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Olfactory adaptations in the deep-sea fishes
Čichové adaptace u hlubokomořských paryb a ryb

Bachelor´s thesis

Supervisor: Mgr. Zuzana Musilová, Ph.D.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 22.04.2024 Jaroslav Kamrla

Poděkování

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Abstrakt

Cílem této práce bylo shrnout znalosti o čichu hlubokomořských paryb a ryb. Výzkumy na toto téma jsou spíše ojedinělé, nicméně pár zajímavých prací nastiňuje důležitost čichu v těchto nehostinných podmínkách. Po krátkém představení obecného fungování čichové soustavy se tato práce zabývá jednotlivými adaptacemi v hlubokém moři. Na základě porovnání jednotlivých částí mozku, které jsou zodpovědné za recepci jednotlivých smyslů, lze získat představu o důležitosti jednotlivých smyslů. Ta byla zkoumána u mesopelagických a demersálních druhů. Mezi adaptace patří například ontogenetický posun ve smyslové orientaci. Dále také sexuální dimorfismus čichové soustavy. Zvětšené olfaktorické orgány u samců slouží pravděpodobně k jednodušší lokalizaci samice. Co se týká bohatosti repertoáru olfaktorických receptorů, tak genom hadální ryby z Mariánského příkopu ukázal ztrátu spousty čichových genů, ale zdůraznil jejich specificitu. Vlastní data, která jsou součástí této práce, odhadují počet čichových genů u hlubokomořských ryb, pro které nemáme kvalitní genom, ale jen zdrojová data ze sekvenace genomu. Odhady s velkým konfidenčním intervalem ukazují spíše nižší počty genů, ale jsou i výjimky s větším repertoárem, které jsem označil za kandidáty pro další studium.

Klíčová slova: hluboké moře, ryby, paryby, čich, sexuální dimorfismus, ontogenetický posun, adaptace, olfaktorické geny

Abstract

The goal of this work was to summarize the present knowledge about olfaction in deep-sea fishes. The research on this topic is seldom, however, few interesting studies suggest the importance of olfaction in these inhospitable conditions. After a short introduction of how the olfaction works, individual description of deep-sea adaptations follows. Implications on the importance of a specific sense can be made based on comparisons between individual brain areas responsible for the input of each sense. The importance of individual senses was investigated in mesopelagic and demersal species. Ontogenetic shift in sensory importance is one of the adaptations. Furthermore, sexual dimorphism in the olfactory system. Enlarged olfactory organs in males are most likely responsible for easier localisation of a female. Regarding the olfactory receptor repertoire, a whole genome of a hadal fish from the Mariana trench marked a massive loss of olfactory genes but highlighted their specificity. My own data included in this thesis estimate the number of the olfactory genes in deep-sea fishes for which we lack the high-quality genome. The estimates (albeit with large confidence intervals) suggest rather smaller numbers of genes in most of the species, while there are some species with putatively expanded gene number, which I identify hereby as candidates for the future genome research as verification.

Key words: deep sea, fish, olfaction, sexual dimorphism, ontogenetic shift, adaptation, olfactory genes

Contents

INTRODUCTION

Fishes have successfully penetrated all the aquatic environments, whether marine, freshwater, or brackish. Currently, there are 36 759 valid species of fish. However, the term 'fish' comprises of all fish-like vertebrates. This work will focus on cartilaginous fishes (Chondrichthyes) and bony fishes (Osteichthyes). Class Chondrichthyes (1 300 species) can be divided into two subclasses Holocephali and Euselachii (sharks and rays). Class Osteichthyes splits into subclasses Sarcopterygii (8 species) (lobe-finned fishes) and Actinopterygii (35 314 species) (ray-finned fishes) (Nelson, Grande, and Wilson 2016; CAS Eschmeyers Catalog of Fishes).

Deep sea is characterized by extreme conditions. Crushing pressure, scarce food supplies, low temperatures, and the absence of solar light. Due to these factors, it would seem impossible for any living creature to withstand this hard setting and yet, organisms thrive in the depths. Challenger Deep represents the deepest place on Earth going down to almost 11000 m in the Mariana Trench. Life is present even in these greatest depths. *Halice sp.*, an amphipod, has been retrieved from 10908 m (Li et al. 2019). Various barophilic bacterial strains have been isolated from 10898 m (Kato et al. 1998). However, fish have never been observed this deep. The deepest fish observation has been recorded at 8336 m. Species of this fish has not been assigned due to the unavailability of tissue sample. Nevertheless, it belongs to the Liparidae family (Jamieson et al. 2023). The hypothesis, that fish do not occur below 8400 m due to the physiological constraints has not been disproven yet (Yancey et al. 2014). Cartilaginous fishes, on the other hand, might be even more limited by their physiological constraints (Treberg and Speers-Roesch 2016) as no species has ever been found deeper than 4156 m (Imants G Priede et al. 2006).

The depth of 200 m marks the end of the epipelagic zone and the start of the deep sea. The upper layer of the deep sea is the mesopelagic zone, which reaches down to 1000 m. Bathypelagic zone follows up until 4000 m. Abyssal zone terminates at around 6000 m (Sutton et al. 2008). The ocean's deepest parts belong to the hadal zone (Bruun 1956). Under the right conditions, solar light can penetrate in the mesopelagic layer. Below that, there is no other source of light other than bioluminescent organisms. Hence, vision might become of a less importance and other sensory mechanisms must emerge. Olfaction might be one of them because of its crucial role in receiving chemical cues from the environment. Organisms use chemical cues for various occasions – localisation of prey, recognition of a conspecific or a predator, migration, asserting of dominance, communication, protection of embryos, defence mechanisms (Hay 2009). In the land of eternal darkness, the olfactory system must be

undoubtedly vital and hence, well-adapted. This thesis presents the actual knowledge on deep-sea olfaction from the morphological, anatomical, and molecular perspective.

OLFACTORY SYSTEM

Olfactory chamber

The olfactory system of fish consists of several parts. The paired nostrils are located dorsally on the snout of the head. There is the anterior nostril, through which the water flows in, also called the incurrent nostril, and the posterior nostril (or the excurrent nostril), that serves as the exit of the water. A piece of skin that lies between these two openings is called nasal bridge. By separating the anterior and posterior nostril with the nasal bridge, a cavity called the olfactory chamber is formed. At the bottom of the olfactory chamber, the sensory epithelium called olfactory rosette is situated (Figure 1) (Zeiske, Theisen, and Breucker 1992).

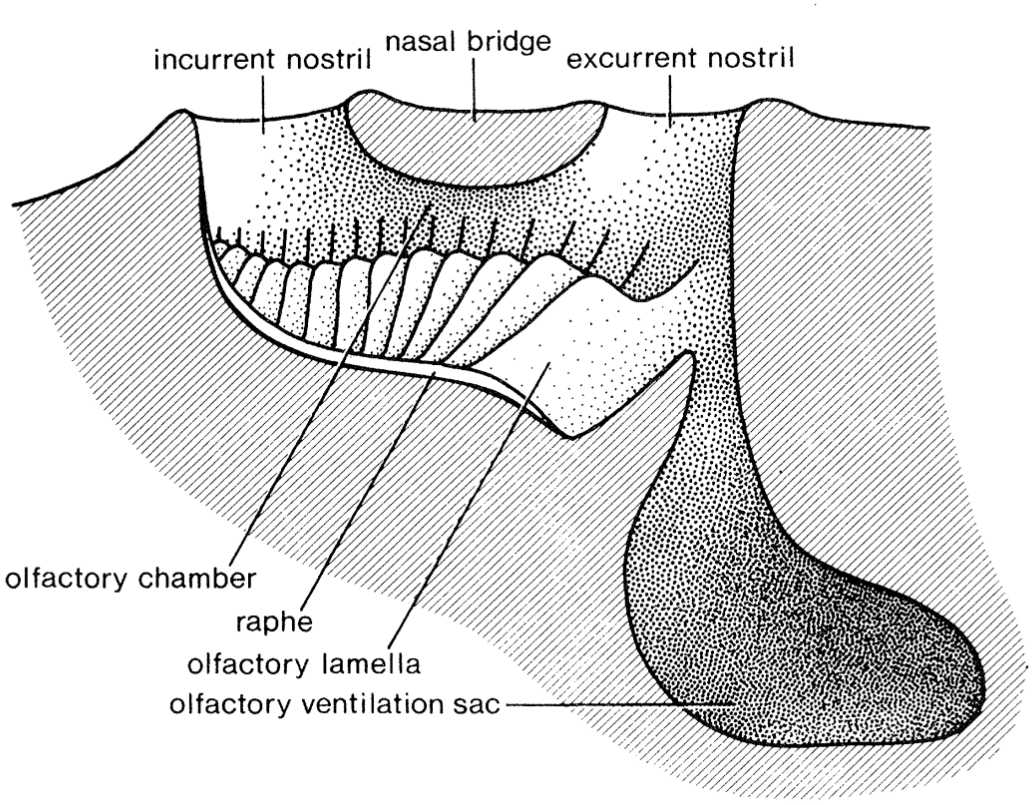

Figure 1 - Morphology of the olfactory chamber (Zeiske, Theisen, and Breucker 1992)

Nostrils and the olfactory organs of elasmobranchs are placed on the ventral side of their snouts (Zeiske, Theisen, and Breucker 1992). Holocephali have their incurrent nostrils ventrally adjacent to their mouth, while the excurrent nostrils can be placed either internally or externally. Connection exists between the oral cavity and internal excurrent nostrils (Howard et al. 2013). The shape, size and position of nostrils can be diverse amongst fishes (Cox 2008; Rutledge

2022; Timm and Fish 2012). The flow of the water into the olfactory chamber is very important for olfaction as it brings the odorants from the external environment. Active movement of water through the chamber is provided by two mechanisms, either via accessory sacs (in Figure 1 – olfactory ventilation sac) or ciliary beats. A group called cyclosmates are fishes that own and use the accessory sacs, while isosmates use ciliary beats (Doving et al. 1977).

The structure of the olfactory rosette comprises of a central raphe and of rows of olfactory lamellae on each side (Figure 1 and Figure 2 – B and C). The shape and size of the rosette can vary between the species and many different types were created (Yamamoto 1979). The number of lamellae differs between species and even intraspecifically, where the number increases with size of the body, until it reaches its maximum lamellae counts. In several species, secondary folds occur and their function is to increase the surface area of the olfactory epithelium (Yamamoto 1982; Pfeiffer 1963; Hara 1975). In Chondrichthyes, the percentage of the increase was investigated and it was found that secondary folds expand the surface area by 70 to 495 % (Ferrando et al. 2019). The sensory epithelium can be distributed on the lamellae in 4 different patterns. Sensory epithelium can be continuous, except for the edges where non-sensory epithelium is located. Sensory epithelium can be separated by the non-sensory epithelium into several large areas. Another type of sensory epithelium can be mingled unevenly with the non-sensory epithelium. Lastly, sensory epithelium can be isolated and form islets, while surrounded by non-sensory epithelium (Yamamoto 1982).

Cells of the epithelium

There are several types of cells located in the epithelium, each with a different function. Basal cells, located near the basal lamina at the bottom of the olfactory epithelium, have the ability to give rise to new sensory neurons. In the event of damage, this ability proves to be beneficial (Graziadei and Metcalf 1971; Costanzo 1991; Graziadei and Graziadei 1979; Iqbal and Byrd-Jacobs 2010). Goblet cells are found in the non-sensory part and their function is to produce mucus (Theisen, Zeiske, and Breucker 1986; Chakrabarti and Ghosh 2009). Other cells found in the non-sensory epithelium are the ciliated cells which provide the propulsion of the fluid via their beats. It has been discussed whether they propel mucus or water and it has been concluded that in elasmobranchs and Holocephali, ciliated cells propel mucus, whereas in bony fish, they thrust water (Cox 2008; 2013; Howard et al. 2013). Olfactory receptor neurons (ORNs), along with supporting cells, are located in the sensory epithelium. The supporting cells form contact with ORNs and probably provide them with insulation and physical support (Morrison and Costanzo 1990). There are several types of ORNs – ciliated, microvillous and crypt RNs (Figure

2 - D). Their common feature are axons that terminate in the glomeruli of the olfactory bulb. In cartilaginous fishes, ciliary RNs are missing. Crypt RNs can appear seasonally. For ciliated and microvillous RNs, another common attribute is an olfactory knob, which protrudes slightly over the epithelium. The olfactory knob bears either cilia in the case of ciliated RNs or microvilli in the case of microvillous RNs. In crypt RNs, both cilia and microvilli are present, however, the knob is absent (Zeiske, Theisen, and Breucker 1992; Hansen and Finger 2000; Ferrando et al. 2006; 2010; Hamdani and Doving 2006). Fourth ORN has been identified in zebrafish (*Danio rerio*), they were named kappe neurons. Its function and existence in other bony fish remains to be determined (Ahuja et al. 2014). More recently, another, fifth, ORN type has been described in zebrafish, pear neuron. They are stimulated by ATP and the gene *A2c*, most likely expressed in the pear ORNs, is probably responsible for the recognition of adenine nucleotides and adenosine. *A2c* orthologues have been identified in the genomes of other fishes and even amphibians (Wakisaka et al. 2017).

Olfactory receptor families

Four different olfactory receptor gene families are present in fishes (Figure 2 - A) - olfactory receptor (OR), trace amine-associated receptor (TAAR), vomeronasal type 1 receptor (V1R) and vomeronasal type 2 receptor (V2R) genes (Niimura and Nei 2005; Hashiguchi and Nishida 2007; Saraiva and Korsching 2007; Hashiguchi and Nishida 2006). All of the receptors belong to the G-protein-coupled receptors (GPRCs) superfamily. The signals for these receptors vary from neurotransmitters to chemokines and hormones, photons of light and most importantly for this work, odorants. The characteristic feature of GPCRs is their seven-transmembrane domain (Pierce, Premont, and Lefkowitz 2002). GPCRs can be divided into separate families, A-F (Kolakowski 1994). While ORs, TAARs and V1Rs belong to family A, V2Rs fall within the family C (Alioto and Ngai 2006; Pin, Galvez, and Prezeau 2003). Family C receptors are distinguished by a large extracellular domain, where binding of ligands takes place (O'Hara et al. 1993; Okamoto et al. 1998). Simple way of how GPCRs signalling works: ligand binds to a receptor extracellularly evoking a conformational change, which activates the intracellular heterotrimeric G-protein. G-protein consists of subunits α, ß and γ. In inactive state, GDP is bound to α subunit. After activation, GDP is swapped for GTP, which subsequently causes fragmentation of the heterotrimeric G-protein into α+GTP and ßγ units. These units act as effectors and further cause, for example, enzyme activation or desensitization of a membrane (Kobilka 2013; Tuteja 2009; Antunes and Simoes de Souza 2016). The Gα-proteins can be divided into three classes. Within a class, α subunits exhibit sequential and functional

similarities (Strathmann and Simon 1990).

Research conducted by Hansen et al. (2003;2004) using immunochemistry, in situ hybridization and electron microscopy in the olfactory epithelium of channel catfish (*Ictalurus punctatus*) and goldfish (*Carassius auratus*) revealed association between cell type and Gα-proteins. In the channel catfish, $G\alpha_{olf/s}$ antiserum labelled the ciliated RNs and therefore confirmed $G\alpha_{olf/s}$ presence in this cell type. Microvillous RNs showed $G\alpha_{q/11}$ expression. The third type of cells, crypt RNs, was highlighted by $G\alpha_o$ antibodies (Hansen et al. 2003). This finding was further supported and expanded by using the same methods in goldfish. Ciliated RNs were labelled with $G\alpha_{olf}$ antisera. Microvillous RNs displayed reactivity against $G\alpha_o$, $G\alpha_{i-3}$ and $G\alpha_q$ antibodies. Crypt RNs were marked by $G\alpha_q$ and $G\alpha_o$ antisera. Whereas microvillous RNs exhibited responsiveness against three distinct Gα subunits, each of them relates to only one cell. Crypt RNs on the contrary showed coexpression of both Gα subunits (Hansen, Anderson, and Finger 2004).

From the previous paragraph, we can see that each cell type is associated with a specific Gα subunit, however, that is not all; olfactory receptor genes are also related to a specific cell type. ORs and TAARs are expressed in the ciliated RNs, while V2Rs are expressed in the microvillous RNs (Buck and Axel 1991; Liberles and Buck 2006; Hansen, Anderson, and Finger 2004). V1Rs have been proposed to belong to either microvillous RNs or crypt RNs (Pfister, Rodriguez, and Buck 2005). Since cartilaginous fishes lack the ciliated RNs (Theisen, Zeiske, and Breucker 1986), it has been discussed whether and how, if present, are ORs and TAARs expressed. V2Rs dominate the olfactory genes in cartilaginous fishes, it should be noted, however, that a small and stable repertoire of ORs, TAARs and V1Rs exists. In small-spotted catshark (*Scyliorhinus canicula*), expression of olfactory genes has been investigated. Major expression of V2Rs, minor expression of V1Rs and no expression of ORs should not be surprising due to the presence of microvillous and the absence of ciliated RNs. TAARs, however, have been found to be minorly expressed as well and it has been hypothesized that they may have acquired olfactory function in microvillous RNs (Syed et al. 2023).

Olfactory bulb

Olfactory bulb (OB) is composed of four different layers. From the surface into its core: olfactory nerve layer, glomerular layer, mitral cell layer and internal cell layer. Axons of the ORNs relay the signal from the olfactory epithelium through the olfactory nerve layer and end in the glomerular layer, where they meet with the dendrites of mitral cells, in a so-called glomerulus. Glomerulus is basically a spherical neuropil enveloped in a glial sheath (Satou

1990; Satou et al. 1983). The soma of mitral cells is positioned in the mitral cell layer. Each mitral cell has two to five thick dendrites that further branch to create glomerular tufts that extend towards the surface of the OB. Axons of mitral cells can be traced into the olfactory tract (Fujita, Satou, and Ueda 1988). Besides mitral cells, ruffed cells are also present in the mitral cell layer (Kosaka and Hama 1979). Ruffed cells seem to be unique to teleosts (Fuller and Byrd 2005). Granule cells soma appearing in the internal cell layer (Satou 1990) form synapses with both mitral and ruffed cells and seem to have an important role in their activation and inhibition. Excitation signals activate the mitral cell, whilst ruffed cell is inhibited via granule cell. On the contrary, by the same mechanism, granule cells will inhibit mitral cells when ruffed cells are excited (Kosaka 1980; Zippel 1998). Olfactory tract transmits signals from the OB to higher brain areas (telencephalon, diencephalon) (Figure 2 - A) (Satou 1990).

An idea of odor specificity and spatial differentiation of OB in fishes was confirmed in two salmonid species, in Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) (Thommesen 1978) and ever since then has been researched in other species. In channel catfish, ciliated RNs projected mainly to ventrally and medially localized glomeruli, microvillous RNs projected to dorsal glomeruli and crypt RNs projection has been detected anteriorly and posteriorly ventralmost extremes of the OB (Hansen et al. 2003). Hansen et al. (2003) concluded based on research of Nikonov and Caprio (2001), that nucleotides are detected by microvillous RNs projecting to the dorsal part of OB, bile acids signal to the medial part through ciliated RNs, amino acids evoked reaction predominantly via ciliated RNs in the rostral, ventral and dorsolateral part, however, it has been suggested that both ciliated RNs and microvillous RNs can detect amino acids (Hansen et al. 2003; Nikonov and Caprio 2001). There results, unfortunately, cannot be interpreted as conclusive for all teleosts, as the chemotopic organization differs between species (Bazaes, Olivares, and Schmachtenberg 2013).

Olfactory Tract

OT divides into two distinct fiber bundles: lateral olfactory tract (LOT) and medial olfactory tract (MOT), which further subdivide (Satou 1990). Functional organization seems to not be restricted only to OB, but also to the olfactory tract (OT). In crucian carp (*Carassius carassius*), different behavioural responses have been shown to be mediated by different parts of the OT. Medial bundle of the MOT is associated with alarm reaction (E.-H. Hamdani et al. 2000). The lateral bundle of MOT mediates the reproductive behaviour (Weltzien et al. 2003). LOT is linked with feeding behaviour (E. H. Hamdani, Kasumyan, and Doving 2001). Different ORNs have been correlated with different bundles in the crucian carp. Based on staining, microvillous

RNs participate in feeding behaviour (E. H. Hamdani, Alexander, and Doving 2001), crypt RNs are connected with reproduction (E. H. Hamdani and Doving 2006), ciliated RNs might possibly provide alarm reaction (E. H. Hamdani and Doving 2002).

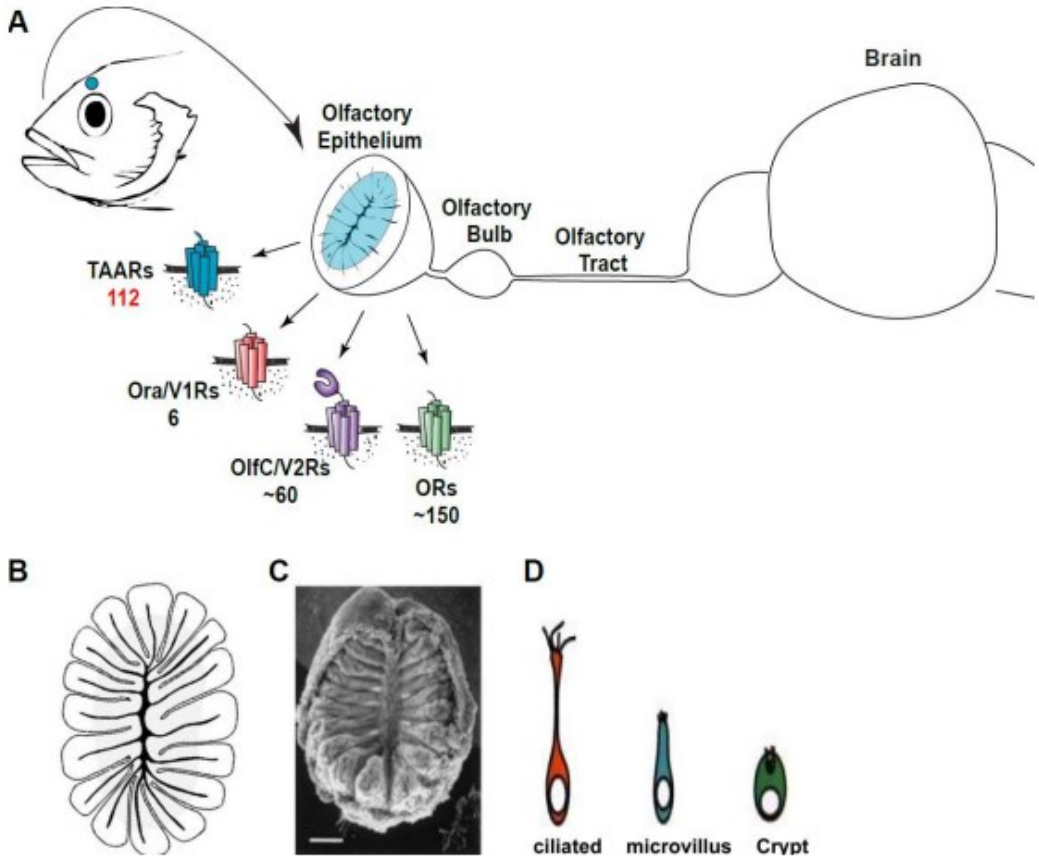

Figure 2 – General organization of the olfactory system in zebrafish, A depicts the organization, B and C show the olfactory rosette and D illustrates the ORNs (Hussain 2011) (kappe and pear RNs were not known at this time)

Indicators of olfactory capability

It is difficult to perform in vivo tests in deep-sea fishes, most of our knowledge comes from rare in situ recordings and from dead specimens, therefore the olfaction has to be assessed differently. The quality of olfactory perception can be estimated based on few traits. Relative size of different brain areas can serve as a reliable predictor of their relative importance, in this context, the volume of OB serves as an indicator of olfactory capability (Kotrschal, Van Staaden, and Huber 1998). This was supported by a study, which compared the convergence ratio between primary and secondary neurons in the olfactory pathway of brownbanded bamboo shark (*Chiloscyllium punctatium*) and goldfish. The convergence ratio was 10 times higher in brownbanded bamboo shark with a ratio of 50:1, compared to 5:1 in goldfish. The relative

volume of OBs of brownbanded bamboo shark was 2.5-fold larger and therefore provides further evidence of relative volume of OBs as a good proxy (Camilieri-Asch et al. 2020). There also seems to be a correlation between the gene repertoire and number of lamellae (Policarpo et al. 2021; 2022) and between gene repertoire and number of OB cells (Burguera et al. 2023). The complexity of the olfactory rosette appears to be influential. Species without multilamellar rosette, such as broadnosed pipefish (*Syngnathus typhle*), had significantly lower OR gene repertoire than species with high number of lamellae, such as zig-zag eel (*Mastacembelus armatus*), 15 OR genes versus 429, respectively (Policarpo et al. 2021). Another study further expanded the species pool and confirmed the correlation between the number of lamellae and number of OR genes, while also observing a significant relationship between number of lamellae and number of V2R genes as well as the total number of olfactory receptors. No correlation was found for TAAR genes (Policarpo et al. 2022).

DEEP-SEA OLFACTORY ADAPTATIONS

Deep-sea brains

As previously mentioned, sensory inferences can be made based on the relative volume of brain areas (Kotrschal, Van Staaden, and Huber 1998). A study focused on mesopelagic fishes examined 67 different species and compared four different brain areas, that are responsible for primary projections of different senses. By comparing relative volume of an individual's brain areas with the average that was calculated from the whole pool, they identified specialists, that had only one sensory area above-average, dominated species in which two senses were above-average and generalists which were characterized by having three above-average areas. It has concluded that because of the occurrence of bioluminescence and residual solar light in the mesopelagic habitat, vision is the most important sense in this layer (Wagner 2001b). This finding is well supported by a study, where axon counts in the visual and olfactory pathway of two *Conocara* species (*Conocara macroptera* and *Conocara murrayi*) were compared. The result was a 41:1 ratio in favor of vision (S. P. Collin, Lloyd, and Wagner 2000). However, one olfactory specialist was found, *Borostomias elucens*. The mean relative volume of OB in the pool was 2.76 %, whereas the optic tectum, responsible for vision, had value of 61.26 %. Although 23 species exceeded the average value of OB, it seems that olfaction is associated with other senses and only plays a minor role in these open waters (Wagner 2001b).

Same approach was used for demersal fishes (i.e. fishes that live near or on the bottom of the deep sea). There were 35 demersal species and 5 of them were identified as olfactory specialists. Olfactory specialist species: *Histiobranchus bathybius*, *Synaphobranchus kaupi*,

Coryphaenoides armatus, *Cataetyx laticeps* and *Barathrites iris*. These findings were well correlated with feeding strategies of these species, along with axon counts in *Coryphaenoides armatus*, where the olfactory fibers exceed the optic ones 5.9-fold. Mean relative volume of OB formed 16.48 % of the primary projection brain areas, whereas optic tectum accounted for 57.56 %. 13 species surpassed the OB average. In conclusion, vision seems to be the most important sense in this habitat as well, however, olfaction and taste combined overshadow vision, therefore the demersal habitat seems to be a richer sensory environment (Wagner 2001a).

Three families, that were both present in mesopelagic and demersal realm were compared. Specifically, grenadiers (Macrouridae), eels (Anguilliformes) and slickheads (Alepocehalidae). In slickheads, vision plays the dominant role irrespectively of the habitat and seems to be a family trait. However, the difference between mesopelagic and demersal slickheads becomes visible when comparing the additional role of other senses. Whereas the octavolateral system seems to be important in demersal slickheads, olfaction secondarily dominates in mesopelagic species. Pelagic eels seem to rely mostly on vision, but olfaction is the most dominant for demersal eel species. This is consistent with their scavenging strategy and their quick and effective localization of their prey. Grenadiers are very diverse in their senses. To conclude, vision seems to be more dominant in the open waters, in contrast to the bottom, where olfaction is of bigger importance (Wagner 2002).

Contrarily, in elasmobranchs, it was found that benthic species have smaller olfactory rosette, lesser amount of lamellae and lower olfactory sensory area than benthopelagic elasmobranchs. There was no significant difference between OBs of benthic and benthopelagic species. However, this was not specifically focused on the deep-sea only elasmobranchs, nonetheless, it suggests that phylogenetic conservation is low and that olfactory morphology seems to be shaped by their diet, habitat and reproductive strategy (Schluessel et al. 2008). Although the OBs were not included by Yopak and Montgomery (2008), in their research which focused on the brain organization and specialization in deep-sea cartilaginous fishes, they concluded that deep-sea brains of chondrichthyans, despite showing some interspecific variation, can be interpreted as a deep-sea cerebrotype (Yopak and Montgomery 2008). When the OBs are compared with a greater species pool including deep-sea species, it shows that all the deep-sea species possess large OBs, irrespective of their lifestyle. The dependence on olfaction in deep-sea chondrichthyans is therefore suggested to be of a greater importance, as the visual cues are reduced or absent (Yopak, Lisney, and Collin 2015). Micro-computed tomography investigated the head anatomy of a deep-sea blackbelly lanternshark (*Etmopterus lucifer*). Due to enlarged number of pores and corresponding brain area responsible for electroreception, this sense seems

to be dominant. But corresponding brain areas for olfaction were also enlarged and hence might accompany electroreception. Whereas electroreception can be used to detect a living prey, olfaction can guide the shark to a dead carcass. Large eyes, however, suggest that vision might as well play some role in this species (Staggl, Ruthensteiner, and Straube 2023).

Ontogenetic shift

As noted earlier, *Coryphaenoides armatus* is an olfactory specialist (Wagner 2001a). Moreover, this species might undergo an ontogenetic shift in its reliance on senses. The relative size of the optic tectum decreases allometrically from about 7 %, while the olfactory bulb enlarges allometrically from about 1 %. After this phase, the brain areas have a more parallel pattern of growth and settle at about 4 % for the optic tectum and 2 % for the olfactory bulb. This shift occurs at the size range of 400 to 500 mm, in terms of weight, at around 1000 g (Wagner 2003). Additionally, study on the axon count in the olfactory tract and optic nerve in differently sized individuals supported this finding. There were on average 338 942 ± 166 029 axons in the olfactory tract with averagely 12.6 ± 7.6 % rate of myelinization across all individuals investigated without size in consideration. The values for the optic nerve were 83 443 ± 36 002 axons, with around 63.6 ± 5.7 % of them myelinated. Whereas the number of both olfactory and optic axons rises with size, the increase is clearly greater in the former (Figure 3).

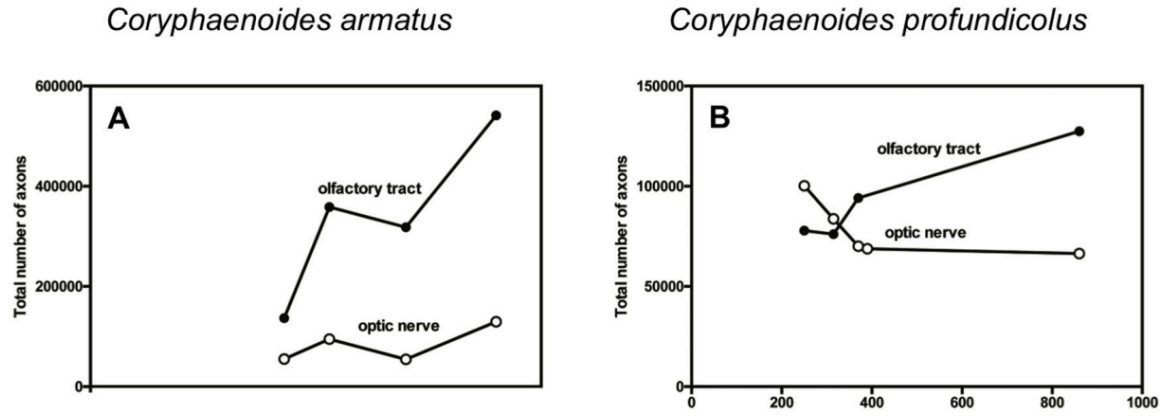

Figure 3 - Number of axons in olfactory (full circles) and optic (open circles) pathway in differently sized individuals, Y axis - total number of axons, X axis - size of the individual (Lisney, Wagner, and Collin 2018)

In an individual of 430 mm, the ratio, where both myelinated and unmyelinated axons are included, of olfactory input is in 2.48:1 favor to optic input. This ratio increases to 4.18:1 in 900 mm specimen. No specimens smaller than 400 mm were investigated, but the ratio would have likely been lower as it tends to increase with size (Lisney, Wagner, and Collin 2018). Concomitantly, notable change can be seen in the rostrum of *C. armatus*. Small individuals possess a longer, sharper rostrum, while on the contrary, as the *C. armatus* grows, the rostrum

tends to be shorter, blunter and broader (McLellan 1977; Wagner 2003). Wagner proposes that this developmental switch in *C. armatus* from a rather visual specialist to an olfactory specialist could be related with changes in behavior and feeding strategies (Wagner 2003) and there is more than enough evidence to support this.

Stable isotope analysis revealed the variety of consumed prey in *C. armatus*. The diet between small and large individuals differed. In small *C. armatus*, the diet consisted predominantly of benthic prey, whereas large ones fed upon pelagic prey, which most likely fell to the bottom of the ocean floor from upper layers. Total weight of the carrion prey in large specimens of *C. armatus* amounted for 69.21 %, but only 3.74 % in small specimens. This would support the scavenging lifestyle in larger individuals (Drazen et al. 2008). Even though there were some very rare observations of young macrourids around the bait, none of them fed on it, opposed to the larger individuals that were feeding and frequent around it (King, Bagley, and Priede 2006). Brilliant sense of smell in *C. armatus* is also indicated by the fact that it only takes minutes to localize the carrion bait. Time of the first arrival increased from 7.5 min to 41 min with increasing depth, however, *C. armatus* was always the first fish species to arrive in this study and also, the most abundant one (Wilson and Smith 1984). Decline in abundancy and frequency of occurrence around bait happened after being exposed to an odor plume from a dead conspecific. *C. armatus* either actively avoids the area infested with the odor plume of conspecific, or might be unresponsive to it (Barry and Drazen 2007). An investigation of the genome of *C. armatus* would be interesting, because in zebrafish, TAAR13c is responsible for the perception of putrescine and cadaverine that are emitted from a dead fish and trigger avoidant behavior (Hussain 2010).

Gustation and touch apparently play a significant role as well. The theory is that olfaction is used to detect cues over long distances, but to localize the prey at close range, gustatory and tactile barbel is deployed. Although very small (12 mm in 695 mm fish), the barbel has been histologically investigated and using tracing techniques, its role in mechanoreception and chemoreception has been confirmed. In the barbel nerve, about 20 000 axons reside and around 450 mm^{-2} taste buds are present in the barbel skin. Head-down swimming in *C. armatus* has been explained to maintain postural stability during low speed as well as to place the barbel in contact with seafloor (David M. Bailey et al. 2007).

Ontogenetic shift has also been indicated in another macrourid, *Coryphaenoides profundicolus*. Its ratio of axons in the olfactory pathway to axons in the optic pathway goes from 0.77:1 in 250 mm long individual to 1.92:1 in 860 mm individual. However, it should be noted that whereas the number of olfactory tract axons increased, the optic nerve axons decreased with

increasing size (Figure 3). The percentage of myelinated axons increased with size in both pathways in this species. The rate of myelinization was far more pronounced for the optic pathway, where it went from 45.3 % to 85.5 %. This high increase in myelinization could be a compensation for the loss of axons and hence maintaining the conduction velocity in the optic pathway. Olfaction may therefore be more important in adults of *C. profundicolus*, however, not to the same extent as in *C. armatus* (Lisney, Wagner, and Collin 2018).

Sexual dimorphism

Many different forms of sexual dimorphism can be encountered in the nature. For example, males can differ from a female in coloration, behavior or size. In the deep sea, sexual dimorphism can happen in the olfactory pathway. In a study conducted by Marshall (1967), different species of bathypelagic fishes exhibited sexual dimorphism in both body size and size of their olfactory organs. Males of *Cyclothone braueri*, *Cyclothone livida*, *Cyclothone pallida* and *Cyclothone acclinidens* all contained very large olfactory organs with numerous pronounced lamellae and wide nostrils, in females, on the contrary, the olfactory organs were regressed and only few relics of lamellae were present. Number of lamellae in males of *Sigmops bathyphilus* were around 25 inside large olfactory organs, whereas females had again smaller olfactory organs. *Sigmops elongatum*, a mesopelagic species, however, did not exhibit sexual dimorphism. There were also bathypelagic species, that did not show signs of sexual dimorphism, as olfactory organs of both genders of *Serrivomer beanii* and *Avocettina infans* were small (Marshall 1967). Both belonging to the order Anguilliformes and hence supporting Wagner's statement that pelagic eels use other senses than olfaction (Wagner 2002). In some species, only females were retrieved, and sexual dimorphism could not have been investigated. One of them being *Eurypharynx pelecanoides* (Marshall 1967). Luckily, a comparison could have been made years later. While the diameter of eyes of relative same sized individuals of different sexes remained unchanged, visible changes in the olfactory rosette were observed. The rosette of a female was barely noticeable, the rosette of a male on the other hand formed a large knob that was close to circular with a diameter of 5.5 mm. There were also differences in the size of nostrils. In both sexes, they were oval, in female, however, the parameters for both nostrils were only 0.4 × 0.3 mm, whereas in male 1.2 × 0.6 mm for the anterior and 2.9 × 1.3 mm for the posterior nostril (Gartner 1983). Marshall (1967) claims sexual dimorphism is present in 80 % of bathypelagic species. Furthermore, he notes that sexual dimorphism in mesopelagic fishes is quite rare, even though he observed it in *Cyclothone microdon* (Figure 4) (Marshall 1967).

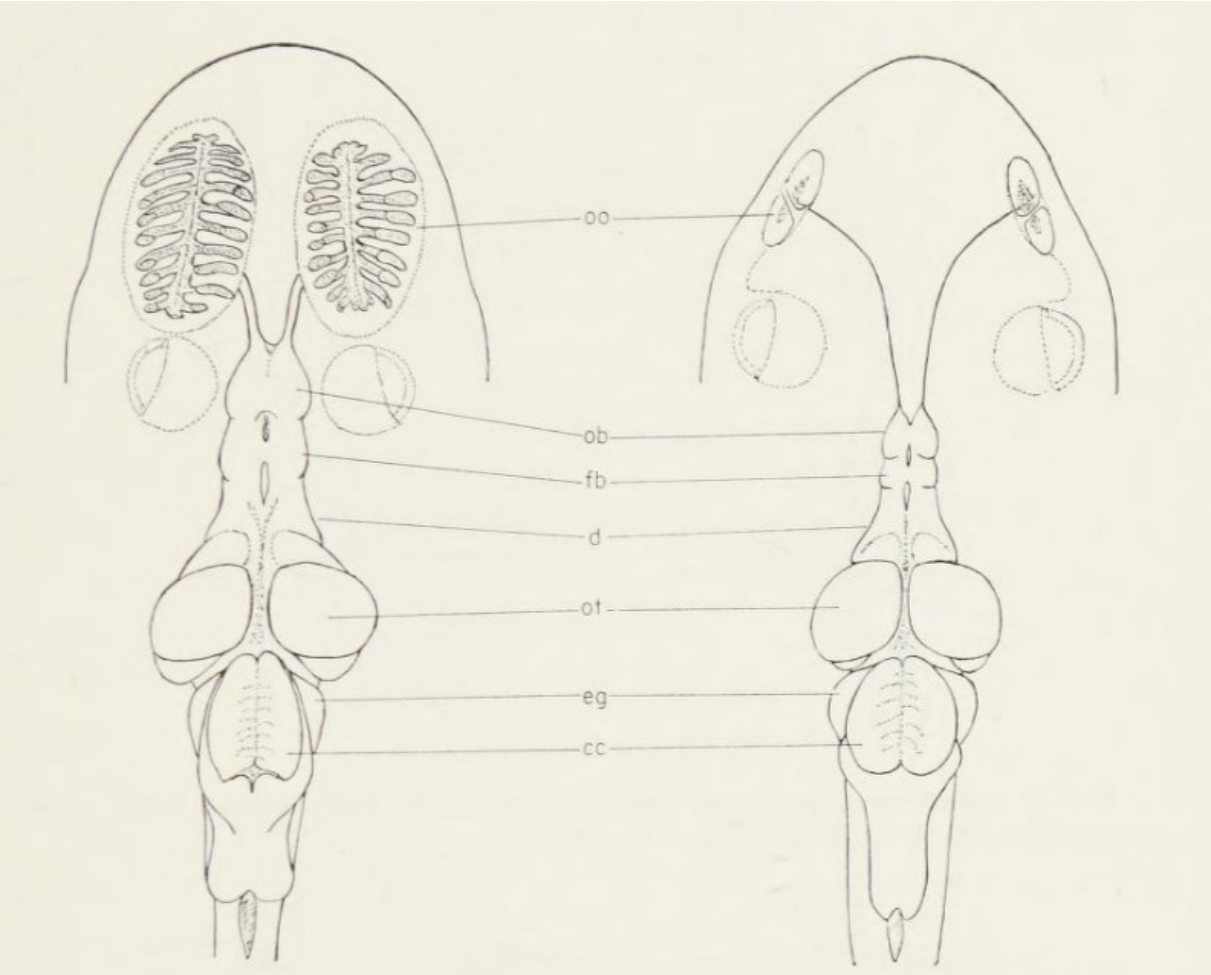

Figure 4 - Sexual dimorphism in Cyclothone microdon. Male is on the left, female is on the right. The olfactory organ (oo), olfactory bulb (ob) and forebrain (fb) are visibly enlarged in male. (Marshall 1967)

In spite of that, sexually dimorphic males have been found in other mesopelagic species. *Argyropelecus hemigymnus* and *Valenciennellus tripunctulatus* of the Sternoptychidae family showed pronounced differences in males and females. In *A. hemigymnus*, the olfactory bulbs were distinctive in their sizes, the diameter of the olfactory nerve was higher in males, as well as the number of lamellae and rosette area. For comparison, number of lamellae in sexually mature males was 19 – 23, whereas in sexually mature females it was only 1 – 14. Area of the rosette for males was calculated to fall in range of 1 – 1.7 mm^2, while in females it was 0.6 – 0.7 mm^2. In *V. tripunctulatus*, same traits except for the OBs were observed. The OBs of *V. tripunctulatus* were closely joined and the olfactory tract seemed like a single bundle, instead of two (Baird, Jumper, and Gallaher 1990). It is important to note that, despite the sexual dimorphism, olfactory organs are well developed in both genders of mesopelagic species compared to bathypelagic (Marshall 1967; Baird, Jumper, and Gallaher 1990). Not all sternoptychids exhibit dimorphic signs. *Sternoptyx diaphana* had no distinctive signs between

the sexes. Their olfactory organs, specifically the rosette was minute compared to *A. hemigymnus*, its area was only 0.2 – 0.4 mm^2. Interestingly, the rosette of *S. diaphana* lacked lamellar folding (Baird and Jumper 1993). Another case of dimorphism was found in the Myctophidae family, concretely in the *Loweina* genus. This genus comprises of only three species, *Loweina interrupta*, *Loweina rara* and *Loweina terminata*. Significantly larger olfactory organs and more numerous lamellae can be distinguished in males of *L. interrupta* and *L. rara*. No female was available for *L. terminata*, however, due to high lamellar count and the large size of olfactory organ in the *L. terminata* male, it can be assumed that dimorphism is present as well (Martin and Smith 2024). Gibbs (1991) investigated another mesopelagic species in her master thesis, with a goal to find evidence of sexual dimorphism, but in none of the species (*Serrivomer sector*, *Bathylagus antarcticus*, *Macropinna microstoma*, *Alepocephalus tenebrosus*, *Sagamichthys abei*, *Chauliodus sloani*, *Stomias boa*, *Lycodapus mandibularis*), there was any indication of it (Gibbs 1991). She makes an interesting point on the conclusion made by Marshall (1967) and says that he might have overestimated the 80 % occurrence of sexual dimorphism in bathypelagic species. Out of six truly bathypelagic species, for which both sexes were available, only four showed signs of dimorphism (Gibbs 1991; Marshall 1967). She concluded, after examining reach of other senses, that olfaction is the most important sense in terms of long distance communication and mate location and that pheromones could be detected even without enlarged olfactory organs (Gibbs 1991).

The most fascinating form of sexual dimorphism occurs in the order Lophiiformes, more specifically in the deep-sea suborder Ceratioidei. Olfactory organs of males are larger than those of females (Bertelsen 1951). They are also much smaller in size, to give an example, female of *Ceratias holboelli* measured 980 mm, whereas the male only 90 mm, for *Cryptopsaras couesii*, it was, 290 mm and 12 mm, respectively (Pietsch 2005). Sexual dimorphism in ceratioids was driven to perfection and created a unique form of symbiosis, sexual parasitism. Males can be either obligatory parasitic, facultatively or free-living (Pietsch 2005). In the obligatory parasitic species, such as *Neoceratias spinifer*, male bites with its jaws into the female's body and fuses with it. Other than pigmented boundary zone, no clear distinction can be found in the fused area and the dermis appears as of one (Munk 2000). The eyes, as well as olfactory organs and jaws start degenerating after attachment (Munk 2000; Pietsch 2009). Although interconnected blood vascular plexuses are found in the fusement area, one in female, one in male, there is no knowledge whether they are actually connected to the female's blood vascular system and brings nourishment for the male, but it is assumed it does (Munk 2000). Munk (2000) found no signs of degenerating heart, digestive system, or gills. The testes, however, were large and

sexually mature (Munk 2000). It is believed that both genders become sexually mature after the connection (Pietsch 2005). In *Cryptopsaras couseii*, as many as 8 dwarf males were found attached to a single female (Saruwatari 2007). This strategy bears an evolutionary advantage in this low density environment, as when the female is ready to spawn, it has one or more males attached, which provides the sperm needed for fertilization (Isakov 2022).

Most likely, sexual dimorphism in olfactory organs of deep-sea fishes has evolved for mate location, hence reproduction. Males with enlarged organs are probably sensitive to a species-specific pheromone emitted by the female (Bertelsen 1951). Deep-sea fish populations are scarce. Sexual dimorphism may therefore be present in species with low population densities, such as *Loweina* (Martin and Smith 2024). For *A. hemigymnus*, individuals are situated approximately 22 – 25 m from each other, for *V. tripunctulatus* distance is 18 – 20 m (Baird, Jumper, and Gallaher 1990). In mesopelagic fishes investigated by Gibbs (1991), she calculated distance between specimens was 20 m (Gibbs 1991). Density of ceratioids is around 30 m between individuals (Bertelsen 1951). *Cyclothone*, more populous than ceratioids, are separated by approximately 3 m (Marshall 1967). Males are likely to be more frequent in populations, in order to increase the chances of mating (Gibbs 1991). In ceratioids, it has been estimated that 55 – 60 % of their population is formed by males and for one receptive female, there's 15 – 30 metamorphosed males (Bertelsen 1951). In bioluminescent species, such as myctophids, kin recognition can be achieved by species-specific bioluminescent patterns. However, the emitted light can reach only a distance of around 10 m. It is therefore expected that chemical cues initialize the communication between individuals over long distances, bring them together and in close range, other sensory mechanisms, such as vision, take over (S. p. Collin, Marshall, and Herring 2000). Ceratioid females possess an illicium (first dorsal-fin spine) that carries an esca, a bioluminescent organ. Esca is used to lure prey and kin. After the male detects the female's scent by olfaction, close-range detection is believed to be ensured by vision (Bertelsen 1951; Pietsch 2005; 2009). Males in some species are believed to cease feeding after maturation. In *Eurypharynx pelecanoides*, the body cavity is mostly filled with well-developed testes and atrophied stomach, indicating that it does not receive any nutrition and potentially dies after spawning (Gartner 1983). Males of the Cetomimidae family undergo a transformation and after becoming mature, they lose their stomach and oesophagus, while only gonads, liver and thin-walled intestine persist. The large liver, nutritioned by the copepod filled intestine, seems to be responsible for keeping the males alive until the spawning event (Johnson et al. 2009). Large liver is also present in the attached ceratioid males, along with the nourishment acquired from the female, it might accountable for the males growth in size even after being permanently fused

to its opposite sex (Bertelsen 1951; Munk 2000).

A mate location model has been created for *A. hemigymnus* by using mathematical formulas. For the use of the model, size of the female was set at 30 mm. It is therefore surprising, that it can release pheromone, that can spread up to 100 m in about 9 hours and dissipates in about a day in the case of single instantaneous release and 283 m in 12 hours in the case of continuous release. Close range detection of photophores (which are not sexually dimorphic in this species) has been assumed to be in a radius of 1 m (30 body lengths). Without olfactory cues, it would take around 8 days for a male to localise a still female. After counting the movement of female at the same pace, time needed for interaction is 5 to 6 days. With olfactory cues present, time for a detection of female is drastically reduced to less than hour and half. After entering the pheromone patch, male begins to search. He chooses a random direction and swims at least one body length, if the concentration declines, he changes direction. He continues this random swimming until he either enters the interaction radius of 1 m, or swims out of the patch. Earlier detection by male increases its success. There is, however, only 1 % chance that the female will have to wait to be found by a male for more than 3 hours. The females must express a species-specific pheromone of a steroidal nature, in order to attract only males of their own kind and not any sympatric species (Jumper, and Baird 1991). High specificity to pheromones has been shown in crucian carp. Only one of four pheromones stimulated the majority of ORNs in males and thus indicating the specificity of the receptors. Females, on the other hand, did not exhibit any specificity (Lastein, Hamdani, and Doving 2006).

Loss of genes

Certain species from the Liparidae family inhabit the most inhospitable places on Earth, the hadal trenches. Genome of the Mariana snailfish (*Pseudoliparis swirei*) showed remarkable traits of adaptation. Other than mechanisms dealing with the immense pressure, specific sensory changes emerged. Indicated by not reacting to the lights of the deep-sea lander, loss of several key photoreceptor genes has been confirmed by genome analysis (Wang et al. 2019). Expression of only *rh1* and *gnat1*, found also in other deep-sea fishes, suggests their importance in this environment (Xu et al. 2023; Musilova et al. 2019a). *Sws2* was also present, however, only as pseudogene (Xu et al. 2023). Degraded vision, however, needs to be compensated by other senses.

Using comparative analysis with shallow-living species, OR and TAAR repertoire has been analysed and showed interesting results. One of the compared species was the shallow-living relative Tanaka's snailfish (*Liparis tanakae*) (Jiang et al. 2019), inhabiting depths of only 50 –

121 m (Froese, R. and D. Pauly 2024 - Fishbase). Mariana snailfish and Tanaka's snailfish diverged around 20.22 million years ago (Mya) (Wang et al. 2019). The closest known relatives of the Mariana snailfish live at depths around 1000 m and come from the genera *Paraliparis*, *Careproctus*, *Crystallias* and *Rhodichthys* and the estimation of the divergence time is 9.9 Mya (Xu et al. 2023). Mariana snailfish possess 53 OR genes and 31 TAAR genes, in comparison to Tanaka's snailfish with 75 OR genes and 26 TAAR genes. However, it should be noted that the rate of pseudogenization is much higher in Mariana snailfish. Out of 53 OR genes, 10 of them are pseudogenes, whereas Tanaka's snailfish has 3 pseudogenes and 2 truncated genes, giving only 43 functional OR genes in Mariana snailfish and 70 in Tanaka's snailfish. Considering TAAR repertoire, Mariana snailfish has 22 functional TAAR genes and 9 pseudogenes in its genome, while Tanaka's snailfish 20 functional, 1 truncated and 5 pseudogenes. Common ancestor of these two snailfishes had by estimation 51 OR and 26 TAAR genes. Mariana snailfish therefore gained 16 OR and 4 TAAR genes and lost 24 OR and 8 TAAR genes. On the other hand, Tanaka's snailfish gained 23 OR genes, while losing only 4, and gained just 2 TAAR genes and lost 8. OR genes of these two snailfishes have been separated into 15 subfamilies. Contraction occurred in 10 subfamilies in Mariana snailfish. The most notable difference happened in the subfamilies 120 and 121, where Mariana snailfish had barely 1 and 2 genes, respectively, on the other hand, Tanaka's snailfish had 11 and 12 genes, respectively. In only one subfamily, Mariana snailfish had more genes, the subfamily 126. 22 OR genes were assigned to the subfamily 126 in Mariana snailfish, whereas Tanaka's snailfish had only 13 genes in it. TAAR subfamilies are bit more complicated. They can be divided into two clades, Class I and Class III. Both Class I subfamilies 21 and 27 are present in both snailfish species. Contradictorily, there are only 3 Class III subfamilies (29, 25 and 30) present in Mariana snailfish, whereas Tanaka's snailfish possesses 6 of them (13, 23, 24, 25, 29, 30). The only notable difference in the number of genes appeared in the subfamily 29, where Mariana snailfish possessed 4 TAAR genes, but Tanaka's snailfish only 1.

These results indicate that the contraction in the OR repertoire might correlate with decreased sensitivity to specific odorants of different subfamilies, except for subfamily 126, where expansion suggests higher sensitivity to a certain, unknown, odorant. The loss of TAAR subfamilies offers a similar explanation, Mariana snailfish is less capable of detecting amines. However, due to expansion in subfamily 29, Mariana snailfish may be more sensitive to certain amines. Whereas the loss of the OR genes could be argued with living in a chemically poor environment, the conservation of the TAAR genes could be explained by carrion falls, one of the main sources of food in the hadal zone. While being microbiologically decomposed, range

of amines is emitted, which could attract Mariana snailfish (Jiang et al. 2019). The majority of the Mariana snailfish's stomach was filled with crustaceans, mostly with *Hirondellea gigas* (Wang et al. 2019). In situ observations showed, that with 98 % predation rate, Mariana snailfish targeted amphipods that were feeding on a bait, ingesting them by suction feeding. Accidentally ingested bait was ejected (Linley et al. 2017). Mariana snailfish therefore locates the carrion, which is occupied by the amphipods and the suction feeds on them (Jiang et al. 2019; Linley et al. 2017). OR and TAAR gene repertoire of the Mariana snailfish, although simplified, appear to be very specialized to its environment (Jiang et al. 2019).

Yap trench is occupied by tentatively named Yap hadal snailfish. The divergence time between Yap hadal snailfish and Mariana snailfish occurred around 0.92 Mya (Mu et al. 2021), however, a newer study suggests that these two hadal snailfishes diverged only 0.044 Mya (Xu et al. 2023). In Yap hadal snailfishes, 40 OR genes were predicted, 25 of them functional and 15 as pseudogenes (Mu et al. 2021). However, looking into the S20 table of this study (Mu et al. 2021), the same number of the genes was predicted for the Mariana snailfish, which varies from the number of OR genes in Jiang et al. (2019), which identified totally 53 OR genes, 43 of them functional and 10 pseudogenes. Different method might have been used, but, given the number of predicted genes was the same for these two hadal snailfishes and their short divergent time, their olfactory repertoire could be the same. It would be also interesting to investigate the V1R and V2R repertoires in these fishes.

CONCLUSION

In this thesis, I have summarized the knowledge on olfaction in the deep-sea fishes. Although sparse, there has been some research conducted on the subject. Based on the relative volume of the brain areas, olfactory specialists have been identified in both mesopelagic and demersal fishes, and the importance of the olfaction in these environments has been highlighted. The same has been listed also for the deep-sea cartilaginous fishes. The ontogenetic shift in *C. armatus* and *C. profundicolus* has been recognized, shifting from the visually oriented juveniles to the olfactory oriented adults. Sexual dimorphism has been identified in both mesopelagic and bathypelagic species. Location model in the deep-sea has been outlined. And lastly, the whole genome of the hadal snailfishes showed us the specific OR and TAAR repertoire in these extreme depths. Mysteries on the olfaction in deep-sea fishes, however, remain to be explored.

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OWN DATA

Introduction

High-quality genomes, i.e. the chromosome-level assemblies, of the deep-sea fishes are barely available, whereas there are several raw-read data sets around (from the public database or previously produced by the research group I am a member of). The idea is to test a simple method to estimate the number of olfactory genes based on raw genomic reads of 11 different deep-sea fish species. This could potentially reveal candidate species of interest for the future studies.

Methods

First, I mapped the genome raw data sets of the species with the high-quality genome assembly available and with the known number of olfactory genes. Then I used the same mapping method for the deep-sea raw genome data sets with unknown/poor genome assembly and I tried to estimate the number of the olfactory genes in the genome. I used the mapping results from the known genomes as a proxy to do so.

Sequences of the OR, TAAR, V1R and V2R genes from *Anguilla anguilla*, *Danio rerio* and *Oreochromis niloticus* have been used as a reference for the genome reads mapping. In total, I mapped 45 genome assemblies of 32 different species (Table 1) acquired from NCBI GenBank using sratoolkit package. I used Geneious software (Geneious Prime 2023.2.1) "Map to reference" option with Medium sensitivity/Fast settings. The selected data sets used for mapping were produced by the Illumina sequencing platform, and the minimum and maximum length of sequences within one data set have been always the same. The overlap of the mapped reads with the reference has been set to 100 bp in case of 150 bp long sequences, and to 75 bp in case of 100 bp long reads. The BUSCO genes have also been used to get a comparative data set of single-copy genes. For the 32 species with a high-quality genome, the olfactory gene repertoire (=number of genes) has been retrieved from Burguera et al. (2023). Based on that, linear regression model has been created, with the known number of genes on the X axis, and fragments per million (FPM) sequences ((number of mapped reads/total number of sequences in the data set) * 1000000) on the Y axis. Another linear regression model has been constructed using the BUSCO genes, with number of genes on the X axis again, and the normalized reads (number of mapped reads/mapped BUSCO genes) on the Y axis. These models have been created for every olfactory gene family, i.e. OR, TAAR, V1R and V2R, independently. Further, an alternative method of estimation was based on the simple ratio. For every genome, a ratio of mapped reads against the known number of genes was calculated. The averaged value for all 45 genome assemblies was applied as a proxy to calculate the putative number of olfactory genes in the deep-sea fish genomes. Accordingly to the linear models, the ratio has been calculated for each olfactory gene family and for both methods based on the FPM or normalized reads. The lower and upper bounds of confidence intervals for the linear models were calculated with the use of confint function from the stats R package. The lowest and highest coefficients for the slope of the line along with their intercepts were obtained from confint function and then put into the formula as in the estimation of individual olfactory gene families. Confidence intervals for the ratio method were calculated with the use of CONFIDENCE function in

Microsoft Excel (version 2403). The lower and upper bounds were obtained by using the same formulas as in for the estimations, but with ratio values that were either reduced or enlarged, respectively, by the number acquired from the CONFIDENCE function.

For the deep-sea fish genomic reads, the mapping has been done using the same mapping settings. The equations from the aforementioned linear regression models, as well as the average ratios have been used to calculate the number of genes (and their confidence intervals) in these species. In total, 14 genome assemblies of 11 deep-sea species have been utilized. There was, however, no simple way to exclude truncated genes, pseudogenes or false positive sequences.

Species	Accession	N. of sequences
Colossoma macropomum	SRX10122101	218 168 058
Phyllopteryx taeniolatus	SRX15891812	705 279 938
Pygocentrus nattereri	SRX1487139	172 644 946
Channa maculata	SRX9604232	188 869 174
Anguilla anguilla	SRX14353623	151 709 846
Mastacembelus armatus	SRX18302040	212 703 872
Thymallus thymallus	ERX2073365	451 228 148
Syngnathus acus	ERX4023158	764 331 910
Anabas testudineus	ERX2465797	144 052 186
Periophthalmus modestus	SRX11378326	352 521 790
Sparus aurata	ERX3666106	140 242 860
Gadus morhua	ERX1622647	322 158 668
Thallasophryne amazonica	ERX3647544	924 599 866
Vimba vimba	SRX13920995	410 269 238
Proterorhinus semilunaris	SRX12587195	482 952 296
Takifugu rubripes	DRX021131	278 642 344
Takifugu rubripes	DRX021132	244 796 700
Oreochromis niloticus	SRX14028757	122 090 106
Oreochromis niloticus	SRX8393299	65 349 616
Oreochromis niloticus	SRX8393298	53 317 110
Oryzias latipes	SRX12775939	241 742 368
Oryzias latipes	DRX248128	635 184 560
Scomber japonicus	DRX300068	197 665 426

Species	Accession	N. of sequences
Thunnus albacares	ERX6465926	99 808 482
Thunnus albacares	ERX6465924	122 849 358
Lampris incognitus	SRX18366552	21 071 332
Coregonus clupeaformis	SRX14636782	128 854 504
Coregonus clupeaformis	SRX14636783	144 136 320
Electrophorus electricus	SRX7427113	195 146 880
Electrophorus electricus	SRX7427114	223 444 120
Esox lucius	SRX5245295	272 812 190
Esox lucius	SRX5245298	259 442 270
Danio rerio	SRX9605334	60 621 160
Danio rerio	SRX9605331	65 767 508
Chanos chanos	SRX10107514	448 115 174
Scleropages formosus	ERX3490068	180 190 592
Anguilla anguilla	SRX14353621	80 445 692
Lepisosteus oculatus	SRX091942	84 429 654
Lepisosteus oculatus	SRX091938	95 331 446
Myripristis murdjan	ERX3475847	208 130 852
Mola mola	SRX1665387	194 400 168
Anabas testudineus	ERX4142762	76 406 180
Anabas testudineus	ERX1545067	96 800 404
Solea senegalensis	ERX6700259, ER	171 715 340
Sebastes schlegelii	ERX5044832	141 995 218

Table 1 - High-quality genome assemblies used for both methods of estimation

Results

The plotted linear models based on the 45 teleost high-quality genomes with the known number of olfactory genes are Figure 5, along with their coefficient of determination and p-values. The 95% confidence intervals were plotted as well. The estimates of the deep-sea fish olfactory gene numbers are listed in Table 2. Total number of the olfactory genes was calculated by the equation from the linear models. (Normalized reads+3.7435)/0,056 was used for the total number of genes in the method using BUSCO genes. Equation for the OR genes was: (Normalized OR reads+5.4068)/0.0603, for TAARs: (Normalized TAAR reads-0.3916)/0.0407, for V1Rs: (Normalized V1R reads-0.6503)/0.1092 and for V2Rs: (Normalized V2R reads+0.1626)/0.0708. For the method without the usage of BUSCO genes, following equations were used: (FPM+33.238)/0.3218 for the total number of genes, (FPM OR reads+30.357)/0.3238 for the number of OR genes, (FPM TAAR reads+4.1728)/0.2723 for TAARs, (FPM V1R reads-3.4839)/0.3836 for V1Rs and (FPM V2R reads+1.8754)/0.3695 for V2Rs. Ratios for the method with the use of normalized reads via BUSCO were 0,041930302 for total number of genes, 0,025409342 for OR genes, 0,042584429 for TAARs, 0,240894923 for V1Rs and 0,066302956 for V2Rs. Ratios for the method without BUSCO genes, i.e. FPM were 0,196439455 for total number of genes, 0,124834299 for ORs, 0,178076902 for TAARs, 1,253131539 for V1Rs and 0,323647857 for V2Rs. The calculation was as following, total

Species	Accession	N. of sequences	N. of genes	OR genes	TAAR genes	V1R genes	V2R genes	N. of genes FPM	FPM OR	FPM TAAR	FPM V1R	FPM V2R
Chauliodus danae	Unpublished	5 662 218	98 (10-239)	92 (29-190)	0 (0-31)	6 (0-77)	6 (0-19)	129 (32-286)	96 (36-194)	15 (0-45)	7	9 (0-36)
Chauliodus danae	SRX11964133	132 301 178	133 (38-284)	98 (34-207)	0 (0-32)	16 (5-142)	12 (2-25)	133 (35-291)	98 (37-196)	16 (0-46)	7	10 (0-37)
Coccorella atlantica	SRX11968797	157 915 148	122 (29-270)	106 (40-218)	0 (0-32)	0 (0-43)	22 (11-36)	141 (42-302)	106 (44-207)	16 (0-45)	0	20 (1-50)
Scopelarchus michaelsarsi	SRX11972411	146 235 760	121 (28-269)	113 (45-227)	0 (0-31)	3 (0-62)	11 (1-24)	135 (37-293)	108 (46-210)	15 (0-45)	0	11 (0-38)
Guentherus altivela	ERX1544984	101 711 814	125 (31-273)	122 (53-239)	0 (0-33)	0 (0-35)	12 (2-25)	120 (25-274)	104 (42-204)	16 (0-45)	0	8 (0-35)
Melanonus zugmayeri	ERX1544998	132 521 692	314 (185-519)	123 (54-241)	0 (0-34)	3 (0-59)	153 (127-188)	276 (151-479)	119 (54-224)	17 (0-47)	1	121 (83-185)
Melanonus zugmayeri	ERX1544999	46 703 810	303 (176-504)	125 (55-243)	0 (0-34)	1 (0-48)	146 (121-179)	285 (158-491)	123 (58-229)	18 (0-48)	0	127 (87-193)
Regalecus glesne	ERX1545038	183 856 558	91 (4-230)	100 (35-210)	0 (0-35)	0 (0-15)	7 (0-19)	122 (27-277)	102 (41-202)	19 (0-49)	0	9 (0-36)
Borostomias antarcticus	ERX1544982	131 926 864	141 (44-294)	111 (44-224)	0 (0-32)	6 (0-81)	23 (11-37)	134 (37-293)	103 (42-204)	16 (0-46)	0	15 (0-43)
Borostomias antarcticus	ERX10375717	101 579 116	174 (71-337)	97 (33-206)	0 (0-31)	39 (18-281)	11 (1-24)	137 (39-297)	96 (36-194)	15 (0-45)	14	8 (0-35)
Acanthochaenus luetkenii	ERX1545046	129 405 710	112 (21-257)	105 (39-216)	14 (4-49)	0 (0-17)	8 (1-21)	130 (33-288)	103 (42-204)	28 (6-60)	0	9 (0-36)
Chatrabus melanurus	ERX1545050	333 029 402	485 (325-740)	254 (158-418)	173 (171-232)	0 (0-42)	79 (61-101)	222 (107-408)	144 (74-257)	60 (33-101)	0	29 (9-62)
Brotula barbata	ERX1545047	131 794 732	181 (77-347)	134 (63-256)	13 (2-49)	0 (0-21)	38 (25-55)	256 (135-453)	157 (85-274)	42 (18-78)	0	57 (31-100)
Diretmus argenteus	ERX3138036	234 364 500	392 (249-620)	144 (70-269)	291 (262-401)	0 (0-36)	33 (21-49)	263 (140-461)	122 (57-228)	142 (101-203)	0	22 (3-53)

Species	Accession	N. of sequences	N. of genes	OR genes	TAAR genes	V1R genes	V2R genes	N. of genes FPM	FPM OR	FPM TAAR	FPM V1R	FPM V2R
Chauliodus danae	Unpublished	5 662 218	42 (37-48)	6 (5-8)	0 (0-0)	5 (4-8)	4 (4-5)	42 (35-53)	6 (4-8)	0 (0-0)	5 (3-12)	4 (4-6)
Chauliodus danae	SRX11964133	132 301 178	88 (78-102)	20 (16-26)	1 (2-1)	10 (8-15)	10 (9-12)	48 (40-60)	10 (8-14)	1 (1-1)	5 (3-12)	5 (4-7)
Coccorella atlantica	SRX11968797	157 915 148	74 (65-85)	39 (32-51)	1 (1-1)	3 (2-4)	21 (18-24)	62 (51-77)	31 (25-42)	1 (0-1)	2 (1-5)	17 (13-22)
Scopelarchus michaelsarsi	SRX11972411	146 235 760	73 (64-84)	54 (44-70)	0 (0-0)	4 (3-6)	10 (8-11)	51 (43-64)	37 (29-49)	0 (0-0)	3 (2-7)	6 (5-8)
Guentherus altivela	ERX1544984	101 711 814	77 (68-89)	77 (62-99)	2 (2-1)	2 (2-3)	10 (9-12)	27 (23-34)	26 (21-35)	1 (1-1)	1 (0-2)	3 (3-5)
Melanonus zugmayeri	ERX1544998	132 521 692	330 (291-381)	80 (65-103)	3 (4-3)	4 (3-6)	161 (142-186)	283 (236-353)	65 (52-88)	3 (3-4)	3 (2-8)	133 (107-174)
Melanonus zugmayeri	ERX1544999	46 703 810	315 (278-364)	84 (68-109)	3 (4-3)	3 (2-5)	153 (135-177)	298 (249-373)	76 (60-102)	3 (3-4)	3 (2-7)	139 (113-182)
Regalecus glesne	ERX1545038	183 856 558	32 (29-37)	25 (20-32)	5 (7-4)	1 (1-1)	5 (4-6)	31 (26-39)	22 (18-30)	6 (5-7)	1 (0-2)	5 (4-6)
Borostomias antarcticus	ERX1544982	131 926 864	98 (87-114)	50 (41-65)	1 (2-1)	6 (4-8)	22 (19-25)	51 (43-64)	25 (20-33)	1 (1-1)	3 (2-6)	11 (9-14)
Borostomias antarcticus	ERX10375717	101 579 116	143 (126-165)	18 (15-24)	0 (0-0)	20 (15-30)	9 (8-11)	56 (47-70)	7 (5-9)	0 (0-0)	7 (5-18)	3 (3-5)
Acanthochaenus luetkenii	ERX1545046	129 405 710	61 (53-70)	36 (29-46)	23 (29-19)	1 (1-1)	6 (6-7)	45 (37-56)	25 (20-34)	19 (15-24)	1 (0-2)	5 (4-6)
Chatrabus melanurus	ERX1545050	333 029 402	559 (493-645)	391 (319-506)	175 (220-145)	3 (2-4)	81 (72-94)	194 (162-243)	130 (103-175)	68 (56-87)	1 (1-2)	27 (22-36)
Brotula barbata	ERX1545047	131 794 732	153 (135-176)	106 (86-137)	22 (28-18)	1 (1-2)	38 (33-44)	250 (208-312)	165 (131-222)	40 (33-51)	2 (1-4)	59 (48-78)
Diretmus argenteus	ERX3138036	234 364 500	435 (383-501)	128 (105-166)	287 (362-238)	2 (2-3)	33 (29-38)	261 (217-326)	73 (58-99)	193 (159-246)	1 (1-3)	19 (15-25)

Table 2 – Estimated numbers of olfactory genes in deep-sea fish species. Upper table represents linear models (blue – with the use of BUSCO genes, green – without BUSCO genes, i.e. FPM). Bottom table shows the estimations made from ratios (yellow – with the use of BUSCO genes, orange – without BUSCO genes, i.e. FPM) (confidence interval for V1R genes predicted from FPM method not shown as the estimates fall outside of it, FPM V1R is the only insignificant linear model)

number of genes using the normalized reads: normalized reads mapped in the deep-sea fish divided by ratio from known species, i.e. 0,041930302. Estimations were made for both methods for the total number of genes and for each olfactory gene family.

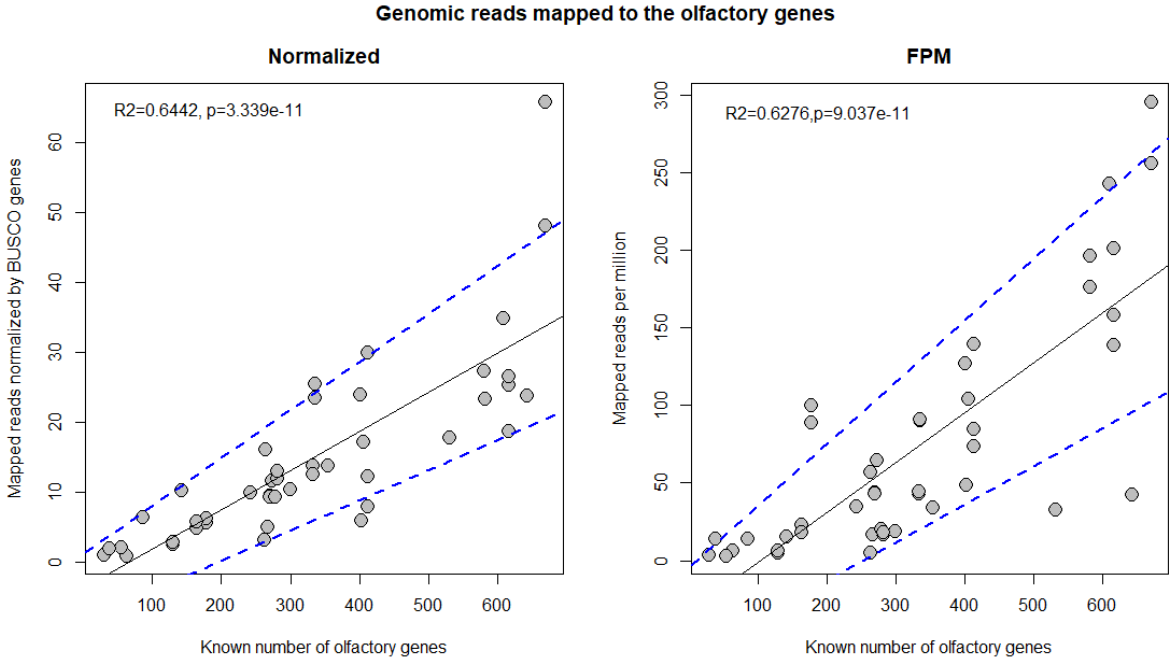

Figure 5 - Plots of linear models with their confidence intervals

Discussion

My results indicate that 8 out of 11 studied deep-sea fish species have rather lower number of olfactory genes (below 200), while three species exceed the total number of 300 at least in one counting method.

The number of the olfactory genes correlates with complexity of the olfactory rosette and number of lamellae and hence, individual level of olfactory ability can be assumed (Policarpo et al. 2021; 2022). *Chauliodus sloani* and *Chauliodus danae* are closely related, their most recent common ancestor lived 7.6 Mya to 20.6 Mya (Kenaley, DeVaney, and Fjeran 2014). *C. sloani* was classified as a visionary specialist, as its relative volume of the brain area responsible for the vision input was the only part of the brain above average. It lives in the mesopelagic zone, where vision, due to bioluminescence and residual solar light, dominates other senses (Wagner 2001b). The diet of *C. sloani* consists of fish only (Carmo et al. 2015). Piscivorous habits and life in the mesopelagic zone in the closely related species, should therefore not present any surprise in the low olfactory gene count in *C. danae*. *Melanonus zugmayeri*, *Scopelarchus michaelsarsi* and *Diretmus argenteus* were also investigated in Wagner's study. *S. michaelsarsi* did not have above-average OB, low olfactory count aligns well with this. *M. zugmayeri*, however, had OB above-average along with the input area responsible for the lateral line system (Wagner 2001b). Olfaction, based on Wagner's results and results of this study's estimation, might hence play an important role in this species. Lateral line system has been investigated in *M. zugmayeri* and it has been greatly developed. Although not having any body canal, canals on the head are widened and neuromasts are also placed on a papillae extending

slightly above the skin (Marshall 1996). These findings, along with the life in the bathypelagic zone, where food is scarcer, might suggest sit-and-wait strategy (I. G. Priede 2017). Fishes with sit-and-wait strategy are also, although not to the same extent as active foragers (scavengers), attracted to the bait and the stronger the odor plume, the further it gets, and the higher number of individuals are attracted to the bait. The fishes stay in the area for prolonged periods (D. M. Bailey and Priede 2002). Higher olfactory genes could suggest that *M. zugmayeri* could well detect the odor, enter the area and then sit-and-wait, until nearby prey stimulates its lateral line system. The very high olfactory gene count in *D. argenteus* is very surprising and could as well be a mistake. Based on the brain areas, *D. argenteus* was classified as vision specialist (Wagner 2001b). Important role of vision in this species is also indicated by the anatomy of its eye and by the expanded opsin repertoire, having 38 RH1s, that are important in dim light conditions (Musilova et al. 2019b). High gene count in *Chatrabus melanurus* could be argued with its lifestyle. *C. melanurus* belongs to order Batrachoidiformes, which mostly occupies benthic areas near the shore (Biston Vaz 2020). Based on data from FishBase, its depth range is 120 – 600 m, however, it was usually observed only to 250 m (Froese, R. and D. Pauly 2024 - FishBase). Possessing only two cone opsin genes supports life in dim light conditions (Musilova and Cortesi 2023) and high olfactory gene count indicates importance of olfaction, rather than vision. *Brotula barbata* is not considered as a deep-sea fish, but reef-associated, although it can occur down to 600 m (Froese, R. and D. Pauly 2024 - FishBase).

Demian Burguera used the same pipeline, as in his study (Burguera et al. 2023), from which the number of genes for shallow-water species were taken. In a rare high-quality genome of a deep-sea fish, more specifically *Borostomias antarcticus*, he unravelled 51 ORs, 6 TAARs, 4 V1Rs and 19 V2Rs, in total 80 genes. Comparing this with the estimated results in the same genome assembly, 174 olfactory genes (97 ORs, 0 TAARs, 39 V1Rs, 11 V2Rs) with normalized reads from the linear model and 138 without BUSCO genes using FPMs (96 ORs, 15 TAARs, 14 V1Rs, 8 V2Rs) and from the ratio method, 143 olfactory genes were obtained from the normalized reads approach (18 ORs, 0 TAARs, 20 V1Rs, 9 V2Rs) and 56 using FPMs (7 ORs, 0 TAARs, 7 V1Rs, 3 V2Rs). Results of every method differ strikingly and there is no clear indication of which one is the best. Number of genes in each olfactory family does not sum up to the total number of olfactory genes. That is due to different residuals in each linear model and due to ratios of each olfactory gene family, that did not sum up to equal the ratio of total number of genes. In some cases, negative values emerged due to residuals and in that scenario, they were replaced with 0. Also, the confidence intervals (Figure 5) are vast and ranged widely. For example, in *B. antarcticus*, as previously mentioned, total number of genes equalled 174 in the method with normalized reads, the lower bound of the confidence interval was 71 genes and the upper bound was 337 genes. FPMs method confidence interval ranged from 39 to 298 genes, while the equation estimated 138 genes. For the ratio method, with normalized reads, the estimation was 143 and its confidence interval ranged from 126 to 165, for the FPM approach, the value for the total number of genes was 56 and the confidence interval spanned from 47 to 70. Results should be therefore taken with caution.

There were also other imperfections in the methods used. Firstly, only Illumina sequences were used. PacBio and Oxford Nanopore sequences were utilized at first, they did not, however,

combine well with each other and therefore only Illumina data sets were considered. Secondly, false positive sequences were not excluded. Some results may therefore be overestimated as the sequences used to detect olfactory genes might have detected other genes, that were similar in sequences. This might have happened due olfactory gene receptors belonging to the family of GPCRs (Pierce, Premont, and Lefkowitz 2002). Truncated genes and pseudogenes were also not excluded.

The simple methods utilized in this work might not be precise, they could, however, relatively estimate the number of genes and could potentially reveal olfactory specialists. Also, findings that Wagner (2001) presented in his study for few of the species that are in common with this work align with the results presented in here (except for *D. argenteus*). Conclusively, for most of the species, lower number of olfactory genes was encountered. It should be noted, on the other hand, that in one of the deepest-living fish, the Mariana snailfish (*Pseudoliparis swirei*), the ORs had only 53 genes and TAARs had 31 genes, showing its compacted, but specific gene repertoire (Jiang et al. 2019). No V1Rs nor V2Rs were investigated, however, V1R repertoire is generally small in fishes (Burguera et al. 2023), so unless there has been an expansion of V2Rs, the total olfactory gene count in *P. swirei* will not be high. Results of this study might correspond and could indicate that due to chemically poor environment, highly specific gene repertoire was created, which would be suggested by lower numbers in most species. Olfaction in the deep-sea is mainly used for feeding and reproduction, therefore only genes necessary for these activities could have been maintained during evolution and the receptors just pick up specific odorants. In this work, however, there were 3 species, that had high gene counts across all the methods – *M. zugmayeri*, *C. melanurus* and *D. argenteus*. Although some further investigations must be made, these species could rely more on olfaction and might be potential research candidates for high-quality sequencing.

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