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Anatomy

Distribution of recesses in the olfactory organ of African lungfish *Protopterus aethiopicus*

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ABSTRACT. In the olfactory organ of lungfish, recesses at the bases of lamellae comprise sensory and nonsensory epithelia. The sensory epithelium of the recesses, the recess epithelium, is distinguished from the olfactory epithelium covering the lamella by the absence of ciliated olfactory receptor cells. Therefore, it has been suggested that the recess epithelium is a primordium of the vomeronasal organ of tetrapods. However, developmental changes in the number and distribution of recesses in the olfactory organ of lungfish were unknown. We examined four *Protopterus aethiopicus* specimens of body lengths 215–800 mm to determine the localization of recesses in their olfactory organs. Histological examination showed recesses at the bases of lamellae in all individuals examined. The recesses were localized mainly in the medial and caudal parts of the olfactory organs, especially in juveniles. Compared to smaller fish, larger fish had a larger number of recesses, distributed more broadly in their olfactory organs. Significance of the recess localization and its relationship to the function of lungfish olfactory organ warrants further investigation.

KEYWORDS: Dipnoi, histology, vomeronasal

Several tetrapods have two types of olfactory organs: the olfactory epithelium (OE) and the vomeronasal organ (VNO). The OE contains ciliated olfactory receptor cells (ORCs) expressing odorant receptors, whereas the VNO contains microvillous ORCs expressing vomeronasal receptors [10, 11]. Fish have a single olfactory organ containing ciliated and microvillous ORCs [2]. Thus, it has been postulated that the ciliated ORCs and microvillous ORCs were intermingled in a single olfactory organ in the common ancestor of tetrapods, as in the OE of fish, and subsequently separated to give rise to the OE and VNO of tetrapods [3, 11].

The lungfish is phylogenetically closest to tetrapods, and its olfactory organ is situated in the nasal sac, communicating with the external environment via the anterior nostril and with the oral cavity via the posterior nostril [5]. The olfactory organ of lungfish consists of multiple folds, known as lamellae. Recesses—also termed epithelial crypts [1] or vomero-like epithelial crypts [6]—are situated at the base of lamellae in lungfish species reported, three of the genus Protopterus (*P. annectens*, *P. amphibius*, and *P. dolloi*) and one of the genus Lepidosiren (*L. paradoxa*) [1, 6, 8, 9, 12]. Ultrastructural and immunohistochemical studies [1, 8] have shown that the lamellar OE, which covers the surface of lamellae, resembles the OE of other fish, whereas the recess epithelium, the sensory epithelium constituting the recess, resembles the VNO of tetrapods. In addition, axons originating from the recess epithelium and the lamellar OE have been shown to project separately to the olfactory bulbs [1, 8]. Nevertheless, functional distinction between two epithelia remains uncertain.

Previously, we reported that juvenile *Protopterus annectens* (approximately 300 mm total body length) has recesses mainly in the medial and caudal parts of the olfactory organ [9]. However, whether the distribution of recesses varies among developmental stages is unclear. To address this question, we histologically analyzed the olfactory organs of juvenile and adult *Protopterus aethiopicus*.

MATERIALS AND METHODS

The animals used in this study (n=4) were of total body length 215–800 mm (Table 1) and were purchased from commercial suppliers or kindly gifted by Dr. Hideaki Kato (Shizuoka University). Developmental stage was estimated based on total body length [7]; fish of total body length less than and more than 400 mm were regarded as juveniles or adults, respectively. The fish were anesthetized with tricaine methane sulfonate and euthanized by decapitation. All procedures were approved by the Animal Experiment Committee and conformed to the guidelines of Iwate University (approval number A202003). Olfactory organs were dissected from the heads of the lungfish, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and divided into two

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halves through the midline raphe (Fig. 1). The medial and lateral halves of the olfactory organs were cryoprotected using a sucrose gradient, embedded in Optimal Cutting Temperature compound (Sakura Finetek, Tokyo, Japan), and cryosectioned at a thickness of 20 µm. Sagittal sections of the olfactory organs were stained with hematoxylin and eosin (HE) and visualized and photographed using a light microscope (BX-50; Olympus, Tokyo, Japan).

RESULTS

The olfactory organs of *P. aethiopicus* were elliptical, and their longitudinal axis extended almost rostrocaudally. In addition, the length and width of the olfactory organ were larger in larger individuals (Fig. 1, Table 2), consistent with observations in other species of lungfish (e.g., *P. annectens* and *L. paradoxa* [12]), implying that the olfactory organ increases in size with development. Moreover, as reported in other species of lungfish, including *P. annectens* and *L. paradoxa* [12], more lamellae were found in the olfactory organs of relatively large individuals than in those of smaller individuals (Table 2).

Recesses were found at the base of lamellae in all individuals examined. Examination of HE-stained serial sections showed that the histological properties of the recess in *P. aethiopicus* were similar to those in other lungfish [1, 6, 8, 9, 12]. The recesses were approximately 220–240 µm in diameter, consisted of glandular epithelium and recess epithelium, and were separated from neighboring epithelia by non-sensory epithelium at the entrance to the lumen (Fig. 2). Furthermore, relatively large individuals had more recesses in their olfactory organs compared to smaller animals (Table 2).

We next evaluated whether the distribution of recesses in the olfactory organ of *P. aethiopicus* varied among individuals (Fig. 3). In all specimens examined, the medial half of the olfactory organ contained more recesses than the lateral half (Fig. 3A), and the caudal half of the olfactory organ contained more recesses than the rostral half (Fig. 3B). This is consistent with our previous report of the biased distribution of recesses towards the medial and caudal regions of the olfactory organs of juvenile *P. annectens* [9].

Recess localization was analyzed by dividing the olfactory organ of each animal into caudomedial, rostromedial, caudolateral, and rostrolateral regions (Fig. 4). Recesses were mainly situated in the caudomedial region in small individuals, particularly in the smallest sample (total body length 215 mm), and were distributed across the four regions in large individuals, particularly in the largest sample (total body length 800 mm).

Table 1. Animals used in the present study

Total body length (mm)	Body weight (g)	Sex	Developmental stage*
215	34.9	Male	Juvenile
355	151	Female	Juvenile
500	349	Female	Adult
800	2,400	Female	Adult

*Developmental stage was estimated by the total body length according to Mlewa and Green (2004) [7].

Table 2. Morphometric features of the olfactory organ

Total body length (mm)	Length of olfactory organ (mm)	Width of olfactory organ (mm)*	Number of lamellae	Number of recesses
215	5.1	2.20	25	39
355	6.9	2.92	26	105
500	9.0	3.64	33	232
800	15.0	4.80	54	377

*Width of the olfactory organ was calculated by multiplying the number of sections by the thickness of sections.

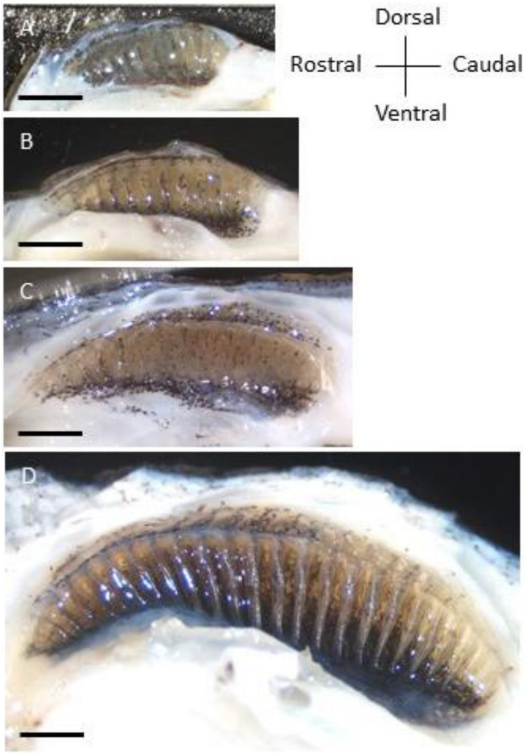

Fig. 1. Sagittally sectioned olfactory organs of *Protopterus aethiopicus*. Medial views of the lateral half. A: 215 mm, B: 355 mm, C: 500 mm, and D: 800 mm in total body length. Scale bars=2 mm.

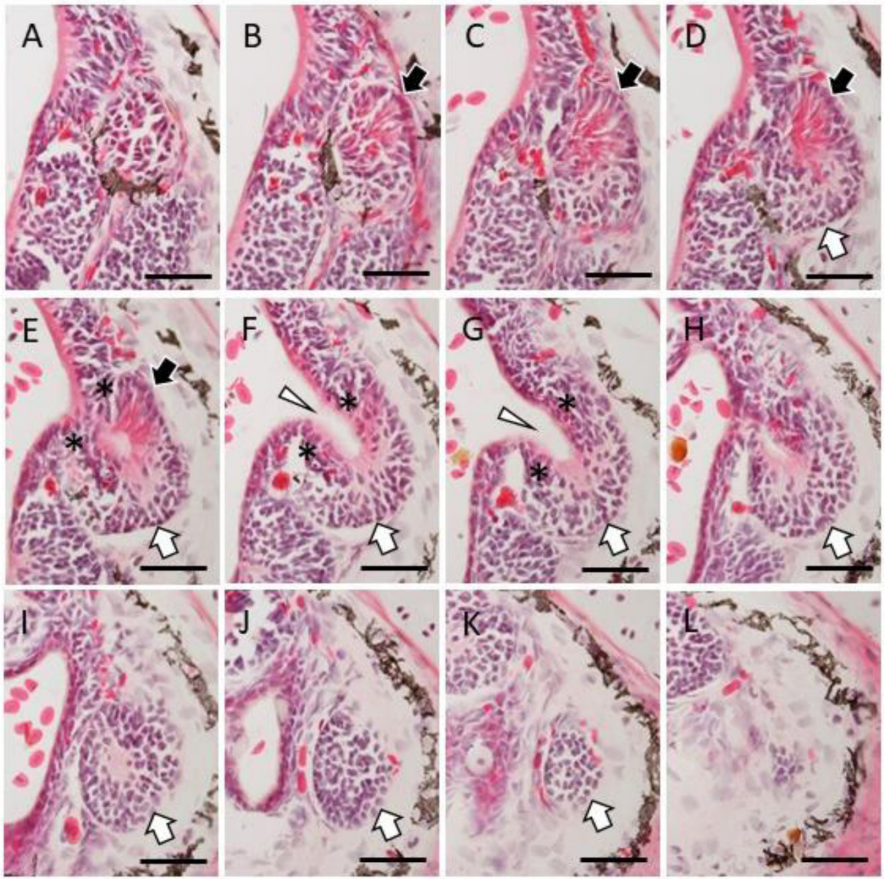

Fig. 2. Hematoxylin and eosin-stained, paraformaldehyde-fixed frozen sections showing a recess constituted by the recess epithelium (white arrows) and glandular epithelium (black arrows), separated from the neighboring epithelia by nonsensory epithelium (asterisks) at the entrance to the lumen (arrowheads). Scale bars=100 μm.

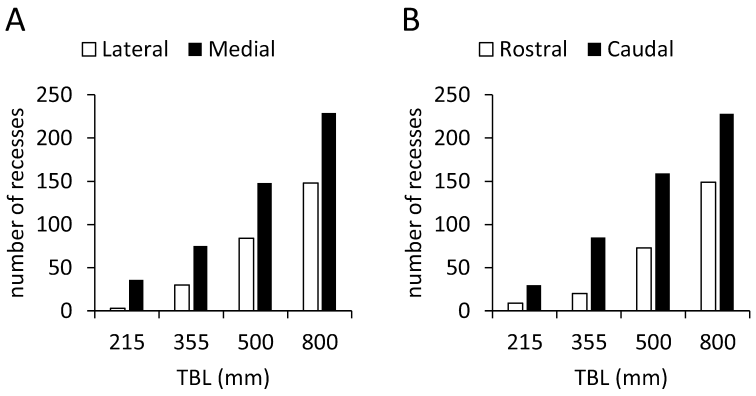

Fig. 3. Distribution of recesses in the olfactory organ of *Protopterus aethiopicus* of four different body sizes.

DISCUSSION

Collectively, our results indicate the presence of recesses in the olfactory organ of *P. aethiopicus*. Recesses were mainly situated caudomedially in juveniles and were present in greater numbers and distributed more broadly in adults.

Unlike other fish, the lungfish is characterized by the nasal cavity which is connected to the oral cavity via the posterior nostrils [5]. This enables the lungfish to pass air as well as water through the nasal cavity. Thus, the olfactory organ of lungfish is expected to have an ability to detect both waterborne odorants and airborne odorants. In one possibility, the recess epithelium might be involved in the detection of airborne odorants. In that case, the lamellar OE is supposed to detect waterborne odorants. In another

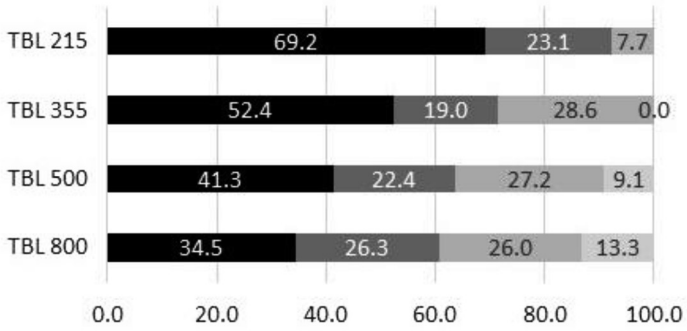

Fig. 4. Relative abundance of the recesses in the caudomedial (black), rostromedial (darkgray), caudolateral (gray), and rostrolateral (lightgray) regions of the olfactory organ (%) of *Protopterus aethipicus* of four different body sizes.

possibility, the detection of airborne odorants might be accomplished by the lamellar OE. In that case, the recess epithelium is supposed to detect waterborne odorants. In any case, function of the recess epithelium is not known at present. It is also unknown if the recess epithelium and the lamellar OE are functionally segregated from each other. Although the segregation of axonal projections to the olfactory bulbs [1, 8] suggests the function of recess epithelium distinct from that of the lamellar OE, it awaits to be determined by the future study whether the recess epithelium and lamellar OE have totally different functions or partially overlapping functions.

The habitats and feeding habits of juvenile *P. aethiopicus* differ from those of adults [4]. Juvenile *P. aethiopicus* (total body length 200–400 mm) inhabit swamp lagoons and feed on insects, small mollusks, and crustaceans, whereas adult *P. aethiopicus* inhabit open water (deep water far from shore) and feed mainly on fish [4]. Because the function of the lungfish olfactory organ is thought to vary according to habitat and feeding habit, as well as by the reproductive and/or social status of individuals, significance of the recess localization and its relationship to the function of lungfish olfactory organ warrants further investigation.

In all individuals examined, more recesses were found in the caudal and medial regions of the olfactory organ than in the rostral and lateral regions. Especially, the recesses were concentrated in the caudomedial region of the olfactory organ in juveniles. *Protopterus aethiopicus* is the largest of the African lungfish, reaching 1.5–2.0 m in length in adulthood. The total body lengths of the lungfish in this study were 215–800 mm. Further analysis of smaller and larger fish than those used in this study is required to evaluate the number and distribution of recesses in the olfactory organ.

Recesses in the olfactory organ of *P. aethiopicus* imply their presence in all extant lungfish except the Australian lungfish [1, 6, 8, 9, 12, this study]. Extant lungfish are categorized into those with two lungs (order Lepidosireniformes) and those with a single lung (order Ceratodontiformes). The African lungfish and South American lungfish belong to the former, and the Australian lungfish to the latter. The Australian lungfish is distinguished from other species of lungfish in terms of its anatomical and physiological properties. For instance, African lungfish can survive for a long period with little water by aestivation, whereas Australian lungfish cannot because they do not aestivate [4]. It would be of interest to determine whether the olfactory organ of Australian lungfish has recesses.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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