EPITHELIAL GILL CELLS IN THE ARMORED CATFISH, *Hypostomus* CF. *plecostomus* (LORICARIIDAE)

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ABSTRACT

Epithelial gill cell morphology and distribution were investigated in the armored catfish, *Hypostomus* cf. *plecostomus*, which lives in soft ion-poor Brazilian freshwaters. Pavement cells are the most abundant type of cell on both filament and lamellar epithelia and there are a great number of mucous and chloride cells between them. Mucous cells are almost covered by adjacent pavement cells and have large packed granules showing electrondense differences. No mucous cells were found on the lamellar epithelium. Chloride cell were distributed throughout both epithelia and usually have large apical surface facing the external medium and may exhibit short and sparsely distributed microvilli. The presence of chloride cells on the lamellar epithelium may be an adaptation to low ion concentrations in the water, allowing for improved ion-transport capacity of the gill. The large size of these cells increases the water-blood barrier and may affect the transference of respiratory gases. However, the negative effect on the respiratory process may be minimized by this species' ability to resort to atmospheric air to fulfill its oxygen requirements.

Key words: gills, epithelium, teleost, loricariid, *Hypostomus* cf. *plecostomus*.

RESUMO

Epitélio branquial de cascudo, *Hypostomus* cf. *plecostomus* (Loricariidae)

A morfologia e a distribuição das células do epitélio branquial foram analisadas no cascudo *Hypostomus* cf. *plecostomus*, peixe que vive em água doce, caracterizada por ser pobre em íons, no Brasil. As células pavimentosas são as mais abundantes em ambos os epitélios, do filamento e lamelar. Entre essas células há um grande número de células mucosas e de células de cloreto. As células mucosas são quase totalmente encobertas pelas células pavimentosas adjacentes e possuem grandes grânulos que mostram eletrondensidades diferentes. Não foram encontradas células mucosas no epitélio lamelar. As células de cloreto estão distribuídas em ambos os epitélios e, em geral, têm uma extensa superfície, que pode exibir poucas microvilosidades curtas em contato com o meio externo. A presença de células de cloreto no epitélio lamelar pode indicar uma adaptação à reduzida concentração de íons na água, a fim de aumentar a capacidade de transporte ativo de íons por meio das brânquias. O tamanho e a forma dessas células aumenta a barreira água–sangue e pode afetar a troca de gases para a respiração. Entretanto, o efeito negativo no processo respiratório pode ser minimizado pelo fato de essa espécie possuir respiração aérea facultativa e poder utilizar o ar atmosférico para suprir sua necessidade de oxigênio.

Palavras-chave: brânquias, epitélio, teleósteos, loricarídeo, *Hypostomus* cf. *plecostomus*.

INTRODUCTION

Gills are multifunctional organs with a complex internal organization that is similar in most teleosts (Hughes, 1984; Laurent, 1984). Gill epithelia consist of several cell types. Pavement, chloride and mucous cell distribution and morphology have been intensively investigated to understand the integration of several of their functions, such as gas exchange, ion and acid-base regulation, and nitrogen excretion. The role of different cell types in marine fish gill epithelia has been established and cell distribution has been clearly defined (Foskett *et al*., 1981; Perry & Laurent, 1993). The physiological functions of the gill cells of freshwater fish, however, appear to be more complex (Evans, 1984; Perry & Wood, 1985; Avella *et al*., 1987; Perry & Laurent, 1989, 1993; Brown, 1992; Goss *et al*., 1992a, b, 1994). Freshwater environments vary from almost distilled to high ion concentration waters and, therefore, the epithelial gill cell types in freshwater fish show morphological and functional adaptations that allow the species to live in such environments. Most Brazilian freshwaters are ion-poor and soft except for a few saline lakes close to the Brazilian coast.

In this context, the present study reports on the morphology and distribution of epithelial gill cells in the armored catfish, *Hypostomus* cf. *plecostomus* L., relating them with the environments where these fish live. This species is a facultative air-breathing fish that uses the stomach to breathe atmospheric air (Carter, 1935) only when the O_2 tension in the water is low (Perna & Fernandes, 1996). Although *H*. cf. *plecostomus* has a reduced gill respiratory surface area, as do most air-breathing teleosts (Mattias *et al*., 1996; Perna & Fernandes, 1996), the afferent arterial vasculature of its gill shows modifications that may facilitate lamellae perfusion during the respiratory cycle (Fernandes & Perna, 1995). During environmental hypoxia, deprived of access to atmospheric air, this species behaves as an oxygen regulator with moderate to high tolerance to decrease oxygen in the water, depending on its body size (Perna & Fernandes, 1996).

MATERIAL AND METHODS

Adult specimens of the armored catfish, *Hypostomus* cf. *plecostomus* (n = 10; W = 46-82 g; Lt = 12-19 cm) were collected in the Monjolinho Reservoir, São Carlos, SP (water composition in mM L^{-1}: Na^+ 0.034, Ca^{2+} 0.043, K^+ 0.023, Mg^{2+} 0.073, Cl^- 0.014; conductivity = 1.8 μs; titration alkalinity = 13.32; hardness = 9.70 as mg L^{-1} $CaCO_3$; pH ≅ 6.8). The fish were anaesthetized with 0.01% benzocaine, killed and their gill arches excised and processed for light, scanning and transmission electron microscopy.

Gill filaments from dorsal, middle and ventral portions of the each gill arch were cut off with a razor blade. Most of the arch tissue was removed but the anterior and posterior rows of filaments remained attached to the septum of the arch. Samples consisting of 1-5 gill filaments were fixed in 2.5% glutaraldehyde buffered to pH 7.3, with 0.1 M phosphate buffer for 2 h at 4°C.

Fixed tissue samples were dehydrated for scanning electron microscopy (SEM), using a graded ethanol series till absolute ethanol, soaked in two successive baths of 1,1,1,3,3,3-hexamethyldisilazane (Aldrich) and then air dried, according to the Laurent & Hebibi (1989) procedure. Filament pairs were glued with silver paint onto the specimen stub, coated with gold in a vacuum sputter and examined under a DSM 940 ZEISS Scanning Microscope at 25 kV. Epithelial surfaces on the leading and trailing edges of the filaments near the base of the lamellae and from the lamella were randomly photographed with 3000-fold magnification (4 noncontiguous fields). The apical surface of individual chloride and mucous cells and their density on the filament and lamellar epithelia were determined by tracing cell perimeters using a morphometric software program (Sigma-Scan, Jandel Scientific, Inc.). From these measurements, the mean chloride and mucous cell fractional area mm^{-1} epithelium were calculated.

For transmission electron microscopy (TEM), fixed pieces of individual filaments (~ 1 mm long) were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer pH 7.3 at 4°C, dehydrated by a graded acetone series and embedded in Araldite 6005 (Ladd Research). Semi-thin sections were stained with toluidine blue and examined under an Olympus-Micronal photomicroscope. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100 CX transmission electron microscope at 60 or 80 kV.

The lamellae's water-blood barrier thickness (τ_h) were calculated from the harmonic mean

intercept length (l_h) of random probing lines crossing the barrier, according to the equation $\tau_h = 2/3\ l_h$ (Weibel & Knight, 1964). The *l* values were determined by superimposing a grid for layered structure (Gundersen *et al*., 1988) on randomized electron micrographs (magnified x 3000) of lamellar cross sections.

All values are presented as means ± SE. The statistical significance, where applicable, was determined using unpaired Student's *t*-tests between appropriate sample means within 95% confidence limits.

RESULTS

Two types of epithelia were clearly identified in the gill filaments of *H.* cf. *plecostomus*: primary or filament epithelium and secondary or lamellar epithelium (Fig. 1). The filament epithelium is non-respiratory. It is stratified and consists of 4-10 cell layers involving the leading and trailing edges of the filament and the interlamellar space. The lamellar epithelium is structurally adapted to gas exchange. It is highly vascularized and consists of two epithelial cell layers separated from the pillar cell flanges by the basement membrane.

Filament epithelium

The outermost layer of the filament epithelium consists mainly of pavement cells (PVC) with numerous mucous (MC) and chloride cells (CC) spread between them. The distribution of these cells however, showed a spatial organization. Most of the mucous cells were found on the lateral leading and trailing edges of the filament, while the chloride cells were found close to the onset of secondary lamellae on both leading and trailing edges of the filament and on the interlamellar space. No mucous cells were found in the interlamellar space. Pavement cells are polygonal in shape, have an almost smooth surface with sparse and irregular microridges (0.2-0.4 μm height; 0.8-6.0 μm length), and cell limits are well defined by long and circular microridges (Fig. 2A). PVC are thin, with small number of mitochondria, numerous ribosomes, abundant rough endoplasmic reticulum and a 400 nm long junctional complex which exhibits intense interdigitation between lateral cell surfaces (Fig. 2B, C).

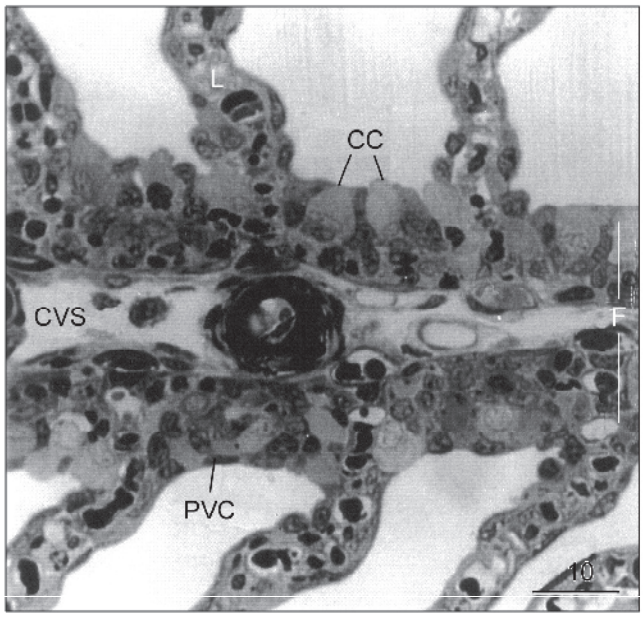

Fig. 1 — Filament (F) and lamellar (L) epithelium of *H.* cf. *plecostomus.* Semi-thin longitudinal section of gill filament stained with Toluidine blue. CC = chloride cell; PVC = pavement cell; CVS = central venous sinus. Scale bar is in μm.

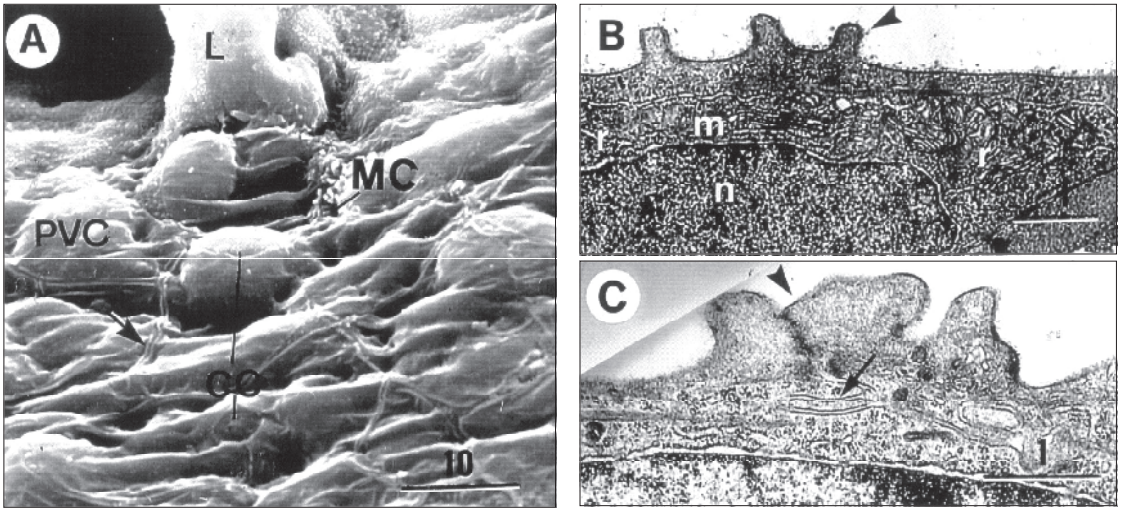

Fig. 2 — *H.* cf. *plecostomus*. **A.** SEM photomicrograph of filament epithelium. Note the smooth surface of pavement cells (PVC) and the long microridges bordering the cell limits (arrow). CC = chloride cell; L = lamellae; MC = mucous cell. **B.** TEM photomicrograph of PVC showing large nuclei (n), abundant endoplasmic reticulum (r), mitochondria (m) and small microridges (arrowhead). **C.** TEM photomicrograph of PVC showing large microridges bordering the cell limits (arrowhead) and the junctional complex characterized by interdigitation on the lateral cell surface (arrow). Scale bars are in μm.

Chloride cells are large and round with a large number of mitochondria (Figs. 1 and 3). Chloride cells (CC) were characterized by an electronlucent cytoplasmic matrix showing a well developed tubular system with a constant diameter and had a large apical surface (23.3 ± 1 μm^2) contacting the external environment (Fig. 3A). The surface of CCs are generally smooth but some cells displayed short microvilli (~ 0.3 μm in height) on their apical surface however, 3% to 4% of CCs showed an apical pit and were partially covered by pavement cells. Some CC having an electrondense cytoplasmic matrix and exhibiting signs of apoptosis were randomly spread on the filament epithelium (Fig. 3A). CCs with degenerated features were about 1% and were found contacting the external environment or dispersed among cells of the inner epithelial layer. These cells showed large vesicles and structural disorganization of the mitochondria and tubular system (Fig. 3B).

Mucous cells are round (4-9 μm diameter). These cells are almost totally covered by adjacent pavement cells and their surface facing the aquatic environment were very reduced (0.10 to 0.11 μm^2) (Fig. 4A). MCs were found on both filament edges and their incidence (17 ± 0.2 cells/$\mu m^2.10^3$ of filament) was higher than CCs. However, their total filament's fractional area was below 1%. Several *H.* cf. *plecostomus* MCs have large packed granules showing different electrondensities (Fig. 4B).

Inner cell layers of the filament epithelium consisted of several cells showing different electrondensities.

A thin basal lamina interfaces the filament epithelium and the connective tissue, which were reduced close to the primary artery.

Lamellar epithelium

The lamellar epithelium surrounds the vascular space formed by the pillar cell flanges (Fig. 5A). The lamellae are small and the harmonic mean between the lamellae's water and blood barrier (consisting of epithelial layers and pillar cell flanges) was estimated as 6.42 ± 0.32 μm. Three cell types were abundant in the epithelial layers in *H.* cf. *plecostomus*: PVC and CC in the outer layer and undifferentiated cells in the inner layer (Fig. 5B).

The surface architecture of pavement cells of the lamellar epithelium was characterized by short microvilli (0.03-0.4 μm) throughout the cell surface, with unclear definition of cell boundaries (Fig. 5A, C). CCs were distributed throughout the lamellar surface (Fig. 5C) and CCs exhibiting electrondense and electronlucent cytoplasmic matrix were easily identified.

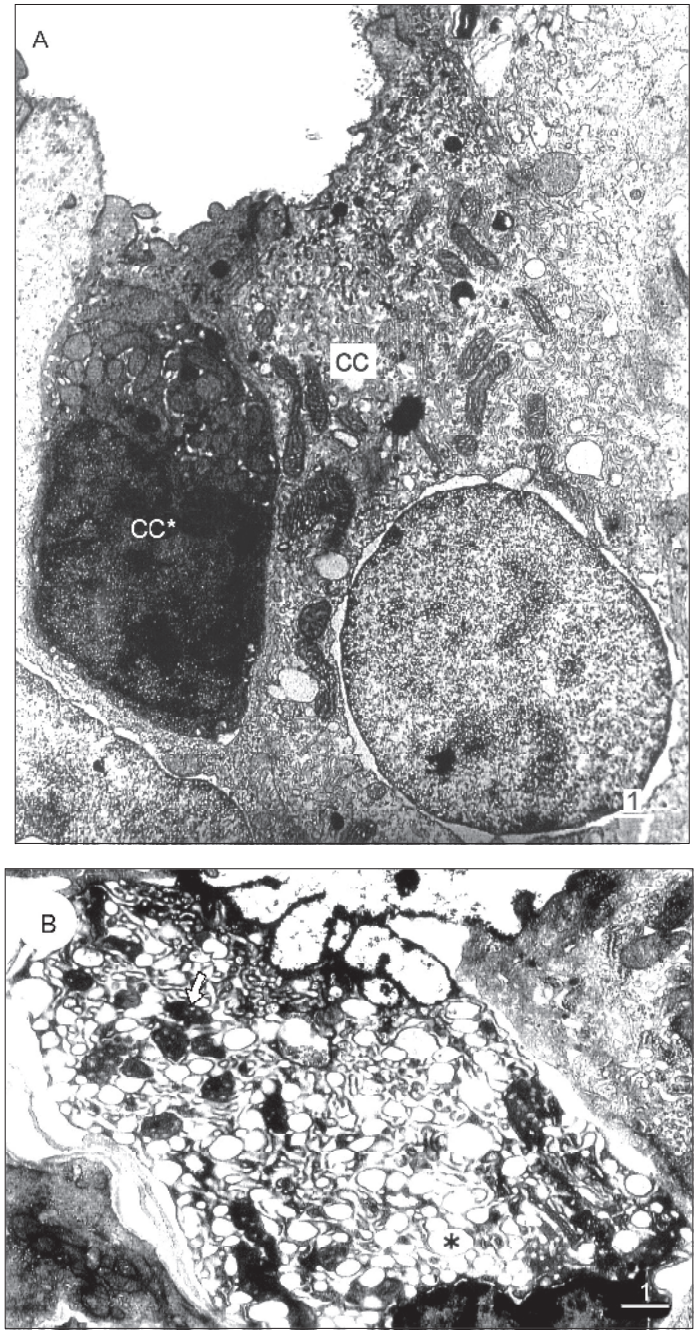

Fig. 3 — *H.* cf. *plecostomus*. **A.** TEM photomicrograph of normal chloride cell (CC) and a chloride cell in early stage of apoptosis (CC*) on filament epithelium. **B.** TEM photomicrograph of chloride cell showing degeneration (necrosis). Note mitochondria (arrow) features and large vesicles (*) in the cytoplasm. Scale bars are in μm.

Apical surfaces of CCs are similar to those found in the filament epithelium. Significant differences were found in the number of CCs between the filament (interlamellar region = 13 ± 2 CC/$\mu m^2.10^3$) and the lamellar epithelium (9 ± 1 CC/$\mu m^2.10^3$).

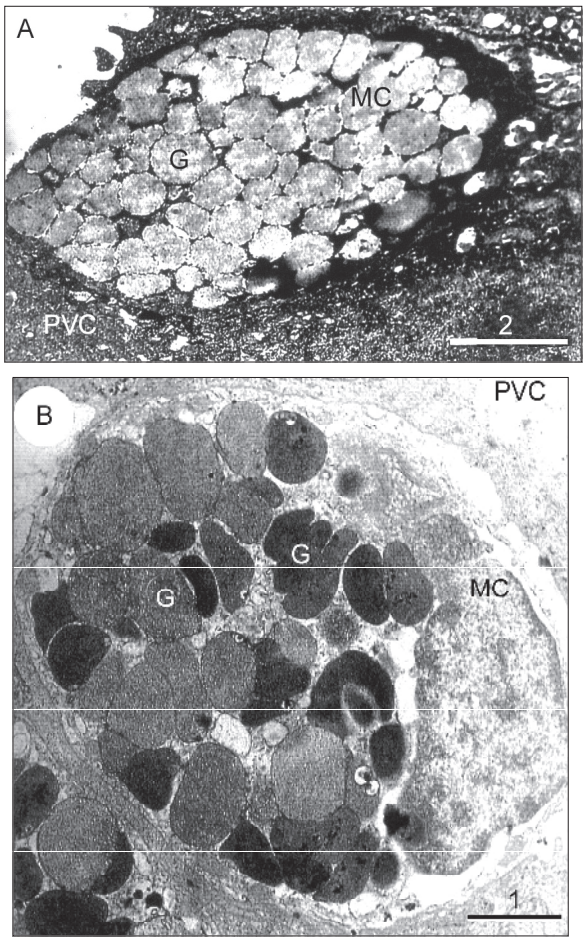

Fig. 4 — *H.* cf. *plecostomus*. TEM photomicrograph mucous cells (MC). **A.** MC with granules (G) of same electrondensity. **B.** MC showing granules (G) with different electrondensity. PVC = pavement cell. Scale bars are in μm.

The inner layer's cells were close to the basal lamina and were poor in organelles, having only a small number of mitochondria and ribosomes. These cells' nuclei tend to appear in a position overlying the pillar cell bodies. No junctional structures were identified between the basal membrane of PVC and CC and the cells of the inner layer; large intercellular spaces were found between these epithelial layers, mainly when leukocytes were present (Fig. 5A).

DISCUSSION

Typically, the filament (multilayered) and lamellar (two cell layers) epithelia of *H.* cf. *plecostomus* gills are stratified with cell types consisting of the same cells described for other teleosts (Morgan & Tovell, 1973; Laurent, 1984), that is, PVC, MC and CC facing the external environment and undifferentiated or incompletely differentiated cells facing the basal lamina.

Neuroepithelial cells were not identified on the leading edge of the filament epithelium of *H.* cf. *plecostomus*, as was found in *Oncorhynchus mykiss, Stizoztedion lucioperca, Ictalurus melas, Anguilla anguilla* and *Micropterus dolomieui* (Dunel-Erb *et al*., 1982). This may be due to the particular location of these cells on the epithelium or the orientation of the sections.

PVC ultrastructure is similar to other teleosts (Laurent, 1984). The well-developed Golgi complex, abundant rough endoplasmic reticulum and the number of mitochondria indicate an active cell (Laurent, 1989).

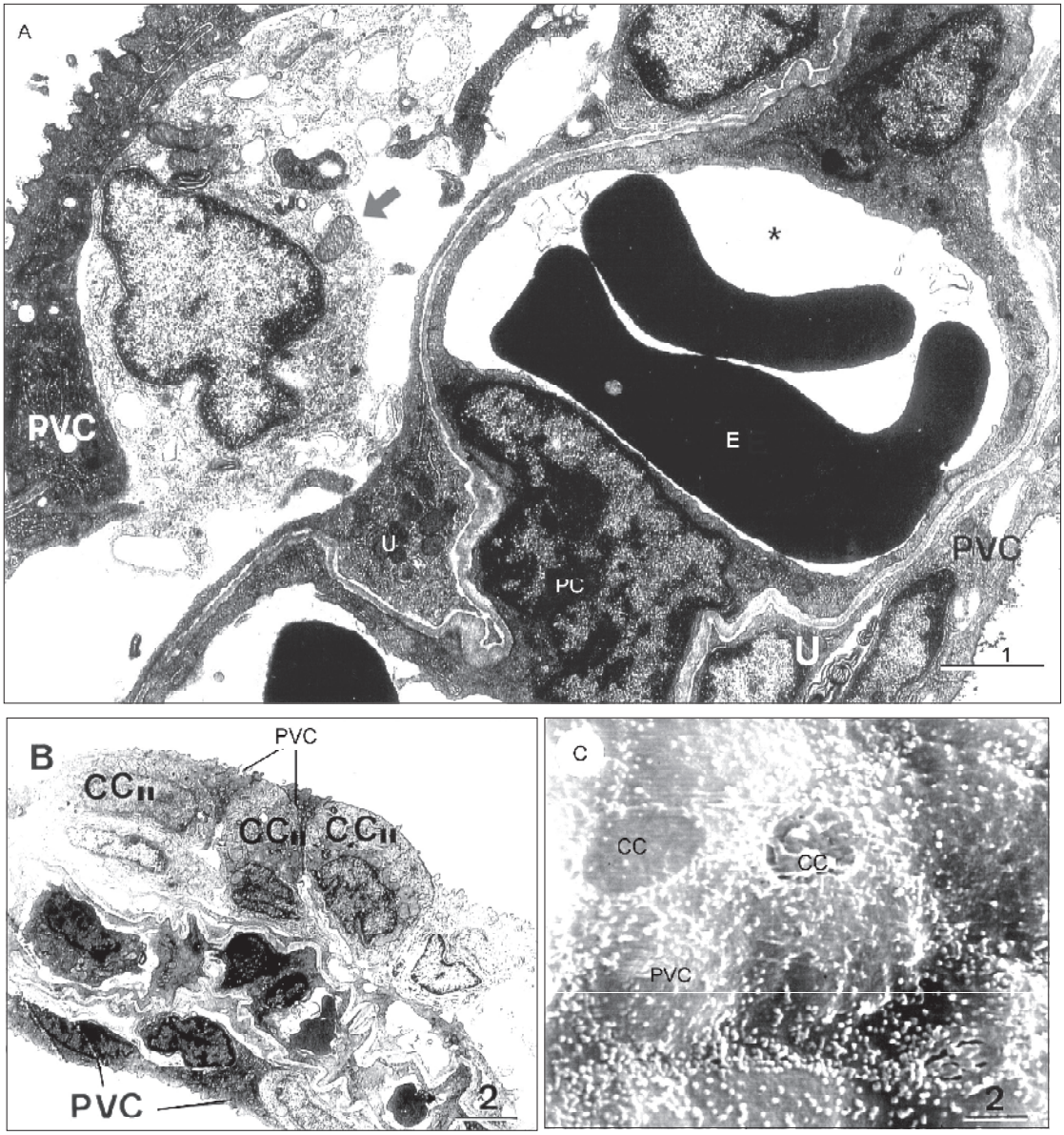

Fig. 5 — *H.* cf. *plecostomus*. **A.** TEM photomicrograph of lamellae showing the pillar cell (PC) and vascular space (*). Note the presence of leukocyte between the two epithelial layers (arrow) and the increased water–blood barrier. E = erythrocyte; U = undifferentiated cell; PVC = pavement cell. **B.** Chloride cells in the outer layer of lamellae. Note the large distance between water and blood. PVC = pavement cell. **C.** SEM photomicrograph of lamellae showing the surface architecture of pavement cells (PVC). Note the chloride cells (CC) between them and their large surface area. Scale bars are in μm.

Recent studies have suggested that lamellar pavement cells could be involved in acid-base regulation (Na^+ uptake/H^+ excretion) in freshwater fish (Perry *et al*., 1992; Goss *et al*., 1992a, b, 1994; Perry & Laurent, 1993). The functional role of the differences in the surface architecture between filament and lamellar epithelium cells are unknown; however, their ornamentation may be important in anchoring mucus to the epithelial surfaces. The mucus is believed to form a protective layer (McCahon *et al*., 1987) and to facilitate ion regulation (Handy *et al.*, 1989).

The MCs of *H.* cf. *plecostomus* in the filament epithelium are quite similar to those found in the epidermis of teleosts (Roberts *et al*., 1973; Takashima & Hibiya, 1995). Although their distribution and number in gill epithelia vary from one species to another (Laurent, 1984), MCs in *H.* cf. *plecostomus* were found in similar numbers in the leading and trailing edges of the gill filament. The presence of these cells on the leading filament edge appears to aid in more effective distribution of mucus over the gills. Saboya-Moraes *et al*. (1996) recently reported histochemical differences in the MC population of *Poecilia vivipara*, a euryhaline species, depending on their location on the gill epithelium. No histochemical analysis was done of MCs in this study and, although differences on electrondense packet granules were found in some MCs, as reported for *Periophthalmus vulgaris* (Welch & Storch, 1976), the MCs of the *H.* cf. *plecostomus* filament epithelium were randomly distributed and did not show any particular location on the filament epithelium. The differences in electrondense packet granules may be related to their degree of development.

The main features of the epithelial gill cells of *H.* cf. *plecostomus* are related to CC morphology and distribution. The apical morphology and surface area of CCs exposed to the environment is related to pH, salinity and low Ca^{2+} concentrations in water (Laurent *et al*., 1985; Leino *et al*., 1987). The large apical surface area of CCs such as that found in *H.* cf. *plecostomus* is typical of fish living in low NaCl and Ca^{2+} concentrations, characteristic of most Brazilian freshwaters. *H.* cf. *plecostomus* shows a sharp reduction of its apical surface through the development of an apical pit when exposed to distilled water, which may be a response it triggers to prevent ion loss or to favor ion uptake by providing a microenvironment different from the more exposed epithelial surfaces (Fernandes *et al*., 1998).

The distribution of CCs is normally restricted to the interlamellar region of the filament epithelium at the base of the lamellae, being more abundant in the trailing edge (afferent side) of the filament (Laurent, 1984, 1989). In *H.* cf. *plecostomus*, CCs are dispersed throughout the lamellae and are found in the leading and trailing edges of the filament. The presence of CCs in the lamellar epithelium of freshwater fish varies according to the species; they are absent in guppy (Pisam *et al*., 1987) and frequently observed in goldfish (Kikuchi, 1977). Several studies suggest that it may depend on the salinity of the external environment, proliferating in ion-poor water (Laurent & Hebibi, 1989; Laurent, 1989; Franklin, 1990; Perry & Laurent, 1993). Moreover, a correlation between the number of CCs and the Ca^{2+} or NaCl concentration in water and gill ion uptake suggests that they may be the sites for such ion transport (Perry & Laurent, 1989). Fish acclimated to a high ion concentration in the water do not present CCs on the lamellar epithelium, while fish living in ion-poor water frequently show CC proliferation on the lamellar epithelium (Mattheij & Stroband, 1971; Laurent & Hebibi, 1989; Perry & Laurent, 1993). Since the Monjolinho Reservoir water where the *H.* cf. *plecostomus* were collected is ion poor and soft, the presence of CCs on the lamellar epithelium may be an adaptation to the low ion concentration in the water, which is presumably beneficial to increase the ion transport capacity of the gill.

The large size of CCs increases the water–blood barrier and has been found to affect the transference of respiratory gases, implying decreased resistance to a hypoxic environment (Thomas *et al*., 1988; Bindon *et al*., 1994; Greco *et al*., 1995; Fernandes *et al*., 1998). However, as *H.* cf. *plecostomus* is a facultative air-breathing fish that resorts to atmospheric air when the oxygen in water is low (Perna & Fernandes, 1996), the presence of CCs on the lamellar epithelium may improve ion transport in ion-poor water without having a drastically negative effect on the respiratory process.

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