[REVIEW]

Evolutionary Changes in Developmental Potentials of the Embryo Proper and Embryonic Membranes in Hexapoda: A Synthesis Revised

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1. Introduction

Machida and Ando (1998) demonstrated that the evolutionary changes in embryonic membranes and evolutionary transition of developmental potentials (*involving dorsal closure and secretion of the cuticular egg envelope*) in the embryo proper and embryonic membrane are clearly recognized in Atelocerata (=Myriapoda+Hexapoda) and that the amnioserosal fold–amniotic cavity system shown in Dicondylia (=Zygentoma+Pterygota) could be rationally elucidated in this context.

The concept presented by Machida and Ando (1998) is as follows. 1) The most primitive condition is when no definite functional specialization has taken place between the embryo and embryonic membrane (serosa) concerning dorsal closure and secretion of the cuticular egg envelope. Such a condition, shown in Myriapoda, is regarded as the plesiomorphic state in the Atelocerata. 2) In Hexapoda, the embryonic membrane (serosa) renounces participation in the definitive dorsal closure, and consequently this task is exclusively performed by the embryo: this is an autapomorphy of this group. 3) Further, in Ectognatha, the following events occur as autapomorphies of this group: i) the renouncement of the ability to secrete the cuticular egg envelope by the embryo, ii) the formation of the embryonic membrane fold (serosal or amnioserosal) for cuticular secretion beneath (ventrally to) the embryo, iii) the differentiation of a second embryonic membrane or the amnion, and iv) the temporal specialization of "dorsal closure in the embryonic period" or provisional dorsal closure by the serosa and amnion. 4) Finally, Dicondylia acquire the following autapomorphies: i) the close linkage of the production of the amnion to the formation of the embryonic membrane fold, and ii) the resultant amnioserosal fold–amniotic cavity system and the assignment of functional aspects concerned, to the embryonic membranes (serosa and amnion). The original and primary role of the amnioserosal fold (and amnioserosal fold–amniotic cavity system) must have been the secretion of a cuticular layer (cuticular egg envelope) beneath the embryo, in lieu of the embryo which had lost this ability.

The above-mentioned scheme of Machida and Ando (1998) was reconstructed, premising that Atelocerata are monophyletic and that the embryonic membrane and functional specialization involving the embryo and embryonic membrane are comparable in Collembola and Diplura, entognathous hexapod representatives for which embryogenesis has been investigated. Recently, an argument, however, has been put forward supposing a closer affinity and the monophyly of Crustacea and Hexapoda (see Dohle, 1997; Reiger and Schultz, 1997; Schultz and Reiger, 2000): this kind of argument mainly comes from the molecular evolutionary biology, developmental biology, sense organ morphology and developmental neurology. Also, a recent embryological study on Diplura (Ikeda and Machida, 2001) revealed that their embryonic membrane system significantly differs from that of Collembola. The present paper investigated the revisions to these recent findings and information, focusing on Hexapoda, considering the above-mentioned Machida and Ando's (1998) scheme on the evolutionary changes in embryonic membranes and functional specialization in the

embryo and embryonic membrane.

The monophylies of Myriapoda and Crustacea are still controversial (see Ax, 1987; Dohle, 1988; Reiger and Schultz, 1997; Wilson *et al.*, 2000; Schultz and Reiger, 2000). However, for both groups, such an argument may be premature, and details of paraphyly proposed are highly variable. Thus, we adopted the traditional framework supposing both groups to be monophyletic.

2. Embryonic membranes and functional specialization in the embryo and embryonic membrane in Crustacea

Most previous crustacean embryological studies have made little reference not only to the functional specialization of the dorsal closure and secretion of the cuticular egg envelope in the embryo and embryonic membrane, which the present paper focuses on, but also to the differentiation of embryonic and extraembryonic areas and the characterization of the embryonic membrane. The reason may be as follows. First, because (related to the conclusion of this section) the crustacean extraembryonic area maintains the potential for participation in body formation or definitive dorsal closure, this makes determination whether it is embryonic or extraembryonic very difficult, and it is hard to understand the functional specialization between the embryonic and extraembryonic areas and between the embryo and embryonic membrane. In primitive forms of crustaceans, furthermore, the extraembryonic area seems to be practically lacking (Bensch, 1969). Second, crustaceans are usually aquatic, with some representatives having different breeding habits, and in some cases the embryonic period is very short. It may readily be imagined that such arthropods often lack the cuticular egg envelope, which may have a protective or homeostatic role. That is, in several crustacean forms, the potential for secretion of the cuticular egg envelope cannot be analyzed. Third, the ambiguousness of the embryonic and extraembryonic areas in some cases makes it practically impossible to determine whether it is the cuticular egg envelope or embryonic/larval cuticle.

Crustacean embryological studies were surveyed by covering more taxa (Dohrn, 1870; Grobben, 1881; Brooks, 1882; Weldon, 1892; McMurrich, 1895; Kühn, 1908, 1913; Taube, 1909, 1915; Fuchs, 1914; Cannon, 1924; Heath, 1924; Manton, 1928, 1934; Terao, 1929; Tokioka, 1936; Hickman, 1937; Goodrich, 1939; Shiino, 1942, 1950; Vagin, 1947; Nair, 1949; Weygoldt, 1958, 1960; Oishi, 1959, 1988; Barker, 1962; Sanders, 1963; Scholl, 1963; Strömberg, 1965, 1967, 1971; Anderson, 1969, 1973; Bensch, 1969; Zilch, 1974). Most of these studies, as in the case mentioned above, proved to refer little to the issues concerned, so relevant data will be extracted from them as much as possible in order to generalize the embryological features in crustaceans.

2.1. Embryonic membranes in Crustacea

First, the extraembryonic area in Crustacea is defined, referring to Anderson (1973). In primitive crustaceans, eggs are small and perform typical holoblastic cleavage (cf. Bensch, 1969). All resultant blastomeres, including 1a–1d, 2a–2d, 3a–3d and 4d destined to ectoderm, develop into individual tissues and organs, and contribute to the formation of body structures. In advanced crustaceans such as malacostracans, however, eggs are generally far larger because of heavy accumulation of yolk, and perform either superficial or holoblastic cleavage with cytoplasmic division retarded or suppressed, and the blastoderm forms (Manton, 1928, 1934; Shiino, 1942, 1950; Weygoldt, 1958; Strömberg, 1965, 1967, 1971; *etc.*). Then, a germ disc differentiates in a small area of the blastoderm, and an extensive area other than the germ disc is represented by an attenuated cellular layer directly derived from the original blastoderm. The germ disc is more or less comparable to the whole embryo or all the derived blastomeres of small-sized eggs in primitive crustaceans, because the former and the latter are accurately coincident with each other in distribution of presumptive areas. This implies that the germ disc of large-sized eggs of advanced crustaceans should be equivalent to the whole embryo of small-sized eggs of primitive crustaceans. Thus, the germ disc can be designated as the embryonic area or embryo proper. Hence, any extensive surface area other than the germ disc, or the area occupied by the attenuated cellular layer directly derived from the original blastoderm, which does not differentiate in the small-sized eggs with a typical holoblastic cleavage, may be regarded as being newly acquired in advanced crustaceans as a covering of egg surface expanded in association with the accumulation of yolk (*i. e.*, "dorsal closure in the embryonic period"), and designated as the extraembryonic area regardless of its fate, that is, whether or not it participates in the formation of body structures (*e. g.*, the definitive dorsal closure). Eggs of Cirripedia perform holoblastic cleavage typical of small-sized crustacean eggs (Anderson, 1969). With progressive embryogenesis, cells of a small dorsal area are attenuated to

become a thin cellular layer. The area occupied by this cellular layer is comparable to the extraembryonic area shown in malacostracan eggs, as Anerson (1969, 1973) thought. We find a wide variation in the extension of the extraembryonic area in Crustacea, in accordance with the amount of yolk accumulated: most extensive in malacostracans, a condition found in cirripedians, but which is much less extensive or practically lacking in branchiopods (Bensch, 1969).

Thus expansion of the egg surface occurs in crustaceans, although there is a wide variation according to the amount of yolk, and the extraembryonic area differentiates. The extraembryonic area is represented by an attenuated cellular layer directly derived from the original blastoderm (or a superficial layer of blastula). This cellular layer is accurately comparable to the serosa shown in Hexapoda and is designated as such, categorized as the embryonic membrane (cf. Machida and Ando, 1998).

A second embryonic membrane is reported for isopod malacostracans. In Oniscoidea (*Porcellio, Armadillidium*), development proceeds and all the serosal cells fall into the yolk and degenerate (Goodrich, 1939). Then, the area from which the serosa regressed is occupied by a thin cellular layer originating from the embryonic margin. This newly appeared cellular layer, prior to the final stage of organogenesis, concentrates dorsally, and finally degenerates without participating in definitive dorsal closure. A similar cellular layer is reported in Valivifera (*Idotea*), but it does not degenerate, taking part in definitive dorsal closure, as does the serosa (Strömberg, 1965). Goodrich (1939) called this cellular layer, or second embryonic membrane, as an amnion. This amnion could and should be recognized as a specialized structure derived in a part of advanced malacostracan crustaceans. Therefore, further reference to the isopod amnion may be unnecessary in the present paper (it is not referred to in Table 1 and Fig. 1), because information on the crustacean embryonic membrane selected here is relevant to the determination of the ancestral state of Hexapoda. Consecquently, the crustacean embryonic membrane system may be, in the present paper, generalized as follows. The embryonic membrane differentiates in Crustacea and is basically represented by the serosa.

Ectognathous hexapods develop a structure formed by folding of the embryonic membrane or the embryonic membrane fold, *i. e.*, the serosal or amnioserosal fold (cf. Machida and Ando, 1998). It deserves a special mention that crustaceans, even isopods with amnions, do not form any structures comparable to the embryonic membrane fold, the same as myriapods (see Goodrich, 1939; Strömberg, 1965; Anderson, 1973).

2.2. Dorsal closure and secretion of the cuticular egg envelope in Crustacea

In a primitive syncarid malacostracan, *Anaspides* (Anaspidacea), the cuticular egg envelope is reported to be secreted during the egg-nauplius stages (Hickman, 1937) (Fig. 1A1). In an isopod malacostracan, *Limnoria*, Strömberg (1967) also reported a structure, which may be identifiable as being cuticular, although he designated it an "embryonic membrane," to be segregated in the stages prior to blastokinesis. It is also well known that in decapod malacostracans, the secretion of the cuticular egg envelope begins from around the dorsal organ (Shiino, 1950; *etc.*). The cuticular egg envelopes shown in these malacostracans are apparently derived both from the embryo and serosa, and they may be categorized as the blastoderm cuticle, according to Machida and Ando's (1998) definition. In an anostracan branchiopod, *Artemia*, in which differentiation of the extraembryonic area is hardly discernible, some layers of cuticular egg envelopes are reported to be segregated (Bensch, 1969).

As for participation by the serosa in definitive dorsal closure, it has been clearly asserted in some crustaceans, while other papers and/or their figures allude to this possibility: Cirripedia (Anderson, 1969); Anaspidacea (Hickman, 1937); Thermosbaenacea (Barker, 1962); Isopoda (Strömberg, 1965, 1971); Tanaidacea (Scholl, 1963) (Fig. 1A2, 3). In crustaceans with larger eggs such as decapod malacostracans, it is reported that an excessive part of the serosa is cast off, forming a dorsal organ (Manton, 1928, 1934; Shiino, 1950; *etc.*) (Fig. 1A4), which may be comparable to the secondary dorsal organ of ectognathous hexapods. Similar partial degeneration of the serosa is also shown in a chilopodan myriapod, *Scolopendra* (Heymons, 1901; Heymons and Heymons, 1905) (Fig. 1A4). Anyway, we can say that the crustacean embryonic membrane or serosa achieves definitive dorsal closure, in cooperation with the embryo, besides functioning as a "dorsal closure in the embryonic period" or as a covering of the egg surface expanded in association with the accumulation of yolk (Fig. 1A1, 2).

2.3. Conclusion

The embryonic membrane, which is basically represented by the serosa (see the above section 2.2), is also
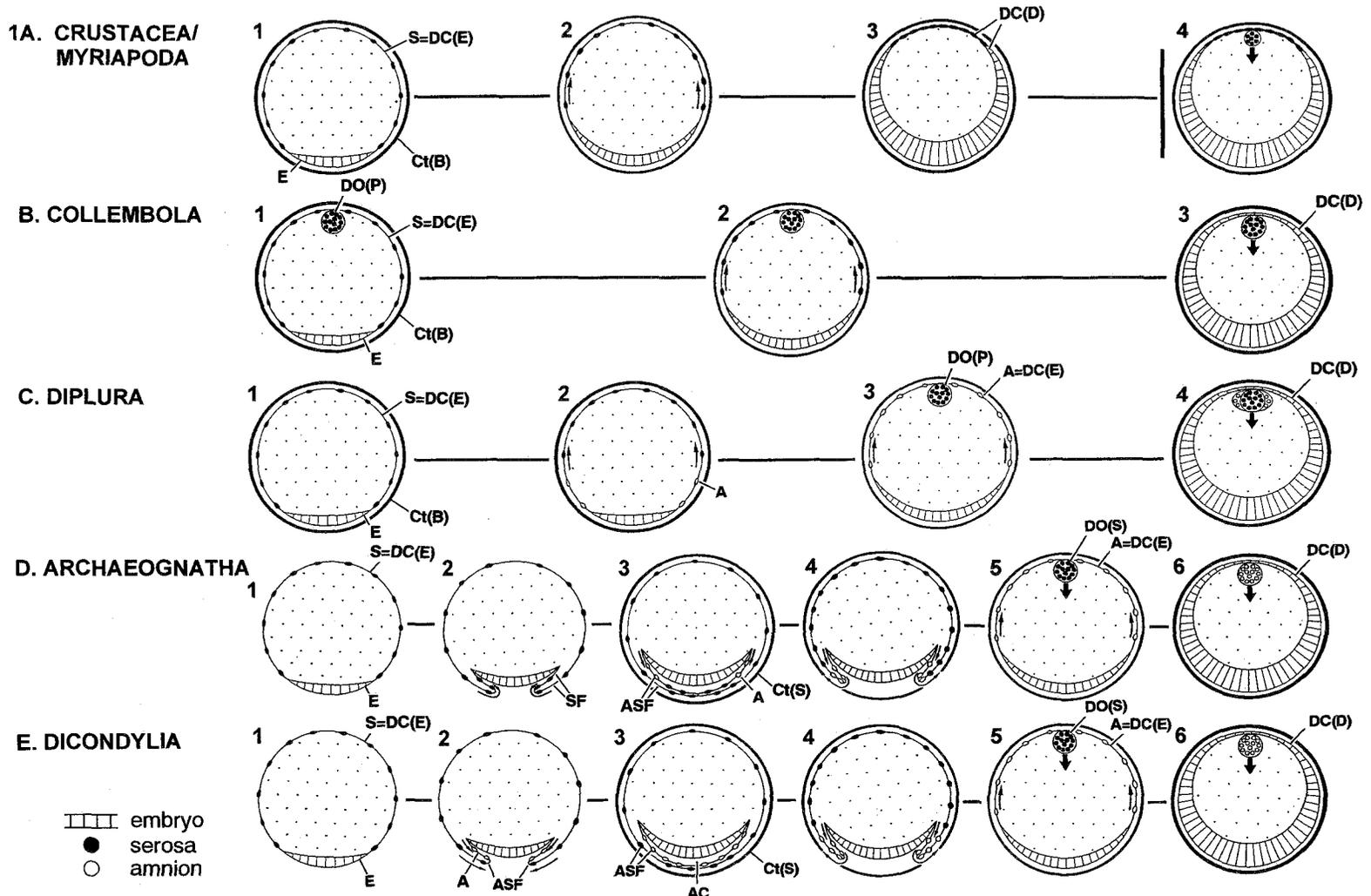
Figure 1

differentiated in Crustacea. The serosa, which originally functions as a "dorsal closure in the embryonic period," has the ability to differentiate into the definitive dorsal wall or participate in definitive dorsal closure, and the embryo has the ability to secrete the cuticular egg envelope, as well as the serosa. Thus, specialization regarding the functions concerned is not definite between the embryo and embryonic membrane (serosa).

The embryonic membrane system and functional specialization in the embryo and embryonic membrane in Crustacea are exactly comparable to those in Myriapoda [Table 1; Fig. 1A; see also Machida and Ando (1998)]. Therefore, even if Crustacea are classed as a sister group of Hexapoda instead of Myriapoda, the scheme that Machida and Ando (1998) developed, at least regarding the elucidation of the ancestral state of Hexapoda, could be accepted without revision.

[In some crustacean forms, an aggregation of "glandular" cells, closely resembling the primary dorsal organ of Collembola and Diplura, is formed in the dorsal area of the serosa in the early developmental stages (Shiino, 1950; Weygoldt, 1958; Strömberg, 1965, 1967; Zilch, 1974; etc.), as well as in a symphylan myriapod, *Hanseniella* (Tiegs, 1940) and pauropodan myriapod, *Pauropus* (Tiegs, 1947) (not shown in Fig. 1, because the structure does not always appear in all Crustacea and Myriapoda). In the present paper, the structure is simply dealt with as a structure derived from the serosal area, and further reference to it is not made.]

Fig. 1 Embryonic development of different mandibulate groups. Protura on which we have no available embryological data concerned are excluded. A: Myriapoda and Crustacea. 1. Blastoderm differentiates into the embryo and serosa, and a cuticular egg envelope or blastoderm cuticle is secreted. 2, 3. With the progression of embryogenesis, definitive dorsal closure proceeds and completes, and the serosa, which was functioning as a "dorsal closure in the embryonic period," also participates. 4. In some forms of crustaceans and myriapods, excessive serosal cells degenerate without participating in definitive dorsal closure. B: Collembola. 1. Blastoderm differentiates into the embryo and serosa, in which a primary dorsal organ develops at its dorsal side, and a cuticular egg envelope or blastoderm cuticle is secreted. 2, 3. With the progression of embryogenesis, the definitive dorsal closure proceeds and completes. The serosa, which was functioning as a "dorsal closure in the embryonic period," is concentrated dorsally, to degenerate in the same way as the primary dorsal organ, without participating in the definitive dorsal closure. C: Diplura. 1. Blastoderm differentiates into the embryo and serosa, and a cuticular egg envelope or blastoderm cuticle is secreted. The serosa is functioning as a "dorsal closure in the embryonic period." 2, 3. Serosal cells migrate dorsally to form a primary dorsal organ, and the area from which the serosa receded is occupied by an amnion segregated from the embryonic margin. The amnion now functions as a "dorsal closure in the embryonic period," instead of the serosa. 4. With the progression of embryogenesis, definitive dorsal closure proceeds and is completed, but the serosa (primary dorsal organ) and amnion do not participate in it. D: Archaeognatha. 1. Blastoderm differentiates into the embryo and serosa. 2. Serosa invades beneath (ventrally to) the embryo, to form a serosal fold. 3. The serosal fold closes beneath the embryo, and generally, soon becomes an amnioserosal one with the formation and supplementation of amnion. Now, the whole egg surface is covered by the serosa, and a cuticular egg envelope or serosal cuticle is secreted. 4, 5. After the secretion of a serosal cuticle, the embryonic membrane fold (an amnioserosal one but rarely a serosal one) is soon regressed, and the serosa, which was functioning as a "dorsal closure in the embryonic period," is condensed as a secondary dorsal organ, to degenerate. The amnion replaces the serosa and functions as a second "dorsal closure in the embryonic period." 6. With the progression of embryogenesis, definitive dorsal closure proceeds and completes, but the amnion does not take part in it. E: Dicondylia. 1. Blastoderm differentiates into the embryo and serosa. 2. Accompanying the production of amnion from the embryonic margin, the serosa invades beneath (ventrally to) the embryo, to form an amnioserosal fold. 3. The amnioserosal fold closes beneath the embryo, and an amniotic cavity appears between the fold and embryo, to form the amnioserosal fold–amniotic cavity system. Now, the whole egg surface is covered by the serosa, and a cuticular egg envelope or serosal cuticle is secreted. 4, 5. Katatrepsis occurs. The amnioserosal fold is regressed, and the serosa, which was functioning as a "dorsal closure in the embryonic period," is condensed as a secondary dorsal organ and degenerates. The amnion replaces the serosa and functions as a second "dorsal closure in the embryonic period." 6. With the progression of embryogenesis, the definitive dorsal closure proceeds and completes, but the amnion does not participate in it. A: amnion, AC: amniotic cavity, ASF: amnioserosal fold, Ct: cuticular egg envelope, Ct(B): blastoderm cuticle, Ct(S): serosal cuticle, DC: dorsal closure, DC(D): definitive dorsal closure, DC(E): "dorsal closure in the embryonic period," DO: dorsal organ, DO(P): primary dorsal organ, DO(S): secondary dorsal organ, E: embryo, S: serosa, SF: serosal fold.

3. Evolutionary changes in embryonic membranes and functional specialization in the embryo and embryonic membrane in Hexapoda

As discussed above, whichever Crustacea or Myriapoda are postulated as the sister group of Hexapoda, the ancestral or plesiomorphic state for Hexapoda can be recognized as being completely identical. Therefore, a reexamination of Machida and Ando's (1998) scheme using new information on dipluran embryogenesis (Ikeda and Machida, 2001) begins within the coverage of Hexapoda. Protura, on which we have no available embryological data concerned, are excluded from the discussion.

3.1. Embryonic membrane system in Diplura

Ikeda and Machida (2001), using rhabduran *Lepidocampa weberi* as material, referred to the embryonic membrane system of Diplura as follows. First, the blastoderm differentiates into the embryo and serosa. The embryo and serosa soon cooperate to secrete a cuticular egg envelope, *i. e.*, a blastoderm cuticle [for definition, see Machida and Ando (1998)]. After secretion of the blastoderm cuticle (Fig. 1C1), the serosa commences to move dorsally, and finally concentrates at the dorsal area of the egg, to form a primary dorsal organ (Fig. 1C2, 3). The extraembryonic area from which the serosa receded in association with the dorsal organ formation is now occupied by a cellular layer derived from the embryonic margin (Fig. 1C2, 3). With progressive definitive dorsal closure, this newly formed cellular layer concentrates dorsally toward the dorsal organ, and is fated to degenerate similarly to the serosa or dorsal organ, without participating in the definitive dorsal closure (Fig. 1C4). This cellular membrane is characterized by: 1) being derived from the proliferation of cells of embryonic margin, and 2) not participating in the definitive dorsal closure, like the serosa, although it plays a role in "dorsal closure in the embryonic period," it can be homologized with the amnion of Ectognatha, and be designated as such. The embryonic membranes of Diplura or the serosa and amnion do not form any structures comparable to the embryonic membrane fold shown in Ectognatha, *i. e.*, a serosal or amnioserosal fold (Fig. 1C1–4).

The embryonic membrane system of Diplura differs markedly from that of Collembola, in which the embryonic membrane is represented only by the serosa (Jura, 1972; Uemiya and Ando, 1987) (Fig. 1B). Machida and Ando's (1998) scheme was developed, assuming that the embryonic membrane systems in Diplura and Collembola were comparable with each other.

[It is well known that the primary dorsal organ forms in Collembola the same as in Diplura, although a difference is recognized in its formation between Collembola and Diplura: in the former the structure is developed by partial thickening of the serosa (Jura, 1972) (Fig. 1B1), not by the concentration of the serosa as observed in Diplura. In the present paper, these structures are simply dealt with as structures derived from the serosal area, and further reference to them is not given.]

3.2. Phylogenetic account of Diplura

Diplura acquire the amnion, and temporal specialization concerning "dorsal closure in the embryonic period" occurs between the serosa and amnion: earlier with the former, later with the latter (Table 1; Fig. 1C). According to Machida and Ando (1998), the acquisition of amnion and temporal specialization regarding "dorsal closure in the embryonic period" by the serosa and amnion are regarded as autapomorphies of Ectognatha (Fig. 1D, E). New information on the dipluran embryonic membrane system, however, requires revision of this idea. That is, there being no evidence refuting the homologization of the amnions of Diplura and Ectognatha, "the acquisition of amnion and the temporal specialization regarding dorsal closure in the embryonic period by the serosa and amnion" could and should be attributed to Diplura plus Ectognatha as their autapomorphies. In this respect, Diplura can be recognized as an advanced step when compared to Collembola, in which the embryonic membrane is represented only by the serosa (Table 1; Fig. 1B *vs* 1C).

There still exists, however, a significant difference between Diplura and Ectognatha, in the light of the functional specialization in the embryo and embryonic membrane. Namely, in Diplura the embryo still retains potential for the secretion of a cuticular egg envelope (Table 1; Fig. 1C1), whereas in the Ectognatha the embryo has lost this ability, leaving it exclusively to the serosa, and an embryonic membrane fold develops in order to maintain the secretion of cuticular layer beneath (ventrally to) the embryo (Table 1; Fig. 1D2, 3, E2, 3): thus, in the light of the functional specialization in the embryo and embryonic membrane, Diplura are less advanced than Ectognatha. In Diplura, of

Table 1   Functions of the embryo proper and embryonic membrane in different mandibulate groups.

	Embryo	Embryonic membranes	
		Serosa	Amnion
Crustacea/Myriapoda	1, 3	1, 2, 3	—
Collembola	1, 3	1, 2	—
Diplura	1, 3	1, 2'	2"
Archaeognatha	3	1, 2', 4	2"(,4)
Dicondylia	3	1, 2', 4'	2", 4'

Protura on which we have no available embryological data concerned are excluded. Numerals imply functions shared: 1: secretion of a cuticular egg envelope, 2: "dorsal closure in the embryonic period," 2': "dorsal closure in the embryonic period," until relieved by the amnion, 2": "dorsal closure in the embryonic period," after degeneration of the serosa, 3: definitive dorsal closure (formation of the body wall or participation in definitive dorsal closure), 4: formation of embryonic membrane fold, 4': formation of the amnioserosal fold–amniotic cavity system. For details, see text and Machida and Ando (1998).

which the embryo has potential for the secretion of a cuticular layer, the embryonic membrane fold is unnecessary, and actually they do not develop one (Ikeda and Machida, 2001).

3.3. Conclusion

The above-mentioned is summarized as follows (see also Table 1; Figs. 1, 2). 1) Diplura, in which the amnion is acquired and temporal specialization occurs concerning "dorsal closure in the embryonic period," are one stage more advanced than Collembola. 2) The acquisition of amnion and temporal specialization of "dorsal closure in the embryonic period" by the serosa and amnion are recognized as autapomorphies of Diplura plus Ectognatha. 3) The renouncement of the potential for the secretion of a cuticular egg envelope by the embryo and resultant production of embryonic membrane fold are autapomorphic to Ectognatha: Diplura, of which the embryo retains the potential for secretion of the cuticular egg envelope, and in which the embryonic membrane fold is yet to be acquired, are less advanced than Ectognatha in the light of functional specialization in the embryo and embryonic membrane.

4. Conclusion

Traditionally, the sister group of the Hexapoda has been thought to be Myriapoda, whereas the data recently accumulated may be suggestive of Crustacea (see 1. Introduction), but the argument has not been settled. However, even providing that Crustacea are set as the sister group of Hexapoda, a scheme that, presupposing the sister group relationship of the Myriapod and Hexapoda, Machida and Ando (1998) developed concerning the evolutionary changes of the embryonic membrane and evolutionary transition of the functional specialization in the embryo and embryonic membrane in Atelocerata, does not need to be amended, at least as to the elucidation of the ancestral or plesiomorphic state in Hexapoda (Table 1; Fig. 1).

New information on dipluran embryology requires a revision to Machida and Ando's (1998) scheme in understanding Collembola and Diplura, as discussed above. Their concept in the other respects on Hexapoda, however, remains valid and may be accepted without any amendment. Table 1 summarizes the evolutionary changes of the embryonic membrane and functional specialization in the embryo and embryonic membrane in different hexapod groups plus the Crustacea and Myriapoda as candidates of a hexapod sister group (including those that have not been directly examined and mentioned here), reevaluating and revising Machida and Ando's (1998) concept. Likewise, Figure 1 illustrates the embryogenetic processes of these groups, with special reference to the issues concerned. For details, especially concerning the hexapods not directly examined here, see Machida and Ando (1998); an explanation of Figure 1 is also available. Figure 2 synthesizes in a form of phylogenetic tree the information concerning the evolutionary changes of the embryonic membrane and functional specialization in the embryo and embryonic membrane in Hexapoda.

What Figure 2 shows is summarized as follows. 1) The most plesiomorphic is the condition that the functional specialization between the embryo and embryonic membrane (serosa) concerning dorsal closure and secretion of the
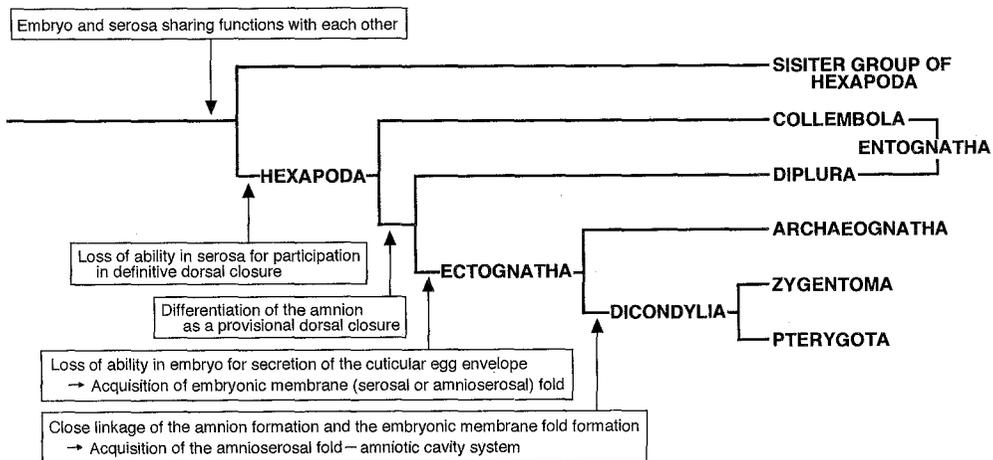
Fig. 2   Hexapod phylogeny reconstructed in the light of evolutionary changes of embryonic membrane and functional specialization in the embryo proper and embryonic membrane. Crustacea or Myriapoda are thought to be a sister group of Hexapoda. Protura, on which we have no available embryological data, are excluded. For details, see text and Machida and Ando (1998).

cuticular egg envelop is not definite (Fig. 1A). Such a condition is seen in the candidates for the hexapod sister group, i. e., Crustacea and Myriapoda. 2) In Hexapoda, the serosa renounces participation in definitive dorsal closure, and consequently it is exclusively performed by the embryo (Fig. 1B–E): this is an autapomorphy of this group. 3) Further in Diplura–Ectognatha, the following occurs as their autapomorphies: i) the differentiation of amnion, and ii) the temporal specialization of "dorsal closure in the embryonic period" (provisional dorsal closure) by the serosa and amnion (Fig. 1C–E). 4) Then, in Ectognatha, the followings are acquired as their autapomorphies: i) the renouncement of the potential to secrete the cuticular egg envelope by the embryo, and ii) the formation of an embryonic membrane fold (serosal or amnioserosal) for cuticular secretion beneath (ventrally to) the embryo (Fig. 1D, E). 5) Finally, in Dicondylia the amnion is produced with closely linked to the formation of the embryonic membrane fold, and an elaborated amnioserosal fold–amniotic cavity system is acquired (Fig. 1E). i) The close linkage of the production of amnion to the formation of the embryonic membrane fold, and ii) the acquisition of amnioserosal fold–amniotic cavity system and the assignment of functional aspects concerned to the embryonic membrane, are recognized as autapomorphies of this group.

The hexapod phylogeny deduced from the present discussion (Fig. 2) rejects Entognatha. "Entognatha" is a taxon established with entognathous mouth-parts regarded as their most important autapomorphy (Hennig, 1981), and have been widely accepted. However, as for the monophyletic status of Entognatha, Kukalová-Peck (1987) from her morphological study on the mouth-parts of an ancient dipluran, Koch (1997, 2000) from his anatomical comparisons on entognathans, and Kraus (1997) from his comprehensive morphological survey of atelocerates cast some doubt. We (Ikeda and Machida, 1998) have already shown a similar trend in light of the comparison between dipluran and collembolan entognathy formation.

5. Remarks

Deducing that the amnion is not a requisite constituent in the amnioserosal fold of Archaeognatha and that the archaeognathan amnion only bears a plan as a provisional dorsal closure instead of regressed serosa (Machida et al., 1994), Machida et al. (1994) and Machida and Ando (1998) concluded that the primary and ancestral amnion plan should have been as in Archaeognatha. The finding of amnion in Diplura (Ikeda and Machida, 2001), which can be recognized as being a group retaining the ancestral state of Ectognatha considering evolution of embryonic membrane and functional specialization in the embryo and embryonic membrane, reinforces their conclusion. That is, in Diplura, notwithstanding deficiency of the structure comparable to the embryonic membrane fold, the amnion does differentiate and functions as a provisional dorsal closure occupying the area from which the serosa has been regressed.

Machida *et al.* (1994) and Machida and Ando (1998) deduced that the embryonic membrane fold should have been acquired in the ectognathous ancestors, close to Archaeognatha, so as to secrete a cuticular egg membrane beneath (ventrally to) the embryo which had renounced the potential of cuticular secretion. Then, the production of amnion being closely linked to the formation of embryonic membrane fold, the embryonic membrane fold as shown in Archaeognatha develops into the elaborated amnioserosal fold–amniotic cavity system of Dicondylia, which is now closely involved in their embryogenesis, and may be maintained under an unknown but surely important developmental plan. Whatever the plan is, Machida and Ando (1998) assumed that the functional role of the system should have originally and primarily lain in the secretion of a cuticular layer as an egg envelope beneath the embryo, the same as in the archaeognathan embryonic membrane fold (see also Tojo and Machida, 1997, 2001). They pointed out that the secretion of a cuticular egg envelope unexceptionally occurs during the period the system or the amnioserosal fold exists in Dicondylia. Recently, our embryological studies revealed that the cuticular egg envelope or serosal cuticle is secreted in the period between the anatrepsis and katatrepsis or when the amnioserosal fold exists, in some dicondylian representatives: *i. e.*, in the most primitive dicondylian Zygentoma (Masumoto and Machida, 2002), in Ephemeroptera (Tojo and Machida, 1997, 2001), which were comparative embryologically substantiated to be the most primitive pterygote group (Tojo and Machida, 2002), and one of the most primitive neopteran pterygotes Notoptera (Uchifune and Machida, 2002).

Appendix: In Machida and Ando (1998) *Proc. Arthropod. Embryol. Soc. Jpn.*, **33**, 1–13, misprintings were found: "Fig. 1c-1" in 11 line from bottom and "Fig. 1c-3" in 6 line from bottom of p. 3 should respectively be "Fig. 1a-1" and "Fig. 1a-3."

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