

Formation of the entognathy of *Dicellurata*, *Occasjapyx japonicus* (Enderlein, 1907) (Hexapoda: Diplura, Dicellurata)

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Abstract

The development of the entognathy in *Dicellurata* was examined using *Occasjapyx japonicus* (Enderlein, 1907). The formation of entognathy involves rotation of the labial appendages, resulting in a tandem arrangement of the glossa, paraglossa and labial palp. The mandibular, maxillary and labial terga extend ventrally to form the mouth fold. The intercalary tergum also participates in the formation of the mouth fold. The labial coxae extending anteriorly unite with the labial terga, constituting the posterior region of the mouth fold, the medial half of which is later partitioned into the admentum. The labial appendages of both sides migrate medially, and the labial subcoxae fuse to form the postmentum, which posteriorly confines the entognathy. The entognathy formation in *Dicellurata* is common to that in another dipluran suborder, Rhabdura. The entognathy of *Diplura* greatly differs from that of *Protura* and *Collembola* in the developmental plan, preventing homologization of the entognathies of *Diplura* and other two entognathan orders.

Keywords: Entognatha, comparative embryology, mouth fold, admentum, postmentum

1. Introduction

The *Diplura*, a basal clade of the Hexapoda, have traditionally been placed within Entognatha [= *Diplura* + *Collembola* + *Protura*], a group characterized by entognathy (Hennig 1969). However, Hennig's 'Entognatha-Ectognatha System', especially the validity of Entognatha, has been challenged by various disciplines. For example, recent research from paleontology (Kukalová-Peck 1987), comparative cephalic morphology (Koch 1997), comparative embryology (Ikeda & Machida 1998, 2001, Machida et al. 2002, Machida 2006) and comparative spermatology (Dallai et al. 2010) suggest a sister-group relationship between *Diplura* and *Ectognatha*, casting doubts on the monophyly of Entognatha. Information on *Diplura* is very significant not only when discussing the status of Entognatha, but also for reconstructing the hexapod groundplan and phylogeny.

Comparative embryology is one of the most useful methods for discussing phylogenetic problems. However, our knowledge of dipluran embryology mainly concerns *Rhabdura* (Heymons 1897, Uzel 1898, Tiegs 1942, Ikeda & Machida 1998, 2001). Information on the embryonic development of another dipluran suborder, *Dicellurata*, remains scanty and fragmented, with only two brief studies by Grassi (1885) and Silvestri (1933). For this reason,

we started the embryological study of Dicellurata, using *Occasjapyx japonicus* (Enderlein, 1907) as a representative of this taxon (Sekiya & Machida 2009). In this study, we describe the entognathy of *O. japonicus*, compare the results with previous studies to reconstruct the groundplan of the entognathy of Diplura, and discuss the homology of entognathous mouthparts among entognathan orders.

2. Materials and methods

Adult *Occasjapyx japonicus* collected in Tsukuba, Ibaraki Prefecture, Japan, were separately reared at room temperature (about 24 °C) in a 10 cm × 10 cm × 3 cm plastic case with a soil bottom on which a 6 cm × 6 cm × 5 mm glass plate was placed for refuge. Females built their nests under the glass plate and laid an egg mass, which comprised 20 to 50 eggs. Eggs were isolated from the egg mass, and the chorion was removed with forceps in Ephrussi-Beadle's solution (0.75% NaCl + 0.035% KCl + 0.021% CaCl₂) and fixed with Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde pH 7.2 0.1 M HCl-sodium cacodylate buffer solution) overnight. The fixed eggs were stored in 70% ethanol.

For light microscopical observations of the embryos, the fixed eggs were stained with Delafield's hematoxylin or borax carmine and observed under a biological microscope equipped with a Nikon (Tokyo) ELWD X10 or 20 extra-long-working-distance objective. For scanning electron microscopical observations of the embryos, the fixed eggs were dehydrated in a graded ethanol series, dried with a critical point dryer, coated with gold and observed under a TOPCON (Tokyo) SM-300 scanning electron microscope.

3. Results

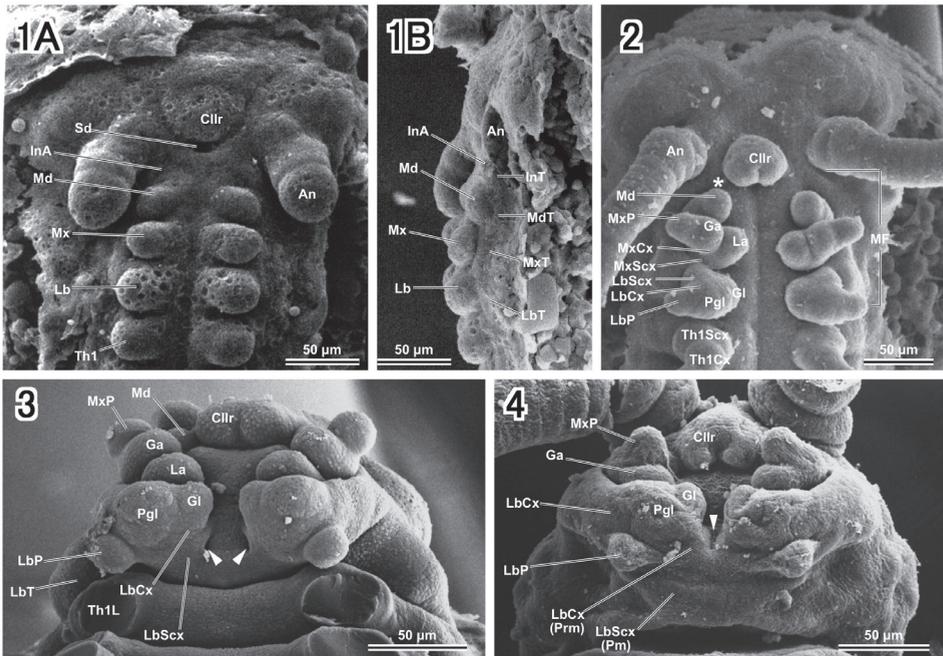
At the stage when the gnathal appendages differentiate, a pair of swellings appears in the intercalary segment. Representing the anlagen of the pair of appendages of the segment (Fig. 1A, B), these structures soon degenerate without further development (Fig. 2). The maxillary and labial appendages divide into (i) the distal telopodites, i.e., the future maxillary palp in maxilla and labial palp in the labium and (ii) proximal coxopodites, which are divided into the subcoxa and coxa (Fig. 2). The two swellings representing the endites bulge out from the medial side of the coxopodite, i.e., the inner and outer swellings being the future lacinia and galea in the maxilla and the future glossa and paraglossa in the labium, respectively (Fig. 2).

The mandibular, maxillary and labial terga start to extend ventrally, to form a longitudinal ridge (Figs 2, 5). This is the precursor of the mouth fold, and the intercalary tergum also contributes to its formation (Fig. 5). The labial appendages start to rotate (Figs 2, 3), with a final 90-degree rotation resulting in a tandem arrangement of the anterior-most glossa, intermediate paraglossa and posterior-most labial palp (Fig. 4). Simultaneously, the labial coxae start to extend anteriorly (Fig. 6) and unite with the labial terga without any demarcation between them, implying that the labial coxae also participate in the formation of the mouth fold as their posterior-most constituent (Fig. 7). Thus, the mouth fold originates from the intercalary tergum, three gnathal terga and anterior labial coxa.

The labial appendages of both sides migrate medially, and finally the labial subcoxae fuse to form the postmentum (Figs 3, 4), which makes the posterior wall of the entognathy (Fig. 4). A pair of depressions, which had appeared medial to the labial appendages (Fig. 3), medially migrates with the migrations of labial appendages and finally unites into a single opening (Fig. 4),

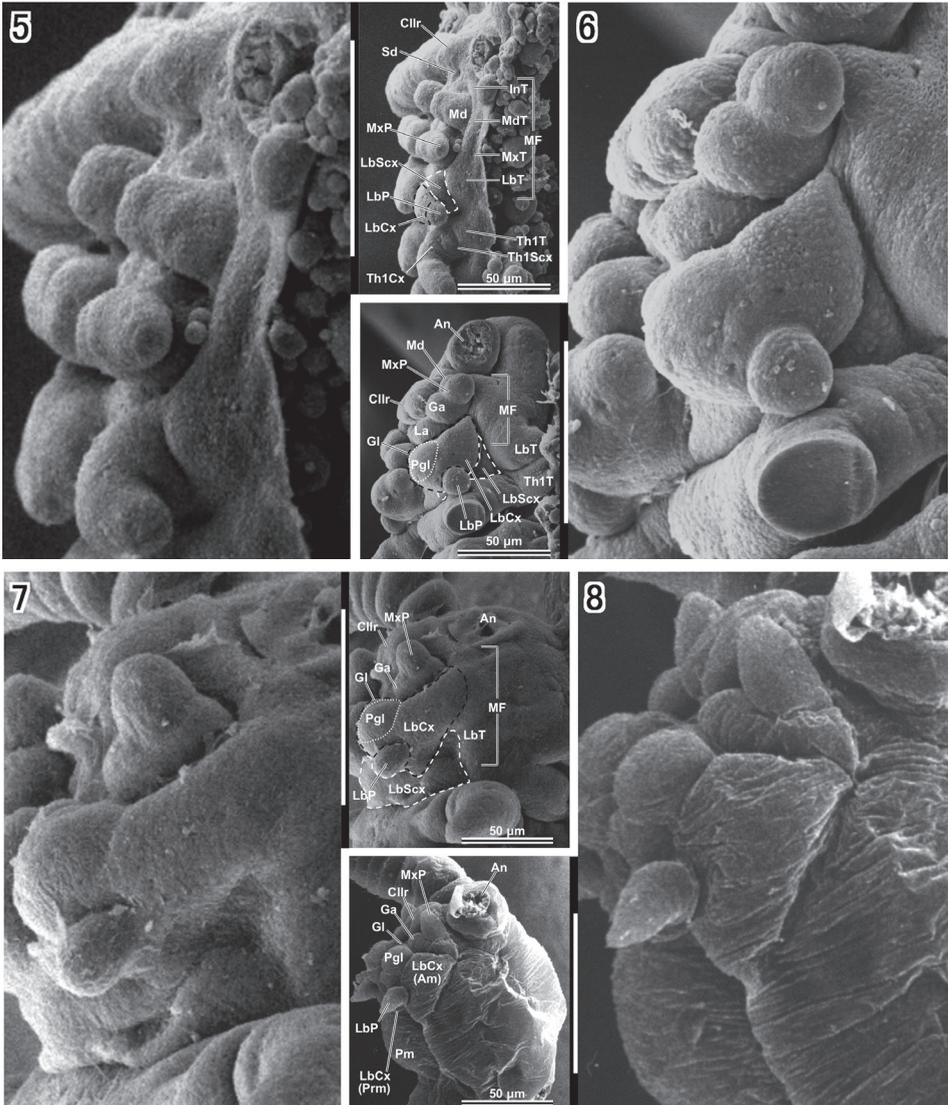
which is the common opening of the salivary glandular ducts. The admentum (Fig. 8), which is a sclerites unique to Diplura, forms just before hatching with the demarcation in the posteromedial area of the mouth fold: this area of the mouth fold is a derivative of the labial coxa, and so the admentum is labial coxal in origin.

The entognathy of *O. japonicus* can be summarized as: 1) the mouth fold is formed by the intercalary tergum, three gnathal terga and anterior labial coxa, 2) the posterior limits of entognathy are established by the postmentum, 3) the labial appendages undergo a 90-degree rotation during the entognathy's formation, and 4) the admentum, which is labial coxal in origin, is partitioned from the posteromedial part of the mouth fold.



Figs 1–4 Cephalic regions of the embryos of *Occasjapyx japonicus* (Enderlein, 1907). **1A, B:** An embryo at the stage when appendages differentiate in the gnathal segments. **A:** Ventral view. **B:** Lateral view. An antenna was removed. **2:** Ventral view of an embryo when the mouth fold starts to form. An asterisk shows where the intercalary appendage was present in the previous stage. **3:** Ventral view of an embryo of which the labial appendages are in rotation. Arrowheads show a pair of ectodermal invaginations giving rise to the sali vary glands. Thoracic appendages were removed. **4:** Ventral view of an embryo when the labial subcoxae (LbScx) fuse to form the postmentum (Pm). An arrowhead shows the common opening of salivary glandular ducts.

Am = admentum, An = antenna, Cllr = clypeolabrum, Ga = galea, Gl = glossa, InA = intercalary appendage, InT = intercalary tergum, La = lacinia, Lb = labium, LbCx = labial coxa, LbP = labial palp, LbScx = labial subcoxa, LbT = labial tergum, Md = mandible, MdT = mandibular tergum, MF = mouth fold, Mx = maxilla, MxCx = maxillary coxa, MxP = maxillary palp, MxScx = maxillary subcoxa, MxT = maxillary tergum, Pgl = paraglossa, Pm = postmentum, Prm = prementum, Sd = stomodaeum, Th1 = first thorax, Th1Cx = first thoracic coxa, Th1L = first thoracic appendage, Th1Scx = first thoracic subcoxa, Th1T = first thoracic tergum, white dotted line = paraglossa, black dashed line = labial coxa, white dashed line = labial subcoxa.



Figs 5–6 Lateral views of the cephalic regions of the embryos of *Occasjapyx japonicus* (Enderlein, 1907). Abbreviations and other information are given below. **5:** The mouth fold starts to form. An antenna was removed. **6:** The mouth fold continues to form. An antenna and a prothoracic appendage were removed.

Figs 7–8 Lateral views of the cephalic regions of the embryos of *Occasjapyx japonicus* (Enderlein, 1907). Abbreviations and other information are given below. **7:** An embryo when the postmentum has formed. An antenna was removed. **8:** An embryo in which the embryonic cuticle was segregated. An antenna was removed.

For abbreviations see Fig. 1–4.

4. Discussion

Silvestri (1933) described some stages of the entognathy of a dicelluratan *Japyx major* Grassi, 1886. We could not find any difference between *J. major* and *O. japonicus* in the entognathy, and it can be safely asserted that the entognathy is formed via the same plan for both species. Thus, the characteristics of the entognathy of *O. japonicus* are regarded as those of Dicellurata.

Ikeda & Machida (1998) studied the entognathy in a rhabduran, *Lepidocampa weberi* Oudemans, 1890, and found that a rotation of about 90 degrees of the labial appendages is involved in the formation of entognathy; the entognathy is posteriorly confined by the postmentum, which is derived from the fused subcoxae; the mouth fold is formed with the extension of the intercalary, mandibular and maxillary terga. Ikeda & Machida (1998) did not refer to the participation of the labial coxae in the formation of the mouth fold, and assigned the origin of the admentum to the maxillary tergum. However, referring to their figures, we could not find any differences between Dicellurata and Rhabdura in the formation of the entognathy, and we may safely assert that they failed to notice the unification of the labial coxa to the labial tergum and to correctly designate the origin of the admentum. In conclusion, we can summarize the development of entognathy in Diplura as: 1) the mouth fold is formed with the extension of the intercalary, mandibular, maxillary and labial terga, the latter of which the labial coxa unites with, 2) the entognathy is posteriorly confined by the postmentum, which is derived from the fused labial subcoxae, 3) a rotation of about 90 degrees of the labial appendages is involved in the formation of the entognathy, and 4) the admentum is partitioned from the posteromedial area of the mouth fold, which mainly originates from the labial coxa.

The entognathy has been regarded as the most reliable autapomorphy of Entognatha. However, we found some notable differences between the entognathy of Diplura and those of Protura and Collembola. In Collembola and Protura, neither substantial rotation of the labial appendages occurs, nor do any structures comparable to the admentum of Diplura form (Collembola: Folsom 1900, Uemiyama & Ando 1987, Tomizuka & Machida 2010; Protura: Fukui & Machida 2006, 2009). Furthermore, there are major differences between Diplura and Collembola plus Protura with respect to the origin of the mouth fold and the posterior limit of the entognathy. The mouth folds of Collembola and Protura are derived from the intercalary and the three gnathal terga only, without the contribution of the labial coxae (Uemiyama & Ando 1987, Fukui & Machida 2009, Tomizuka & Machida 2010). The posterior limit of the entognathy is reported to be established by the posteromedially extended labial terga in Protura and Collembola (Fukui & Machida 2009, Tomizuka & Machida 2010), although Uemiyama & Ando (1987) suggested that the entognathy is posteriorly defined by a postmentum of labial subcoxal origin in Collembola. The entognathy in Diplura is very different in developmental plan from that of either Protura or Collembola. Therefore, at least the entognathy of Diplura may be not unconditionally homologized with that of Protura or Collembola, and the monophyletic status of the Entognatha cannot always be substantiated.

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