Morphology and Histochemistry of the Peripheral Olfactory Organ in the Round Goby, *Neogobius melanostomus* (Teleostei: Gobiidae)

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ABSTRACT This first comprehensive study of the peripheral olfactory organ from a representative of the large and economically important order of teleost fishes, the Perciformes, shows a compact structure with olfactory sensory neurons distributed widely throughout the olfactory chamber. The spatial organization of the nasal cavity in the bottom-dwelling round goby (Gobiidae, *Neogobius melanostomus*) was examined using impression material injection, immunocytochemistry, and transmission electron microscopy. The olfactory chamber contains a single olfactory lamella; prominent dorsocaudal lachrymal and ethmoidal accessory nasal sacs are situated ventrocaudal to the chamber. The location of the olfactory mucosa within the olfactory chamber is novel for teleost fish, as it extends beyond the ventral surface to the lateral and dorsal regions. Microvillar olfactory sensory neurons and ciliated olfactory sensory neurons were identified by transmission electron microscopy and the spatial distribution of these two cell types was assessed through immunocytochemistry against olfactory receptor coupled G-proteins. Both $G_{\alpha olf}$-immunoreactive ciliated olfactory sensory neurons and the $G_{\alpha o}$-immunoreactive microvillar form were located throughout the olfactory epithelium. Ciliated crypt cells were $G_{\alpha o}$ immunoreactive and were found throughout the olfactory epithelium of some specimens. The widespread occurrence of olfactory sensory neurons in the olfactory chamber supports the idea that olfactory signaling is important to the survival of the round goby. The prominence of the lachrymal and ethmoidal accessory nasal sacs indicates the capacity to regulate the flow of odorant molecules over the sensory surface of the olfactory sensory neurons, possibly through a pump-like mechanism driven by opercular activity associated with gill ventilation. J. Morphol. 257:62–71, 2003.

KEY WORDS: $G_{\alpha o}$; $G_{\alpha olf}$; immunocytochemistry; round goby; transmission electron microscopy

The teleost peripheral olfactory organ comprises an anterior naris, olfactory chamber with olfactory sensory neurons (OSNs), and a posterior naris. In many species, accessory nasal sacs assist with water ventilation through the nasal cavity (Burne, 1909; Doving et al., 1977; Melinkat and Zeiske, 1979). During olfactory stimulation, soluble odorants enter the olfactory chamber through the anterior naris and flow over the surface of OSNs, where the molecules interact with G-protein-regulated olfactory receptor proteins. Olfactory mucosa containing the OSNs is typically located on the floor of the olfactory chamber, which is often folded, forming olfactory lamellae (e.g., Kleerekoper, 1969; Hara, 1975). The extent of olfactory lamellar folding within the teleost superorder Acanthopterygii varies considerably from a flat, unfolded surface to a multilamellar rosette with over 100 folds in the morays and eels (Yamamoto, 1982). The large surface area provided by olfactory lamellae enables many OSNs to populate the relatively small teleost olfactory chamber. Fish such as salmonids and cyprinodonts, with multilamellar peripheral olfactory organs, have an acute sense of smell and various aspects of their life history, such as feeding and reproduction, are mediated through olfactory cues (Hara, 1992). On the other hand, in teleosts with a small lamellar surface, OSNs may extend beyond the ventral surface to the lateral and dorsal regions of the nasal cavity. For example, olfactory epithelium extends to the lateral walls of the olfactory chamber of *Spinachia spinachia* (Perciformes; Gasterosteidae) (Theisen, 1982). Some tetrapods, such as amphibians, contain a relatively flat nasal cavity, with olfactory epithelium on the ventral and dorsal surfaces (e.g., *Xenopus laevis*) (Hansen et al., 1998).

In the economically important order Perciformes, the number of olfactory lamellae varies from 0 (*Omobranchus elegans*) to 64 (*Upeneus bensasi*) (Yamamoto, 1982). The gobioid fishes (Perciformes: Gobioidei) are the most speciose of all suborders of bony fishes, with over 2,500 nominal species arranged in at least 300 genera (Miller, 1993). The

Contract grant sponsors: NSERC, the Michigan Great Lakes Protection Fund, and the University of Windsor Faculty of Graduate Studies.

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DOI: 10.1002/jmor.10106

peripheral olfactory organ is unilamellar, with a single fold along the rostrocaudal axis in five species of the family Gobiidae (*Acanthogobius flavimanus, Chasmichthys dolichognathus, Sagamia geneionema, Chaenogobius urotaena, Odontamblyopus rubicundus*) (Yamamoto and Ueda, 1979). Olfaction appears to be important to gobies: in *Gobius niger*, the reaction to food extracts is vigorous (Pipping, 1926, 1927), and in *Bathygobius sporator* sex discrimination and courtship behavior is mediated by olfaction (Tavolga, 1956). The use of pheromonal communication may account for migratory behavior in four riverine Hawaiian gobies, *Lentipes concolor, Stenogobius genivittatus, Awaous guamensis*, and *Sycyopterus stimpsonir* (Sorensen, 2001) and may mediate reproductive behavior in *Gobius jozo* (Colombo et al., 1980). Physiological responses to odorous molecules have been recorded from the nasal cavity, immediately adjacent to the anterior nostril in the round goby *Neogobius melanostomus* (Murphy et al., 2001), implying that OSNs are broadly distributed in the nasal cavity of these fish.

Two morphological forms of OSNs, ciliated and microvillar, have been distinguished in the peripheral olfactory organ of teleost fish through identification of the cilia or microvilli extending from the apical olfactory knob on the OSN mucosal surface (e.g., Evans et al., 1982; Zielinski and Hara, 1988; Hansen et al., 1999). A third receptor form, the crypt cell, is also present in several species (Hansen et al., 2001). Ciliated OSNs have a slender, tall dendrite and perikaryon in the lower region of the olfactory epithelium, whereas the dendrites of microvillar OSNs are shorter, with the perikaryon in the superficial region of the olfactory epithelium (Morita and Finger, 1998). Vertebrate OSNs contain heteromeric guanine nucleotide-binding regulatory proteins (G-proteins) that mediate signaling from a large number of diverse olfactory receptor proteins. The G-protein, $G_{\alpha olf}$, is expressed by OSNs in mammals (Jones and Reed, 1989), amphibians (Mezler et al., 2001), and ciliated OSNs in teleosts (Abogadie et al., 1995). Sensory neurons in the terrestrial vertebrate chemical sensing organ, the vomeronasal organ, express the G-protein $G_{\alpha o}$ (e.g., Jia and Halpern, 1996) as do microvillar OSNs in catfish and goldfish (Hansen et al., 2001). Therefore, $G_{\alpha olf}$ immunoreactivity of ciliated OSNs and $G_{\alpha o}$ immunoreactivity of microvillar OSNs may be useful for determining if these cells extend beyond the ventral surface of the olfactory chamber in Gobiidae.

In some fish the opening and closing of the mouth causes the pumping action of accessory nasal sacs, which aid in the flow of water through the nasal cavity (Parker, 1910; Pipping, 1926; Liermann, 1933; Melinkat and Zeiske, 1979; Nevitt, 1991). The occurrence of accessory sacs in fish has long been associated with a semisedentary life (Kyle, 1899) and may be necessary for unidirectional water flow across the olfactory chamber in stationary bottom-dwelling fish (Burne, 1909; Kapoor and Ojha, 1972). Fish with accessory sacs for the pumping of water through the olfactory organ have previously been designated cyclosomates and fish using only the beating of ciliated cells were termed isomates (Doving et al., 1977). In cyclosomates, ciliary beating and accessory sac ventilation likely have a cooperative role for moving odorant molecules over the olfactory epithelium. Cyclosomate teleosts have either one or two accessory nasal sacs that may either be linked or fused, such as those observed in Pleuronectiformes (Kyle, 1899; Burne, 1909; Chabanaud, 1927; Leirmann, 1933; Kleerekoper, 1969; Applebaum and Schemmel, 1983; Webb, 1993) or distinct, separate structures such as those found in many of the Perciformes (Burne, 1909; Sinha and Sinha, 1990). Species in two families of Perciformes, Trachinidae and Scombridae, contain single accessory nasal sacs (Burne, 1909). When two accessory nasal sacs are present, they generally follow the ethmoidal and lachrymal bone structure of the skeletal system and are thus designated the ethmoidal and lachrymal sacs after Burne's (1909) nomenclature or the dorsomesial and ventromesial sacs (Kapoor and Ojha, 1973). In *Spinachia spinachia* (Gasterosteidae), the lachrymal sac was larger than the ethmoidal sac and both sacs were lined with nonsensory epithelium (Theisen, 1982). There have been no studies on the occurrence of accessory nasal sacs in fish belonging to the Gobiidae family.

In this study, the organization of the round goby nasal cavity, including the accessory sacs, was examined through the use of vinyl polysiloxane impression material. Acetylated tubulin immunocytochemistry was used to examine the extent of ciliation in the nasal cavity. Ciliated and microvillar OSNs were identified by transmission electron microscopy and the spatial distribution of these cells was determined through immunocytochemistry against the G-proteins, $G_{\alpha lf}$ and $G_{\alpha o}$.

The round goby is native to the Ponto-Caspian Region and has colonized the Laurentian Great Lakes since its recent arrival through ballast water transfer by trans-Atlantic ships (Charlebois et al., 2001). This bottom-dwelling teleost has successfully established robust populations that have challenged native fishes for habitat and food resources (Jude et al., 1995). The round goby displays reproductive behavior that may be mediated through intraspecific communication by pheromones. For example, the male occupies a nest chamber, defends it against intrusion by predators, and allows gravid females to enter for spawning (Miller, 1984; MacInnis and Corkum, 2000). Physiological studies have shown that steroidal putative pheromones evoke electro-olfactogram responses from the anterior region of the nasal cavity (Murphy et al., 2001). However, further physiological and behavioral studies have been hampered by lack of information on the spatial organization of the nasal cavity in gobies. For exam-

ple, knowledge about OSN distribution will help with the application of olfactory sensory deprivation during behavioral studies. Therefore, the objective of this study was to determine the morphology and spatial organization of the peripheral olfactory organ in the round goby.

MATERIALS AND METHODS

Reproductive adult male round gobies, *Neogobius melanostomus* (Pallas 1811), were collected between August 2000 and August 2001 by angling and trawling in the Canadian waters of the Detroit River and the western basin of Lake Erie. The animals were between 90–160 mm long and weighed between 10–30 g. All animals were maintained under a constant photoperiod (16L:8D) in holding tanks with a flowing dechlorinated tapwater system (10–15°C) at the University of Windsor holding facilities. Round goby diets consisted of Nutrafin® fish flakes and zebra mussels (*Dreissena polymorpha*).

Nasal molds

Reprosil®, hydrophilic vinyl polysiloxane impression material, was used to make solid molds of the round goby peripheral olfactory organ. The orange base was initially diluted with acetone before adding the solidifier. The impression material was injected into the posterior nostril of an anesthetized fish (MS-222) using a 23-gauge needle. It was allowed to set for 10 min and the mold was carefully dissected from the nasal cavity and viewed using a dissecting microscope.

Immunocytochemistry

Round goby tissues were prepared for immunocytochemistry by cardiac perfusion fixation using 6.3% saline followed by Zamboni's fixative (2% paraformaldehyde, 1.2% picric acid, 0.1 M phosphate buffer, PB) or 4% paraformaldehyde (diluted 50 mL dH_2O and 50 mL 0.2 M PB, pH 7.4) injections in to the nostril; gross dissection of the peripheral olfactory organ followed. The tissue was frozen after cryoprotection using a sucrose gradient and sectioned using a cryostat. For fluorescence immunocytochemistry, sections were immersed for 10 min in cold 0.1 M phosphate buffered saline (PBS), followed by cold acetone, and then washed in cold 0.1 M PBS again, followed by blocking in normal goat serum (0.25%) for 15 min. Tissue was then incubated in primary antibodies against acetylated tubulin (antimouse acetylated tubulin 1:2,000; Sigma, St. Louis, MO, USA), $G_{\alpha olf}$, and $G_{\alpha o}$ (antirabbit; diluted 1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight. This was followed by three 10-min washes in cold 0.1 M PBS. The secondary antibody (Alexa 488 antirabbit and Alexa 488 antimouse IgG; diluted 1:100; Molecular Probes, Eugene, OR, USA) was applied for an hour followed by three 10-min washes in cold 0.1 M PBS and mounted in Vectashield® (Vector Labs, Burlingame, CA). Whole mounts were performed by removing the ventral surface of the nasal cavity and were stained as described above, without cryoprotection. Fluorescence microscopy (postacquisition software: Northern Eclipse) and confocal microscopy (BioRad MRC 1024, Hercules, CA) were used to obtain images. Changes in tissue processing for DAB immunocytochemistry included the use of the ABC Elite Kit (rabbit IgG for G protein antibodies) and DAB Fast tablets with metal enhancers (Sigma Chemical). These sections were dehydrated, mounted in Permount®, then viewed with a Zeiss Axioskop FS.

Spatial Analysis of $G_{\alpha olf}$ and $G_{\alpha o}$ Immunoreactivity

The distribution of olfactory epithelium in the olfactory chamber was assessed from 20 specimens that were serially sectioned in the vertical plane and prepared for $G_{\alpha olf}$ and $G_{\alpha o}$ immunocytochemistry.

Transmission Electron Microscopy

Cardiac perfusion using saline (6.3%) followed by Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, in 0.07 M cacodylate buffer, 0.25% $CaCl_2$) was performed on round gobies anesthetized with MS-222. The nostrils were removed and stored overnight in Karnovsky's fixative, subsequently immersed in 1% osmium tetroxide buffered with cacodylate buffer (pH 7.4), and dehydrated using ice-cold ethanol, followed by propylene oxide infiltration. Tissue was embedded in an epoxy resin, sectioned into 90 nm, and stained with 2% uranyl acetate. Transmission electron microscopy (Philips EM201) was used to view ciliated and microvillar OSNs.

RESULTS

In the round goby, the nasal cavity is narrow at the tubular anterior naris and wider towards the posterior, with two bulbous-shaped accessory nasal sacs adjacent to the posterior nostril (Fig. 1A,B). The ethmoidal sac is positioned at the medial axis and the lachrymal sac is located laterally (Fig. 1B). Both have a bulbous appearance and are connected by duct-like passages to the olfactory chamber. Ciliated epithelium covers the entire dorsal, lateral, and ventral surfaces of the olfactory chamber, from the anterior tubular nostril to the accessory nasal sacs (Fig. 1C). The epithelium lining the accessory nasal sacs is unciliated, but ciliated epithelium covers the surface of the nasal cavity adjacent to the posterior nostril (Fig. 1C). A single low lamella located along the medial axis on the floor of the nasal cavity was seen in dissected preparations and in serially sectioned preparations (Fig. 1D). The presence of acetylated tubulin-immunoreactive fibers extending from the olfactory epithelium on the ventral, lateral, and dorsal surfaces suggested that the spatial pattern for OSNs extends beyond the low olfactory lamella.

Microvillar and ciliated OSNs were identified by TEM. The luminal surface of microvillar OSNs forms a low olfactory knob, with microvillar projections extending into the mucociliary complex, and centrioles were visible in the cytoplasm subjacent to the olfactory knob (Fig. 2A). Cilia extend from the olfactory knob of ciliated OSNs. Basal bodies are located in the cytoplasm below the cilia and microtubules are abundant in the dendrite (Fig. 2B; fig. 1D in Belanger et al., 2002). Ciliated nonsensory cells were recognizable by the flat ciliated apical surface and striated rootlets extending from the ciliary basal bodies (Fig. 2B).

The $G_{\alpha olf}$-immunoreactive cilia of ciliated OSNs were prominent in whole-mount preparations (Fig. 3A). The heavy ciliation of the olfactory epithelium from ciliated OSNs and ciliated nonsensory cells was evident following acetylated tubulin-immunocytochemistry (Fig. 3B). These numerous cilia prevented the viewing of microvilli in whole-mount preparations, as microvilli are considerably shorter

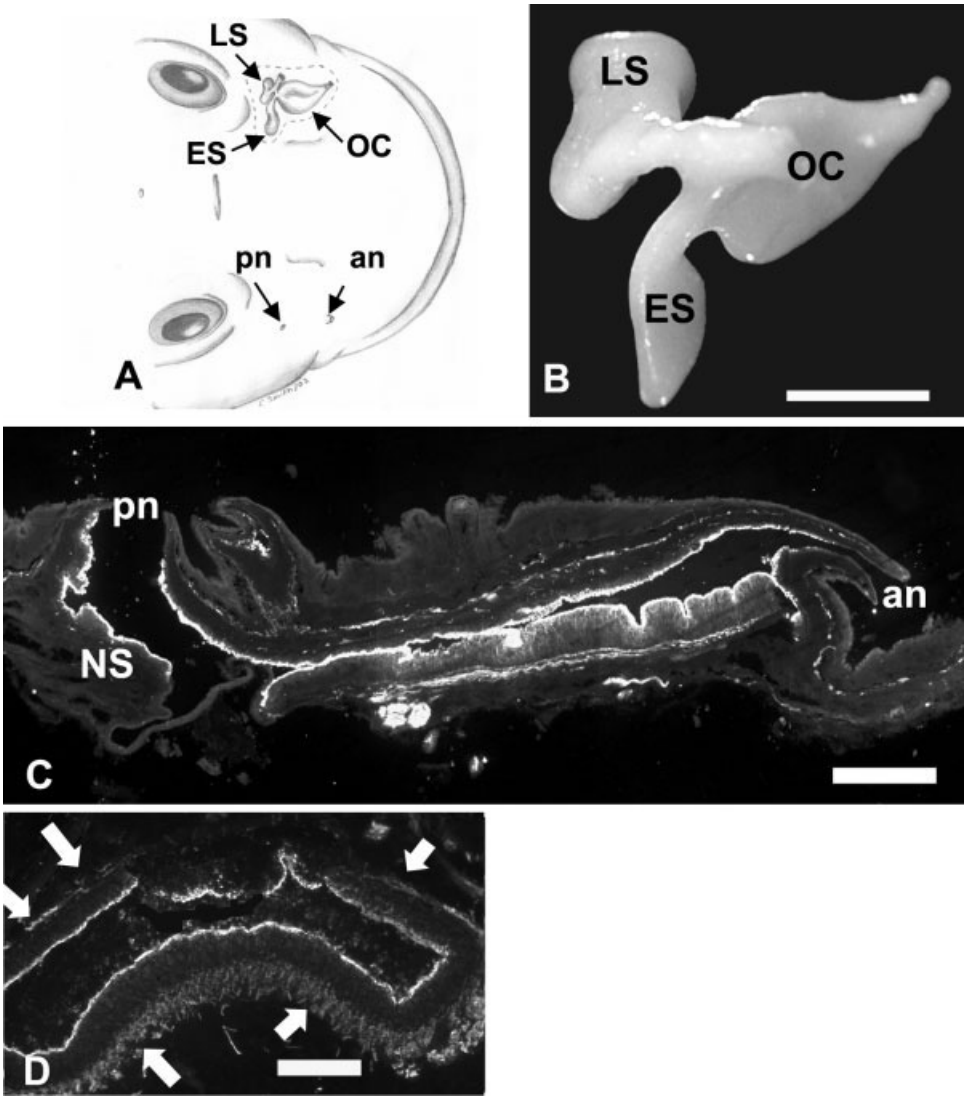

Fig. 1. The nasal cavity of the round goby *Neogobius melanostomus*. **A:** Diagram showing the location of the nostrils and the nasal cavity. The location of the anterior nostril (an) and posterior nostril (pn) on the surface are shown on the lower half of the diagram. The area outlined by a dashed line in the upper portion of the diagram shows the three compartments of the nasal cavity, the olfactory chamber (OC), and two posterior accessory nasal sacs (arrows). **B:** A mold impression of the nasal cavity shows the olfactory chamber (OC) and two accessory nasal sacs. The ethmoidal sac (ES) is located medially and the lachrymal sac (LS) is positioned laterally. Scale bar = 1 mm. **C:** A longitudinal section of the nasal cavity shows intense acetylated tubulin immunoreactivity extending along the dorsal epithelial surface from the anterior (an) to the posterior nostril (pn). The immunoreactivity is prominent on the ventral surface and absent from the accessory nasal sac (NS). Scale bar = 500 μm. **D:** A cross sectional view of the nasal cavity shows intense acetylated tubulin immunoreactivity on the luminal surface of the epithelial lining and small fibers extending into the lamina propria from the olfactory epithelium (arrows). Scale bar = 100 μm.

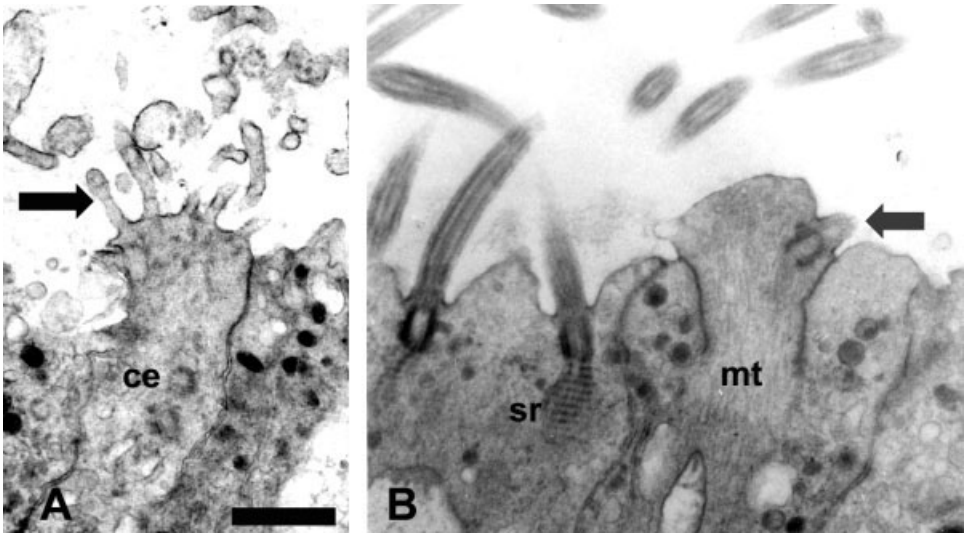

Fig. 2. Transmission electron micrographs of microvillar and ciliated OSNs in the olfactory chamber of the round goby *Neogobius melanostomus*. **A:** A tuft of microvilli (arrow) extends from a low olfactory knob. Two centrioles (ce) are visible in the apical dendrite. Scale bar = 1 μm. **B:** A prominent ciliated olfactory knob is present in the ciliated OSN (arrow). One cilium is seen extending laterally from the olfactory knob. The apical dendrite contains microtubules (mt) and tight junctions separate adjoining cells. Cilia protruding from the adjacent ciliated nonsensory cell are associated with basal bodies attached to striated rootlets (sr). The scale bar is shown in **A**.

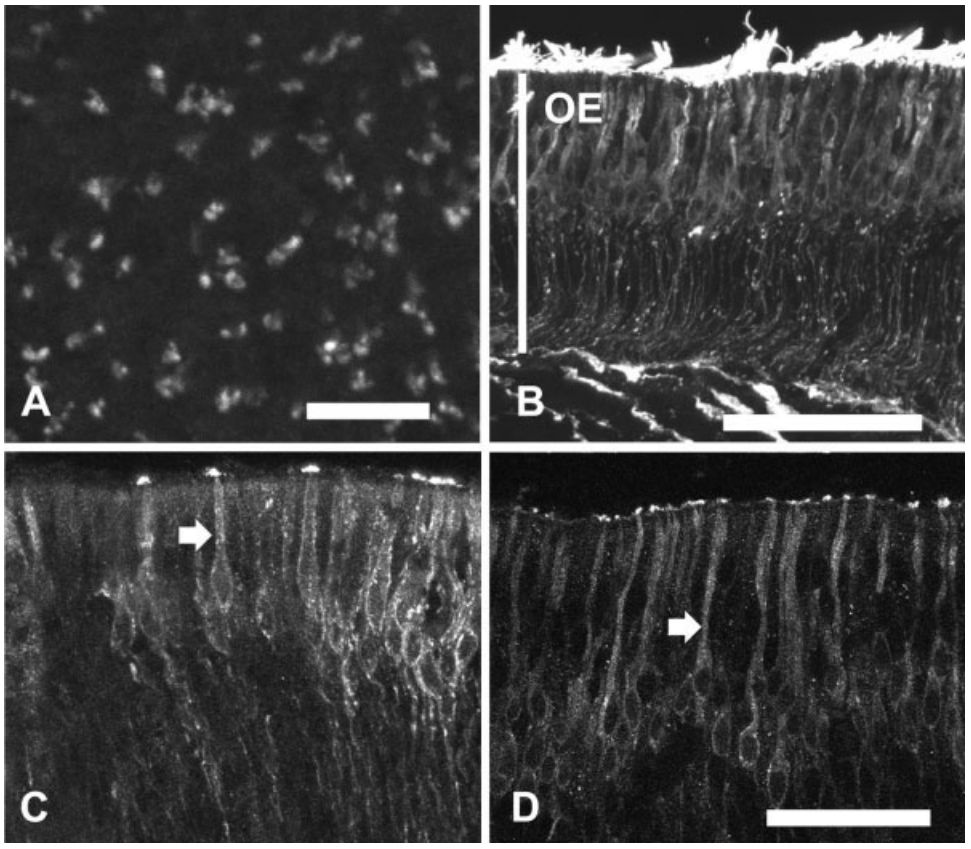

Fig. 3. *Neogobius melanostomus*. High-power confocal images of preparations stained for $G_{\alpha olf}$ and $G_{\alpha o}$ an acetylated tubulin immunofluorescence. **A:** A whole-mount view of the ventral luminal surface of the anterior olfactory chamber shows $G_{\alpha olf}$-immunoreactive cilia. Scale bar = 10 μm. **B:** A section stained for acetylated tubulin immunoreactivity shows the densely ciliated covering of the olfactory epithelium. The acetylated tubulin immunoreactivity also is seen in the OSN dendrites and axons within the olfactory epithelium (OE) and extends to axon fascicles within the lamina propria. Scale bar = 50 μm. **C:** Sectioned olfactory epithelium stained for $G_{\alpha o}$ immunocytochemistry shows the superficial location of the cell bodies of the immunoreactive cells, as well as the staining in the apical microvillar tuft, dendrite, cell body, and axon of presumptive microvillar OSNs. The arrow points to the dendrite of a single $G_{\alpha o}$-immunoreactive OSN. The scale bar is shown in **D**. **D:** Sectioned olfactory epithelium stained for G_{olf} immunocytochemistry shows labeling in the cilia, dendrites, cell bodies, and axons of ORNs. The arrow points to the dendrite of a G_{olf} immunoreactive OSN. The perikarya are in the lower portion of the olfactory epithelium. Scale bar = 25 μm.

than cilia. However, in sectioned tissue the $G_{\alpha o}$-immunoreactive microvilli, dendrites, cell bodies, and axons of microvillar OSNs were prominent (Fig. 3C). The nuclei of the $G_{\alpha o}$-immunoreactive OSNs are positioned within the upper third of the olfactory epithelium in comparison to the more basal perikarya of $G_{\alpha olf}$-immunoreactive OSNs (Fig. 3C,D). This perikaryal distribution implies that the $G_{\alpha o}$-immunoreactive OSNs are microvillar OSNs and the $G_{\alpha olf}$-immunoreactive OSNs are ciliated (Morita and Finger, 1998), and shows that in the round goby the ciliated OSNs are $G_{\alpha olf}$-immunoreactive and the microvillar OSNs are $G_{\alpha o}$-immunoreactive.

Spatial analysis of serially sectioned cross-sectional views of the olfactory chamber revealed the distribution of ciliated $G_{\alpha olf}$-immunoreactive OSNs and microvillar $G_{\alpha o}$-immunoreactive OSNs (Fig. 4). A low ventral lamellar ridge was evident from the anterior to the posterior portions of the olfactory chamber. Adjacent to the anterior nostril, olfactory epithelium containing both $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity lines the ventral surface as well as the lateral edges (Fig. 4A,B). The $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity extended to the lateral and dorsal surfaces (Fig. 4C,D) in over 90% of the serial sections. At the posterior edge of the olfactory chamber, the olfactory epithelium is limited to the ventral olfactory lamella (Fig. 4E,F). High-power views of the dorsal olfactory epithelium confirmed the presence of $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity on the OSN apical olfactory knobs and dendritic regions (Fig. 5). These results show that olfactory epithelium, with ciliated and microvillar OSNs, lines the entire olfactory chamber, from the anterior nostril to the accessory sacs, with the exception of small regions adjacent to the anterior and posterior nostril.

Cells that fit the description of crypt cells (bulbous in shape, with a projection reaching to the apical surface of the olfactory epithelium) (Hansen and Finger, 2000) were present in the olfactory epithelium of some specimens (Fig. 6). These cells were intensely $G_{\alpha o}$-immunoreactive, with a prom-

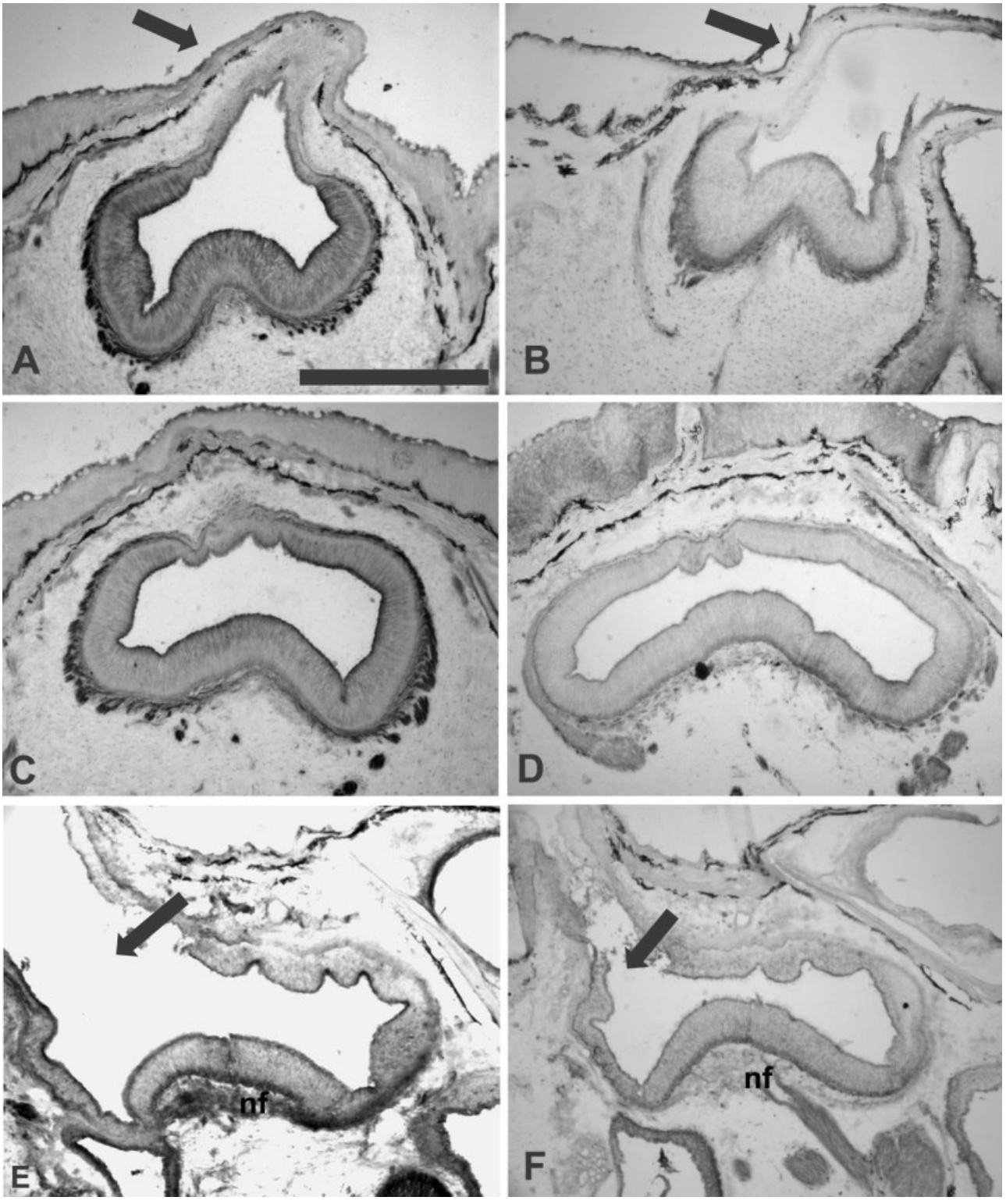

Fig. 4. *Neogobius melanostomus*. Low-power cross-sectional views of the olfactory mucosa in the round goby olfactory chamber stained for $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity. All micrographs have the same magnification. Scale bar in **A** = 200 μm. **A,C,E:** $G_{\alpha olf}$ immunoreactivity. **B,D,F:** $G_{\alpha o}$-immunoreactive. **A,B:** $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity on the ventral and lateral surfaces at the anterior edge of the olfactory chamber, adjacent to the anterior nasal cavity. The arrow points to epidermal tissue covering the anterior nostril. **B,D:** $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity in representative sections from the length of the olfactory chamber. $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity is located on the low ventral olfactory lamella, as well as the lateral and dorsal surfaces. The medial dorsal edge has a scalloped outline and lacks $G_{\alpha olf}$-immunoreactivity in the lamina propria (arrow). **E,F:** $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity at the caudal edge of the olfactory chamber, where staining is limited to the ventral olfactory lamella. The arrow points to the posterior naris. Nerve fascicles (nf) are seen converging ventral to the lamella.

inent apical cilium that was acetylated tubulin-immunoreactive.

DISCUSSION

This first comprehensive study of the peripheral olfactory organ in a perciform teleost demonstrates prominent accessory nasal sacs and a tube-shaped unilamellar olfactory chamber with microvillar and ciliated OSNs covering the ventral, lateral, and dorsal surfaces of the olfactory chamber.

The round goby contains two accessory nasal sacs, the lateral lachrymal sac and the medial ethmoidal sac. The occurrence of one or two accessory nasal sacs in teleost fish (summarized in the Appendix) appears to follow a phylogenetic pattern, with two accessory sacs present in the peripheral olfactory organ of fish in the order Perciformes. Two exceptions are *Trichinus vipera* and *Scomber scombrus*, with a single accessory nasal sac (Burne, 1909). Fish in the order Pleuronectiformes contain a fused pair (Kyle, 1899; Burne, 1909; Chabanaud, 1927; Applebaum and Schemmel, 1983; Liermann, 1933; Webb, 1993) or single accessory nasal sacs (Webb, 1993). The accessory nasal sacs in the round goby are round in shape and comprise half the length of the olfactory tube, as are those of another perciform, *Thynnus thunnina* (Sinha and Sinha, 1990), and the Pleuronectidae (Webb, 1993).

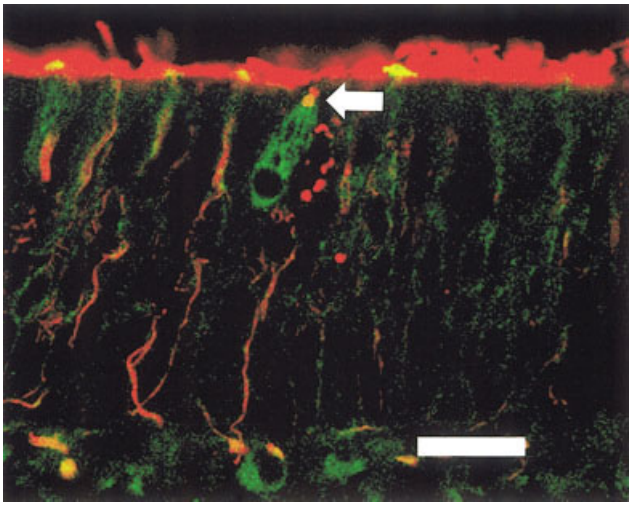

Fig. 6. *Neogobius melanostomus*. A crypt cell double-labeled with $G_{\alpha o}$ immunocytochemistry (green) and acetylated tubulin immunocytochemistry (red). The crypt cell is strongly $G_{\alpha o}$-immunoreactive, with an aceylated tubulin immunoreactive distal tip (arrow). Scale bar = 20 μm.

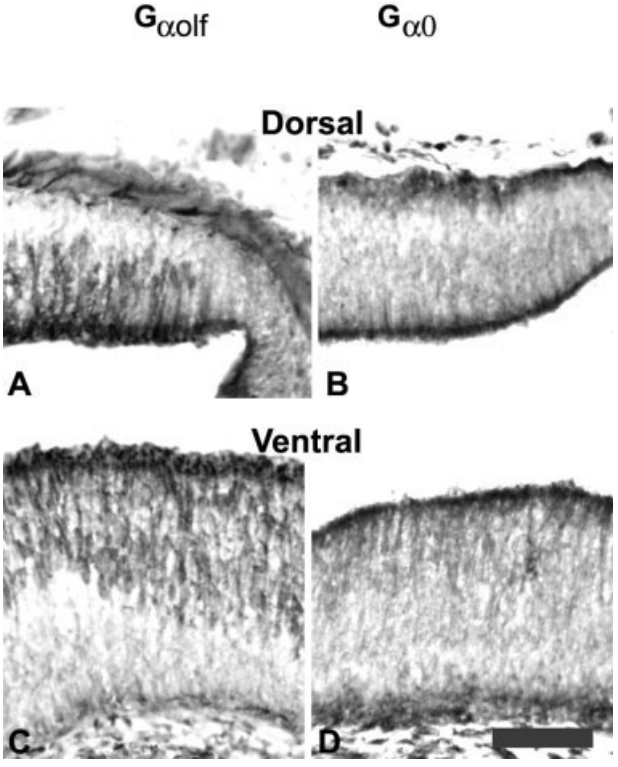

Fig. 5. *Neogobius melanostomus*. High-power views of $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity on the dorsal and ventral surface of the olfactory chamber. Scale bar in D = 50 μm. **A,C:** $G_{\alpha olf}$-immunoreactivity. **B,D:** $G_{\alpha o}$-immunoreactivity. **A,B:** $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactivity on the dorsal surface of the olfactory chamber. The $G_{\alpha olf}$-immunoreactivity extends deep into the olfactory epithelium (**A**) and the $G_{\alpha o}$-immunoreactivity (**B**) is limited to the upper third of the olfactory epithelium. **C,D:** The ventral surface of the olfactory chamber. The olfactory epithelium on the ventral surface is thicker than on the dorsal surface. $G_{\alpha olf}$-immunoreactivity reaches to the lower third of the olfactory epithelium (**C**) and $G_{\alpha o}$-immunoreactivity remains in the upper third of the olfactory epithelium.

The presence of these prominent accessory sacs in the round goby implies that it has the capacity to regulate the flow of water over the surface of the OSNs. A pumping mechanism regulating intake and expulsion of water through the olfactory organ of fish with accessory nasal sacs has been previously demonstrated in other perciforms (e.g., Burne, 1909; Doving et al., 1977; Sinha and Sinha, 1990). This ventilatory mechanism is based on constant compression and decompression of the sacs by contraction of surrounding buccal muscles and the movement of maxillary bones or pressure changes in the lymphatic system (Van den Berghe, 1929; Melinkat and Zeiske, 1979). The occurrence of two accessory nasal sacs in the round goby designates it as a cyclosmate. The heavy ciliation of olfactory and nonsensory epithelium allows the flow of water through the nasal cavity to be regulated by both ciliary beating and accessory sac compression (Doving et al., 1977). The prominent accessory nasal sacs and dense ciliation of the nasal cavity in the round goby suggest that odorant molecules reach the OSNs through "sniffing" (Nevitt, 1991) as well as ciliary beating. As round gobies perch on substrates using fused pelvic fins, the ability to sample surrounding waters for olfactory cues through a pumping mechanism would provide an evolutionary advantage to these small fish. Furthermore, previous studies that linked round goby gill ventilatory responses to olfac-

tory stimulation by putative pheromones of steroidal origin have suggested that the gill ventilatory mechanism is associated with increasing water flow through the olfactory organ (Murphy et al., 2001; Murphy and Stacey, 2002).

In the round goby, olfactory epithelium lines the luminal surfaces of a unilamellar olfactory chamber with a rich supply of ciliated and microvillar OSNs, stretching from the anterior nostril to the accessory nasal sacs. The rostral location of the OSNs, close to the anterior nostril, may assist the goby with odor sampling while remaining stationary. There is a single previous report of olfactory epithelium in anterior and dorsal locations in the bottom-dwelling sea stickleback, *Spinachia spinachia* (Gasterosteiformes, Gasterosteidae) (Theisen, 1982). However, in most teleosts OSNs appear to be restricted to olfactory lamellae that line the floor of the nasal cavity (Yamamoto, 1982). The presence of OSNs on the lateral and dorsal regions of the olfactory chamber is a novel finding. This spatial pattern likely maximizes the surface area for odorant/receptor interaction, as the goby possesses a unilamellar olfactory organ, compared with the multilamellar structures observed in many teleosts (Yamamoto, 1982). Interestingly, in amphibians both dorsal and ventral surfaces of the relatively flat nasal cavity are covered by olfactory mucosa (Hansen et al., 1998).

The use of G-protein immunocytochemistry for $G_{\alpha olf}$ and $G_{\alpha o}$ was effective in assessing the distribution of ciliated and microvillar OSNs. The $G_{\alpha o}$-protein is expressed by vomeronasal receptor neurons in terrestrial vertebrates (Matsuoka et al., 2001) and is localized in microvillar OSNs in catfish and goldfish (Hansen et al., 2001). Both $G_{\alpha olf}$- and $G_{\alpha o}$-immunoreactive OSNs appeared to be equally prominent throughout the olfactory epithelium on the ventral, lateral, and dorsal surfaces of the olfactory chamber. This even distribution pattern of ciliated and microvillar OSNs is common in teleosts. However, other fish, such as Atheriniformes, show specific spatial mapping of ciliated and microvillar cell types (Zeiske et al., 1979; Yamamoto, 1982).

Crypt cells, as described by Hansen and Finger (2000), were stained intensely with the $G_{\alpha o}$ antibody, indicating that crypt cells may share similar antigenic properties to the alpha subunit of the G-protein for microvillar receptor neurons. In the round goby, crypt cells varied in location and were absent in some specimens, while numerous in others, a characteristic also previously observed in diverse teleost species; however, their function is unknown (Hansen and Finger, 2000). Future research on the ciliated and microvillar OSNs and crypt cells is needed to clarify their function in the peripheral olfactory organ.

ACKNOWLEDGMENTS

The authors thank Dr. W. Li (Michigan State University), Dr. T. Hara (University of Manitoba, Department of Fisheries and Oceans), Dr. T. Johnson (Ontario Ministry of Natural Resources, Wheatley), D. Clemente Lopez (University of Salamanca, Spain), and A. Belanger and A. Frontini (University of Windsor). Discussions with Dr. A. Hansen (University of Colorado Health Science Center, Denver, CO) are also greatly appreciated.

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APPENDIX. Summary of the number of nasal sacs in teleost fishes (subdivision Eutelaostei)

Order	Family	Genus species	Number of nasal sacs	Reference
Cypriniformes	Cyprinidae	*Cyprinus carpio*	0	Døving et al., 1977
		Labeo rohita	1	Kapoor and Ojha, 1972
		Rutilis rutilis	0	Døving et al., 1977
		Tinca tinca	0	Døving et al., 1977
Siluriformes	Siluridae	*Siluris glanis*	0	Døving et al., 1977
Atheriniformes	Bedotiidae	*Bedotia geayi*	1	Melinkat and Zeiske, 1979
	Melanotaeniidae	*Chilatherina sentaniensis*	1	Zeiske et al., 1979
		Glossolepis incisus	1	Zeiske et al., 1979
		*Melanotaenia fluviatilis***	1	Zeiske et al., 1979
		*Melanotaenia maccullochi***	1	Zeiske et al., 1979
		*Melanotaenia maculata***	1	Zeiske et al., 1979
	Atherinidae	*Craterocephalus sp.*	1	Zeiske et al., 1979
Beloniformes	Belonidae	*Belone vulgaris*	0	Burne, 1909
Cyprinodontiformes	Poeciliidae	*Xiphophorus helleri*	1	Kux et al., 1988
Beryciformes	Berycidae	*Beryx delphinus*	2	Burne, 1909
Zeiformes	Zeidae	*Zeus faber*	1	Burne, 1909
	Caproidae	*Capros aper*	2	Burne, 1909
Gasterosteiformes	Gasterosteidae	*Spinachia spinachia*	1	Theisen, 1982
Scorpaeniformes	Scorpaenidae	*Scorpaena porcus*	present*	Doving et al., 1977
	Triglidae	*Trigla hirundo*	2	Burne, 1909
	Cyclopteridae	*Cyclopterus lumpus*	2	Burne, 1909
Perciformes	Percidae	*Perca fluviatilis*	2	Burne, 1909; Døving et al., 1977
	Sparidae	*Pagellus centronotus*	2	Burne, 1909
	Mullidae	*Mullus barbatus*	2	Burne, 1909
	Latridae	*Latridopsis ciliaris*	2	Burne, 1909
	Cichlidae	*Tilapia sp.*	present*	Døving et al., 1977
	Labridae	*Crenilabrus cinereus*	present*	Døving et al., 1977
		Lubrus berggylta	present*	Døving et al., 1977
	Bovichthyidae	*Bovichthys variegatus*	2	Burne, 1909
	Trachinidae	*Trachinus vipera*	1	Burne, 1909
	Gobiidae	*Neogobius melanostomus*	2	This study
	Scombridae	*Scomber scombrus*	1	Burne, 1909
		Thynnus thunnina	2	Sinha and Sinha, 1990
	Channidae	*Channa punctatus*	2	Kapoor and Ojha, 1972, 1973
Pleuronectiformes	Scophthalmidae	*Scophthalmus sp.*	2	Kyle, 1899
	Pleuronectidae	*Ammotretis rostratus*	1	Webb, 1993
		Pleuronectes sp.	2	Burne, 1909; Liermann, 1933; Webb, 1993
	Soleidae	*Solea vulgaris*	Fused pair	Burne, 1909; Chabanaud, 1927; Applebaum and Schemmel, 1983
	Cynoglossidae	*Cynoglossus sp.*	Fused pair	Kyle, 1899; Webb, 1993
		Symphurus atricauda	Fused pair	Webb, 1993

*This article shows the presence of accessory sacs but does not indicate the number of sacs.
**Melanotaenia was formerly Nematocentris.
We followed the taxonomic classification scheme described by Nelson (1994). Genus and species names are listed alphabetically within each family.