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Study of the functional morphology of mouthparts of parasitic isopods of marine fishes

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PEER REVIEW

Peer reviewer

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Comments

The paper is essentially sound, and I agree with the functional morphology made by the authors with the parasitic isopods. The author included more information on distinguishing the species from its congeners. It has got value informations about cymothoids. I read this article with curiosity, it contributes scientific data in this field.
(Details on Page 131)

ABSTRACT

Objective: To carry out a comparative study of the mouthparts and the diet of eight isopod fish parasites. **Methods:** A description of the mouthparts, together with their diet nature, was derived both by direct observation and an interpretation of their structure. The three-dimensional study of the mouthparts of the isopod parasites was done to reveal their morphology. **Results:** Observations revealed that these species are wholly carnivorous. Result shows how they are adapted for tearing and bolting fish food material. The mouthparts consist of a labrum, paragnaths, paired mandibles, maxillules, maxillae and maxillipeds. The labrum and the paragnaths are the least developed but peculiarly the mandibles are asymmetrical, large, stout and highly modified. The analysis of gut contents indicated that *Cymothoa indica* and *Joryma brachysoma* diet consisted of 90% to 95% of animal blood. The diet of *Mothocya renardi*, *Ryukyua circularis* and *Joryma hilsae* were mainly composed of mucus (80%-90%). The stomach contents of *Nerocila phaeopleura* and *Nerocila sundaica*, were dominated by body muscles (75%-83%). **Conclusions:** The possible functions of the mouthparts, especially in feeding are discussed in light of their structure. The morphology of the mouthparts of the isopod parasites are heavily modified with their feeding behavior.

KEYWORDS
Mouthparts, Isopods, Morphology, Marine fishes

1. Introduction

Parasites have received considerable scientific attention because they cause serious damage to fishery resources. Isopods associate with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting, or damaging these fishes[1-3]. The relationship of diet with the function morphology of mouth parts have been studied for many crustaceans. In general, macrophagy has been associated with both mouth parts and foregut[4]. Considerable interest in functional morphology of crustacean mouthparts and foreguts has, however, been generated by systematists who are attempting to determine evolutionary relationships and develop meaningful taxonomic systems[5].

In most animals the structure of their mouth parts reflects the type of diet utilized. This was found to be true for isopods by Jones *et al*[6]. Mouthparts of the free-living isopod Jaera nordmanni described by the researchers[7]. Made a detailed SEM study on the gnathiid isopod *Paragnathia formica*[8]. Described three genera within the cirolanid family (*Metacirolana*, *Neocirolana*, and *Anopsilana*) and three other new genera (*Natatolana*, *Politolana* and *Cartetolana*)[9]. Studied the morphology of the body appendages of the gnathiid isopod *Bythognathia yucatunensis*[10]. Examined the unusual endoparasitic isopod *Tiarinion texopallium*, from the majid crab *Tiarinia* sp. and directed special attention to the description of the antennules, antennae and pereopods related to parasitic adaptation[11]. Keable *et al.* described a new species of the cirolanid isopod *Dolicholana*, and redescribed *Dolicholana porcellana* with special reference to their mouthparts and setal types[12]. He revealed the difference between the molar median surfaces of *Dolicholana elongata* and *Natatolana*

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corpulenta by scanning electron micrographs. Investigated the oniscoid isopod *Pentoniscus* and described a new species with details of its mouthparts, pereiopods and pleopods[13]. The mouthparts of the cirolanid isopod *Ceratolana papuae*, and the prominent horns formed by its rostrum and frontal lamina as a diagnostic feature of that species[14]. At present, there is no accurate study on the mouth parts and diet analysis of parasitic isopods. Hence the present attempt was to examine the structure of the mouthparts and relate it to their function in the feeding process.

2. Materials and methods

The specimens were collected from marine fishes of Parangipettai (11 ° 29′ N; 79 ° 46′ E). The heads were removed with a sharp razor blade to avoid any discharge that may conceal surface details. Mouthparts were removed using techniques described by Barnard[15]. Mouthparts were placed in glycerin on glass slides under cover slips.

Foreguts were dissected out of 73 specimens. Heads were gently pulled away from the body of the animals with forceps. The foregut and midgut remained attached to the head. To soften muscle attached to the foregut, the head was soaked in a solution of 10% potassium hydroxide overnight. The foreguts were then separated from the surrounding tissue and placed on a drop of glycerin on a microscope slide. The food contents were estimated by the frequency of occurrence method and the volumetric methods as suggested by Williams[16]. Specimens were observed with a dissection and compound microscopes.

3. Results

3.1. Feeding structures

3.1.1. Cymothoa indica

The mouth appendages were strongly modified for the parasitic habit. The maxillipeds were reduced to small palps of two or three articles, the distal being manifestly smaller than the proximal and the basis of the maxilliped was often enlarged into a flattened plate; The first maxillae (=maxillules) are reduced to slender, unarticulated styles, which lay adjacent to one another in such a manner as to facilitate transfer of the host's blood toward the mouth. The second maxillae was small, bilobed appendages. All these appendages bear strong, recurved, terminal or sub terminal spines that serve to hold the buccal region strongly affixed to the flesh of the host fish. The teeth of the maxillae may assist the mandible in rasping the host's flesh. The first maxillae bear four terminal spines. The mandibles had lost the lacinia mobilis, setal row, molar process, and the incisor region is modified into a sharp, blade-like cutting process presumably capable of slicing through the host's epidermis. The labrum was lamellar and well developed, aiding in preventing loss of the host's blood from the mouth region because there specificity on preference only in the buccal cavity (Figures 1 and 2).

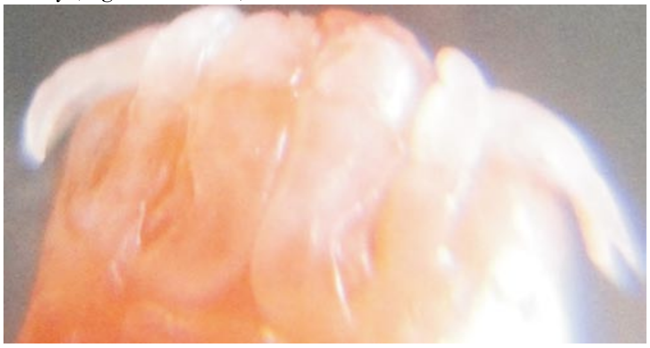

Figure 1. Mouth parts of *Cymothoa indica*.

Figure 2. *Sphyraena jello* on *Cymothoa indica*.

3.1.2. Mothocya renardi

The first segmented of mandibular palp expanded, third segment with three setae at apex. Incisor process of mandible acute. Maxilla 1 with one large staright spine and three smaller recurred apical spines. Distal lobes of maxilla two each with one recurved spine and distal segment of maxilliped palp with three stout recurved spines, apex of second segment was with stout triangular spine (Figure 3). All these appendages were highly modified to hold the gill chamber of host fish strongly (Figure 4).

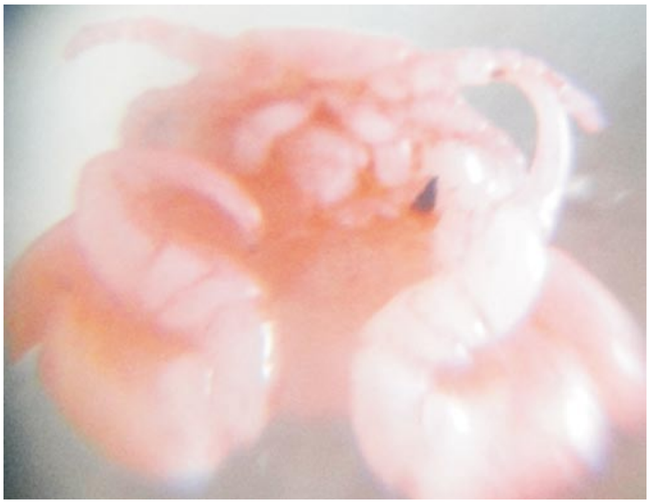

Figure 3. Mouth parts of *Mothocya renardi*

Figure 4. *Strongylura leiura* on *M. renardi.*

3.1.3. *Lobothorax typus*

Articles of mandibular palp all distinct, article 3 with 3 apical setae. Maxillule slender, styli form, with four terminal robust setae. Maxilla broad, endopod and lateral lobes each with two robust setae. Maxilliped composed of 3 articles and obscurely segmented basal articles; palp article 3 rounded, with 4 robust setae (Figure 5). *Lobothorax typus* was collected only from the buccal region of *Trichiurs lepturus* (Figure 6).

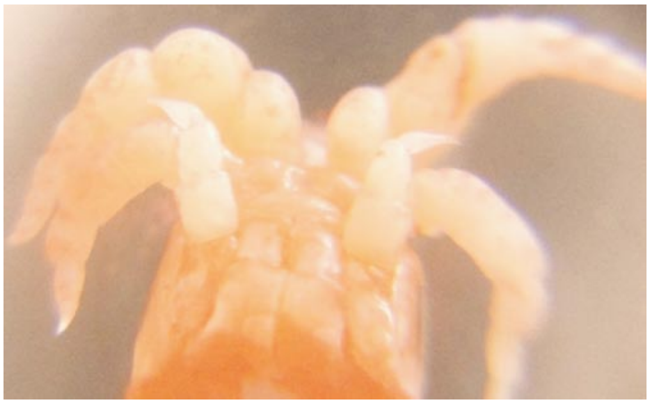

Figure 5. Mouth parts of *Lobothorax typus.*

Figure 6. *Lobothorax typus* in the buccal region *Trichiurs lepturus.*

3.1.4. *Nerocila phaeopleura*

Maxilla I slender with two small apical stylets, bilobed and inner lobe very small, bud like outer lobe squarish apical margin of outer lobe flate serrated and with a stout postero-lateral spine. Mandibles broad triangular with broad, maxilliped 3 segmented, basal segment very long, middle nearly circular and apical acutely bent laterally with three unequal spines (Figure 7). All these appendages are highly modified to hold the body surface and tearing the body muscles of host fish strongly.

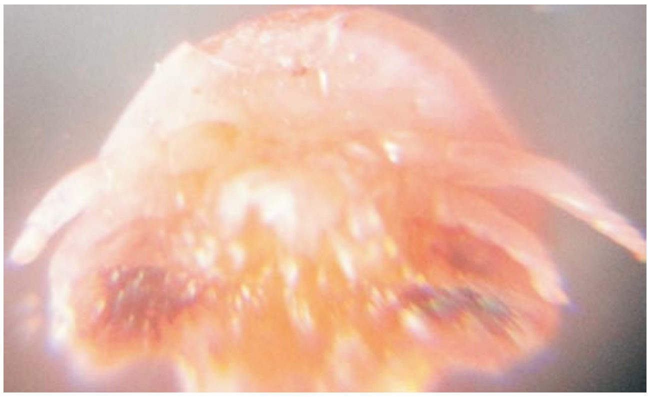

Figure 7. Mouth parts of *Nerocila phaeopleura.*

3.1.5. *Nerocila sundaica*

Maxilla slender with two small apical stylets, bilobed and inner lobe were very small. Outer lobe squarish, apical margin was flate serrated and with a stout postero-lateral spine. Mandibles broad triangular with broad, maxilliped 3 segmented, basal segment very long, middle nearly circular and apical acutely bent laterally with unequal spines (Figure 8).

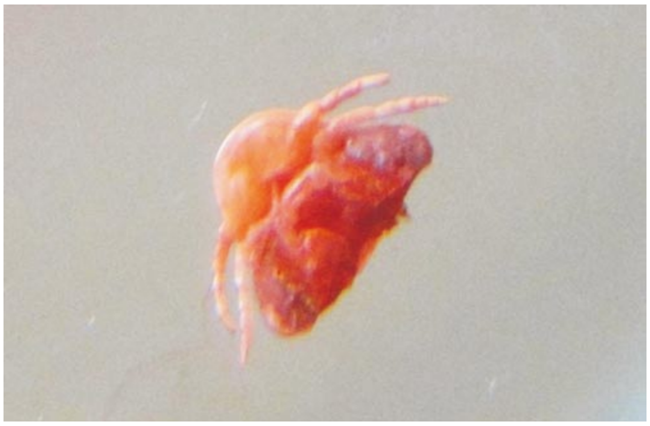

Figure 8. Mouth parts of *Nerocila sundaica.*

3.1.6. *Ryukyua circularis*

Maxilla I slender with five apical stylets. Maxilla II with serrated margins but without spines. Palp stout distinctly 3 segmented narrow and distally terminal finger shaped (Figure 9). All these appendages are highly modified to hold the branchial chamber of host fish strongly.

Figure 9. Mouth parts of *Ryukyua circularis*.

3.1.7. *Joryma brachysoma*

Maxilla I slender with five apical stylets. Maxilla II bilobed, outer lob large and inner lobe small, both with serrated margins but without spines. Incisor process of mandible is chispel–shaped, guarded by a cap. Palp stout, distinctly 3 segmented. Maxilliped indistinctly 3 segmented, terminal finger shaped segment bears three stout apical spines. Incisor region is modified into a sharp, blade–like cutting process presumably capable of slicing through the host's epidermis. The labrum was lamellar and well developed, aiding in preventing loss of the host's blood from the buccal field (Figure 10).

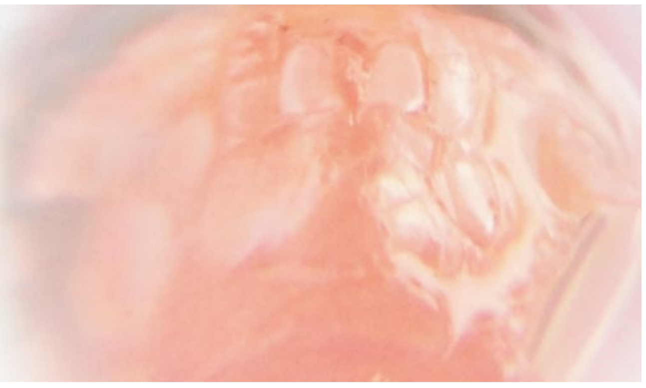

Figure 10. Mouth parts of *Joryma brachysoma*.

3.1.8. *Joryma hilsae*

Maxilla slender with five apical stylets and serrated margins but without spines. Palp stout distinctly 3 segmented narrow and distally terminal finger shaped (Figure 11). All these appendages are highly modified to hold the branchial chamber of host fish strongly.

Figure 11. Mouth parts of *Joryma hilsae*.

3.2. Diet analysis

A total of 73 stomachs was analysed (19 males/54 females). Comparison of the total number of stomachs analysed is shown in Figures 12 and 13, the gut contents of *C. indica* and *J. brachysoma* consisted of frequency 90% to 95% and volumetric 85%–93% of blood. The diet of *M. renardi*, *R. circularis* and *J. hilsae* were mainly composed frequency 80%–90% and volumetric 70%–81% of mucus and similarly the stomach contents of *N. phaeopleura* and *N. sundaica* were dominated by body muscles frequency 75%–83% and volumetric 80%–82%, because they were attached only the outer surface of the body[16].

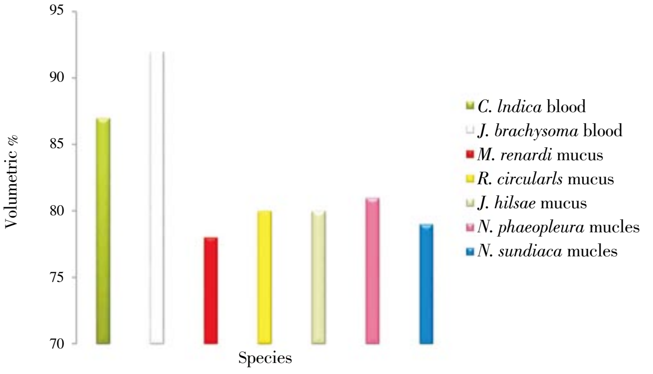

Figure 12. Volumetric percentage of diet in parasitic isopods.

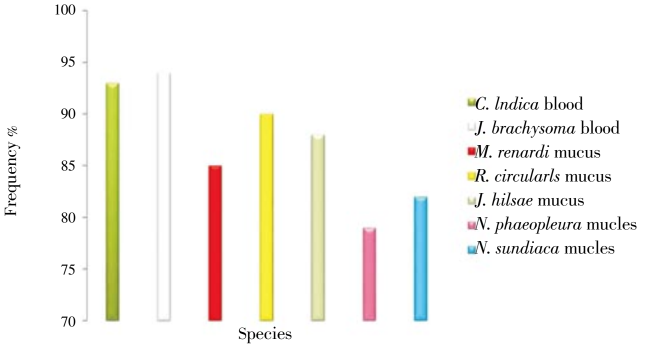

Figure 13. Frequency percentage of diet in parasitic isopods.

4. Discussion

Parasitic isopods exploit different habitats and can consume different foods of their hosts. However, there are also largely free living isopods with a few groups being symbionts on Limpets and on chitons[17,18]. Consequently, isopods have different life styles. This is reflected in the structure of their mouthparts. Generally, crustaceans as a lower arthropod group than insects have the mouth lobes (upper and lower lips) more or less developed or even modified when compared to those of insects. The latter are more potentially developed and highly modified to perform definite functions and have received much attention in research due to their economic importance.

In the present study, isopods like *C. indica* and *J.*

brachysoma, the incisor region is modified into a sharp, blade-like cutting process presumably capable of slicing through the host's epidermis. The labrum is lamellar and well developed, aiding in preventing loss of the host's blood from the buccal field. The mouth parts of *M. renardi, R. circularis* and *J. hilsae* are structurally somewhat similar to one another. The mandibular palp is expanded with three setae at apex. Incisor process of mandible is acute. Maxilla with large staright spine and recurred apical spines. All these appendages are highly modified to hold the gill chamber of host fish strongly. Similarly the mouth parts of *N. phaeopleura* and *N. sundaica* are also morphologically somewhat similar. Mandibles of these species are triangular and broad, maxilliped 3 segmented, basal segments very long, middle nearly circular and apical acutely bent laterally with unequal spines and are highly modified for anchoragement to the body surface. In addition, the diet found in their gut contents indicating their parasitic habit that involves detection, attachment, manipulation and processing of host items before parasitism. Detection of the host may be visual and the mandibles are well equipped for handling the host or large food items. The mandibular medial cuticular surface is elaborated into different forms of hard denticles as a pronounced cutting tooth, molars, and canines for shredding and masticating food; hooked mandibles, for perhaps tightly gripping the host fish, serrated movable plates for sawing hard bits of their hosts and then crushing them between opposite grinding molar surfaces, like stones if needed. The shredded and ground food is pushed into the mouth by perhaps, the stout setae of mobiles aided by the long maxillulary robust setae as well. In *C. indica* and *J. brachysoma*, the labrum was lamellar and well developed, aiding in preventing loss of the host's blood from the buccal field.

C. bovina the incisors of each mandible have three sclerotized cusps instead of four as is the case in *Metacirolana moortgati*[9]. It is also worthy to notice that Hale *et al.* and Jones *et al.* used the form of mandibles as a systematic basis for separating or comparing the different genera such as *Neocirolana* and *Cirolana*[19,6]. The mandibles and maxillipeds of post molt and gravid females of some isopods (*e.g. Paracerceis*) may lose their setae as they stop feeding during this period[20]. Considering the first maxillae, it is found that its inner endite in *Cirolana bovina* has three robust pappose setae as opposed to three plumose ones in *Metacirolana moortgati* and four plumose setae in *Sphaeroma serratum*. In addition, the outer endite of the maxillae is heavily chitinized and denticulate in *Metacirolana*[21] and in *Sphaeroma serratum*[22]. This is reminiscent of the branchiopod phyllopodia used in filter-feeding process. The maxillipedal palp of *Sphaeroma serratum* carries typical filter setae with packed soft setules, while its maxillipedal plumose setae are more numerous with dense setules[22]. In contrast, the maxillipedal plumose setae of *C. bovina* are few and ventral and may be used as a secondary filtering net applied to the primary maxillary one for controlling the mesh size. *C. bovina* has three maxillipedal hooks ,two are distal and longer than the third. These have been examined in some marine isopods and found to be restricted to females. For instance, found one coupling maxillipedal hook in the sphaeromatid Dynamene bidentata[23]. The cuticular surface of Crustacea, like that of other arthropods, shows a wide variety of microstructures as displayed by scanning electron microscopy. The most complex structures are the setae, where as some cuticular structures are only ornamental and others are sensory[23].

The mouthparts of *C. bovina* provides a background for further studies of their ultrastructural features and feeding mechanisms. Moreover, the highly differentiated structure of the mandibles may be used in the construction phylogenies within the isopods, as the morphology of the mouthparts has been the basis of amphipod systematics since the time of Boech[24]. The present observations reveal a great diversity of morphology of mouth parts that may be species-specific. The possible functions of the mouthparts, especially in feeding are discussed in light of their structure. In this respect, the mouth parts are heavily modified with respected to their feeding behavior. In the light of the hypothesis that the structures of the mouthparts are related to diet, it seems obvious that parasitic isopods are mainly carnivorous and feeding on host tissues.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Isopods associate with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting, or damaging these fishes. The mouth appendages are strongly modified for the parasitic habit. The labrum of *C. indica* is lamellar and well developed, aiding in preventing loss of the host's blood from the mouth region because there specificity on preference only in the buccal cavity. All these appendages Joryma hilsae are highly modified to hold the body surface and tearing the body muscles of host fish strongly.

Research frontiers

To carry out a comparative study of the mouthparts and the diet of eight isopod fish parasites. The mouthparts consist of a labrum, paragnaths, paired mandibles, maxillules, maxillae and maxillipeds. The labrum and the paragnaths are the least developed but peculiarly the mandibles are asymmetrical, large, stout and highly modified. The analysis of gut contents indicated that *C. indica* and *J. brachysoma* diet consisted of 90% to 95% of animal blood. The diet of *Mothocya renardi, Ryukyua circularis* and *J. hilsae* were mainly composed of mucus (80%-90%).

Related reports

In contrast, the maxillipedal plumose setae of *C. bovina* are few and ventral and may be used as a secondary filtering net applied to the primary maxillary one for controlling the mesh size. *C. bovina* has three maxillipedal hooks ,two are distal and longer than the third. These have been examined in some marine isopods and found to be restricted to females. For instance, found one coupling maxillipedal hook in the sphaeromatid *Dynamene bidentata*. The cuticular surface of Crustacea, like that of other arthropods, shows a wide variety of microstructures as displayed by scanning electron microscopy. The most complex structures are the setae, where as some cuticular structures are only ornamental and others are sensory.

Innovations & breakthroughs

The innovative outcome of this paper to examine the structure of the mouthparts and relate it to their function in the feeding process.

Applications

Comparison of the total number of stomachs analysed the gut contents of *C. indica* and *J. brachysoma* consisted of 90% to 95% (frequency %) and 85%-93% (volumetric %) of blood. The diet of *M. renardi, R. circularis* and *J. hilsae* were mainly composed 80%-90% (frequency %) and 70%-81% (volumetric %) of mucus and similarly the stomach contents of *N. phaeopleura* and *N. sundaica* were dominated by body muscles 75%-83% (frequency %) and 80%-82% (volumetric %) because they were attached only the outer surface of the body.

Peer review

The paper is essentially sound, and I agree with the functional morphology made by the authors with the parasitic isopods. The author included more information on distinguishing the species from its congeners. It has got value informations about cymothoids. I read this article with curiosity, it contributes scientific data in this field.

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