

## DISTRIBUTION OF THE NERVOUS ELEMENTS IN THE HAPTOR OF FOUR PECULIARLY-CLAMPED MONOGENEAN FISH PARASITES TO MATCH WITH ITS MORPHOLOGY

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### ABSTRACT

The innervations of haptors in four monogenean fish parasites were studied using the enzymatic technique, the acetylthiocholin iodide for choline esterases. The innervations of the clamps in *Gastrocotyle trachuri* is curious since the 15 or so anteriormost clamps are supplied from the clamp-side lateral nerve trunk or cord and the 10 or so posteriormost clamps are innervated from the main haptor nerve. After the fusion of the two main haptor nerves, two distinct nerve arise to supply marginal II in addition to marginal I and pair of small hamuli. The innervation of haptor of *Microcotyle donovani* consists of the usual two pre-haptor ganglia which give rise to 2 main haptor nerves; from each one a separate nerve arises to supply a clamp. From each side of the pre-haptor ganglia, two thin nerves arise one at side of each main haptor nerve. After supplying the two rows of the clamps the 2 haptor nerves fuse together and after their fusion, fine nerves arise possibly to supply the hamuli and 2 pairs of marginal hooks. The innervations of the haptor of *Axine belones* appears to be unusual; the pre-haptor ganglia are formed after the fusion of the ventral and lateral nerve cords only, because the dorsal nerve cord does not extend beyond the anterior third of the body. The non-clamp side pre-haptor ganglion is located near the position of the larval haptor while the clamp-side pre-haptor ganglion is remote from the position of the hamuli. It is interesting to note that even with replication of clamps there are separate nerves supplying each clamp. From the clamp-side pre-haptor ganglion a main nerve arises to supply the additional clamps, and in addition to that another nerve arises to supply some of the anteriormost additional clamps. The haptor innervations of *Pseudaxine trachuri* is asymmetrical as the clamps are innervated from the main haptor nerve, and not from the lateral nerve cords. The interesting feature of *P. trachuri* is the asymmetrical positions of the pre-haptor ganglia. The clamp-side pre-haptor ganglion is situated close to the anteriormost clamps, while the non-clamp side pre-haptor ganglion is situated near the languette. After the fusion of the main nerve trunks of the non-clamp side, prominent nerves arise to supply the languette with its armature, which are the 2 hamuli and marginal I. It can be concluded that the haptor innervations of the four monogenans studied cope with the modification of haptor with its armature.

**KEY WORDS:** enzymatic technique, monogenean fish parasites, nervous system

### INTRODUCTION

In the review of the nervous system of invertebrates, Bullock and Horridge (1965) a chapter was devoted to platyhelminths, which included surveys of classical work on the gross morphology and histology as revealed by histological stains. Some noteworthy studies of individual monogeneans have been published: Halton and Jennings (1964) on *Diplozoon paradoxum*; Bovet (1967: cited by Llewellyn, 1981) on *D. paradoxum*; Rohde (1968) on *Polystomids malayai*, Halton and Morris (1969) on *Diclidophora merlangi* Rahemo and Gorgees (1987) on *Polystoma integerrimum*, using both conventional and enzymatic techniques. More recently a sophisticated technique namely immunomicroscopical method was used proved to be more efficient in tracing the nervous system as this done by Zurawski *et al.* (2001) on adult *Eudiplozoon nipponicum*. Furthermore, Phalloidin fluorescence technique, enzyme cytochemistry and immunocytochemistry in conjunction with confocal scanning laser microscopy have been used for the first time to describe the nervous and muscles of the viviparous monogenean gill parasite, *Macroglyrodactylus clarii* (El-Naggar *et al.* 2004). One Gastrocotylid and three microcotylid were selected to study their nervous systems as they are unique among monogenean as there is peculiarity in clamps distribution i.e. they are distributed approximately equal along margins of cotylophore as in *Microcotyle*, or clamps decidedly unequal in number on the two sides as in *Axine*, or clamps distributed along one margin of the cotylophore as in *Pseudaxine* while in *Gastrocotyle trachuri* the cotylophore is a simple lateral flange bearing a single row of small clamps (see Dawes, 1968). Jack Llewellyn (personal communication) wonder if nervous elements cope with the type of cotylophore especially clamps distributions in the four species selected, as such this investigation was designed.

### MATERIALS AND METHODS

Specimens of monogeneans were collected from the marine fishes at Plymouth in period 1979-1982. *Gastrocotyle trachuri* Benden and Hesse, 1863 collected from the gill of horse mackerel; *Microcotyle donovani* Benden and Hesse, 1863 collected from the gills of ballan wrasse; *Axine belones* Abildgaard, 1794 collected from the gills of Garfish; *Pseudaxine trachuri* Parona and Perugia, 1890 collected from the gills of mackerel. The specimens were fixed in 10% formaline for about half an hour then washed in distilled water then incubated in the working solution of

acetylcholinesterases, the acetylthiocholine iodide method of Gomori (1952) as reported by Jennings and LeFlore (1972). After 3-13 hrs treatment they were washed with 40% sodium sulphate then mounted in glycerine jelly, examined and photographed either by using superimposed illumination (epillumination) or ordinary transmitted light illumination.

## RESULTS AND DISCUSSION

### *Gastocotyle trachuri*

*G. trachuri* is an asymmetrical monogenean but its asymmetry is different from that of *Axine belones*. It shows unilateral symmetry i.e. the clamps only develop on one side of the body while on the opposite side the clamp development is suppressed. The innervations of the clamps in *G. trachuri* is curious (Figure 1 -2) since the 15 or so anteriormost clamps are supplied from the clamp-side lateral; nerve trunk or cord and the 10 or so posteriormost clamps are innervated from the main haptoral nerve i.e. the fused ventral and lateral nerve cords only: the usual fusion of the nerve trunks occurs at the middle of the clamp row and not anterior to the haptor (the usual position of the pre-haptoral ganglia). The other interesting feature of the haptor of *G. trachuri* is the innervations of the languette. After the fusion of the two main haptoral nerves, two distinct nerves arise to supply marginal II in addition to marginal I and pair of small hamuli.

The innervation of *G. trachuri* corresponds to the asymmetry of the haptor. As the clamps are distributed on only one side of the body, the clamp nerves are also present on only one side, and are absent on the other side where the development of the clamps is suppressed. An interesting feature of the innervations of the haptor is that the positions of both the “clamp-side” and the “non-clamp side” pre-haptoral ganglia are curious, the non-clamp side pre-haptoral ganglion being situated near the larval haptor (i.e. in the languette) and the clamp side pre-haptoral ganglion at the middle of the clamp row. As the replication of the clamps occurred in postero-anterior succession, indicated by their sizes (Llewellyn, 1959), the position of the “pre-haptoral” ganglion is in the middle of the clamp row. This could be explained by there being an addition of more clamps anteriorly. The lateral nerve, before its fusion with the ventral nerve, contributed in supplying the anteriormost clamps. Another interesting feature in *G. trachuri* is that the non-clamp side “pre-haptoral” ganglia is not located anterior to the adult haptor, but anterior to the languette, indicating that early stages of development there is a suppression of clamp formation, as indeed was described by Llewellyn (1959). The other feature is the innervations of the languette.

In addition to supply of the 2 hamuli and marginals I there are special innervations to marginal II. The innervations to the persistent marginal II indicates the important role played by marginal II, even if not actually deciduous, appears to be no more than “vestigial” e.g. in *Kuhnia scombri*. In *G. trachuri* however, M II, after being ventrally directed in the newly-hatched oncomiracidium, later becomes dorsally directed and makes a special contribution to the attachment of the parasite by impling a secondary lamella contiguous with the dorsal surface of the haptor and so promoting greater security.

### *Microcotyle donavini*

The organs of attachment of *M. donavini* consist of two rows each containing numerous clamps (Figure 3 -4), one on each side of the haptor. The innervations of haptor consist of the usual two pre-haptoral ganglia which give rise to 2 main haptoral nerves; from each one a separate nerve arises to supply a clamp. In addition to the main haptoral nerves arise from each side of the pre-haptoral ganglia, two thin nerves arise one at side of each main haptoral nerve i.e. one lateral and one medial. These run parallel to the main haptoral nerves. The targets of these slender nerves are not known but they may supply the haptor itself since the main haptoral nerves are mainly involved in supplying the clamps. After supplying the two rows of the clamps the 2 haptoral nerves fuse together and after their fusion, fine nerves arise possibly to supply the hamuli and 2 pairs of marginal hooks.

The interesting features of the haptor innervations of *Microcotyle donavini* is the two main haptoral nerves which arise from the 2 pre-haptoral ganglia to supply the clamps on both sides of the haptor. Two thin nerves also emerge from the pre-haptoral ganglia to supply the haptor proper. Similar nerves supplying the margin of the haptor were also described in *Kuhnia scombri*. The other interesting feature of the clamp nerves is, as there is a replication of clamps, there is a corresponding replication of clamp nerve. The origins of the clamp nerves are symmetrical for both sides of the haptor, but the nerves are of different lengths. Comparing the results obtained in this study (as seen in figs. 3-4) with those of Goto (1894), who described the nervous system of *Microcotyle caudate*, *M. sebastis*, *M. elegans* and *M. reticulate*, the following features can be pointed out. Goto was not able to trace clamp nerves or haptoral ganglia formed after the fusion of the main trunks. All these shortcomings are possibly due to the technique used but might, of course be due to differences in phylogeny.

Comparing the nervous system of *M. donavini* with *M. sebastis* which was described by Bonham and Guberlet(1937), some differences can be seen. These authors were not able to trace the haptor ganglia formed after the fusion of the 3 main trunks and, more curiously, the clamp nerves arising from the main haporal nerves.

### ***Axine belones***

The haptor in *A. belones* is markedly asymmetrical with row of about 60 clamps on one side and apparently no clamps on the other. The morphological posteriormost part of the haptor however, is situated about the middle of the clamp row since in that position there is a pair of small hamuli i.e. the morphologically posteriormost part of the body is not the topographically posteriormost part of the haptor.

The innervations of the haptor (Figure 5 -7) appears to be unusual; the pre-haptor ganglia are formed after the fusion of the ventral and lateral nerve cords only, because the dorsal nerve cord does not extend beyond the anterior third of the body. The non-clamp side pre-haptor ganglion is located near the position of the larval haptor(which is indicated by the position of the small hamuli) while the clamp-side pre-haptor ganglion is remote from the position of the hamuli, or in other words, the clamp-side pre-haptor ganglion has changed its original position. It is interesting to note that even with replication of clamps there are separate nerves supplying each clamp. From the clamp-side pre-haptor ganglion a main nerve arises to supply the additional clamps, and in addition to that another nerve arises to supply some of the anteriormost additional clamps (Figure 6). A row of cells (Figure 7) equal in number to the number of clamps is also present and runs parallel to the main haptor nerve. The axons of these cells are directed towards the clamp nerves. In each clamp, if viewed under high power, a network can be distinguished in both anterior and posterior valves (Figure 7).

The nervous system of *A. belones* is asymmetrical in correspondence with the asymmetry of the body. The most interesting asymmetrical feature is the innervations of the clamps which are situated at both sides (anterior and posterior) of the hamuli, which themselves are situated midway along the clamp-margin. As there is no complete study of the developmental stages of *A. belones*, the sizes of the clamps were taken to be indicators of their time of origin and growth.

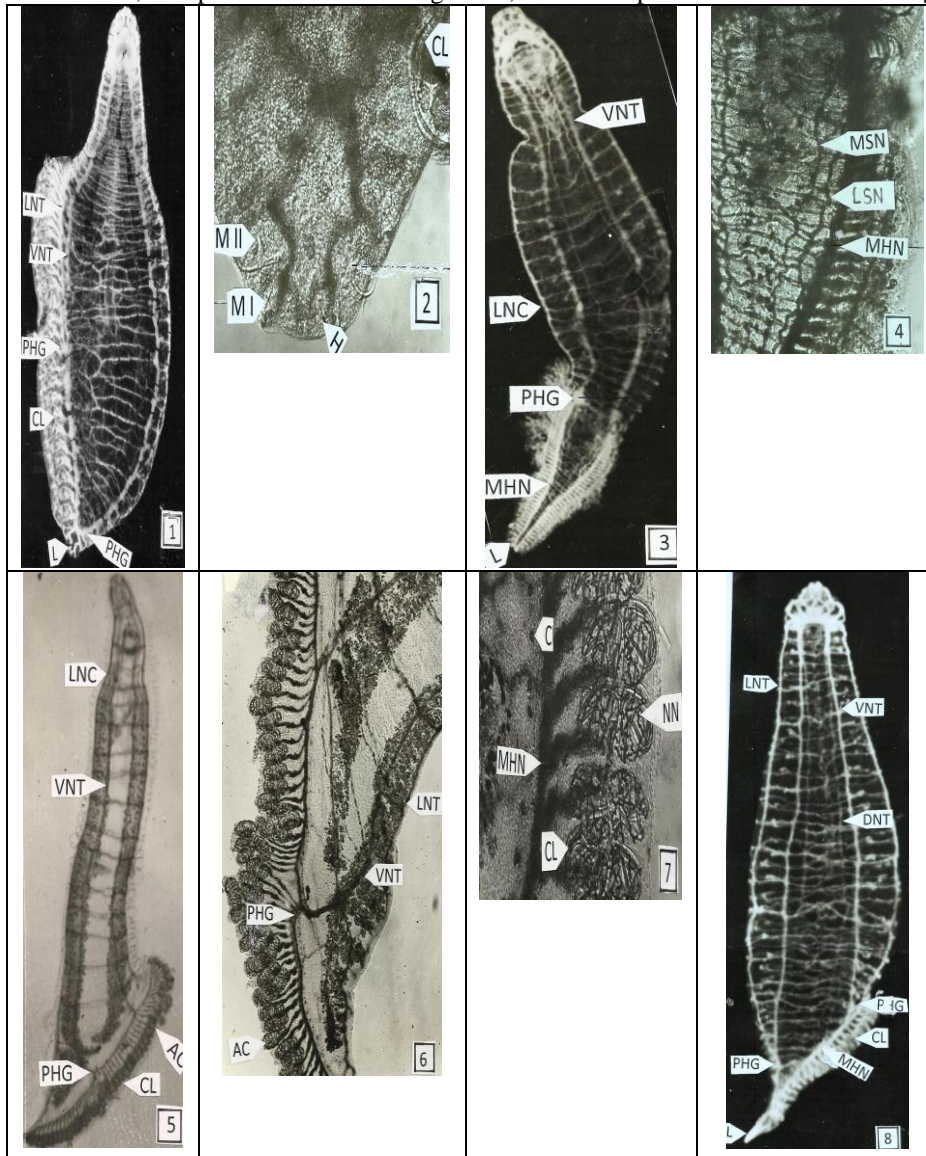
Comparing the positions of the “pre-haptor ganglia” of *A. belones* with those observed in bilaterally symmetrical polyopisthocotyleans, it is clear that the “non-clamp” side “pre-haptor ganglion” remains in its original position, while that of the clamp-side becomes remote from hamuli(see Figs. 5-6). This could be explained by the replication of the clamps having taken place in the area immediately anterior to the hamuli, this having led to a displacement of the ganglion anteriorly. In addition, further clamps are replicated at the extreme anterior border of the haptor and “so” an accessory nerve has developed to supply them. As the non-clamp side “pre-haptor” ganglion remains near the hamuli, it may be assumed that the replication of the clamps occurred at one site, namely at the posterior tip of the haptor and thus there was no “need” for the development of an additional nerve to supply them.

The asymmetrical features of the nervous system of *A. belones* cannot be resolved accurately until further studies have been made such as tracing the nerves at various developmental stages. If the nervous system of *A. belones* as described in this study is compared with that of *A. heterocerca*, described by Goto(1894), some differences emerge: Goto was not able to trace (a) the complete courses of the haptor postero-lateral nerves and their contribution to the formation of the “pre-haptor” ganglia; and (b) the row of the unipolar cells from the clamp frill or the 2 large cells associated with the “pre-haptor” ganglia. Again all these differences could be due to the technique used.

### ***Pseudaxine trachuri***

The haptor innervations of *P. trachuri* (fig.8) is asymmetrical similar to that of *G. trachuri* except that the clamps are innervated from the main haptor nerve as observed in other monogeneans, and not from the lateral nerve cords as it is in *G. trachuri*. The interesting feature of *P. trachuri* is the asymmetrical positions of the pre-haptor ganglia. The clamp-side pre-haptor ganglion is situated close to the anteriormost clamps, while the non-clamp side pre-haptor ganglion is situated near the languette. After the fusion of the main nerve trunks of the non-clamp side, prominent nerves arise to supply the languette with its armature, which are the 2 hamuli and marginal I. The “clamp-side” pre-haptor ganglion is situated close to the anteriormost clamps, i.e. in its expected position. In this respect it is different from that of *Gastrocotyle trachuri*, where it is situated in the middle of the clamp-row. The reason for this difference is not clear, but possibly it indicates that growth or replication of clamps in *P. trachuri* occurred in a postero-anterior direction as described by Llewellyn(1959) which leads to the “pre-haptor” ganglion moving more anteriorly to be located close to the anteriormost clamps. Comparing this situation with that in *G. trachuri*, in *G. trachuri* the replication seems to have happened more anteriorly(parallel to the longitudinal axis of the body), so that the lateral nerve cord or trunk contributed to the innervations of the anteriormost clamps, while in *P. trachuri* the replication

occurred antero-laterally to the main longitudinal axis of the body. The innervations of the languette of *P. trachuri* is similar to that of *G. trachuri*, except that there is no marginal II, the nerves pass to the hamuli and marginal I only.



**Figure 1.** An epillumination photomicrograph of *Gastrocotyle trachuri* after Acetylthiocholine iodide treatment (AcThI) showing the main components of the nervous system: lateral nerve trunk (LNT), ventral Nerve trunk (VNT), pre-haptoral ganglion (PHG), clamps (CL), and Languette (L). X 94.

**Figure 2.** Transmitted light Photomicrograph of *G. trachuri* after AcThI Treatment showing the innervations of the langutte with its Marginal hook II (M II), marginal I (M I), hamuli (H), and clamps (CL). X364.

**Figure 3.** An epillumination photomicrograph of *Microcotyle donovani* after AcThI Treatment showing: the lateral nerve cord (LNC), ventral nerve Trunk (VNT), pre-haptoral ganglion (PHG), main haptoral nerve (MHN) and the languette (L). X 70.

**Figure 4.** An enlarged transmitted light photomicrograph of *M. donovani* after AcThI treatment showing: the main haptoral nerve (MHN), median Slender nerve (MSN), lateral slender nerve (LSN). X 107.

**Figure 5.** A transmitted light photomicrograph of *Axine belones* after AcThI Treatment showing: lateral nerve cord (LNC), ventral nerve cord (VNC), Pre-haptoral ganglion (PHG), clamp (CL), additional clamps (AC). X36.

**Figure 6.** An enlarged transmitted light photomicrograph of *A. belones* after AcThI treatment showing: lateral nerve trunk (LNT), ventral nerve Trunk (VNT), pre-haptoral ganglion (PHG), additional clamps (AC). X 150.

**Figure 7.** Highly enlarged photomicrograph of *A. belones* after AcThI Treatment showing: the main haptoral nerve (MHN), clamp (CL), and nerve Network (NN), Cell. X 250.

**Figure 8.** An epillumination photomicrograph of *Pseuaxine btrachuri* after AcThI Treatment showing: lateral nerve trunk (LNT), ventral nerve Trunk (VNT), dorsal nerve trunk (DNT), pre-haptoral ganglion (PHG), Main haptoral nerve (MHN), clamp (CL), Languette (L). X90.

## REFERENCES

- Bonham K. and Gubertlet J.E. (1937).** Notes on *Microcotyle sebastis* Goto from Puget Sound. *J. Parasitol.* **23**: 281-290.
- Dawes B. (1968).** The Trematoda with Special Reference to British and Other European Forms. Cambridge at the university press.
- El-Naggar M.M., Arafa S.Z., El-Abbasy S.A., Stewart M.T. and Halton D.W. (2004).** Neuromusculature of *Macrogyrodactylus clarii*, a monogenean gill parasite of the Nile catfish *Clarias gariepinus* in Egypt. *Parasitol. Res.* Pages. 1-15.
- Gomori G. (1952).** Microscopical Histochemistry. University of Chicago Press, Chicago. pp.273
- Goto S. (1894).** Studies on the ectoparasitic trematodes of Japan. *J. Coll. Sci. Tokyo.* **8**:1-27.
- Halton D.W. and Jennings J.B. (1964).** Demonstration of the nervous system in the monogenetic trematode *Diplozoon paradoxum* Nordmann by the indoxyl acetate method for esterase. *Nature.* **202**: 510-511.
- Halton D.W. and Morris G.P.(1969).** Occurrence of cholinesterase and ciliated sensory structures in a Fish gill-fluke, *Diclidophora merlangi* (Trematoda: Monogenea). *Zeitschrift fur parasitenkunde.* **33**: 21-30.
- Jennings J.B. and LeFlore W.B(1972).** The histochemical demonstration of certain aspects of cercaria morphology. *Trans. Am. Microscop. Soc.* **91**:56-62.
- Llewellyn J. (1959).** The larvae and larval development of monogeneans. *Advanc. Parasitol.* **1**:287-326.
- Llewellyn J. (1981).** Biology of Monogeneans. In EMOP # workshop no.4, Cambridge.Parasitology. **56**:section II.493-504.
- Rahemo Z.I.F. and Gorgees N.S.(1987).** Studies on the nervous system of *Polystoma integerrimum* as revealed by acetylthiocholine activity. *Parasitol. Res.* **73**:234-239.
- Rohde K.(1968).** Das nervensystem der Gattung *Polystomoides* Ward, 1917(Monogenea).(The nervous system of the genus *Polystomoides*, Ward,1917 (Monogenea). *Zeitschrift fur Morphologie der Tiere.* **62**: 58-76.
- Zurawski, T.H., Mousley A., Mair G.P, Brennan G.P., Maule A.G., Gelnar M. and Halton D.W. (2001).** Immunomicroscopical observations on the nervous system of adult *Eudiplozoon nipponicum* (Monogenea: Diplozoidae). *Inter. J. Parasit.* **31**:783-792.