The lateral line system and its innervation in the boxfish Ostracion immaculatus (Tetraodontiformes: Ostraciidae): description and comparisons with other tetraodontiform and perciform conditions

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The lateral line system and its innervation were examined in the ostraciid Ostracion immaculatus (Tetraodontiformes), and compared with those in the triacanthodid Triacanthodes anomalus (Tetraodontiformes) and the acropomatid Malakichthys wakiyae (Perciformes). The carapace of O. immaculatus was composed of 6 cephalic and 2 trunk lateral lines, all neuromasts being categorized as “superficial.” Triacanthodes anomalus was identical with O. immaculatus in the absence of the mandibular line and its innervating ramus, whereas in M. wakiyae the line and ramus were present. All neuromasts were “superficial” in the former two, but “canal” in the latter. Judging from the essentially identical lateral line topography and innervation patterns in all three species, the superficial neuromasts in the two tetraodontiforms were considered to have resulted from replacement of canal neuromasts. The number of neuromasts in the cephalic lateral lines of O. immaculatus (106) and T. anomalus (91) were similar, being significantly higher than in M. wakiyae (30). However, the reverse was true for the trunk lateral lines, the two tetraodontiforms having fewer neuromasts (39 in O. immaculatus, 47 in T. anomalus) compared with M. wakiyae (59).

Key words: Tetraodontiformes · Ostraciidae · Lateral line system · Nerves · Neuromasts

The lateral line system is a receptive organ unique to anamniotes, being innervated by lateral line nerves developed embryologically from lateral line neurogenic placodes that have been lost in amniotes (Liem et al., 2001). Although descriptions of the system, including the number of lateral line scales, are widespread in systematic ichthyological literature (see Webb, 1989), the details of the descriptive treatment differ significantly from group to group, depending upon their availability as diagnostic characters [e.g., high in Gobiidae; see Ahnelt and Bohacek (2004) for review] and ease of observation (e.g., very inconspicuous in Tetraodontiformes; see following). Regarding neuroanatomy, a few authors (e.g., Freihofer, 1972) have attempted to infer the interrelationships of fishes based on lateral line nerve characters, and this requires delicate dissections. Utilizing the horseradish peroxidase (HRP) protocol, recent studies (e.g., Puzdrowski, 1989; Song and Northcutt, 1991; Piotrowski and Northcutt, 1996; Northcutt et al., 2000) analyzed in detail lateral line innervations and their central projections in some primitive fishes (Cypriniformes, Semionotiformes, Polypteriformes, and Siluriformes). However, in the Percomorpha (sensu Johnson and Patterson, 1993), only a few descriptive accounts have been published in recent decades (see Freihofer, 1978, for review), the classic works (e.g., Herrick, 1899; Cole and Johnstone, 1901; Allis, 1903) having remained as authorities on lateral line nerve patterns. Regarding tetraodontiforms, little attention has been paid to the lateral line system, most likely owing to its inconspicuous nature. Overall, the system has not been utilized positively for either taxonomic or phylogenetic characters, although the statement that infraorbitals are absent in the order (Tyler, 1980) may be interpreted as an indirect reference to the lateral line system. Although Bal (1937) studied the nervous system of Takifugu oblongus, details were omitted in the description.

In this study, the lateral line system and its innervation are described and illustrated in detail for the first time in the Tetraodontiformes. As a first step in tetraodontiform neuroanatomy, we examined two “extremes” to roughly estimate the stability of the lateral line system and its innervation within the order: Ostracion immaculatus (Ostraciidae) for its specialized “scutes” with a three-dimensional collagen network structure (Besseau and Bouligand, 1998) and Triacanthodes anomalus (Triacanthodidae) for its primitive position in the order (Santini and Tyler, 2003). Furthermore, the perciform Malakichthys wakiyae is described and illustrated as a comparative reference to tetraodontiform conditions, a sister-group search not being attempted in this study.

Materials and Methods

Specimens examined in this study are listed after this section. Abbreviations in parentheses refer to the methods of examination: D, dissected; CS, cleared and stained.
Measurements (in mm) are of standard length (SL). Bones were observed on specimens stained by Alizarin Red-S, and nerves on CS specimens prepared by the Sihler technique (Fraser and Freihofer, 1971), with modifications of Nakae and Sasaki (2004), and the Sudan Black B protocol (Filipski and Wilson, 1984). Superficial neuromasts in *Ostracion immaculatus* were observed by scanning electron microscopy (SEM). Neuromast samples (lateral line scales) were taken from the carapace of a 10% formalin-fixed specimen. The samples were washed in distilled water for 2 days and postfixed in 1% osmium tetroxide for 12 h at room temperature. Subsequently, the samples were washed in distilled water for 30s, dehydrated in a graded ethanol series, and transferred to 100% 2-methyl-2-propanol. The samples were then freeze-dried with a JEOL JFD-300 freeze-drying device, sputter coated with gold with a JEOL JFD-1500 ion sputtering device at 200Å, and viewed and photographed with a JEOL JSM-5300LV SEM. Terminology generally follows Tyler (1980) for osteology, Northcutt et al. (2000) for neuroanatomy, and Webb (1989) for superficial lateral lines. Note that because infraorbitals are absent in all recent tetraodontiforms (Tyler and Sorbini, 1996), primary recognition of the “infraorbital line” is more or less arbitrary in the inclusion or exclusion of a certain neuromast. Although the lateral line nerves have been traditionally considered (including Freihofer, 1978) as components of the three cranial nerves [facial (VII), glossopharyngeal (IX), and vagal (X) nerves], recent studies have clearly shown that the lateral line nerves constitute a distinctly separate series of cranial nerves (see Northcutt et al., 2000 and citations therein). This study follows the new homology concept, including terminology (see above).

**Material examined.** Ostraciidae: *Ostracion immaculatus*, BSKU (Laboratory of Marine Biology, Faculty of Science, Kochi University) 70468 (122.8 mm SL, D), BSKU 60471 (2 specimens; 110.3 and 115.6 mm SL, CS), BSKU 71622 (125.8 mm SL, CS), BSKU 72455 (115.4 mm SL, SEM sample). Triacanthodidae: *Triacanthodes anomalus*, BSKU 70467 (62.7 mm SL, D), BSKU 70470 (3 specimens; 79.2–80.3 mm SL, CS), BSKU 71623 (81.5 mm SL, CS). Acropomatidae: *Malakichthys wakiyae*, BSKU 70466 (86.0 mm SL, D), BSKU 70469 (3 specimens; 95.2–107.4 mm SL, CS).

**Results**

**Ostracion immaculatus.** Lateral line system (Figs. 1, 2).—The lateral line system was composed only of superficial neuromasts arranged in shallow open grooves on the external surfaces of the hard platelike scales. Although in CS specimens a neuromast was visible at the position of each stumplike process (Fig. 1), it was not possible to determine from our SEM preparation (see above) (observations up to ×10000) if the dorsal surface of the process represented the sensory macula of a neuromast or if the process simply enclosed it. In both cases, neuromasts were “superficial” in the sense that they were not enclosed in a common canal. The number of neuromasts on a single scale varied from 1 to 8, irrespective of scale size. The lateral line system comprised the following (Fig. 2): supraorbital line (SOL), infraorbital line (IOL), preopercular line (PRL), otic line (OTL), postotic line (POL), supratemporal line (STL), trunk line (TRL), and dorsal trunk line (DTL); a mandibular line (MDL) was absent.

SOL, derived from POL above the posterior margin of the orbit, ran anteroventrally and terminated on the snout immediately in front of the orbit; IOL comprised a transverse groove on the dorsal surface of the snout and a Y-shaped groove below the orbit, the latter crossing downward on the lateral surface of the cheek to be confluent with PRL ventrally; PRL began on the ventrolateral surface of the snout, running backward on the ventral surface of the head to a level below the pectoral fin base, and subsequently looped outward to be terminated immediately below the base; below the orbit, a transverse line was derived dorsolaterally from the longitudinal line; three posteriorly separated neuromasts were also included in PRL; OTL was short, extending between the SOL–POL junction and the posterodorsal rim of the orbit; POL extended between the SOL–OTL and STL–TRL junctions; STL coursed transversely, interconnecting the posteriormost neuromasts of

![Fig. 1. Scanning electron micrographs of anterior part of trunk lateral line in *Ostracion immaculatus*, showing three stumplike processes (A) and enlargement of the anteriormost (B). Anterior to left](image-url)