The setae of the gill-cleaning mechanism in the soldier crab *Mictyris guinotae* Davie, Shih and Chan, 2010 (Decapoda: Brachyura: Mictyridae)

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Abstract.– The setae involved in gill-cleaning in the soldier crab *Mictyris guinotae* were examined using a light microscope and an environmental scanning electron microscope. The setae of the epipods of the first and third maxillipeds are classified into three types: 1) anchor setae, 2) digitate-scale-setule setae, and 3) denticulate setae. The second maxillipeds lack epipods, and this absence can be explained in relation to the presence of the cup-shaped basal part of the third maxilliped epipod. The presence of anchor setae in *M. guinotae* may support the hypothesis that anchor setae are a brachyuran synapomorphy. The full anchor form may result from a fusing of the denticles on denticulate setae. The presence of digitate-scale-setule setae in *M. guinotae* may confirm that digitate-scale-setule setae are the ancestral character.

Introduction

Respiration in decapods requires a constant flow of water across the gills. This can lead to problems with suspended particulate debris, and the intake of potential gill epibionts. To solve this problem, decapods have developed mechanisms to protect their gills from fouling. Several researchers have studied the gill-cleaning mechanisms of brachyuran crabs (Bauer, 1981, 1989; Holmquist, 1989; Pohle, 1989; Batang & Suzuki, 2003; Matsuoka & Suzuki 2011; Matsuoka *et al.*, 2011) and have suggested that they clean their gills with the setae on the epipods of the first to third maxillipeds (EpMp1-EpMp3).

Soldier crabs (Family Mictyridae) inhabit the intertidal zone and play an important ecological role in maintaining the proper functioning of the intertidal flats where they occur (Quinn, 1986; Dittman, 1994; Webb & Eyre, 2004). These crabs belong to one of the eight families in the Superfamily Ocypodoidea. The family Mictyridae contains a single genus *Mictyris* and seven species (Davie *et al.*, 2010; Unno & Semeniuk, 2011). Of these species, only *Mictyris guinotae* Davie, Shih and Chan, 2010, is found in Japan. The mictyrids differ greatly from other ocypodid crabs in overall shape and lifestyle, and they therefore occupy a unique position within the superfamily Ocypodoidea. The gill-cleaning setae of species in the Ocypodidae and Macrophthalmidae have been described previously (Matsuoka & Suzuki, 2011; Matsuoka *et al.*, 2011). Detailed knowledge of the gill-cleaning setae of the family Mictyridae will help to further understand the gill-cleaning mechanisms of brachyuran crabs, including the Ocypodoidea, and more generally may assist in understanding evolutionary patterns and phylogenetic relationships within the brachyurans.

Materials and Methods

Mictyris guinotae specimens were captured from tidal flats on Amami-Oshima Island, the Ryukyu Archipelago, southern Japan (28°17'N, 129°22'E) in August 2007. One adult male specimen was fixed in 10% formalin and then preserved in 70% ethyl alcohol (EtOH). The specimen was dissected to examine EpMp1 to EpMp3 with an

Fig. 1. *Mictyris guinotae*: dorsal view after removal of the carapace. A, location of epipods of first maxilliped; B, location of epipod of third maxilliped (gills removed). White lines show the outline of epipods.

Fig. 2. *Mictyris guinotae*: ventral views of first to third maxillipeds. A, first maxilliped; B, second maxilliped; C, third maxilliped.

environmental scanning electron microscopy (ESEM, Japan FEI XL30, FEI Japan, Tokyo, Japan) and light microscopy (Nikon type 104, Nikon Co. Ltd. Tokyo, Japan). The dissected parts were photographed using a digital camera system (DIGITAL SIGHT, Nikon Co. Ltd. Tokyo, Japan). The specimen was dehydrated for at least 2 hours following an

EtOH series (70%, 80%, 90%, 95%, 100% and 100%), and then air-dried. The specimen was mounted on aluminum stubs, coated with gold with an Eiko ION COATER IB-2 and viewed with Japan FEI XL30.

Results

Fig. 3. *Mictyris guinotae*: frontal view of left Milne-Edwards aperture (MEA). MEA is sealed by cup-shaped basal part of the epipod of third maxilliped.

The EpMp1s were located on the dorsal side of the gills (Fig. 1A), whereas the EpMp3s were located beneath the gills (Fig. 1B). EpMp2 was absent. Setae were present on both EpMp1 and EpMp3 (Fig. 2). EpMp3 has a cup-shaped basal part (Figs. 2C, 3). The Milne-Edwards aperture (MEA) was sealed tightly by the cup-shaped basal part of the EpMp3 (Figs. 2C, 3). The setae on the epipods were classified into three types: digitate-scale-setule setae (DSSS), anchor setae, and denticulate setae (DS). Distally all three types of setae were armed with serrate-shaped denticules (Fig. 4D). One type of anchor setae was found in *M. guinotae*. Anchor setae were the dominant setae on EpMp1 and were the second most abundant setae on EpMp3. The anchor setae were of a full anchor type, with two recurved hooks joined back to back and facing opposite directions (Fig. 4C, E). The anchor setae were equipped with normal setules (Fig. 4C, E). On EpMp1, the anchor setae were located only near the base of the dorsal side. On EpMp3, the anchor setae were located all along the dorsal side. On EpMp1, the DSSS with distal serrate-shaped denticules (Fig. 4A, B, D) were the second most abundant type, and were located on the long, thin part of the epipod (Fig. 2A). On EpMp3, the DSSS with distal serrate-shaped denticules (Fig. 4A, B, D) were dominant and could be located on the ventral side (Fig. 2C). The DS were found only at the base of the dorsal side of EpMp1 (Fig. 2A) and were the third most

abundant type. The DS were equipped with normal setules (Fig. 4F, G). Distally the DS were serrate-shaped denticules (Fig. 4D). The denticules were arranged in the similar form of a full anchor (Fig. 4G).

Discussion

Most ocypodid crabs inhabit intertidal areas and retain water within their branchial chambers for respiration when they are exposed to air. Maitland (1990) described the respiratory water-circulation and water-uptake mechanisms of *Heloeius cordiformis* (H. Milne Edwards, 1837). Some ocypodid crabs are able to replenish water supplies from substratum surface water. They can continue to respire on land by circulating water within the branchial chamber. The water then leaves the exhalant apertures, flowing down and out over the pterygostomial region of the branchiostegite. Water returns to the branchial chambers via an aperture termed the Milne-Edwards aperture (MEA) (see Matsuoka & Suzuki, 2011; Fig. 1) located at the base of the chelipeds.

In *M. guinotae*, the MEA was sealed tightly by the cup-shaped basal part of the EpMp3. Therefore, water containing particulate debris is mostly prevented from entering the branchial chamber. In other crabs, however, the MEA is always open and this allows water with suspended particulate water to easily enter the branchial chamber. Therefore, the crabs must clean the mesial side of the gills with the EpMp2 located at the entrance of the MEA. However, in the case of *M. guinotae* it is assumed that, because the cup-shaped basal part tightly seals the MEA, there is no need for an EpMp2 for cleaning. Anchor setae with their opposing recurved ends may have a high scraping efficiency and help clean and prevent the breakage of other setae, as noted in pinnotherids (Pohle, 1989). It is probable that the anchor setae serve the same function in portunids and xanthids (Batang & Suzuki, 2003; Matsuoka & Suzuki, 2011; Matsuoka *et al.*, 2011). Anchor setae were also found on the epipods of the first and third maxillipeds of *M. guinotae*. Anchor setae seem to occur only in brachyuran crabs (Pohle, 1989; Matsuoka & Suzuki, 2011), and this led Matsuoka & Suzuki (2011) to suggest that anchor setae might be a synapomorphy of the Brachyura. The presence of anchor setae

Fig. 4. *Mictyris guinotae*: setae on the epipod of first to third maxilliped. A, digitate-scale-setule seta with distal serrate-shaped denticules; B, close-up of the digitate-scale setules; C, full anchor seta with normal setules and distal serrate-shaped denticules; D, close-up of the distal part of full anchor seta with normal setules and distal serrate-shaped denticules; E, close-up of the normal setules of full anchor seta with normal setule and distal serrate-shaped denticules; F, denticulate seta with normal setules and distal serrate-shaped denticules. Denticles arranged like anchor; G, close-up of the denticules and normal setules on seta in F.

in *M. guinotae* adds additional support for this hypothesis

The denticulate setae observed here in *M. guinotae* have not been reported by previous gill-cleaning studies in ocyrodoids or other

brachyurans generally (Bauer, 1989; Holmquist, 1989; Pohle, 1989; Batang & Suzuki, 2003; Matsuoka & Suzuki 2011; Matsuoka *et al.*, 2011). The small denticules found in the denticulate setae (DS) of EpMp1 were arranged in a pattern

resembling a full anchor (Fig. 4E). Furthermore, we propose that this full anchor form might have resulted from a fusing of these denticles.

The digitate-scale-setule setae are found from dendrobranchiates to brachyurans (Bauer, 1979, 1989, 1998, 1999; Batang & Suzuki, 1999, 2000; Batang *et al.*, 2001; Pohle, 1989; Suzuki & McLay, 1998; Matsuoka & Suzuki, 2011; Matsuoka *et al.*, 2011); and are again reported here for *M. guinotae*. Therefore, we consider that the digitate-scale-setule setae are most likely to be the ancestral (plesiomorphic) character state.

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