ORIGINAL ARTICLE

# An Introduction to the Comparison Shape of some Cranial Structures in Boleophthalmus Dussumieri and Periophthalmus Waltoni (Teleostei: Oxudercidae) in the Persian Gulf: An Exploratory Analysis with Micro-CT Scanning

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Received: 23 October 2022 / Accepted: 20 November 2022

## Abstract

**Purpose:** The osteological characteristics of fish, especially the head structure, are important in understanding the biological characteristics. Periophthalmus waltoni and Boleophthalmus dussumieri are among the mudskippers and are distributed along the coasts of the Oman Sea and the Persian Gulf.

**Materials and Methods:** After catching and fixing the samples in 96% ethanol, the samples were then sent to the preclinical laboratory (Lotus-InVivo) for micro-CT scanning (TPCF, in Tehran university of medical sciences) for imaging.

**Results:** In B. dussumieri, the skull is rudimentary and a high percentage of the bones is still cartilaginous. In this species, despite the larger head size, the braincase is small. In P. waltoni, the braincase is larger, but the skull tissue is completely bony and has very little cartilaginous. The jaws have also undergone drastic changes, corresponding to the change from a nearly fixed biting mouth to a flexible sucking mouth. In both species, the teeth are sharp and in two parts in the jaws. In P. waltoni, there are three pairs of sharp teeth for hunting in the upper jaw, the number of these teeth in B. dussumieri is four passes and it is less curved.

**Conclusion:** In this report, for the first time, the skull structure of the Persian Gulf was investigated. Micro CT technique has also been used for the first time. Mudskippers have developed special adaptations to live in mud in terrestrial and aquatic conditions. These adaptations are greater in P. waltoni, which shows greater degrees of terrestrialization, and requires detailed studies in this field.

**Keywords:** Cranial Skeleton; Neurocranium; Mudskipper; Micro-Computed Tomography Scanning, Persian Gulf.




DOI:

## 1. Introduction

Ecological and behavioral information about the first tetrapods that moved from water to land is not available, but this may be indicated by some extant fish. Osche (1962) suggested that mudskippers may be considered a model to explain the transition of organisms from water to land, in fact, mudskippers were the first vertebrates to make this transition in the late Devonian period (approximately 365-385 million years ago) have done and are known as tetrapodomorphs [1]. Mudskippers are considered organism models for transfer from water to land. Their transfer was independent and unmediated. They show many modifications in anatomical and physiological traits in their bodies that allow them to live and search for food on land [1].

The Gobioidei subclass with many species distributed throughout the tropics and is usually featured by small specimens with low mobility and resistance to temperature and salinity changes [2, 3]. The term ‘mudskippers; amphibious fish’ typically refers to four genera, namely Periophthalmodon, Periophthalmus, Boleophthalmus, and Scartelaos were part of the oxudercinae gobies (Gobiidae) in the past [4, 5]. Currently, they are isolated from the Gobiidae family and classified as Oxudercidae: Oxudercinae [6, 7], are distributed in mangroves and mudflats of the Indo-West Pacific and the western coast of Africa [8]. They represent the full range of terrestrialization from shallow waters to intertidal and supra-coastal areas [9]. Periophthalmus and Periophthalmodon spend most of their time out of the water, although their degree of terrestriality varies gradually between species within each genus. In contrast, Scartelaos is mainly aquatic and spends less time out of the water, and consequently, Boleophthalmus is less dependent on water [10].

According to the report [11, 12], three species of mudskippers are distributed in the Persian Gulf and the Sea of Oman (Iraq to Mumbai), which are: (Periophthalmus waltoni Koumans, 1941), (Boleophthalmus dussumieri Valenciennes, 1837) and (Scartelaos tenuis (Day, 1876)).

The amphibian ability of mudskippers makes them comparable with primitive quadrupeds. The skeletons of early tetrapods such as Tiktaalik and Pandarichthys show significant deviations from the modern fish skeleton design, while the skeleton of the mudskippers preserves the basic architecture of the early fish skeleton, in such a way that their body is supported by a cartilaginous skeleton and an extended notochord [13].

The adaptations that have made the mudskippers have the ability to move fast and accelerated on Muddy beaches [14] include various biological modifications, e.g., vision [15], skeletal division [12, 14, 16], fins, and the muscle system associated with them. For example, Scartelaos can stand on their tails and Periophthalmus can climb the roots and stems of mangrove rocks [14]. They also demonstrate diverse social behaviors with the mobility of the cranium during territorial defense and terrestrial feeding. For example, Boleophthalmus dussumieri Valenciennes, 1837, Boleophthalmus boddarti (Pallas, 1770), Periophthalmus modestus Cantor 1842, and Periophthalmus argentilineatus Valenciennes, 1838 show the cranial elevation and depression during territorial displacement. B. dussumieri, B. boddarti, P. modestus, P. argentilineatus, and Periophthalmodon schlosseri (Pallas, 1770) show the cranial elevation at sharp angle and yawn during mating or aggression [17, 18]. As these fishes interact with a substrate like benthic fishes, without the aid of buoyancy, modifications to their fin rays would be hypothesized to provide a functional benefit during terrestrial locomotion [19, 20].

We anticipate that the morphological detail and analytical power that come with the technique we here employed will assist us in this task. The skeletal structure of mudskippers is still poorly understood. Hence, there may be a possible link between the skeletal structure of the skull and their amphibious life. In order to clarify this hypothesis, we examined the anatomical configurations of the cranial vertebral joint and the arrangement of teeth in two species of P. waltoni and B. dussumieri, which show different degrees of terrestrial and feeding habits.

## 2. Materials and Methods

The study was conducted on three specimens of B. dussumieri and P. waltoni collected from the shores of the Musa estuary. Specimens were fixed in ethanol (96 %). Two to three years old adult specimens d were examined and the osteological study of the skull structure was carried out on them. The samples were then sent to the preclinical laboratory (Lotus-InVivo) for micro-CT scanning (TPCF, in Tehran university of medical sciences) for imaging. Micro-CT or Micro-Computed Tomography is a three-dimensional imaging device using X-rays. This device uses the same imaging method as a hospital CT scan, but compared to it, it has a much higher resolution.


**Figure 1.** A: *Periophthalmus waltoni.* B: *Boleophthalmus dussumieri* from Khor Jafari, Musa estuary, Persian Gulf, Iran. Scale bars = 1 cm

With this device, real 3D microscopic images of the internal structure of *B. dussumieri* and *P. waltuni* species were prepared in a completely non-invasive manner, without causing any change or destruction in the samples. Continuous imaging of the samples with 40 to 90 kV voltage, and 10-micron magnification, was prepared in two-dimensional and three-dimensional forms.

The geometry of this scanner is such that the sample inside the device was placed on a fixed bed and the X-ray source and the detector rotated around it on a ring so that they were facing each other and on both sides of the sample. The entire imaging process was done automatically with the console that was provided to the user next to the device.

## 3. Results

The skull can be divided into five parts, which include: the neurocranium (neural skull), its appendages (suspensorium), jaws, gill cover bones, and lamellar gill apparatus. The neural skull, which forms the brain compartment, is the most rigid part of the skull, which typically includes 40 to 50 bones. The connection between the neural skull and the jaws is made through a row of bones (laminar, simple, quadrate, metatarsals, etc.) in general, they are called appendages (suspensorium). From the point of view of evolution, these bones have undergone drastic changes in terms of shape, size, and position, from the lineage fish to today's teleost fish and quadrupeds.

In bony fishes, the jaws have undergone drastic changes, which are related to the change from a fixed biting mouth to a flexible sucking mouth. Regarding the details related to this change, it should be mentioned that in the upper jaw, the premaxilla bone is considered the main bone instead of the maxilla bone, and the major bones that contain teeth are also changed. On the contrary, the main bones of the lower jaw (teeth) have remained almost unchanged.

The neurocranium in B. dussumieri is wide in the posterior part and slightly narrow in the middle and anterior parts. In P. waltoni, it is wide in the ventral part and narrow in the middle and anterior parts, so it has a slightly triangular shape in Figure 2.

In the Branchiocranium, the upper jaw includes a pair of Maxilla and Premaxilla bones.

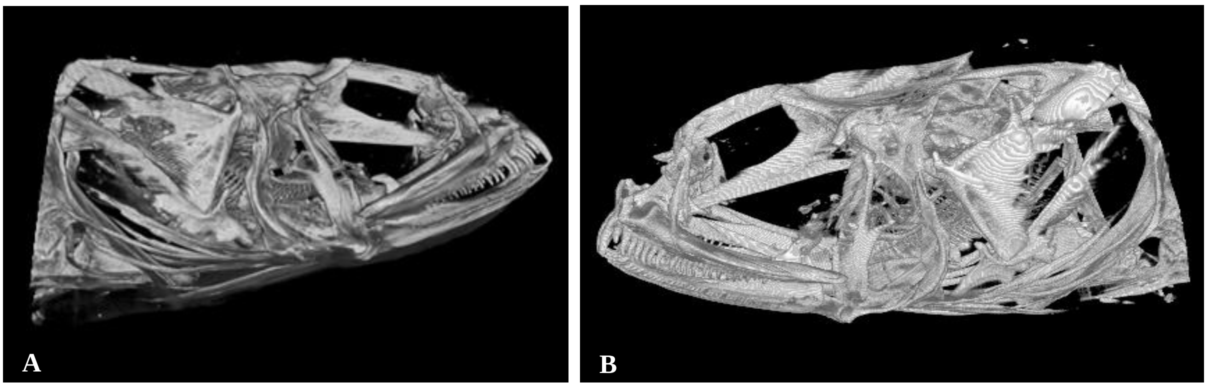


**Figure 2.** Three-dimensional view of Periophthalmus waltoni (A) and Boleophthalmus dussumieri (B), cranial skeleton. (Number 1: Primaxilla, 2: Maxilla, 3: Supermaxilla, 4: Nasal, 5: Prefrontal, 6: Frontal, 7: Postfrontal, 8: Parietal, 9: Vomer, white arrow: Opercular)

In B. dussumieri, the premaxilla is wide in the anterior part and narrow in the posterior part. In P. waltoni, the premaxilla is wide in the anterior part and slightly narrower and slightly convex in the posterior part. In addition, it seems that the thickness of this bone is greater in P. waltoni in Figure 3.

On the premaxilla in both species, there is only one row of canines, which are directed vertically. The front four pairs are larger in B. dussumieri and the front three pairs are larger in P. waltoni, while the others are reduced posteriorly, less so in P. waltoni. On the dentary, in both species, there is a row of teeth in the margin, which are directed horizontally. In P. waltoni, they have found a slight inward deviation, which is due to their predatory behavior. However, in B. dussumieri, the horizontal position is forbidden, and this is because of their vegetarian diet. In B. dussumieri, there are approximately 65 teeth in the Premaxilla and 69 teeth in the dentary. In P. waltoni also, there are 26 teeth in the Premaxilla and 26 teeth in the dentary. Overall, P. waltoni has a stronger skull with a narrower snout and a more developed premaxilla than B. dussumieri, despite having a smaller skull. Compared to the small and almost triangular P. waltoni skull, the B. dussumieri skull is significantly wider and has a rhomboid shape. The frontal bone is also much stronger and more defined in P. waltoni than in B. dussumieri. It is weaker and thinner in B. dussumieri in Figure 4.

Eventually, in B. dussumieri, the skull is rudimentary. As it is still cartilaginous-osseous, a high percentage of the structure is still cartilaginous. This state is in such a way that the mass in the middle remains cartilaginous and the surrounding area is becoming ossified. In this species, despite the larger head size, the brain is small. In P. waltoni, the brain area is larger, but the skull tissue is completely bony and has very little cartilaginous. The floor of the skull is spongy. The connection between the neurocranium and the jaws is made through a row of bones, one pair of each (hyomandibular, symplectic, quadrate, suspensorium, etc.), [21]. In terms of shape, size, and position, these bones have undergone more severe changes in P. waltoni than in B. dussumieri. The jaws have also undergone drastic changes, corresponding to the change from a nearly fixed biting mouth to a flexible sucking mouth. In both species, the teeth are sharp and in two parts in the jaws. In P. waltoni, there are two pairs of sharp teeth for hunting in the upper jaw, the number of these teeth in B. dussumieri is one pair and it is less curved.

## 4. Discussion

The skull of a bony fish is a skillful puzzle of articulated bones that differ in many ways from the skulls of other fish [22]. Mudskippers have many morphological adaptations that enable them to cope with the different conditions encountered in and between aquatic and terrestrial habitats [18]. In comparison with other gobies, the pre- orbital shortening of mudskipper skulls is evident [12, 23], as is the reduction in bones [23].



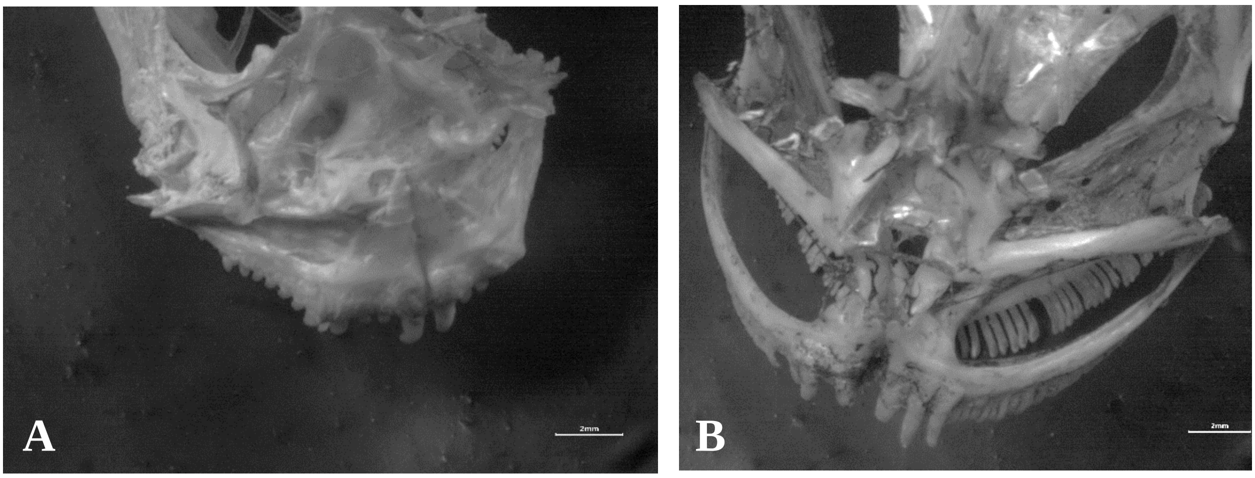


**Figure 3.** Two dimensional view of Perimaxilla and maxilla form in *Periophthalmus waltoni* (A) and *Boleophthalmus dussumieri* (B). (Black arrow: Primaxilla, Red arrow: Maxilla, white arrow: Nasal, Blue arrow: Jugal, Yellow arrow: Suspensorium)

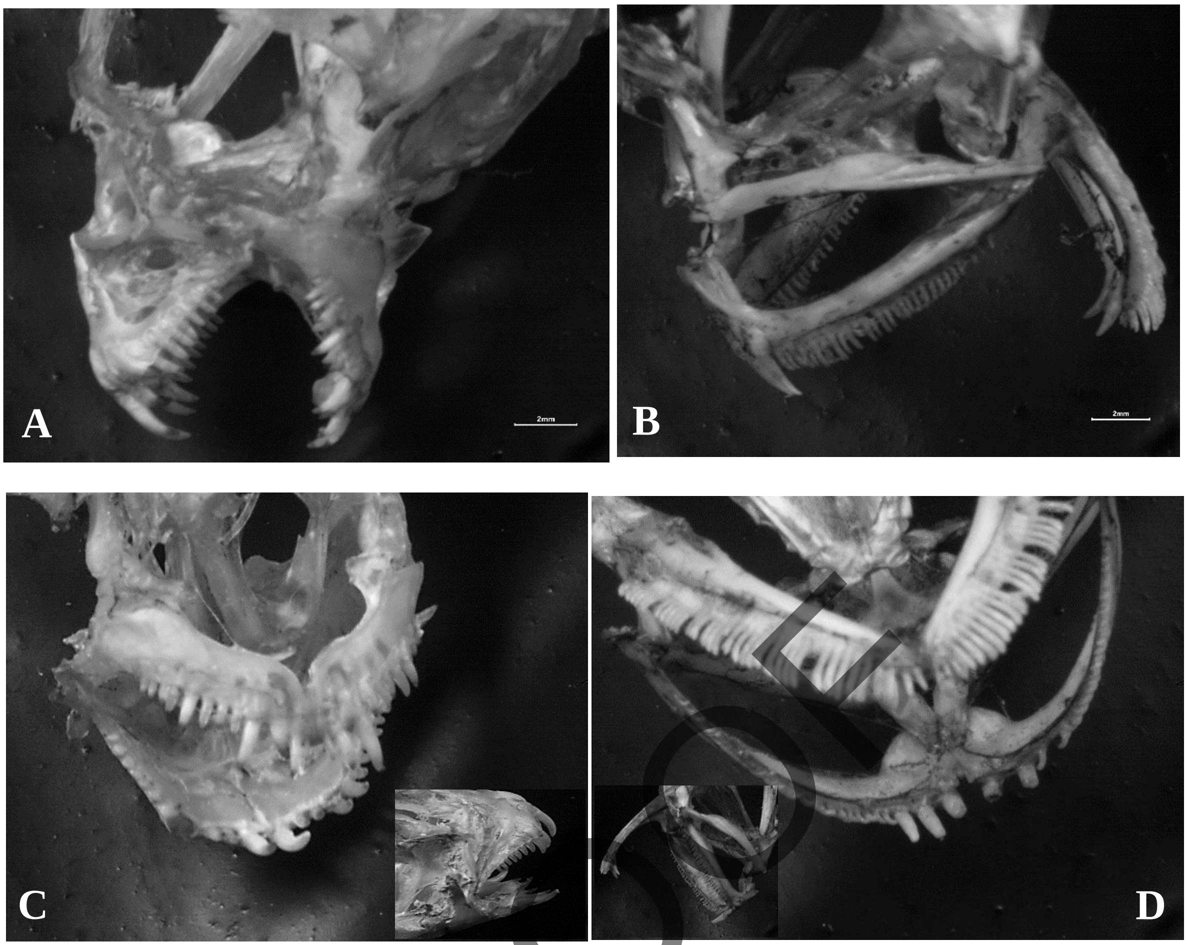


**Figure 4.** A two-dimensional image of the shape of teeth and jaws in *Periophthalmus waltoni* (A, C) and *Boleophthalmus dussumieri* (B, D). (Red arrow: Dentary, White arrow: Quadrate, Yellow arrow: Hyomandibula)

Morphologically, little attention has focused on the pharyngeal plates. The marked differences in the dentition of these plates relate to different feeding strategies [24] but they may also have taxonomic value.

Our preliminary analysis reveals specialized anatomical features of the cranial apparatus that may indicate the adaptation of mudskippers to feeding and living on land. For the first time in the world, we investigated the morphology of the P. waltoni and B. dussumieri skulls using the micro-CT scanning technique. Thus, in this study, we clearly observed P. waltoni quite osseous and B. dussumieri chondro-osseous skull. The changes that were observed in the bone collection of P. waltoni in accordance with its more terrestrial lifestyle, including the strengthening of bone fragments in P. waltoni compared to Dasomeri, which shows the specialization of P. waltoni compared to B. dussumieri, which has preserved the characteristics of its fish species. On the other hand, changing the shape of the teeth to protrude and then bend inward (like a scythe), is suitable for its hunting life. Also, the change in the location of the eye sockets from around to straight and up, which is for the proper vision of the fish on the muddy shores, on the other hand, the number of teeth in B. dussumieri is not proportional and is different in the two upper and lower jaws, but in P. waltoni, the number of teeth is different. The teeth are reduced and the number of teeth is equal in the upper and lower jaw, and of course, it may be different, but it was equal in the samples we examined.

P. waltoni has a stronger and shorter skull (Approximately, the P. waltoni skull is 25 mm and the B. dussumieri skull is 34 mm), and a narrower snout (for convenience when hunting) with more developed premaxillae compared to B. dussumieri, mostly due to the greater strength of its dorsal cranial skeleton. Also, we think it is due to more specialization and movement of P. waltoni towards land.

In order to eat food with fewer mud particles, they must remove the mud surface. Boleophthalmus boddarti with head depression and lateral rotation allows the teeth to cover a thin layer of mud [20].

In herbivores and omnivores mudskippers, e.g., O. nexipinnis, Pa. serperaster, Pd. elongatus, S. histophorus, and B. boddarti, it has been reported that they graze in very shallow waters or mudflats. Their food items are richest in the upper layer of the mud surface, all on a dentary with horizontal teeth [8, 18]. We also observed this condition in the species P. waltoni and B. dussumieri, although in P. waltoni this amount of horizontalization was slightly less than in B. dussumieri. We hypothesize that P. waltoni spends more time in the mud than B. dussumieri, as well as the forward-protruding position of the canines, which suggests that the head burrows into the mud to find food. However, whatever the feeding behavior, in order to eat, the head must be pressed and the dentary teeth placed at a low angle to the flower [18]. On the other hand, the study of the dental and skeletal system of B. dussumieri herbivores showed that the feeding apparatus of the fish has been modified presumably to transport diatom cells from the mudflat surface to the digestive tract with a minimum admixture of mud particles [8]. Generally, the fish further needs to effectively separate diatom cells from mud particles. Each feeding bout takes place with a mouthful of water, and therefore, somewhat resembles pump filtration of some aquatic fishes in which food particles are ingested sporadically by a series of suctions created by the expansion of the buccal and opercular cavities, while a fish is stationary, but unlike ram filtration, in which a large volume of water continuously passes through the buccal cavity, while a fish is swimming with mouth agape and opercles flaring [18].

Since the examination of the skeletal structure of the skull of the mudskippers has undergone various changes, in the present study, we only examined an introduction to their skeletal structure. In future studies, we will do a detailed qualitative and quantitative analysis of all the skeletal structures of the skulls of the mentioned species.

## Acknowledgments

Special appreciation to the respected officials of the preclinical laboratory (Lotus-InVivo) for micro-CT scanning (TPCF, in Tehran university of medical sciences) who prepared the images used in this research with high precision and with the best quality and cooperated with us to the fullest. Also, gratitude to Dr. Rasoul Karamiani for our help and guidance in naming the bone fragments.

All experimental procedures involving animals were conducted in accordance with the Razi University of Kermanshah, Iran Regulation on Ethics in Animals.

Experimentation (Number: IR.RAZI.REC.1400.064).



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