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Morphology of the circulatory system in the sea spider, *Ammothella biunguiculata* (Pycnogonida, Ammotheidae), with special reference to the cephalic region

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Abstract
The morphology of the circulatory system in the pycnogonid, *Ammothella biunguiculata* was investigated by means of serial semithin sections. The basic design of the system is similar to that reported in previous studies from other pycnogonid species, with the exception of some differences and new discoveries in the cephalic region. The embracement of the brain by the anterior part of the dorsal vessel is considered here to be a synapomorphy of the Chelicerata (Pycnogonida + Euchelicerata).

Key words: brain, circulatory organs, dorsal vessel, horizontal septum, ostium

INTRODUCTION

Pycnogonids or sea spiders are a small group of marine arthropods whose phylogenetic position is rather isolated. While in most textbooks affinities to euchelicerates (merostomes + arachnids) are assumed (e.g. Meglitsch & Schram 1991; Ax 2000; Brusca & Brusca 2003), they are suggested to be the sister group of all other arthropods in some recent molecular (Wheeler 1998) and combined molecular and morphological analyses (Zrzavý et al. 1997; Giribet et al. 2001). These alternative phylogenetic conceptions suggest a critical re-examination of all relevant morphological characters.

The circulatory system in pycnogonids has been only partly studied. It seems at first glance to be very simple, in consisting of a longitudinal dorsal vessel lying above a double-layered horizontal septum (King 1973; Arnaud & Bamber 1987). According to Firstman (1973), however, the system is rather complex especially in the cephalic region: the dorsal vessel lumen is described as being directly connected with a sinus between the two layers of the horizontal septum by a channel embracing the circumoesophageal connectives. These observations, however, are based on analysis by dissections only, and preliminary investigation revealed that some important morphological features have been overlooked (Miyazaki 1998). Therefore, we examined in detail the structure of circulatory system in the pycnogonid, *Ammothella biunguiculata* (Dohrn, 1881) by serial semithin sections.

MATERIAL AND METHODS

Adult specimens of *A. biunguiculata* were collected from the intertidal zone around the Seto Marine Biological Laboratory (SMBL) at the Kii Peninsula, Pacific side of central Japan. After fixation with mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer (pH 7.3), or Bouin's solution,

they were dehydrated and embedded in Spurr's resin. The serial semithin sections (1 μm thick) were stained with a mixture of 1% azure II and 1% methylene blue in 1% borax solution (for methodological details see Pernstich et al. 2003).

RESULTS AND DISCUSSION

The basic design of the circulatory system in *A. biunguiculata* is similar to that reported in previous studies for some other pycnogonid species (reviewed in King 1973; Arnaud & Bamber 1987). It consists of a longitudinal dorsal vessel lying directly above a double-layered horizontal septum. The dorsal vessel has a single ostium at the posterior end and two pairs of lateral ones in the first and second trunk segments. The posterior unpaired ostium is present in at least some species of pycnogonids (Arnaud & Bamber 1987). In the cephalic region, the dorsal vessel splits into two channels which surround the muscle bundles just behind the eye tubercles (Fig. 1A), and which are united anteriorly to form an extensive sinus where it encloses the supraoesophageal ganglion, or brain (Fig. 1B). Contrary to the observation of Firstman (1973), there are no channels at the circumoesophageal connectives (Fig. 1C), and no direct connection between the dorsal vessel and the sinus between the layers of horizontal septum. The dorsal vessel lumen, however, opens into the cephalic haemocoel through a paired channel in a more anterior region just in front of the eye tubercle (Fig. 1B). A part of the dorsal vessel enclosing a nerve cord originating from the brain extends anteriorly and opens into the haemocoel near the tip of proboscis (Fig. 1D).

The embracement of the brain by the dorsal vessel in *A. biunguiculata* (present study) and some other species of pycnogonids (Firstman 1973) is a feature occurring also in merostomes and some (mostly basal) arachnids (Firstman 1973). It is probably a synapomorphy of the Chelicerata (Pycnogonida + Euchelicerata), as such an embracement does not occur in other arthropod groups (Horridge 1965) as well as in Onychophora (Pass 1991), a probable sister taxon with euarthropods. In Tardigrada, another candidate of the sister taxon, no circulatory system is developed (Brusca & Brusca 2003). Thus this character does not support the recently proposed hypothesis that the pycnogonids are the sister group of all other arthropods (Zrzavý et al. 1997; Wheeler 1998; Giribet et al. 2001), but is concordant with the alternative hypothesis that the Chelicerata are a monophyletic group (Wheeler & Hayashi 1998; Edgecombe et al. 2000; Waloszek & Dunlop 2002).

For further clarification of the functional and evolutionary morphology of the circulatory system in pycnogonids, detailed observations on the trunk and pedal regions of *A. biunguiculata* are in progress. In addition, observations on other species in different body sizes, habitats, and phylogenetic positions, are required.

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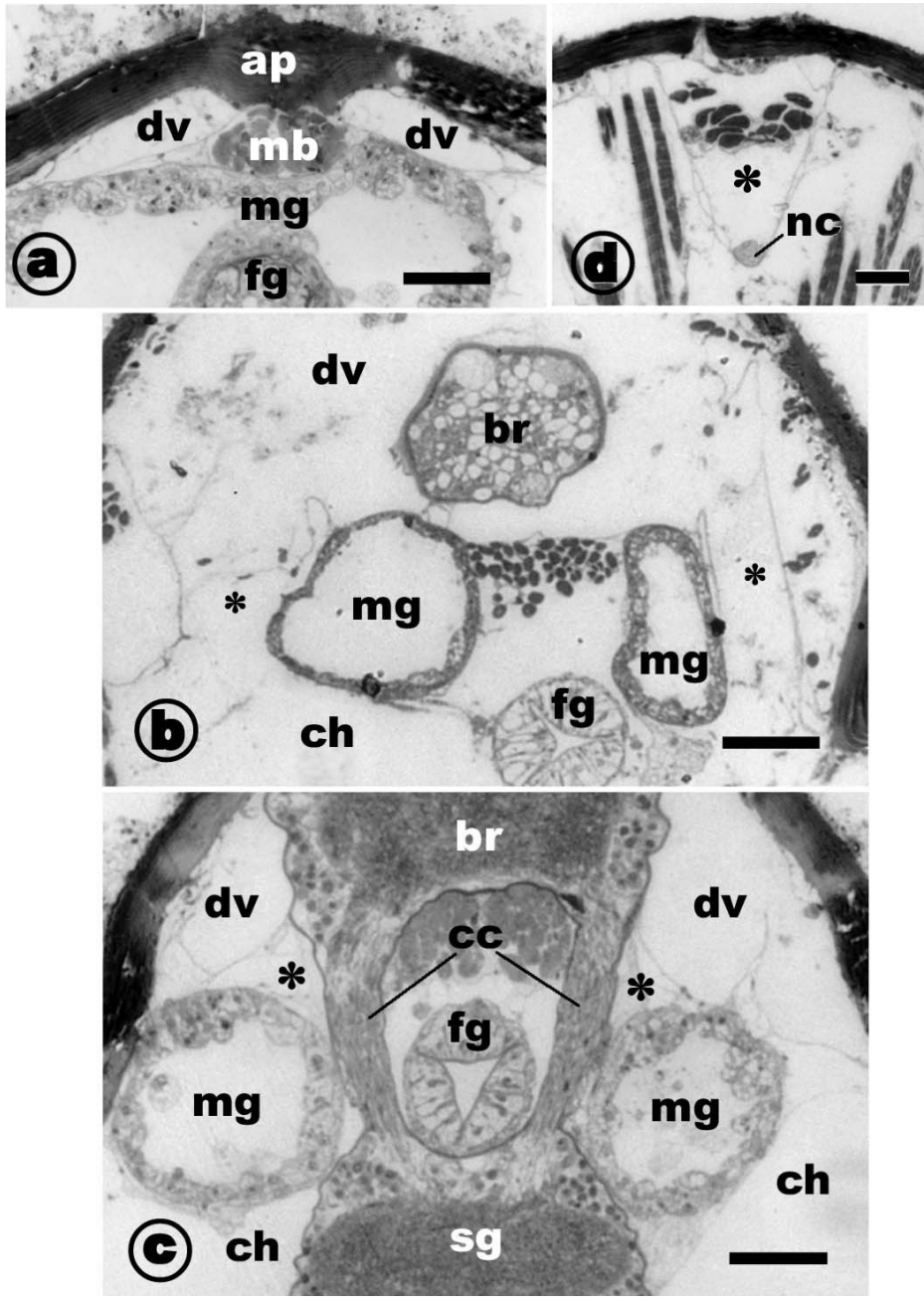

Fig. 1. Transverse semithin sections of *Ammothella biunguiculata*. (**A**) Cephalic region just behind the eye tubercle showing the divided dorsal vessel. Bar = 150 µm. (**B**) Cephalic region at the front of eye tubercle showing the brain inside the dorsal vessel, and the channels (*) between the dorsal vessel and the cephalic haemocoel. Bar = 150 µm. (**C**) Cephalic region at the circumoesophageal connective showing the thickened cellular layer (*) sealing the dorsal vessel. Bar = 150 µm. (**D**) Dorsal part of proboscis showing the extended dorsal vessel (*) enclosing the nerve cord. Bar = 100 µm. ap: apodeme; br: brain; cc: circumoesophageal connectives; ch: cephalic haemocoel; dv: dorsal vessel; fg: foregut; mb: muscle bundles; mg: midgut; nc: nerve cord; sg: suboesophageal ganglion.

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