Functional dynamics observations of haptoral armature in *Mizelleus indicus* (Jain 1957) Pandey et al. 2003

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Abstract The negative effect of monogenea on piscine host may vary in the host species. Genus *Mizelleus* (Monogenea: Ancyrocephalidae) was reported for the first time from the freshwater shark, *Wallago attu* (Bl. and Schn.) at Lucknow by Jain (1957). Haptor or opisthohaptor of monogenea which is more or less discoid muscular structure situated posteriorly, is the chief adhesive organ. It is usually provided with anchors and marginal hooklets. Anchors, ventral transverse bar, together with the additional supporting dorsal bars (patches) are active elements of haptor while, marginal hooklets serve as secondary supportive participants, whereas, the dorsal transverse bar can be treated as an inactive element in the whole organ of attachment. Functional dynamics detail of haptoral armature is almost unattended. The anchors in case of *M. indicus* (Jain 1957) Pandey et al. (2003) are disposed one against the other and used to act like four lobed grapnel like a spreader. All components of haptoral armature contribute in one way or another in the process of functional process. Interestingly the worms do not follow the same pattern of sequences during their movements. Various elements and factors are involved in the process. The authors tried to pinpoint the changes in the activity of one major element *i.e.*, dorsal anchor. The movement and change in position/orientation of various elements with reference to dorsal anchors were observed

and summarized. Present paper is rare effort in unexplored aspect in monogenea biology.

Keywords Monogenean · Adduction and abduction · Haptor · Functional morphology

Introduction

Monogeneans evolved from Rhabdocoela like or Temnocephala like ancestors. These organisms probably occupied the body surface or the inner side of the operculum of the ancient fishes. These organisms attached themselves to their host by the posterior haptor acting as a sucker (Bychowsky 1957). The negative effect of monogeneans on piscine host may vary between the host species. Besides, direct tissue destruction by the parasite is obvious, when extensive hyperplasia and destruction of gill-lamellae, bacteria, viruses and variety of other microbes may also enter the host through the injuries, inflicted by monogeneans, with the help of their anchor and feeding apparatus (Cusack and Cone 1985, 1986). It was reported that due to presence of monogeneans on the gills, a significant decrease in respiratory surface area takes place, which adversely affects the health of piscine host (Molnár 1994). Genus *Mizelleus* (Monogenea: Ancyrocephalidae) was reported for the first time from the freshwater shark, *Wallago attu* (Bl. and Schn.) at Lucknow by Jain (1957). Since then, different species of this parasite has been reported by various workers. *M. indicus* was recently re-described by Pandey et al. (2003). Haptor or opisthohaptor is the chief adhesive organ which is situated posteriorly and usually provided with anchors. In majority of monogenea the anchors serve as the main organ for attachment for the parasite. The marginal hooklets, together with the adhesive secretions of the head organs, serve to hold the

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worm, on to their host during the interval between ‘abduction’ and ‘adduction’ of anchors, during their movement along the gills and skin. In case of the *M. indicus* (Monogenea : Ancyrocephalidae) the dorsal anchors are much larger as compared to the ventral anchors. Functional dynamics of haptor armature in case of *M. indicus* is not addressed by previous workers.

Materials and methods

Collection and identification of the piscine hosts

The fish host *Wallago attu* (Bl. and Schn.) for the present study were obtained from local fish markets of Meerut (29:01N; 77:45E). Identification of host was carried (Srivastava 1968; Day 1994). The fishes were immediately examined at the Laboratory, Department of Zoology, Ch. Charan Singh University Meerut.

Collection of the parasitic monogeneans

Due to presence of large amount of mucus on gill filaments (Mizelle 1936, 1938) freezing technique methods (Putz and Hoffman 1963; Rogers 1967; Thatcher and Kritsky 1983) were used.

Functional dynamics/morphology of haptor

Specimens live/fixed (n = 350 approx) were observed for the present study. Initially functional dynamics was observed in live specimens by hanging drop method keeping live parasite along with water drop on the coverslip and then hanging this drop in the cavity and observed with compound microscope under various magnifications. Rough hand drawings were made, in order to get a finer understanding. Parasites were fixed using 10 % neutral formalin in different movement positions. Temporary glycerine mounts were also made in different movement orientations. Since, various elements and factors are involved in the process. In order to explain the phenomenon the authors tried to pinpoint the changes in the activity of one major element *i.e.*, dorsal anchor. Relevant microscopic images were also taken at different magnifications with the help of Motic microscope which were later analysed using Motic Images 2002 1.3 and Motic Images Plus 2.0 software provided by the supplier along with the microscope.

Results and discussion

Interestingly fact that the worms do not follow the same pattern of sequences during their movements. Since,

various elements and factors are involved in the process. Authors tried to pinpoint the changes in the activity of one major element *i.e.*, dorsal anchor. The movement and change in position/orientation of various elements with reference to dorsal anchors were observed. The dorsal anchors of the *M. indicus* are much larger in comparison to the ventral anchors. Situated mid dorsally, with their points directed outwards. Dorsal transverse bar is attached to the conical protuberance present on the inner side of the base of the dorsal anchor. Beneath this protuberance, a cavity is found harboring the rounded upper knob of the dorsal transverse bar, lower knob of dorsal transverse bar is also rounded. The supporting dorsal bars have a sharp pointed and curved end. The paired ventral anchors are situated posteriorly in the lateral lobes of the haptor, points directed outwardly. Each half is ‘J’ shaped and is connected to each other through a small filamentous ligament in the centre which facilitates the flexible movements. The marginal hooklets are fourteen in number and are of peculiar shape and different sizes, they may facilitate the worm position in place during the pendulum like movement of the gill filaments caused by alternating water currents.

The anchors disposed against one another and act as a four lobed grapnel (Fig. 1r) on the principle of a spreader. The dorsal transverse bar acts as an inactive element in the whole process of attachment. The ventral anchors spread the gill filaments during abduction. Ventral transverse bar which is oriented like ‘V’ (Fig. 1p) during the adduction and ‘Λ’ like during abduction (Fig. 1l). The dorsal anchors show a restricted movement due to the presence of straightened type fixed dorsal transverse bar. But the dorsal supporting bars are oriented ‘Λ’ like (Fig. 1g) during abduction and ‘V’ like (Fig. 1o) during adduction thus providing additional support to the anchors. However, they remain parallel to the dorsal transverse bar during clenching.

During the resting state or unattachment stage the dorsal anchors remain in crossed positions with each other like a pair of tongs. The shafts of the dorsal anchor are overlapped in distance between the points of two dorsal anchors. In this position, the base of the dorsal anchors are at maximum distance with each other (Fig. 1a). During the resting phase, the conical protuberance on the inner root of dorsal anchor remains fixed into the gap between the upper and lower outgrowth of the dorsal transverse bar (Fig. 1a, e). Dorsal transverse bar, ventral transverse bar and the supporting dorsal bars remain almost parallel to each other in different planes. Sometimes it was also observed that the inner arm of the lateral pieces of ventral transverse bar rests in the curvature of the spine of supporting dorsal bar. The median filamentous piece of ventral transverse bar remain in relaxed state. Whereas, the ventral anchors do follow the same plan as the dorsal anchor by remaining in the position where the distance between the two outwardly oriented

Fig. 1 Different steps during adduction and abduction in *M. indicus* (Jain 1957) Pandey et al. (2003). **a** Haptoral armature in resting position. Dorsal anchors relaxed and ventral transverse bars resting on curvature of dorsal supporting bars (*patch*). **b & c** Ventral transverse bars exhibit activity resulting points of ventral anchors to open up. **d–h** Ventral transverse bars facilitates the anchorage of ventral anchor which initiates the outward movement in the points of dorsal anchors. **i–m** Marginal hooklets appears to facilitate the entanglement, dorsal

transverse bar appears as an inactive element but it allows to give support while dorsal anchor point is on move resulting outward movement of points and firmness in piercing into the host tissue. **n–t** Median piece of ventral transverse bar allows consolidation steps. Ventral transverse bars further make points of ventral anchor to penetrate deep into host tissue and points of dorsal anchor enters further deep into the host tissue. Marginal hooklets still playing supportive role by entangling to the surrounding host tissue

points remains the minimum. The marginal hooklets, which play secondary supportive role during attachment, remain variously oriented. Since the orientation of marginal hooklets could not be observed during study of movements of worms in live conditions due to magnification problems the observations related to marginal hooklets are primarily based upon the study of fixed specimens.

The process of achieving attachment, with the host tissue or any other suitable substrate, involves the movements of ventral transverse bar along with ventral anchors. This movement is attained with or without the aid of supporting dorsal bars, which move upward and downwards resulting in the spreading of points of dorsal anchors. Besides this, at the same time reduction in the gap between the broad base of the dorsal anchors takes place. Supporting dorsal bars play major role in the process of movement of the major element *i.e.*, dorsal anchor. The upward and downward movement of pointed ends of dorsal supporting bar together with it lifts the lateral pieces of ventral transverse bar (Fig. 2d, o, q). During the lifted state the lateral pieces form ‘^’ shape, with terminal ends placed apart (Fig. 1l). The median filamentous piece may be in most stretched state (Fig. 1l). The entire set of events in coordination with muscular activity results in spreading of points of dorsal anchor. During this process dorsal transverse bar attached to spine of dorsal anchor on the inner side sets dorsal anchor in position. In the mean time during the process of ‘V’ and ‘^’ form like orientation of lateral pieces of ventral transverse bar, the ventral anchors hold the worm on to the host tissue like a pair of hooks, boring into the host tissue by their points. Similarly, the points of the dorsal anchor also fits into the host tissue by boring into it. During the course of establishment, marginal hooklets also get fixed into the soft tissue. Subsequently, the process goes on and ultimately the maximum portion of points of dorsal as well as ventral anchors get embedded deep into the host tissue causing deep damages to the host, whereas, the parasite is benefited by the firm attachment.

After the parasite has anchored itself comfortably into the host tissue, the anchor points are displaced to the maximum possible distance. This minimizes the distance between the bases of the two dorsal anchors (Fig. 1t). Similarly, for the ventral anchor it may or may not be minimum depending upon the attachment orientation. The upper outgrowth of the dorsal transverse bar remain fixed in the protuberance of dorsal anchor on the inner side. The gap between the lower outgrowth of dorsal transverse bar and protuberance on the dorsal anchor appears to be maximum in this position. The supporting dorsal bars are oriented almost parallel to the dorsal transverse bar (Fig. 1s). The median piece of ventral transverse bar may be found in relaxed condition. Whereas, the lateral pieces together may be aligned in straight or ‘V’ or ‘^’ form

(Fig. 1n, r, s). The ventral transverse bars appears to be the most active element in the movement of worm. Here the activity of lateral pieces of ventral transverse bar appears to be related to the activity of the worm, and water current. Most of the marginal hooklets remain in the attached condition within the surrounding host tissue depending on the availability of the host tissue in close association.

Discussion

Monogenean neuromuscular systems are structurally and functionally well-differentiated, as evidenced by research on the fish-gill parasite (Halton et al. 1998). In spite of the biological interest negligible attention has been paid by the workers to study the attachment mechanism of the dactylogyridean type in general and *Mizelleus* in particular. But as far as our knowledge about the functional dynamics or functional morphology is concerned it is undoubtedly mechanically diverse (Halton et al. 1998; Unnithan 1971; Kearn 1998). The anchors in case of *M. indicus* are disposed one against the other and used to act like four lobed grapnel like a spreader. In Ancyrocephalines the anchors acts on the principle of spreader (Gussev 1976). The rapid movement of the ventral anchors could be due to the fact that the intrinsic muscles responsible for their operations might be striated type (in case of *Chauhanelus australis* (Kearn, 1994)). In the attachment process the authors perceived that the attachment of dorsal anchor is more firm as compared to ventral anchors. Because these anchors are capable of turning to a less angle (acute angle). More firm attachment is achieved when anchors are turned to a less angle in case of *Actinocleidus*, *Bychowskyella*, *Quadriacanthus* and others (Gussev 1973). The greater span and more turning capacity of ventral anchors could not be correlated with any previous findings but the author feels that this could be due to the presence of a set of transverse bars attached to each other with the help of a flexible filament. In unattached condition (resting state) dorsal anchors are found in crossed positions like a pair of tongs. Dorsal transverse bars, ventral transverse bars and supporting dorsal bars remain almost parallel to each other in different planes of the body. It is felt that since both the transverse bars together with supporting dorsal bars add to strengthening and flexibility of system by means of a multi articular levers. Since, at the time of detachment no power is required to reinforce the anchor in the gill lamellae thus this articulating levers are perpendicular to the body axis. During the time of attachment, uplifting of supporting dorsal bars provide an additional strength to ventral transverse bar to give better span to ventral anchors. So that they are able to spread the gill lamellae within which the movements and fixation of dorsal anchors becomes easy.

Fig. 2 (Photomicrographs) Different steps during adduction and abduction in *M. indicus* (Jain 1957) Pandey et al. (2003). **a** Haptoral armature in resting position. Dorsal anchors relaxed and ventral transverse bars resting on curvature of dorsal supporting bars (*patch*). **b & c** Ventral transverse bars exhibit activity resulting points of ventral anchors to open up. **d–h** Ventral transverse bars facilitates the anchorage of ventral anchor which initiates the outward movement in the points of dorsal anchors. **i–m** Marginal hooklets appears to facilitate

the entanglement, dorsal transverse bar appears as an inactive element but it allows to give support while dorsal anchor point is on move resulting outward movement of points and firmness in piercing into the host tissue. **n–t** Median piece of ventral transverse bar allows consolidation steps. Ventral transverse bars further make points of ventral anchor to penetrate deep into host tissue and points of dorsal anchor enters further deep into the host tissue. Marginal hooklets still playing supportive role by entangling to the surrounding host tissue

Earlier it was also pointed out more or less similar events in the functioning of haptor in an American genera *Muretytrema* (Monogenea) (Gushev 1973). It is found that marginal hooklets use to bore across the branchial tissue within the area squeezed by the anchors. Which provide the additional support during the attachment and it also helps at times when parasite use to change their place and withdraw the anchors (Gushev 1973). However, marginal hooklets are typically present in adult dactylogyrideans but the contribution of these hooklets to the attachments seems to be minimum as compared to the massive hamuli (Kearn 1998). In case of *Chauhanelus australis*, the marginal hooklets are specially active immediately after the haptor moves to new inter lamellar niche and by pinning the haptor to the gill lamellae they may facilitate the penetration of hamuli into the gill lamellae (Kearn 1994). During present study marginal hooklets were observed in fixed specimens only where they appears to have various orientations.

Conclusions

The anchors in case of *M. indicus* are disposed one against the other and used to act like four lobed grapnel like a spreader. In the attachment process the authors perceived that the attachment of dorsal anchor is more firm as compared to ventral anchors. Because these anchors are capable of turning to a less angle (acute angle). The greater span and more turning capacity of ventral anchors could be due to the presence of a set of transverse bars attached to each other with the help of a flexible filament. Both the transverse bars together with supporting dorsal bars add to strengthening and flexibility of system by means of a multi articular levers. During the time of attachment, uplifting of supporting dorsal bars provide an additional strength to ventral transverse bar to give better span to ventral anchors. So that they are able spread the gill lamellae within which the movements and fixation of dorsal anchors becomes easy. Marginal hooklets use to bore across the branchial tissue within the area squeezed by the anchors provide the additional support during the attachment and it also helps at times when parasite use to change their place and withdraw the anchors. During present study marginal hooklets could be observed in fixed specimens only where they appears to have various orientations.

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