

Occipito-Vertebral Fusion in Ocean Sunfishes (Teleostei: Tetraodontiformes: Molidae) and Its Phylogenetic Implications

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ABSTRACT We describe the ontogeny of the occipital skull and anterior vertebrae of the molids *Ranzania laevis* and *Masturus lanceolatus* and compare it with that of the ostraciid *Lactophrys* sp. The first vertebra fuses to the basioccipital in early ontogeny in the two molids and previous authors thus confused that vertebra with the back of the basioccipital, so that all previous counts of their vertebral numbers are incorrect by one vertebra. As evidenced by *Lactophrys* sp., ostraciids are the only other tetraodontiforms with similar occipito-vertebral fusion. In contrast to the molids, additional anterior vertebrae fuse with this complex in ostraciids. We conclude that the shared occipito-vertebral fusion in molids and ostraciids and its otherwise extremely rare occurrence among teleosts provide support for a sister-group relationship of the two families. *J. Morphol.* 266:74–79, 2005. © 2005 Wiley-Liss, Inc.

KEY WORDS: basioccipital; first vertebra; phylogeny; ontogeny; homology; Molidae; Ostraciidae

The ocean sunfishes of the family Molidae are a small group of bizarre, relatively enormous, marine tetraodontiforms with a pelagic lifestyle and a world-wide distribution (Fraser-Brunner, 1951; Nelson, 1994). Because of their unique appearance, ocean sunfishes have fascinated naturalists and scientists for centuries. Although the earliest anatomical descriptions date from the 1800s (Meckel, 1828; Wellenbergh, 1840; Goodsir, 1841; Cleland, 1862; Steenstrup and Lütken, 1898) and several monographs appeared in the 20th century (Gregory and Raven, 1934; Raven, 1939a,b; Winterbottom, 1974; Tyler, 1980), much of the unusual, highly derived anatomical structure of molids is still poorly understood. During our study of the development and homology of the molid clavus or pseudocaudal fin (Britz and Johnson, 2004; Johnson and Britz, 2004), we discovered a previously overlooked and highly unusual character of the vertebral column that characterizes the Molidae: ontogenetic fusion of the first vertebra with the occiput. Such fusion is rare in teleosts and occurs elsewhere among tetraodontiforms only in the boxfishes, family Ostraciidae (Tyler, 1963). The goal of the present article is to

describe the ontogeny of this unusual character and comment on its systematic significance.

MATERIALS AND METHODS

This study is based on the following cleared and stained (c&s) (Taylor and van Dyke, 1985) material:

Family Molidae: *Ranzania laevis* (Pennant, 1776), USNM, uncataloged, 35 specimens from 2.3 mm notochord length (NL) to 26 mm standard length (SL), alizarin stained only. *Masturus lanceolatus* (Liénard, 1840), USNM uncataloged, one specimen, 5.8 mm SL, alizarin stained only; MCZ 157343, one specimen, 11.5 mm SL.

Family Ostraciidae: *Lactophrys* sp. SEAMAP No. 25817, three specimens 3.2 mm NL, 3.3 mm NL, 4.0 mm SL; SEAMAP No. 25776, one specimen, 4.3 mm SL; SEAMAP uncatalogued, 11.2 mm SL.

Developmental stages of the different taxa were photographed with a Leica camera or a ProgRes digital camera attached to a Zeiss Tessovar.

RESULTS

Ontogeny of Occipital Skull and Anterior Vertebrae in *Ranzania laevis*

2.3 mm (Fig. 1A,B). The chorda extends far into the base of the skull. The basioccipital is ossified as two elongate perichondral ossifications around the parachordals connected with each other through a perichordal ossification. Further posteriorly the paired elongate and neural arch-like exoccipitals are perichondrally ossified. The neural arches are developed in membrane bone, and left and right halves remain widely separated in the dorsal midline.

4.0 mm (Fig. 1C,D). The basioccipital has paired anterior arms that extend well forward of the middle portion. Posteriorly, it is expanded into paired lateral, triangular processes that approach the exoccipitals posterolaterally and the first centrum pos-

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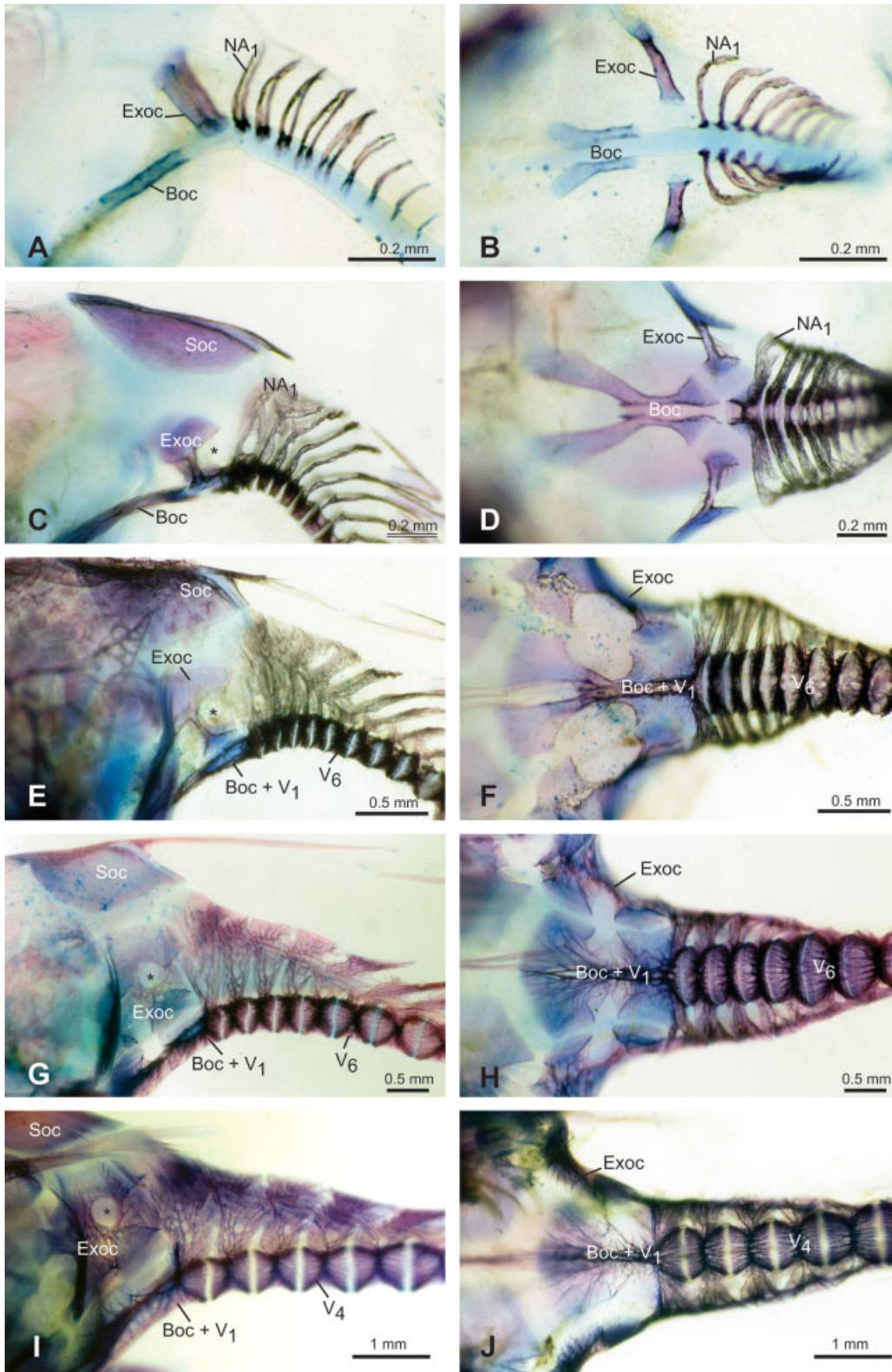


Fig. 1. Development of occiput and anterior vertebrae in *Ranzania laevis*, cleared and alizarin-stained specimens in lateral (left column) and ventral view (right column). Foramen of spinooccipital nerves marked with an asterisk. **A,B**: 2.3 mm NL. **C,D**: 4 mm SL. **E,F**: 6.4 mm SL. **G,H**: 16 mm SL. **I,J**: 26 mm SL. Boc, basioccipital; Exoc, exoccipital; NA, neural arch; Soc, supraoccipital; V, vertebra.

teriorly in the ventral midline. The exoccipitals have grown and exhibit a dorsal expansion that surrounds larger areas of the cartilage. The ventral base of each is also slightly expanded and bears a lamina of membrane bone. The centra of the anterior vertebrae have formed and are relatively narrow, except that of the first, which projects anteriorly toward the basioccipital. The first three neural arches are laterally expanded, with the extent of the expansion decreasing posteriorly. The posterior edge of the ventral third of the first neural arch has a large incisure that represents an incompletely closed foramen for the first spinal nerve. A similar, although smaller incisure is present on the second and third neural arches. The anterior four neural arches remain separated in the dorsal midline, but the succeeding ones are closed. Anterior neural arches are straight and relatively upright proximally, then sharply curve posteriorly at an angle. The point of curvature starts in the distal third of the first and moves successively more proximally on posteriorly sequential arches. As a result of the expansion and curvature of the neural arches, the anterodorsal edge of neural arch 2 overlaps the posterodorsal edge of neural arch 1. Neural arches 2 and 3 have an anterodorsally directed pointed process at the corner of the curvature. Anterior centra 2–5 are narrower than more posterior ones, being about two-thirds of their length.

6.4 mm (Fig. 1E,F). The basioccipital has expanded laterally, especially at its anterior arms and posterior processes, while the middle part remains relatively narrow, resulting in a pair of conspicuous incisures. The chorda is still present between the two anterior arms. The exoccipital has an unusual hourglass shape with dorsal and ventral expansions and a narrow middle part, so that an anterior incisure faces that of the basioccipital incisure, and a posterior one faces that of the first neural arch. The first centrum is fused anteriorly with the posterior edge of the basioccipital, where a thin line still demarcates the area of fusion. The anterior neural arches are further expanded and the first four overlap each other along almost their entire length. Centra 2–5 are much narrower, only about half the length of more posterior ones.

16 mm (Fig. 1G,H). The basioccipital has grown extensively, and the cranial part of the chorda has been resorbed so that the anterior arms have grown together in the ventral midline. The pair of incisures in its middle part have also started to close through development of membrane bone. The fusion of the first centrum and the basioccipital is complete so that the two components are indistinguishable. The posterior incisure of the exoccipital has been bridged by a lamina of membrane bone extending between the posterior ends of the dorsal and ventral expansions of the former hourglass-shaped perichondral ossification, thus enclosing a foramen for passage of the vagus and spinooccipital nerves. All anterior

neural arches have expanded further and their overlap is so extensive that they form an almost complete roof over the neural canal. The anteriorly directed pointed processes on the lateral sides of the neural arches have enlarged and overlap with the preceding arch, where they are housed in a shallow groove.

26 mm (Fig. 1I,J). This stage closely resembles the previous one. The anterior neural arches are further expanded, and their anteriorly directed lateral processes much more elongated. The exoccipital has grown further posteriorly so that the vagal/spinooccipital foramen is located in the middle of the bone. The anterior centra are more elongated and are now only slightly shorter than the more posterior ones.

Ontogeny of Occipital Skull and Anterior Vertebrae in *Masturus lanceolatus*

5.8 mm (Fig. 2A). The basi- and exoccipitals are ossified and the latter shows a large posterior incisure for exit of the occipito-spinal nerve. The first vertebra comprises a relatively large centrum that surrounds the chorda and bears a narrow neural arch that widens distally. This widened wing sits atop the occipital roof of the skull. The second and third vertebra have a similar appearance, with even wider distal portions of the neural arches. The left and right neural arches of vertebrae 1–5 are separated in the dorsal midline. The sixth vertebra is the first with a neural spine.

11.5 mm (Fig. 2B). The incisure in the posterior edge of the exoccipital has closed to form a complete foramen for the occipito-spinal nerve. The first centrum has started to fuse with the basioccipital along the dorsal and ventral borders of the chorda, but not at its sides. Its neural arch is much broader and encloses a foramen for the first spinal nerve. The neural arches of subsequent centra have also expanded laterally and bear spinal nerve foramina. The sixth vertebra is the first with a neural spine, as in the previous stage.

Ontogeny of Occipital Skull and Anterior Vertebrae in *Lactophrys* sp.

3.3 mm (Fig. 2C,D). The occiput of the chondrocranium is still mostly cartilaginous. A nodular process extends from the base of the occipital region along the side of the chorda. The developing exoccipital is present as a perichondral ossification extending from this process dorsally around the cartilage that limits the foramen magnum laterally. Its posterior lamina of membrane bone forms a dorsal hook-like process that surrounds the foramen for the spinooccipital nerves only incompletely at this stage. The anterior neural arches are well ossified. The bases of the left and right halves of the anterior three neural arches are connected to each other by a dorsal half-centrum. The subsequent centra surround the chorda completely.

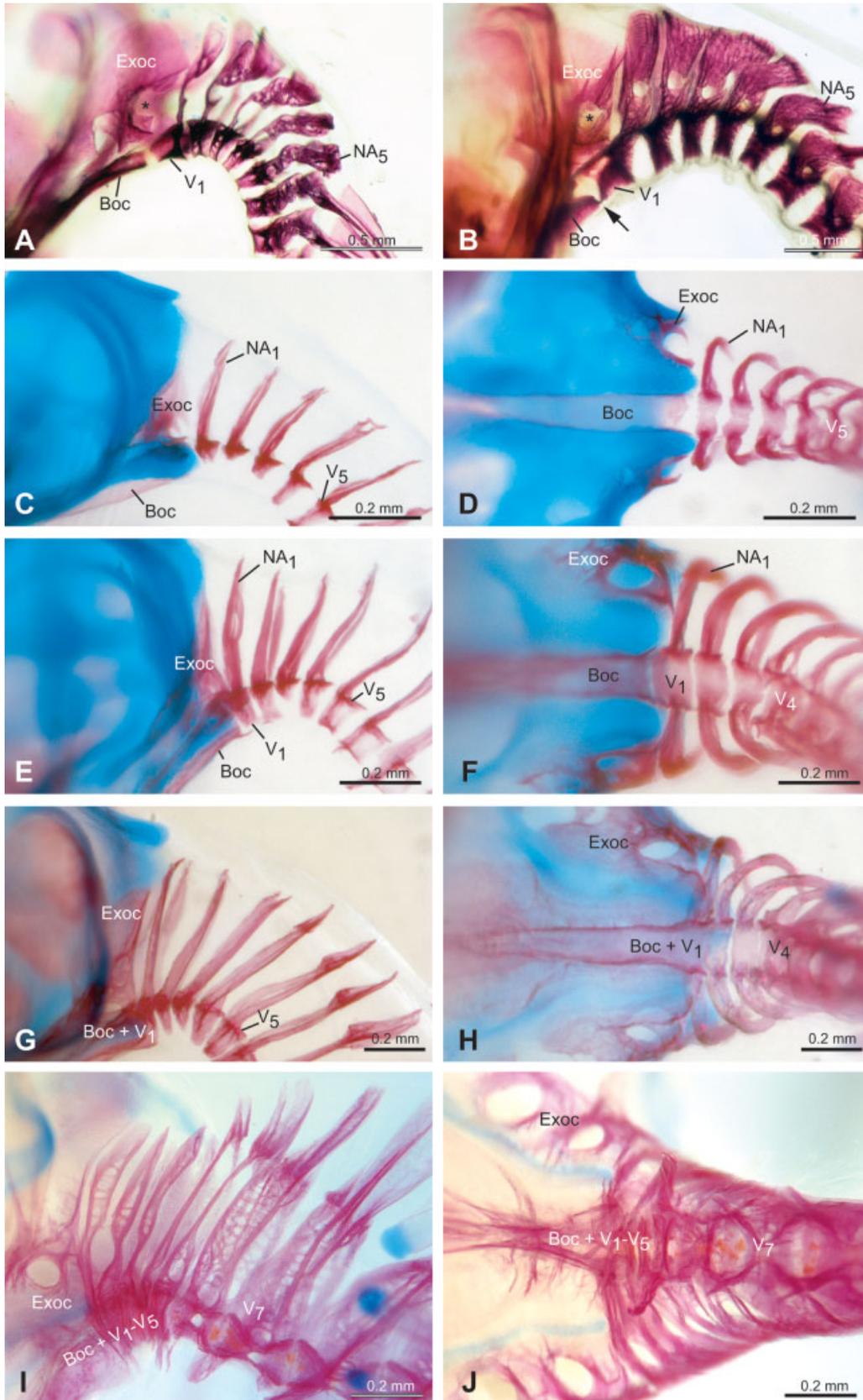


Fig. 2. Development of occiput and anterior vertebrae in lateral and ventral view. **A,B:** *Masturus lanceolatus*, lateral view of cleared and alizarin-stained specimens. Foramen of spinooccipital nerves marked with an asterisk. **A:** 5.8 mm SL. **B:** 11.5 mm SL. **C–J:** *Lactophrys* sp., cleared and double-stained specimens in lateral (left column) and ventral view (right column). **C,D:** 3.2 mm NL. **E,F:** 3.3 mm NL. **G,H:** 4.0 mm SL. **I,J:** 4.3 mm SL. Boc, basioccipital; Exoc, exoccipital; NA, neural arch; V, vertebra.

4.0 mm (Fig. 2E,F). The exoccipital with its posterior lamina of membrane bone has grown further, so that the foramen for the spinooccipital nerves is completely enclosed by bone. The anterior centrum has fully formed and approaches the basioccipital closely, but is clearly separated from it by the occipito-vertebral gap. The basioccipital is still mostly restricted to the thin perichordal ossification, but has a short thin posterior flange of membrane bone that covers the first centrum ventrally. The second centrum is also complete, but the third is still only a half centrum.

4.3 mm (Fig. 2G,H). All chondral bones of the occiput have grown further and cover larger areas of the occipital chondrocranium. The basioccipital in particular has invaded the lateral and more anterior areas close to the chorda, where it covers the cartilaginous base of the neurocranium perichondrally. The first centrum has fused with the basioccipital, but the line that marks the occipito-vertebral gap is still partially visible. Although this stage is further developed than the previous, the centra of vertebrae 2 and 3 are only developed as half centra.

11.2 mm (Fig. 2I,J). The anterior three centra are fused with each other and the basioccipital. They bear narrow neural arches that remain separate in the dorsal midline. The two subsequent centra are very narrow and very closely attached to the anterior three. The neural arches of these two vertebrae taper distally, are joined in the dorsal midline, and each bear a spatulate neural spine. Neural arches 3–5 have a pointed lateroventrally directed process at their bases. That of the sixth vertebra is directed ventrally and comes off the centrum. A thick pad of connective tissue extends between this process and the basioccipital, thus obscuring the view onto the ventral side of the more anterior centra. Centrum 6 is also relatively narrow, but has a broad neural arch and spine. The seventh and more posterior vertebrae appear normal.

DISCUSSION

Our developmental study of the occipital neurocranium and the anterior vertebrae of the two molids, *Ranzania laevis* and *Masturus lanceolatus*, demonstrates clearly that the first vertebra fuses indistinguishably with the basioccipital during ontogeny.

In one of the earliest accounts of molid anatomy, Cleland (1862, p. 173) stressed that the basioccipital “projects so far behind the other bones of the skull that it is liable on a cursory glance to be mistaken for the first vertebra...,” a fact also noted by Steenstrup and Lütken (1898, p. 90). Tyler (1980, pgs. 368, 369) described the basioccipital as “prolonged into a pair of sturdy prongs much like those of the first vertebra.” Although these authors were already aware of the great resemblance of the posterior part of the putative basioccipital with an anterior verte-

bra, they were unable to resolve its identity as a true but fused first vertebra, because they did not examine early developmental stages.

The neural arches of the first vertebra in molids are tightly bound to the dorsolateral sides of the posterior occipital region of the skull. In later development, they articulate with the exoccipitals exactly as they do in most tetraodontiforms (see Tyler, 1980). This led to Tyler’s confusion of the first vertebra with the posterior end of the basioccipital and his erroneous statement that the “exoccipital does not articulate with the first vertebra.”

This previous misinterpretation of the first vertebra of molids has further ramifications. Santini and Tyler (2002, 2003) used the unusual structure of the basioccipital and the lack of articulation of the exoccipitals with the first vertebra as two of the numerous synapomorphies of the extant Molidae, but those two are invalid.

Furthermore, because of the occipito-vertebral fusion in molids all previous counts of vertebral numbers are incorrect by one vertebra. Raven (1939b) and Tyler (1980) reported 8 abdominal and 10 caudal vertebrae for *Ranzania laevis*, but Fraser-Brunner (1951) provided a count of 8 abdominal and a range of 10 or 11 caudal vertebrae in his key, without indicating the source or basis for this count. Whatever the number of caudal vertebrae, *Ranzania* has 9, not 8, abdominal vertebrae.

Similarly, Raven (1939a) and Tyler (1980) reported 8 abdominal and 8 caudal vertebrae in *Masturus*, but it has actually 9 + 8, as evidenced by our two developmental stages.

Vertebral counts for *Mola mola* were reported as 8 abdominal plus 8 caudal by Cleland (1862), Steenstrup and Lütken (1898), and Kaschkaroff (1914), but Tyler (1980) reported 8 + 9 in his two specimens. Because of the occipito-vertebral fusion, *Mola* actually has 9 abdominal vertebrae. We were surprised by the difference in number of caudal vertebrae reported by Tyler (1980) and that by Cleland (1862) and Steenstrup and Lütken (1898). We checked Tyler’s material and confirmed his caudal count. Assuming that the counts of the latter two authors are correct, there are several possible explanations for this discrepancy: 1) Cleland’s (1862), Steenstrup and Lütken’s (1898), and Kaschkaroff’s (1914) “*Mola*” specimens were actually *Masturus*; 2) the number of caudal vertebrae is variable in a population; or 3) the Atlantic and Pacific populations of *Mola mola* differ in this character. To resolve this issue, we need additional information on vertebral numbers and their variation from many more specimens of *Mola* worldwide.

Strangely, and unlike any previous author, Fraser-Brunner (1951) gave the vertebral numbers for *Mola* and *Masturus* as 9 + 8, but again, as with *Ranzania*, he provided no source or basis for this count. Tyler (1980, p. 379) speculated that “Fraser-Brunner’s counts of nine abdominal vertebrae ... were made from

radiographs and that the dorsal prongs of the basioccipital were mistaken for the first vertebra.”

Fusion of anterior vertebrae with the occipital skull region is a rare event among teleosts (Patterson and Johnson, 1995; Bemis and Forey, 2001). The only other acanthomorph group we know of that has a similar occipito-vertebral fusion is the Ostraciidae, as nicely and clearly demonstrated by Tyler (1963, figs. 1–4). As shown above in the two molids, the first vertebra of *Lactophrys* is separate from the occiput in very early ontogeny (see Fig. 2C,D) up to 4.0 mm, but is fused with the basioccipital by 4.3 mm. During subsequent development additional vertebrae fuse into this complex. Unlike *Acanthostracion* (Tyler, 1963), in which the first two vertebrae fuse to each other before that complex fuses to the basioccipital, our material of *Lactophrys* has the first vertebra fused to the basioccipital before the second fuses to that complex. We do not know if this difference is taxon-specific or if the pattern of fusion varies intraspecifically.

The unique occurrence of occipito-vertebral fusion in the Molidae and Ostraciidae among acanthomorphs clearly warrants a detailed reexamination of the phylogenetic relationships of the two taxa, which we are currently undertaking. The present and accepted consensus about the phylogeny of tetraodontiforms is mainly based on articles by Winterbottom (1974), Tyler (1980), and Winterbottom and Tyler (1983). Ostraciids are classified along with their putative sister group, the family Balistidae and the Triacanthidae and Triacanthodidae, as members of a monophyletic clade, the Sclerodermi or Balistoidei. Molids, on the other hand, are hypothesized to be part of a monophyletic assemblage, the Gymnodontes or Tetraodontoidae, which also comprises the Triodontidae, Tetraodontidae, and Diodontidae. Among recent authors, only Leis (1984) held a different view. On the basis of external larval characters, he proposed that the ostraciids form a monophyletic group with the tetraodontids, diodontids, and molids, in which they are more closely related to the latter two families. The results of this study and our ongoing project on tetraodontiform relationships indicate that Leis' hypothesis is correct and that ostraciids most likely are the sister group of the molids.

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