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Observations of the Development of the Post-Embryo of the Shrimp, *Palaemon Paucidens*

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Abstract

Post-embryonic development was observed for the freshwater shrimp, *Palaemon paucidens*, under 19-25℃. The lst antenna showed a biramous type at the egg-nauplius period of 60-80 hours after spawning. It returned to an uniramous type after that and continued its development as it was since appearances of the lst maxilla and successors at 104 hours after spawning . The 2nd antenna showed also as the lst antenna the biramous at the egg-nauplius period, however, continued its development without returning to the uniramous after that. Both of the antennae developed mainly their length towards the posterior pole during their early period of development until about 6th day, showing almost no expansion of width. Since about 7th day, especially at the distal ends of antennae, expansive development was recognized, accompanying with the annulus formation. The formation of annuli began surely at the distal at least in the case of antennae, and at 17th day just before hatching the lst and 2nd antennae possessed 3 and 2 annuli, respectively. Each of the head ganglions differentiated in the proximal areas of the optic lobe, lst and 2nd antennae at 50 hours after spawning. The fore-brain became to be formed firstly by approaching and fusion of each paired ganglion of the proximal of the optic lobe at 176 hours after spawning. At 14th day, the mid- and hind-brains were formed by the same procedure as the former except their origin. The mid- and hind-brains derived from each proximal of the lst and 2nd antennae, respectively. The hind-brain held the fore-gut between its paired posterior portion. It took about 18 days during the embryonic development.

Recent prosperity of the fishing as a leisure sport has produced a deficiency of baits. Some kinds of shrimps as important and useful merchandises have been borne a role of the fishing bait. However, in addition, increasing pollutions due to the industrial drainings and urban drains or sewages or reclaimation works have ruined widely each tiny habitat of shrimps. As for the freshwater shrimps, since farmers used agricultural chemicals for managements of rice fields shrimps disappear gradually and nowadys inhabitants which have been commonly seen at one time are almost vanishing out of sight. According to the above mentioned reasons, shrimp cultures especially of freshwater species become by slow degrees to engage in an artificial production. This experiment owes partly its commencement to such a background of recent years. In this, embryological observations were performed for the freshwater shrimp, *Palaemon paucidens*, especially concerning with the development of post-embryo. As for this species, there are previously some reports by KAJISHIMA[1)2)3)]. His works which traced all procedures of

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developmental stages are almost complete, but some obscurity also remains especially at the post-embryonic period. In some cases, the time intervals used by him are not suitable for detection of appearing sequence of appendage annuli or vicissitude of diverging pattern at the appendage tip. Therefore, this experiment was carried out specially to take care of such an obscurity on the post-embryonic organogenesis of head region as the lst, 2nd antennae and brain.

Materials and Method

The shrimp, *Palaemon paucidens*, was collected at the Ikeda Lake of Kagoshima Prefecture. During rearing in laboratory aquariums at 19-25℃, females have repeatedly spawned taking advantage of an opportunity. The spawned eggs of the shrimp were elliptical shaped as about 1.6 mm and 1.0 mm in long and short axial length, respectively. They adhered to the abdominal appendages of the female until hatching. For experimental materials, 10-20 of such bred eggs were picked off at a time by degrees according to each adequate time interval after spawnig. Each crowd of collected eggs at different times was fixed with 70% ethanol solution. In each crowd, the developmental rate showed usually individual differences among members, especially conspicuous later since the embryonic period of the abdominal folding over the head. Therefore, the most developed ones were represented for standard features of their group. Number of bred eggs was reported by KUBO and MIYAKE[4] as about 60-260 for the shrimp from the Towada Lake of North Japan. In this experiment, its value was counted as about 50-200. Eggs before embryonic formation were prepared for the histological preparation. Eggs proceeding along the embryonic development were supplied for the external observation under a binocular, using properly a weak solution of methylene blue. Further, as for the recognition of brain formation, samples of corresponding stages were decolorized in 50% glycerin after excess-staining, letting only unrelated portions be transparent.

Results

Over than 10 hours after spawning, an aggregation of larger cells than in other area is recognized at the presumptive area of blastopore invagination and its periphery. These cells are regarded as the endo- and mesoderm cells[1]. They engage in the emboly at 15 hours after spawning. At 30 hours after spawning, the invagination is almost finished and the remains of larger cells show an aggregation like as surrounding the blastopore to which small cells as ectodermal accumlate forming each of two arms of the letter of V (**Plate 1-1**). The large cells are observed also at each tip of arms of the V which is the presumptive area of the optci lobe rudiment. At 40 hours after spawning, the invagination is completely finished. A ventral plate appears at the bottom of the V, similarly a paired optic lobe rudiment at each tip of the arms (**Plate 1-2**). From the histological observations, the lining of mesodermal large cells was recognized inside the ventral plate and optic lobe rudiment. Also at the middle portion of each

arm connecting the ventral plate with the optic lobe rudiment, the lining of them was provided and it seems that it is an area which would develop each rudiment of head appendages in near future. At 50 hours after spawning, each rudiment of the lst, 2nd antennae and mandible appears in the middle area between the optic lobe rudiment and ventral plate. The ventral plate as a thoracic abdominal plate begins to expand forwards (towards the area of the optic lobe rudiment). Stomodaeum is formed in the middle area between both of paired lst antennae, and an anus rudiment is recognized further at the distal end of the thoracic abdominal plate. Formation of ganglions is recognized proximally at each rudiment of head appendages (**Plate 1-3**). At 60 hours after spawning, each appendage as the lst, 2nd antennae and mandible engages in elongation showing a flat-tubular shaps. At this stage, both of antennae show a biramous type. Labrum appears anteriorly near by the stomodaeum. The above mentioned ganglions develop as the lst, 2nd, 3rd and mandibular ganglions (**Plate 1-4**). This stage is named as the egg-nauplius period because of its correspondence to the nauplius period in Penaeidae. Since this stage, the embryo becomes to be slightly compact setting its movement towards the posterior pole. At 80 hours or about 4 days after spawning (spawnings occurred generally at about 4 o'clock a.m. and the day was regarded as lst day after spawning), the embryonic movement has been finished and the embryo situates itself at the posterior pole. Successive development occurs, therefore, forwards from this position. The optic lobe, lst, 2nd antennae, mandible and thoracic abdominal plate develop extensively. The labrum elongates backwardly covering over the stomodaeum. Until now, each of the lst and 2nd antennae shows a biramous type (**Plate 1-5**). At about 90 hours after spawning, the thoracic abdomianl plate becomes to be somewhat constricted proximally showing a flat-tubular shape and expands its superior portion forwards. Its tip comes to a halt posterior to the labrum (**Plate 2-6**). At 104 hours or 5 days after spawning, such appendages as the lst, 2nd maxillae and lst maxilliped are newly formed. Embryo itself develops forwards and distal portions of the lst and 2nd antennae elongate backwardly. At this stage, prevous lst antenna which has showed a biramous type returns its type to the uniramous. On the contrary, the 2nd antenna stays as the biramous (**Plate 2-7**). At 128 hours or 6 days after spawning, the 2nd and 3rd maxillipeds are newly formed. The optic lobe expands largely and the embryo elongates to the equator level. Each distal portion of the lst and 2nd antennae continues to elongate backwardly. As for the thoracic abdominal plate, its tip doesn't yet reach to the labrum (**Plate 2-8**). At 152 hours or 7 days after spawning, its width and length of the embryo are well developed. The optic lobe provided with melanoid pigment engages to develop as a compound eye. The previous mentioned ganglions as the lst, 2nd and 3rd aggregate one another to the proximal of the optic lobe beginning to form a brain rudiment. The lst and 2nd maxillipeds which have been formed at previous stage (6 days after spawning) develop larger than the two paired maxillae. Each appendage later than the lst maxilla is provided with the endo- and exopodites (**Plate 3-9**). At 176 hours or 8 days after spawning, the anterior end of the embryo reaches to the anterior pole. At this stage, the lst ganglion which is situated at the proximal of the optic lobe connects its

paired component forming a fore-brain. The mid- and hind-brains are not yet formed completely, but each brain with the former and labrum are recognized easily externally as a rised plate above surrounding area. As for the thoracic abdominal plate, its tip reaches posteriorly near to the labrum and its 6 segments become to be visible. Its tip portion, telson, can be seen to be especially separated from the previous segments (**Plate 3-10**). At 248 hours or 11 days after spawning, a rostral formation is observed although new appendage doesn't appear. Each of the lst, 2nd and 3rd maxillipeds elongates considerably. Abdomen as the developed thoracic abdominal plate is provided with still more developed telson which covers over the labrum completely and of which tip possesses some differentiated setae. An outline of carapace becomes to be seen externally (**Plate 4-11**). At 320 hours or 14 days after spawning, the compound eye develops enough and the annulus of its peduncle is distinct. The abdomen expands still more and its tip attains to the anterior pole. Further, in the former, a digestive tract can be traced partly. Cardiac organ is obseved on the dorsal position within the carapace to the compound eye (**Plate 4-12**). At 392 hours or 17 days after spawning, elongation of the abdomen proceeds and its telson turns along the rostrum and the dorsal area of carapace between paired peduncles of the eye. Yolk has been almost absorbed until this stage, and its remnant is a little observed around the cardiac organ. Further, at this stage, the lst and 2nd pereiopods of the biramous type are formed posteriorly to the 3rd maxilliped, although its recognition is difficult externally (**Plate 5-13**). At 416 hours or 18 days after spawning, the embryo prepares the same morphological organization as that of the hatching larva. The telson attains to the equator level, passing beyond the cardiac position of the dorsal carapace. No formation of new appendages is recognized (**Plate 5-14**). Hatching has occurred as such a state of the embryo. This stage corresponds to the proto-zoea III period in Penaeidae. Larva as the hatched embryo swims moving the three paired maxillipeds of the biramous type that possess 2-4 setae at each tip. Two paired appendages are counted as the pereiopod.

Especially as for each development of the lst, 2nd antennae and brain, somewhat detailed observations were conducted. The lst antenna shows a round verrucous shape at its early period of differentiation. After 60 hours, it becomes to be the biramous type (**Plate 6**). It continues development as the type until 80 hours after. However, it returns to an uniramous type at about 100 hours after spawning when the embryo engages in elongation and appendages later than the lst maxilla appear. Antennal elongation at 128 hours after spawning continues possessing newly differentiated setae at its tip (**Plate 6**), but its early development depends mainly on the axial elongation. However, since about 152 hours or 7 days after spawning, its distal end begins to enlarge flatly. Next 176 hours or 8th day, an annulus formation undertakes its start from the tip of antenna. At 248 hours or 11th day, one annulus is distinctly observed, then, the lst segment has separated. Successive annulus appears at 320 hours or 14th day. The 3rd annulus appears at 392 hours or 17th day. At this stage, the distal end of the 2nd segment is provided with a large seta. This seta seems to correspond to the medial flagellum as one of both flagella which are formed completely after hatching. The lst segment possesses 2-4 setae of middle size, and it

seems that one of them continues its development to become the lateral flagellum of larva. The 2nd antenna of early period shows the verrucous shape as the same with the lst antenna (**Plate 7**). It becomes to be the biramous type at 60 hours after spawning. However, contrary to the state of the lst antenna, it continues development as it is without returning to its primary type. Its exopodite rudiment differentiates at the early period as 60-104 hours after spawning (**Plate 7**). It develops faster than the trunk which is the endopodite at this period, then, its tip is before that of the latter. This relation reverses at 128 hours or 6th day, but its state is temporary and at 152 hours or 7th day, previous state becomes to be restored. The 2nd antenna shows also its development as the axial elongation mainly during the early period, however, since about 7th day its development of distal end begins to expand. The exopodite especially expands flatly seeming to become a squama in future. At 176 hours or 8th day, the annulus formation occurs, and two annuli can be recognized at 392 hours or 17th day. The 2-4 and 9 setae differentiate at each end edge of the endo- and exopodites, respectively. As for the former, one of the setae seems to develop as the antennal flagellum in future. Finally, the fundamental procedure of brain formation is as follows. At early period, each ganglion situated at the proximal area of related head appendage develops as a paired condition. At 90 hours after spawning, each of paired lst ganglions is observed to separate from the optic lobe, approaching each other (**Plate 2-6b**). The 2nd and 3rd ganglions continue to develop at their proper positions (**Plate 8-1**). At 128 hours after spawning, each posterior half of the lst ganglion connects each other (**Plate 8-2**). At 176 hours after spawning, the fore-brain becomes to be formed by complete fusion of each half of the lst ganglion, and also each of the paired ganglions of the 2nd approaches each other (**Plate 8-3**). At this period, the external recognition of ganglion arrangement is possible as a bulging plate separated from the surrounding tissue. At 320 hours after spawning, the brain formation proceeds further and except the hind-brain, their connection and fusion of the fore- and mid-brains are almost completed at least externally (**Plate 8-4**). The hind-brain continues its development holding a fore-gut between its paired posterior portions. In the embryo just before hatching, the brain formation shows externally no more development compared with the previous period, but it is observed at least that the ventral of the hind-brain is provided with the large oval mass of neuropile at each proximal area of the 2nd antenna (**Plate 8-5**).

Discussion

As for the lst antenna, there have been hitherto no reports indicating the biramous appearance at its early period of development[1)3)]. In this experiment, it was recognized that the lst antenna showed the biramous during 60-80 hours after spawning, then, returned to the uniramous after that. Its returning period is considered by an aspect of successive appearances of such appendages as the the lst, 2nd maxillae and lst maxilliped to the transient period from the nauplius to the proto-zoea I in Penaeidae, and therefore it seems that it may show only the biramous at the egg-nauplius period corresponding to the nauplius in Penaeidae. The 2nd

antenna showed in this experiment its biramous type since the same stage as that of the lst antenna, that is, 60 hours after spawning, contrary to the result by KAJISHIMA[2] who recognized its appearance since 6th day. This result obtained here is considered as the reasonable appearance because of its correspondence to the nauplius in Penaeidae. The fore-brain formation proceeded slowly compared with the results by the former. In this experiment, each paired ganglion became at last to form the fore-brain after approaching each other at 8th day, contrary to the former result in which it formed the fore-brain according to the same procedure at 5th day. In this stage of 8th day, the embryonic development was remarkable, and the optic lobe had attained to the anterior pole. Such aspects mean a comparatively slower rate of the development in this case than that of the former. These differences would be caused by the temperature difference of 3-9℃, for one of reasons. However, other factors may be well considered because during this experiment each egg from not only different parent under the same temperature but also same parent showed a time lag of 2-3 days one another for hatching contrary to the results by the former, although its early development being almost simultaneous. Physiological factors may be, therefore, important to the embryonic development. At the post-period of embryonic development, the posterior of the hind-brain held the fore-gut. It is because of the formation of a circumoesophageal nerve ring with the paired mandibular ganglion. However, in this experiment its connection between the hind-brain and mandibular ganglion couldn't be recognized. At the final period of the embryonic development, that is, at 17th day or 392 hours after spawning, two paired appendages had been newly formed. KAJISHIMA[2][3] named them as the lst and 2nd pleopods. However, they were situated at the thoracic region and just posterior to the 3rd maxilliped. They were, therefore, considered here to be the lst and 2nd pereiopods. Finally, early embryo or blastoderm until 40 hours after spawning showed its body axis, that is, the median line of the letter V, turning aside in some cases. If its phenomenon had any meaning, reason for its purpose is at present inexplicable and whichever cases its cause expects an inherent explication in future.

References

1) 梶島孝雄（1950）：スジエビ *Leander pacificus* STIMPSON の発生Ⅱ．初期発生，動雑，**58,** 7-11.
2) 梶島孝雄（1950）：スジエビ *Leander pacificus* STIMPSON の発生Ⅲ．胚子の後期発生，*ibid,* **59,** 9-12.
3) 梶島孝雄（1950）：スジエビ *Leander pacificus* STIMPSON の卵の胚葉分化の機構，実験形態学，**6,** 19-38.
4) 久保伊津男・三宅貞祥（1973）：節足動物：甲殻類エビ類．in "川村日本淡水生物学" (ed. by 上野益三)，北隆館，東京，489-495.

Abbreviations in **Plates**

abd.: abdomen, bl.: blastopore, br.: brain, car.: carapace, com.e.: compound eye, end.: endopodite, ex.: exopodite, f. br.: fore-brain, h. br.: hind-brain, lab.: labrum, man.: mandible, m. br.: mid-brain, opt. l.: optic lobe, r. opt.l.: rudiment of optic lobe, rost.: rostrum, stom.: stomodaeum, tel.: telson, th. abd.pl.: thoracic abdominal plate, ven. pl.: ventral plate, y.: yolk, lst. ant.: lst antenna, 2nd.ant.: 2nd antenna, lst. gan.: lst ganglion, 2nd. gan.: 2nd ganglion, 3rd. gan.: 3rd ganglion, lst. ml.: lst maxilla, 2nd. ml.: 2nd maxilla, lst. mp.: lst maxilliped, 2nd. mp.: 2nd maxilliped, 3rd. mp.: 3rd maxilliped, 3rd. mp. end.: endopodite of 3rd maxilliped.

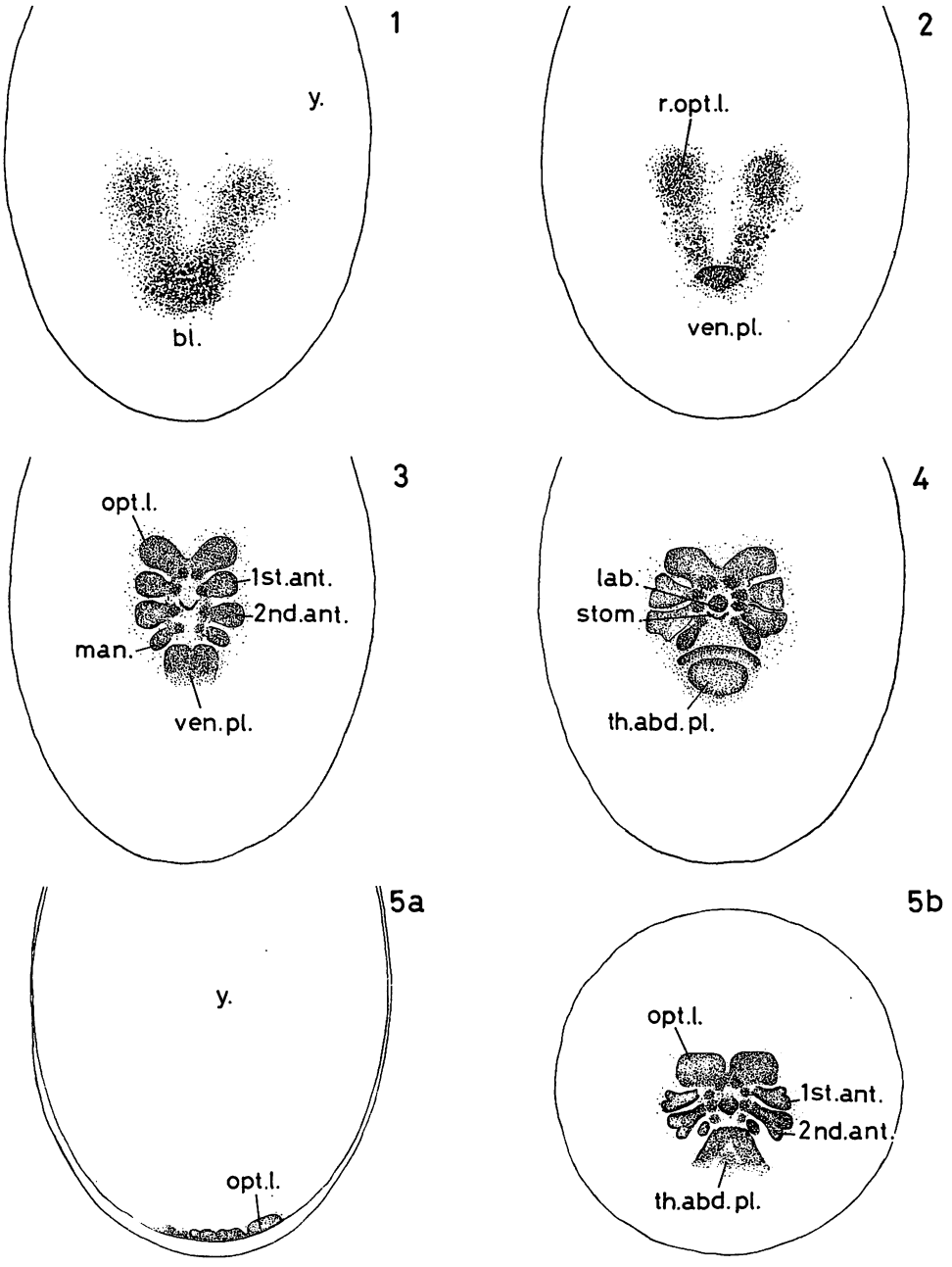

Plate 1. Early stages of the embryonic development from the blastopore to completion of the embryonic movement towards the posterior pole. Each number in the plate corresponds to the successive post-time after spawning as follows. 1 : 30hrs., 2 : 40hrs., 3 : 50hrs., 4 : 60hrs., 5 : 80hrs.

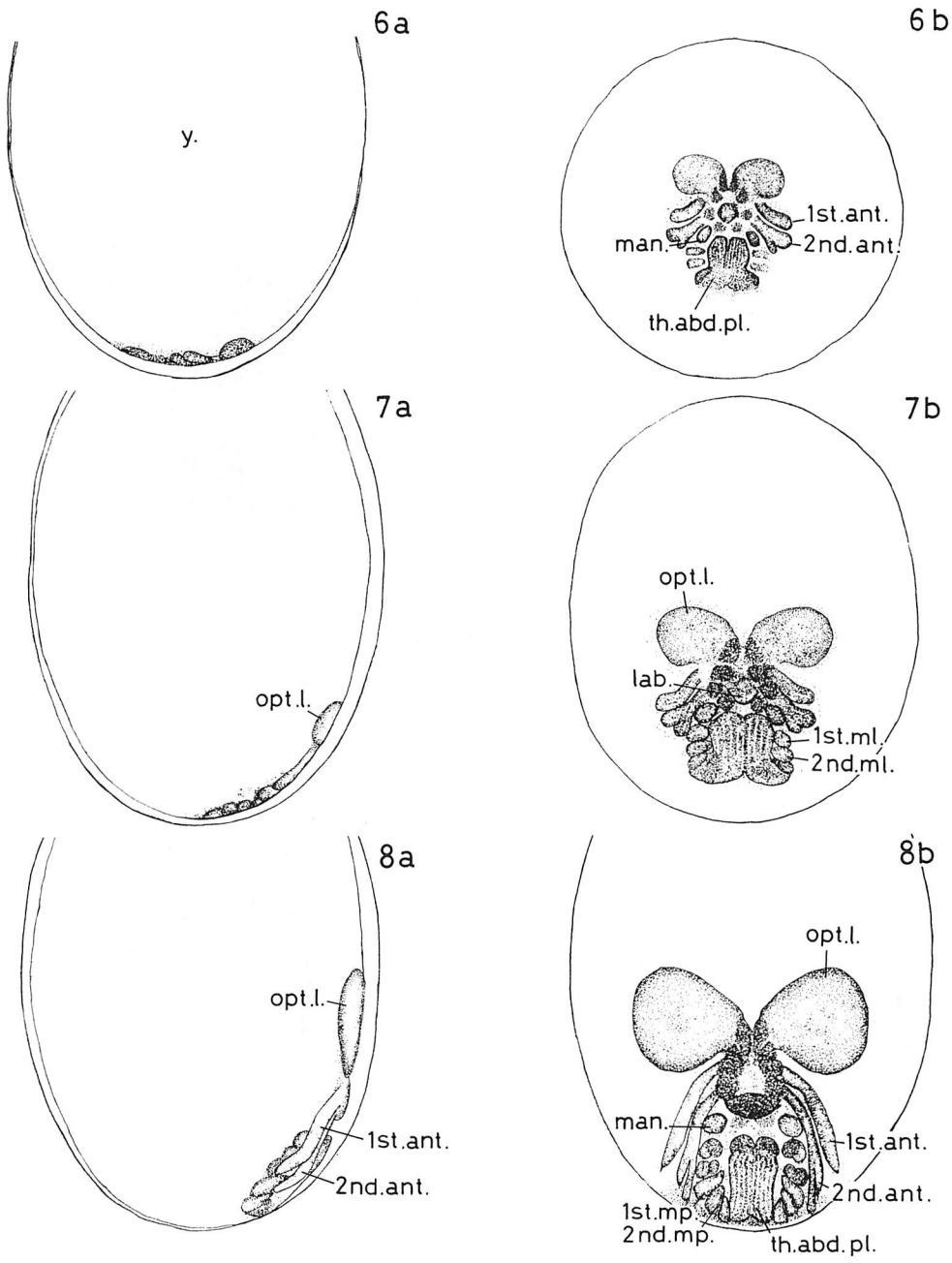

Plate 2. Developmental stages from the polar position to appearances of the lst and 2nd maxillipeds. The letter **a** and **b** in the plate mean the right-lateral and ventral views, respectively. Also, each number corresponds to the successive post-time after spawning as follows. 6 : 90hrs., 7 : 104hrs., 8 : 128hrs.

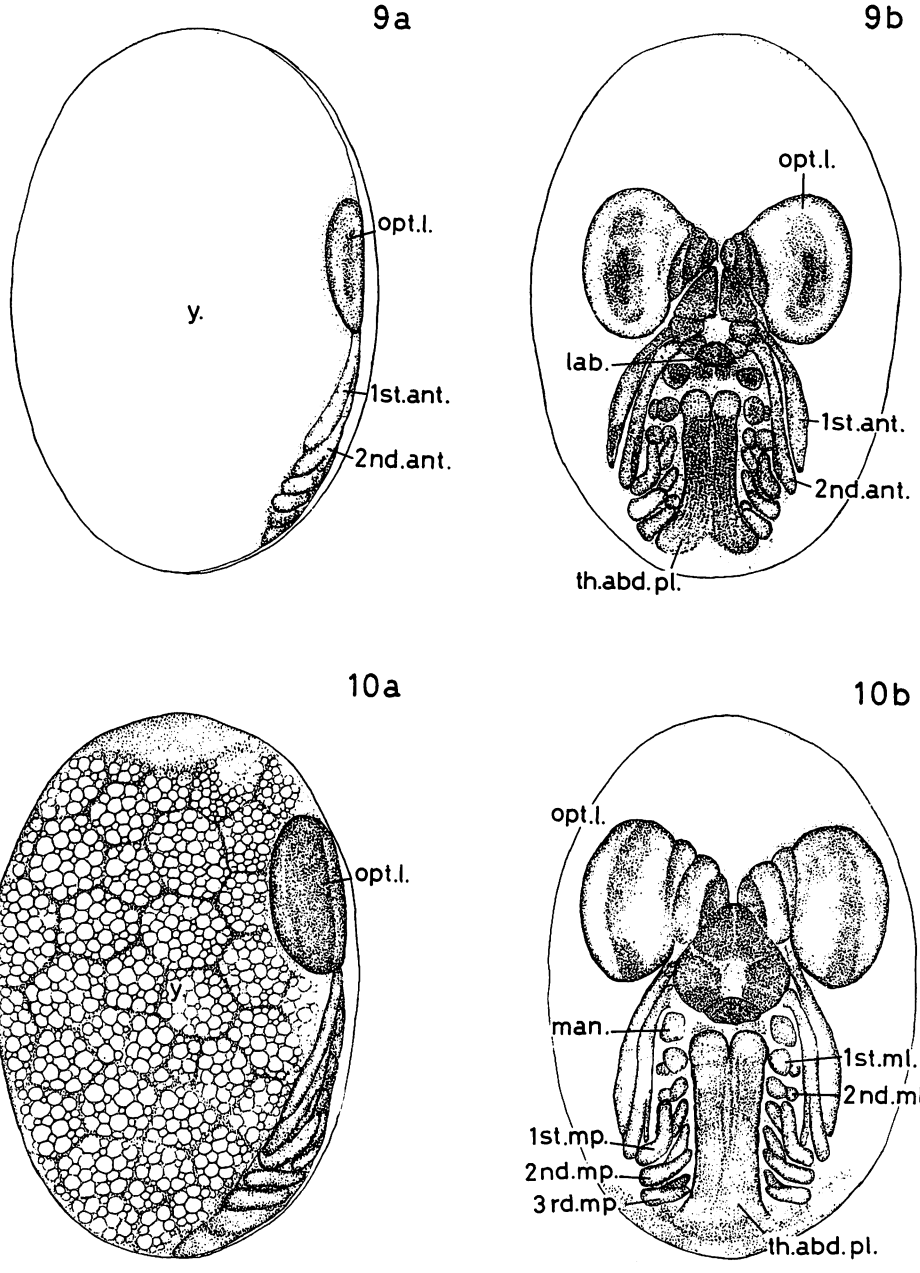

Plate 3. Developmental stages from an appearance of pigment in the optic lobe to the thoracic abdominal plate's attaining to the labrum. The letter **a** and **b** in the plate mean the right-lateral and ventral views, respectively. Also, each number corresponds to the successive post-time after spawning as follows. 9 : 152hrs., 10 : 176hrs.

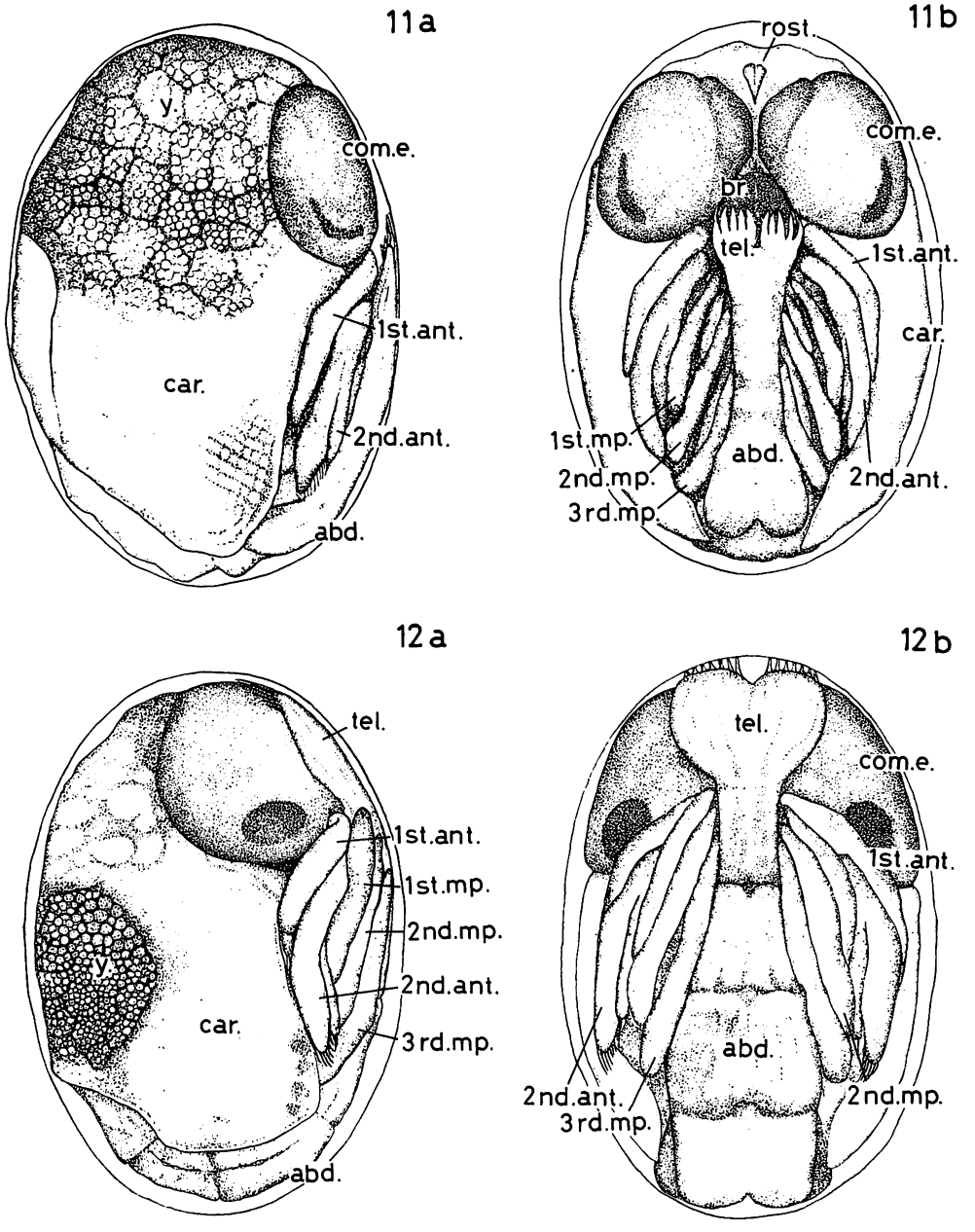

Plate 4. Developmental stages from the rostral formation to the thoracic abdominal plate's attaining to the anterior pole. The letter **a** and **b** in the plate mean the right-lateral and ventral views, respectively. Also, each number corresponds to the successive post-time after spawning as follows. 11 : 248hrs., 12 : 320hrs.

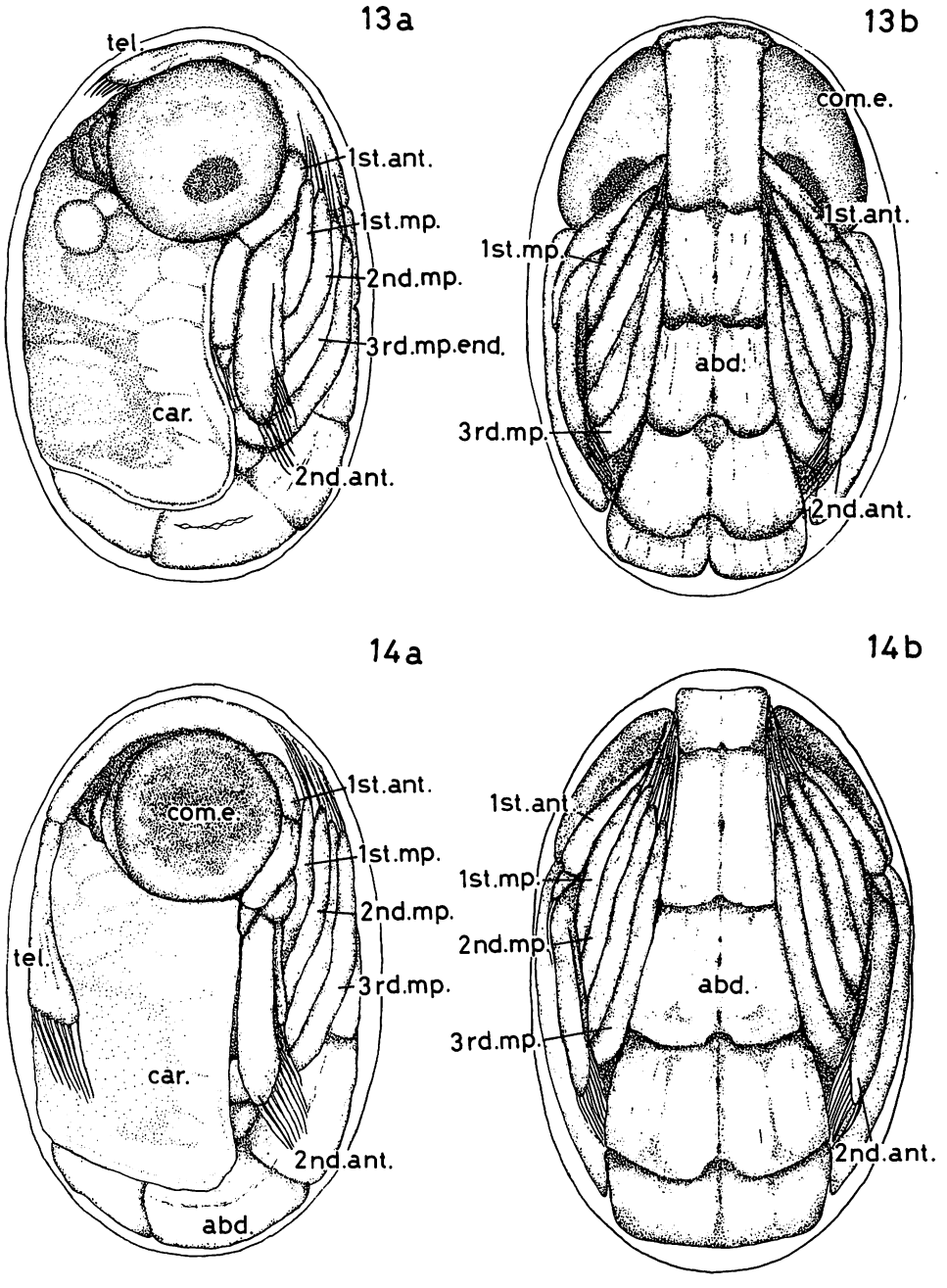

Plate 5. Final developmental stages from the yolk exhaustion to the telson's attaining to the equator. The letter **a** and **b** in the plate mean the right-lateral and ventral views, respectively. Each number in the plate corresponds to the successive post-time after spawning as follows. 13 : 392hrs., 14 : 416hrs.

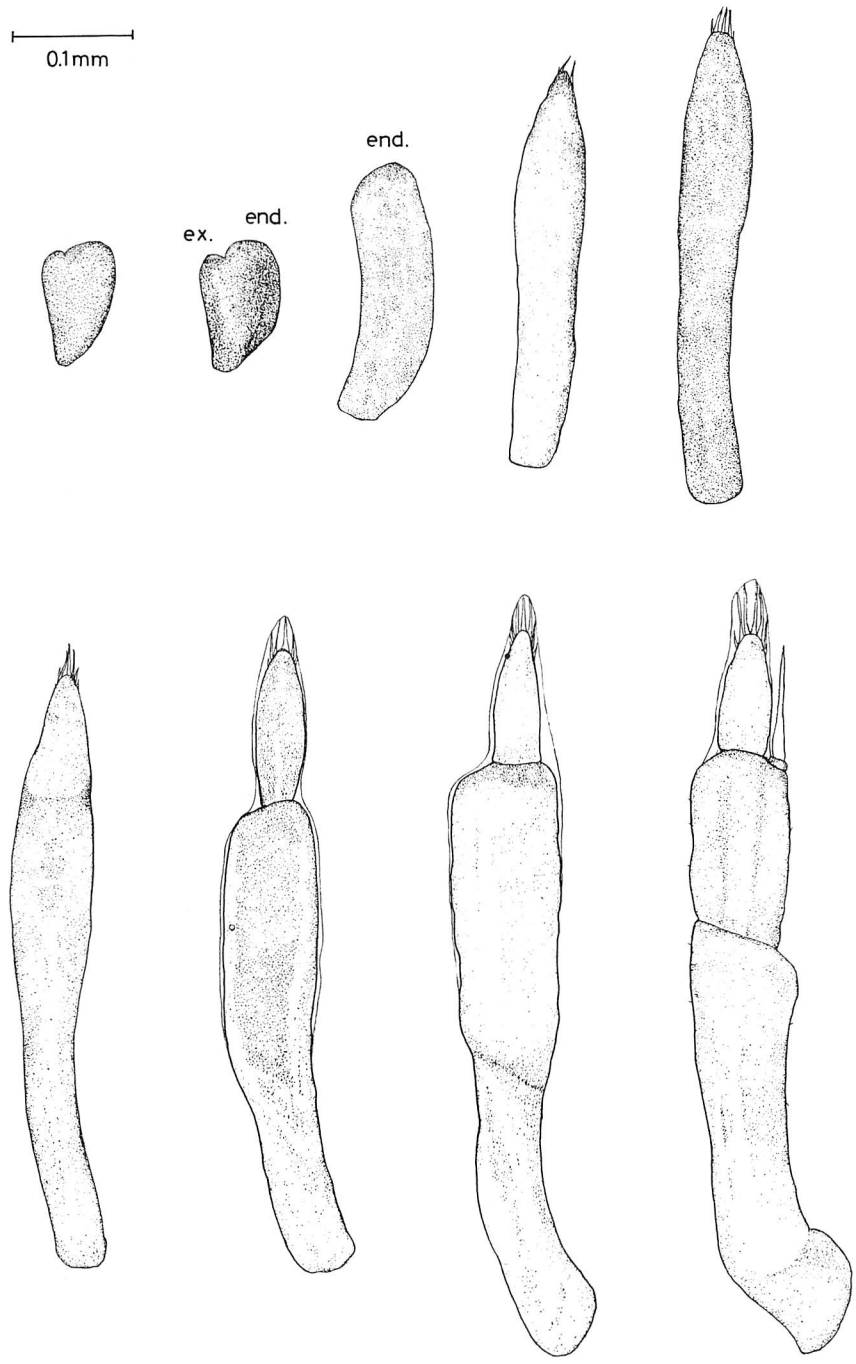

Plate 6. Developmental procedure of the lst antenna, showing a temporary biramous condition at its early period. Each drawing corresponds to 60, 80, 100, 128, 152, 176, 248, 320 and 392 hours after spawning, respectively.

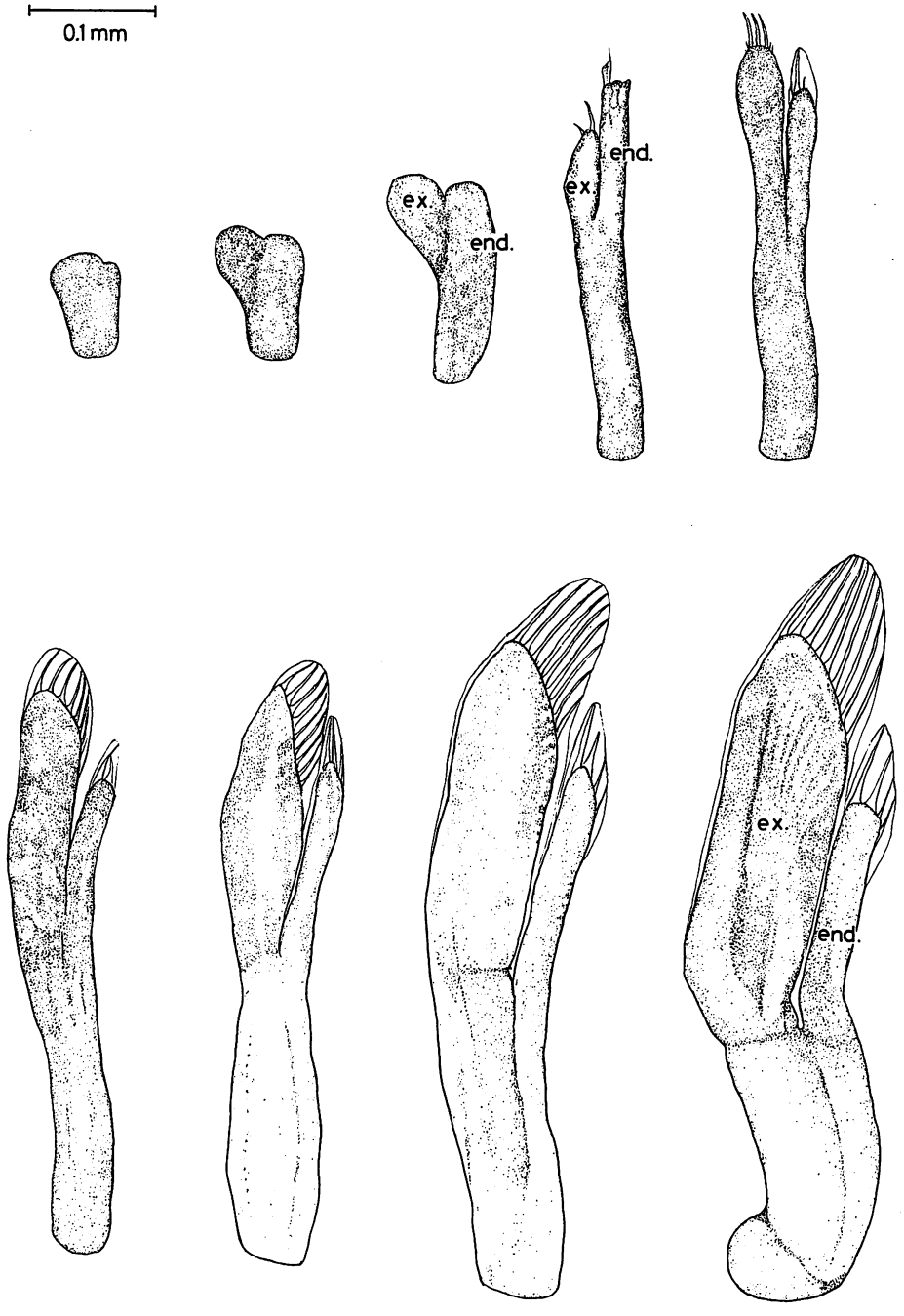

Plate 7. Developmental procedure of the 2nd antenna, showing a temporary reversion of the length between the endo- and exo-podites at their early differentiation. Each drawing corresponds to 60, 80, 100, 128, 152, 176, 248, 320 and 392 hours after spawning, respectively.

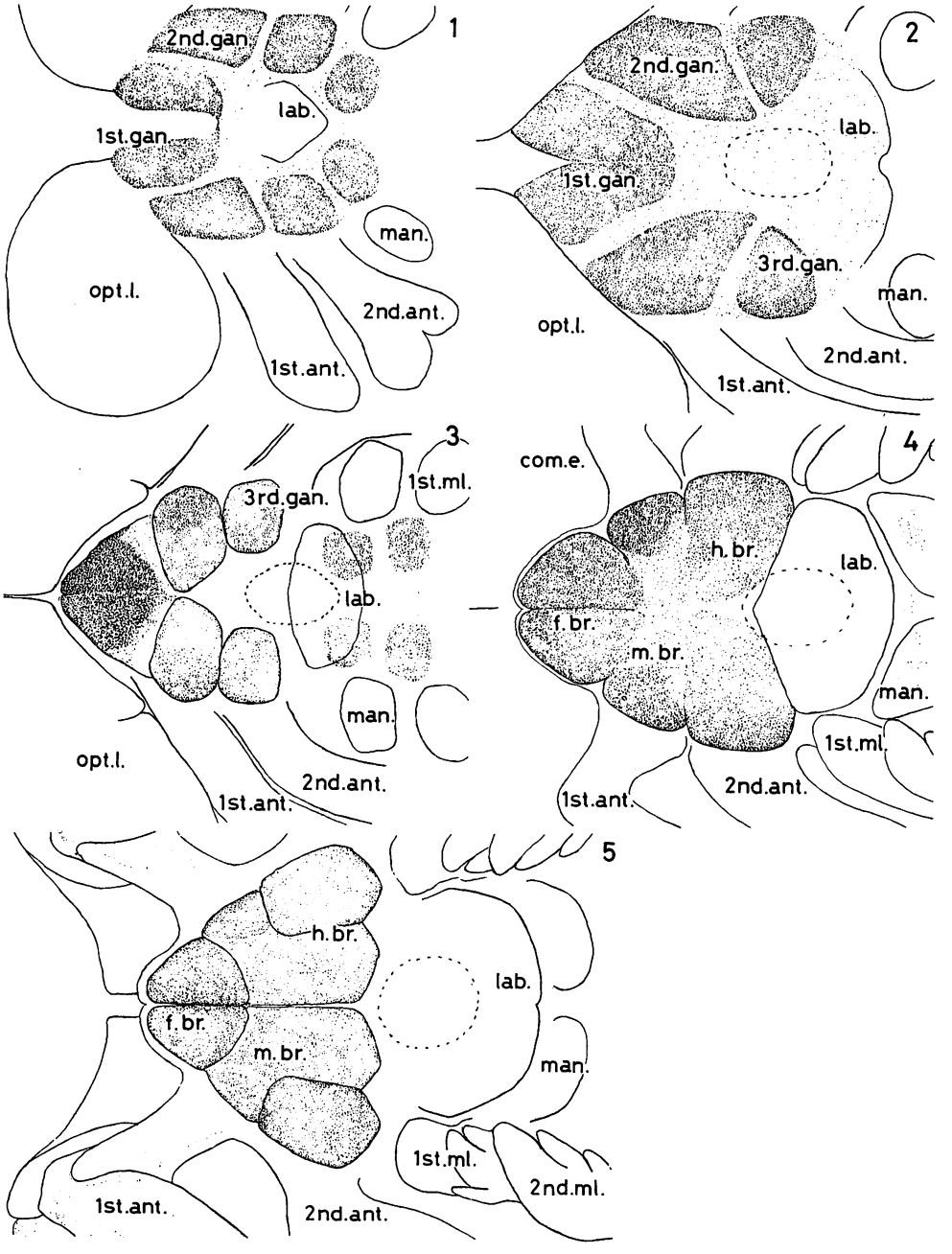

Plate 8. Fundamental pattern of the brain formation, observed externally. The lst ganglion situated at the proximal of the optic lobe forms the fore-brain. The 2nd and 3rd ganglions which are situated at the proximals of the lst and 2nd antennae form the mid- and hind-brains, respectively. Each number in the plate corresponds to the successive post-time after spawning as follows. 1 : 90hrs., 2 : 128hrs., 3 : 176hrs., 4 : 320hrs., 5 : 392hrs.