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Age specific anatomy and cytological studies on unilamellar olfactory structure of a teleost (*Pseudapocryptes lanceolatus*)

Swaraj Kumar Sarkar, Shreya Jana and Subrata Kumar De*

Ultrastructure and Fish Biology Research Unit, Department of Zoology, Vidyasagar University, Midnapore (West), West Bengal, India

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ABSTRACT

The age-specific macro- and micro-anatomical changes within the olfactory structure in two different age groups [young (total body length: 70mm to 150mm) and adults (total body length: 150mm to 200mm)] of Pseudapocryptes lanceolatus (Bloch and Schneider, 1801) has been examined under light microscope (LM). P. lanceolatus possess single olfactory lamella that connects anterior and posterior nares on the either side of the snout. The distance between nares (Y) and the length of olfactory lamella (Z) are significantly related in accordance with increasing total body length (X) (70mm to 150mm) of P. lanceolatus (calculated correlation coefficient r_{XY} = 0.962 and r_{XZ} = 0.962 respectively). In adults, the length of olfactory lamella and distance between the nares are remain constant and does not show any significant correlation with the changing total body length. The microanatomical study indicates continuous proliferation of basal cell within the olfactory neuroepithelium of both young and adults but differs in frequency of occurrence of degenerating sensory receptor cell within the olfactory neuroepithelial system. Thus, the constant proliferation of basal cell is the only factor which is responsible for enlarging the surface area of olfactory neuroepithelium (in young stage) as well as replacing degenerating sensory receptor cells in adult olfactory structure. This base line data might helps to explore details regarding age-related neurodegenerative disorders in higher vertebrates.

Keywords: *P. lanceolatus*, olfactory, pseudostatified, neurodegenerative, *etc.*

INTRODUCTION

Olfaction is one of the oldest sensory modality of vertebrates [1]. This sense is mediated through olfactory organ [2]. The anatomical organization of olfactory organ shows a wide range of diversity in vertebrate phylogeny [3-14]. In teleosts, olfactory neuroepithelium is generally raised from the floor of olfactory chamber and subsequently folded to form olfactory rosette [15]. The number of the olfactory lamella within the rosette is variable among the teleosts which may depend on the species and age of the fish concerned [16]. During the post natal development of multilamellar olfactory apparatus, the number of olfactory lamella is gradually amplified through subsequent folding of olfactory neuroepithelium according to the increasing body length or body weight for a certain period of life and reflects age specific anatomical variation within the species [15, 17-20]. Teleosts that are devoid of multilamellar rosette, the age-specific anatomical changes in olfactory structure are still not addressed. *Pseudapocryptes lanceolatus* (Bloch and Schneider, 1801) is a common mudskipper of Gangetic Bengal, India and possess unilamellar olfactory apparatus [12, 14]. This species also shows distinct sexual maturity in respect to increasing total body length (TL) [adults: 15.4cm (in female) and 16.3cm (in male) respectively] [21-22]. The present study emphasized on the macro- and micro-anatomical details of the olfactory structure of *P. lanceolatus* to explore the age-specific anatomical changes of the concerned organ.

MATERIALS AND METHODS

a. Experimental specimen

Pseudapocryptes lanceolatus is an air breathing coastal gobiid of South East Asia. It is generally found in the mudflats of Gangetic Delta of South 24 Parganas, West Bengal, India. Live specimens of *P. lanceolatus* having variable total body length [*i.e.,* 70mm to 150mm (young) and 150mm to 200mm (adults)] were collected and brought to the laboratory for acclimatization [temperature: 20˚ to 25˚C, humidity: >40%, *etc.*]. The specimens were anaesthetized by using MS – 222 (dose: 100mg/L – 20 0mg/L) for anatomy, morphometry and microanatomical studies respectively.

b. Anatomy and morphometry of olfactory apparatus

The olfactory apparatus of *P. lanceolatus* were dissected out from the dorsal surface of the head and separately fixed in aqueous Bouin's solution. The gross anatomical structures of the olfactory apparatus in *P. lanceolatus* of variable total body length (TL) were examined under binocular light microscope (LM). The distance between the nares and length of the olfactory lamella were noted. The nonparametric correlation between distance between nares, length of olfactory lamella with respect to increasing total body length (TL) were analysed in *P. lanceolatus* through statistical methods using Microsoft Office Excel 2007 and SPSS 10.0 (for windows).

c. Microanatomy

The olfactory apparatus of young and adult *P. lanceolatus* were dissected out from the antero-dorsal side of the head and separately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH. 7.2-7.4) at 4°C for 2 hours . After primary fixation, the olfactory tissues were rinsed in the same buffer and then fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH. 7.2-7.4) for 1 hour at 30°C . The olfactory tissues were then rinsed in the same buffer and dehydrated in chilled acetone. The tissues were embedded in Araldite CY212 (TAAB, UK) and resin polymerized for 48 hours at 60°C. Transverse sections of the ol factory lamella (thickness: 1µm) were cut with an u ltramicrotome (Leica ultracut UC6), stained with 1% toluidine blue and examined under trinocular microscope (Primo Star; Carl Zeiss Microscpy, GmbH, Germany) and image analyzed by Axio Vision LE (version 4.3.0.101) [Carl Zeiss Vision, GmbH, Germany].

RESULTS

Anatomy and morphometry of olfactory apparatus

Pseudapocryptes lanceolatus (Fig. 1A) possess two pairs of nares [tube-shaped anterior naris and oval-shaped aperture of posterior naris] (Fig. 1B). The anterior naris is present at the apical tip of the snout where as the posterior naris is located at the anterior edge of the eye (Fig. 1B). The olfactory apparatus of *P. lanceolatus* is comprised of single olfactory lamella along with accessory nasal sacs, olfactory chambers, olfactory nerves, olfactory bulbs and brain (Figs. 1C and 1D). The olfactory lamella is an elongated tube-like structure and associated with ethmoidal and lacrimal sac at the caudal part (Fig. 1E). The length of the olfactory lamella and distance between anterior and posterior nares shows considerable variation according to the increasing total body length (young: 70mm to 150mm) of *P. lanceolatus* (Table I). The morphometric relationship between the total body length (X), the distance between the nares (Y) and length of the olfactory lamella (Z) of *P. lanceolatus* are calculated (Table I). The calculated correlation coefficient (r_{XY} = 0.962) is found significant at the 0.01 level (two tailed). The length of the olfactory lamella (Z) is also shows significant correlation coefficient (r_{XZ} = 0.962) at the 0.01 level (two tailed) according to increasing total body length (X) of the species (Tables I, II & III). Interestingly, the distance between the anterior and posterior nares (Y´) as well as length of the olfactory lamella (Z´) remains constant in the adult stages of *P. lanceolatus* (TL: 150mm to 200mm) (X´) (Table IV). Therefore the distance between the nares (Y´) and length of the olfactory lamella (Z´) are not showing any significant correlation with the increasing total body length at the adult stages of the concerned species (Table IV).

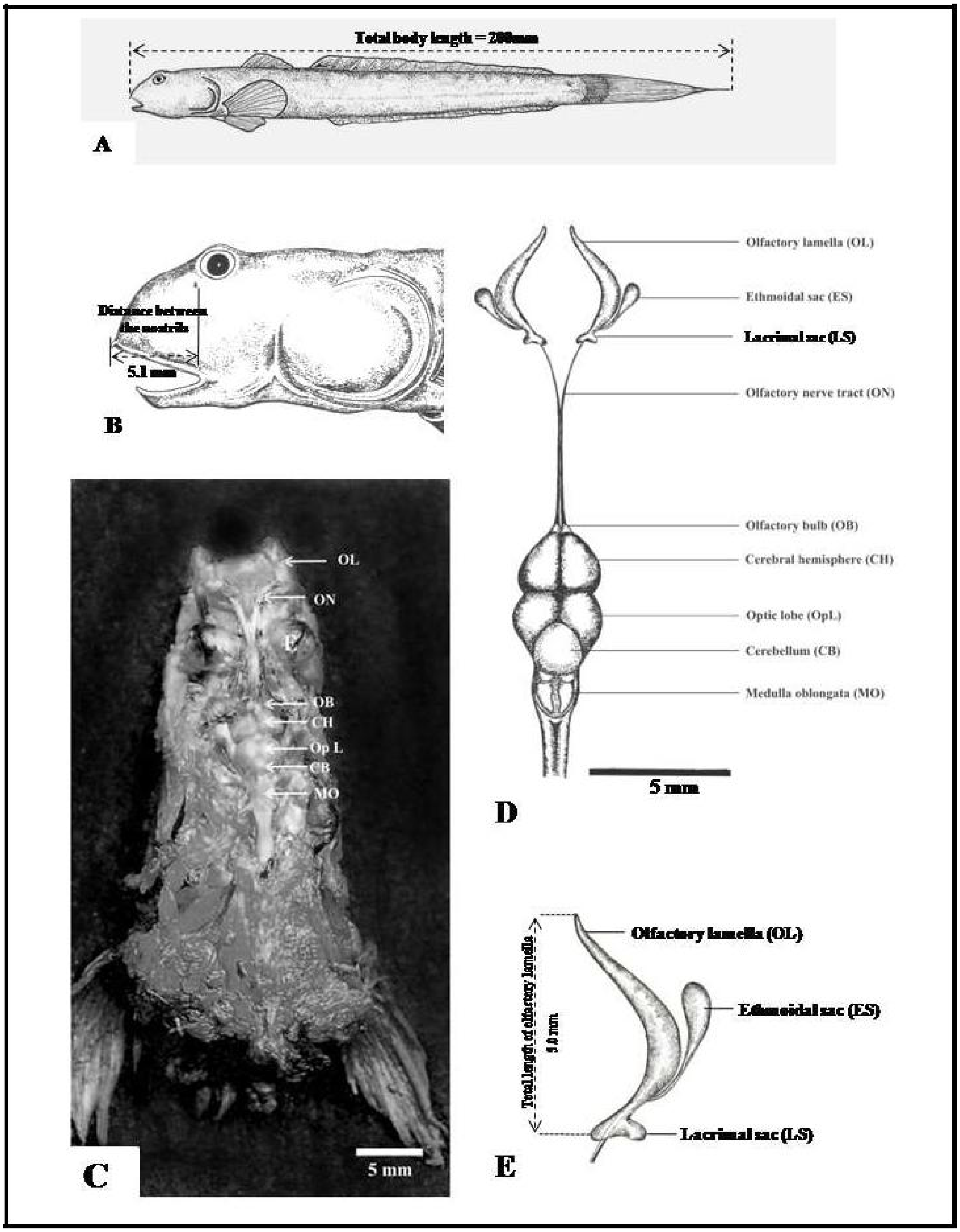


Figure 1: The morphoanatomy of the olfactory apparatus in *P. lanceolatus* A – The diagrammatic representation of adult *P. lanceolatus* [Not to scale]. B – The length of the snout is meas ured 5.1mm (approx.) in adult *P. lanceolatus* species [Not to scale]. C- The macroanatomy of olfactory apparatus in *P. lanceolatus* shows olfactory lamella (OL), olfactory nerve (ON), olfactory bulb (OB), cerebral hemisphere (CH), optic lobe (OpL), cerebellum (CB) and medulla oblongata (MO). D- The diagrammatic representation of the olfactory apparatus in adult *P. lanceolatus.* E- The olfactory lamella is an elongated tube-like structure (length: 5mm approx. in adult *P. lanceolatus*) and well associated with ethmoidal sac and lacrimal sac at the different position of the caudal part [Not to scale].

Table: I

Sl. No.	Total body length of *P. lanceolatus* (in mm.) (X)	Distance between the nostrils (in mm.) (Y)	Standard deviation between X and Y	Correlation value between X and Y	Length of the olfactory lamella (in mm.) (Z)	Standard deviation between X and Z	Correlation value between X and Z
1	70	3.1	±49.81360494	0.962273654	3.0	±49.86225737	0.962273654
2	75	3.3			3.2		
3	80	3.4			3.3		
4	85	3.5			3.4		
5	90	3.7			3.6		
6	95	3.9			3.8		
7	100	4.1			4.0		
8	105	4.4			4.3		
9	110	4.6			4.5		
10	150	5.1			5.0		

Table I. The morphometric variation of the distance between the nostrils (Y) and length of the olfactory lamella (Z) in respect to increasing total length [70mm. to 150mm.] of *P. lanceolatus* (X) using SPSS 10.0 (for windows)

Table : II

Correlations

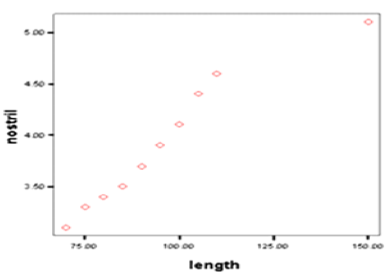
Correlations

		LENGTH	NOSTRIL
LENGTH	Pearson Correlation	1.000	.962**
	Sig. (2-tailed)	.	.000
	N	10	10
NOSTRIL	Pearson Correlation	.962**	1.000
	Sig. (2-tailed)	.000	.
	N	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

A

Interactive Graph



B

Table : III

Correlations

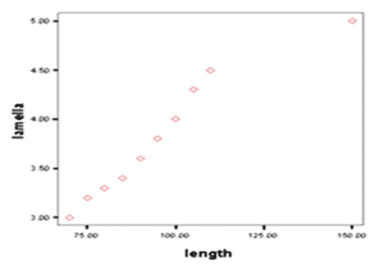
Correlations

		LENGTH	LAMELLA
LENGTH	Pearson Correlation	1.000	.962**
	Sig. (2-tailed)	.	.000
	N	10	10
LAMELLA	Pearson Correlation	.962**	1.000
	Sig. (2-tailed)	.000	.
	N	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

A

Interactive Graph



B

Table II. The table shows significant correlation between the increasing total length of *P. lanceolatus* (X) and distance between the nostrils (Y)
Table III. The table shows significant correlation between the increasing total length of *P. lanceolatus* (X) and length of the lamella (Z)

Microanatomy

The olfactory lamella of *P. lanceolatus* is externally lined by the pseudostratified olfactory neuroepithelium and possesses sensory receptor cells, supporting cells and basal cells (Figs. 2A and 2B). Among the other type of neuroepithelial cells, sensory receptor cells are bipolar neuron but the supporting cells are columnar in nature (Fig. 2B). Basal cells are polygonal in shape and loosely arranged just above the basal lamina (Fig. 2C). The mitotic stages of basal cells are frequently observed within olfactory neuroepithelium of juvenile, young and adult stages (Figs. 2C, 2D and 2E). The dividing basal cells are located just above the layer of basal cell and shows gradual morphological changes in subsequent differentiating stages (Figs. 2C and 2D). The olfactory neuroepithelium in adult *P. lanceolatus* also shows proliferating and differentiating stages of basal cells, degenerating sensory receptor cells respectively (Fig. 2E). The perikaryon of degenerating sensory receptor cells become shrunken in nature and the nuclear elements are less compact (Fig. 2E). The cellular artifacts within the cytoplasm of the degenerating perikaryon are clearly noted under light microscope (LM). The frequency of degenerating sensory receptor cell is higher in adults of *P. lanceolatus* (Fig. 2E). It is the only microanatomical fact that is noted under light microscope (LM) during the age-specific study on the olfactory neuroepithelium of *P. lanceolatus*.

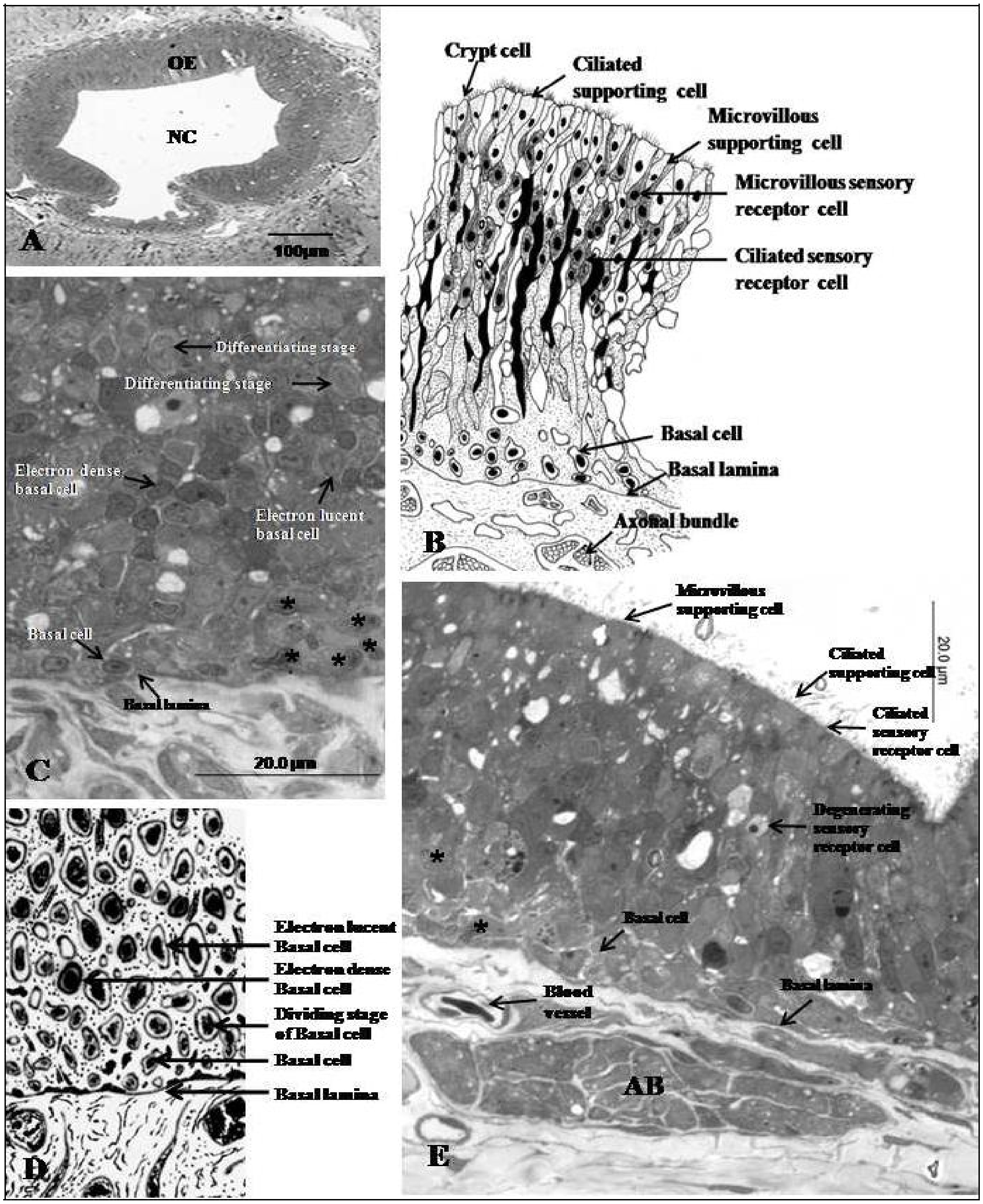


Figure 2: The microanatomy of olfactory neuroepithelium in *P. lanceolatus*. A – The transverse section of the olfactory lamel la shows pseudostratified olfactory neuroepithelium (OE) enclosing the central nasal cavity (NC). B – The diagr ammatic representation of olfactory neuroepithelium showing sensory receptor cells (includes ciliated sensory receptor cell, microvillous sensory receptor cell and crypt cell), supporting cells (*i.e.*, ciliated supporting cell and microvillous supporting cell) and basal cell. Axonal bundles are also noted [Not to scale]. C- The olfactory neuroepithelium of young *P. lanceolatus* indicates the polygonal basal cells, mitotic stages of dividing basal cells, differentiating stages of electron lucent and electron dense basal cells, *etc.* D- The diagram indicates gradual changes of basal cells, electron lucent and electron dense basal cell within the olfactory neuroepithelium [Not to scale]. E – The micrograph shows several degenerating stages of sensory receptor cells at the different depth of olfactory neuroepithelium in adults. Various cellular components [*viz.*, ciliated and microvillous sensory receptor cell, ciliated and microvillous supporting cell, basal cell and differentiating stages of electron lucent basal cell (stars)] are also marked.

Table: IV

Sl. No.	Total body length of *P. lanceolatus* (in mm.) (X´)	Distance between the nostrils (in mm.) (Y´)	Correlation value between X´ and Y´	Length of the olfactory lamella (in mm.) (Z´)	Correlation value between X´ and Z´
1	150	5.1	_	5.0	_
2	155	5.1		5.0	
3	160	5.1		5.0	
4	165	5.1		5.0	
5	170	5.1		5.0	
6	175	5.1		5.0	
7	180	5.1		5.0	
8	185	5.1		5.0	
9	190	5.1		5.0	
10	200	5.1		5.0	

Table IV. The increasing total body length [150mm. to 200mm.] of *P. lanceolatus* (X´) shows constant values of the distance between the nostrils (Y´) and length of the olfactory lamella (Z´). Therefore, no significant correlation is found among the observed numeric data.

DISCUSSION

Olfaction in fishes is the most highly developed senses of all vertebrates [23]. This sense is mediated through olfactory apparatus (Burne, 1909). The structural organization of olfactory apparatus in *P. lanceolatus* is very unique (devoid of olfactory rosette) which may be an indicative of species specific variation [12, 14]. A tube-like olfactory lamella is present at the snout region and connects the nares. From the functional point of view, the paired nares are responsible for incurrent and excurrent of water during nasal ventilation for olfaction [16]. In *P. lanceolatus*, olfactory lamella connects the nares for unidirectional water ventilation over the olfactory neuroepithelium [24]. The cellular proliferation within the olfactory neuroepithelium is an essential cytological process for the continuous turnover of new olfactory sensory receptor cells that originate from stem cells [25 - 27]. The basal cells of olfactory neuroepithelium may acts as progenitor of sensory receptor cell [28, 29]. This cell divides several times and their progeny differentiate into mature sensory receptor cells [30]. The cell division of progenitor cell is followed by specification and differentiation into post mitotic neuron. It is the major cellular event of olfactory neurogenesis [31]. The basal cell is residing at a fixed position near to the basal lamina [32] and divide to form electron lucent and electron dense basal cell within the olfactory neuroepithelium of *P. lanceolatus*. The electron lucent basal cell (*i.e.*, the progeny of basal cell) may differentiate and transform into immature olfactory sensory receptor cell [27]. In *P. lanceolatus*, the event of proliferation of basal cells may involve in the enlargement of surface area of olfactory neuroepithelium in young stages along with increasing length of olfactory lamella among the individuals of different total body length. This relationship may not persist in adults having total body length of 150mm to 200mm. Moreover, the cellular proliferation may also regulate and maintains a constant density of sensory receptor cells within the olfactory sensory neuroepithelium at adult stage [32].

CONCLUSION

Ageing is thought to be a stochastic process which leads to cellular degeneration as well as weakening the cellular compensatory mechanism [33]. In young stages of *P. lanceolatus*, the proliferation of olfactory basal cell is not only increasing the number of sensory receptor cell but also involve in enlarging the surface area of olfactory neuroepithelium. In contrast, the proliferation of basal cell is only involved to replace and compensate the sensory receptor cells within the adult olfactory neuroepithelium. This base line data might helps to explore details age-related changes in olfactory system in relation to neurodegenerative disorders in higher vertebrates.

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