

# Olfactory Sensory Neuron Morphotypes in the Featherback Fish, *Notopterus notopterus* (Osteoglossiformes: Notopteridae)

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## KEY WORDS

Olfactory sensory neurons  
Polymorphism  
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## ABSTRACT

**Background:** As in other vertebrates, olfactory sensory neurons (OSNs) in fishes are the main components of sensory part of olfactory epithelium that relay olfactory information (smell and taste) to the brain. **Purpose:** Objective of the present study was to analyze if any polymorphism occurs in the OSNs in a featherback fish, *Notopterus notopterus* as far as the teleost lineage is concerned. **Methods:** With the help of neuronal staining technique, polymorphism of OSNs in *N. notopterus* was studied. **Results:** Three polymorphic forms of OSNs were identified which are ciliated OSNs, microvillus OSNs and crypt OSNs. These morphotypes were identified on the basis of location of their somata within the depth of olfactory epithelium and resulting length of their dendrites. The ciliated OSNs have basally situated somata and long, thin dendrites with a few apically arranged cilia while microvillous OSNs have somata located midway in the epithelium and thick moderate-length dendrites with microvilli. Third cell type is crypt OSNs which are spherical or pear-shaped, located apically just close to the epithelial surface having cilia and microvilli in an invagination and devoid of any dendrite. **Conclusion:** *N. notopterus* belongs to order Osteoglossiformes which is a representative of an early evolutionary lineage of teleost fishes. OSN polymorphism reported in the present work indicates that it is a fairly conserved trait throughout the evolution of teleosts. To our knowledge, we are the first ones to report OSN polymorphism in a member of the order Osteoglossiformes.

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## Introduction

Olfactory system is one of the crucial chemosensory systems for fishes functioning in almost all aspects of their life including food-finding, nest-finding, detecting and avoiding predators or other perilous situations,<sup>1</sup> feeding,<sup>2</sup> kin discrimination,<sup>3</sup> reproduction<sup>4</sup> and to identify natal streams during spawning migration by salmon.<sup>5-7</sup> Fish communication is often accomplished via the use of pheromones.<sup>8</sup> In fish and other vertebrates, this system consists of a peripheral part called olfactory rosette (OR) including olfactory nerve formed by the axons of olfactory sensory neurons (OSNs) and a central part comprising olfactory bulb and higher brain areas involved in processing of olfactory information.<sup>9</sup> The olfactory epithelium consists of lamellae whose surfaces include sensory and non-sensory regions. Main components of sensory region are the OSNs.

In teleosts, OSNs exhibit three polymorphic forms - ciliated OSNs, microvillous OSNs and crypt receptor cells.<sup>10-19</sup> These morphotypes are distinguished by location of their somata within the depth of olfactory epithelium and resulting length of their dendrites. These polymorphs differ in relation to their shape and position within the OE and also functionally.<sup>20</sup> They have differential projections to the olfactory bulb,<sup>18,19,21,22</sup> different molecular<sup>23,24</sup> and physiological properties.<sup>18,25-28</sup>

Among the teleosts, OSN polymorphism has been reported in many species including the members of cypriniformes - goldfish, *Carassius auratus*;<sup>29</sup> zebrafish, *Danio rerio*;<sup>13</sup> *Aplocheilichthys lineatus* and *Xiphophorus helleri*;<sup>30</sup> siluriformes - channel catfish, *Ictalurus punctatus*<sup>29,31</sup> and salmonid fishes.<sup>16,32</sup>

In the present study using the Kluver and Barrera (1953)<sup>33</sup> neuronal staining technique, we sought to analyze OSN polymorphism in the olfactory epithelium of a freshwater teleost, featherback fish, *Notopterus notopterus* belonging to the order Osteoglossiformes.

## Methods

### Teleost phylogeny

Our study on olfactory sensory neuron morphotypes makes use of fish taxonomy by Nelson (1994)<sup>34</sup> and the phylogenetic tree of evolution of teleosts is presented in Fig. 1.

### Animals

Adult featherbacks (*N. notopterus*) of either sex (n = 6) with body weight ranging between 125g to 150g and length 27 ± 2cm were obtained from a single freshwater body, Telangkhedi-Futala lake, Nagpur City from 2009-2011. After transport, fishes were maintained in well-aerated glass aquaria (3×2×1.5). Animal

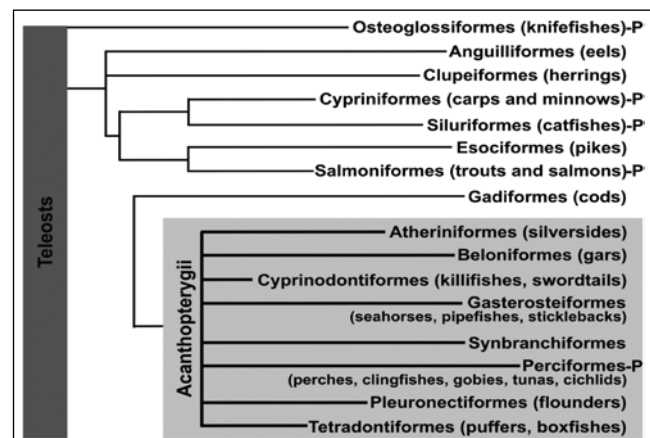


Fig. 1: Phyletic tree of the Teleostei, based on Nelson (1994) showing the occurrence of olfactory sensory neurons polymorphism (P) (For references see the discussion section). The grey box/overlay depicts the group of Acanthopterygii.

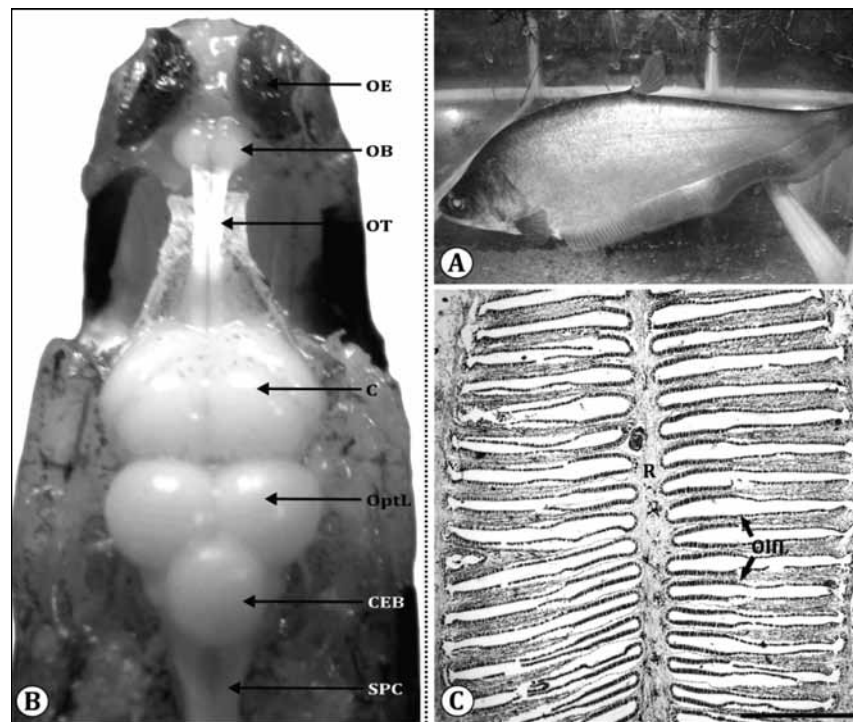


Fig. 2: (A) Photograph of *Notopterus notopterus*. (B) *In situ* photograph of olfactory organ with brain of *N. notopterus* showing; olfactory epithelium (OE), olfactory bulb (OB), olfactory tract (OT), cerebrum (C), optic lobe (OptL), cerebellum (CEB) and spinal cord (SPC). (C) Horizontal section of the olfactory epithelium showing olfactory lamellae (OlfL) radiating from the central raphe (R). Scale bar = 500  $\mu\text{m}$ .

care protocols were approved by the Institutional Animal Care and Use Committee.

#### Neuroanatomical analysis

Fishes were anaesthetized with an aqueous solution of 2 phenoxyethanol (0.03%; P 1126; Sigma), decapitated, olfactory system with the brain was dissected out and immediately fixed in aqueous Bouin's fixative for 24 hrs, dehydrated in graded series of alcohol and embedded in paraffin wax after clearing in xylene. For neuroanatomical studies, sections of olfactory epithelium were cut at 10  $\mu\text{m}$  thickness in horizontal as well as saggital planes on a rocking microtome, mounted on Mayer's albumin coated slides, and then subjected to Kluver and Barerra (1953)<sup>33</sup> staining. The stained sections were analyzed on a Nikon Eclipse E200 photomicroscope (Japan) and different cellular groups were identified according to their characteristic size, shape, staining intensity of the perikaryon and packing density as well as distribution pattern of the cell bodies. Cell and nuclear diameter were measured with an oculometer. All the numeral data in the results were presented as mean values  $\pm$  standard deviations (SD).

#### Analysis of OSN morphotypes

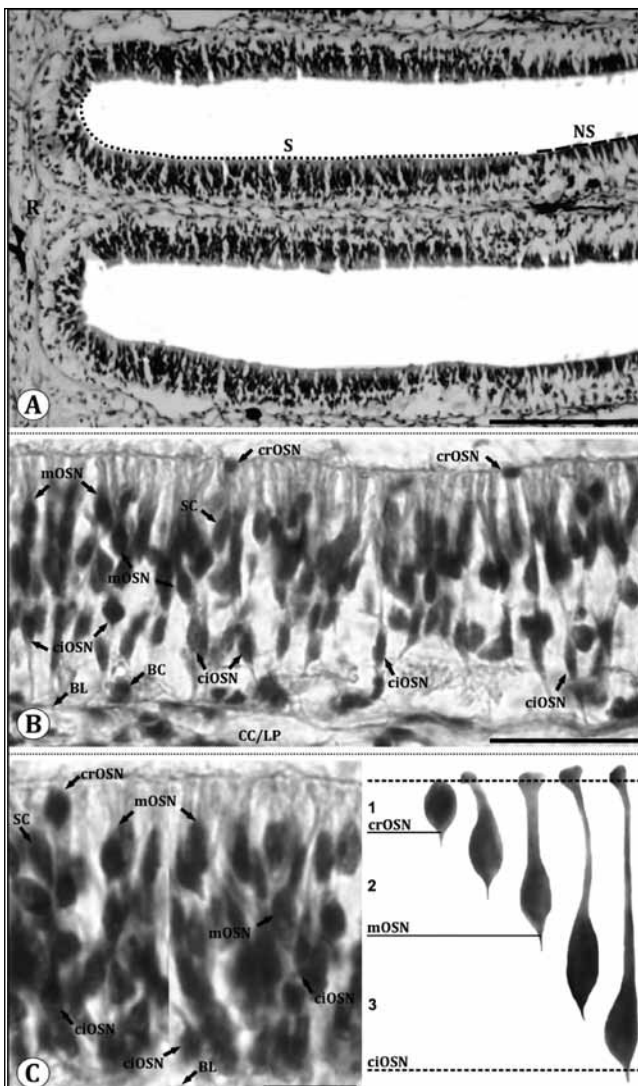
OSN morphology was classified based on depth of soma within the olfactory epithelium and resulting length of their dendrites.<sup>20,26</sup> To determine the depth, olfactory epithelium was divided into 3 arbitrary horizontal layers, most apical layer being layer 1 and the most basal being layer 3 (Fig. 3C). With these criteria, OSNs were grouped into one of the three overall types: ciliated, microvillous and crypt types.

#### Imaging

Desired fields from various sections were photographed using Nikon (E8400) camera at different magnifications and adjusted for size, contrast and brightness in Adobe Photoshop 7.0 and Corel Photo-Paint X4 software. Photo plates were prepared using Corel Draw X4 (version 14) software. Different types of identified OSNs from the photographs were isolated and presented/illustrated according to their distribution in different zones within the olfactory epithelium (Fig. 3B, C). Scale bars were expressed in terms of  $\mu\text{m}$  and measurements were taken by using an oculometer.

#### Results

In the featherback fish, *N. notopterus* (Fig. 2A), paired olfactory organs are situated on snout region in a cavity called olfactory pits or olfactory chambers connected to the telencephalic hemispheres of brain by a long olfactory tract and are thus pedunculated (Fig. 2B). Each olfactory organ is a cup shaped elongated structure possessing a series of  $74 \pm 2$  lamellae radiating from a central raphe on both the sides (Fig. 2B, C). The lamellae in the middle of rosette (on both sides) are the largest and they gradually taper towards anterior and posterior ends of the rosette (Fig. 2C). Olfactory epithelium (OE) is a thick sheet (30–35  $\mu\text{m}$ ) of pseudo-stratified ciliated epithelial cells which is folded to form olfactory lamellae (Fig. 3A). Each lamella is divisible into sensory and nonsensory regions (Fig. 3A). The sensory region is located at the base of lamellae consisting of bipolar OSNs, supporting cells and basal cells (Fig. 3B, C).



**Fig. 3:** (A) Part of horizontal section of olfactory epithelium showing location of sensory (S) and nonsensory (NS) regions of olfactory lamellae. Scale bar = 100  $\mu$ m. (B) Magnified view of sensory region of olfactory lamellae showing; basal cell (BC), basal lamina (BL), central core (CC)/lamina propria (LP), crypt olfactory sensory neurons (crOSNs), ciliated olfactory sensory neurons (ciOSNs), microvillous olfactory sensory neurons (mOSNs) and supporting cell (SC). Scale bar = 50  $\mu$ m. (C) Magnified view of sensory region of olfactory lamellae (left) showing location of different cell types including; crOSNs, ciOSNs and mOSNs in different zones of olfactory epithelium. Scale bar = 25  $\mu$ m. Illustrations (right) of different types of olfactory sensory neurons found in different layers of olfactory epithelium (Upper dotted line represents epithelial surface and lower dotted line represents basal lamina).

### OSN Morphotypes

In *N. notopterus*, three types of OSNs are noted- ciliated, microvillous and crypt cells (Fig. 3B, C). Each morphotype is characterized by a cell soma in a specific layer of the olfactory epithelium, variable length of dendrite and extension of axonal process towards basal lamina. OSN morphotypes and their properties are summarized in Table 1.

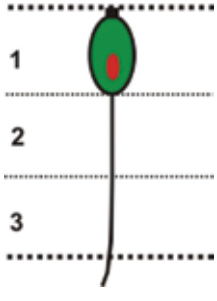
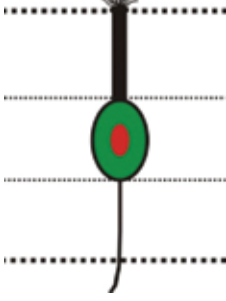
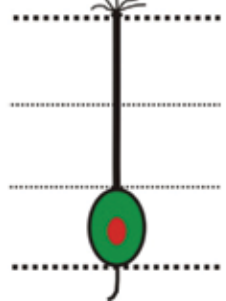
1. **Ciliated olfactory sensory neurons (ciOSNs):** These are longest ( $18.78 \pm 1.2 \mu\text{m}$ ) among all the types of OSNs distributed throughout the sensory epithelium. These are characterized by their columnar, bipolar organization having basally located somata within layer 3 of the epithelium and their thin, long dendrites reaching upto epithelial surface. They have pronounced olfactory knob with cilia projecting into olfactory mucosa and at the opposite pole axonal process extends towards the basal lamina (Fig. 3B, C). Their cell body containing a round prominent nucleus is situated deep in the epithelium, cytoplasm is highly granular and intensely stained (Fig. 3B, C).
2. **Microvillous olfactory sensory neurons (mOSNs):** These have moderate ( $12.06 \pm 0.81 \mu\text{m}$ ) length and are characterized by their columnar, fusiform, bipolar organization having cell body located in the mid region within layer 2 of the epithelium. Their thick, moderate length dendrite reaches upto epithelial surface having less pronounced olfactory knob with microvilli at the top. At the opposite pole, axonal process extends towards the basal lamina (Fig. 3B, C). The cell body is more superficial in the epithelium than the ciOSNs and possesses a round nucleus. Cytoplasm is granular and stained intensely (Fig. 3B, C). Population of these cells dominates over the ciOSNs.
3. **Crypt olfactory sensory neurons (crOSNs):** These are the shortest ( $2.9 \pm 0.22 \mu\text{m}$ ) OSNs. These neurons are characterized by their spherical, pear-shaped or ovoid structure having somata situated apically in the most superficial layer 1 of the olfactory epithelium, devoid of any dendrite and with a axonal process extending toward the basal lamina (Fig. 3B, C). These cells bear submerged cilia and microvilli in the upper portion of the cell and are intensely stained (Fig. 3B, C). They occur regularly in all the lamellae but their absolute number appears to be low as compared to the other two cell types.

### Discussion

Olfactory epithelium in *N. notopterus* is a continuous thick sheet of pseudo-stratified columnar epithelial cells; 30–35  $\mu\text{m}$  in thickness. It is 35  $\mu\text{m}$  in piranha, *Serrasalmus nattereri*,<sup>35</sup> 35–55  $\mu\text{m}$  in European eel, *Anguilla anguilla*,<sup>36</sup> and 60–75  $\mu\text{m}$  in swordtail, *Xiphophorus helleri*,<sup>37</sup> which is folded to form olfactory lamellae. Number, shape and arrangement of lamellae vary considerably among different teleosts ranging from flat unfolded surface to multi-lamellar rosette.<sup>9,38-43</sup> Olfactory epithelium in *N. notopterus* is a multi-lamellar rosette comprising large number ( $74 \pm 2$ ) of lamellae. Generally lamellae are arranged laterally around a central raphe in Zebrafish, *Danio rerio*,<sup>13</sup> Catfish, *Clarias batrachus*,<sup>44,45</sup> Carp, *Cirrhinus mrigala*,<sup>46</sup> Cichlid, *Oreochromis mossambicus*,<sup>47</sup> *A. Anguilla*,<sup>14</sup> *Mugil parisi*,<sup>48</sup> Indian major carp, *Labeo rohita*,<sup>49,50</sup> *Wallago attu*,<sup>51</sup> and *Macrogathus aculeatus*,<sup>52</sup> same arrangement is observed in *N. notopterus*. However, in some other teleosts, olfactory lamellae are arranged at the top of raphe, parallel to each other and in rostro-caudal orientation as in *Channa punctatus*<sup>53</sup> and *Channa gachua*.<sup>54</sup>

In *N. notopterus*, each olfactory lamella comprises sensory and nonsensory regions. Location of these regions varies in different species.<sup>42</sup> In *N. notopterus*, sensory region is at the proximal end and basal region of lamellae and non sensory region at the middle of lamellae. Sensory region is at the middle of

Table 1: Summary of olfactory sensory neuron morphotypes and their properties.

OSN Morphotypes	Crypt Cell	Microvillous	Ciliated
Structural organization			
Apical surface	Submerged crypt with cilia and microvilli	Short olfactory knob with microvilli	Olfactory knob with cilia
Dendrite	Absent	Thick	Thin
Location of cell body in OE	Upper third	Mid region	Lower third
Size	2.9±0.22 μm	12.06±0.81 μm	18.78±1.2 μm

lamellae and nonsensory region is at the proximal and basal regions of lamellae in a Cyprinid, *L. rohita*<sup>49,50</sup> but in *Rhodeus amarus*, sensory region is at the base and middle of lamellae and nonsensory region is at the proximal end.<sup>55</sup> Sensory region comprises olfactory sensory neurons, supporting cells and basal cells.<sup>13,14</sup> Same cell types are observed in *N. notopterus*.

OSNs in *N. notopterus* exhibit polymorphisms similar to that observed in other teleosts. Three OSN morphotypes (ciliated, microvillous and crypt) vary in their shape and position within the epithelium. First type, the ciliated OSN, has a cell body located deep in the OE (in layer 3) near the basal lamina. Its dendrite is long and also quite thin, with a bulbous ending in the olfactory mucosa known as the olfactory knob, which bears cilia.<sup>13,14,16,17,20,26,56</sup> Same types of ciOSNs are observed in *N. notopterus*. Second morphotype, the microvillous OSN, have their cell bodies located in the middle third (in layer 2) of OE having thick and moderately long dendrites bearing microvilli at the top which are also reported in other teleosts.<sup>13,14,16,17,20,26,56</sup> These two polymorphic forms are seen even in the rainbow trout embryos (*Salmo gairdneri*), where ciliated OSNs appear 8 days earlier than the microvillous type.<sup>57</sup> Third OSN morph is the crypt cells.<sup>11-13</sup> It is devoid of any dendrite. It has a cell body located superficially (in layer 1) in the OE bearing apically located cilia and microvilli both, in a crypt-like invagination and longest axonal process extending towards the basal lamina.<sup>13,14,20,26,56</sup> Their number in *N. notopterus* is less as compared to the other two types.

In the sensory region, two morphologically distinct types of OSNs, ciliated and microvillous are prevalent in teleosts.<sup>57</sup> In addition to the teleosts, expression of both ciliated and microvillous sensory cells is seen in a primitive extant ray-finned fishes, the bichir (*Polypterus senegalus* and *P. ornatipinnis*)<sup>58</sup> and the sturgeons of genus *Acipenser*.<sup>59</sup> They occur together but in varying proportions in different species.<sup>59</sup> In the OE of *N. notopterus*, mOSNs visually seem to dominate over ciOSNs, same is observed in *C. punctatus*<sup>53</sup> whereas ciOSNs are domi-

nant over mOSNs in *Labeo bata*.<sup>60</sup> In channel catfish, density of mOSNs is highest in the medial part of lamellae while that of ciOSNs is highest in the lateral areas of lamellae.<sup>61</sup> In goldfish, mOSNs are most abundant in dorso-medial areas of the lamella close to the midline raphe.<sup>24</sup> Third type of cells, crypt OSNs are also observed in *N. notopterus* but are very few in number. These are however widespread in number of teleost fishes.<sup>10,12,58,59,62-66</sup> They are reported in Cyprinodonts,<sup>37</sup> catfish, swordtail and needlefishes<sup>11</sup> and in Zebrafish, *Danio rerio*.<sup>13</sup>

Expression of OSN polymorphism appears to follow an evolutionary pattern in those species that have been investigated. In an ancient jawless fish (superclass Agnatha), the sea lamprey (*Petromyzon marinus*), only ciliated OSNs are seen<sup>67,68</sup> but three morphotypes are reported in *P. marinus* based on dendrite length and position of cell body within the OE.<sup>15</sup> In elasmobranchs, these sensory cells bear only microvilli<sup>65,66,69-71</sup> and crypt cell also appears in the OE.<sup>65,66</sup> With the divergence of rayfined fishes in different habitats OE seems to be populated by all the three OSNs.<sup>58,59,64</sup> Overall, in fishes displaying all the three morphotypes, ciliated cells predominate with microvillous OSNs being fewer in number.<sup>16</sup> Generally, crypt cells tend to be rare,<sup>63</sup> and in some species, these do not appear in all the specimens<sup>10</sup> and their number may also vary with season<sup>72</sup> and sex.<sup>63</sup> Ciliated OSNs are tuned toward bile salts and microvillous OSNs toward amino acids.<sup>16</sup> However, recent electrophysiological studies concluded that ciliated OSNs might be termed as generalists which respond to varying species of odorants including amino acids, bile salts and other odorants whereas microvillous OSNs might be called as specialist, which respond specifically to amino acids and nucleotides.<sup>27,73</sup>

In addition to Agnatha<sup>15</sup> and Elasmobranchs, OSN polymorphism has been reported in many Teleostomi such as bichir- *Polypterus senegalus* and *P. ornatipinnis*<sup>58</sup> and sturgeon, *Acipenser*.<sup>59</sup> In the teleosts it is reported in order Cypriniformes- goldfish, *Carassius auratus*,<sup>29</sup> *Aplocheilichthys lineatus* and *Xiphophorus helleri*;<sup>30</sup> zebrafish, *D. rerio*,<sup>13</sup> Siluriformes- channel

catfish, *Ictalurus punctatus*,<sup>29,31</sup> Salmonid fishes,<sup>16,32</sup> and among Perciformes in round goby, *Neogobius melanostomus*.<sup>10</sup>

Order Osteoglossiformes is a representative of an early evolutionary lineage of teleost fishes. *N. notopterus* belongs to this order. In this fish, polymorphous OSNs are noted similar to those reported among the members of other teleosts including orders-cypriniformes, siluriformes and salmoniformes which are highly evolved orders. This indicates that OSN polymorphism is a conserved trait throughout the evolution of teleosts. Present study will be helpful to study the expression of receptor subtypes on each receptor cell and to know the physiological role assigned to each of the receptor cell types. Possibly these polymorphs respond to different odors, as seen in other teleosts.

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