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## [SHORT COMMUNICATION]

# Formation of Subcoxae-1 and 2 in Insect Embryos: The Subcoxal Theory Revisited*

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**Abstract**

In their embryological study of the carabid ground beetle *Carabus insulicola*, Kobayashi *et al* (2013) revealed that the basalmost region of thoracic appendages, or subcoxa, can be subdivided into the subcoxae-1 and 2, and they form the larval pleuron. The study also showed that the subcoxae-1 and 2 are the equivalents of the anapleural and katapleural rings, respectively, of the larval pleuron. The study thus provided tangible evidence for the subcoxal theory whose validity has been much debatable. However, the applicability of the subdivision of the subcoxa (subcoxae-1 and 2) to other hexapod embryos remains unverified. The present paper reveals that the subdivision could be discernible in the embryos of several holometabolous orders, *i.e.*, Coleoptera, Megaloptera, Neuroptera, and Trichoptera, thus suggesting the applicability of the subcoxal theory at least to holometabolous insects. In non-holometabolous orders, however, we have not yet convincing evidence for the subdivision of the embryonic subcoxa.

### History of the subcoxal theory

The subcoxal theory postulates that the basalmost podomere of the thoracic appendage, or subcoxa, participates in the formation of the larval pleuron. The theory assumes that all the pleural areas between the tergum and sternum are derived only from the subcoxa. This idea was pioneered by the embryological observations by Heymons (1899) in the hemipteran *Naucoris cimicoides*. He identified the subcoxa in the region proximal to the coxa of the embryonic thoracic appendage (Fig. 1A, scx), and interpreted that the region forms the larval pleuron. Roonwal (1937) also described that the basalmost region of the thoracic appendage in the embryo of the locust *Locusta migratoria* forms the episternum and epimeron in the larval pleura (Roonwal, 1937, Figs. 70, 71, 72). He also regarded the region as the subcoxa. The Roonwal's subcoxa in each of the meso- and metathoracic segments contains a spiracular opening near its anterodorsal corner, but the Heymons' subcoxa does not contain it and the opening is located at the dorsal outside of his subcoxa, or on the underside of the paratergal lobe (Fig. 1A, ptl). Thus, as will be discussed later, the 'subcoxa' *sensu* Heymons and that *sensu* Roonwal are different in the range included.

Heymons' idea was subsequently elaborated as the subcoxal theory by Snodgrass (1927, 1935) and Weber (1928, 1952), based on the comparative morphology of the larval and adult thorax. They recognized two series of sclerites concentrically arranged and proximal to the coxa in broad hexapod taxa, and assumed that these sclerites are formed by fragmentation of the subcoxal rings, that is, the inner (distal) katapleural ring (katapleurite) and the outer (proximal) anapleural ring (anapleurite) (Fig. 1B, apl, kpl). In pterygote insects, the two rings are generally divided into the anterior and posterior sclerites by the pleural suture running dorsoventrally. Thus, the katapleurite is divided into the anterior katepisternum and posterior katepimeron, and the anapleurite into the anepisternum and anepimeron (Fig. 1C, aepm, aeps, kepm, keps, pls). Weber (1928, 1952) also presumed that not only the pleuron but also the sternum (basisternum and furcasternum) are derived from these rings (Fig. 1C, bs, fs). However, Snodgrass (1958) subsequently rejected his own theory by concluding that only the katapleurite is subcoxal in origin, but the anapleurite does not exhibit any signs of an appendicular nature. Nonetheless, Matsuda (1956a, 1956b, 1960, 1963, 1970) reevaluated and refined the theory by including many of his own observations of thoracic sclerites in a wide range of insect taxa. In particular, he stressed the importance of the paracoxal suture (pleural costa of Ferris, 1940) that separates the pleural walls into the katapleural and anapleural rings, because the suture forms a pleural apodeme that provides surface for the attachment of many thoracic muscles. He also pointed out that the anepisternum of many insect orders is further divided by the

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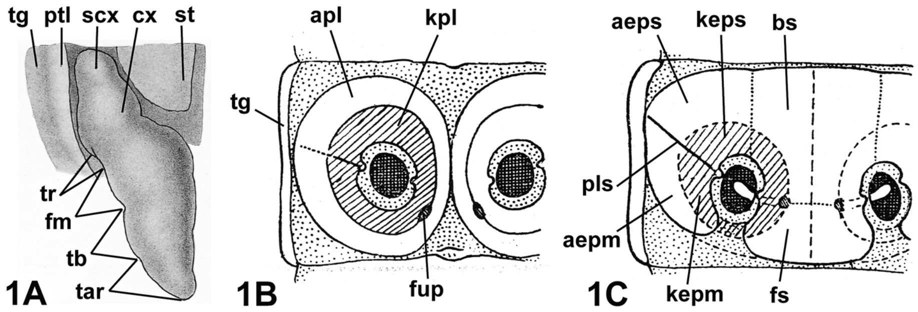

Fig. 1 History of the subcoxal theory. A. Heymons' theory. *Naucoris cimicoides* (Hemiptera). Ventral view of the right mesothoracic leg of an embryo, showing the subcoxal region (scx) proximal to the coxa (cx) (modified from Heymons, 1899). B, C. Weber's theory on the origin of the pleuron and sternum (modified from Weber, 1952). Ventral views of a schematic thoracic segment in the prototype (B) and the orthopteroid type (C).
aepm: anepimeron, aeps: anepisternum, apl: anapleural ring: bs: basisternum, fm: femur, fs: furcasternum, fup: furcal pit, kepm: katepimeron, keps: katepisternum, kpl: katapleural ring, pls: pleural suture, ptl: paratergal lobe, st: sternum, tar: tarsus: tb: tibia: tg: tergum, tr: trochanter.

anapleural suture, which distinguishes a ventral preepisternum anterior to the katepisternum (cf. Matsuda, 1970, Fig. 14). Matsuda (1970) naturally accepted the possibility that these sclerites are derived from the subcoxa, but also pointed out that the embryological data for supporting the subcoxal theory is still very insufficient. Strangely enough, however, according to both the original subcoxal theory as well as the refinements of Matsuda (1970), no consideration is given to whether the spiracle could be included in the proximal anapleural ring or not.

Even assuming the katapleural and anapleural rings, which are identified only from morphological observations, are derived from the subcoxa, there is no distinction between these rings in the original embryonic 'subcoxa' of Heymons and Roonwal. In recent years, Uchifune and Machida (2005) clearly demonstrated that the embryonic subcoxa of *Galloisiana yuasai* (Grylloblattodea) forms the sclerites of both pleuron and sternum, but the distinction of the katapleural and anapleural rings is not shown in the embryonic subcoxa.

## Subcoxal theory revisited

Recently Kobayashi *et al* (2013) recognized the embryonic subcoxa at the basalmost region of each of the thoracic appendages in the young embryo of the carabid ground beetle *Carabus insulicola* (Fig. 2A, B, scx1, scx2). As development proceeds, the region is divided into the proximal subcoxa-1 and distal subcoxa-2 by the paracoxal suture (Fig. 2C, pcxs). In the stage of katatrepsis, the two subcoxae are further subdivided anteroposteriorly into several regions, namely the prospective preepisternum, anepisternum, anepimeron, katepisternum, katepimeron, and pleural apophyseal pit (Fig. 2D, peps, aeps, aepm, keps, kepm, pla), and they differentiate into the larval thoracic pleurites (Fig. 2E). It was thus confirmed for the first time in the embryonic development that the subcoxae-1 and 2 are the equivalent of the anapleural ring and katapleural ring, respectively. Also, as in the case of *G. yuasai* (Uchifune and Machida, 2005), the spiracle of *C. insulicola* is located in the preepisternum, and thus the spiracle belongs to the anapleural ring, or the subcoxa-1 (Fig. 2D, E, spr).

According to the subcoxal theory *sensu* Weber (1928, 1952) and *sensu* Matsuda (1970), the ventral part of the subcoxa is involved in the formation of the sternum. In the first instar larva of *C. insulicola*, except for the large semicircular presternum in the prothorax, most of the regions of the thoracic sterna are membranous (Fig. 2E), which means that boundaries between the supposed sclerites are indiscernible. However, since the anterior part of the subcoxa-1 of each thoracic segment extends ventro-medially, it is highly probable that the definitive sterna are derived from the subcoxal element.

Despite the complete absence of abdominal appendages in the larvae of *C. insulicola,* two pairs of appendage-like swellings, the medial and lateral ones, temporarily arise in each of the first eight abdominal segments (Fig. 2B, msw, lsw). In the ninth abdominal segment, only the medial swellings arise. The lateral swellings soon degenerate in the subsequent stage (Fig. 2C). The medial swellings are presumed to be serially homologous to thoracic appendages, and thus regarded as abdominal appendages. The appendages (medial swellings) in the first segment develop into pleuropodia which can be divided into the proximal subcoxa and coxa and distal telopodite (Fig. 2B, pp). The appendages in segments II to IX are composed of only the subcoxa and coxa. As development proceeds, the subcoxa in each abdominal segment is divided into the subcoxae-1 and 2 like those of thoracic segments (Fig. 2D). The abdominal subcoxae-1 and 2 correspond to the prospective tergopleurite and epipleurite of the larval abdominal pleuron (Fig. 2D, E, tpl, epl). The coxal part of each appendage flattens out and participates in the formation of the hypopleurite of the larval pleuron (Fig. 2D, E, hpl). Therefore, the larval abdominal pleuron originates from both the subcoxa and coxa. Regarding the formation of the abdominal sternites, observations by Kobayashi *et al* (2013) strongly suggested that the three sternites, the mediosternite,

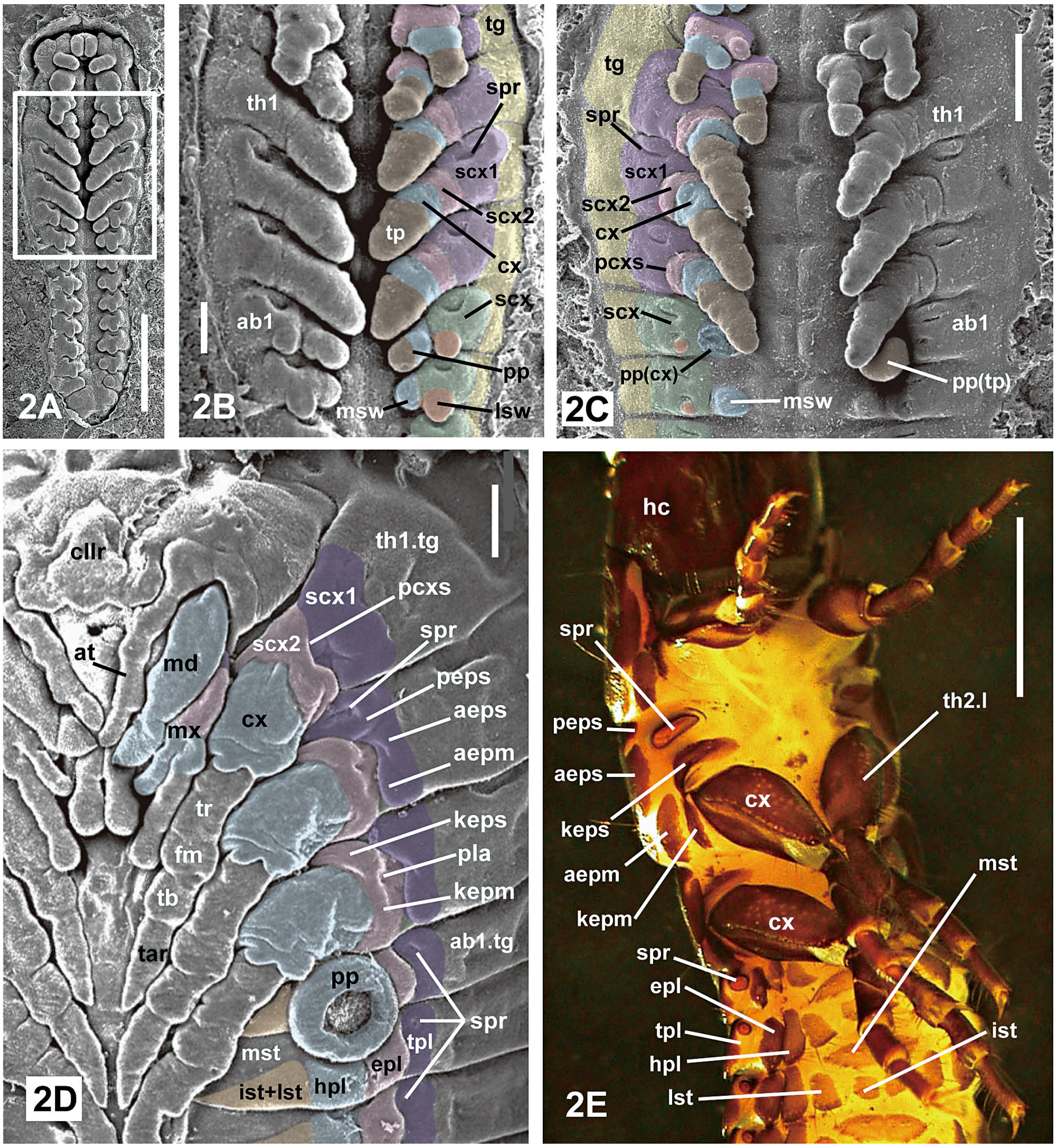

Fig. 2 A–F. SEM micrographs of developing embryos (ventral views) of the carabid ground beetle *Carabus insulicola* (modified from Kobayashi *et al*., 2013). A. Embryo at the stage of 33% DT. B. High magnification of rectangle in A, showing thoracic segments. C. Embryo at the stage of 40% DT, showing thoracic segments. D. Embryo at the stage of about 60% DT, showing thoracic segments. E. Light micrograph of the first instar larva (ventral view) of *C. insulicola* fixed with alcoholic Bouin's fluid several hours after hatching (modified from Kobayashi *et al*., 2013). The regions corresponding to the subcoxa (scx), subcoxa-1 (scx1), subcoxa-2 (scx2), coxa (cx), and telopodite (tp) are highlighted in green, purple, red, blue, and brown, respectively.
ab1: first abdominal segment, ab1.tg: first abdominal tergum, aepm: anepimeron, aeps: anepisternum, at: antenna, cllr: clypeolabrum, epl: epipleurite, fm: femur, hc: head capsule, hpl: hypopleurite, ist: inner sternite, kepm: katepimeron, keps: katepisternum, lst: laterosternite, lsw: lateral swelling (highlighted in orange), md: mandible, mst: mediosternite, msw: medial swelling, mx: maxilla, pcxs: paracoxal suture, peps: preepisternum, pla: pleural apophyseal pit, pp: pleuropodium, pp(cx): coxal part of pleuropodium, pp(tp): telopodal part of pleuropodium, spr: spiracle, tar: tarsus, tb: tibia, tg: tergum (highlighted in yellow), th1: prothoracic segment, th1.tg: prothoracic tergum, th2.l: mesothoracic leg, tpl: tergopleurite, tr: trochanter. Scales = A: 500 μm, B: 100 μm, C, D: 200 μm, F: 2 mm.

laterosternite, and inner sternite, are formed by medial extension of the subcoxal element (Fig. 2D, E, mst, lst, ist).

In summary, observations by Kobayashi *et al* (2013) convincingly demonstrated that the origin of the larval anapleural and katapleural rings of *C. insulicola* can be traced back to the embryonic subcoxa-1 and 2, respectively. In this species, moreover, the coxa and subcoxae-1 and 2 are identified in the proximal region of the abdominal appendage, and these three regions participate in the formation of abdominal pleuron. Thus, at least in this species, the subcoxal theory is applicable to both thoracic and abdominal segments.

The applicability of the subcoxal theory to other hexapod taxa largely depends on whether the subdivision of the subcoxa into the subcoxae-1 and 2 could be discernible in their

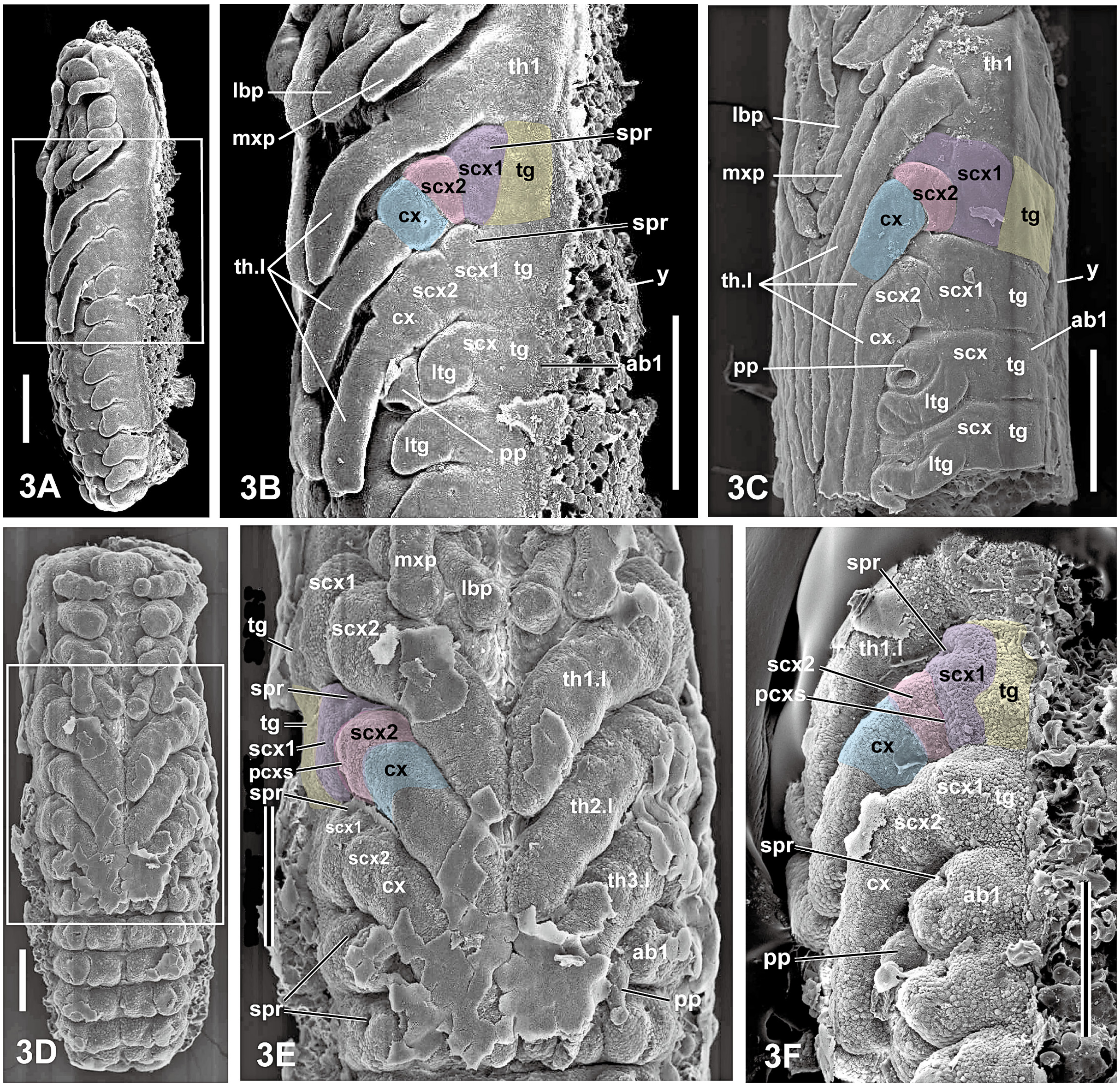

Fig. 3 A–C. SEM micrographs of developing embryos (lateral views) of the whirligig beetle *Dineutus mellyi* (modified from Komatsu and Kobayashi, 2012). A. Embryo at the stage of 52% DT. B. High magnification of rectangle in A, showing thoracic segments. C. Embryo at the stage of 60% DT, showing thoracic segments. D–F. SEM micrographs of developing embryos of the dobsonfly *Protohermes grandis* (Komatsu and Kobayashi, unpublished data). D. Ventral view of an embryo at the stage of about 40% DT. E. High magnification of rectangle in D, showing thoracic segments. F. Lateral view of an embryo at the stage of about 40% DT. The regions corresponding to the tergum (tg), subcoxa-1 (scx1), subcoxa-2 (scx2), and coxa (cx) are highlighted in yellow, purple, red, and blue, respectively.

ab1: first abdominal segment, lbp: labial palp, ltg: lateral tracheal gill, mxp: maxillary palp, pcxs: paracoxal suture, pp: pleuropodium, scx: subcoxa, spr: spiracle, th1: prothoracic segment, th.l: thoracic leg, th1.l: prothoracic leg, th2.l: mesothoracic leg, th3.l: metathoracic leg, y: yolk. Scales = A–C: 200 $\mu$m, D–F: 100 $\mu$m.

embryonic stage or not. For the discrimination of the delicate boundary between the two subcoxae, it is necessary to inspect the data obtained by SEM observations. To date I have found two regions corresponding to the subcoxae-1 and 2 in the proximal part of thoracic appendages in the following published or unpublished data; *Dineutus mellyi* in Coleoptera (Komatsu and Kobayashi, 2012), *Tribolium castaneum* in Coleoptera (Coulcher, 2011), *Protohermes grandis* in Megaloptera (Komatsu and Kobayashi, unpublished data), *Chrysopa perla* in Neuroptera (Konopová and Zrzavý, 2005), and *Nemotaulius admorsus* in Trichoptera (Kobayashi and Ando, 1990).

For example, in the embryo of the whirligig beetle *D. mellyi* at the stage of 52% DT (percent developmental time), Komatsu and Kobayashi (2012) identified the subcoxa at the basalmost region of the thoracic appendage (Komatsu and Kobayashi, 2012, Fig. 5A, B, scx). They also regarded the region just distal to the 'subcoxa' as the coxa. However, their 'subcoxa' actually corresponds to the subcoxa-1, because, in meso- and metathoracic appendages, the 'subcoxa' (subcoxa-1) houses a spiracular opening near the anterior corner (Fig. 3A, B, scx1, spr), although the openings close in the subsequent stage (Fig. 3C). Their interpretation of the coxa (Komatsu and Kobayashi, 2012, Fig. 5A, B, cx) is also incorrect; that is, their 'coxa' is actually the subcoxa-2 (Fig. 3B, C, scx2), because the same region is identified as the 'subcoxa' in more advanced stages (60% DT, 80% DT) (Komatsu and Kobayashi, 2012, Figs. 7B, 9, scx).

In the embryo of the red flour beetle *T. castaneum*, the basalmost region of the thoracic appendage is regarded as the subcoxa which can be identified by both the SEM observation and expression patterns of marker genes (*Distal-less* and *Serrata*) (Coulcher, 2011, p. 97. Fig. 3.10). In my interpretation, the Coulcher's 'subcoxa' probably corresponds to the subcoxa-2, because the region does not include a spiracle. In this species, the region which is more basal to his 'subcoxa' and clearly delimited by a deep suture (paracoxal suture) could be interpreted as the subcoxa-1 housing a spiracle.

In the embryo of the dobsonfly *P. grandis*, SEM observations reveal the presence of subcoxae-1 and 2 which are divided by the paracoxal suture at the basalmost region of thoracic appendages (Fig. 3D, E, F, scx1, scx2, pcxs). In another megalopteran species, *Sialis lutaria*, Bäcker *et al.* (2008, Fig. 9C) demonstrated the presence of katapleural and anapleural rings (their 'trochantinopleurite' and 'eupleurite', respectively) in the pleural region of the larval thorax, and assumed those rings being subcoxal in origin. Although the authors did not refer to the embryonic subcoxa of this species, I believe that their 'eupleurite' and 'trochantinopleurite' correspond to the subcoxae-1 and 2, respectively.

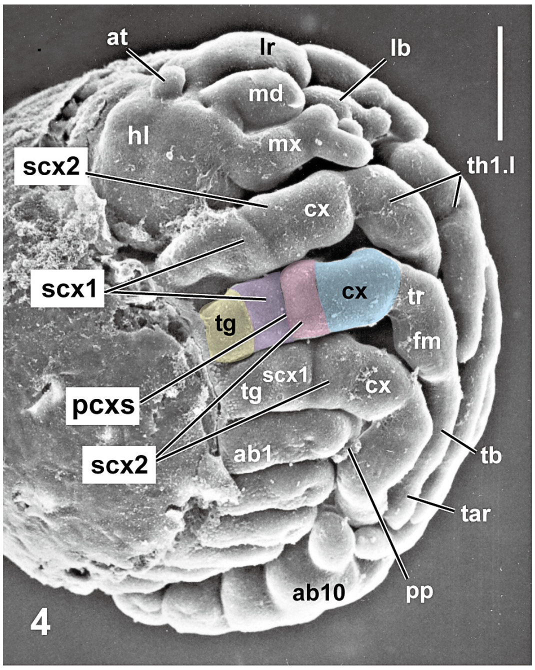

Fig. 4 SEM micrograph of an embryo of the caddisfly *Nemotaulius admorsus* at the stage of about 60% DT, showing the proximal parts of thoracic appendages (ventral view) (Kobayashi and Ando, unpublished data). The specimen of this micrograph is the same specimen used for Fig. 38 by Kobayashi and Ando (1990). The regions corresponding to the tergum (tg), subcoxa-1 (scx1), subcoxa-2 (scx2), and coxa (cx) are highlighted in yellow, purple, red, and blue, respectively.
ab1: first abdominal segment, ab10: tenth abdominal segment, at: antenna, fm: femur, hl: head lobe, lb: labium, lr: labrum, md: mandible, mx: maxilla, pcxs: paracoxal suture, pp: pleuropodium, tar: tarsus, tb: tibia, th1.l: prothoracic leg, tr: trochanter. Scale = 100 $\mu$m.

In the closely related order Neuroptera, or in *C. perla*, an SEM micrograph of a middle stage embryo by Konopová and Zrzavý (2005, Fig. 8a) also strongly suggests the presence of subcoxae-1 and 2 just ventral to the prospective tergum.

In the embryo of a caddisfly, *N. admorsus*, Kobayashi and Ando (1990) regarded the basalmost region of the appendage as the subcoxa, and furthermore interpreted the region between the subcoxa and the prospective tergum as the epicoxa *sensu* Kukalová-Peck (1983, 1987) (Kobayashi and Ando, 1990, Fig. 38, ecx). However, their 'subcoxa' and 'epicoxa' correspond to the subcoxa-2 and subcoxa-1, respectively, which are clearly divided by the paracoxal suture (Fig. 4, scx1, scx2, pcxs). As pointed out by Kobayashi *et al* (2013), their subcoxa-1 almost corresponds to the Kukalová-Peck's epicoxa, which she postulated as the basalmost podomere of the insect leg. However, they do not employ the term epicoxa, because, unlike her epicoxa, which is used only for the pleural region, the subcoxa-1 is assumed to participate in the formation of the sternum.

These examples thus suggest that the subdivision of the subcoxa into subcoxae-1 and 2 in the embryonic stage could widely occur at least in such holometabolous orders as Coleoptera, Megaloptera, Neuroptera, and Trichoptera. In non-holometabolous orders, however, we have not yet convincing data showing the subdivision of the subcoxa. The 'subcoxa' in the embryonic thoracic legs of *G. yuasai* indicated by Uchifune and Machida (2005, Fig, 15A, Scx) is most likely to correspond only to the subcoxa-1, and the real subcoxa-2, which may be very small area, may be concealed in the proximal part of the coxa (Uchifune and Machida, 2005, Fig. 15A, Cx). The same interpretation is probably applied to the Roonwal's (1937) 'subcoxa' in *L. migratoria*: that is, his 'subcoxa' is actually the subcoxa-1 housing a spiracle. Inversely, as mentioned before, the Heymons' (1899) 'subcoxa' in *N. cimicoides* probably corresponds to the subcoxa-2, because his subcoxa does not include a spiracle. Matsuda (1960) pointed out that the paracoxal suture is frequently poorly defined in non-holometabolous insect orders, such as Plecoptera, Dermaptera, and Isoptera. Thus it may be more difficult to discriminate the two subcoxae in the embryonic period in the non-holometabolous taxa cited above than in Holometabola.

In conclusion, in order to evaluate the applicability of the subcoxal theory in a wide range of insect taxa, it is necessary to accumulate the detailed embryological data on the development of the proximal part of appendages especially in non-holometabolous orders.

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