

Embryonic Development of *Occasjapyx japonicus* (Enderlein): Notable Features (Hexapoda: Diplura, Dicellurata)

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Abstract

Notable embryological features of Dicellurata are described using *Occasjapyx japonicus*. Dicelluratan embryogenesis is characterized by 1) embryogenesis of short germ band type, 2) abdominal metamerism with 10 segments, 3) formation of a primary dorsal organ by the concentration of serosal cells in the later stage of embryogenesis, 4) production of amnion, and 5) entognathy formation accompanied with the rotation of labial appendages. Features 2 to 5, all of which are not found in Collembola and Protura, are shared by Rhabdura, and features 2, 3 and 5 are regarded as being synapomorphies of Dicellurata and Rhabdura, strongly supporting the monophyly of Diplura. The production of amnion (feature 4) should be elucidated as being a synapomorphy of Diplura and Ectognatha, as postulated by Ikeda and Machida (2001: *Journal of Morphology*, **249**, 242–251). Embryogenesis of the short germ band type shown in Dicellurata (feature 1) is regarded as being apomorphic to the group in Diplura.

Introduction

Recent research provides evidence discounting the monophyletic status of Entognatha, which include Diplura, suggesting a sistergroup relationship between Diplura and Ectognatha (Kukalová-Peck, 1987; Koch, 1997; Ikeda and Machida, 1998, 2001; Machida *et al.*, 2002; Machida, 2006). In addition, the comparative morphology of ovarian (Biliński, 1994) and spermatozoal structures (Jamieson, 1987) casts some doubt on the monophyly of Diplura, which are composed of two suborders Rhabdura and Dicellurata. Thus, Diplura contribute significantly to reconstructing hexapod basal clades. Comparative embryology is one of the most useful methods for elucidating phylogenetic problems; however, most of our knowledge on dipluran embryology mainly concerns Rhabdura, and information on dicelluratan embryology remains scanty and fragmented: we have only Grassi's (1885) and Silvestri's (1933) brief studies.

For this reason, we initiated an embryological study of Dicellurata, using *Occasjapyx japonicus* (Enderlein), and have outlined dicelluratan embryogenesis. In this paper, we refer to some notable embryological features of Dicellurata, comparing them with those reported for Rhabdura and other hexapods.

Materials and Methods

We collected adult *Occasjapyx japonicus* (Enderlein) in Tsukuba, Ibaraki Prefecture from May to August 2006 and 2007. They 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 nest under the glass plate, and laid their eggs as an egg mass, which comprised 20 to 50 eggs. Eggs were isolated from the egg mass, punctured with a fine needle and fixed in Carl's fluid (acetic acid : formalin : ethyl alcohol : DW = 2 : 6 : 15 : 30) or Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde pH 7.2 0.1 M HCl-sodium cacodylate buffer solution) overnight. Fixed eggs were stored in 70% ethyl alcohol.

For external observation, fixed eggs were stained with DAPI, and observed under a fluorescence stereomicroscope (LEICA MZ FL III + FLUOCOMBI, UV-excitation), or the chorion was removed with forceps, stained with Delafield's hematoxylin or borax carmine, and observed under a biological microscope equipped with ELWD X 10 or 20 objective (Nikon). For histological observation, the fixed eggs were processed into methacrylate resin serial sections of 2 µm thickness [Technovit 7100 (Külzer, Wehrheim) : styrene = 4 : 1], according to Machida *et al.* (1994a, b), and stained with Delafield's hematoxylin and eosin.

Observations

The egg period of *Occasjapyx japonicus* ranges from 16 to 19 days at room temperature (about 24°C). The earliest observed embryo was dumbbell-shaped, about 700 µm long and 300 µm wide, constricted in its middle (Fig. 1). The embryo soon starts to elongate posteriorly

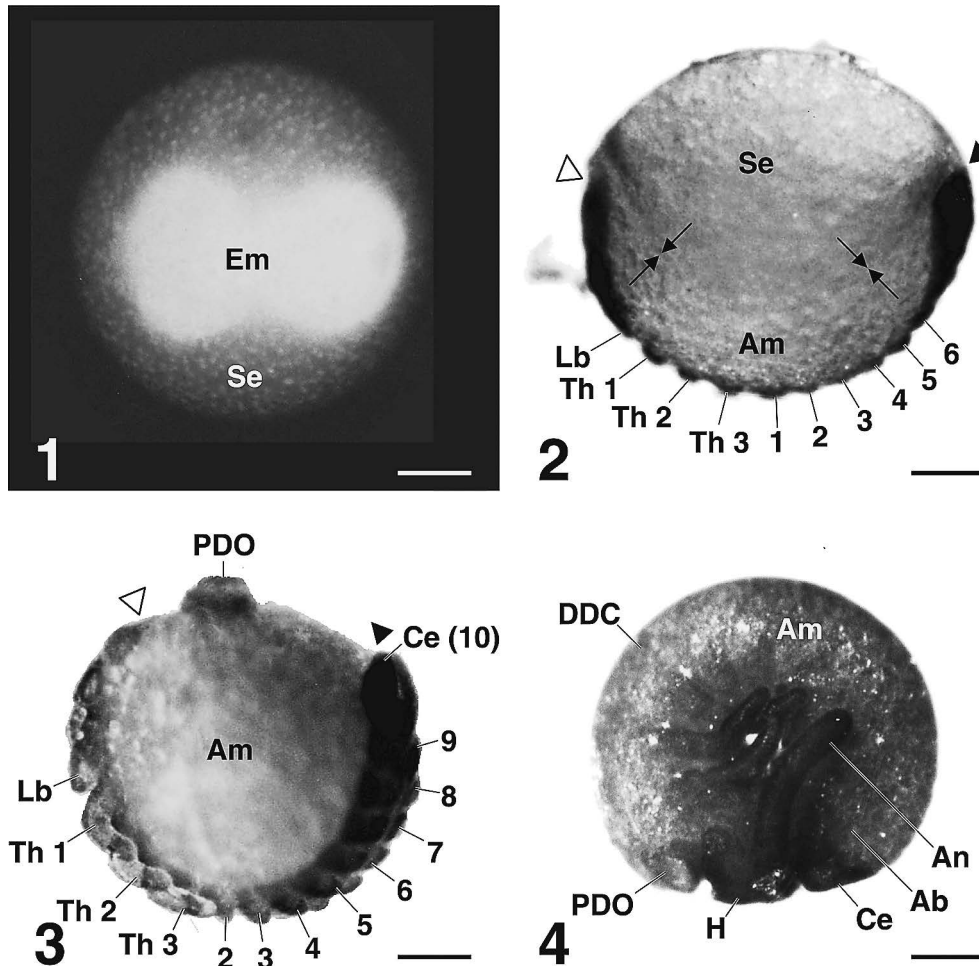
with progressive segmentation (Figs. 1, 2), and in embryos of which segmentation is completed, the abdomen is composed of 10 segments (Figs. 3, 6A). The germ band type of *Occasjapyx japonicus* is categorized as of the short germ band type.

The blastoderm area that did not participate in embryo formation is the serosa (Fig. 1). With progressive segmentation of the embryo, serosal cells concentrate dorsalwards (Figs. 2, 5, 6A, B), finally, after the completion of the abdominal segmentation, into a hemispherical structure. This is the primary dorsal organ, which is about 100 μm in diameter and is initially situated midway between the embryo's cephalic and caudal ends (Figs. 3, 7). Simultaneously with the concentration of serosal cells, a thin cellular membrane

is produced and segregated from the embryonic margin to occupy the egg surface from which the serosa has receded. This thin cellular membrane is a second embryonic membrane or amnion (Fig. 5).

After the primary dorsal organ is completed, blastokinesis occurs. The blastokinesis of *Occasjapyx japonicus* is a simple flexion of the embryo. The embryo bends ventrally at the thorax to the anterior abdomen. Blastokinesis is completed within half a day, with the cephalic and caudal ends facing each other (Fig. 4).

The definitive dorsal closure starts and proceeds, replacing the amnion, which diminishes and finally degenerates. The primary dorsal organ, placed between cephalic and caudal ends in the previous stages, is pushed away towards and situated just behind the head



Figs. 1-4 Eggs of *Occasjapyx japonicus*.

Fig. 1 Ventral view of an egg at the earliest stage observed, DAPI staining, UV excitation.

Fig. 2 Lateral view of an egg at the stage where the embryo is segmented up to the anterior abdomen, the chorion removed. The serosa has started to concentrate dorsally and the amnion begins to segregate from the embryonic margin: arrows show the boundary of the serosa and amnion, borax carmine staining.

Fig. 3 Lateral view of an egg when segmentation is complete, the chorion and antenna removed. Primary dorsal organ is complete, Delafield's hematoxylin staining.

Fig. 4 Lateral view of an embryo when blastokinesis has just completed, the chorion removed. Due to the completion of blastokinesis, the cephalic and caudal ends face each other, Delafield's hematoxylin staining.

Ab: abdomen, Am: amnion, An: antenna, Ce: cercus, DDC: definitive dorsal closure, Em: embryo, H: head, Lb: labium, PDO: primary dorsal organ, Se: serosa, Th 1-3: first to third thoracic segments, 1-10: first to tenth abdominal segments, ∇ : cephalic end, \blacktriangledown : caudal end. Scales = 100 μm .

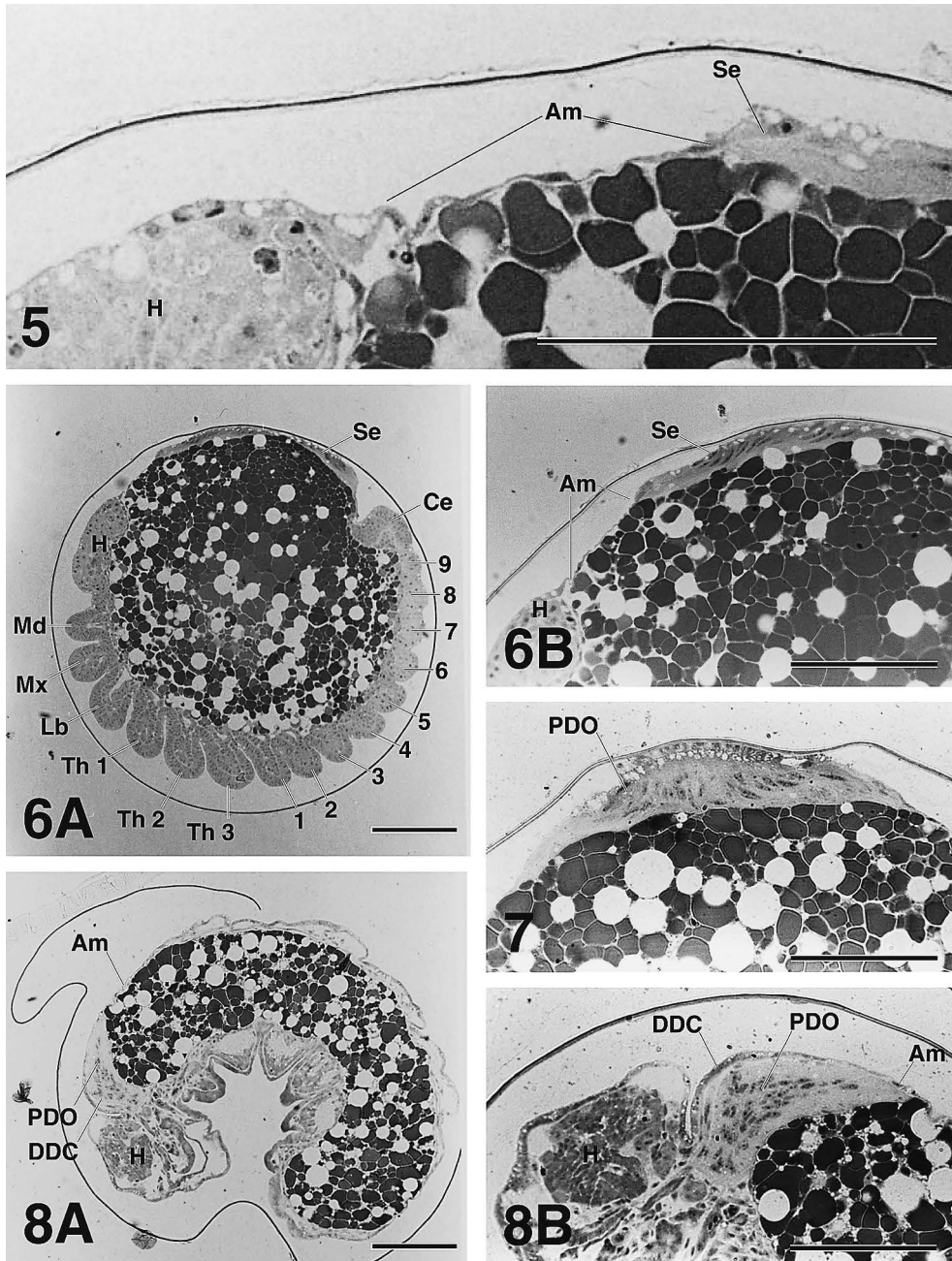
(Fig. 4), and finally covered by progressive definitive dorsal closure (Fig. 8A, B). The embryo extends by progressive development, taking a spiral posture.

Around the stages of blastokinesis, entognathly formation occurs. The mouth folds are formed by ventral extension of the mandibular and maxillary terga, and an element of the intercalary segment is also involved. Keeping step with the formation of the mouth folds, the

labial appendages, which were laterally directed in previous stages (Fig. 9A, B), rotate by about 90 degrees, and consequently the labial palpi take their definitive position in the posterior part of the labial segment and face posteriorly (Fig. 10A, B).

Discussion

In *Occasjapyx japonicus*, the abdomen was revealed



Figs. 5-8 Sagittal sections of eggs of *Occasjapyx japonicus*.

Fig. 5 An egg when the amnion starts to be produced, enlargement of dorsal region.

Fig. 6 A. An egg when segmentation has just completed. B. Enlargement of concentrated serosa.

Fig. 7 Completed primary dorsal organ.

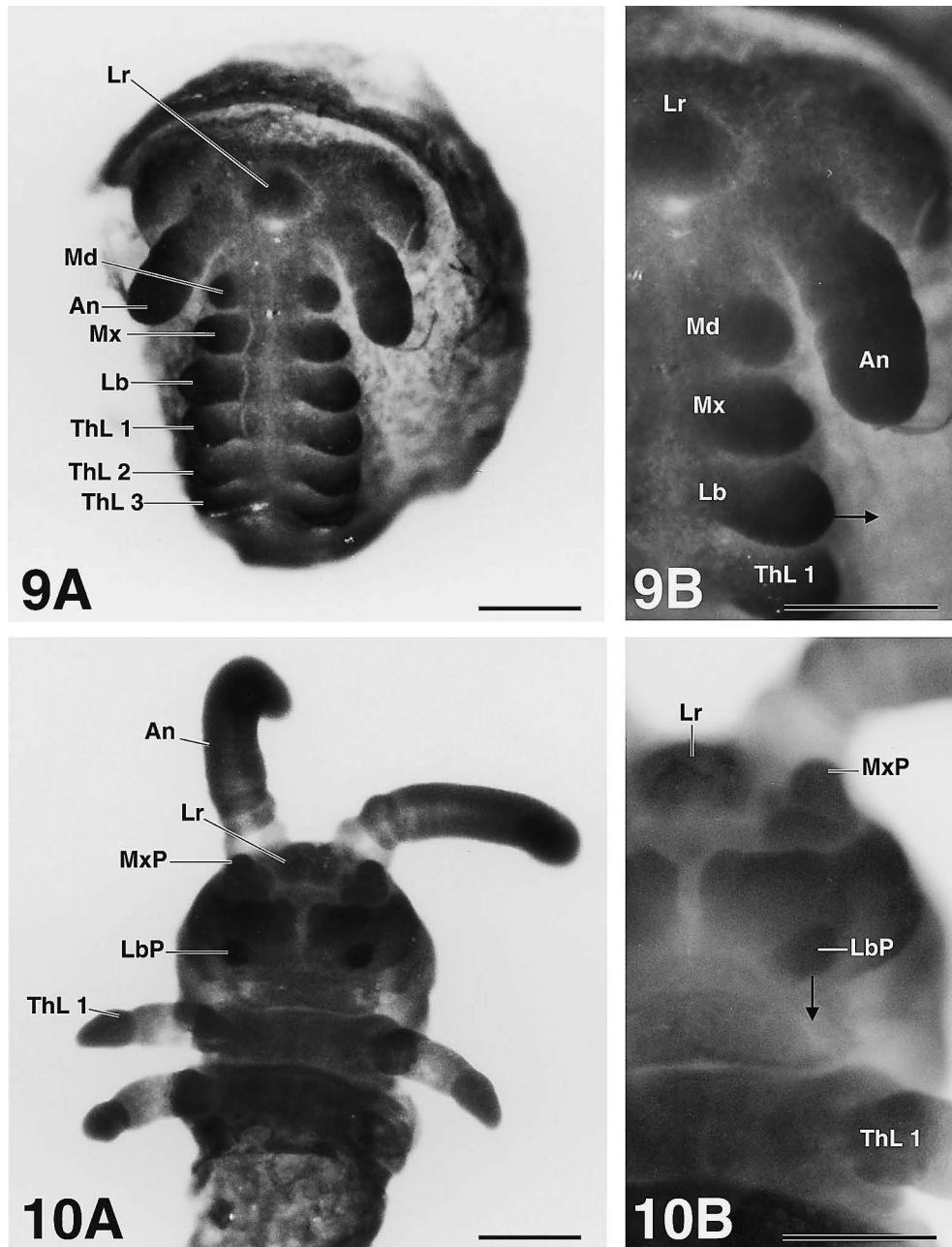
Fig. 8 A. An egg at the early postblastokinesis stage. B. Primary dorsal organ, of which the posterior part has been covered by the progressive definitive dorsal closure.

Am: amnion, Ce: cercus, DDC: definitive dorsal closure, H: head, Lb: labium, Md: mandible, Mx: maxilla, PDO: primary dorsal organ, Se: serosa, Th 1-3: first to third thoracic segments, 1-9: first to ninth abdominal segments. Scales = 100 μm.

to be composed of 10 abdominal segments, as in Rhabdura (Uzel, 1898; Ikeda and Machida, 1998). In Hexapoda, there is no representative with 10 abdominal segments: other Entognatha, *i. e.*, Collembola have six abdominal segments and Protura, the prelarvae of which hatch with nine abdominal segments, increase their abdominal segments up to 12 during postembryonic development; in Ectognatha, the number of abdominal segments is fixed as 11. Abdominal metamerism with 10

segments is a common important characteristic for Dicellurata and Rhabdura.

The primary dorsal organ of *Occasjapyx japonicus* is formed by the concentration of serosal cells after germ band formation. In its manner and timing, the formation of the primary dorsal organ in *Occasjapyx japonicus* completely agrees with that in Rhabdura (Ikeda and Machida, 1998), but differs greatly from in Collembola and Protura. Collembolan and proturan primary dorsal



Figs. 9, 10 Ventral views of embryos of *Occasjapyx japonicus*, illustrating the rotation of labial appendages during entognathy formation, Delafield's hematoxylin staining.

Fig. 9 A. An early embryo equivalent in stage to that shown in Fig. 2. B. Enlargement of gnathal region. Note that the labial appendage faces laterally (an arrow).

Fig. 10 A. A full-grown embryo. B. Enlargement of gnathal region. Note that the labial appendage takes its position at the posterior part of the segment and faces posteriorly (an arrow).

An: antenna, Lb: labium, LbP: labial palp, Lr: labrum, Md: mandible, Mx: maxilla, MxP: maxillary palp, ThL 1-3: first to third thoracic legs. Scales = 100 μ m.

organs were directly formed from the blastoderm, before the stage of germ band differentiation (Jura, 1972; Fukui and Machida, 2006). Grassi (1885) made a sketchy report of the primary dorsal organ in *Japyx solifugus*.

With the progressive formation of the primary dorsal organ, the amnion is produced from the embryonic margin in *Occasjapyx japonicus*, as in Rhabdura (Ikeda and Machida, 1998). In Collembola and Protura, the embryonic membranes are represented only by serosa, and the secondary embryonic membrane or amnion never differentiates (Uemiyama and Ando, 1987; Fukui and Machida, 2006).

The rotation of labial appendages by about 90 degrees was revealed to be involved in the entognathy formation of *Occasjapyx japonicus*, as in Rhabdura (Ikeda and Machida, 1998): observing the embryos of limited stages in *Japyx major*, Silvestri (1933) briefly described the movement of labial appendages. Such a rotation of the labial appendages never occurs in Collembola and Protura, (Uemiyama and Ando, 1987; Fukui and Machida, 2009), and this can be counted as a unique feature of Diplura.

Some comparative reproductive biologists such as Jamieson (1987) and Biliński (1994) have disputed the monophyly of Diplura; however, the present study revealed that Dicellurata and Rhabdura share significant embryological features, as addressed above: *i. e.*, 1) abdominal construction of 10 segments, 2) formation of primary dorsal organ by the concentration of serosal cells in a later stage of embryogenesis: its completion being after the abdomen has been completely segmented, 3) production of amnion, and 4) entognathy formation accompanied with the rotation of labial appendages. From outgroup comparison, features 1, 2 and 4 can be safely asserted as being synapomorphies of Rhabdura and Dicellurata, and the monophyly of Diplura is strongly supported.

Based on their embryological studies of Rhabdura, Ikeda and Machida (1998, 2001) mentioned that 1) the monophyly of Entognatha is not always substantiated, because the entognathy is formed in a different manner in Collembola and Diplura, and that 2) the possession of an amnion in Diplura is significant enough to postulate their sistergroup relationship with Ectognatha. The present study revealed that the entognathy in Dicellurata is formed as in Rhabdura (feature 4 in the previous paragraph) and that the amnion is also produced in Dicellurata (feature 3 in the previous paragraph). Thus, Ikeda and Machida's (1998, 2001) idea has been further reinforced.

The present study revealed that Dicellurata differ greatly from Rhabdura in the germ band type (cf. Krause, 1939). In *Occasjapyx japonicus*, a small embryo is initially formed, and it elongates, accompanied with sequential differentiation of segments, as Silvestri (1933) described

in *Japyx major*, and so the embryogenesis of Dicellurata is categorized as the short germ band type. On the other hand, in Rhabdura, the embryos are initially proportioned similarly to definitive embryos in length, and the full set of segments differentiates in the earliest stage of embryogenesis; hence, rhabduran embryogenesis is categorized as the long germ band type (Uzel, 1898; Ikeda and Machida, 1998). It is well known that Collembola and "Myriapoda" exhibit embryogenesis of the long germ band type (Johannsen and Butt, 1941; Jura, 1972; Anderson, 1973), and recently, Protura were revealed also to undergo embryogenesis of the long germ band type (Fukui and Machida, 2006). Although the embryogenesis of higher crustaceans is apparently of the short germ band type, the germ band type of crustaceans might be regarded as basically and originally of the long type (cf. Anderson, 1973; Machida *et al.*, 2002). Therefore, the long germ band type shown in Rhabdura may be considered to be plesiomorphic, and the short germ band type found herein for Dicellurata should be regarded as apomorphic in Diplura: acquisition of the short germ band type in Dicellurata and Ectognatha should be elucidated as a homoplasy, because the monophyly of Diplura has been strongly supported herein.

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References

- Anderson, D.T. (1973) *Embryology and Phylogeny in Annelids and Arthropods*. Pergamon Press, Oxford.
- Biliński, S. (1994) The ovary of Entognatha. In J. Büning (ed.), *The Insect Ovary*, pp. 7–30. Chapman & Hall, London.
- Fukui, M. and R. Machida (2006) Embryonic development of *Baculentulus densus* (Imadaté): An outline (Hexapoda: Protura, Acerentomidae). *Proceedings of Arthropodan Embryological Society of Japan*, **41**, 21–28.
- Fukui, M. and R. Machida (2009) Formation of the entognathy in *Baculentulus densus* (Imadaté) (Hexapoda: Protura, Acerentomidae). *Proceedings of the Arthropodan Embryological Society of Japan*, **44**, 25–27.
- Grassi, B. (1885) I progenitori degli insetti e dei miriapodi. *L'Japyx e la Campodea*. *Atti della Accademia Gioenia di Scienze Naturali in Catania*, *Ser. 3*, **19**, 1–83.
- Ikeda, Y. and R. Machida (1998) Embryogenesis of the dipluran *Lepidocampa weberi* Oudemans (Hexapoda, Diplura, Campodeidae): External morphology. *Journal of Morphology*, **237**, 101–115.
- Ikeda, Y. and R. Machida (2001) Embryogenesis of the dipluran *Lepidocampa weberi* Oudemans (Hexapoda, Diplura, Campodeidae): Formation of dorsal organ and related phenomena. *Journal of Morphology*, **249**, 242–251.
- Jamieson, B.G.M. (1987) *The Ultrastructure and Phylogeny of Insect Spermatozoa*. Cambridge University Press, Cambridge.
- Johannsen, O.A. and F.H. Butt (1941) *Embryology of Insects and Myriapods*. McGraw-Hill, New York.
- Jura, Cz. (1972) Development of apterygote insects. In S.J. Counce and C.H. Waddington (eds.), *Developmental Systems: Insect*, Vol. 1, pp. 49–94. Academic Press, London.
- Koch, M. (1997) Monophyly and phylogenetic position of the Diplura

- (Hexapoda). *Pedobiologia*, **41**, 9–12.
- Krause, G. (1939) Die Eitypen der Insekten. *Biologisches Zentralblatt*, **59**, 495–536.
- Kukalová-Peck, J. (1987) New Carboniferous Diplura, Monura, and Thysanura, the hexapod ground plan, and the role of thoracic side lobes in the origin of wings (Insecta). *Canadian Journal of Zoology*, **65**, 2327–2345.
- Machida, R. (2006) Evidence from embryology for reconstructing the relationships of hexapoda basal clades. *Arthropod Systematics and Phylogeny*, **64**, 95–104.
- Machida, R., T. Nagashima and H. Ando (1994a) Embryonic development of the jumping bristletail *Pedetontus unimaculatus* Machida, with special reference to embryonic membranes (Hexapoda: Microcoryphia, Machilidae). *Journal of Morphology*, **220**, 147–165.
- Machida, R., T. Nagashima and T. Yokoyama (1994b) Mesoderm segregation of a jumpig bristletail, *Pedetontus unimaculatus* Machida (Hexapoda, Microcoryphia), with a note on an automatic vacuum infiltrator. *Proceedings of Arthropodan Embryological Society of Japan*, **29**, 23–24. (in Japanese).
- Machida, R., Y. Ikeda and K. Tojo (2002) Evolutionary changes in developmental potentials of the embryo proper and embryonic membranes in Hexapoda: A synthesis revised. *Proceedings of Arthropodan Embryological Society of Japan*, **37**, 1–11.
- Silvestri, F. (1933) Sulle appendici del capo degli "Japygidae" (Thysanura Entotropha) e rispettivo confronto con quelle dei Chilopodi, dei Diplopodi e dei Crostacei. *Compte Rendu Ve Congrès International d'Entomologie*, 329–343.
- Uemiya, H. and H. Ando (1987) Embryogenesis of a springtail, *Tomocerus ishibashii* (Collembola, Tomoceridae). *Journal of Morphology*, **191**, 37–48.
- Uzel, H. (1898) *Studien über die Entwicklung der Apterygoten Insecten*. Friedländer & Sohn, Berlin.