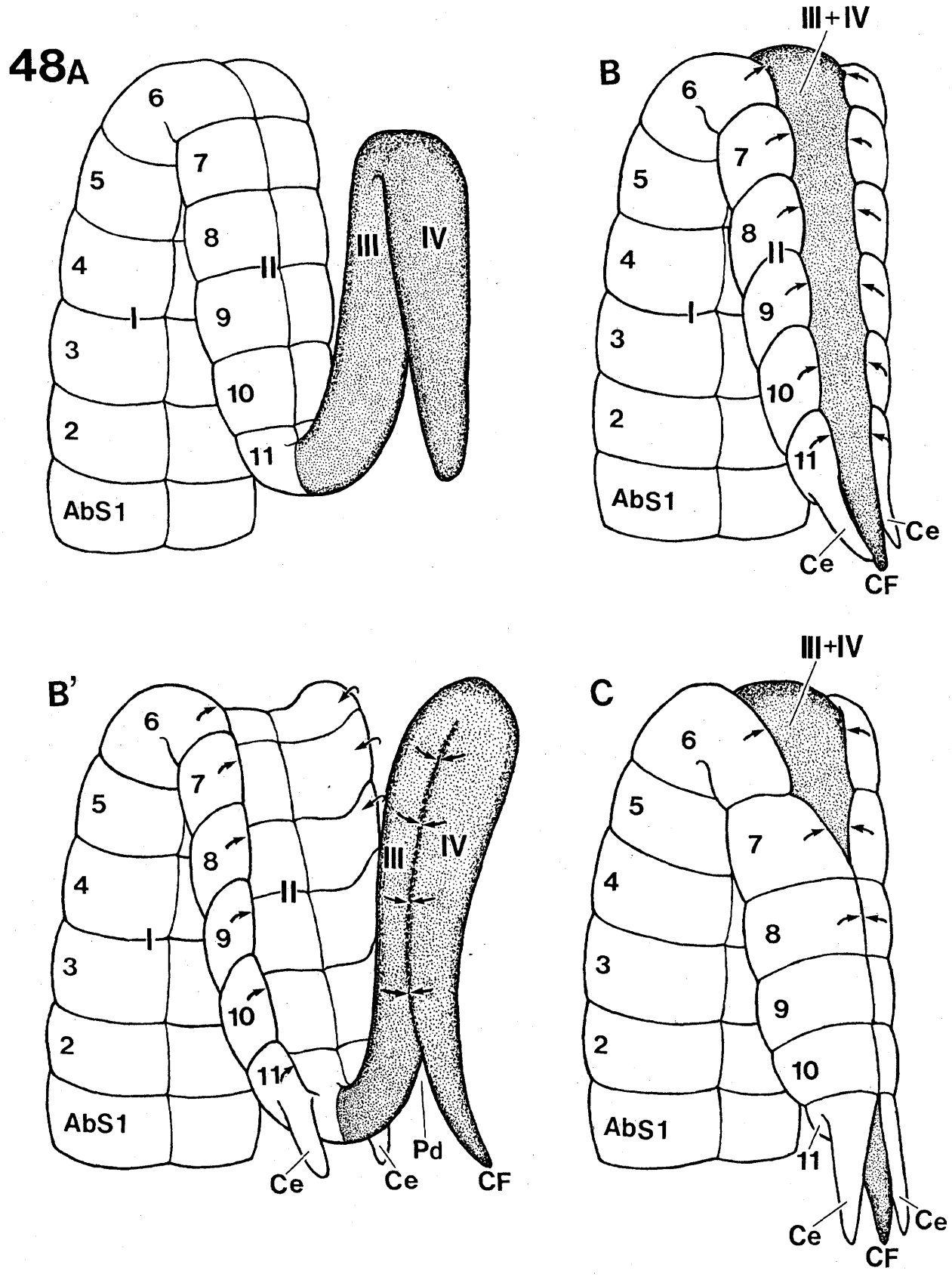

48A
6
7
5
8
4
II
I
9
3
10
2
11
AbS1
III
IV
B
III+IV
6
7
5
8
4
II
I
9
3
10
2
11
AbS1
Ce
Ce
CF
B'
6
7
5
8
4
II
I
9
3
10
2
11
AbS1
III
IV
Ce
Ce
Pd
CF
C
III+IV
6
5
7
4
8
3
9
2
10
AbS1
11
Ce
Ce
CF

Fig. 49. Precocious revolution (katatrepsis). Embryos of stage 8 are in the course of katatrepsis. See text.

49A, B. Ventrolateral and ventral views of an egg. Bar = 20 μm.

49C. A photograph of an egg, of which embryo is stained with thionin. Bar = 20 μm.

I-IV, abdominal regions I to IV; An, antenna; Cllr, clypeolabrum; HL, head lobe; IcS, intercalary segment; Lb, labium; Md, mandible; Mx, maxilla; PsAm, presumptive amnion; ThL1, proleg.

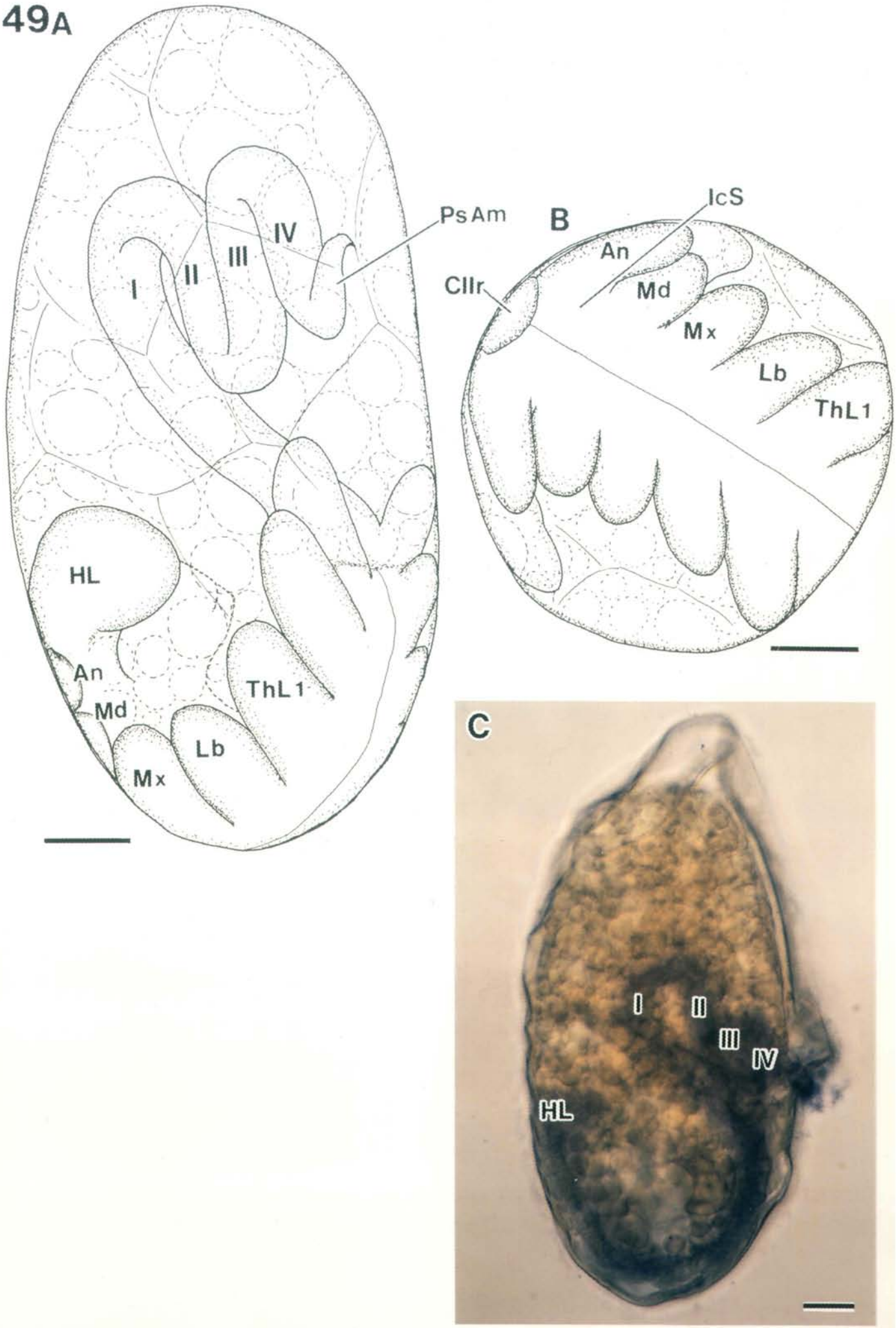

49A
I
II
III
IV
PsAm
HL
An
Md
Mx
Lb
ThL1
B
IcS
An
Md
Mx
Lb
ThL1
CIIr
C
I
II
III
IV
HL

Fig. 50. A cross section of head through mandibular segment, at stage 11. Bar = 20 μm.

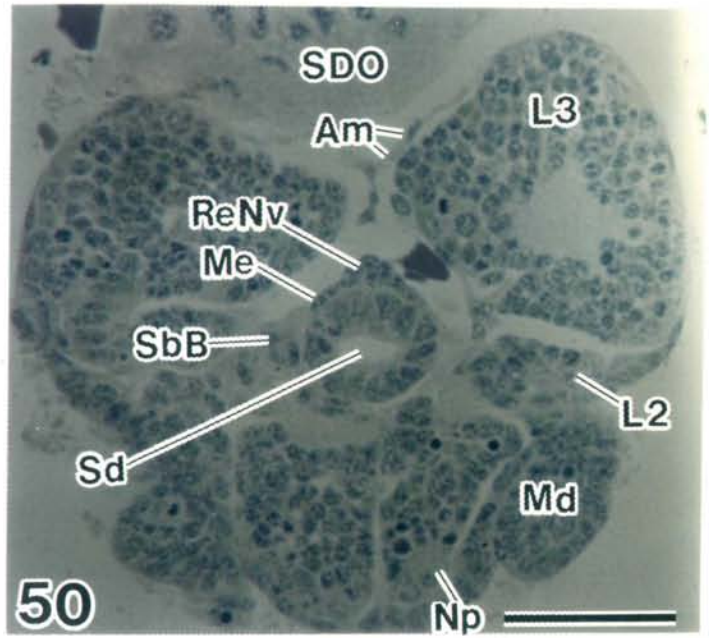
Fig. 51. A cross section of head through mandibular segment, at stage 9. Arrows show the anteiror tentorial invaginations. Bar = 20 μm.

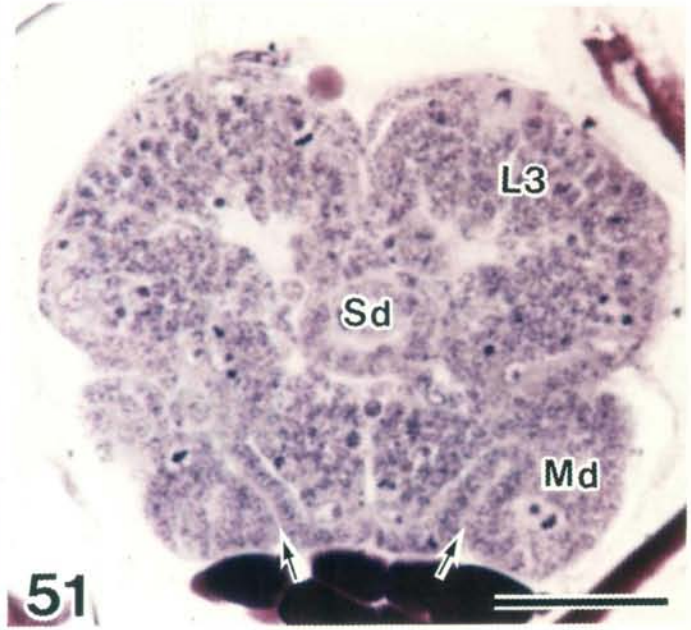
Fig. 52. A cross section of head through labial segment, at stage 9. Bar = 20 μm. Arrows 1 and 2 respectively show the invaginations of the posterior tentorium and the salivary gland.

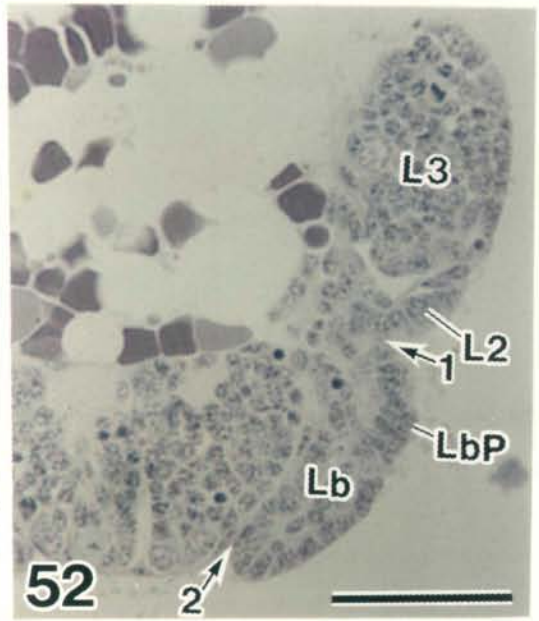
Fig. 53. A cross section of prothorax. Neuroblasts appeared in the ventral region of the segment give rise to daughter cells or ganglion cells. Bar = 20 μm.

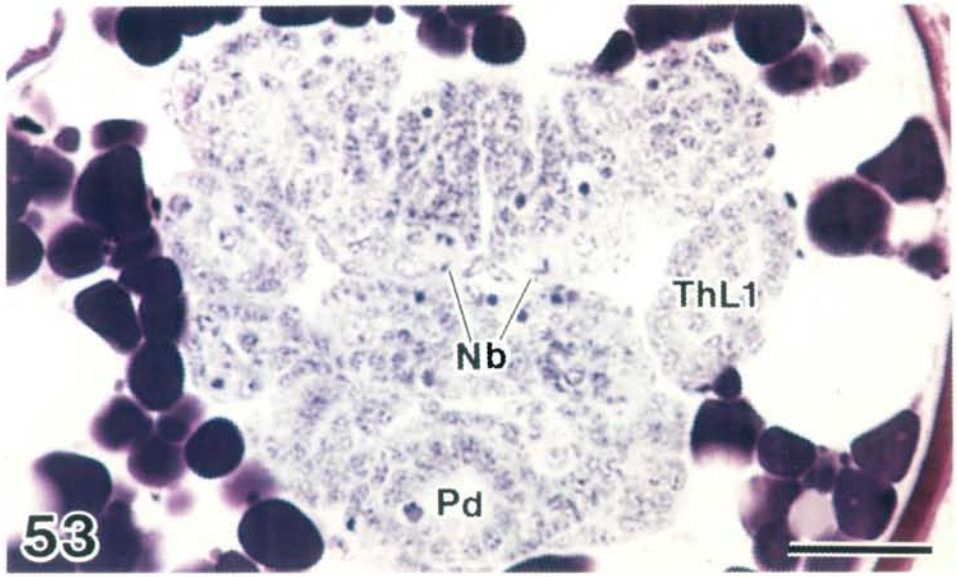
Fig. 54. Tentorial structure of the 1st instar nymph. Bar = 20 μm.

Am, amnion; AT, anterior tentorium; CB, central body; L2, 3, lobi 2 and 3; Lb, labium; LbP, labial palp; Md, madible; Me, mesoderm; Nb, neuroblast; Pd, proctodaeum; PT, posterior tentorium; ReNv, recurrent nerve; SbB, suboesophageal body; Sd, stomodaeum; SDO, secondary dorsal organ; ThL1, prothorax.


SDO
Am
L3
ReNv
Me
SbB
L2
Sd
Md
Np
50


L3
Sd
Md
51


L3
1
L2
LbP
Lb
2
52


ThL1
Nb
Pd
53

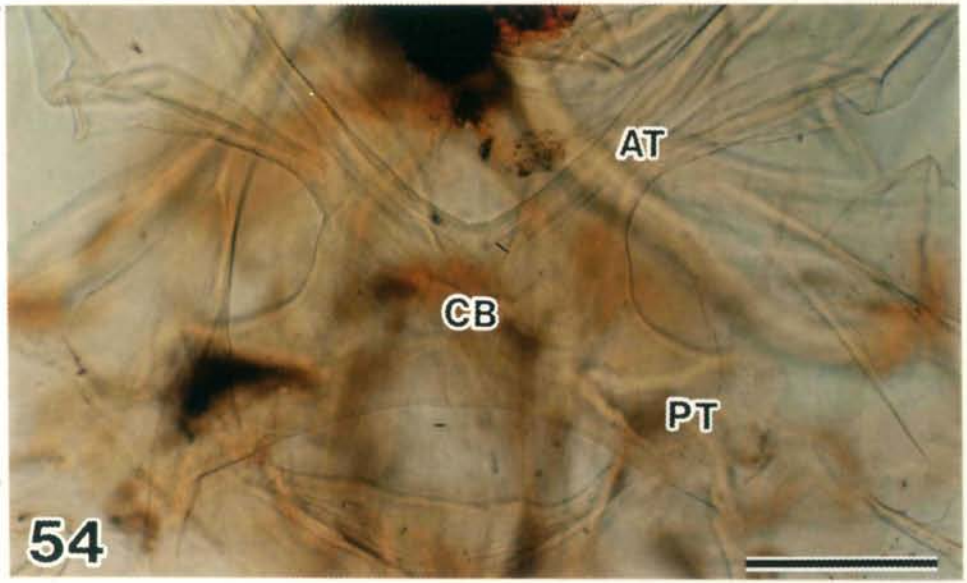

AT
CB
PT
54

Fig. 55. Sagittal sections of an embryo at stage 8. Bar = 10 μm.

55A. A mass of ectodermal cells (AMER) differentiates near the stomodaeal blind end.

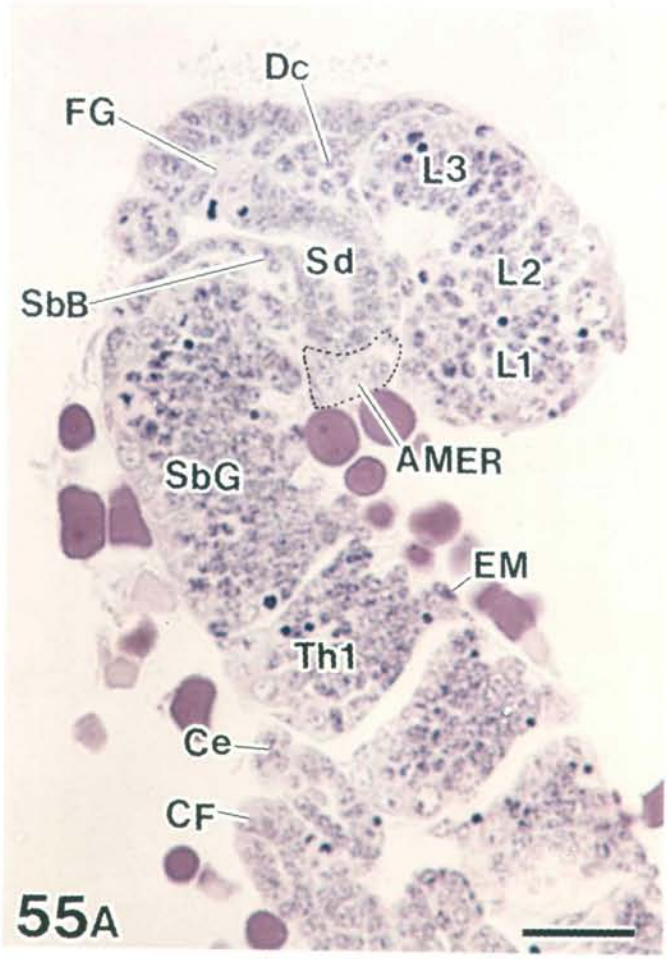
55B. A mass of ectodermal cells (PMER) differentiates near the proctodaeal blind end.

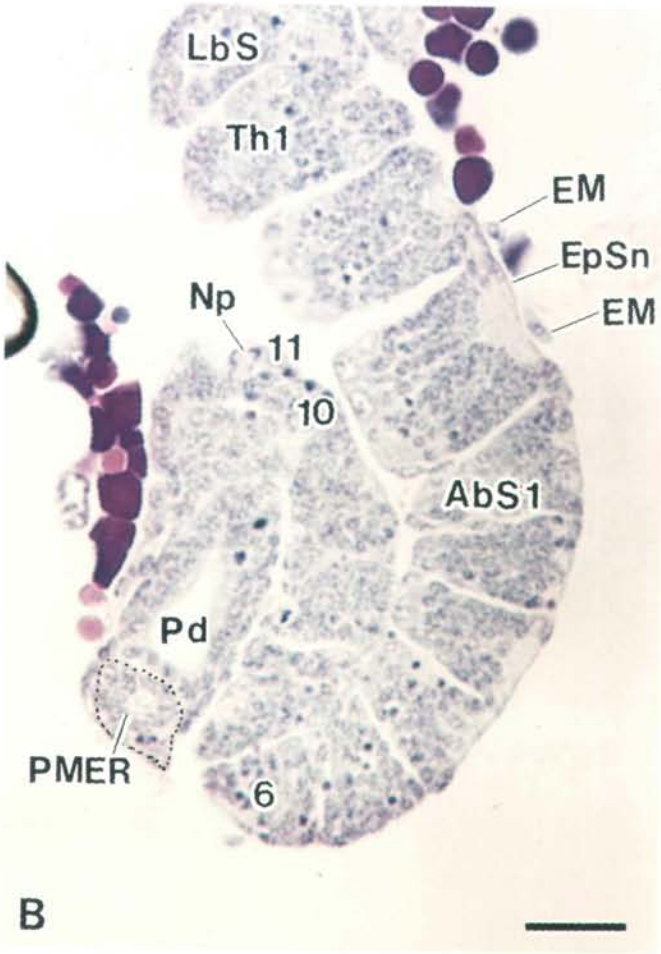
Fig. 56. Sagittal sections of an egg at stage 11. Bar = 20 μm.

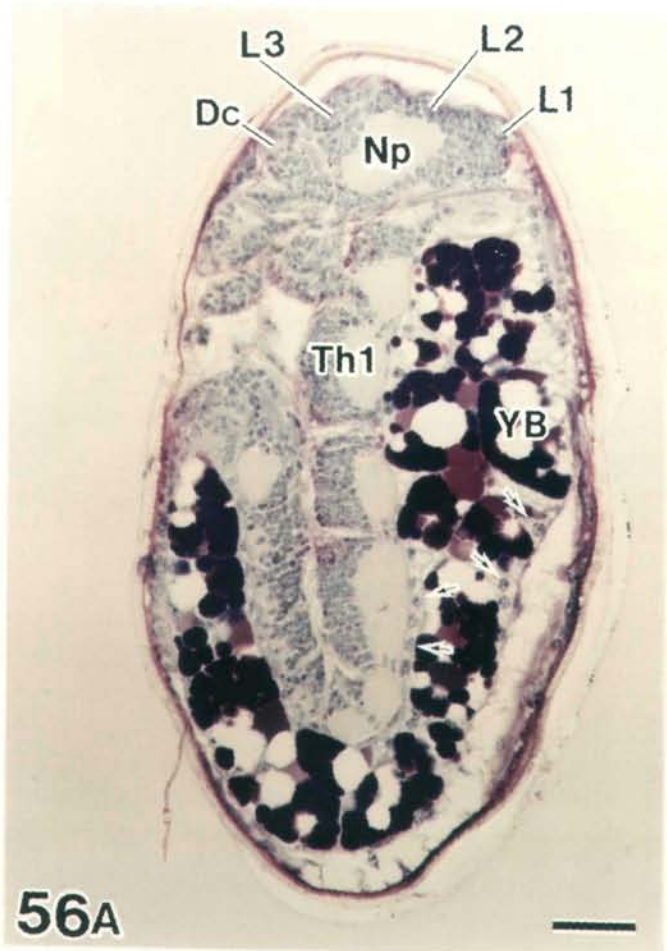
56A. Yolk cells migrate onto the ental membrane and settle themselves there (e.g., arrows).

56B. Enlargement.

AbS1, 5, 6, 8, 10, 11, 1st, 5th, 6th, 8th, 10th and epithelium rudiment; Ce, cercus; CF, caudal filament; Dc, deutocerbrum; EM, ental membrane; FB, fat body; EpSn, epineural sinus; FG, frontal ganglion; L1-3, lobi 1 to 3; LbS, labial segment; Np, neuropile; MME, developing midgut epithelium; Pd, proctodaeum; SbB, suboesophageal body; SbG, suboesophageal ganglion; Sd, stomodaeum; PMER, posterior midgut epithelium rudiment; Th1, prothorax; ThL1, proleg; YB, yolk block.


Dc
FG
L3
Sd
L2
SbB
L1
AMER
SbG
EM
Th1
Ce
CF
55A


LbS
Th1
EM
EpSn
Np
EM
11
10
AbS1
Pd
PMER
6
B


L3
L2
Dc
L1
Np
Th1
YB
56A

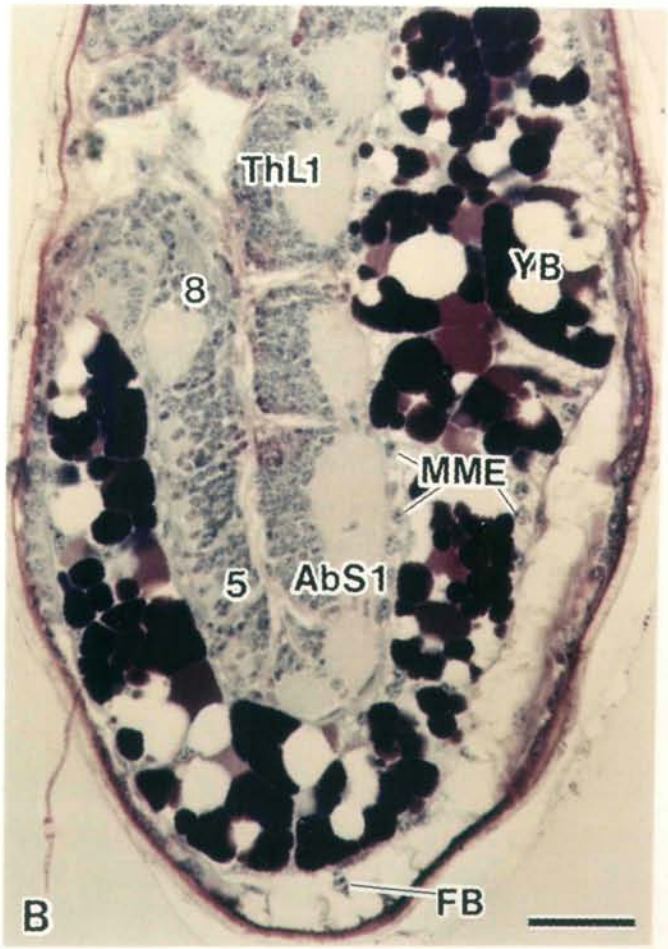

ThL1
YB
8
MME
5
AbS1
FB
B

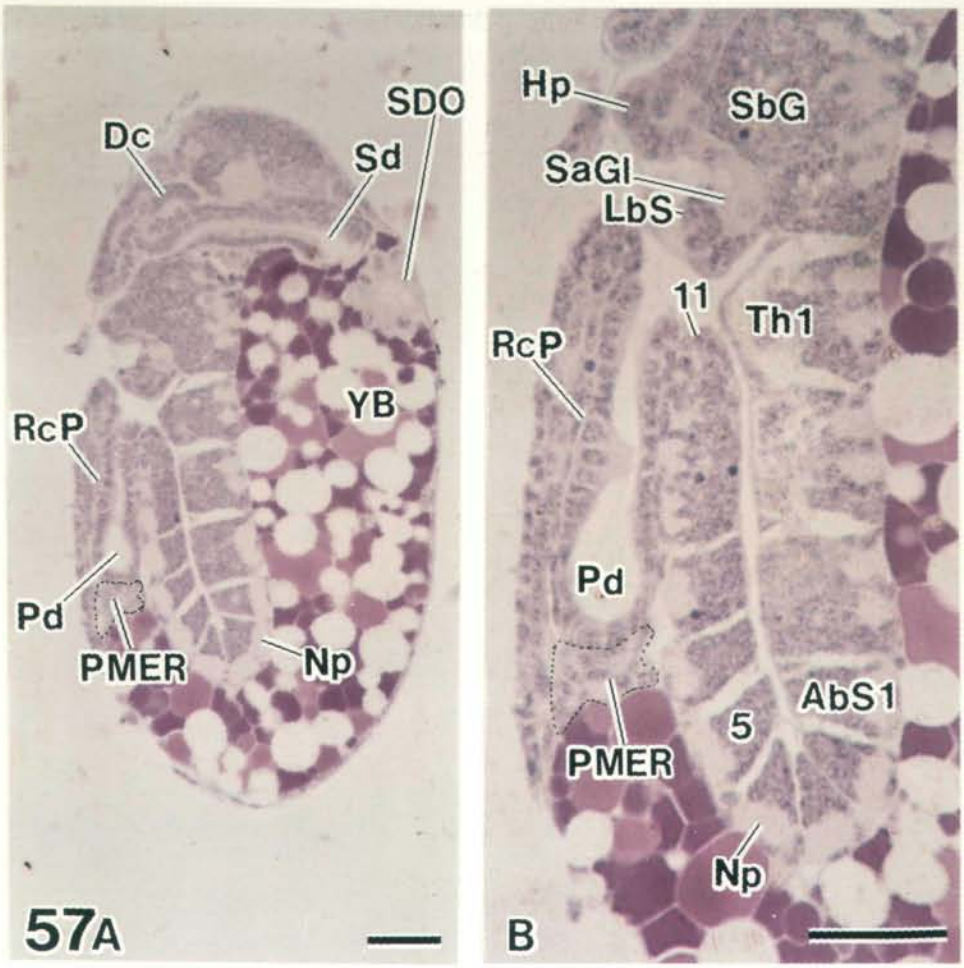
Fig. 57. Sagittal sections of an egg at stage 11.

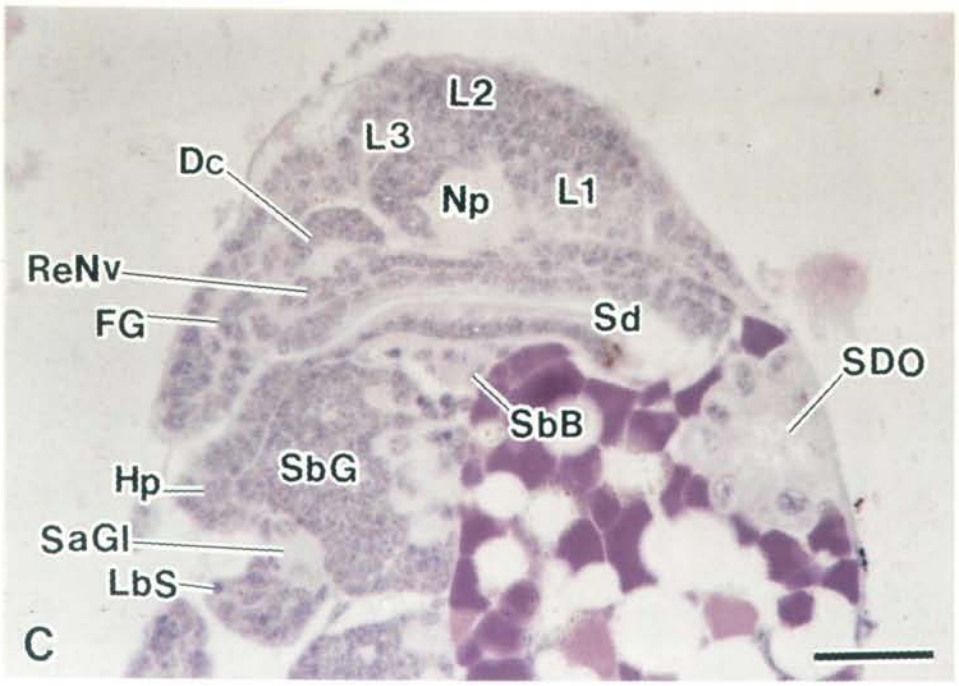
57A. A sagittal section. Bar = 20 μm.

57B. Enlargement of abdomen. Bar = 20 μm.

57C. Enlargement of head. Bar = 20 μm.

AbS 1, 5, 11, 1st, 5th and 11th abdominal sements; Dc, deutocerebrum; FG, frontal ganglion; Hp, hypopharynx; L1-3, lobi 1 to 3; LbS, labial segment; Np, neuropile; Pd, proctodaeum; PMER, posterior midgut epithelium rudiment; RcP, rectal pad; ReNv, recurrent nerve; SaGl, salivary grand; SbB, suboesophageal body; SbG, suboesophageal ganglion; Sd, stomodaeum; SDO, secondary dorsal organ; Th1, prothorax; YB, yolk block.


Dc
SDO
Sd
YB
RcP
Pd
PMER
Np
57A
Hp
SbG
SaGl
LbS
11
Th1
RcP
Pd
PMER
5
AbS1
Np
B


L2
L3
Dc
Np
L1
ReNv
FG
Sd
SDO
SbB
Hp
SbG
SaGl
LbS
C

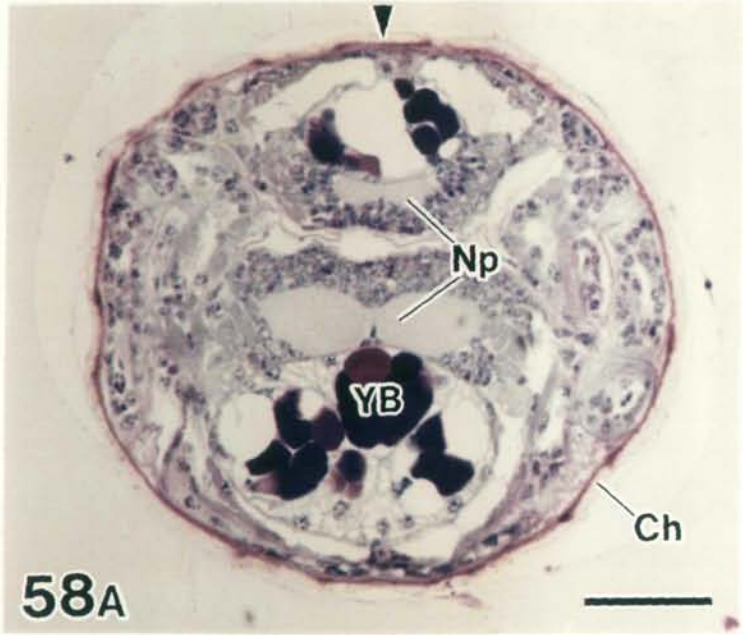
Figs. 58, 59. Cross sections of an egg at stage 11.

58A. A cross section through metathorcic (dorsal side of egg) and 4th-5th abdominal segments (ventral side of egg, arrowhead). Bar = 20 μm.

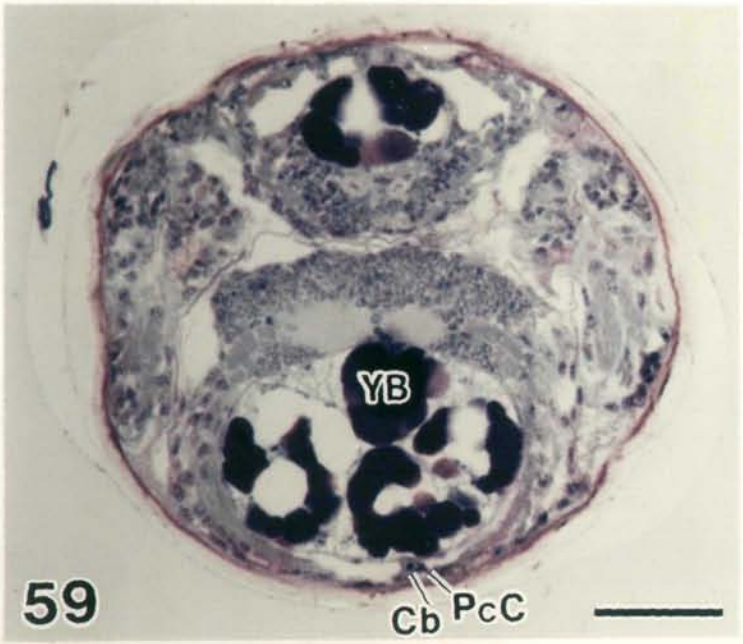
58B. Enlargement.

Fig. 59. A cross section 6 μm apart from Fig. 58. Bar = 20μm

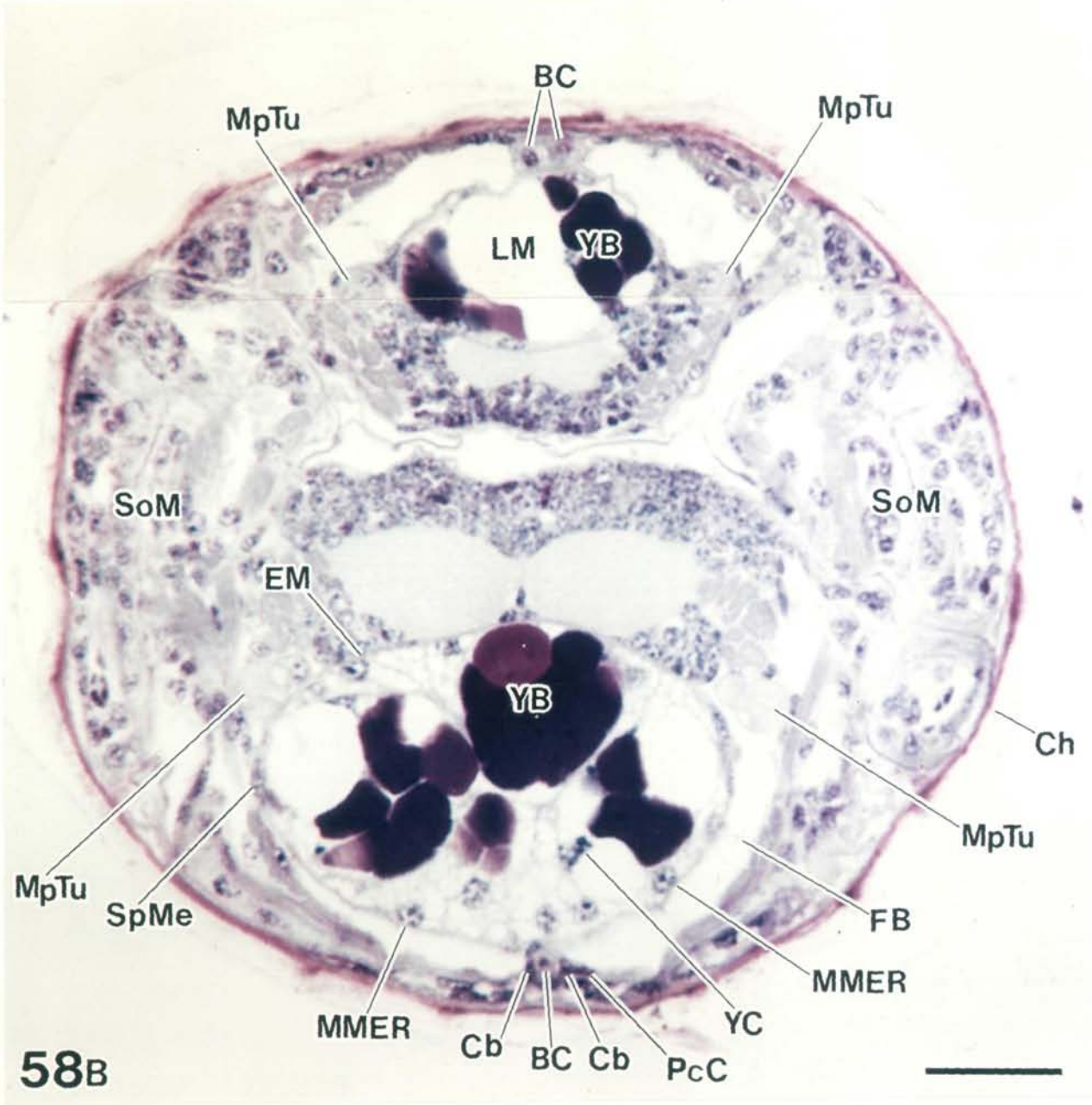
BC, blood cell; Cb, cardioblast; Ch, chorion; EM, ental membrane; FB, fat body; LM, lumen of midgut; MMER, middle region of developing midgut epithelium derived from yolk cells; Mptu, malpighian tubule; Np, neuropile; PcC, pericardial cell; SoM, somatic muscle; SpMe, splanchnic mesoderm; YB, yolk block; YC, yolk cell.


Np
YB
Ch

58A


YB
Cb
PcC

59


BC
MpTu
MpTu
LM
YB
SoM
SoM
EM
YB
Ch
MpTu
MpTu
SpMe
FB
MMER
MMER
YC
Cb
BC
Cb
PcC

58B

Fig. 60. Head region of a sagittal section of an egg at stage 12.

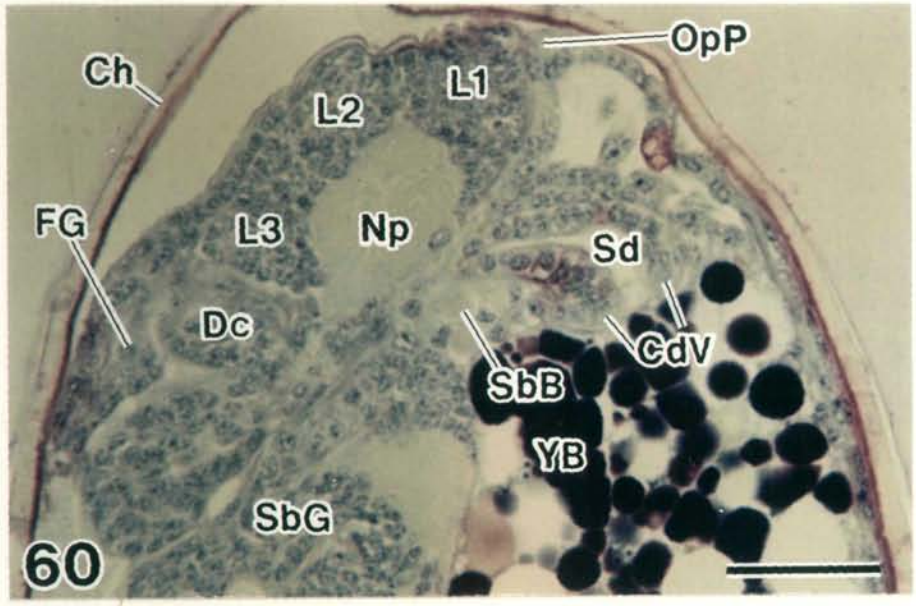
Fig. 61. Sagittal sections of 1st instar nymph.

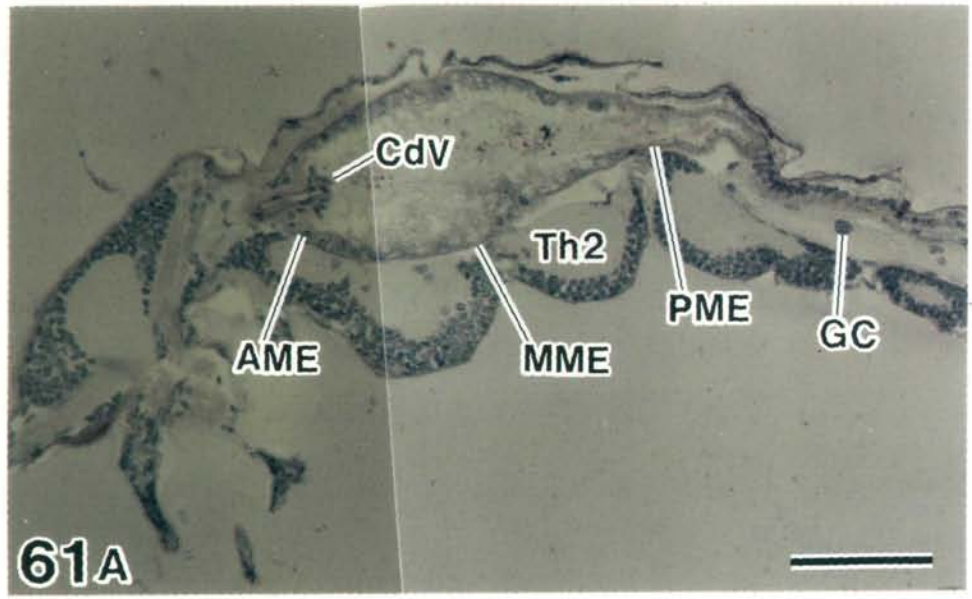
61A. Head to 2nd abdominal segment. Bar = 50 μm.

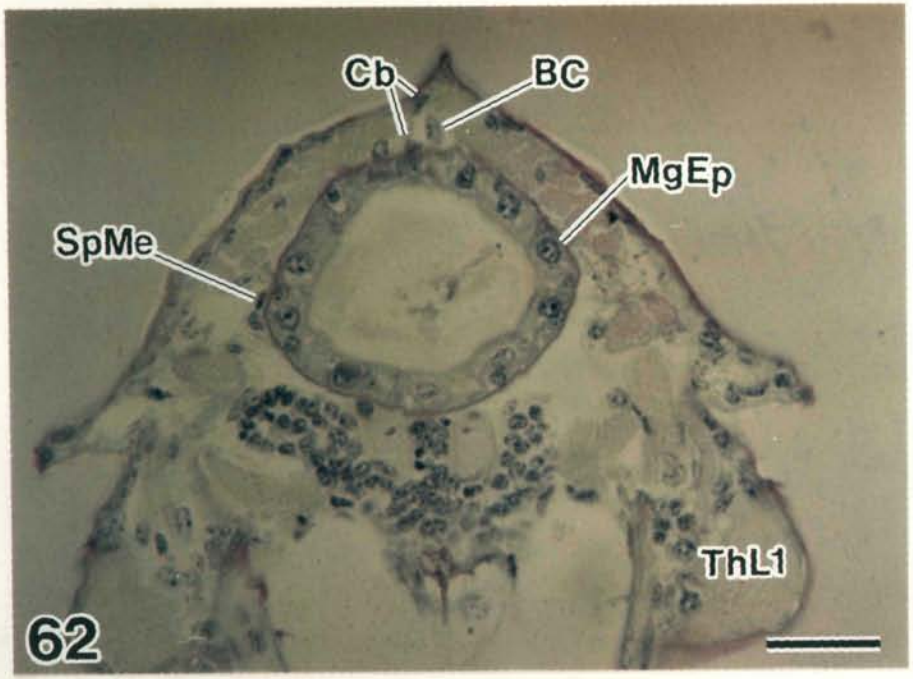
61B. Enlargement. Bar = 20 μm.

Fig. 62. A cross section of 1st instar nymph through mesothorax. Bar = 20 μm.

AME, anterior regions of midgut epithelium; BC, blood cell; Cb, cardioblast; CdV, cardiac valve; Ch, chorion; Dc, deutocerebrum; FG, frontal ganglion; GC, germ cell; Hg, hindgut; L1-3, lobi 1 to 3; Mg, midgut; MgEp, midgut epithelium; MME, middle region of midgut epithelium; MpTu, malpighian tubule; Np, neuropile; OpP, optic plate; PME, posterior region of midgut epithelium; SbB, suboesophageal body; SbG, suboesophageal ganglion; Sd, stomodaeum; SpMe, splanchnic mesoderm; Th2, 3, meso- and metathorax; ThL1, preleg; YB, yolk block.


Ch
OpP
L1
L2
FG
L3
Np
Sd
Dc
CdV
SbB
YB
SbG
60


CdV
Th2
PME
GC
AME
MME
61A


Cb
BC
MgEp
SpMe
ThL1
62

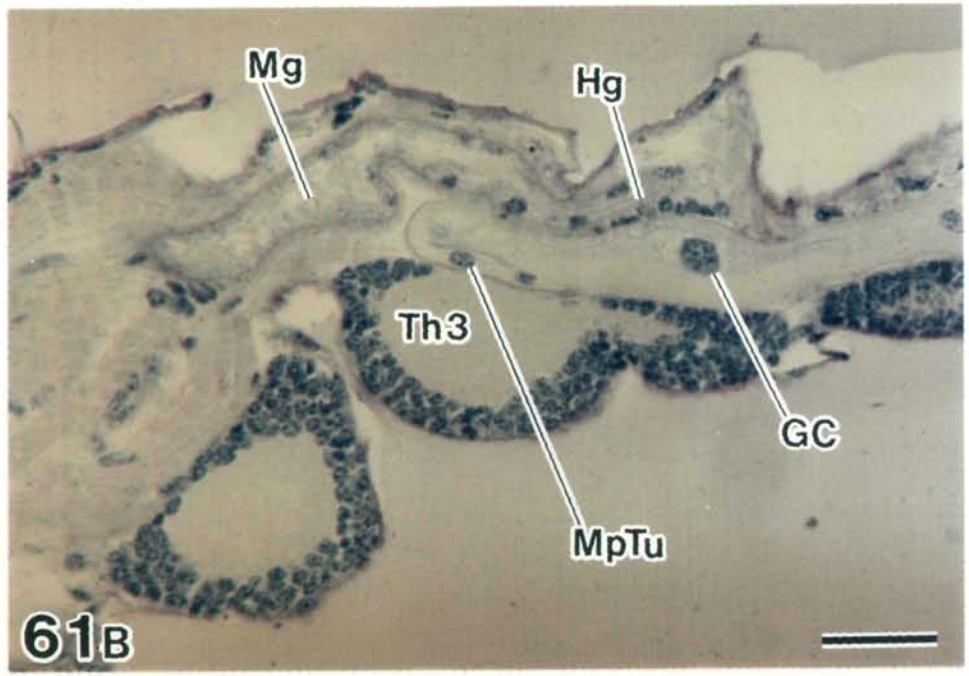

Mg
Hg
Th3
GC
MpTu
61B

Fig. 63. Diagrammatic representation showing the development of midgut epithelium. See text.

63A. Anterolateral views of developing midgut (anterior to the left, dorsal to the top).

63B. Sectional diagrams of midgut formation.

AME, anterior region of midgut epithelium; AMER, anterior midgut epithelium rudiment; Fg, foregut; Hg, hindgut; Mg, midgut; MME, middle region of midgut epithelium; MMER, rudiment of midgut epithelium of middle region; PME, posterior region of midgut epithelium; PMER, posterior midgut epithelium rudiment; YB, yolk block; YC, yolk cell.

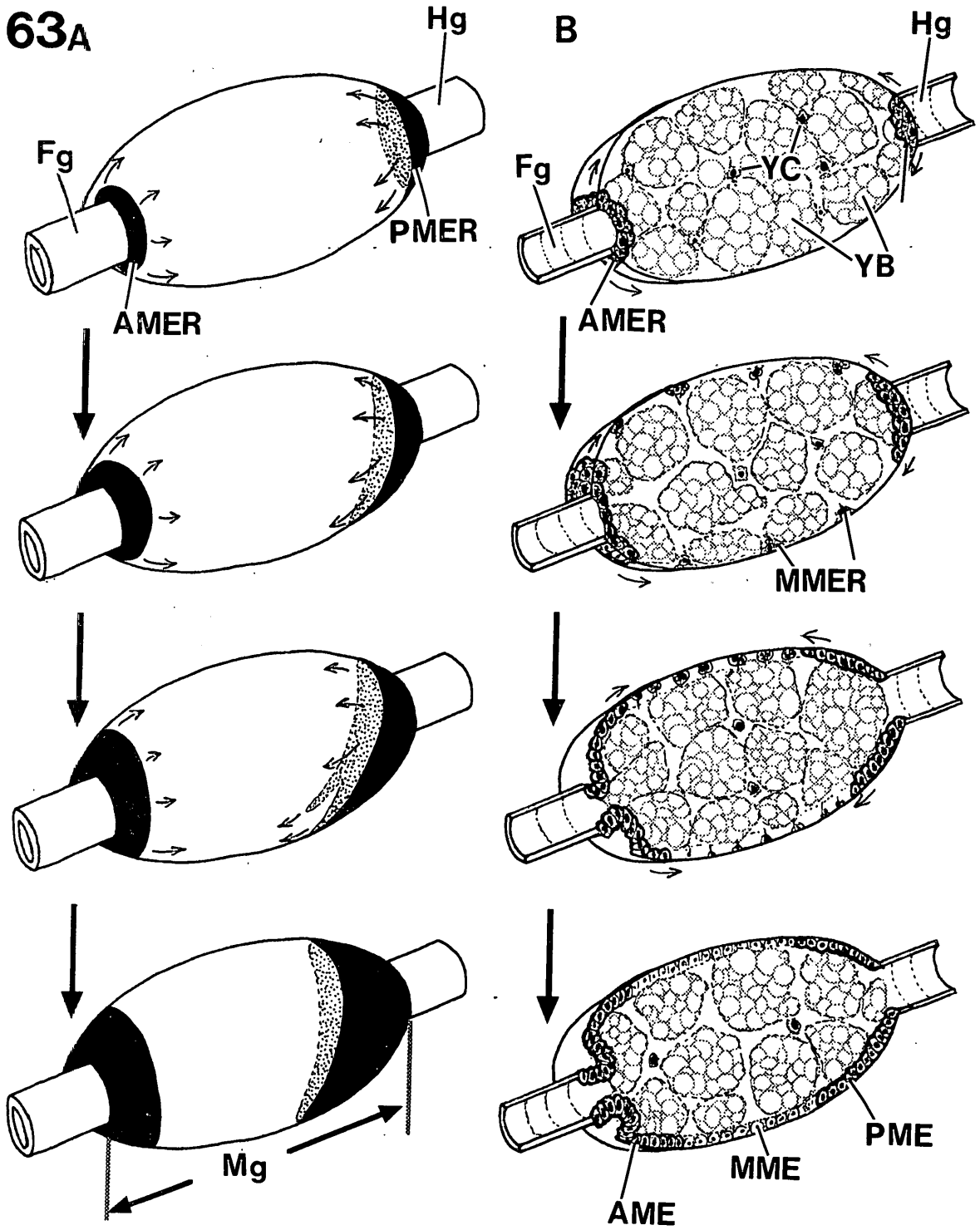
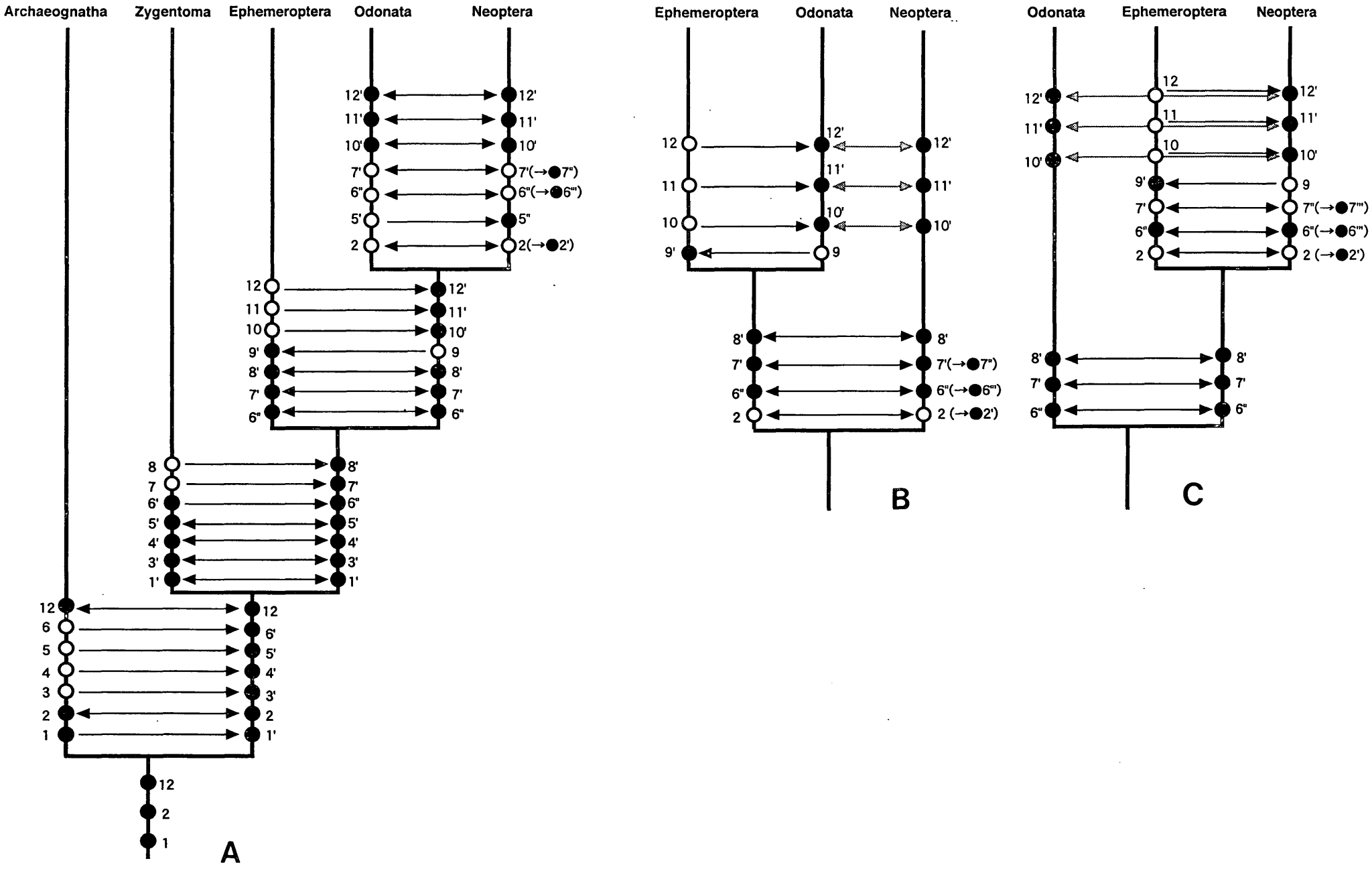

63A
Hg
Fg
PMER
AMER
B
Hg
Fg
YC
YB
AMER
MMER
Mg
PME
MME
AME

Fig. 64. Mapping the embryological characters on the currently proposed phylogenesis of Ectognatha/Pterygota (A-C). See text.


Archaeognatha
Zygentoma
Ephemeroptera
Odonata
Neoptera
12'
11'
10'
7'
6"
5'
2
7'(→●7")
6"(→●6"')
5"
2(→●2')
12
11
10
9'
8'
7'
6"
9
8
6'
5'
4'
3'
1'
6
5
4
3
2
1
A
12
2
1
Ephemeroptera
Odonata
Neoptera
12
11
10
9'
12'
11'
10'
9
8'
7'
6"
2
7'(→●7")
6"(→●6"')
2 (→●2')
B
Odonata
Ephemeroptera
Neoptera
12'
11'
10'
12
11
10
9'
9
7'
6"
2
7"(→●7"')
6"(→●6"')
2 (→●2')
8'
7'
6"
C