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Palaeoptera or Metapterygota?: A Comparative Embryological Approach*

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Abstract

The phylogenetic relationships between the major three pterygote groups, *i. e*., Ephemeroptera, Odonata, and Neoptera, were examined in light of comparative embryology, focusing on the following characters: [1] the amnioserosal fold, [2] the germ band type, [3] the cleavage type, [4] the egg tooth, [5] the formation of the midgut epithelium, [6] the mode of embryonic invagination, [7] the formation of the proctodaeum, [8] the micropyle, [9] the formation of germ rudiment, [10] the formation of clypeolabral rudiment, [11] the development of hypopharynx, [12] the caudal filament, and [13] the exite structures on thoracic appendicular bases. The embryological and morphological groundplans in insects or pterygotes were also discussed as well as their path of evolutionary transition. The pterygote phylogenies currently proposed were reviewed from the comparative embryological standpoint, and the following phylogenetical relationship was supported: [Ephemeroptera + Metapterygota (= Odonata + Neoptera)].

Introduction

Insects account for three quarters of all animal species, and more than 99 percent of them are of the wing-acquired type or the Pterygota. The evolution of insects or pterygotes, which have attained spectacular diversity and radiation, is an interesting subject.

The phylogeny of the basal clades of pterygotes remains controversial. Three phylogenetical hypotheses have been proposed concerning the interrelationships between the major pterygote groups, *i. e*., the Ephemeroptera, the Odonata, and the Neoptera. Hennig (1953, 1969) from morphological evidence and Rohdendorf *et al*. (1962) based on paleontology supported the phylogeny [Palaeoptera (= Ephemeroptera + Odonata) + Neoptera]. Kristensen (1975, 1991) mainly from morphological evidence and Wheeler and Carpenter (1996) from an overall analysis supported the phylogeny [Ephemeroptera + (Odonata + Neoptera)]. Lemche (1940) and Matsuda (1981) through studies of comparative morphology, and Boudreaux (1979) based on functional morphological evidence and the development of wing buds supported the phylogeny [Odonata + (Ephemeroptera + Neoptera)]. Recently, a range of molecular phylogenetical approaches have been adopted to further examine this problem. However, solutions to the "Palaeoptera" or "Metapterygota" problem remain controversial (Wheeler *et al*., 2001; Hovmöller *et al*., 2002; Ogden and Whiting, 2003; Kjer, 2004; Regier *et al*., 2005; Terry and Whiting, 2005; Kjer *et al*., 2006; Mallat and Giribet, 2006). Thus, the study of Ephemeroptera appears to be critical to attempts to elucidate the various evolutionary transitions and changes to the basic body plan and the groundplan of morphogenesis in insects or pterygotes and to reconstruct their phylogeny.

A comparative embryological approach is useful for understanding the groundplan, elucidating the evolutionary transition of the body plan, and speculating on phylogeny. Despite a number of studies such as Joly (1876), Heymons (1896a, b, c), Murphy (1922), Ando and Kawana (1956), Wolf (1960), Bohle (1969) and Tsui and Peters (1974), ephemeropteran embryology is still not well understood. I have been conducting a comparative embryological study of the Ephemeroptera, using several species as materials: primarily the ephemerid mayfly *Ephemera japonica* (*e. g*., Tojo and Machida, 1996, 1997a, b, 1998a, b, 2001, 2003; Miyairi and Tojo, 2007).

In this paper, based on comparative embryological studies, I will discuss the evolutionary transitions of the body plan and groundplan of embryogenesis of insects or pterygotes, so as to re-examine the pterygote phylogeny, with special reference to the affinity of the three major pterygote groups, the Ephemeroptera, Odonata and Neoptera.

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Re-evaluation of embryological characters

The following 13 embryological characters were used to elucidate the phylogeny of basal clades of the Insecta, and the relationships between Ephemeroptera, Odonata and Neoptera: [1] the amnioserosal fold, [2] the germ band type, [3] the cleavage type, [4] the egg tooth, [5] the formation of midgut epithelium, [6] the mode of embryonic invagination, [7] the formation of proctodaeum, [8] the micropyle, [9] the formation of germ rudiment, [10] the formation of clypeolabral rudiment, [11] the development of hypopharynx, [12] the caudal filament, and [13] the exite structures on thoracic appendicular bases.

[1] The amnioserosal fold

In myriapods and entognathan insects, the embryonic membrane is represented by the serosa, and neither the amnion nor the amnioserosal fold is developed. These structures first appeared in the Ectognatha [=Archaeognatha + Dicondylia (= Zygentoma + Pterygota)] as an autapomorphy (cf. Machida *et al*., 1994, 2002; Machida and Ando, 1998; Masumoto and Machida, 2006).

In the Dicondylia, the amnioserosal fold is completely fused, forming the amnioserosal fold-amniotic cavity, which had been considered an autapomorphy of the group. The amnioserosal fold was inherited by the archaeognathan and dicondylian (including the Ephemeroptera) lineages as a synapomorphy, and in the latter it transformed itself into the amnioserosal fold-amniotic cavity system.

As to the function of this system, certain mechanical advantages, such as protection of the embryo, have been assumed (Sharov, 1966; Ando, 1970, 1988; Zeh *et al*., 1989), but nothing definite is known (cf. Anderson, 1972a). However, Machida *et al*. (1994, 2002) and Machida and Ando (1998) have extended discussion on the functional role of the amnioserosal fold, based on the evolutionary transition of functional specialization between the embryo proper and embryonic membranes in the Atelocerata (= Myriapoda + Insecta) or Pancrustacea (= Crustacea + Insecta). They concluded that the fold was acquired during the evolution of insects in order to secrete the serosal cuticle beneath the embryo, a function being lost in the course of atelocerate or pancrustacean evolution. It has been confirmed that in the ephemeropteran *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 surface of the egg is occupied by the serosa (Figs. 1, 2; Tojo and Machida, 1997a, 2001). This may support the assumption of Machida *et al*. (1994, 2002) and Machida and Ando (1998) that the principal functional role of the amnioserosal fold and the amnioserosal fold - amniotic cavity system in its advanced form lies in the secretion of the serosal cuticle beneath the embryo.

Traits 1 and 1' in figure 12 refer to the amnioserosal fold and the amnioserosal fold-amniotic cavity system, respectively.

[2] The germ band type

The embryo of Ephemeroptera can be categorized as a typical short germ band type, characterized by the sequential proliferation of segments anterior to posterior (cf. Krause, 1939) [*e. g*., baetids *Baetis rhodani* and *Baetis vernus* (Bohle, 1969); ephemerids *Ephemera strigata* (Ando and Kawana, 1956) and *Ephemera japonica* (Fig. 1; Tojo and Machida, 1997a, b, 1998a, b); polymitarcyids *Tortopus incertus* (Tsui and Peters, 1974), *Ephoron shigae* and *Ephoron eophilum* (Tojo and Sekiné, unpublished data)].

The germ rudiment of the short germ band type first appeared in the Archaeognatha (Machida, 1981) and was inherited by the dicondylian lineage [*e. g*., odonates *Epiophlebia superstes* (Ando, 1962) and *Euphaea yayeyamana* (Suzuki *et al*., 2008); plecopteran *Kamimuria tibialis* (Kishimoto and Ando, 1985); higher orders such as paraneopterans (Anderson, 1972a)]. It may be regarded as an autapomorphy of Ectognatha (cf. Sander, 1984).

Traits 2 in figure 12 represents the short germ band type.

Appendix: In the higher neopterans, the germ type is transformed into the long form, which is recognized as an apomorphy (shown by 2'; cf. Krause, 1939).

[3] The cleavage type

Cleavage is fundamentally holoblastic in arthropods, and total cleavage is predominant in primitive insects, at least in the initial stage [*e. g*., proturan *Baculentulus densus* (Fukui and Machida, 2006); collembolans *Isotoma cinerea* (Philiptschenko, 1912) and *Tetrodontophora bielanensis* (Jura, 1965); archaeognathan *Pedetontus unimaculatus* (Machida *et al*., 1990)]. The fertilized eggs of the ephemerid mayfly *Ephemera japonica* (Fig. 1; Tojo and Machida, 1998a, b) and the polymitarcyid mayfly *Ephoron shigae* and *Ephoron eophilum* (Tojo and Sekiné, unpublished data), however, undergo a typical superficial cleavage, which is characteristic of dicondylian insects (Johannsen and Butt, 1941; Sharov, 1966). The superficial cleavage in insects is recognized as an autapomorphy of Dicondylia (cf. Machida *et al*., 1990).

Traits 3 and 3' in figure 12 indicate total and superficial cleavage, respectively.

[4] The egg tooth

In the final embryonic stage of Ephemeroptera, the larval cuticle is secreted beneath the embryonic cuticle, and a sclerotized egg tooth appears in the frontal

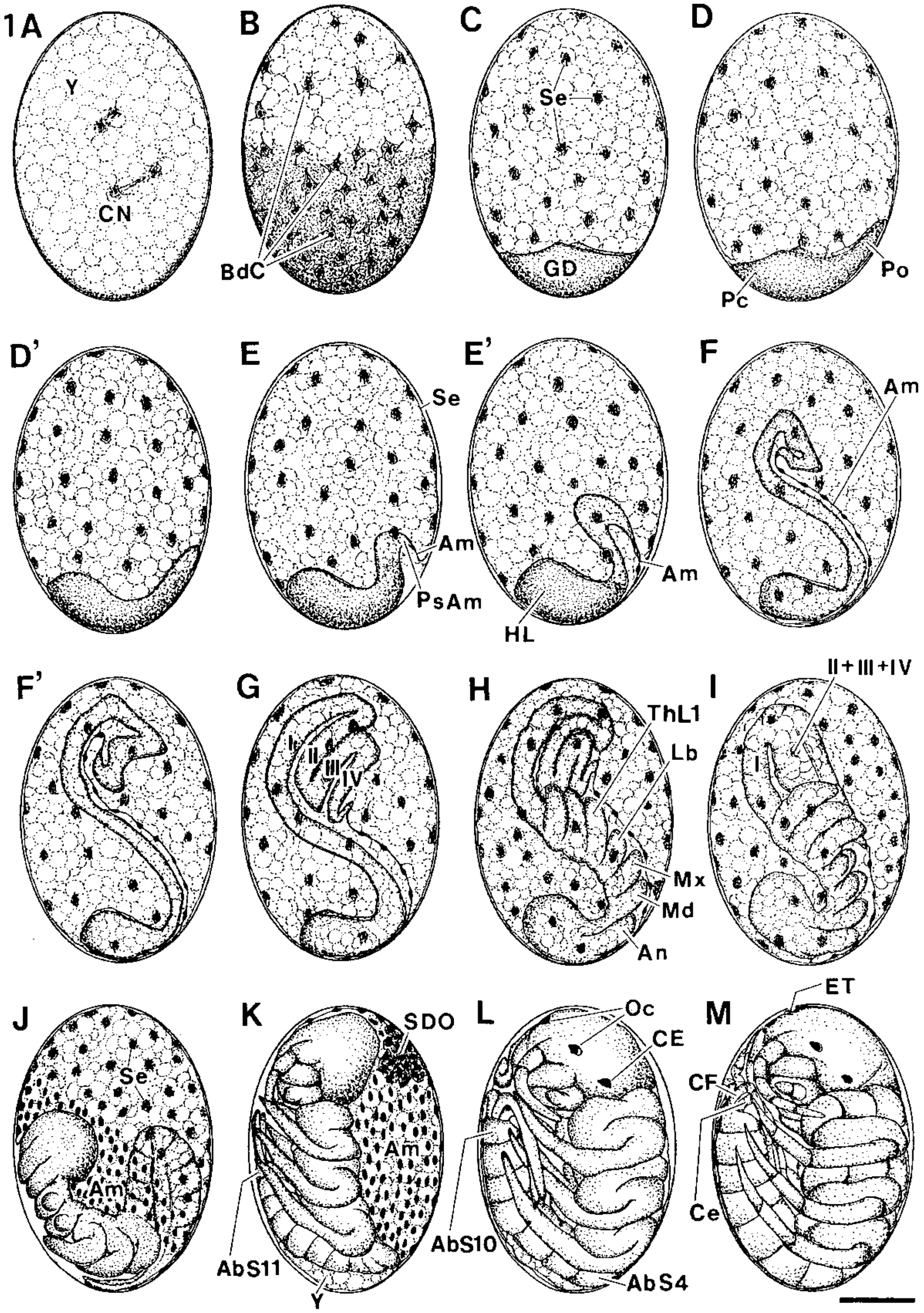


Fig. 1 Successive stages of embryonic development in the ephemerid mayfly *Ephemera japonica*. Lateral views. A. Stage 1 (egg cleavage). B. Stage 2 (blastderm formation). C. Stage 3 (germ disc formation). D, D'. Early (D) and late (D') stage 4 (pear-shaped embryo). E, E'. Early (E) and late (E') stage 5 (start of invagination of germ band). F, F'. Early (F) and late (F') stage 6 (S-shaped embryo). G. Stage 7 (longest embryo). H. Stage 8 (segmentation of embryo). I. Stage 9 (proctodaeum formation). J. Stage 10 (revolution). K. Stage 11 (postrevolution I). L. Stage 12 (postrevolution II). M. Stage 13 (postrevolution III).

AbS4, 10, 11: fourth, tenth, and 11th abdominal segments, Am: amnion, An: antenna, BdC: blastoderm cell, CE: compoud 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, I–IV: abdominal regions I–IV. Bar = 50 μm.

region [*e. g.*, siphlonurid *Siphlonurus lacustris*, heptageniids *Heptagenia sulphurea* and *Heptagenia lateralis* (Degrange, 1960); polymitarcyid *Tortopus incertus* (Tsui and Peters, 1974) ; ephemerid *Ephemera japonica* (Fig. 5; Tojo, 1999)].

The egg tooth is not found in entognathan insects and archaeognathans (cf. Jura, 1972; Machida, 1981), but is present in the zygentomans (cf. Sharov, 1953). Thus it can be recognized as an autapomorphy of the dicondylian lineage (cf. Sharov, 1966; Ando and Kobayashi, 1996).

Traits 4 and 4' in figure 12 indicate the absence and presence of an egg tooth, respectively.

[5] Formation of the midgut epithelium

The midgut epithelium is exclusively derived from yolk cells in 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, 1897; Ikeda, 2001) and Archaeognatha (Machida and Ando, 1981). On the other hand, in the pterygotes, more strictly the neopterans, the midgut epithelium is entirely derived from the midgut epithelial rudiments arising from the blind ends of the stomodaeum and proctodaeum, and is ectodermal in origin (cf. Johannsen and Butt, 1941; Anderson, 1972a, b; Ando and Kobayashi, 1996).

Tojo (1999) revealed that the midgut epithelium in the ephemeropteran *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 originates from yolk cells (Fig. 6).

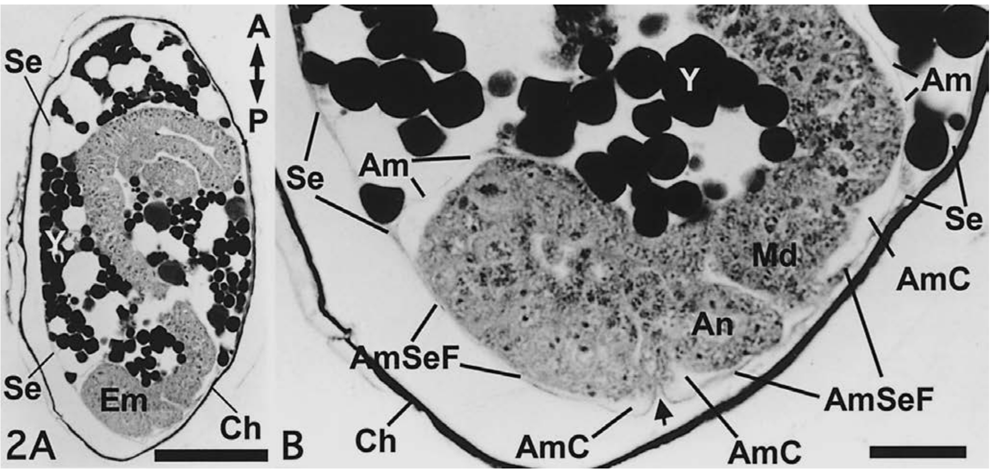
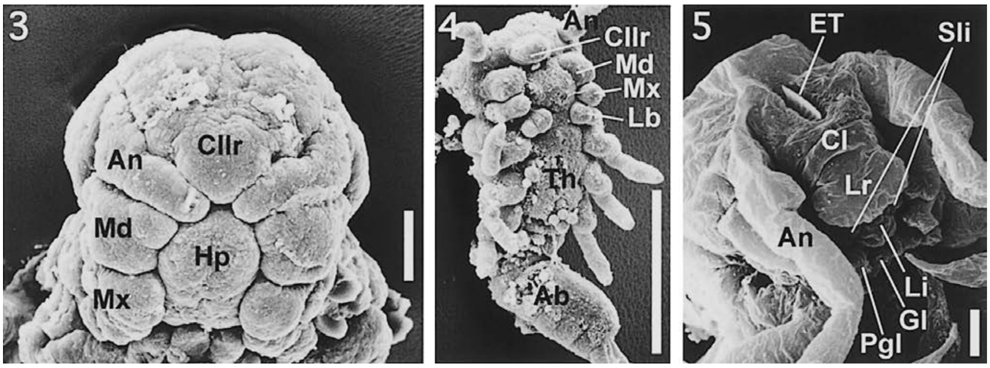


Fig. 2 Formation of the amnioserosal fold in the ephemerid mayfly *Ephemera japonica*. A. Sagittal section of an egg, at late stage 6, in which anatrepsis is just completed. B. Enlargement. The amnioserosal folds fuse with each other beneath the embryo at the level of the labrum (arrow), and anatrepsis is completed.

Am: amnion, AmC: amniotic cavity, AmSeF: amnioserosal fold, An: antenna, A-P: anterior-posterior axis, Ch: chorion, Em: embryo, Md: mandible, Se: serosa, Y: yolk. Bars = A: 50 μm; B: 10 μm.



Figs. 3–5 SEMs showing the cephalic morphogenesis in the ephemerid mayfly *Ephemera japonica*.

Fig. 3 Embryo at stage 9, just before katatrepsis, ventral view.

Fig. 4 Embryo at stage 11, just after katatrepsis, ventro-lateral view.

Fig. 5 Embryo at stage 13, just before hatching, ventro-lateral view.

Ab: abdomen, An: antenna, Cl: clypeus, Cllr: clypeolabrum, ET: egg tooth, Gl: glossa, Hp: hypopharynx, Lb: labium, Li: lingua, Lr: labrum, Md: mandible, Mx: maxilla, Pgl: paraglossa, Sli: superlingua, Th: thorax. Bars = 3, 5: 10 μm; 4: 100 μm.

Such a formation of the midgut epithelium has also been reported for the Zygentoma (*e. g., Lepisma saccharina*: Sharov, 1953) and Odonata (*e. g., Epiophlebia superstes*: Ando, 1962), that is, the most ancestral dicondylian Zygentoma, and another representative of the most primitive pterygotes, the Odonata.

An anagenetical transition in the formation of the midgut epithelium occurred in insects: the midgut epithelium is formed exclusively by yolk cells in entognathan insects and in archaeognathans, whereas it is ectodermal in origin in neopterans. In the zygentomans, ephemeropterans and odonates, it is formed by both yolk cells and the ectoderm.

This anagenetical transition can be interpreted in terms of the character states. First, the formation of the midgut epithelial formation by yolk cells is recognized as a plesiomorphic event within insects (cf. Machida and Ando, 1981), because it is basic to the myriapods and crustaceans (Johannsen and Butt, 1941; Anderson, 1973; Machida and Ando, 1981). The participation of the ectoderm in the formation of the midgut epithelium is apomorphic for Dicondylia. In the Neoptera, the midgut epithelium is exclusively ectodermal in origin, implying a loss of ability to differentiate by the yolk cells, a character which can be recognized as being apomorphic to the group.

Traits 5, 5' and 5" in figure 12 indicate, respectively, that the midgut epithelium is formed exclusively by yolk cells, the ectoderm plays a role in the formation, and the midgut epithelium is formed exclusively by the ectoderm, *i. e.*, the yolk cells lost the ability to differentiate into the midgut epithelium.

[6] The mode of embryonic invagination

The invagination of the embryo into the yolk is considered to have first occurred in the Dicondylia, because it appears to be closely linked to the acquisition of the amnioserosal fold-amniotic cavity system (cf. Tojo and Machida, 1997b; Machida and Ando, 1998). Thus, the invagination of embryos can be recognized as a synapomorphy of Zygentoma and Pterygota. The Archaeognatha (*e. g., Pedetontus unimaculatus*: Machida *et al*., 1994; Machida and Ando, 1998) have an amnioserosal fold, but it does not develop into a cavity system.

The deep invagination with S-shaped embryos of the ephemeropterans [*e. g.*, ephemerid *Ephemera japonica* (Figs. 1, 2; Tojo and Machida, 1996, 1997a, b); polymitarcyid *Ephoron eophilum* (Tojo and Machida, 2001); potamanthid *Potamanthus formosus* (Tojo and Machida, 2001); caenid *Brachycercus japonicus* (Tojo and Machida, 2001)] also occurs in representatives of primitive pterygotes such as odonates (cf. Ando, 1962) and polyneopterans (cf. Anderson, 1972a) [as well as in some higher pterygotes such as the paraneopteran orders (cf. Anderson, 1972a)], whereas in the Zygentoma (*e. g., Lepisma saccharina*: Sharov, 1966), the invagination is not so extensive. It may be safely assumed that the

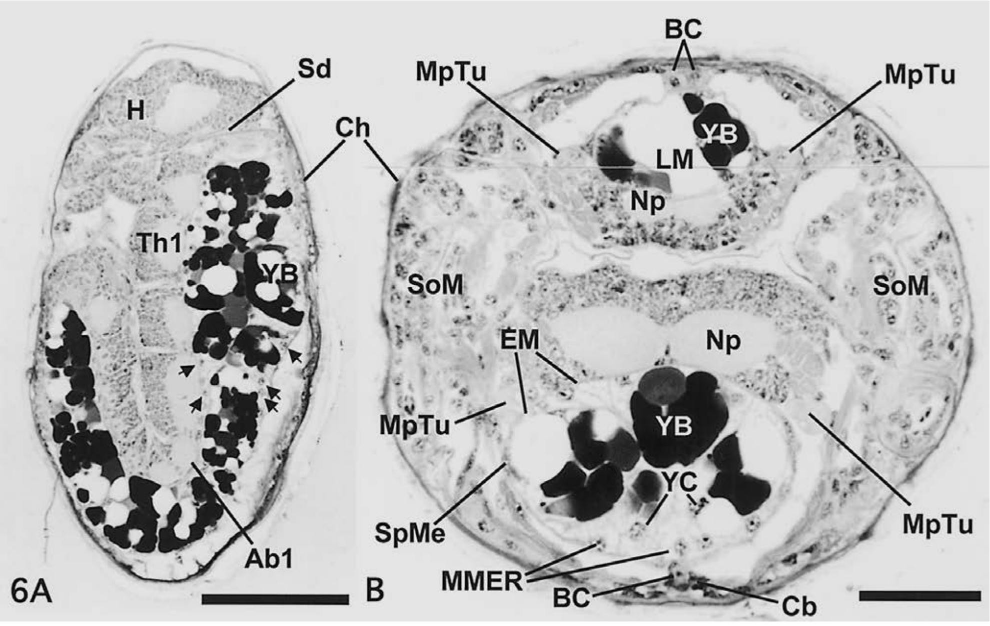


Fig. 6 Formation of the midgut epithelium in the ephemerid mayfly *Ephemera japonica*. A. A sagittal section of an egg at stage 11. B. A cross section of an egg at stage 11, through the metathoracic segment (dorsal side of egg: bottom) and about the 4th to 5th abdominal segment (ventral side of egg: top). Yolk cells migrate onto the ental membrane and settle themselves there (*e. g*., arrows).
Ab1: first abdominal segment, BC: blood cell, Cb: cardioblast, Ch: chorion, EM: ental membrane, H: head, LM: lumen of midgut, MMER: middle region of developing midgut epithelium derived from yolk cells, MpTu: malphigian tubule, Np: neuropile, Sd: stomodaeum, SoM: somatic muscle, SpMe: splanchnic mesoderm, Th1: prothorax, YB: yolk block, YC: yolk cell. Bars = A: 50 μm; B: 20 μm.

deep invagination is basic to the pterygotes and apomorphic to their stem.

Traits 6, 6' and 6" in figure 12 respectively indicate the primitive condition found in Archaeognatha, the invagination of embryos in Zygentoma, and the deep invagination of S-shaped embryos.

Appendix: In some higher neopterans, the invagination-type of embryo is transformed into other forms, which are recognized as apomorphic (shown by 6'"; cf. Johannsen and Butt, 1941).

[7] Formation of the proctodaeum

In apterygotes, *i. e.*, the entognathans, archaeognathans and zygentomans, the proctodaeum is formed as a simple ectodermal invagination like in myriapods and crustaceans. This feature is recognized as plesiomorphic within insects.

In pterygotes, however, the proctodaeum is formed by the fusion of belt-like proctodaeal rudiments, such as in the Ephemeroptera [*e. g.*, baetid *Baetis rhodani* (Bohle, 1969); ephemerids *Ephemera danica* (Heymons, 1896a), *Ephemera strigata* (Ando and Kawana 1956) and *Ephemera japonica* (Figs. 1, 2, 7; Tojo and Machida, 1996, 1997a, b); polymitarcyids *Tortopus incertus* (Tsui and Peters, 1974) and *Ephoron eophilum* (Tojo and Machida, 2001); caenid *Brachycercus japonicus* (Tojo and Machida,

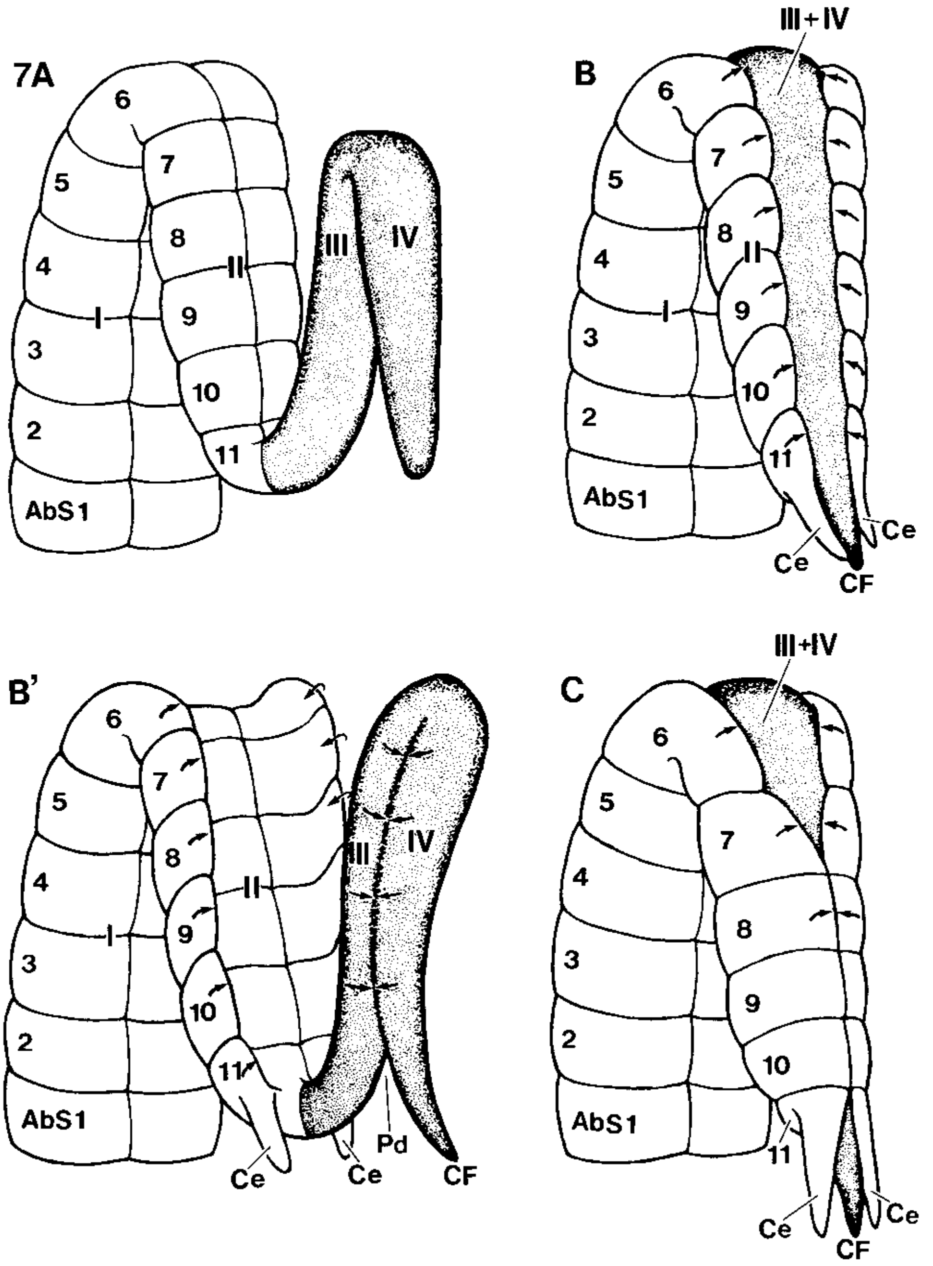


Fig. 7 Diagrammatic representation of the proctodaeal formation in the ephemerid mayfly *Ephemera japonica*. A. Stage 8. B, B'. Early stage 9. In B', regions III and IV are pulled apart from region II to show the developing proctodaeum. C. Late stage 9. The proctodaeum is enclosed by the definitive dorsal closure of region II.

AbS1–11: first to 11th abdominal segments, Ce: cercus, CF: caudal filament, Pd: proctodaeum, I–IV: abdominal regions I–IV.

2001)], Odonata (*e. g., Epiophlebia superstes*: Ando, 1962; *Euphaea yayeyamana*: Suzuki *et al.*, 2008) and some neopteran groups [*e. g.*, hemipterans *Pyrrhocoris apterus* (Seidel, 1924), *Oncopeltus fasciatus* (Butt, 1949) and *Pyrilla perpusilla* (Sander, 1956); mecopteran *Panorpa pryeri* (Suzuki and Ando, 1981); trichopteran *Stenopsyche marmorata* (Miyakawa, 1975); lower lepidopterans *Endoclita signifer* (Kobayashi *et al.*, 1981) and *Neomicropteryx nipponensis* (Kobayashi and Ando, 1988)]. This manner of proctodaeal formation can be regarded as basic in pterygotes and apomorphic to their stem.

Traits 7 and 7' in figure 12 represent a proctodaeum formed by simple invagination and by the fusion of belt-like rudiments, respectively.

Appendix: In some groups of neopterans, the proctodaeum is formed by simple ectodermal invagination, which may be regarded as a new character of each group (shown by 7"; cf. Johannsen and Butt, 1941).

[8] Micropyle

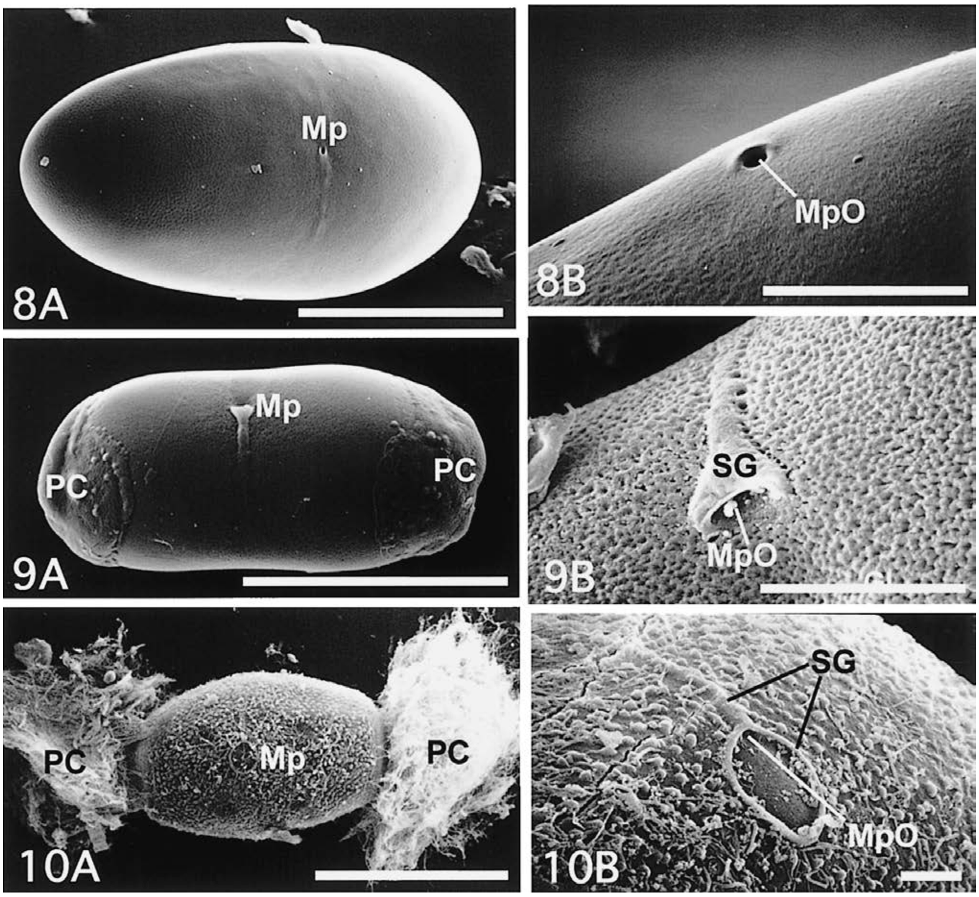
Many ephemeropterans develop micropyles (Figs. 8–10; Degrange, 1960; Koss, 1968; Koss and Edmunds, 1974; Hinton, 1981; Gaino and Mazzini, 1984, 1987, 1988; Gaino *et al.*, 1987, 1989; Tojo and Machida, 1998b, c; Tojo and Matsukawa, 2003; Tojo *et al.*, 2006), as in other pterygote insects (Hinton, 1981; *e. g.*, plecopteran *Perlodes microphala*; phasmid *Carausius morosus*; hemipterans *Triatoma infestans, Belostoma* sp. and *Lethocerus indius*; mecopteran *Harpobittacus australis*; lepidopterans *Crambus pascuellis* and *Lycaena phlaeas*; dipterans *Psila rosae* and *Fannia canicularis*).

Micropyles have not been reported in apterygotes as in myriapods, and the absence of micropyles may be regarded as plesiomorphic.

Traits 8 and 8' in figure 12 indicate the absence and presence of micropyles, respectively.

[9] Formation of germ rudiment

In the Ephemeroptera, a very broad embryonic area is formed, to produce a small germ disc by condensation [*e. g.*, baetid *Baetis rhodani* (Bohle, 1969); ephemerids *Ephemera strigata* (Ando and Kawana, 1956) and *Ephemera japonica* (Fig. 1; Tojo and Machida, 1997a, b, 1998a, b); polymitarcyids *Ephoron shigae* and *Ephoron*



Figs. 8–10 Eggs and micropyles of three ephemeropterans.
Fig. 8 Egg (A) and micropyle (B) of the ephemerid mayfly *Ephemera japonica*.
Fig. 9 Egg (A) and micropyle (B) of the caenid mayfly *Caenis* sp.
Fig. 10 Egg (A) and micropyle (B) of the potamanthid mayfly *Potamanthus formosus*.
Mp: micropyle, MpO: micropylar opening, PC: polar cap (attachment apparatus), SG: sperm guide. Bars = 8A, 9A, 10A: 100 μm; 8B, 9B, 10B: 10 μm.

eophilum (Tojo and Sekiné, unpublished data)]. This broad embryonic area, in which condensation results in the formation of germ rudiments, is unique to insects, and is regarded as an autoapomorphy of the Ephemeroptera.

Traits 9 and 9' in figure 12 indicate the embryonic area common to ectognathans and the broad embryonic area found in the Ephemeroptera, respectively.

[10] The formation of clypeolabral rudiment

In myriapods and apterygotes, the clypeolabrum arises as a single structure [*e. g.*, chilopodan *Scolopendra cingulata* (Heymons, 1901); symphylan *Hanseniella agilis* (Tiegs, 1940); pauropodan *Pauropus silvaticus* (Tiegs, 1947); diplopodan *Glomeris margirata* (Dohle, 1964); collembolan *Tomocerus ishibashii* (Uemiya and Ando, 1987); diplurans *Japyx major* (Silvestri, 1933) and *Lepidocampa weberi* (Ikeda and Machida, 1996, 1998); archaeognathan *Pedetontus unimaculatus* (Machida, 1981); zygentoman *Lepisma saccharina* (Sharov, 1966)], the same as in the Ephemeroptera (*e. g.*, ephemerid *Ephemera japonica*: Figs. 3, 4; Tojo, 1999), and the clypeolabrum as a single unpaired structure is regarded as plesiomorphic within the insects. In the Odonata (*e. g., Epiophlebia superstes*: Ando, 1962) and Neoptera [cf. Eastham, 1930; Rempel, 1975; *e. g.*, raphidiodean *Inocellia japonicus* (Tsutsumi and Machida, 2006); lepidopteran *Endoclita signifer* (Kobayashi *et al.*, 1981) and *Endoclita sinensis* (Tanaka *et al.*, 1985); mecopteran *Panorpa pryeri* (Suzuki, 1990); trichopterans *Nemotaulius admorsus* (Kobayashi and Ando, 1990) and *Stenopsyche marmorata* (Miyakawa, 1974)], the clypeolabrum is formed by the fusion of paired rudiments, and this is to be regarded as apomorphic to the Odonata and Neoptera.

Traits 10 and 10' in figure 12 indicate clypeolabral rudiments formed as a single unpaired structure and as a paired structure, respectively.

[11] The development of hypopharynx

In myriapods, apterygotes and ephemeropterans, the hypopharynx differentiates into the superlinguae and lingua [*e. g.*, pauropodan *Pauropus silvaticus* (Tiegs, 1947); symphylan *Hanseniella agilis* (Tiegs, 1940); collembolan *Tomocerus ishibashii* (Uemiya and Ando, 1987); dipluran *Lepidocampa weberi* (Ikeda and Machida, 1996, 1998); archaeognathan *Pedetontus unimaculatus* (Machida, 1981); ephemeropteran *Ephemera japonica* (Figs. 3-5; Tojo, 1999)], whereas in the odonates and neopterans, superlinguae do not develop. The differentiation of superlinguae is to be recognized as plesiomorphic within the insects, and the loss of differentiation as synapomorphic to the odonates and neopterans.

Traits 11 and 11' in figure 12 indicate the differentiation of superlinguae and the loss of their differentiation, respectively.

[12] The caudal filament

Previous studies have revealed that the caudal filament of the Ephemeroptera is an elongation of the telson (Fig. 7; Tojo and Machida, 1996, 1997b). Machida (1981) made a similar interpretation for the caudal filament of the archaeognathan *Pedetontus unimaculatus*. Structures closely resembling the caudal filament of Ephemeroptera are present in the Zygentoma and Paleozoic monuran *Dasyleptus* spp., the origin of which could be similar to that in the Ephemeroptera: the Monura constitutes the Dicondylia together with the Zygentoma and Pterygota (Kukalová-Peck, 1987). Structures resembling the caudal filaments in these insects are not found in any other atelocerates, so the caudal filament can be recognized as an autapomorphy of the Ectognatha.

Ectognathans other than the Archaeognatha, Monura, Zygentoma and Ephemeroptera, namely, the Odonata and Neoptera, do not develop the caudal filament, and this condition, *i. e.*, the loss of the caudal filament, may be recognized as a synapomorphy of the Odonata and Neoptera.

Traits 12 and 12' in figure 12 represent the acquisition and the loss of caudal filaments, respectively.

[13] Exite structures on thoracic appendicular bases

Recently, structures regarded as the remnants of subcoxal and coxal exites were observed in the heptageniid mayfly *Bleptus fasciatus* (Fig. 11; Miyairi and Tojo, 2007). Coxal exites are observed on the pro-, meso-, and metalegs, and subcoxal exites were also observed on the meso- and metalegs. These coxal and subcoxal exite structures developed during the embryonic stage.

These exite structures are considerd as significant indicators of the groundplan of pterygote insects and establish firmly the origin of insect wings, and the 'gill origin theory (= epicoxal origin theory; Kukalová-Peck, 1978, 1991)' or presently the most accepted theory is well explained. Furthermore, recent developmental biological evidence also supports this theory when a comparison is made between the expression of genes found in crustacean gills with the corresponding genes found in the wings of insects (Averof and Cohen, 1997).

Among modern pterygote insects, only ephemeropterans have these 'exite' structures. Similar (probably homologous) structures are observable in some Permian fossils of insects (Kukalová-Peck, 1978, 1991), *i. e.*, these structures are recognized as plesiomorphic. In addition, the dorsal projection found in the dorso-middle part of the coxal segment of the apterygote archaegnathans' meso- and metalegs (*i. e.*, stylus) may also be homologous to the coxal exite found on *Bleptus fasciatus* (see trait 13* in figure 12A; cf. Tillyard, 1932).

Solid-lined open oblongs and dotted-lined open oblongs for trait 13 in figure 12 represent the possession and the subsequent loss of exite structures, respectively. However, the subsequent loss of this character could not be considered a synapomorphy (*i. e.*, it should be treated as the parallel deletion of a plesiomorphic character).

Affinities between the major three pterygote groups evidenced by embryological data

The states of 13 characters were examined and determined. In figure 12, these characters are mapped on the three phylogenies currently proposed: Phylogeny A is supported by authors such as Kristensen (1975), Wheeler and Carpenter (1996). Phylogeny B is supported by authors such as Hennig (1969). Phylogeny C is supported by authors such as Boudreaux (1979). The lineages leading to the Archaeognatha, the Zygentoma and the stem of Pterygota are shown only in figure 12A, because these phylogenies support the monophylies of Ectognatha, Dicondylia and Pterygota.

Although the characters examined here may not be sufficient to positively elucidate these three groups' relationships phylogenetically, they are useful to indicate their phylogeny cladistically. The results support that both Dicondylia (= Zygentoma + Pterygota) and Pterygota (= Ephemeroptera + Odonata + Neoptera) are monophyletic, characterized by five (characters 1, 3–6) and three (characters 6–8) autapomorphies, respectively.

Regarding the interrelationships within Pterygota, phylogeny A in figure 12 proves to be the most parsimonious. Both phylogenies B and C suppose three parallel acquisitions, characters 10–12, *i. e.*, the clypeolabral rudiment, the superlingua and the caudal filament (these characters are marked by large oblongs with gray shading): no supposition of the parallel acquisition of characters is needed in phylogeny A.

Consequently, the comparative embryological examination presented here strongly supports phylogeny A (Fig. 12A) formulated as [Pterygota (= Ephemeroptera + Metapterygota (= Odonata + Neoptera))], preferred by authors such as Kristensen (1975) and Wheeler and Carpenter (1996).

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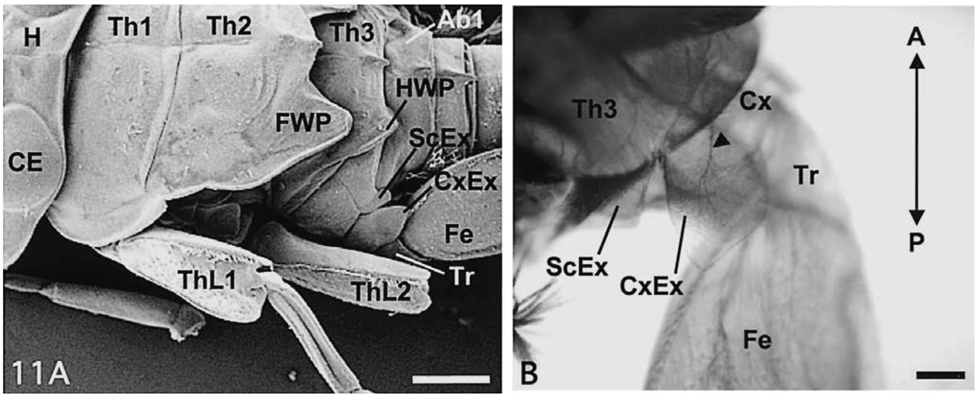

Fig. 11 Coxal and subcoxal exites of a nymph of the heptageniid mayfly *Bleptus fasciatus*. A. An SEM micrograph, dorso-lateral view. Note the coxal exite (CxEx), the subcoxal exite (ScEx) and the hind wing pad (HWP='epicoxal' exite?). B. A light micrograph of the base of the right metaleg, dorsal view. Tracheation (arrowhead) is clearly observed in the coxal exite.
Ab1: first abdominal segment, A-P: anterior-posterior axis, CE: compound eye, Cx: coxa, CxEx: coxal exite, Fe: femur, FWP: fore wing pad, H: head, HWP: hind wing pad, ScEx: subcoxal exite, Th1–3: pro-, meo-, and metathorax, ThL1, 2: pro- and mesoleg, Tr: trochanter. Bars = A: 1 mm; B: 100 μm.

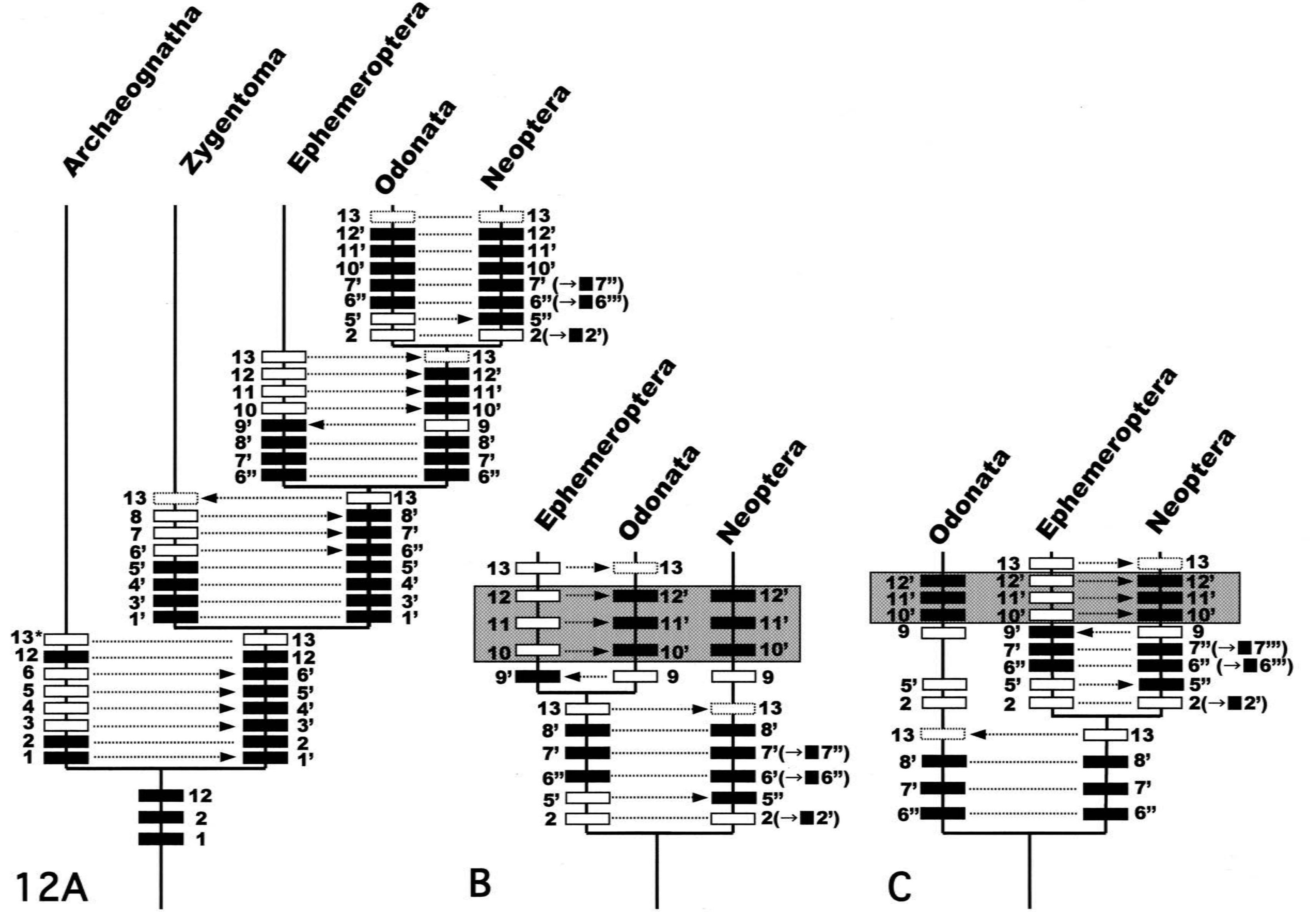

Fig. 12 Map of the embryological characters for the currently proposed phylogenies of Ectognatha / Pterygota (A–C). Numerals indicate the characters or character states, as designated in the text: the transformation of each character is shown by prime-signs (', '', '''). Open and solid oblongs represent plesiomorphic (ancestral) and apomorphic (derived) character states, respectively. Dotted line and dotted arrows indicate synapomorphic (or symplesiomorphic) relationships and the transformation of a character, respectively. The characters marked by large oblong frames with gray shading show a parallel acquisition. The arrowed numerals in parentheses show that the character was transformed within one or more groups of the lineage concerned. See the text for an explanation of solid-lined and dotted-lined open oblongs and an asterisk for trait 13.

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