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Vasculature of the respiratory organs in a bimodally breathing freshwater crab-*Paratelphusa spinigera*, Wood Mason of Kawar Lake wetland (Begusarai), North Bihar.

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Abstract- The freshwater crab, *Paratelphusa spinigera* is abundantly found in/on the mud soil of the bank of Kawar Lake Wetland. These crabs live in the coastal littoral zone of the wetland having shallow water. These are not only aquatic but also terrestrial in habit and crawl on the land and take their food. These animals are not only aquatic but also terrestrial in habit and respire bimodally. The respiratory circulation was investigated in the bimodally breathing crab *Paratelphusa spinigera* by analysis of the distribution of carmine solution injected into the haemocoel. The gills and branchial membrane (branchiostegite and membranous thoracic walls), both trap this carmine solution. The main blood supply to the branchiostegites is from the venous sinuses, which shows its involvement in gas exchange. In the air-breathing crab a greater proportion of the total venous return is directed to the branchiostegite. The air- haemolymph barrier in the branchiostegite was found to be 3.232 μm . *Paratelphusa spinigera* has seven pairs of phyllobranchiate gills contained in the branchial chamber. From central axis of the gills arise bilaterally situated thin flaps, the lamellae. The afferent branchial vessel (the epibranchial vessel) is located on the dorsal side of the gill arch and the efferent vessel (the hypobranchial vessel) on the ventral side. Between these two blood vessels the blood percolates through the lamellar vascular channels, where it is oxygenated. The lamellae consist of an epithelial cell layer covered by thin cuticle. The epithelial cells approach each other at regular intervals and fuse in the middle of lamellar sinus delineating the vascular channels.

Key words: Freshwater crab, *Paratelphusa spinigera*, branchiostegite membrane, counter current principle

INTRODUCTION

The respiration involves the exchange of gases between the body and the environment. The oxygen from the surrounding medium enters within the body and carbon dioxide comes out of the body to the environment. Either entire surface or at least some parts of the body act as respiratory surface. Bimodally breathing crabs have universally retained gills, but may also developed accessory breathing organs, branchial epithelium or lungs formed

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from the inner lining of the carapace and branchiostegites, some researchers have also stated that in aerial respiration important role is played by the thin epithelium that lies beneath the branchiostegites.^{1,2}

The vascularised branchiostegal membranes lining the branchial chambers of air- breathing crabs have clearly become the major site of oxygen uptake, providing an evolutionary parallel to the lungs of pulmonate molluscs formed by vascularisation of the mantle chamber.^{3,4} However unlike the pulmonates, air- breathing crabs have retained their gills, which have also become modified for aerial gas exchange.

Farrelly and Greenaway (1993)⁵ reported several circulatory patterns in the lungs of land crabs, using corrosion casting techniques. Till date there is no data available on the morphology and vascular organisation of respiratory organs of *Paratelphusa spinigera*, which are abundantly found in and on the mud soil of ponds, puddles, wetlands and swamps of the North Bihar. The present study has been contemplated to evaluate morphology and vascular organisation of respiratory organs (both gills and branchiostegal membrane) of a freshwater crab *Paratelphusa spinigera*, Wood Mason.

MATERIALS & METHODS

For morphological study of the respiratory organs of *Paratelphusa spinigera*, preserved specimens were washed under running tap-water and they were dissected by following standard method adopted by Whitehouse and Grove (1956)⁶ in the case of Cray fish. For this the different part of the respiratory organs was observed externally and was measured with the help of a divider and mm-graduated scale, whichever required. For the observation of gills, they were removed from the attachment point of branchial chamber and examined under magnified hand lens.

Another method was also used to study the vascularisation of the branchiostegal membrane, which has been reported by Diaz and Rodriguez (1977)⁷. A solution of methylene blue (1.5 mg/ml, approximately) was prepared in boiled distilled water and then reduced by addition of a saturated solution of sodium sulphite, all blue colour disappeared. From 1 to 5 μ l of this reduced solution were injected every four hours into living crabs, through the coxopodite of each pereopod. Reduced methylene blue recovers its original colour by oxidation and stains the tissues dark or black where oxygen uptake occurs. Crabs were sacrificed at different times after injection and the entire branchial chambers were inspected.

The path of the water flow through branchial chamber was followed by observing streams of Indian ink, which were pipetted near the respiratory openings above the cheliped and pereopods. The water flow pattern was observed by this method which was used by Arudpragasam and Naylor (1964a)⁸.

To study the circulatory system, the specimen was narcotized by ether and carapace was removed by cutting its internal border. The membrane present beneath the carapace was removed carefully, which made visible the

heart of the organism. The aqueous borax carmine solution was injected into the heart by fine sharp pointed needle. After 15 minutes the arteries were traced carefully.

RESULTS & DISCUSSION

The respiratory system of *Paratelphusa spinigera* basically consisted of seven pairs of gills, one pair of branchial chamber or gill chamber, branchiostegal membrane or lungs and two pairs of epipodites.

Branchial chamber: A pair of branchial chambers (gill chamber) was present, one on either side of the thoracic region. The branchial chamber was covered by part of carapace and branchiostegite on the outer side and thoracic wall on the inner side. The inner layer of branchial chamber was thin and membranous and remained in direct contact with water current and air. The gill chamber was drawn anteriorly into a tube, which opened to the exterior by an exhalent opening. Seven gills and one pair of epipodites were found enclosed within each branchial chamber (fig-1).

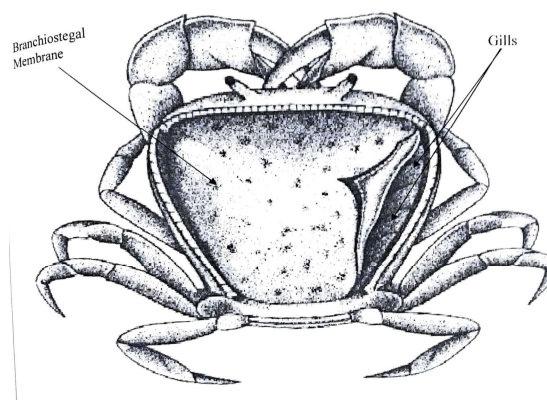


Fig-1 : Diagram showing the branchiostegal membrane after removing the outer carapace (dorsal side) of *Paratelphusa spinigera*.

Epipodites: Two pairs of simple leaf-like epipodites were present inside the branchial chamber. These were outgrowth of the coxal podomers of the 2nd and 3rd maxillipedes.

Gills: The aquatic respiratory system of *Paratelphusa spinigera*, basically consisted of seven pairs of gills contained within well- developed branchial chambers formed by the overhanging carapace and branchiostegites (fig-2). *Paratelphusa spinigera* possesses seven pairs of gills, equal to that reported in the terrestrial crab *Holthuisana*⁹ and in the Indian freshwater crab

*Paratelphusa masoniona*¹⁰. Maina (1990)¹¹ observed seven pairs of gills, in *Potamon niloticus*. The gills of the *Paratelphusa spinigera* are phyllobranchiate (Lamellar) in form, consisting of central axis bearing serially arranged plate like lamellae (Except 1st pair of gills) The first gill also consist of a central axis bearing serially arranged plate-like lamellae on one side i.e., posterior side, but on other side of the central stem lamellae are absent. Thus, the first gill is hemibranch and rests are holobranch.

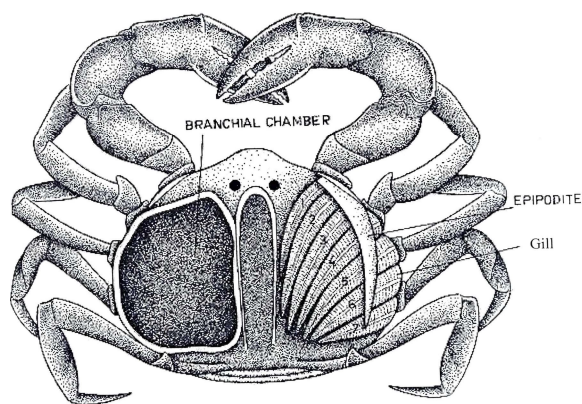


Fig-2 : Diagram showing the arrangement of the seven gills (right hand side) and branchial chamber after removing the gills and epipodites (left hand side).

The lamellae are semicircular in shape and have a relatively smooth surface. At regular intervals more or less in alternate fashion, the central gill raphe (axis) gave rise to lamellae, which decreased in size towards the apex of the gill and finally they fuse. The afferent and efferent branchial vessels are located on the dorsal (epibranchial) and ventral (hypobranchial) aspect of the central gill raphe respectively. These blood vessels may be grossly distinguishable by having regular knobs on the top surface of afferent branchial vessel and the efferent vessel with sharp spikes that decreased in size and eventually disappeared towards the tip (fig-3).

The afferent vessel gave rise to the channels that profuse the lamellar flaps. The epithelial cells that at intervals extended and traversed the lamellar sinus delineated these channels. The morphology of the gills of *Paratelphusa spinigera* is basically similar to that of the other decapods like *Potamon niloticus* as reported by Maina (1990)¹¹. In case of *Paratelphusa spinigera* gills are also structurally well adapted for aquatic as well as aerial mode of life. The swelling tips or margins of the gills help the rest part of the adjacent lamellae to remain separate, when

the animal is out of water and make the total surface area available for gas exchange. Pandey (1997)¹² also reported the presence of swollen tips on the gills, might help the aerial gas exchange in *Paratelphusa spinigera*.

On each side of the branchial chamber there are seven-phyllobranchiae i.e., their axes bear rows of heart shaped leaf like filaments or lamellae. These gills may be distinguished into following two categories depending on their attachment.

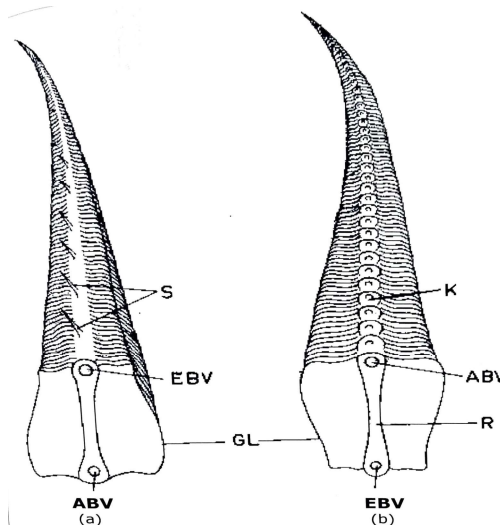


Fig-3 : Diagram showing the dorsal (b) and ventral (a) view of the gills to show the position of spikes (on ventral side) and knob (on dorsal side), over the efferent (hypobranchial) vessel and afferent (epibranchial) vessels respectively.

Abb: ABV – Afferent Branchial vessel, EBV – Efferent Branchial vessel, GL- Gill Lamellae, K- Knob, R- Raphe, S-Spike.

Arthrobranch: The gills, which are attached with the arthroidal membrane of the appendages, are known as arthrobranch. Out of seven pairs of gills, five pairs are of arthrobranch (1st, 2nd, 3rd, 4th and 5th pairs). Along the main axis of each gill the blood flows through lamellae in a direction from epibranchial (Afferent branchial vessel) space to the hypobranchial (Efferent branchial vessel) space. The water flows in the opposite direction, showing counter current principle (fig-7). Among these 5 pairs, first pair was attached with 2nd maxillipeds, second and third pairs with 3rd maxillipeds and 4th and 5th pairs with the chelipeds.

Pleurobranch: The pleurobranch is the gill, attached to the lateral wall of the thoracic segment. Among 7 pairs of gills, last two pairs were pleurobranch, 6th pair of gills were attached with 5th thoracic segment, while the last 7th pair were attached with the 6th thoracic segment.

Branchiostegal membrane (Branchial epithelium): *Paratelphusa spinigera* can survive about 5-10 days out of water. In the present observation it has been found that its gills are modified for aerial gas exchange. Besides these gills, branchiostegal membrane is also modified for aerial respiration, which is highly vascularised. It is the inner lining of the branchial chamber. The branchiostegites of terrestrial and semiterrestrial crabs from several other families are also highly vascularised.^{7,13} Morphological specialization of the inner surface, which appear adapted to facilitate gas exchange, have prompted several authors to suggest that these structures, may function as lungs.¹⁴⁻¹⁶

The circulatory pattern observed in the branchiostegal membrane of *Paratelphusa spinigera* has been drawn in fig-4. The afferent pulmonary vessel supplies venous blood to the branchiostegal membrane from the thoracic sinus. This afferent pulmonary vessel flows around the perimeter of branchiostegite. This afferent system gives rise to interdigitating portal system (branching networks, arising and terminating in the lacunar bed). Blood flowing through these portal systems is oxygenated and collected by efferent pulmonary vessel and oxygenated blood is poured into the pericardial sinus, through branchiopericardial vessels.

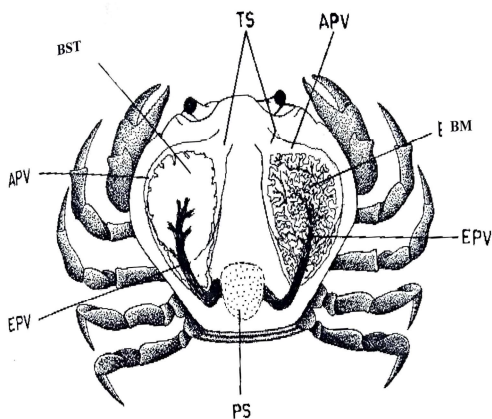


Fig-4 : Diagrammatic representation of Branchiostegal Membrane in *Paratelphusa spinigera*, Abb: APV- Afferent pulmonary Vessel, BM- Branchiostegal Membrane, BST- Branchiostegite, EPV- Efferent Pulmonary Vessel, PS-Pericardial Sinus, TS-Thoracic Sinus.

Water flow pattern: Water enters to the branchial chamber mainly (70-80%) through the Milne Edwards openings above the chelae (Fig.-6). Some water also enters via the small opening above each of the other four pereopods. The majority of the water passes into the hypobranchial space and then flows between the gill lamellae and into the epibranchial space before it moves forwards and comes out through the exhalant opening (fig.-6). The inhalant openings above the 4th and 5th pereopods are imperfectly separated. And water entering through these two opening mixes and passes under the last gill into the hypobranchial space and then up between the proximal parts of gills. However, some water entering via these openings passes around the posterior edge of the last gill and directed into the epibranchial space before passing diagonally downwards across the surface of the anterior gills. After entering of water from Milne Edwards opening, it flows mainly through the posterior part before passing anteriorly. The lamellae of the gills of terrestrial crabs appear to be strengthened with chitinous ridges, which presumably keep them erect and functional in air.¹⁴ The airflow inside the branchial chamber apparently occurs by the beatings of the scaphognathite¹⁷, which provides the oxygen taken by diffusion through the existing water film on the lamellae.¹⁸ Taylor and Greenaway (1984)¹⁹ have reported in bimodally breathing crab, *Holthulsana transversa* that during aquatic ventilation, water entering the branchial chambers at the leg bases, passes first over the gills and these are therefore likely to be the main sites of aquatic gas exchange. However, water leaving the gill then passes through the epibranchial chambers and it is likely that the thin inner integument of the branchiostegites and medial walls would also function in aquatic gas exchange in *H. transversa*.¹⁹

General organization of the circulation in *Paratelphusa spinigera*: The main circulatory routes within *Paratelphusa spinigera* are schematically illustrated in fig.5, 8 & 9. After leaving the heart, blood enters the arterial system, which supplies the musculature and all of the principal organs. Blood then passes through a series of narrow capillaries intimately associated with the individual tissues and these lead into larger haemocoelomic lacunae. Deoxygenated blood then drains into a system of sinuses within the thorax. The whole thoracic sinus system is intercommunicated. From these blood passes to infrabrachial sinuses which run along each side of the thorax at the base of the gills and interconnect the afferent

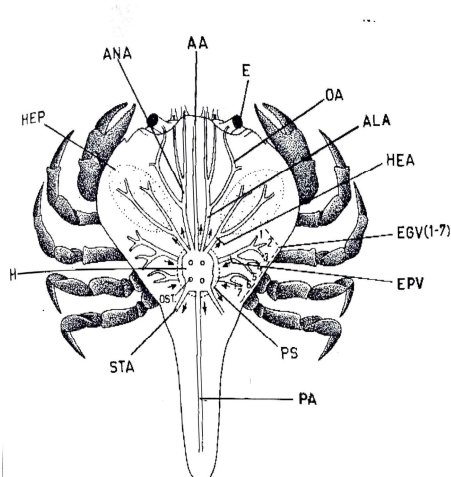


Fig-5 : Dorsal view of *Paratelphusa spinigera* showing blood circulation.

Abb. AA-Anterior Aorta, ALA-Anterolateral Artery, ANA- Antenary Artery, E-Eye, EBV (1-7) Efferent Branchial Vessels (1-7), PV- Pericardial vessel. H-Heart, HEP- Hepatopancrease, HEA- Hepatic Artery, OA –Optic Artery, P.S- Pericardial Sinus, P.A. – Posterior Aorta, STA –Sternal Artery, OST-Ostia.

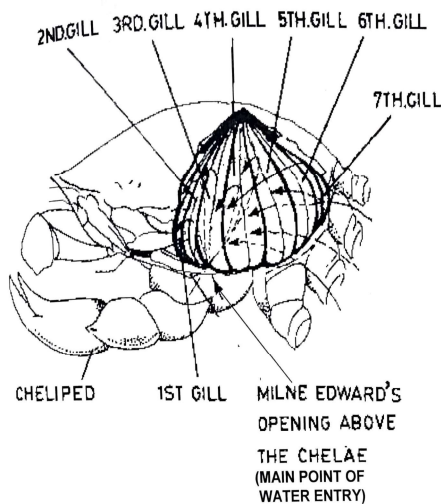


Fig-6 : Diagram illustrating the main water currents within the left branchial chamber of *Paratelphusa spinigera*, solid and dashed arrows represent the direction of water flow in the epibranchial and hypobranchial spaces respectively.

branchial vessels. In *P. spinigera* seven pairs of efferent branchial vessels have been observed, which collect oxygenated blood from the gills. These efferent branchial vessels open into branchiopericardial vessels, which supply the oxygenated blood to the heart by opening into pericardial sinus. The efferent branchial vessels from 1st, 2nd, and 3rd gills join to form a single vessel and efferent

branchial vessels from 4th and 5th gills unite to form another vessel, both of these branches unite to form a common branchiopericardial vessel. Efferent branchial vessels from 6th and 7th gills also unite to form another branchiopericardial vessel. These two branchiopericardial vessels from both sides run towards the heart through thoracic wall and open into the pericardial sinus. Taylor and Greenaway (1984)¹⁹ also reported similar type of observations in *H. transversa*. However, Pandey (1997)¹² had reported to be six afferent and six efferent branchial channels in *Paratelphusa spinigera*.

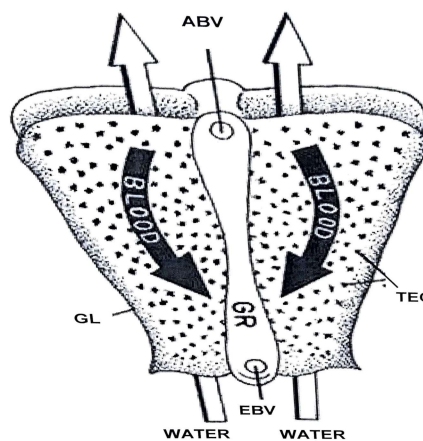


Fig-7 : Diagram showing counter current principle (i.e., opposite flow of water and blood) through the lamellae surface.

Abb. BC- Blood Channel, ABV- Afferent Branchial Vessel, GL-Gill Lamellae, GR- Gill Raphe, MC-Marginal Channel, TEC- Translamellar Epithelial Cells.

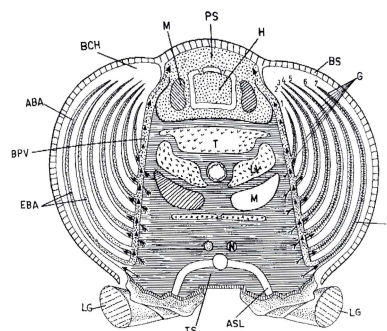


Fig-8 : Diagrammatic view of transvers section of *Paratelphusa spinigera* a showing afferent and efferent circulatory pattern. Abb. ASL-Arterial Supply, ABV-Afferent Branchial Vessel, BCV-Branchiopericardial Vessel, BS- Branchiostegite, G- Gills, GL- Gill Lamellae, H-Heart, LG-Leg, LV-Liver, M-Muscle, N-Nerve, PS- Pericardial Sinus, T- Testis, T.S- Thoracic Sinus.

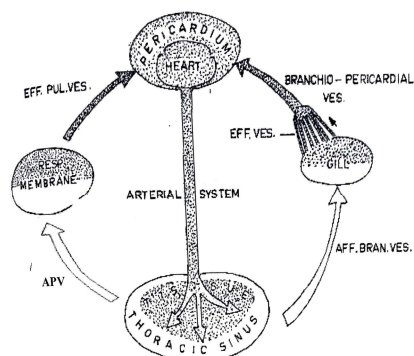


Fig-9 : Schematic representation of circulatory routes in bimodally breathing crab *Paratelphusa spinigera* (dotted regions contain oxygenated blood). Abb. APV- Afferent Pulmonary Vessel. AFF. BRAN. VES- Afferent Branchial Vessel. EFF. PUL. VES- Efferent Pulmonary Vessel.

The left-hand circuit of Fig-9 represents an alternative route for venous return through the branchiostegites, which is also potential respiratory pathway in *Paratelphusa spinigera*. The afferent venous circulation to the branchiostegites consists of main channels extending into the anteroventral region of the branchiostegites from the thoracic sinus. When viewed from the internal surface of the branchiostegites, each of the main channels is seen to be the trunk from which afferent pulmonary vessels arise to supply the deoxygenated haemolymph to the branchiostegal membrane. In the posterior ventral half of each branchiostegite, efferent channels arise and collect oxygenated haemolymph in the a clearly defined pulmonary vessel, which runs around the posterior margin of the branchiostegite on each side and leads directly into the pericardial sinus.

The observations made here demonstrate that the bimodally breathing crab *Paratelphusa spinigera* possesses dual routes for the return of venous haemolymph to the heart. Thus, deoxygenated haemolymph of the thoracic sinus system may return either via the gills, as in primarily aquatic crabs or via vascular bed within the branchiostegites. Taylor and Greenway, (1984)¹⁹ also reported this type of dual routes for the return of venous haemolymph to the heart in bimodally breathing crab, *Holthuisana transversa*.

In *Paratelphusa spinigera*, the blood is not everywhere confined within the tubular vessels. From the central heart, arteries radiate, but they do not end in the

capillaries, instead the blood passes from them into blood spaces or sinuses, in which the organs of the body lie. Such a vascular system is called as open vascular system. The series of sinuses, in which the viscera lie, constitute the haemocoel. The pericardial cavity surrounding the heart is a blood sinus, which is the part of the haemocoel. The contraction of the heart is followed by a relaxation due to which the blood is drawn from the pericardial cavity into the heart through four ostia, present on it. The sinuses leading to the pericardial cavity are filled with oxygenated blood coming from the gills and branchiostegal membrane. The heart therefore, contains only oxygenated blood (Fig-8). Farrelly and Greenaway (1993)⁵ have also reported that in brachyuran crabs, the branchial and pulmonary circulation run in parallel with each other and each with separate vessels return to the pericardial sinus. All haemolymph must pass through either the gills or the lungs before entering the pericardial sinus. Therefore, the pericardial haemolymph is a mixture of haemolymph from these two systems. Thus, if one system has a low oxygen concentration and the other a high one, the final oxygen concentration will be less than that of the higher one. Taylor and Greenaway (1984)¹⁹ also reported this type of dual routes for the return of venous haemolymph to the heart in the bimodally breathing crab, *Holthuisana transversa*. Both, gills and branchial epithelium must therefore be considered as capable of contributing significantly towards the overall gas exchange in the animal. It has also been reported that the relative venous flow to the lungs and gills changes depending upon whether the animal is breathing air or water.¹⁹

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