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Original Article

Structural characterization of the olfactory epithelium of freshwater olive barb, *Puntius sarana* (Hamilton, 1822)

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Abstract: The cellular organization of the olfactory epithelium of *Puntius sarana* (Hamilton, 1822) was explored by means of optical and scanning electron microscopy. The oval shaped olfactory rosette was composed of 26-28 primary lamellae distributed from both sides of the central raphe. The sensory epithelium confined chiefly on the linguiform processes of the lamella and rest of the portion consisted of non-sensory epithelium. The sensory epithelium was embossed with morphologically distinct three types of sensory cells: ciliated, rod and microvillus receptor cells. The non-sensory epithelium was made up of mainly stratified epithelial cells and mucous cells. Different cells lining the olfactory epithelium were discussed in relation to mode of life and living of the fish concerned.

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Introduction

The olfactory organs are of special attention in fish biology because they are basically chemoreceptors, efficient to ascertain water soluble compounds and afford the fish with information about the nearby environment. In fish, water with dissolved chemicals flow from anterior to posterior nostril over the olfactory organ, covered with olfactosensory epithelium which performs an important role in olfaction (Hara, 1971). Olfactory information is extremely important in the lives of fishes including feeding, prey detection, predator avoidance, species and sex recognition, and migration (Camacho et al., 2010). Teleostean olfactory organs exhibit a respectable diversity, reflecting different developmental strategies and ecological habitats (Zeiske et al., 1992). The microstructure and function of olfactory organ in several fishes have been widely described by several workers (Mandal et al., 2005; Sawad et al., 2006; Bhute and Baile, 2007; Kumari, 2008; Ma and Wang, 2010; Chakrabarti and Ghosh, 2011; Ghosh and Chakrabarti, 2012; 2013; 2014). However, no attempt has been made to correlate the functional significance of various cells lining the olfactory epithelium in relation to the species feeding adaptations.

Puntius sarana (Cypriniformes, Cyprinidae) is freshwater, omnivorous, bottom dwelling teleost and distributed throughout the Indian subcontinent (Chetia Borah, 2012). Its food primarily consists of algae, unicellular and single celled animals, larvae of aquatic insects and sands (Shafi and Quddus, 2001). The aim of the present work is to examine the distribution of different cell types and their functional aspects in the olfactory epithelium of important food fish, *P. sarana* by histological and ultrastructural examination.

Materials and methods

Living mature specimens of *P. sarana* (14 to 16 cm in total length) were obtained from the local freshwater body of Burdwan, West Bengal, India. The specimens were anaesthetized with tricane

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methone-sulphonate (MS 222; Sigma Chemical Co.) solution (100 mg/L) and sacrificed following the guidelines given by the Institutional Ethical Committee. The olfactory organs were carefully dissected out from the floor of the nasal cavity under a stereo microscope and immediately processed for the histological and scanning electron microscopic studies.

Histological study: The olfactory tissues were fixed in aqueous Bouin's fluid for 16-18 hour. After fixation the tissues were washed repeatedly in 70% ethanol and dehydrated properly through ascending series of ethanol. Then they were cleaned with xylene and embedded in paraffin wax of 56-58°C under a thermostat vacuum paraffin-embedding bath for a period of 1 hour and 30 minute. Serial sections were cut at 4 µm thick using a rotary microtome (Weswox). After routine histological procedure deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. The prepared histological sections were examined and photographed by Olympus-Tokyo PM-6 compound microscope.

Scanning electron microscopic (SEM) study: The olfactory rosettes were perfused in vivo with 2.5% glutaraldehyde solution in 0.1 M Na-cacodylate buffer (pH 7.4) for 15 minute. The entire olfactory rosettes were dissected out and rinsed with heparinised saline (heparin sodium salt 10,000 IU dissolved in 0.67% NaCl solution) and 1% Tween 40 mixture to remove the adhering mucus. After rinsing in 0.1 M Na-cacodylate buffer, the tissues were again fixed with 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) for 24 hour at 4°C. After primary fixation the tissues were rinsed in the same buffer and post-fixed in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer (pH 7.4) for 2 hour. The tissues were washed thoroughly in the same buffer and dehydrated through graded series of ethanol followed by acetone and isoamyl acetate. The samples were dried with critical point drier (Hitachi 8CP2), mounted on metal stubs, coated with gold palladium by vacuum gold coater. The tissues were examined under a Hitachi S-530 scanning electron microscope.

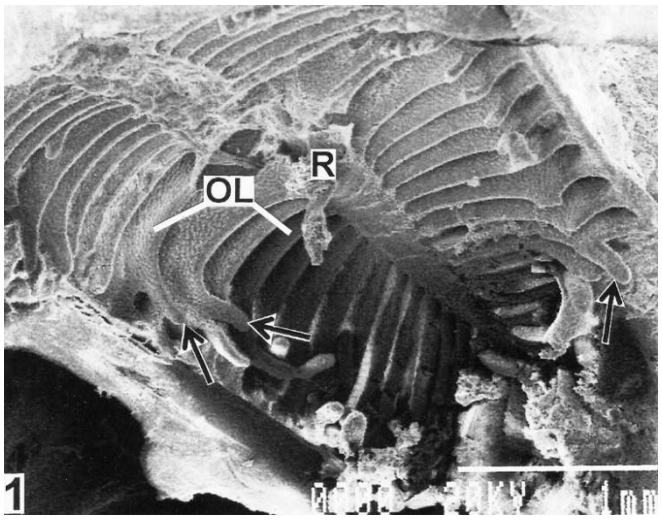

Figure 1. Photomicrographs of the olfactory epithelium of *P. sarana*. Oval shaped olfactory rosette exhibits olfactory lamellae (OL) radiating from central median raphe (R). Arrows indicate linguiform processes of OL (SEM × 50).

Results

According to SEM observations, the olfactory rosette of *P. sarana* is nearly oval in outline with a convex ventral and a concave dorsal appearance. The olfactory rosette consists of a series of 26 to 28 more or less flattened lamellae, distributed in a feather like style on both sides of the central raphe and occupies the entire cavity of the olfactory chamber (Fig. 1). The outer edges of the lamellae are adhered to the olfactory chamber, while the inner edges are attached to the median raphe. The lamellae are capacious in the middle portion of the rosette and diminish in sizes towards the preceding and succeeding ends. The middle dorsal part of the lamella is characterized by free well-developed linguiform process, grows from the distal edge (Fig. 1). The sensory epithelium is restricted mainly on the linguiform processes of the lamella, while rest of the portion is consisted of non-sensory epithelium. Histologically, the olfactory lamellae are radiated from raphe and composed of two layers of thick olfactory epithelium separated from the narrow central lamellar space, central core by a well-developed basement membrane (Fig. 2). The central core is packed with connective tissue fibers, nerve fibers and blood vessels (Figs. 3, 4, 8). The sensory

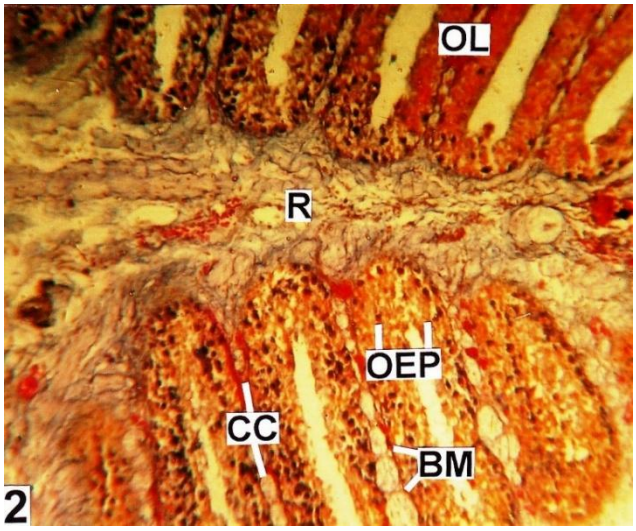

Figure 2. Histological sections of the olfactory epithelium of *P. sarana*. Olfactory lamellae (OL) based on raphe (R) shows olfactory epithelium (OEP) separated from central core (CC) by basement membrane (MT × 100).

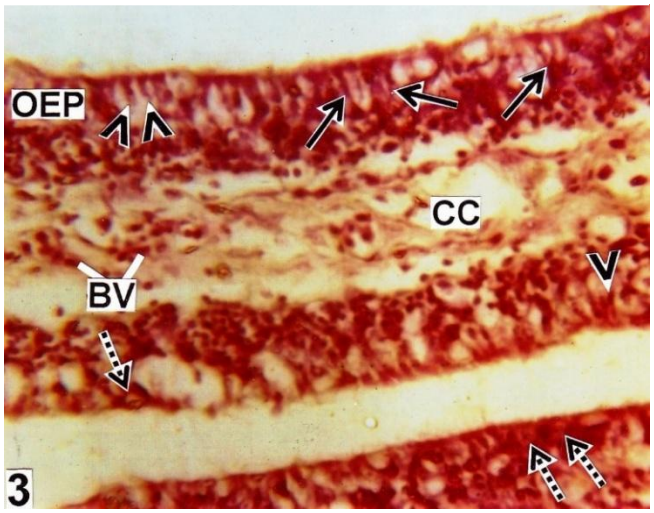

Figure 3. Histological sections of the olfactory epithelium of *P. sarana*. Sensory olfactory epithelium (OEP) lined with receptor cells (RC) (solid arrows), rod cells (arrow heads) and microvillous cells (broken arrows). Note the presence of blood vessels (BV) in CC (HE × 400).

epithelium is typified with mainly receptor cells having long dendrite reaching up to the free epithelial surface and few rod cells and microvillous cells (Fig. 3). Rod cells are ovoid in appearance, extend as a spike like texture and characterized with basally situated deeply stained nuclei. Microvillous cells are situated in more superficial layer of the epithelium, possess a round nucleus and without cilia (Fig. 3). Receptor cells are the sensory elements of the olfactory epithelium and differentiated into primary and secondary receptor cells. The primary

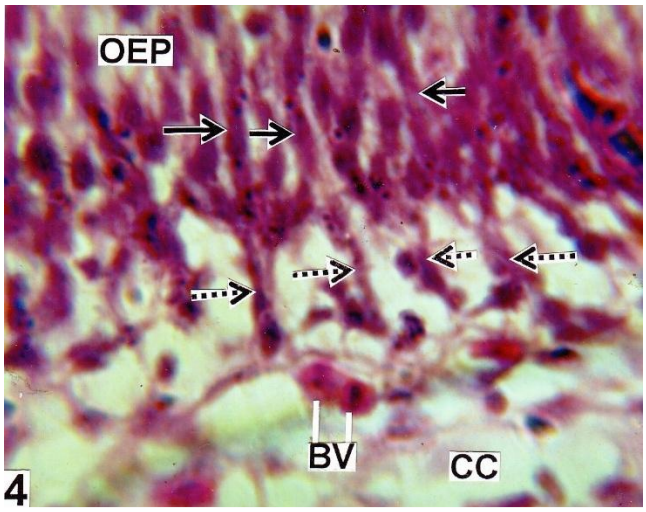

Figure 4. Higher magnification of sensory olfactory epithelium (OEP) of *P. sarana* shows the arrangement of primary receptor cells (solid arrows) and secondary receptor cells (broken arrows). Note presence of blood vessels (BV) in the central core (CC) (MT × 1000).

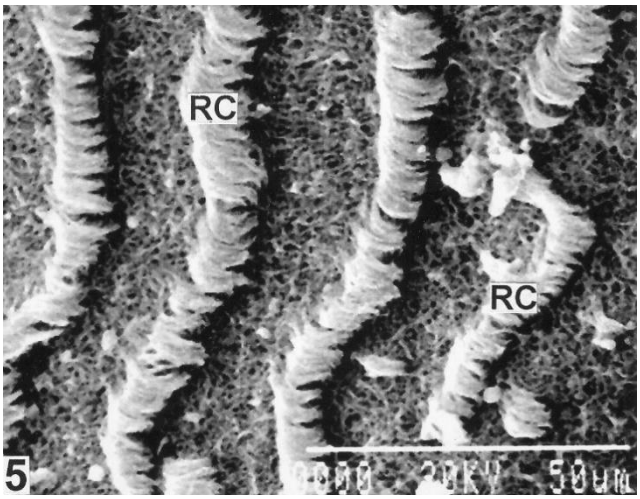

Figure 5. Photomicrographs of the olfactory epithelium of *P. sarana*. Sensory epithelium exhibits bunch of ciliated receptor cells (RC) (SEM × 3500).

receptor cells are highly basophilic with almost rounded and deeply stained nuclei in the distal portion of the cell. The dendrite process of each receptor cell runs to the superficies of the lamellae as a spare cylindrical process (Fig. 4). The secondary receptor cells are distributed below the primary receptor cells and marked by their oval and elongated nuclei. The axonal ends of primary receptor cells synapse with the dendrite tips of secondary receptor cells (Fig. 4). Under SEM observations, the linguiform process of olfactory lamella has a spongy structure due to the appearance of compact ciliated receptor cells (Fig. 5). The

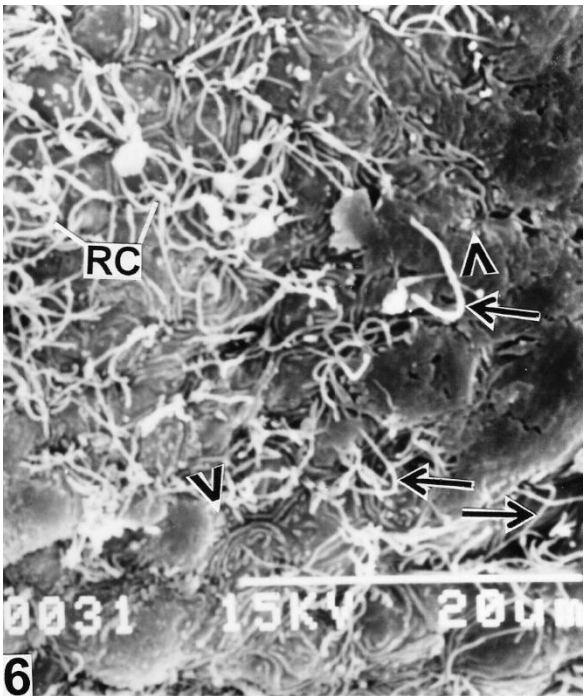

Figure 6. Photomicrographs of the olfactory epithelium of *P. sarana*. Sensory epithelium displays dendrite processes of receptor cells (RC), rod cells (solid arrows) and microvillous cells (arrow heads) (SEM × 4000).

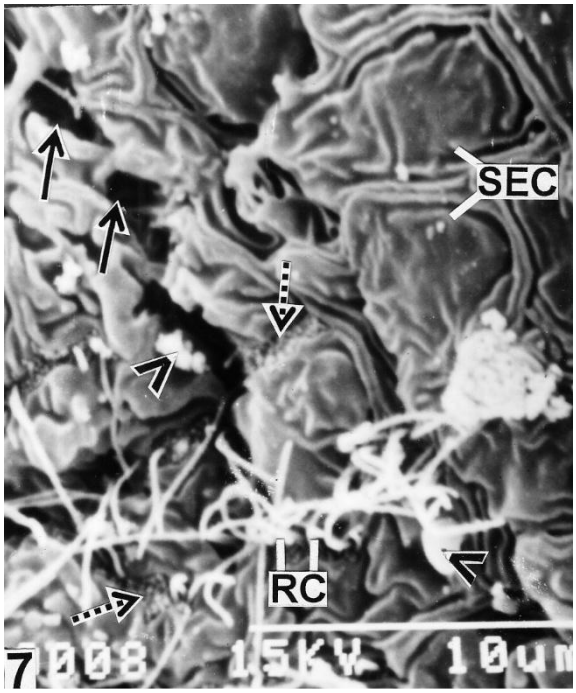

Figure 7. Photomicrographs of the olfactory epithelium of *P. sarana*. Transitional zone of sensory and non-sensory epithelium shows ciliated receptor cells (RC), microvillous cells (broken arrows), stratified epithelial cells (SEC) with complex microridges and mucous cells (solid arrows). Note mucin droplets over the SEC (SEM × 4500).

receptor cells are distinguished by the distal free end of the dendrites and categorized into ciliated, rod and

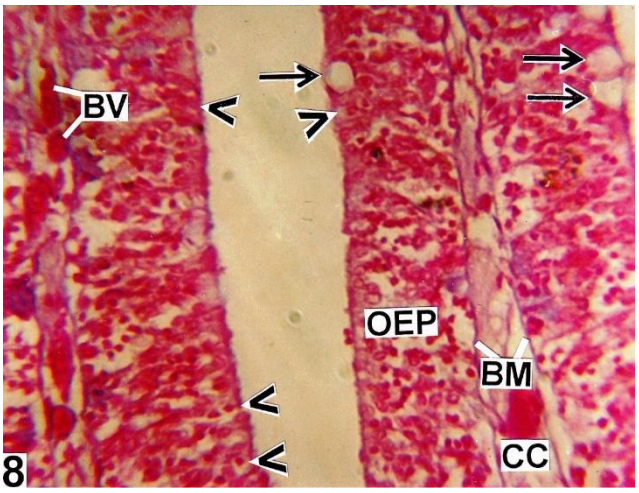

Figure 8. Histological sections of the olfactory epithelium of *P. sarana*. Non-sensory olfactory epithelium (OEP) shows mucous cells (solid arrows) and stratified epithelial cells (arrow heads). Note presence of blood vessels (BV) in the central core (CC) which is detached from OEP by basement membrane (BM) (HE × 400).

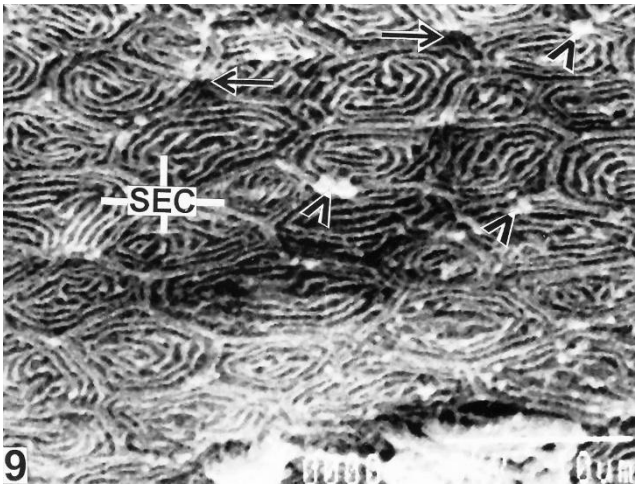

Figure 9. Photomicrographs of the olfactory epithelium of *P. sarana*. Surface epithelium of raphe characterized with stratified epithelial cells (SEC) having labyrinth pattern microridges. Note the presence of opening of mucous cells (solid arrows) in between SEC and mucin mass (arrow heads) over the SEC (SEM × 4000).

microvillous receptor cells. Ciliated receptor cells are furnished by tuft of long cilia, rise to the epithelial surface and dominant over both rod cells and microvillous cells (Fig. 6). The rod cells are few in number and their apical end protrude as a simple rod like structure, distributed randomly in the epithelial surface. The microvillous cells are embossed with tuft of microvilli and dipped into the thickness of the ciliated receptor cells (Fig. 6). The transitional zone of sensory and non-sensory

epithelium is provided with stratified epithelial cells intercalated with the opening of mucous cells leaving microvillous cells adjacent to the ciliated receptor cells (Fig. 7). Histologically, the non-sensory epithelium is lined with chiefly stratified epithelial cells with prominent basophilic nuclei and mucous cells (Fig. 8). Under SEM study, the non-sensory epithelium and raphe is represented by tightly packed stratified epithelial cells having labyrinth pattern microridges and opening of mucous cells. Secreted mucin masses are placed over the microridges of stratified epithelial cells (Fig. 9).

Discussion

Olfactory mucosa comprising the sensory neurons is typically placed on the floor of the olfactory chamber, which is frequently folded, forming olfactory lamellae (Hara, 1975). The feeding habits of fishes are reflected on the structure and cellular organization of the olfactory organ (Hara, 1994). The oval shaped olfactory rosette of *P. sarana* with two rows of olfactory lamellae present on both side of the median raphe belongs to Burne's (1909) rosette column I or Bateson's (1889) rosette type III. Teichmann (1954) classified this oval type of olfactory organ under the category of eye-nose fish, which means that this kind of fish possess similarly developed optic and olfactory senses. The olfactory organ of *P. sarana* holds 26-28 lamellae arranged on both left and right side of the rosette, adapted maximum to the space availability and is similar to other cyprinid olfactory organ can be placed to the type VI by Yamamoto and Ueda (1979). The multilamellar arrangement increases the sensitivity and efficiency of the olfactory organ (Zeiske et al., 1976). The distribution of sensory and non-sensory areas in the olfactory epithelium is variable among teleosts (Yamamoto, 1982). In *P. sarana* the sensory epithelium is bounded in the free linguiform processes, while the rest portion of lamellae is comprised of non-sensory epithelium. This disposition may be due to the fact that the linguiform process with sensory cells faces the flow of incoming water current and the receptor cells interact with the water soluble chemicals during olfaction. Similar findings were also reported by Ojha and Kapoor (1973) in the olfactory apparatus of *Labeo rohita*.

The most interesting characteristics of the olfactory epithelium of *P. sarana* is the histological existence of secondary receptor cells in addition to primary receptor cells and the presence of synaptic connection between these two kinds of receptor cells. This is in conformity with the findings of Goel (1978) in the olfactory epithelium of *Notopterus notopterus*. Buck and Axel (1991) reported that olfaction commences at the apical tip of the receptor cells. In the present observation, the sensory epithelium chiefly consists of three morphologically distinct types of receptor cells: ciliated, rod and microvillous cells; intermingled in different proportions. The ciliated receptor cells correspond to the type I cells of Yamamoto and Ueda (1978), the rod cells similar to the type IV cells of Ichikawa and Ueda (1977), whereas the microvillous cells are harmonized with the type II cells of Muller and Marc (1984). The present study reveals that the ciliated receptor cells dominating over the rod cells and microvillous cells. The cilia are the site of transduction process, stimulated by odour bearing substances and also enable the fish to detect food. Sparsely distributed rod cells in the olfactory epithelium of *P. sarana* are considered as a sensory receptor cells. Yamamoto (1982) opined the rod cells as a sign of aging of ciliated receptor cells and terminate with single cilium at the free surface of the epithelium. Reite and Evensen (2006) believed that these are non-specific immune cells, rolled up in immunity as their number proliferates during parasitic infection. However, on the basis of experimental work and developmental studies, Zielinski and Hara (1988) and Moran et al. (1992) have established that the rod shaped process of these cells represent the dendritic apical processes of olfactory receptor cells. In *P. sarana* the microvillous receptor cells probably form a different olfactory transduction mechanism for pheromones in the regulation of reproductive activities. This is in compliance with the findings of Biju et al. (2003) in

the olfactory epithelium of *Cirrhinus mrigala*. Bhute and Baile (2007) also advocated that the microvillous receptor neurons perceive and process signals of pheromone, which is an important step of breeding in *Labeo rohita*. On contrary Bakhtin (1977) and Bannister (1965) considered that microvillous cells in the olfactory surface of *Squalus acanthias* and teleostean fishes are predecessors of ciliated receptor cells.

In the transitional zone of sensory and non-sensory epithelium few ciliated receptor cells in between stratified epithelial cells are responsible for better monitoring of the water quality even up to this zone. In *P. sarana*, the non-sensory cells lining the olfactory epithelium may help in mechanical assist of sensory cells. Furthermore, the non-sensory epithelium and the raphe consist of stratified epithelial cells provided with labyrinth pattern microridges on their apical surface. Such microridges may enhance the epithelial surface and serve as holding the mucus film over the epithelial membrane and protect the epithelium from mechanical abrasion. The mucin secreted by mucous cells over the lamellae probably forms a suitable medium for diffusion of odorants. The mucus layer may also help in ion trap, which obstructs the penetration of salts and heavy metals to underlying organs (Banerjee, 1993). In addition the mucin secreted from the mucous cells of raphe probably helps the smooth flow of water through the olfactory chamber by binding microscopic debris which is ejected through the posterior nostril. This is in confirmed with the findings of Bandyopaghyay and Datta (1998) in the olfactory organ of *Heteropneustes fossilis*.

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