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Novel Nasal Anatomy Suggests Enhanced Olfactory Acuity in Auchenoglanidid Catfishes (Siluriformes: Auchenoglanididae)

Allison W. Bronson[1] and Melanie L. J. Stiassny[2]

We describe the olfactory chamber and histology of the olfactory epithelium in the auchenoglanidid catfish, *Parauchenoglanis punctatus*. Unlike the olfactory anatomy of non-auchenoglanidid catfishes, in all auchenoglanidids the olfactory rosette is elevated on a membrane suspended over the anterior portion of a large accessory sac. Scanning electron microscopy and histology show an array of long non-sensory cilia are present, likely maintaining flow of water over the rosette. Microvillar and ciliary sensory cells are regionalized in the folds of the lamellae. Combining dissection with reconstruction of the olfactory chamber, membrane, rosette, and accessory sac using contrast-enhanced CT scanning of soft tissue and supporting osteology suggests a novel "sniffing" mechanism may represent the primary means of drawing water and odorants into the olfactory chamber, and that specializations of the olfactory anatomy in this family function in flow augmentation into and around the olfactory chamber, maximizing the entrainment of odorants over the olfactory epithelium.

IN what is widely believed to be the first descriptive reference to the olfactory system of fishes, Monro writes, "In all fishes, external openings or nostrils for smell are very evident, generally two on each side in the osseous fishes, which, on each side of the head, lead to a complex organ, the surface of which is of considerable extent" … and "There can, therefore, be no doubt that they enjoy the sense of smelling; nay, there is great reason to believe, that, suited to their surrounding element, they are much more sensible of odorous bodies dissolved in water, and applied by its medium, than we should be, if the application of the object was to be made to our organ of smell by the same medium" (Monro, 1785: 44).

Catfishes (Siluriformes), particularly the channel catfish *Ictalurus punctatus* and a few other closely related ictalurids, are model organisms for the study of olfaction (Ngai et al., 1993; Mombaerts, 1999; Nikonov et al., 2005; Nikonov and Caprio, 2007; Valentincic et al., 2011). Given the relative simplicity of olfactory systems of fishes compared with those of tetrapods (Nikonov et al., 2005), and the available behavioral, neuroanatomical, genomic, and sensory physiological data for the channel catfish, these studies have served as a template for a broad understanding of vertebrate olfaction (Wilson, 2004). The piscine system, with its singular olfactory function, has been advocated as a candidate biomimicry model for the development of artificial sensors ("electronic noses") for olfactory trace detection with potential in a range of security and medical applications (Settles, 2005; Cox, 2008).

An obvious difference between fish olfaction and that of terrestrial vertebrate models is that fish only detect odorants that are water-soluble, mostly amino acids and nucleotides (initiating feeding behaviors), and bile salts, steroids, and prostaglandins (serving a social context). Although the odorant receptor repertoire and extent of (water-soluble) odorants is limited for fishes (Nikonov et al., 2005), ictalurids at least are known to locate and discriminate among food items, recognize conspecifics and socially dominant individuals, discriminate between the sexes, and assess female maturity by olfaction (Hargreaves and Tomasso, 2004). Diffusion of waterborne odorants, even over very short distances, is extremely slow when compared with aerial diffusion rates of volatile lipophilic molecules (Ngai et al., 1993; Cox, 2008). Consequently, hydrodynamic aspects of flow assume a particular importance for understanding the potential role of specialized nasal anatomy in fish olfaction. The nature of water flow over the olfactory epithelium, and how that flow is harnessed to ventilate the olfactory surfaces, in fishes is poorly understood, but Cox (2008) provides an excellent overview of many hydrodynamic aspects of fish olfaction and olfactory anatomy. He notes, however, that despite many recent advances in the understanding of olfactory neurophysiology, ultrastructure of sensory surfaces, and gene expression of odorant receptors, for most fishes, basic information on gross nasal anatomy is lacking.

Catfishes exhibit a wide array of anatomical configurations (e.g., Fig. 1), each with different effects on the intake and entrainment of odorants over olfactory surfaces (Cox, 2008). Despite considerable variation in morphological details, the "typical" catfish nose is considered highly effective for odorant entrainment in usually having elongate, lamellate olfactory rosettes (Theisen et al., 1991). Additionally, the tubular nostrils present in some species are thought also to contribute to a reduced boundary layer of water traveling over the head and enhanced water flow over the olfactory surfaces (Stoddard, 1980; Denny, 1993; Cox, 2008). However, because they generally rely primarily on a current generated by cilia within the olfactory chamber, most catfishes are somewhat limited in odorant intake (Cox, 2008). In contrast to the simple cilia-driven intake of odorants, some bony fishes have developed accessory sacs adjacent to

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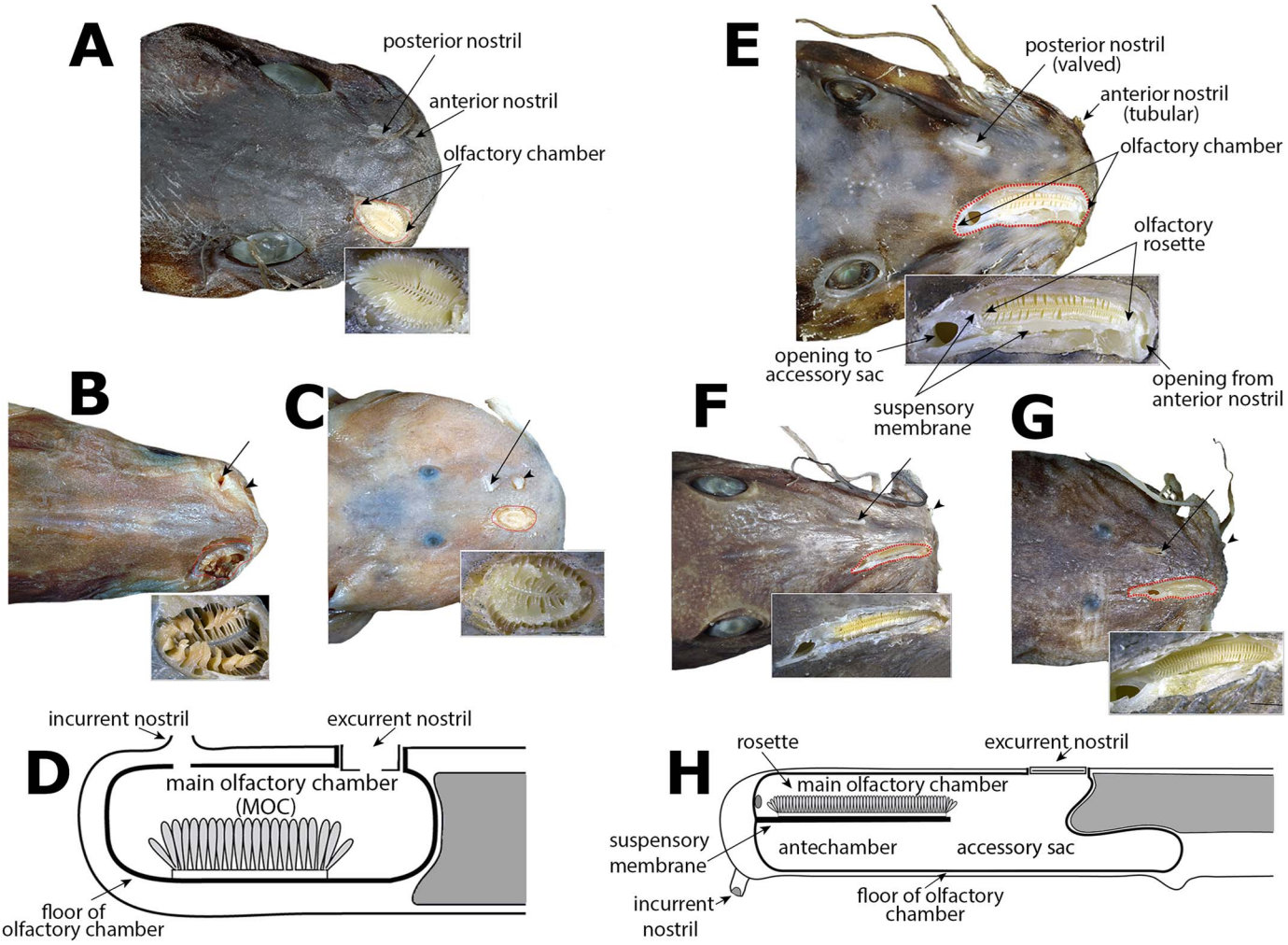

Fig. 1. Head and snout (dorsal view) with the integument removed to expose the right olfactory rosette and associated structures in: (A) *Chrysichthys cranchii*, AMNH 241154 (Claroteidae), (B) *Pangasius nasutus*, AMNH 57267 (Pangasiidae), (C) *Amphilius zairensis*, AMNH 240442 (Amphiliidae), (E) *Parauchenglanis cf. balayi*, AMNH 261037 (Auchenoglanididae), (F) *Auchenoglanis occidentalis*, AMNH 240922 (Auchenoglanididae), (G) *Notoglanidium pallidum*, AMNH 258979 (Auchenoglanididae). Schematic representation in sagittal view of the main components of (D) "typical" catfish olfactory chamber and (H) derived auchenoglanidid system.

the main olfactory chamber (MOC) that function to facilitate the active pumping of water into and over the olfactory rosette and chamber (Holl and Meinel, 1968; Zeiske et al., 1976; Theisen, 1982; Theisen et al., 1991; Cox, 2008). Pumping odorants into the MOC increases efficiency of odorant transport and ensures odorant molecules are consistently and specifically distributed over the olfactory epithelium (OE; Howard et al., 2013). The olfactory epithelium is also variously distributed over the surface of rosettes or lamellar arrays, which may include secondary or tertiary folds (Hansen and Zielinski, 2005; Cox, 2008).

Through histology, CT scanning, and electron microscopy, the present study has revealed a series of soft tissue (Fig. 1E–G) and osteological (Fig. 2) specializations of the olfactory chamber that, based on comparative dissection and CT scanning of representatives of all auchenoglanidid genera, uniquely characterize the Auchenoglanididae. Interestingly, many features of the architecture of the auchenoglanidid olfactory chamber and associated structures appear to mirror those predicted by Cox (2008) to function in flow augmentation into and around the olfactory chamber and to maximize the entrainment of odorants over the olfactory epithelium. In this study, a description of the gross anatomy and histology of the olfactory chamber and associated structures in the representative auchenoglanidid catfish *Parauchenoglanis punctatus* is provided and contrasted with the seemingly less specialized system of non-auchenoglanidid catfishes.

Auchenoglanidids are a small component of the African catfish fauna currently estimated at 20+ species in three genera (Fricke et al., 2024), yet the group has a complex taxonomic history. Mo (1991) removed them from the Afro-Asian Bagridae and established the family Claroteidae to accommodate two subfamilies, Auchenoglaninae and Claroteinae. Although morphological evidence for the monophyly of each proposed subfamily is strong, support for the monophyly of the family is less convincing, and subsequent morphological studies have challenged claroteid monophyly (de Pinna, 1993; Diogo, 2004). Sullivan et al. (2006) undertook a Sanger sequenced molecule-based investigation of higher-level relationships among catfishes and found strong support for the monophyly of both the Auchenoglaninae and Claroteinae, but Claroteidae was rendered paraphyletic by the placement of Auchenoglaninae as sister to a

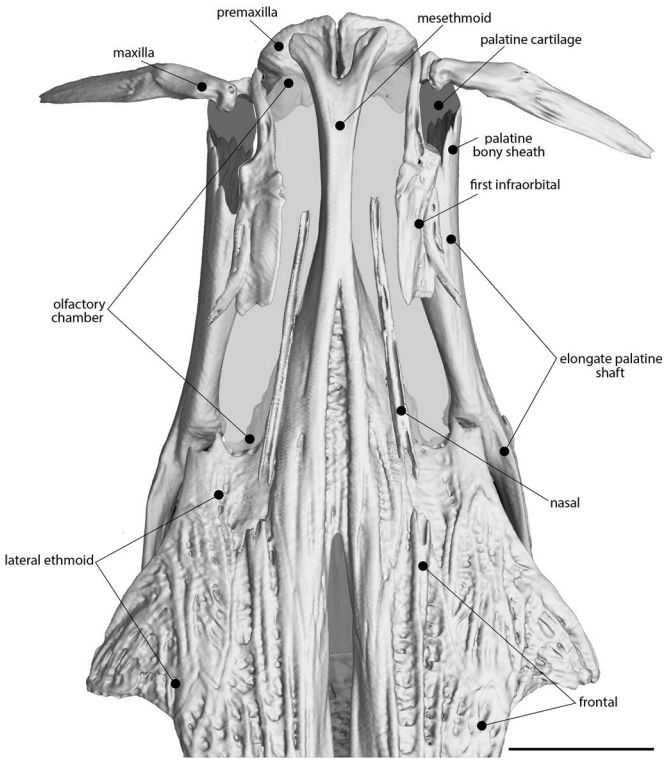

Fig. 2. Main osteological components supporting the auchenoglanidid olfactory chamber, represented here by *Parauchenoglanis ngamensis* (AMNH 255274). Scale bar = 3.5 mm.

clade comprised of the African schilbeids + Claroteinae. A subsequent study by Lundberg et al. (2007) resolved the meso-American Chiapas catfish (*Lacantunia enigmatica*: Lacantuniidae) as the sister to the Claroteinae (= Claroteidae), with Auchenoglaninae (= Auchenoglanididae) resolved as sister to that clade. Here we incorporate examination of the olfactory anatomy in a range of catfishes to assess the distribution of the derived olfactory features reported here as diagnostic for the Auchenoglanididae.

MATERIALS AND METHODS

Specimens of *Parauchenoglanis punctatus* (AMNH 251825) were collected from the Ngampoku River (Mbali River), Democratic Republic of the Congo (2°24′53.7″S, 16°30′55.7″E). Initial anatomical information was gathered by dissection of museum specimens, formalin fixed and preserved in 70% ethanol.

Two specimens of *Parauchenoglanis punctatus*, both from AMNH 251825, were stained in 0.3% phosphotungstic acid (PTA; Metscher, 2009) and CT scanned at California State Polytechnic University Humboldt, using a Nikon XTH 225 microCT scanner. The smaller specimen (11.6 cm total length [TL]) was designated AMNH 251825A, and the larger specimen (12.4 cm TL) was designated AMNH 251825B, for purposes of differentiation throughout this paper. Both specimens were scanned using a tungsten target. The head of AMNH 251825A was scanned at 76 kV and 171 μA at a voxel size of 9.495 μm, generating 4,476 projections. The head of AMNH 251825B was scanned at 81 kV and 172 μA with a voxel size of 15.998 μm, also generating 4,476 projections.

Anatomical structures were then segmented in *Mimics* software (version 24, *Materialise*). Image stacks and reconstructions of olfactory rosettes are available via MorphoSource under project number 00000C925. A series of measurements of the dimensions of the olfactory rosette, and aspects of the olfactory chamber were made by measuring both histological sections and CT-scanned individuals. Measurements of the relative volume of each olfactory sac and rosette were calculated using Meshlab (Cignoni et al., 2008) using a convex hull filter to create a volume enclosing the rosette and another enclosing the olfactory sac.

Details of the ultrastructure of the olfactory rosette and lamellae were investigated under scanning electron microscopy using Hitachi S-4700 Field Emission system (AMNH) and FEI Quanta 250 (Cal Poly Humboldt) microscopes.

Serial histological sections were used to assess the placement and extent of ciliated sensory surfaces and other relevant anatomy. Three specimens of *P. punctatus* AMNH 258089 were prepared using the following methods: Each was individually cut into three segments (head, midsection, and caudal region), and these were decalcified using RDO Rapid Decalcifier Solution (aqueous acidic decalcifier, APX Engineering Products). Midsection and caudal region were stored for future study. Each head was then dehydrated in ethanol and embedded in Paraplast (Sigma Aldrich) and mounted on blocks either for transverse or longitudinal sectioning according to protocol modified from Humason (1962). Specimens were then serially sectioned at 10 μm, using a microtome. The sections were mounted, in sequence, on glass slides that were passed through a stain regime of Gill's hematoxylin and eosin Y (Humason, 1962), according to staining protocol developed by John O. Reiss (pers. comm., modified from Humason, 1962). Slides were then photographed in series and in detail for measurement using FIJI/ImageJ (Schindelin et al., 2012).

RESULTS

***Olfactory anatomy of* Parauchenoglanis punctatus**.—Dissection, CT scanning, and study of *Auchenoglanis biscutatus* (AMNH 230631), *A. occidentalis* (AMNH 240922), *Notoglanidium macrostoma* (AMNH 251807), *N. pallidum* (AMNH 258979), *N. pembetadi* (AMNH 243475), *N. thomasi* (AMNH 276323), *Parauchenoglanis balayi* (AMNH 258806), *P. cf. balayi* (AMNH 261037), *P. ngamensis* (AMNH 255274), *P. pantherinus* (AMNH 258980), and *P. punctatus* (AMNH 251825, AMNH 258089) revealed similar gross olfactory anatomy across auchenoglanidids. Externally, auchenoglanidids possess prominent tubular anterior incurrent nostrils located in the upper lip near the mouth, and posterior slit-like, valved excurrent nostrils on the dorsum of the head, posteriorly displaced and located about halfway between the eye and the anterior margin of the snout (Fig. 1E–G). These excurrent nostrils are inferred to facilitate unidirectional flow based on the presence of a connective tissue sheath with a median slit—water can actively be forced out through the median slit, but is not able to back-flow into the olfactory chamber.

Internally, the olfactory chamber houses a notably elongate olfactory rosette of pseudostratified epithelium and a ventroposterior accessory sac. All examined auchenoglanidids have an opening posterior to the rosette that is

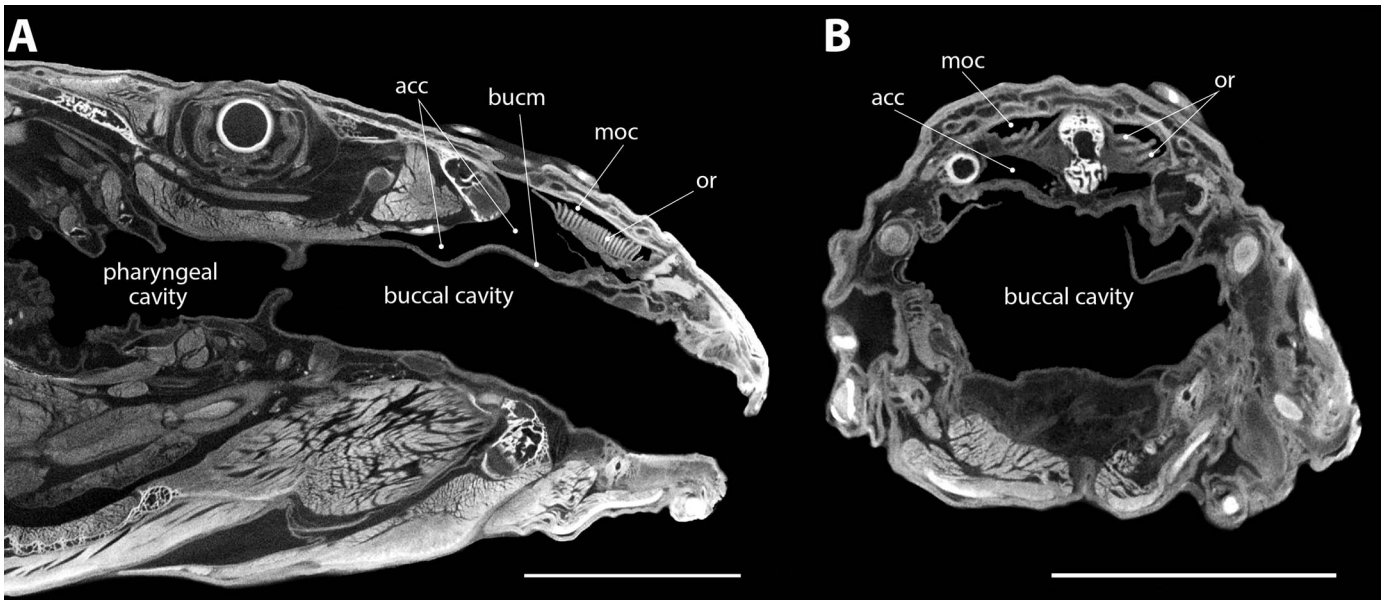

Fig. 3. PTA-stained *Parauchenoglanis punctatus*, AMNH 251825. (A) Sagittal section through the buccal cavity and rosette showing the main olfactory chamber (moc) and accessory (acc) sac, the buccal and pharyngeal cavities, and the rosette suspended above the accessory sac on a thin membrane. (B) Coronal section through the olfactory region and buccal cavity, showing the rosettes within the MOC dorsal to the accessory (acc) sac. Abbreviations: bucm = buccal membrane; or = olfactory rosette. Scale = 5 mm.

contiguous with a ventrally located accessory sac. Beneath the rosette, an open space extends for approximately 1/3 the length of the rosette, confluent with the accessory sac (Figs. 3, 4) but for a very fine, flexible membrane. The rosette curves gently, becoming concave toward the medial part of the head, with an opening to a large accessory sac at the ventroposterior margin of the rosette's subtending membrane (Fig. 5A). We call the anterior portion the 'antechamber' space, since it is separated from the accessory sac by that thin membrane.

As in other teleosts, the number of primary lamellae varies with body size (Olivares and Schmachtenberg, 2019). The largest specimen examined in this study (12.4 cm TL) had 46 pairs of primary lamellae, while the smaller specimen (11.6 TL) had 35 pairs. In *P. punctatus*, the lamellae are shorter at the proximal and distal ends of the rosette and longer toward the center. At the end of each primary lamella, the rounded tip slopes downward and tapers to join the wall of the MOC, with ciliated olfactory neurons lining that most distal part of the lamella (Fig. 5B).

The volumetric ratio of olfactory chamber to rosette in AMNH 251825A is approximately 10:1, specifically a volume calculated in Meshlab of 10.61 mm^3 (chamber) to 1.07 mm^3 (rosette). This measure includes the size of the entire chamber, inclusive of the accessory sac, compared with the rosette itself sitting atop a thin membrane. In Meshlab, the surface area of the rosette of the specimen was calculated as 34.18 mm^2. The accessory sac extends underneath 30.9–34.7% of the posterior portion of the rosette, calculated by measuring the length of the space ventral to the suspensory membrane.

The raphe and dorsal apex of the primary lamellae are bare of cilia, and actin ridges are present on their surface (Fig. 5E). In more dorsal regions the lamellae are covered by extremely dense cilia of non-sensory epithelial cells (nse; Fig. 5C), obscuring much of the epithelial surface. Non-sensory cilia measured near the raphe are 6.8–6.9 μm long. Water-propelling cilia in other taxa are typically 10–20 μm in length while mucus-propelling cilia tend to be considerably shorter (Sleigh, 1989; Cox, 2008). In view of their intermediate length, and the absence of obvious mucus or goblet cells, we suggest that these cilia in *P. punctatus* are likely functioning in water propulsion over the lamellae rather than mucus transport. Distal and ventral to the non-sensory epithelium, two types of olfactory receptor neurons (ORNs) are present: Microvillous cells are located at the base of the rosette and near the apex of the raphe (mn), while ciliated cells (cn) are present on the lamellae (Figs. 4C–D, 5B, D, F). Ciliated sensory cells are absent from the primary and secondary raphe of the rosette, appearing only distally on the apical surface of the primary lamellae and in the folds between the lamellae (Figs. 4C–E, 5B, D, F), where their average cilia length was calculated to be 1.08 μm. At the margins of this dense mat of ciliated cells, as well as distributed sporadically within, microvillar receptor neurons are visible (Fig. 5D, F) and are present, without neighboring ciliated cells, at the deepest part of the lamellar folds (Fig. 4C, D, E). No goblet cells were observed in the epithelium, and the identity of crypt or supporting cells was not readily discernible from histology or scanning electron microscopy, though future investigations with transmission electron microscopy could confirm the identity of the few putative crypt cells identified (Fig. 4D, E, arrowheads).

Consistent with recent observations in tetras (Characiformes; Pintos et al., 2020) and other teleosts (Zeiske et al., 1994), the epithelium is regionalized across the surface of the lamellae: Olfactory receptor cells are likely intermixed with non-sensory cilia in the medial zone of the lamellae, while the proximal zone (at the base of the folds) is exclusively sensory (in this case, dominated by microvillar receptor cells), and the apex (Pintos et al.'s "distal zone") accommodates the non-sensory ciliated cells.

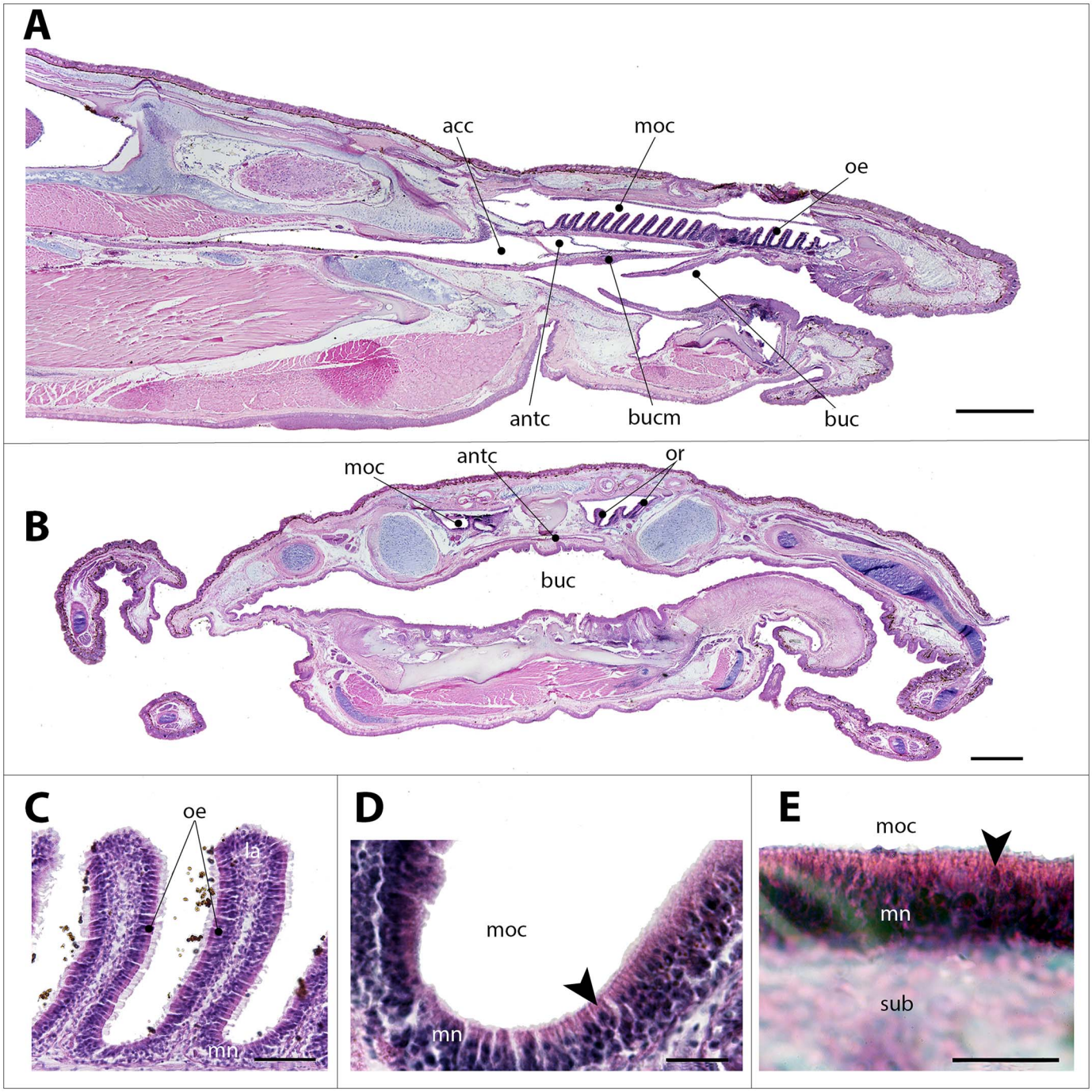

Fig. 4. Histological sections through the nose of *Parauchenoglanis punctatus* (AMNH 258089), H&E stain. (A) Longitudinal section through the left main olfactory cavity (moc), showing the olfactory epithelium (oe) covering the rosette, and the ventroposterior accessory sac (acc) adjacent to the anterior chamber (antc) subtending the membrane on which the rosette sits above the buccal membrane (bucm). Scale = 0.5 mm. (B) Cross section through the head showing the buccal cavity (buc), roughly midway down the length of the olfactory rosette (or), which, at this point, is still only above the anterior chamber (antc). Scale = 0.5 mm. (C) Longitudinal section through the olfactory rosette showing structure of the olfactory epithelium (oe) covering primary lamellae (la), note long and very dense cilia on the lamellae, while at the base of the lamellae, cilia are largely absent and replaced by what appear to be microvillar sensory neurons (mn). Scale = 0.05 mm. (D) Cross-sectional view of the base of lamellar folds, showing microvillar receptor neurons (mn) and a possible crypt cell (arrowhead, though at an unusual position in the rosette). Scale = 0.02 mm. (E) High magnification view of the microvillar receptor cell "patch" at the base of the lamellae, with another possible crypt cell (arrowhead), and the subtending submucosa (sub). Scale = 0.02 mm.

DISCUSSION

Novel nasal anatomy of the Auchenoglanididae.—The order Siluriformes is highly diverse both morphologically and ecologically, and varying groups of catfishes have evolved disparate approaches to the problem of odorant capture and entrainment. For example, the Channel Catfish, *Ictalurus punctatus* (Ictaluridae), a model organism in studies of vertebrate olfaction, has an elongate rosette with 60+ lamellae,

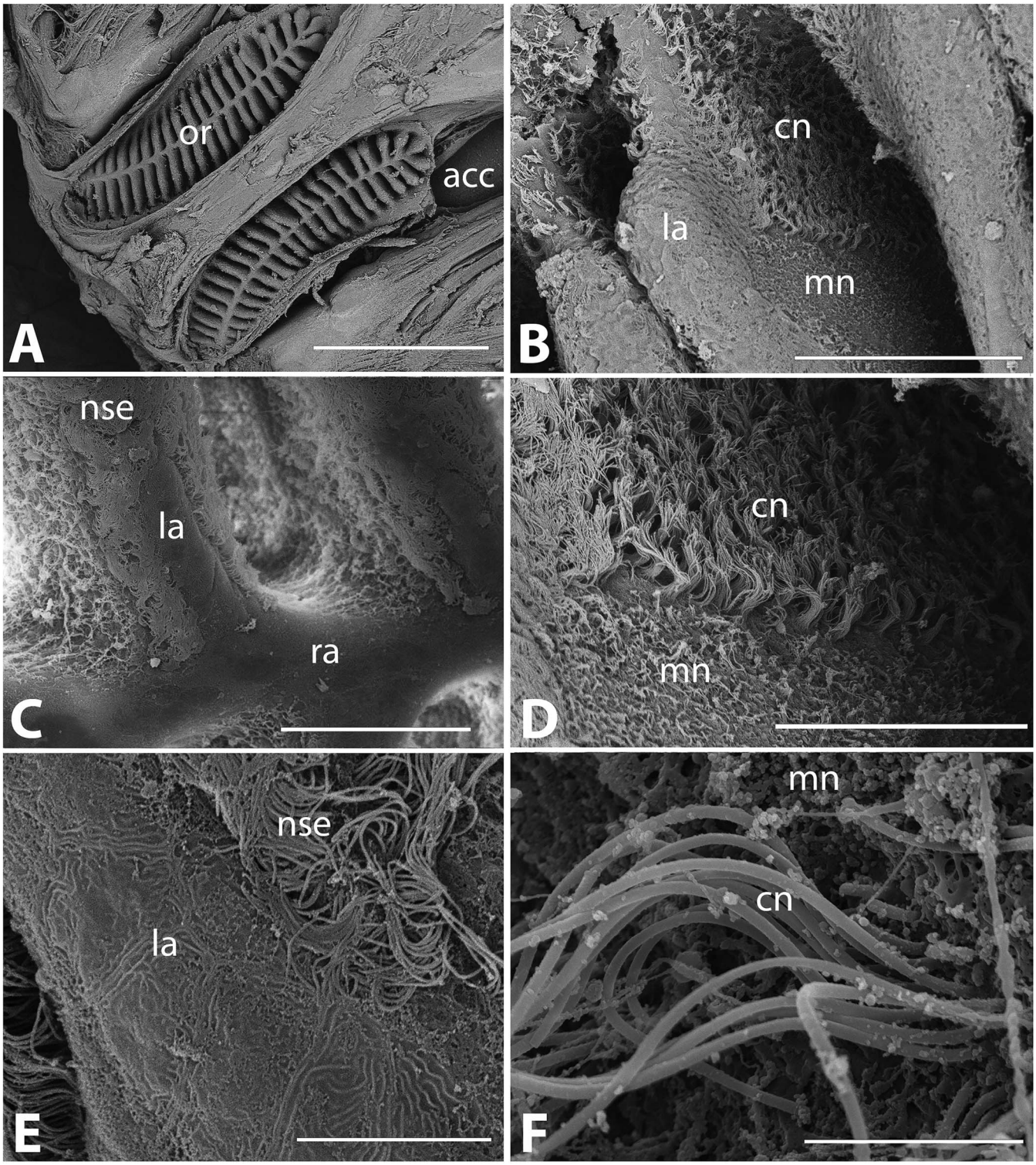

Fig. 5. Scanning electron micrographs of the surface of the olfactory rosette (*Parauchenoglanis punctatus*, AMNH 258089). (A) Dorsal surface of the snout, with integument dissected from the dorsum of the main olfactory cavity (MOC) to reveal the olfactory rosette (or) and opening to the accessory sac (acc). Scale = 1 mm. (B) Distal end of a primary lamella (la) where it joins the wall of the MOC, region of microvillar neurons (mn) in the proximal fold, dense ciliated neurons (cn) lining the lamella near its junction with the periphery of the MOC. Scale = 100 μm. (C) Surface of the rosette where lamellae (la) branch from the raphe (ra), showing lack of cilia on the apical surface and dense non-sensory epithelium (nse) at distal ends of the dorsal surface of lamellae. Scale = 50 μm. (D) Close-up of the region shown in B, with extremely dense ciliated neurons (cn) covering the surfaces of the lamellar folds, adjacent to microvillar neurons (mn). Scale = 5 μm. (E) Higher magnification of actin microridges on the surface of the lamella (la) adjacent to non-sensory epithelium (nse). Scale = 10 μm. (F) Region within sensory epithelium, at highest magnification, showing clear microvillar cells (mn) adjacent to the ciliated cells (cn). Scale = 3 μm.

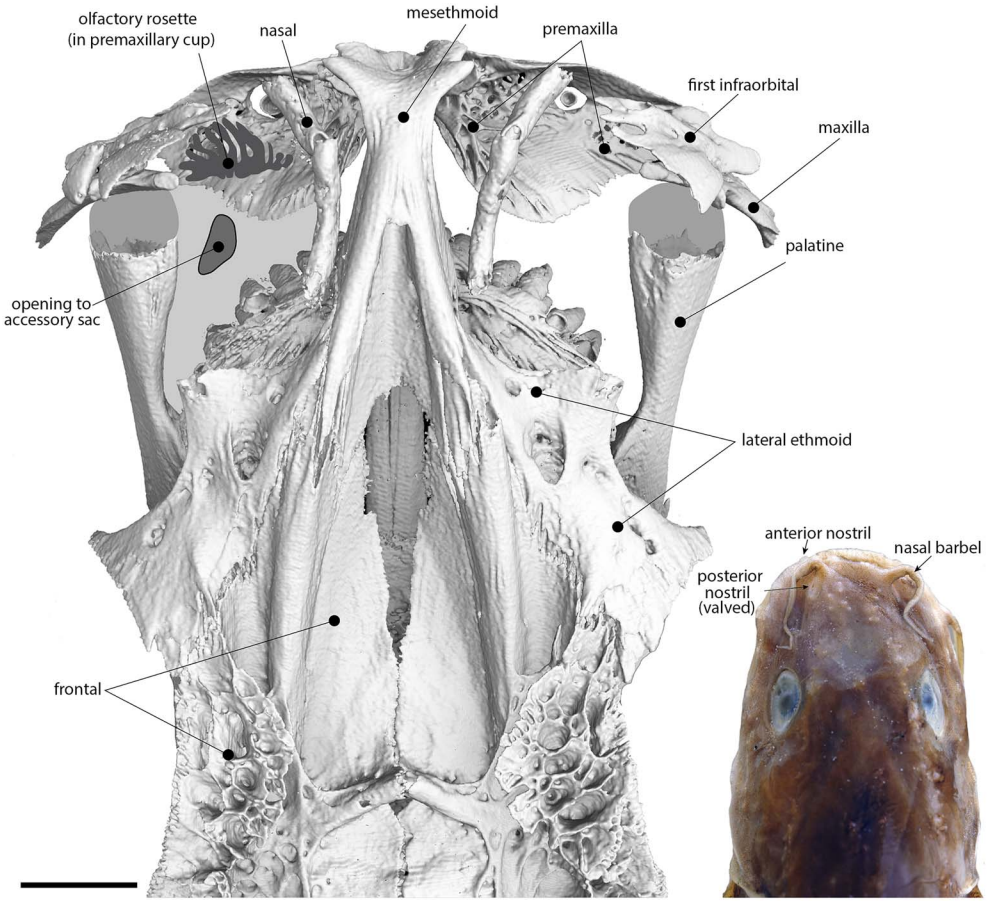

Fig. 6. Main components of the olfactory anatomy of *Plotosus lineatus* (AMNH 35804). Scale bar = 4 mm.

with water flow through the MOC driven by the beating of 15–20 μm cilia in the non-sensory epithelium (Cancalon, 1978; Caprio and Raderman-Little, 1978). In the Hardhead Sea Catfish, *Ariopsis felis* (Ariidae), an elongate rosette, similar that of auchenoglanidids, has an array of 40 lamellae, but flow is externally induced primarily by a Pitot mechanism in which water enters through a funnel-like anterior nostril perpendicular to the flow of water traveling over the fish's head, in addition to beating of cilia of the sensory epithelium (Zeiske et al., 1994; Cox, 2008). While only distantly related to auchenoglanidids (Lundberg et al., 2007; Schedel et al., 2022), the Striped Eel Catfish, *Plotosus lineatus* (Plotosidae), has an olfactory system incorporating an accessory sac. Entrance to the sac is located just distal to a compact olfactory rosette which bears just 10–12 lamellae. Unlike the configuration in auchenoglanidids, in *Plotosus* the rosette is located anteriorly in a bony cup formed by expansion of the anterodorsal face of the premaxilla (Fig. 6) and not suspended above the floor of the olfactory chamber as in auchenoglanidids (Fig. 1H). According to Yamamoto and Ueda (1978a) and Theisen et al. (1991), water flow in *Plotosus* is likely driven entirely by expansion and contraction of the accessory sac.

As in other teleosts, in catfishes the paired olfactory chambers are located dorsally on the snout, and each chamber communicates with the exterior via an anterior incurrent and posterior excurrent nostril. However, as noted by Mo (1991), auchenoglanines (= auchenoglanidids) are unique among catfishes in possessing tubular anterior nostrils displaced far forward on an elongate snout and located on the anteroventral margin of the upper lip (Fig. 1E–G). These tubular nostrils serve to project the opening of the olfactory chamber directly into the boundary layer, reducing a major barrier to odorant transfer into the MOC (as the nonuniform flow of fluid in the boundary layer delays odorant detection in fishes), and the anterior position of these nostrils is also the area of the fish with the thinnest boundary layer (Denny, 1993). The nostrils' slight lateral displacement may additionally serve to increase directionality of the fishes' sense of olfaction (Cox, 2008). Interestingly, the bichir *Polypterus* also appears to have a Pitot-tube-like extension of the anterior naris (Settles, 2005), though its function has yet to be fully investigated.

Auchenoglanidid posterior nostrils are slit-like and positioned posteriorly, midway between the anterior nostrils and the eyes and each has a pair of lip-like valves opposed in the midline and occluding the opening, and a nasal barbel, when present, is reduced to a small bulb on the anterior margin of the posterior nostril. The roof of the elongate and capacious olfactory chamber is bounded dorsally by the nasal and first infraorbital (lachrymal), and supported laterally by greatly elongated palatine shafts, and medially by an equally elongated mesethmoid (Fig. 2). Ventrally, the entire chamber is bounded by the dense mucosa of the buccal cavity (Fig. 3C). The elevation of the olfactory rosette on a suspensory membrane, well above the floor of the olfactory chamber is, as far as we have been able to ascertain, also a unique feature diagnostic of this catfish family. The main features of the auchenoglanidid system are schematically

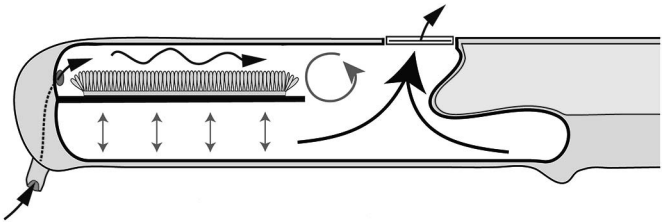

Fig. 7. Hypothetical model of water flow through the auchenoglanidid olfactory chamber.

represented in Figure 1H and contrasted with the more "typical" system found in most catfishes examined (Fig. 1D; see also Zeiske et al., 1994).

The markedly elongate rosette and longitudinally arrayed series of olfactory lamellae extends the surface area and decreases the distance between odorants and the olfactory epithelium, resulting in more efficient capture of odorants by olfactory receptor neurons (Cox, 2008). Little mucus was present in the fishes prepared for histology, and goblet cells were not observed within the olfactory epithelium, so movement of the numerous cilia more likely moves water over the lamellae rather than mucus (Cox, 2013; Howard et al., 2013).

Fishes with especially acute olfaction have a continuous lamellar sensory area, as observed here in *Parauchenoglanis*, while fishes that rely more on vision than olfaction have dispersed sensory areas without many non-sensory cilia (Hara, 1975). In halfbeaks, killifishes, and silversides (Atheriniformes), no ciliated non-sensory cells have been observed; instead, they have densely packed receptor cells that form islands of sensory epithelium with intervening indifferent epithelium (Yamamoto and Ueda, 1978b). No such "islands" are apparent in *Parauchenoglanis*, but "indifferent epithelium" (non-sensory cells) is regionalized along the surface of the rosette, with sensory cells further regionalized into ciliated and microvillar regions in the folds of the rosette.

Muscular contractions have been reported in some other teleosts as a means of olfactory sampling (Døving et al., 1977; Nevitt, 1991). Where present, accessory sacs likely expand and contract to circulate water over the olfactory lamellae; in the black scabbardfish, *Aphanopus carbo* (Trichiuridae), the mechanism of accessory sac expansion is hypothesized to be movements of the buccal jaws (Holl and Meinel, 1968; Cox, 2008; Howard et al., 2013). Pumping of the accessory sac agitates the olfactory chamber in flatfish, and respiration agitates the MOC of sticklebacks (Solger, 1894; Theisen, 1982; Nevitt, 1991) and European perch (Liermann, 1933). Mechanical agitation of the olfactory chamber is also reported in snakeheads (Channidae; Burne, 1909; Kapoor and Ojha, 1973), where an accessory sac extends beneath the MOC, as in auchenoglanidids. In the catfish *Sisor rabdophorus* (Sisoridae; Ojha and Kapoor, 1974), movement of the palatine during respiration agitates the MOC, without involvement of any accessory sac. In these fishes, similar to auchenoglanidids, valves have been described as thin lips on the posterior nostril (Burne, 1909) facilitating unidirectional flow through the chamber.

Based on the anatomy of the olfactory apparatus in auchenoglanidids, we hypothesize that the large accessory sac and suspensory membrane may aid auchenoglanidids in

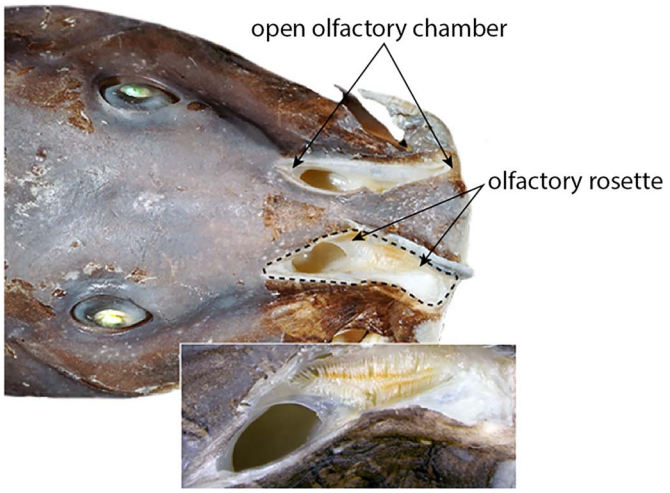

Fig. 8. Head and snout in dorsal view of a sexually mature male specimen of *P. cf. balayi* (AMNH 261037) from the Louesse River (Republic of Congo). The specimen is undissected: the main olfactory chamber and rosette are exposed, both nostrils are absent.

'sniffing' as suggested for the trichiurid *Aphanopus*. Essentially, as a co-option of a generalized suction-feeding mechanism (Westneat and Olsen, 2015), auchenoglanidids, with closed buccal jaws, may rapidly expand the buccopharyngeal chamber, thereby creating a negative pressure in the MOC and "sniffing" water in through the tubular anterior nostrils. Water would then be entrained over the olfactory rosette, moving posteriorly and forming small eddies where the rosette ends, before being forced out of the posterior nostril by contraction of the buccopharyngeal cavity (Fig. 7).

Potential for sexual dimorphism.—Sexual dimorphism in the olfactory apparatus of fishes has been described in numerous taxa: Ceratioidei (Bertelsen, 1951, 1980), Eurypharyngidae (Nielsen and Bertelsen, 1985), Lophiidae (Caruso, 1975), Monognathidae (Bertelsen and Nielsen, 1987), Myctophidae (Martin and Smith, 2024), and Stomiiformes (Marshall, 1967), groups which spend much of their lives in darkness and therefore rely heavily on olfaction for mate and food resources. Muted sexual dimorphism has also been noted in several families within the Characiformes, wherein males have larger nostrils and more lamellae on the olfactory rosettes than do females (Abrahão et al., 2019). In a population of *Parauchenoglanis cf. balayi* from the Louesse River (Republic of Congo), large mature males develop a seemingly degenerate olfactory system (Fig. 8), in which the MOC is entirely open to the surrounding water and the rosettes are smaller, and with fewer and less well-organized lamellae than those of females from the same river system (Fig. 1E; Stiassny and Mamonekene, 2013). As the larger specimens examined in the present study are both female, and if divergence of olfactory anatomy occurs on a similar ontogenetic timeline to that observed in *P. cf. balayi*, then this reduction would not yet have occurred in the small *P. punctatus* that we assessed microscopically. Dissection and study of additional specimens is therefore warranted to determine whether sexual dimorphism of the olfactory apparatus occurs in other members of the genus, or among auchenoglanidids generally.

MATERIAL EXAMINED

Amphiliidae: *Amphilius zairensis* (AMNH 240442), *Paramphilius baudoni* (AMNH 264254).

Auchenoglanididae: *Auchenoglanis biscutatus* (AMNH 230631), *A. occidentalis* (AMNH 240922), *Notoglanidium macrostoma* (AMNH 251807), *N. pallidum* (AMNH 258979), *N. pembetadi* (AMNH 243475), *N. thomasi* (AMNH 276323), *Parauchenoglanis balayi* (AMNH 258806), *P. cf. balayi* (AMNH 261037), *P. ngamensis* (AMNH 255274), *P. pantherinus* (AMNH 258980), *P. punctatus* (AMNH 251825, AMNH 258089).

Bagridae: *Bagrus caeruleus* (AMNH 247170), *B. ubangensis* (AMNH 256256).

Clariidae: *Clarias fuscus* (AMNH 10213), *C. gariepinus* (AMNH 235815), *Dinotopterus cunningtoni* (CU 82794), *Gymnallabes typus* (AMNH 246688), *Platyallabes tihoni* (AMNH 249975).

Claroteidae: *Chrysichthys cranchii* (AMNH 241154), *C. nigrodigitatus* (AMNH 275433), *C. punctatus* (AMNH 227599), *C. velifer* (AMNH 59407), *Rheoglanis dendrophorus* (AMNH 245532).

Diplomystidae: *Diplomystes chilensis* (AMNH 55328).

Ictaluridae: *Ameiurus nebulosus* (AMNH 254336), *Ictalurus punctatus* (AMNH 80538).

Malapteruridae: *Malapterurus microstoma* (AMNH 249889), *Paradoxoglanis caudivittatus* (AMNH 274090).

Mochokidae: *Atopodontus adriaensi* (AMNH 242763), *Chiloglanis congicus* (AMNH 277680), *Euchilichthys boulengeri* (AMNH 251306), *Synodontis contracta* (AMNH 257984).

Pangasiidae: *Pangasius nasutus* (AMNH 57267).

Plotosidae: *Plotosus lineatus* (AMNH 35804).

Schilbeidae: *Schilbe grenfelli* (AMNH 252672), *S. mystus* (AMNH 226448).

DATA ACCESSIBILITY

Image stacks, STL files, and scanning parameters are available on MorphoSource under project ID 00000C925. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

AI STATEMENT

The authors declare that no AI-assisted technologies were used in the design and generation of this article and its figures.

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