Proc. Indian natn. Sci. Acad. B52 No. 2 pp 274-279 (1986)

Structure of Pseudobranch of Two Clupeoid Fishes, *Hilsa ilisha* and *Gadusia chapra*

O N SINGH, T K GHOSH and J S DATTA MUNSHI
Ichthyology Research Laboratory, Post-Graduate Department of Zoology, Bhagalpur University, Bhagalpur 812007

(Received 7 November 1985; after revision 31 January 1986)

The light and scanning electron microscopic observations have been made on the pseudobranch of two clupeoid fishes, *Hilsa ilisha* and *Gadusia chapra*. Though the pseudobranchs of both species appeared alike *in situ*, the SEM studies showed their morphology to be different. In *H. ilisha*, the lamellae are free on the filament but in *G. chapra* fusion of lamellae have taken place except in the tip region. Two types of cell openings belonging to the mucous cells and the chloride cells have been observed at the cell surface. The SEM studies have been correlated with the light microscopic observations.

Key Words: Pseudobranch, *Hilsa ilisha, Gadusia chapra*

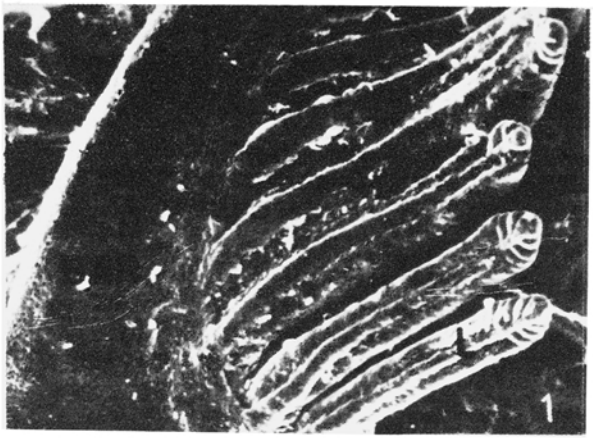
Introduction

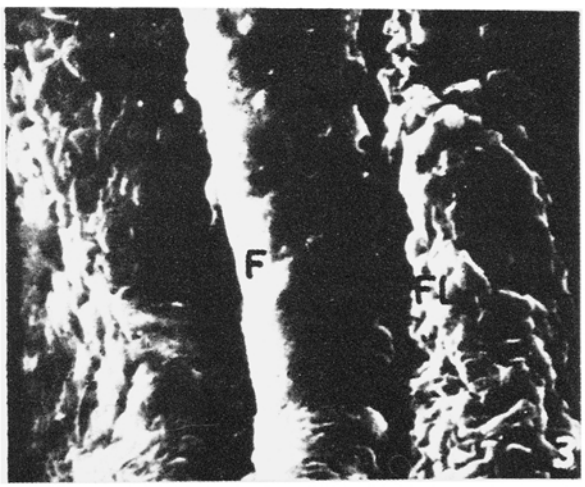
The pseudobranch in fish is a remnant of the mandibular gill, ranging from a free gill like structure situated in the opercular cavity to a glandular structure burried in a mass of connective tissue. This structure was first described by Broussonet (1785). Since then the structure and function of the pseudobranch has attracted the attention of many workers (Maetz 1956, Parry & Holliday 1960, Laurent 1974). Recently Mattey et al. (1980) studied the surface architecture of the pseudobranch of two marine teleosts, *Dicentrachus labrux* and *Liza ramoda* (*Mugil capito*). Munshi and Hughes (1981) have also described the gross and fine structure of glandular psuedobranch of climbing perch, *Anabas testudineus*. Though the precise function of the pseudobranch is uncertain, this organ has been assigned diverse functions, viz., endocrine functions (Vialli 1926, Parry & Holliday 1960), enzyme production (Leiner 1938, Maetz 1956), ionic regulation (Copeland 1948) and sensory (Laurent 1974).

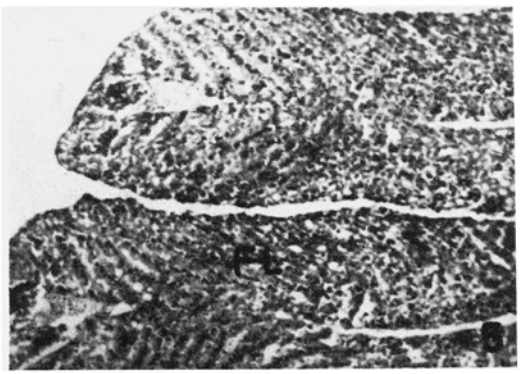
Hilsha ilisa is a euryhaline teleost while *Gadusia chapra* inhabits the freshwater bodies. In the present study, the histology and surface ultrastructure of pseudobranch in these two herrings have been described.

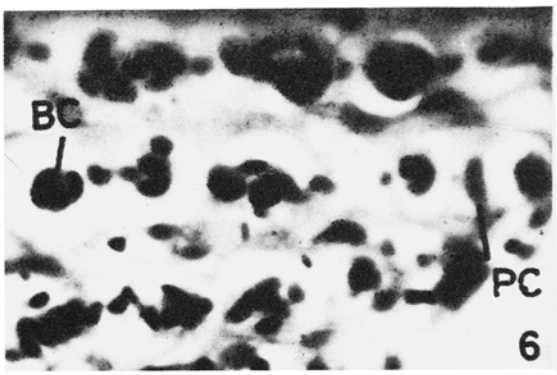
Materials and Methods

Hilsa ilisha were collected from the river Ganga at Farakka (West Bengal) and *Gadusia*

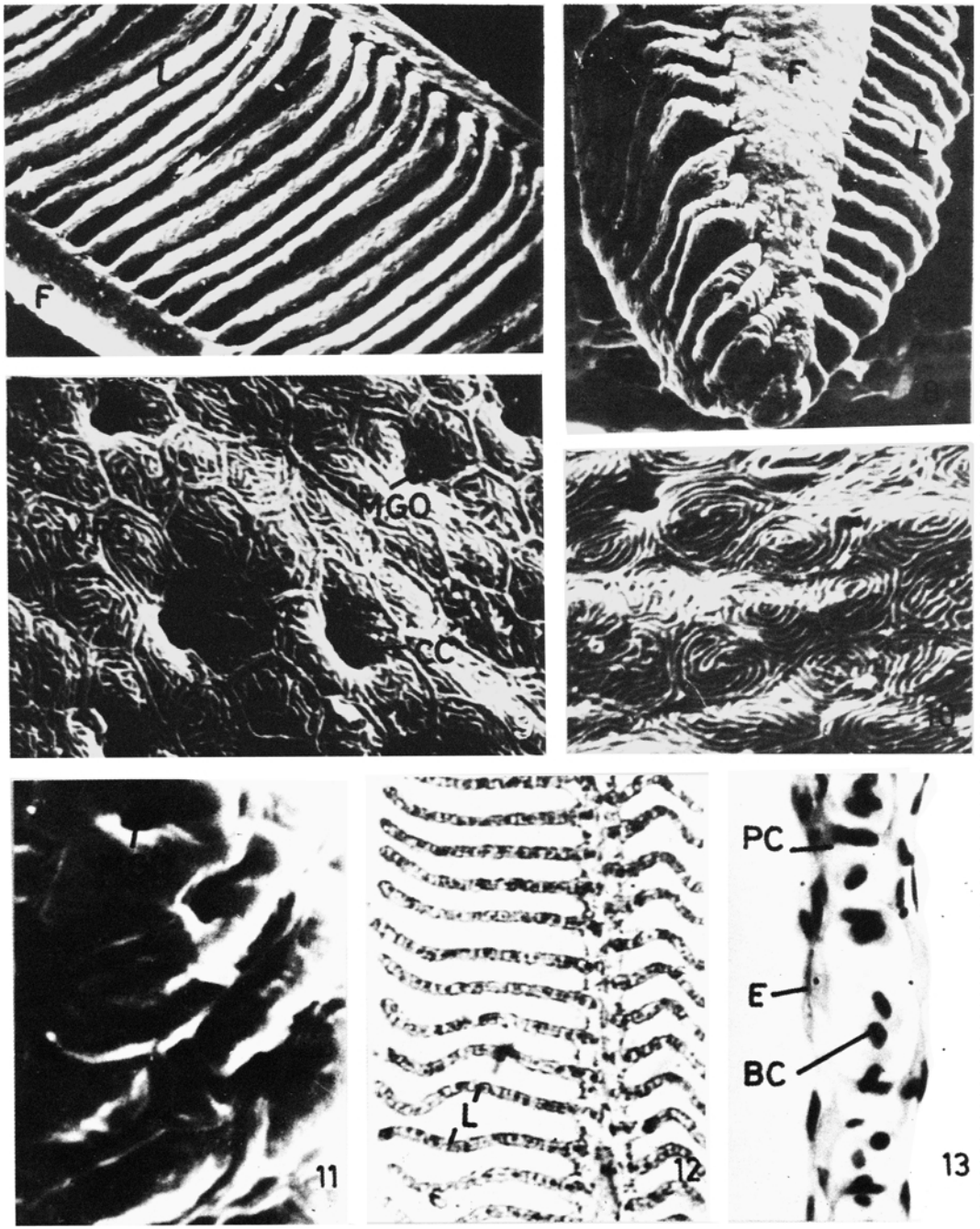








Figures 1–6, SEM and LM photomicrographs of the pseudobranch of *Hilsa ilisha*. **1,** A single filament showing lamellar arrangement (×200); **2,** Tip region of a filament (×400); **3,** Surface of the pseudobranch bar showing both mucous and chloride cell openings (×3200); **4,** Filament surface showing microridged epithelial cells (×3200); **5,** View of the lamellar suface (×6400); **6,** LS of a filament showing arrangement of the lamellae (×100)). *BC,* Blood capillary; *CCO,* Chloride cell opening; *E,* Epithelium; *F,* Filament; *L,* Lamella; *MGO,* Mucous gland opening; *MRC,* Microridged cell; *PC,* Pillar cell



Figures 7–13, Oil immersion photomicrograph of a lamella (×1000) SEM and LM photomicrographs of the pseudobranch of *Gadusia chapra*. **8,** Part of the pseudobranch showing arrangement of filaments (×100); **9,** Tip of a single filament showing lamellar arrangement (×800); **10,** Part of the filament showing lamellar fusion (×800); **11,** Pseudobranch bar surface showing microridged epithelial cells and mucous gland opening (×3200); **12,** L.S. of few filaments showing the fusion of lamellae (×100); **13,** Oil immersion photomicrograph of fused lamellae (×1000)

BC, Blood capillary; *F*, Filament; *FL*, Fused lamellae; *L*, Lamella; *MGO*, Mucous gland opening; *MRC*, Microridged cell; *PC*, Pillar cell; *PSB BAR*, Pseudobranch bar

chapra from the tributary of river Ganga near Bhagalpur. Pieces of pseudobranchs were fixed in 6.25% glutaraldehyde in 0.1 M phosphate buffer (*p*H 7.4) at 4°C for 24 hrs. After fixation tissues were washed thrice in the phosphate buffer and dehydrated in graded alcohol series followed by alcohol acetone series and were finally put into anhydrous acetone. The tissues were critically point dried using carbon dioxide as the transitional fluid, mounted on brass stub and gold coated in a sputter coater (Edwards-150, England) and examined under PSEM 500 (Phillips, Holland) at RSIC, Bose Institute, Calcutta.

For histological studies, tissues were fixed in Bouin's fluid, paraffin sections were cut and stained with haemotoxylin, eosin and mallory's triple stains and examined under light microscope.

Results

The gross morphology of pseudobranch in both *H. ilisha* and *G. chapra* is quite similar *in situ* but histological and SEM studies revealed differences in their anatomy and morphology.

In both the cases pseudobranch has a row of parallel filaments attached with a well defined arch termed here as pseudobranch bar. The pseudobranch bar remains attached to the epithelium of opercular cavity.

SEM Observations

Scanning electron microscopic studies reveal the presence of a row of parallel filaments attached with the pseudobranch bar in both species.

In *H. ilisha* each filament bears numerous leaf like lamellae (figures 1 & 2) arranged on both sides throughout its length. The lamellae are smaller in size towards the tip region (figure 2). Pseudobranch bar surface is covered by a mosaic of ridged epithelial cells (figure 3). These cells are pentagonal or hexagonal in shape and their average diameter measures 6.1 μm. Two types of openings are discernible on the pseudobranch bar, which represent the openings of unicellular mucous glands and chloride cells. The average diameter of the mucous cell and the chloride cell opening was measured to be 1.04 μm and 2.58 μm, respectively. Filament surface was also covered by microridged epithelial cells, average size being 5.44 μm (figure 4). Mucous cell openings are also present which are about 1.4 μm in diameter. The surface of epithelial cells covering the lamellae are devoid of microvilli or microridges. However, mucous cell openings are discernible.

In *G. chapra* except the tip region where there are few free lamellae, fusion of lamellae has taken place in rest of the portion (figures 8 & 10). The pseudobranch bar surface is covered with epithelial cells measuring about 8.78 μm and are provided with short and abbreviated microridges (figure 11). These cells are either pentagonal or hexagonal in shape. Mucous cell openings measuring about 1.55 μm in diameter are present between the epithelial cells. Many pores could be seen on the surface of filaments which probably are the openings of chloride cell. Under high magnification filament surface is found to be covered with smooth surfaced epithelial cells. The surfaces of the epithelial cells of both fused and free lamellae are devoid of any ridges or villi like structures. However, the fused lamellae give an uneven appearance.

Histological Observations: The histological observations confirm that in case of *H. ilisha* each filament bears many lamellae free from each other on both sides (figure 6). In a fish weighing 740 g the number of lamellae per mm was 65 on both sides of the filament. Each lamella is composed of a middle vascular layer followed by a thin basement membrane and an outer epithelial coverage on both sides (figure 7). The epithelium is double-layered and varies from 2.63–5.88 μm in thickness.

In *G. chapra* the filaments bear a few free lamellae at their distal ends. However, in the remaining part of the filament, the lamellae remain fused with each other. Histologically, the basic structure of the pseudobranch and gill lamellae are similar.

Discussion

It has been recognised for a long time that this "gill" is often supplied with blood that has already been oxygenated in the hemibranch of the hyoid or first branchial arch, therefore it is non-respiratory and as such called the pseudobranch (Hughes 1984). Muller (1839) distinguished two types of teleostean pseudobranch, the first with lamellae projecting into the subopercular cavity, and the second of the glandular type, where the organ is sunk below a covering of epithelium as in *Anabas testudineus*. The pseudobranch of *H. ilisha* and *G. chapra* belongs to the first type but the organ of *G. chapra* could be considered to be of more specialized nature as it shows the fusion of lamellae except at the tip region. Granel (1923) and Leiner (1938) have mentioned four types of pseudobranch that vary in their degree of isolation from the external environment.

In the lamellae of pseudobranch well defined blood channels are present between typical pillar cells. Thus, the basic organization of pseudobranch is similar to the gills. The presence of pillar cells is indicative of their homology. Singh (1982) suggested that the catfish carotid labyrinth has been derived from the pseudobranch. Hughes (1984), however, emphasized that the carotid labyrinth in bony fishes (Siluriformes) and the carotid labyrinth and carotid body in tetrapod have different origin and structurally show no trace of the pillar cells.

The presence of microridges and microvilli like structures on the gill epithelia has been noticed (Olson & Fromm 1973, Lewis & Potter 1976, Hughes & Munshi 1978, Hossler et al. 1979, Hughes 1979, Kendall & Dale 1979, Rajbanshi 1977). Close similarities have been found between the gill epithelial surfaces and that of the epithelial surfaces of the pseudobranchs. Similar observation has also been made by Mattey et al. (1980). Though the functions of microridges are still not certain, they have been assigned various functions. The view of Olson and Fromm (1973) and Lewis and Potter (1976), that microridges increase the respiratory surface area do not hold good in the case of pseudobranch, since it is supplied with arterial blood and hence non-respiratory in function. According to Hughes and Wright (1970) and Hughes (1979) primary function of microridges is for the anchorage of mucus. The pseudobranch in these two clupeoid fishes may have osmoregulatory function as they possess many chloride cells. Microridges are likely to cause a region of microturbulence with continual mixing of water layers immediately above the chloride cell openings which would facilitate the ionic exchange rather than gas transfer (Mattey et al. 1980).

Acknowledgement

The authors wish to thank the National Science Foundation, USA (Grant No. INT 830072) for financial support.

References

Broussonet A 1785 Mémoire pour servia à l'histoire de la respiration des poissons; *Mém. Acad. Roy. Sci.* (Paris) 174–196

Copeland D E 1948 The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus; J. Morph.* **82** 201–218

Granel F 1923 Etude histologique et embryologique sur la pseudobranchie des teleosteans; *Arch d'Anat. Histol. et. Embr. Tome* **2** 153–195

Hossler F E, Ruby J R and McIlwain T D 1979 The gill arch of the mullet, *Mugil cephalus* 1. Surface ultrastructure; *J. exp. Zool.* **208** 379–398

Hughes G M 1979 Scanning electron microscopy of the respiratory surfaces of trout gills; *J. Zool. Lond.* **187** 443–453

—— 1984 General anatomy of the gill; in *Fish Physiology* Vol XA pp. 1–72 eds. W S Hoar and D J Randall (London: Academic Press)

—— and Munshi J S D 1978 Scanning electron microscopy of the respiratory surfaces of *Saccobranchus* (=*Heteropneustes*) *fossilis* (Bloch); *Cell and Tissue Res.* **195** 99–109

—— and Wright D E 1970 A comparative study of the ultrastructure of the water-blood pathway in the secondary lamellae of teleost and elasmobranch fishes-benthic forms; *Z. Zellforsch. mikrosk. Anat.* **104** 478–493

Kendall M W and Dale J E 1979 Scanning and transmission electron microscopic observations of rainbow trout (*Salmo gairdneri*) gill; *J. Fish. Res. Bd. Can.* **36** 1072–1079

Laurent P 1974 Pseudobranch receptors in teleosts; in *Hand-Book of Sensory Physiology* eds Autrum, Jung, Loewenstein and Mackay (Berlin: Springer-Verlag)

Leiner M 1938 Die Augenkiemendruse (pseudobranchie) der knochenfische. Experimentelle untersuchungen uber ihre physiologische Bedeutung; *Z. Vergl. Physiol.* **26** 416–466

Lewis S V and Potter I C 1976 A scanning electron study microscope of the gills of the lamprey *Lampreta fluviatilis* (L.); *Micron* **7** 205–211

Maetz J 1956 Le role biologique de l'anhydrase carbonique chez quelques teleosteens; *Bull. Biol. Fr. Belg. Suppl.* **40** 1–129

Mattey D L, Morgan M and Wright D E 1980 A scanning electron microscope study of the pseudobranch of two marine teleosts; *J. Fish. Biol.* **16** 331–343

Muller J 1839 Vergleichende Aantomie der Myxinciden. III. Fortsetzung. Ueber das Gefassystem; Abh. K. bayer Acad. Wiss. Berlin

Olson K R and Fromm P O 1973 A scanning electron microscopic study of secondary lamellae and chloride cells of rainbow trout *Salmo gairdneri*; *Z. Zellforsch. microsk. Anat.* **143** 439–449

Parry G and Holliday F G T 1960 An experimental analysis of the function of the pseudobranch in teleosts; *J. Exp. Biol.* **37** 344–353

Rajbanshi V K 1977 The architecture of the gill surface of the catfish, *Heteropneustes fossilis* (Bloch.) SEM study; *J. Fish Biol.* **10** 325–329

Singh M 1982 Studies on the structure and development of the pseudobranch in certain fishes with particular reference to their innervation; Ph.D. thesis, Allahabad University, Allahabad

Vialli M 1926 La pseudobranchie de pesci; *Archo ital. Anat. Embroil.* **23** 49–117