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Source: Zoological Science, 27(8) : 673-677

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.27.673>

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Ontogeny and Function of the Fifth Limb in Cypridocopain Ostracods

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The exoskeleton of arthropods undergoes reformation at every molting. Accordingly, external morphology can metamorphose through molting. In some crustaceans, the function of appendages is modified through ontogeny. These morphological modifications require accordant modification of the correlation between different body parts because the morphological function depends on the combined correlation between different parts. In the case of crustacean morphology, exoskeleton and muscles are correlated to each other. The functional morphology of the fifth limb of cypridoid ostracods transforms from “walking leg + mouthparts (+ possibly respiratory parts)” to “mouthparts + respiratory parts + grasping hook (in males only)” through ontogeny. In this study, the three-dimensional structures of the exoskeleton and muscular systems were observed by confocal laser-scanning microscopy in some species of suborder Cypridocopina. The muscular system is reportedly not changed by the ontogeny of appendages in females, but it does change in males. Furthermore, regional cell proliferation, which was detected previously, represented the causal factor of exoskeletal modification. I therefore conclude that the enlarged endite in the female fifth limb is produced by exoskeletal modification based on regional cell proliferation, rather than by a change in the muscular system. In contrast, modification in the male requires a change in the muscular system in addition to exoskeletal modification.

Key words: functional morphology, maxilliped, ontogeny, muscular system, Ostracoda

INTRODUCTION

Locomotor function is transferred from the anterior appendages to the posterior appendages during the ontogenetic process in several taxa of Crustacea (e.g., Branchiura: see Møller et al., 2007; Branchiopoda: Barlow and Sleight, 1980; Walossek, 1993; Møller et al., 2003, 2004; Olesen and Grygier, 2003; Olesen, 2004. Cirripedia: Walley, 1969; Moyse, 1984; Lagerrson and Høeg, 2002). This transfer is achieved when a newly formed appendage takes over the locomotor function of the pre-existing appendage. During this time, the pre-existing appendage loses its locomotor function and adopts a different function (e.g., mouthpart function).

This functional transfer is also observed in the appendages of cypridoid ostracods (Smith and Martens, 2000; Fig. 1). In the fifth instar, the fifth limb functions as a locomotor as well as a mouthpart (and probably a respiratory part as well). The locomotor function is subsequently lost at the sixth instar and the respiratory function is clearly achieved by the branchial plate at the eighth instar. At the same time when the fifth limb loses its locomotor function, a sixth limb appears with locomotor function. The modification of the exoskeleton in arthropods is achieved by the molting process (Brusca and Brusca, 2002). Accordingly, the morphological/functional modification of cypridoid appendages must occur during the molting period.

Functions of an organism are based on the correlation between body parts (Rudwick, 1998). In the case of arthropod appendages, muscles move the joint (Manton, 1977), although the movement axis of the joint is determined by the exoskeletal structure. Namely, the functional morphology of an appendage is based on the correlation between the exoskeleton and muscle. This indicates that functional modification is impossible without a corresponding modification of these inter-relationships. Accordingly, the exoskeletal and muscular structures of the fifth limb in Cypridoidea must be described to understand the functional modifications.

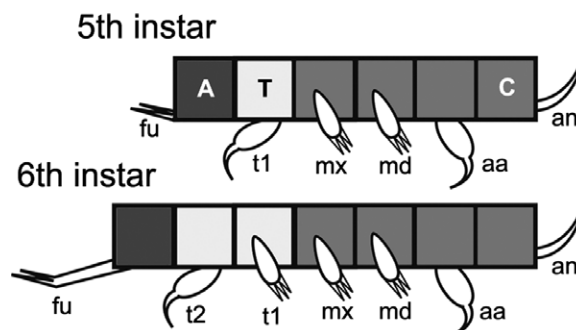


Fig. 1. Morphological configuration of body segments and appendages of Cypridoidea. Each box indicates a body segment. Gray box indicates cephalic segments. Light gray indicates the thoracic segment. Dark gray indicates the abdominal segment. aa, antenna; an, antennula; fu, furca; md, mandible; mx, maxillula; t1, first thoracic leg; t2, second thoracic leg.

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doi:10.2108/zsj.27.673

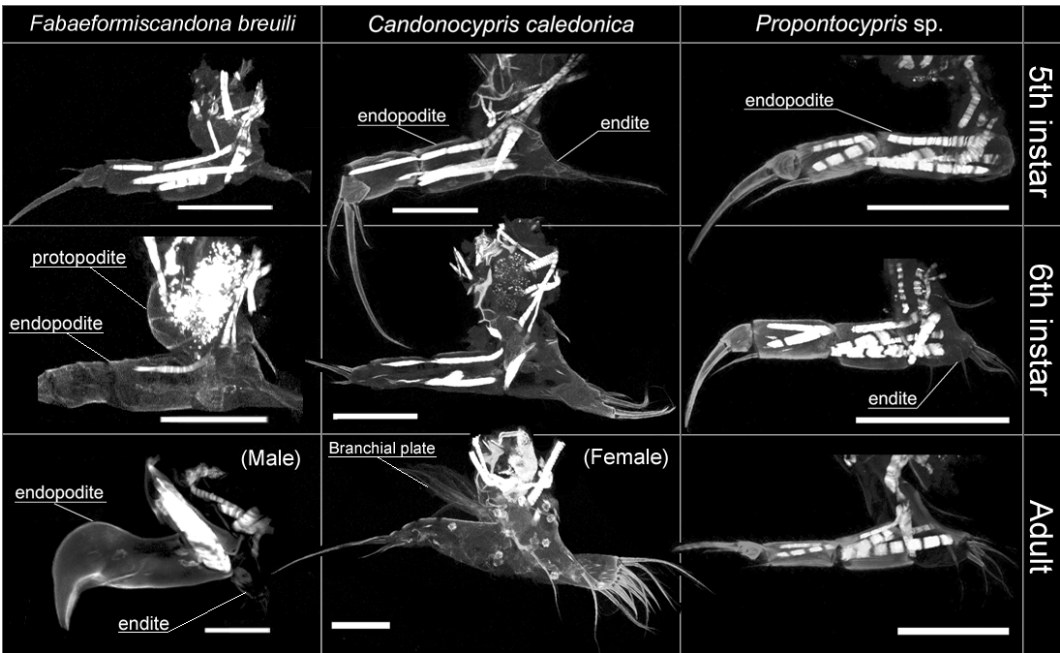


Fig. 2. Confocal images of exoskeletal and muscular structures of the first thoracic leg. Gray area is the exoskeleton detected by autofluorescence. White saturated area is muscle stained by phalloidin. Scale bar: 50 μ m for *C. caledonica* and *P. sp.*, and 30 μ m for *F. breuili*.

In the present study, the developmental sequence of the exoskeletal and muscular structures of the fifth limb of Cypridoidea was observed using a scanning electron microscope (SEM) and a confocal microscope to describe ontogeny. For comparison, the ontogeny of the fifth limb of Pontocypridoidea was also observed. My observations suggest that functional modification of males is based on the modification of exoskeleton as well as muscular system, but that of females is based only on exoskeletal modification rather than a change in the musculature. Furthermore, the mitotic nuclei that were detected represented the causal factor of exoskeletal modification. These results suggest that the modification of the functional morphology in females is derived from exoskeletal modification based on regional cell proliferation. I conclude that exoskeletal modification can be functional for feeding only on the precondition of the adjacent arrangement between the fifth limb and mouthparts.

MATERIAL AND METHODS

The species observed in this study were: *Candonocypris caledonica* collected from Shizuoka, Japan (34°57.36'/138°25.25') and *Propontocypris* sp. collected from Shimoda, Japan (34°39.6'/138°57.50'). For observations using a laser scanning confocal microscope (Leica TCS-SL), appendages were fixed for approximately 5 h in ice-cold 4% paraformaldehyde in phosphate-buffered saline [PBS; 130 mM NaCl, 7 mM Na2HPO4.2H2O, 3 mM NaH2PO4.2H2O (pH 7.0)] and then washed in 0.3% Triton-X 100 in PBS (PBT). F-actin in fixed specimens was stained with rhodamine-phalloidin (Molecular Probes) at a concentration of 100 nM in PBT, and some appendages were stained with anti-phospho-histone H3

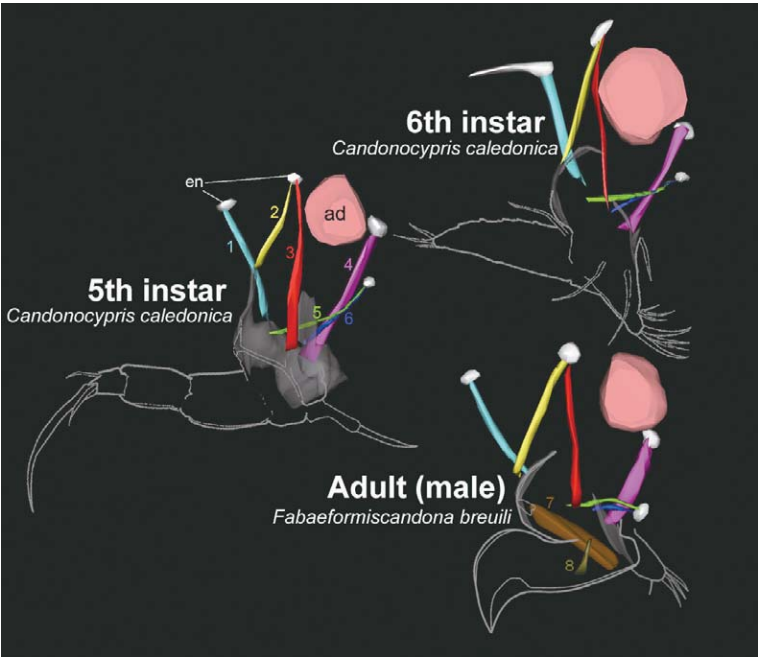


Fig. 3. Three-dimensional structure of muscle arrangement of the fifth limb. Gray area is a portion of the proximal part of the first thoracic leg. Gray line represents the appendage. ad, carapace adductor muscle; en, the part of endoskeleton. Numbers 1–6 represent extrinsic muscle 1–6 and numbers 7–8 represents intrinsic muscle 7–8.

(Upstate Biotechnology) to identify mitotic nuclei, then washed five times with PBT. Appendages were mounted in Vectashield (Vector Laboratories). Three-dimensional reconstructions were performed using Winsurf software (Surfdriver software). For observations using SEM, the specimens were fixed in either 5% formaldehyde or 70% ethanol solution. They were dehy-

drated in an ethanol series, freeze-dried, and then coated with the osmium plasma coater (Nippon Laser & Electronics Lab OPC 40).

RESULTS

Exoskeletal structure and intrinsic muscles of the fifth limb (Fig. 2)

Candonocypris caledonica (Cypridoidea): In the fifth instar, the endopodite has three podomeres and the terminal segment has three claws. Each joint of the endopodite has correlated intrinsic muscles. The endite projects from the basis and has narrow setae. No branchial plate is present on the basis. These morphological features suggest that the limb functions as a walking leg, as well as mouthpart. In the sixth instar, the articulation of the endopodite is degenerate. The terminal claw also degenerates to setae. The muscles show the beginning of degeneration. The endite is enlarged and elongated and has numerous terminal setae. These morphological features suggest that the limb loses the function of a walking leg in the sixth instar. In the adult (female), the endopodite is more degenerate and the muscle is completely missing, the endite is further enlarged, and the branchial plate, which functions in respiration, appears on the basis.

Fabaeformiscandona breuili (Cypridoidea): In the fifth instar, the exoskeletal and muscle structures are very similar to those in *C. caledonica*. In the sixth instar, all intrinsic muscles but one are degenerate. This muscle configuration was found in all specimens of this instar that were examined. The exoskeletal structure is similar to that of *C. caledonica*. In the adult (male), the endopodite is transformed to a grasping hook. The endite remains. The muscle in the protopodite is newly emerged.

Propontocypris sp. (Pontocypridoidea): In the fifth instar, the jointed endopodite has a terminal claw. Each joint of the endopodite has a corresponding muscle. These morphological features imply that the limb functions as a walking leg. There is no distinct endite. In the sixth instar, the components of the endopodite are not degenerate and maintain the functional morphology for walking. The endite is slightly enlarged and bears some terminal setae. These morphological features suggest that the limb functions both as a walking leg and a mouthpart. In the adult, the functional morphology of the walking leg is maintained and the endite is more elongated.

Three-dimensional structure of the muscle arrangement of the fifth limb (Fig. 3)

C. caledonica: In the fifth instar, muscle 1 originates on the endoskeleton and is

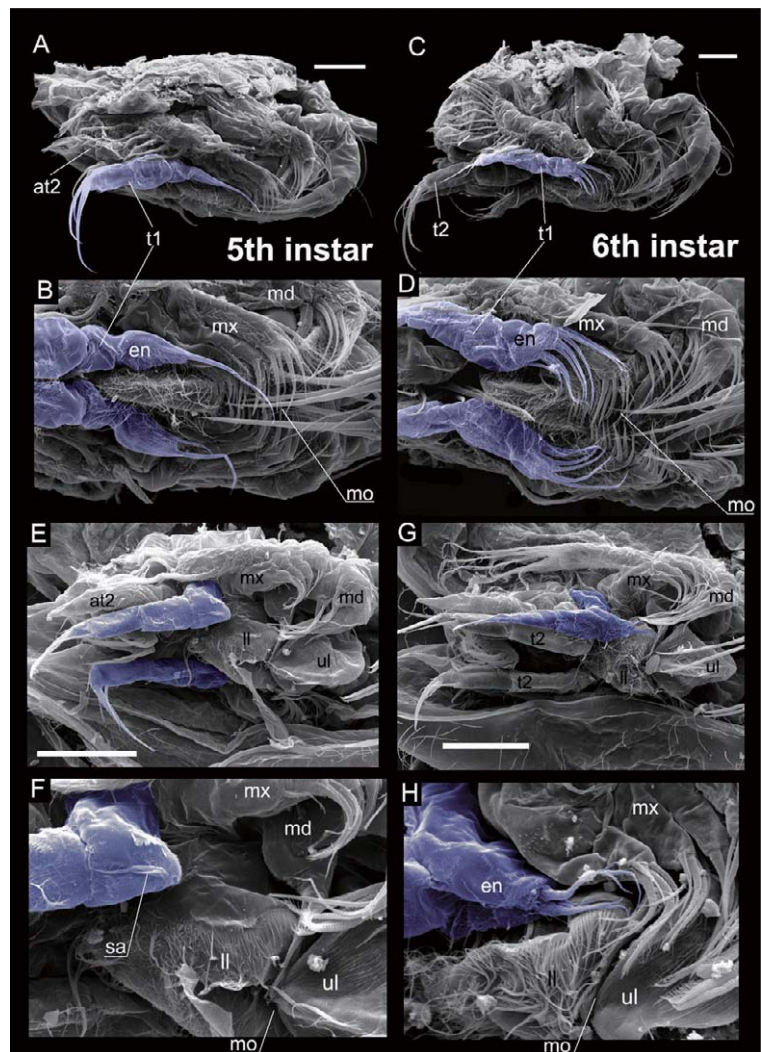


Fig. 4. External microstructure of appendage configuration around mouthparts. (A) Lateral view of *Candonocypris caledonica* (fifth instar). (B) Magnified view of mouthparts in *C. caledonica* (fifth instar). (C) Lateral view of *C. caledonica* (sixth instar). (D) Magnified view of mouthparts in *C. caledonica* (sixth instar). (E) Dorsolateral view of *Propontocypris* sp. (fifth instar). (F) Magnified view of mouthparts in *Propontocypris* sp. (fifth instar). (G) Dorsal-lateral view of *Propontocypris* sp. (sixth instar). (H) Magnified view of mouthparts in *Propontocypris* sp. (sixth instar). at2, anlage of second thoracic leg; en, endite; ll, lower lip; md, mandibula; mo, mouth cleavage; mx, maxillula; sa, setal anlage; t1, first thoracic leg; t2, second thoracic leg; ul, upper lip. Scale bar, 50 μ m.

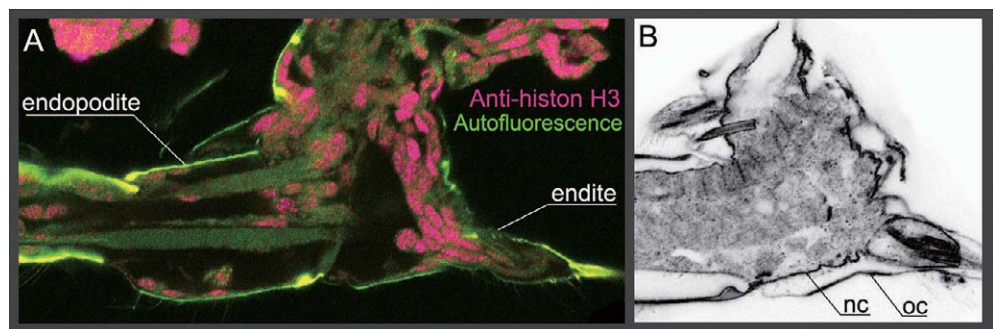


Fig. 5. Confocal section of first thoracic leg of *Candonocypris caledonica*. (A) Arrangement of mitotic nuclei around the endite at the intermolt stage. (B) New cuticle and old cuticle in and around the endite immediately before molting. nc, new cuticle; oc, old cuticle.

inserted in the proximal inner part of the fifth limb. This arrangement suggests that muscle 1 is an adductor muscle. Muscle 2 originates on the endoskeleton and is inserted in the proximal-posterior part of the fifth limb. This arrangement suggests that muscle 2 is a remotor muscle. Muscle 3 originates on the endoskeleton and is inserted in the proximal-outer part of the fifth limb. This arrangement suggests that muscle 3 is an abductor muscle. Muscle 4 originates on the endoskeleton and is inserted in the proximal-anterior part of the fifth limb. This arrangement suggests that muscle 4 is a promotor muscle. Muscles 5 and 6 originate on the endoskeleton and are inserted in the proximal-outer part of the fifth limb. This arrangement suggests that muscles 5 and 6 are remotor as well as abductor muscles. In the sixth instar, the arrangement of the extrinsic muscles is the same as in the fifth instar.

Propontocypris sp.: This exhibits muscular arrangement and ontogeny similar to that of *C. caledonica*.

Adult males of *F. breuili* have the same arrangement of extrinsic muscle. Muscles 7 and 8 are intrinsic muscles. Muscle 7 originates in the posterior wall of the protopodite and is inserted in the frontal-proximal part of the endopod. Muscle 8 originates in the external wall of the protopodite and is inserted in the internal wall of the endopodite.

Connectivity of the mouthparts and the endite of the fifth limb (Fig. 4)

C. caledonica: In the fifth instar, only one seta on the endite reaches the mouthparts (Fig. 4A, B). In the sixth instar, several setae on the enlarged endite reach the mouthparts (Fig. 4C, D). These setae are associated with the setae of the maxillula.

Propontocypris sp.: In the fifth instar, an anlage of setae is visible on the endite (Fig. 4E, F), although the setae do not reach the mouthparts. In the sixth instar, some setae on the enlarged endite reach the mouthparts (Fig. 4G, H).

Endite-specific cell proliferation (Fig. 5)

In *C. caledonica*, localized mitotic nuclei are observed around the endite and the posterior region of the protopodite (Fig. 5A). A few mitotic nuclei are also present in the endopodite, but the density is distinctly lower than that in the endite region. Immediately before the molting period, the newly formed cuticle region that will form the endite has several wrinkles (Fig. 5B). The wrinkles stretch after the molt, forming the enlarged endite.

DISCUSSION

The results of this study show that the arrangement of extrinsic muscles associated with the fifth limb is same in the fifth instar (walking leg + mouthparts; probably with respiratory parts), adult females (mouthparts + respiratory parts), and adult males (mouthparts + grasping hook) (Fig. 3). This suggests that these functions can be achieved using the same moving axis, as the moving axis of the proximal part of the appendage is determined by the arrangement of extrinsic muscles and is related to the exoskeletal structure. The similarity of the moving axis is shown in the fifth limb of adult *Propontocypris* sp., which has double functional morphology (both walking leg and mouthpart) (Fig. 2). These functions are facilitated by the corresponding exoskeletal

modification based on the conserved muscular arrangement. Furthermore, the male grasping hook has entirely new intrinsic muscles. The facts suggest that the function of the hook requires both a rotating movement around the axis and independent movement of the endopodite. Of course, the function of the hook requires exoskeletal modifications to attain the appropriate morphology.

But what is exoskeletal modification? This study shows that the setae on the endite of the fifth limb reach the mouth, allowing the fifth limb to perform the function of a mouthpart (Fig. 4). Previous research has also shown that the function of mouthparts is performed by the former's endite, which is used to sweep food particles into the mouth (Cannon, 1926). The positional change of these setae is caused by enlargement and elongation of the endite. That is, the mouthpart function of the fifth limb is enhanced by the enlargement and elongation of the endite, which in turn is caused by localized cell proliferation around the endite (Fig. 5) during morphogenesis, as "Differential growth determines the shape or curvature of a surface (Stevens, 1974)." Accordingly, it is suggested that localized cell proliferation is a causal factor of enhanced mouthpart function in the fifth limb.

The causal factor is determined by the position of mouthparts. If the mouth is not in front of the endite, the endite cannot assume the function of a mouthpart. Accordingly, the causal factor can only occur when the fifth limb and mouth are connected. Thus, the mouthpart function of the fifth limb is enhanced by the elongation of the endite caused by localized cell proliferation. Preconditions for this are connectivity of the mouthparts and the muscle arrangement.

ACKNOWLEDGMENTS

The authors express deep gratitude to Dr. Hiroshi Wada and Tuoya Yao (Tsukuba University) for their valuable discussion and technical advice. Deep thanks to Dr. Akira Tsukagoshi (Shizuoka University) for valuable suggestions and continuous encouragement.

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(Received December 22, 2009 / Accepted March 26, 2010)