Anatomical Studies of the Olfactory Epithelium of Two Cave Fishes Sinocyclocheilus jii and S. furcodorsalis (Cypriniformes: Cyprinidae) from China

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Abstract.- The olfactory epithelium of cavefish, Sinocyclocheilus jii and S. furcodorsalis were studied on a scanning electron microscope. Twenty six lamellae are in the rosette of S. jii and twenty four lamellae in S. furcodorsalis. The olfactory epithelium of S. jii visualizes two types of receptor cells (i) Ciliated sensory with microvillus (ii) Ciliated non-sensory cells. Rod cells and chloride cells were recognized in the olfactory epithelium of S. furcodorsalis, also having receptor cells of two types, ciliated sensory with microvillus and ciliated non-sensory cells in the form of patches. Supporting cells, rod cells, chloride cells, and blood cells were frequently occurring. On the surface of the olfactory lamellae cells correlate with the functional significance of the fish concerned.

Keywords: Olfactory epithelium, cavefishes, Sinocyclocheilus jii, Sinocyclocheilus furcodorsalis, rod cells, chloride cells.

INTRODUCTION

Cave organisms are given much attention for their diverse troglomorphic characters, such as enlargement of some special sensory organs such as appendages, reduction or loss of eyes, and pigmentation (Culver et al., 1995; Romero et al., 2001). The evolution of cave animals has focused on the process of cave colonization and the mechanisms underlying these troglomorphic characters (Dowling et al., 2002).

More than one hundred cavefish species belonging to three families are found in China. Mostly the hypogean fishes described in the last two decades are from Asia, the majority from China (Zhao et al., 2009). Sinocyclocheilus is one of 50 cave-dwellers species belonging to the family Cyprinidae. These cave fishes are fresh-water fishes and endemic to China. Originally they occur in the central eastern Yungui Plateau and its neighboring region, Guangxi, in southwestern China (Xiao et al., 2005; Chen et al., 2009; Wu et al., 2010). Sinocyclocheilus is significant in its high species diversity and troglomorphic character variation within the genus. Therefore, Sinocyclocheilus is an ideal model for investigating the evolutionary biology and natural selection in troglobionts. Sinocyclocheilus jii has functional eyes, a golden lateral line and brownish color with a black stripe along (a little above) the lateral line, no one troglomorphic character is distinguished in Karst cave (Zhang and Dai, 1992). Sinocyclocheilus furcodorsalis has lost its eyes and have a crossed-forked horn on the back. Jastania and Abbasi (2003a,b) studied the ultrastructure of brain cells and epidermis of herring, Clupea harengus L. on transmission electron microscope and scanning electron microscope and discuss the changes in the cells exposed to the mercury.

Olfactory organs play an important role in fish biology because they are basically chemoreceptors, able to detect water soluble compounds providing information about the nearby environment. Olfaction plays a significant role in mediating behaviors that range from feeding and predator detection to social interaction and reproductive synchrony (Sorensen et al., 1998). In teleosts, there is immense variation in olfactory organ characters including size, shape, sensory and nonsensory areas and lamellar arrangement (Hara,
Scanning electron microscopy (SEM) revealed broad knowledge about the characteristic features of the olfactory epithelium in different teleosts (Mana et al., 2002; Arvedlund et al., 2007; Bhute et al., 2007; Chakrabarti et al., 2010).

The present research explores the changes on the surface ultrastructure of the peripheral olfactory organ for cave fish. The SEM is used to assess the microstructure of the olfactory organ of cave species *S. jii* and *S. furcodorsalis*.

**MATERIALS AND METHODS**

Specimens of *S. jii* and *S. furcodorsalis* were collected in March and April 2011 from Gongcheng County and Tiane County, Guangxi, China, respectively. Fish samples were preserved in 10% formalin in the field and brought to the Zoological Museum of the Institute of Zoology, Chinese Academy of Sciences, Beijing. Environmental Scanning Electron Microscope Quanta 200 with a Zeiss stereoscope binocular microscope was used for the present study. Heads of the fishes were dissected from the dorsal side and examined under the Zeiss stereoscope binocular microscope for the study of olfactory rosettes. The olfactory organs were exposed to count lamellae and then dissected out immediately and fixed in 70% alcohol. Olfactory organs were divided into three portions. Each portion of both lamellae was fixed in 70% alcohol for the SEM. The dehydrated samples were dried with liquid carbon dioxide (CO$_2$) using the critical point dryer CPD 030 and sputter coater SCD 005 prior to observation.

**RESULTS**

Olfactory organs of the two species *S. jii* and *S. furcodorsalis* were compared structurally and the distributions of cell types present in the olfactory organ are described. Cup shape olfactory organ of *S. jii* consists of 12-14 primary lamellae on both sides along the axis of each lamellae. The inner part of the lamellae is attached to the raphe while the outer side is attached to the wall of olfactory chamber. Posterior lamellae are larger than anterior lamellae. The complete structure of the fish *S. jii* is showed in the (Fig. 1a). The head region shows the anterior and posterior nostril openings (Fig. 1b) and dissected olfactory rosette shows the complete olfactory organ (Fig. 1c) lying in the chambers. The lamellae differ in shape and size due to their position in the rosette (Fig. 1c). SEM observation show the oval shaped olfactory rosette of *S. jii* showing lamellae (L) and raphe (R), the lamellae near water inlet are smaller than the lamellae near outlet (Fig. 2a). The ciliated non-sensory cells (CN), were observed in all portions of the olfactory lamellae but the olfactory lamellae near to the water inlet (anterior nostril) and middle portion of the olfactory lamellae displayed a thick number of ciliated non-sensory cells, compared to the posterior portion (posterior nostril) of the olfactory lamellae (Fig. 2b-f). A few microvillus cells (MV), were observed in the space between the ciliated non-sensory cells in all portions of the olfactory lamellae (Fig. 2b,c,d and f). The supporting cells were observed on the surface of the lamellae in the middle and posterior regions (Fig. 2d,a,f). Rod receptor cells were observed only in the posterior region of the olfactory lamellae (Fig. 2e,f). A few receptor cells (RC) were present on the surface of the lamellae belonging to the posterior portion (Fig. 2f). The surface of the olfactory lamellae of *S. jii* displayed a very few number of ciliated non sensory cells (CN) on the posterior portion of the olfactory...
Fig. 2. Scanning Electron Micrograph (SEM) showing (a) full picture of olfactory organ with lamellae (L) and raphe (R); and surface of the lamellae (b-f). Note the ciliated non-sensory cells (CN), microvillus (MV), and supporting cells (S) and rod cells (RD).
organ which are scattered on the surface of the olfactory lamellae (Fig. 3a,b,c,e,f). Chloride cells (CC) were observed scattered in between ciliated non-sensory cells (Fig.3c,e,f). A few ciliated receptor cells (CR) are found on the surface of lamellae (Fig. 3d). The fingerprints like micro-ridge cells (MR) are ordinary epidermal cells. The MR are unbranched and arranged in a concentric spiral, leaving long deep channels in between mucus openings, with dense microvillus cells and few non receptor cells (Fig. 3c and f). A considerable number of the rod cells are found on the surface of the olfactory lamellae (Fig. 3a,b,d,e,f). In between the ciliated non-sensory cells the microvillus cells (MV) are scattered on the surface of the olfactory epithelium (Fig. 3a,b,c,d,f). The surface of the olfactory epithelium shows a very few number of supporting cells (S) (Fig. 3a,f).

The olfactory organ of *Sinocyclocheilus furcodorsalis* is an oval-shape consisting of 11 to 13 primary lamellae in both sides. Each lamella is attached to the central raphe. The lamellae near the water current are smaller than posterior. The complete structure of *S. furcodorsalis* is shown in figure 4a. The head region shows the anterior and posterior olfactory openings (Fig. 4b). The dissected olfactory openings shows the structure of olfactory organ (Fig. 4c) which is lying in the chambers. The shape and size of the lamellae in both species differ according to their position in the rosette (Fig. 4c). In *S. furcodorsalis* the surface of olfactory organ is showing lamellae and raphe (Fig.4c). SEM images of the olfactory organ show the whole structure of the olfactory organ (Fig 5a). The surface of the olfactory lamellae displays different cell types. The surface of the lamellae consists of the non-sensory epithelium and ciliated non-sensory cells. A few numbers of ciliated non-sensory cells were scattered on the surface of the epithelium (Fig. 5b-f). Rod cells were observed in anterior and middle regions which are in few numbers (Fig. 5b,d).

A very few number of supporting cells were observed in anterior and middle portion of the olfactory lamellae (Fig. 5b,d,e). The microvillus and chloride cells were observed only in the middle portion of the olfactory lamellae (Fig. 5c,e). The epithelium of the lamellae of the anterior, middle and posterior portions of the olfactory organ is composed of ciliated non-sensory cells among which the rod cells (RD) are visible (Fig. 6a,b). Chloride cells were observed in between ciliated non-sensory cells (Fig. 6a,c). Supporting cells are clearly visible among the ciliated non-sensory cells (Fig. 6a,d). Microvillus cells are scattered in between the ciliated non sensory cells (Fig. 6c and d). In *S. furcodorsalis* surface of the olfactory epithelium consist blood cells (Fig. 6a,d,e). The ciliated non-receptor epithelium is made up of thick patches of ciliated non-sensory cells (Fig. 6b-f). Some ciliated receptor cells are visible in between the ciliated non sensory cells (Fig. 6f). In addition to the ciliated non-sensory cells and ciliated receptor cells a considerable amount of the rod cells were observed in the ciliated sensory region (Fig. 6a-f). Mucus cells were present on the surface of sensory epithelium which is surrounded by dense nonsensory cells (Fig. 6b-f).

**DISCUSSION**

Our study about the surface of the olfactory lamellae consists of ciliated non-sensory cells, microvillus cells, ciliated receptor cells, rod cells, supporting cells, chloride cells and mucus cells in both *S. jii* and *S. furcodorsalis*. However, the structural organization and shape of the cells differ in both species. Yamamoto (1982) suggested an ecomorphological system of classification that the olfactory organs of fish are classified by the distribution pattern of the sensory epithelium, number of lamellae, and their shape.

In the present study of *S. jii*, the ciliated nonsensory cells were sparse on the olfactory epithelium which is quite similar to earlier studies (Yamamoto, 1982). Other than ciliated non-sensory cilia, cell types, receptor cells, microvillus, rod cells and few supporting cells were observed in the olfactory lamellae of the *S. jii*. In early reports, there was confusion in the identification of the receptor cells, but it has confirmed that both ciliated and microvillus cells are the olfactory receptor cells in the teleosts. Through the retrograde degeneration these two types of olfactory receptor cells were identified as genuine in *Carassius auratus* Ichikawa *et al.* (1977). Olfactory receptor cells first reported in a minnow, *Phoxinus phoxinus*, fall into three
Fig. 3. Scanning Electron Micrographs showing Lamellae consisting of (a) the rod cells (RD), microvillus (MV), supporting cells (S), and ciliated non-sensory cells (CN); surface of the lamellae. Note the microvillus cells (MV), ciliated non-sensory cells (CN) and rod cells (RD); (c) surface of the epithelium showing chloride cells (CC), supporting cells (S), microridges (MR), microvillus (MV) and ciliated non-sensory cells (CN); (d) Surface of the epithelium showing rod cells (RD), ciliated receptor cells (CR) and microvillus (MV); (e) surface of the olfactory epithelium. Microvillus cells (MV), ciliated non-sensory cells (CN), chloride cells (CC) and rod cells (RD); (f) showing surface of the olfactory epithelium consists ciliated non-sensory cells (CN), rod cells (RD), microridges (M), few supporting cells (S), chloride cells (CC) and arrow showing mucus openings. (Scale bar a. 50.0 µm; b. 10.0 µm; c. 10.0 µm; d. 10.0 µm; e. 20.0 µm; f. 20 µm).
categories distinguished by the form of the distal free end of the dendrite: cells bearing cilia (ciliated receptor cells), cells bearing microvilli (microvillus receptor cells) and cells bearing a simple rod called rod cells (Bannister, 1965).

Chloride cells were identified in the olfactory epithelium of both species. The olfactory receptor cells are bipolar neurons with cylindrical dendrites which terminate at the free surface of the epithelium. Our study demonstrates that the sensory epithelium of *S. jii* exhibits three distinct types of receptor cells (morphologically): microvillus, ciliated, and rod cells which are lying intermingled in different proportions. Over the ciliated and microvillus receptor cells, the rod receptor cells were dominant. The occurrence of rod-shaped neurons in the olfactory might be a new physiological condition (Hernadi, 1993). Furthermore, the ciliated and microvillus receptor cells have a special interest because they may form a different olfactory transduction mechanism stimulated by odor-bearing substances. In genus *Acipenser*, ciliated and microvillus receptor cells were found together but in different portions in different species (Zeiske et al., 2003).

The sensory epithelium of *S. jii* consisted of rod-shaped cells, which are dominant in the ciliated sensory area. Yamamoto (1982) reported the occurrence of rod cells as a symbol of aging of ciliated receptor cells and having tubercle on the top with a single cilium. It is perhaps a transitional type non-ciliated and rod cells. The presence of the microvillus cells on the lamellae of *S. jii* may have a significant role in reproduction. In the olfactory epithelium of *Cirrhinus mirgala* the presence of microvillus cells played a significant role in the regulation of reproduction (Biju et al., 2003; Chakrabarti et al., 2011). In *T. jarbua* the olfactory epithelium microvillus cells aid in the transduction of environmental signals stimulating the pituitary and gonads.

The eyeless *S. furcodorsalis* olfactory epithelium surface consists of ciliated receptor cells, ciliated non-sensory cells, microvillus cells, rod cells, supporting cells, and mucus cells. According to the classification given by Yamamoto (1982), the arrangement of olfactory organ in *S. furcodorsalis* is G-pattern, type I. The density of the non-sensory cells on the surface of the olfactory epithelium varies with the species (Figs. 2, 4). The distribution of the sensory and non-sensory epithelia on the surface of the olfactory lamellae shows an immense diversity in different fish species. In *S. furcodorsalis*, the non-receptor epithelium is made up of patches of ciliated non-sensory cells surrounded by the glandular duct cells that classically show the whole part of the lamellae covered with ciliated non-sensory cells.

![Fig. 4. Sinocyclocheilus furcodorsalis, (a), full size picture; (b) dorsal view of the head showing the anterior nostril (ANO), posterior nostril (PNO) and nasal flap (NF); (c) Micrograph from binocular compound microscope showing the cup-shaped olfactory organ raphe (R) and Lamellae (L)](image)

The surface of the olfactory cilia was not smooth, but showed irregular knob-like profiles (Fig. 4 b–f). In the ciliated non-sensory area, a few of ciliated receptor cells are seen intercalating with freely distributed ciliated receptor cells (Fig. 4f). The densely arranged ciliated cells in the olfactory lamellae of *Triplophysa dalica* are responsible for circulation of the water in the interlamellar space as well as in the olfactory chamber. Waryani et al. (2013) reported that the cilia streams incoming solutions of water with dissolved chemicals between the lamellae and over the mucous layer. In the present study, the mucus openings form the pits lubricate the surface of the lamellae suggesting they protect the epithelium from the incoming water
Fig. 5. Scanning Electron Micrograph (SEM) showing (a) full picture of the olfactory organ lamellae (L) and raphe (R); (b) showing the ciliated non-sensory cells (CN), rod cells (RD), and supporting cells (S); (c) showing the ciliated non-sensory cells (CN), chloride cells (CC), microvillus (MV); (d) showing the ciliated non-sensory cells (CN), rod cells (RD) and supporting cells (S); (e) showing the ciliated non-sensory (CN), chloride cells (CC), supporting cells (S) and microvillus (M); (f) showing the ciliated non-sensory cells (CN)
Fig. 6. Scanning Electron Micrograph (a) showing the ciliated non-sensory cells (CN), rod cells (RD), ciliated receptor cells (CR), chloride cells (CC), supporting cells (S) and arrow mucus openings, blood vessels; (b) showing the ciliated non-sensory cells (CN) and rod cells (RD); (c) Surface of the olfactory lamellae showing the ciliated non-sensory cells dense, chloride cells (CC), rod cells (RD), supporting cells (S) and arrow showing the mucus openings; (d) showing surface of the olfactory lamellae. Ciliated non-sensory cells (CN), Microvillus cells (MV), rod cells (RD), blood cells (*) and Mucus openings arrow; (e) showing surface of the olfactory lamellae. Ciliated non-sensory cells (CN), rod cells (RD) and blood cell; (f) showing surface of the olfactory lamellae. Ciliated non-sensory cells (CN), rod cells (RD) and receptor cells (RC).
Scale bar a. 20.0 µm; b. 10.0 µm; c. 10.0 µm; d. 20.0 µm; f. 50.0 µm
during ventilation. In *Triplophysa dalica* mucus from the pits lubricates the surface of the raphae, which protects the epithelium from the incoming water thrust during ventilation (Waryani *et al.*, 2013). The mucus layers may also serve as an ion trap, which delays the access of heavy metal and salts to underlying organs (Banerjee, 1993). In the present study, the surface of the olfactory epithelium of *S. furcodosails* consist rod cells of pear-shaped and occur in between the ciliated non-sensory area. They are surrounded with a distinct capsule and contain an elongate structure called rodlets (Fig. 4a-f). Rodlet cells are found in a variety of tissues of many teleosts both in sensory and indifferent epithelia in the olfactory lamellae (Morrison *et al.*, 1978; Wilson *et al.*, 1967; Zeiske *et al.*, 1976; Zeiske *et al.*, 1979; Waryani *et al.*, 2013). The supporting cells provide the basic structure to the sensory lamellae with microridge system on their surface. Jastania and Abbasi (2003) found microridge in epidermis cells functioning for respiratory and excretory system and also sensitive to stimuli from the surrounding environment. In the present study only one supporting cell on the surface of the olfactory lamellae was observed (Fig. 4c).

The most interesting features of the present study are that the ciliated non-sensory cells are the key type of non-sensory cells in the sensory epithelium on the surface of the olfactory lamellae of the *S. jii*. On the olfactory epithelium a few of the ciliated non-sensory cells were scattered. Low density of the cilia in the sensory epithelium favors a water-propelling function and may help propel mucus in the shortness of cilia. The microvillus receptor cells were dominant on the ciliated non-sensory cells. A few supporting cells were found compared to the receptor cells in surface epithelium. Rod receptor cells are dominant over the microvillus and receptor cells, might change some new physiological conditions. Moran *et al.* (1992) suggested that the rod cells can be considered as affecting non-sensory and sensory cells. Others have interpreted this as degenerative cells (Theisen *et al.*, 1980; Muller *et al.*, 1984). In sea bass rod cells were high in frequency in the olfactory epithelium, which is unlike in wild bass. Less numerous ciliated non-sensory cells supports the hypothesis that ciliary agglutination reflects a degenerative process of the ciliated non-sensory cells Diaz *et al.* (1987). In the present study *S. jii*, display major alteration of the olfactory epithelium that the rod cells occur in high frequency, but ciliated non-sensory were of less number.

In the *S. furcodosails*, the olfactory epithelium is characterized by ciliated non-sensory cells, ciliated receptor cells, microvillus cells and rod cells. In *S. jii*, the ciliated non-sensory cells were not found predominant of indifferent epithelium with rod cells and microvillus cells. The patches of ciliated non-sensory cells and predominance of the mucus cells in the surface epithelium of *S. furcodosails* is a clear feature. The sensory epithelium in the *S. furcodosails* is very rich in ciliated non-sensory cells similar to previous studies of several teleost fish (Thiesen, 1972; Lowe *et al.*, 1975; Ichikawa *et al.*, 1977; Yamamoto *et al.*, 1977; Hansen *et al.*, 1993). Ciliated non-sensory cells are apparently used to create a current of water along the olfactory epithelium (Theisen, 1970, 1972; Bertmar, 1973; Verraes, 1976; Appelbaum *et al.*, 1983).

In *S. jii* and *S. furcodosails* the production of the mucus on the lamellae proves that the cilia boost and are considered goblet cells arise from the mucus openings. According to Jonathan (2012) neoselachian cilia present on the olfactory epithelium propels mucus and are active to protect the sensory epithelium from damage and water flow through the main olfactory channel. Olfactory epithelium of cave fish *S. jii* and *S. furcodosails* are similar and support Jonathan (2012).

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