Proc. Arthropod. Embryol. Soc. Jpn. 40, 1–4 (2005)
© 2005 Arthropodan Embryological Society of Japan
ISSN 1341–1527

Clypeolabrum Formation of a Centipede *Scolopocryptops rubiginosus* L. Koch (Chilopoda: Scolopendromorpha)

Masashi SAKUMA and Ryuichiro MACHIDA

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan
Current address: Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386–2201, Japan
E-mail: sakuma@sugadaira.tsukuba.ac.jp (MS)

Abstract

The clypeolabrum of a centipede *Scolopocryptops rubiginosus* is dual in origin and is formed by the fusion of a single structure as the clypeal element and a paired structure as the labral element. This finding may help to reconcile the alternative interpretations of the origin of the arthropod clypeolabrum.

Introduction

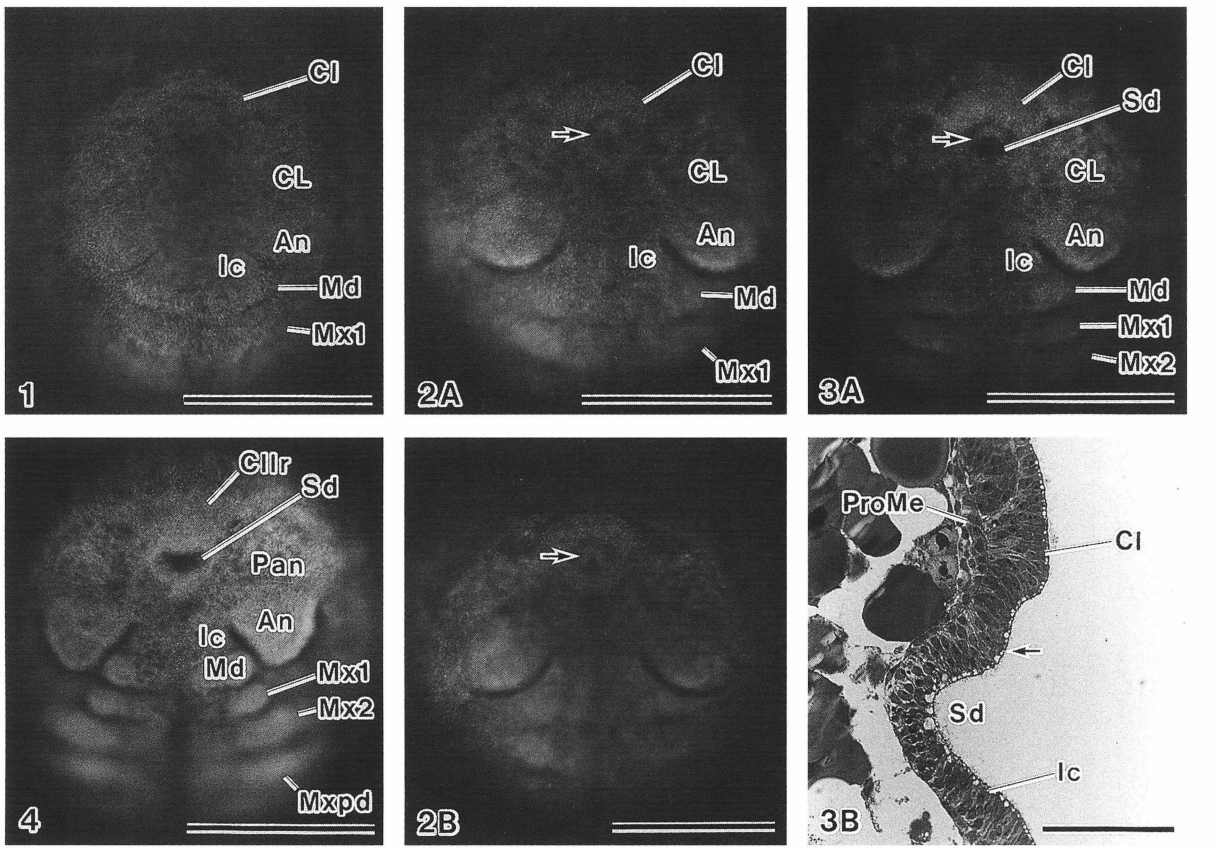
In reconstruction of the groundplan of Arthropoda, cephalic construction is one of the most interesting but controversial subjects, particularly concerning the clypeolabrum, which is often designated simply as the labrum. There has been much debate over the nature and origin of the clypeolabrum, and two interpretations exist (cf. Anderson, 1973). One recognizes the clypeolabrum as merely an ectodermal swelling in front of the stomodaeum (*e. g.,* Snodgrass, 1935; Tiegs, 1947; Manton, 1960; Matsuda, 1965). The other bestows an appendicular nature to the clypeolabrum (*e. g.,* Butt, 1960; Sharov, 1966; Rempel, 1975; Haas *et al.*, 2001). In clarifying this issue, it is crucial to determine whether the arthropod clypeolabrum is originally a paired structure or not. In Chelicerata (*e. g.,* Brauer, 1895; Yoshikura, 1955), Crustacea (*e. g.,* Weygoldt, 1958; Abzhanov and Kaufman, 1999) and higher hexapods Pterygota (*e. g.,* Eastham, 1930; Rempel and Church, 1971), the clypeolabrum is formed as a paired structure, and has been regarded to represent the labral segment as its appendicular constituent (*e. g.,* Sharov, 1966; Rempel, 1975), although some authors attribute the paired appendicular anlagen to the intercalary (= premandibular or second antennal) segment (*e. g.,* Butt, 1960; Haas *et al.,* 2001). On the other hand, in myriapods (*e. g.,* Heymons, 1901; Pflugfelder, 1932; Tiegs, 1940, 1947) and lower hexapods Apterygota (*e. g.,* Uemiya and Ando, 1987; Ikeda and Machida, 1998), no signs suggestive of a paired nature of clypeolabrum have been detected.

We have been studying the embryogenesis of a centipede *Scolopocryptops rubiginosus* L. Koch, and obtained observations significant enough to develop the argument and characterization of arthropod clypeolabrum, which we refer to in the present paper.

Materials and Methods

Females of *Scolopocryptops rubiginosus* were collected at Minami-izu and Shimoda, Shizuoka Prefecture, and Ueda and Sanada, Nagano Prefecture, Japan. The eggs, which the females deposited in the form of an egg mass in rearing conditions, were isolated from maternal care and fixed with Karnovsky's fixative (2% paraformaldehyde + 4% glutaraldehyde in pH 7.2 HCl-sodium cacodylate buffer) in an automatic vacuum infiltrator (cf. Machida *et al.*, 1994b) for 1 day, being punctured with a fine needle. The chorion was removed with forceps. The fixed specimens stored in HCl-sodium cacodylate buffer at 4℃ were stained with DAPI (4', 6-diamidino-2-phenylindole dihydrochloride, diluted about 1 μg/ml with PBS) and observed under a fluorescence stereomicroscope (Leica MZ FL III + FLUOCOMBI, UV-excitation).

Some specimens were dehydrated through a graded ethanol series, replaced in acetone, then embedded in epoxy



Figs. 1–4 Cephalic regions of *Scolopocryptops rubiginosus* germ bands 11–12 days after oviposition. Arrows show labral anlagen. See the text.

Fig. 1 Anteroventral view of the cephalic region of an embryo, stained with DAPI, UV excitation.

Fig. 2 Anteroventral (A) and ventral (B) views of the cephalic region of a slightly more developed embryo than that shown in Fig. 1, stained with DAPI, UV excitation.

Fig. 3 A. Anteroventral view of the cephalic region of a slightly more developed embryo than that shown in Fig. 2, stained with DAPI, UV excitation. B. Sagittal section of the cephalic region of an embryo equivalent in stage to that shown in A.

Fig. 4 Anteroventral view of the cephalic region of a slightly more developed embryo than that shown in Fig. 3, stained with DAPI, UV excitation.

An: antenna, CL: cephalic lobe, Cl: clypeus, Cllr: clypeolabrum, Ic: intercalary segment, Md: mandible, Mx1: first maxilla, Mx2: second maxilla, Mxpd: maxilliped, Pan: preantennal appendage, ProMe: preoral mesoderm, Sd: stomodaeum. Scale bars = 500 μm.

resin (TAAB Spurr's Resin) and processed into 2-μm-thick sections using a semi-thin microtome holding a diamond knife (cf. Machida *et al.*, 1994a). The sections, from which the resin was removed by immersion in a 28% sodium methoxide methanol solution for 30 min, were coated with celloidin and stained with Delafield's hematoxylin and eosin.

Results and Discussion

In the anterior area of *Scolopocryptops rubiginosus* germ bands 11–12 days after oviposition, the cephalic lobe (the region anterior to the antennal segment), the large antennal and the following segments, *i. e.*, intercalary (= premandibular), mandibular, first maxillary, second maxillary and maxillipedal (= first trunk), are distinguished. First, a single large swelling as the anlage of clypeus differentiates at the center of the cephalic lobe (Fig. 1), and soon after just posteriorly to it, paired anlagen of the labrum are distinguished (Fig. 2A, B). The paired appearance of the labral anlagen is ephemeral and soon obliterated due to the unification of the anlagen on both sides just after the commencement of stomodaeal invagination (Fig. 3A), which appears as a shallow depression just posterior to the labrum (Fig. 3A, B). Subsequently, the stomodaeum becomes conspicuous, being crescent in shape, and the labral anlagen fuses with the clypeus into a single clypeolabrum (Fig. 4). A pair of long and wide, transverse swellings appear just anteriorly to the antennal anlagen, and they represent the preantennal segment as its appendicular constituents (Fig. 4).

The present study clearly reveals that the clypeolabrum of *Scolopocryptops rubiginosus* should be formed by the fusion of a clypeus appearing as a single swelling and a labrum arising as a paired structure between the clypeal anlage and stomodaeum: namely, the clypeolabrum is not formed as is but has two origins. This may be meaningful to the debate on the arthropod clypeolabrum, with the potential to reconcile the alternative interpretations on the nature and origin of the arthropod clypeolabrum. In the case where the clypeal element is exaggerated, the clypeolabrum under development could be recognized as a single structure. On the other hand, in the case where the labral element predominates and is well defined, the clypeolabrum could be regarded as being formed in pairs. The following references may be noteworthy: in *Pedetontus unimaculatus* of the most primitive euinsects Archaeognatha, the clypeolabral anlage usually appears as a single structure but occasionally as a paired structure (Machida, 1981); in a diplopod *Glomeris marginata*, the clypeolabrum is observed to be distally bilobed, although only temporarily (Dohle, 1964).

What is the didymousity revealed in the clypeolabrum or labral element derived from? Taking into consideration that a pair of well-defined appendicular structures does exist independently of the clypeolabrum in the preantennal region of some arthropods including chilopods (Fig. 4; cf. Heymons, 1901; Wiesman, 1926), it may not logical to assign the didymousity of the clypeolabrum to the preantennal segment as its appendicular constituent, as has been done by some authors [see Rempel (1975) for a review]. Special mention should be made of the argument developed by Haas *et al.* (2001) that the labrum should be derived from intercalary appendicular endites. Actually, in *Scolopocryptops rubiginosus*, the labral anlagen take their positions on the frontal wall of the stomodaeum (Fig. 3B), and it may well be that the "intercalary appendicular elements" (although in this animals, these appendages never develop) would anteriorly reach there as well, passing medially to the territories of the antennal segment.

Acknowledgments: We thank Dr. K. Yahata and our colleagues at the Sugadaira Montane Research Center, the University of Tsukuba, for their help with collecting materials. The present study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (15570071) to R.M. Contribution No. 196 from the Sugadaira Montane Research Center, the University of Tsukuba.

References

Abzhanov, A. and T. Kaufman (1999) Homeotic genes and the arthropod head: Expression patterns of the *labial, proboscipedia,* and *Deformed* genes in crustaceans and insects. *Proc. Natl. Acad. Sci. USA.*, **96**, 10224–10229.

Anderson, D.T. (1973) *Embryology and Phylogeny in Annelids and Arthropods*. Pergamon Press, Oxford.

Brauer, A. (1895) Beiträge zur Kenntnis der Entwicklungsgeschichte des Skorpions. II. *Z. Wiss. Zool.*, **59**, 351–433.

Butt, F.H. (1960) Head development in the arthropods. *Biol. Rev.*, **35**, 43–41.

Dohle, W. (1964) Die Embryonalentwicklung von *Glomeris marginata* (Villers) im Vergleich zur Entwicklung anderer Diplopoden. *Zool. Jb. Anat.*, **81**, 241–310.

Eastham, L.E.S. (1930) The embryology of *Pieris rapae*. Organogeny. *Phil. Trans. R. Soc. Lond., B*, **219**, 1–50.

Haas, M.S., S.J. Brown and R.W. Beeman. (2001) Pondering the procephalon: The segmental origin of the labrum. *Dev. Genes Evol.*, **211**, 89–95.

Heymons, R. (1901) Die Entwicklungsgeschichte der Scolopender. *Zoologica (Stuttg.)*, **33**, 1–244.

Ikeda, Y. and R. Machida (1998) Embryogenesis of the dipluran *Lepidocampa weberi* Oudemans (Hexapoda, Diplura, Campodeidae): External morphology. *J. Morphol.*, **237**, 101–115.

Machida, R. (1981) External features of embryonic development of a jumping bristletail, *Pedetontus unimaculatus* Machida (Insecta, Thysanura, Machilidae). *J. Morphol.*, **168**, 339–355.

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). *J. Morphol.*, **220**, 147–165.

Machida, R., T. Nagashima and T. Yokoyama (1994b) Mesoderm segmentation of a jumping bristletail, *Pedetontus unimaculatus* Machida (Hexapoda, Microcoryphia), with a note on an automatic vacuum infiltrator. *Proc. Arthropod. Embryol. Soc. Jpn.*, **29**, 23–24. (in Japanese).

Manton, S.M. (1960) Concerning head development in the arthropods. *Biol. Rev.*, **35**, 265–282.

Matsuda, R. (1965) *Morphology and Evolution of the Insect Head*. American Entomological Institute, Michigan.

Pflugfelder, O. (1932) Über den Mechanismus der Segmentbildung bei der Embryonalentwicklung und Anamorphose von *Platyrrhacus amauros* Attems. *Z. Wiss. Zool.*, **140**, 650–723.

Rempel, J.G. (1975) The evolution of the insect head: The endless dispute. *Quaest. Entomol.*, **11**, 7–25.

Rempel, J.G. and N.S. Church (1971) The embryology of *Lytta viridana* Le Conte (Coleoptera: Meloidae). VII. Eighty-eight to 132h: The appendages, the cephalic apodemes, and head segmentation. *Can. J. Zool.*, **49**, 1571–1581.

Sharov, A.G. (1966) *Basic Arthropodan Stock with Special Reference to Insects*. Pergamon Press, Oxford.

Snodgrass, R.E. (1935) *Principles of Insect Morphology*. McGraw-Hill, New York.

Tiegs, O.W. (1940) The embryology and affinities of Symphyla, based on a study of *Hanseniella agilis*. *Q. J. Microsc. Sci.*, **82**, 1–225.

Tiegs, O.W. (1947) The development and affinities of the Pauropoda, based on a study of *Pauropus silvaticus*. Part I. *Q. J. Microsc. Sci.*, **88**, 165–267.

Uemiya, H. and H. Ando (1987) Embryogenesis of a springtail, *Tomocerus ishibashii* Yosii (Collembola, Tomoceridae): External morphology. *J. Morphol.*, **191**, 37–48.

Weygoldt, P. (1958) Embryonalentwicklung des Amphipoden *Gammarus pulex pulex* (L). *Zool. Jb. Anat.*, **77**, 51–110.

Wiesman, R. (1926) III. Entwicklung und Organogenese der Cölomblasen. *In* H. Leuzinger, R. Wiesman and F. E. Lehman (eds.), *Zur Kenntnis der Anatomie und Entwicklungsgeschichte der Stabheuschrecke* Carausius morosus *Br.*, pp. 123–328. Gustav Fischer, Jena.

Yoshikura, M. (1955) Embryological studies on the liphistiid spider, *Heptathela kimurai*. II. *Kumamoto J. Sci., Ser. B*, **2**, 1–86.