

The Gas Bladder of Puffers and Porcupinefishes (Acanthomorpha: Tetraodontiformes): Phylogenetic Interpretations

Bruno Chanet,^{1*} Claude Guintard,² and Guillaume Lecointre¹

¹Département Systématique et Evolution, ISYEB, UMR 7205 CNRS-MNHN-UPMC-EPHE, Muséum national d'Histoire naturelle, CP 50, 57 rue Cuvier, 75005 Paris, France

²Laboratoire d'Anatomie Comparée, ONIRIS – Ecole Nationale Vétérinaire de l'Agroalimentaire et de l'Alimentation, Nantes Atlantique, Route de Gachet, CS 40 706, 44307 NANTES Cedex 03, France

ABSTRACT The anatomy of the gas bladder of Diodontidae (porcupinefishes) and Tetraodontidae (pufferfishes) was studied on the basis of dissections and magnetic resonance imaging. Among the examined taxa of Tetraodontiformes, only puffers and porcupinefishes possess a thick walled and dorsally U-shaped or crescent-moon-shaped gas bladder. In the tetraodontid genus *Lagocephalus* the gas bladder is reduced to a rudiment. The species belonging to the genera *Canthigaster*, *Arothron*, and some species of *Tetraodon* differ in the positioning of their crescent-moon-shaped gas bladder. These observations confirm the close relationship of: (i) Diodontidae and Tetraodontidae and (ii) *Canthigaster*, *Arothron*, and some species of *Tetraodon*. The heterogeneity of the genus *Tetraodon* is supported by the gas bladder morphology, as previously suggested by molecular studies. *J. Morphol.* 275:894–901, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: gas bladder; anatomy; phylogenetics; Diodontidae; Tetraodontidae; Tetraodontiformes

INTRODUCTION

Diodontidae (porcupinefishes) and Tetraodontidae (pufferfishes) are families of Tetraodontiformes composed of six genera, 19 species and about 18 genera, 98 species, respectively, (Nelson, 2006). They are well known at the alpha level, and their biology, morphology, and osteology have been described in great detail in numerous articles documenting their unusual structure (Winterbottom, 1974; Tyler, 1980), their toxicity (Arakawa et al., 2010), and their ability to inflate their body (Braidner, 1994). These two family clades are considered as sister groups. This close relationship has been successively established by the study of their myology (Winterbottom, 1974), their osteology (Tyler, 1980; Tyler and Sorbini, 1996; Santini and Tyler, 2003, 2004), their otoliths (Nolf and Tyler, 2006), and by the comparison of DNA sequences (Holcroft, 2004, 2005; Alfaro et al., 2007; Yamanoue et al., 2008, 2011; Near et al., 2012; Near et al. 2013; Santini et al., 2013a). Nevertheless, although the organization of the genome of some tetraodontiform species has been deciphered

(Aparicio et al., 2002; Jaillon et al., 2004), the internal soft anatomy of these fishes, with the exception of muscles (Winterbottom, 1974), has only been incompletely described (Saint-Hilaire, 1809; Lacépède, 1836; Gregory and Raven, 1934; Breder and Clark, 1947; Johnson and Britz, 2005; Chanet et al., 2012; Chanet et al., 2013) and the anatomy of the gas bladder only rarely detailed. The aim of this work is to describe the diversity of the structure of the gas bladder in Tetraodontiformes and to point out similarities and possible synapomorphies based on this organ. The study of the structure of the gas bladder of puffers and porcupinefishes is based on dissections of both fresh and alcohol preserved (AP) specimens and examinations of images obtained by noninvasive imaging techniques (magnetic resonance imaging [MRI]).

MATERIAL AND METHODS

Following Harder (1975) and McCune and Carlson (2004), we use the term “gas bladder” rather than “swim bladder” as this organ is a gas-filled sac not only used for swimming and control of buoyancy but also, at least in some groups of fishes, in breathing, auditory reception, and sound production (Kasumyan, 2008).

The anatomy of 21 fresh (F) or AP specimens of 17 tetraodontiform species was studied. The examined fresh specimens were fished in the eastern European seashores or came from public aquariums where they died naturally. Standard Length (SL), is indicated for each examined specimen. For practical

Contract grant sponsor: Fondation TOTAL (project “Classification des poissons marins : les téléostéens acanthomorphes”).

*Correspondence to: Bruno Chanet, Département Systématique et Evolution, ISYEB, UMR 7205 CNRS-MNHN-UPMC-EPHE Muséum national d'Histoire naturelle, CP 50, 57 rue Cuvier 75231 Paris Cedex 05, France. E-mail: chanet@mnhn.fr

Received 15 October 2013; Revised 17 January 2014; Accepted 21 February 2014.

Published online 13 March 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jmor.20266

purposes, these specimens after classical dissection (CD) were not in a state that enables their preservation in a systematic collection. They do not then possess catalog number.

The AP specimens are from the collections of the USNM (National Museum of Natural History, Smithsonian Institution, Washington, DC), except for a single specimen of a longhorn cowfish *Lactoria lactoria* (Linnaeus, 1758), Ostraciidae, from the collections of the Department of Comparative Anatomy (ENVN-ONIRIS, Nantes, France). A MRI examination (1.5 T) was conducted on a fresh gray triggerfish *Balistes capriscus* in the Unité d'imagerie médicale (ONIRIS, Nantes). MRI examinations were performed using a 1-T supraconductor magnet (Harmony Siemens). A standard body coil was used along with a three planes localizer. T_1 and T_2 ponderations were performed. The T_1 (TR = 516–656 ms and TE = 13 ms) and T_2 (TR = 3840–5170 ms and TE = 91–115 ms) sequences in three planes (virtual sagittal, horizontal [= coronal], and transverse) extending from the anterior extremity of the snout to the distal tip of the caudal fin were acquired after the specimens were thawed [more technical information for these MRI examinations can be found in Chanet et al. (2012). CD and parasagittal mechanical sections (PMS) on frozen large fishes using an electric meat saw (La Bovida, BG) were performed on the following specimens:

Balistidae

Balistes capriscus Gmelin, 1789, fished in the eastern Atlantic Ocean, F, 300 mm SL, MRI, CD.

Balistes capriscus Gmelin, 1789, fished in the eastern Atlantic Ocean, F, 267 mm SL, PMS.

Melichthys vidua (Richardson, 1845), F, from the Aquarium Mare Nostrum (Montpellier, France), 123 mm SL, CD.

Diodontidae

Diodon holocanthus Linnaeus, 1758, F, from the Aquarium de Vannes (Vannes, France), 232 mm SL, CD.

Diodon liturosus Shaw, 1804, F, from the Aquarium Mare Nostrum (Montpellier, France), 185 mm SL, CD.

Molidae

Mola mola (Linnaeus, 1758), F, two specimens, 124 cm TL (fished in the English Channel) and 142 cm TL (fished in the eastern Atlantic Ocean), MRI (Chanet et al., 2012) and CD.

Ostraciidae

Acanthostracion quadricornis (Linnaeus, 1758) USNM 110416,¹ 340 mm SL, (dry skeleton with dry gas bladder preserved).

Lactoria cornuta (Linnaeus, 1758), AP, collection Anatomie comparée ENVN-ONIRIS spécimen no. PV2011-007, 150 mm SL, from the Aquarium Mare Nostrum (Montpellier, France).

Lactoria cornuta (Linnaeus, 1758), F, from the Aquarium Mare Nostrum (Montpellier, France), 283 mm SL, CD.

Ostracion cubicus Linnaeus, 1758, F, from the Aquarium Mare Nostrum (Montpellier, France), 147 mm SL, CD.

Tetrosomus gibbosus (Linnaeus, 1758), F, from the Aquarium Mare Nostrum (Montpellier, France), 74 mm SL, PMS.

Tetraodontidae

Arothron hispidus (Linnaeus, 1758), AP, USNM 374361,¹ 138 mm SL, CD.

Arothron nigropunctatus (Bloch and Schneider, 1801), F, from the Aquarium Mare Nostrum (Montpellier, France), 256 mm SL, CD.

Canthigaster compressa (Marion de Procé, 1822), F, from the Aquarium Mare Nostrum (Montpellier, France), 64 mm SL, CD.

Lagocephalus lagocephalus (Linnaeus, 1758), F, fished in the eastern Atlantic Ocean, 393 mm SL, CD.

Lagocephalus laevigatus (Linnaeus, 1758), AP, USNM 122000,¹ 228 mm SL, CD.

Lagocephalus laevigatus (Linnaeus, 1758), AP, USNM 289361,¹ 165 mm SL, CD.

Tetraodon cutcutia Hamilton, 1822, F, from the Aquarium Mare Nostrum (Montpellier, France), 68 mm SL, CD.

Tetraodon mbu Boulenger, 1899, F, from the Aquarium de la Porte Dorée (Paris, France), 316 mm SL, CD.

Tetraodon Palembangensis Bleeker, 1852, F, from the Aquarium Mare Nostrum (Montpellier, France), SL: 88 mm SL, CD.

These observations were compared to descriptions present in the literature and to MRI images available at the digital fish library (DFL), <http://www.digitalfishlibrary.org>. The specimens are deposited at the Marine Vertebrate Collection, Scripps Institution of Oceanography (SIO), University of California, San Diego, CA. The specimens examined through the DFL are:

Balistidae

Balistes polylepis Steindachner, 1876, SIO 07-163, 174 mm SL, AP, MRI, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=103>

Diodontidae

Chilomycterus reticulatus (Linnaeus, 1758), AP, SIO 07-187, 370 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=211>

Diodon holocanthus Linnaeus, 1758, SIO 60-76, 226 mm SL, AP, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=5>

Molidae

Masturus lanceolatus (Liénard, 1840), AP, SIO 89-79, 139 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=80>

Mola mola (Linnaeus, 1758), AP, SIO 47-188, 290 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=93>

Ranzania laevis (Pennant, 1776), AP, SIO 70-250, 314 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=94>

Monacanthidae

Pervagor spilosoma (Lay and Bennett, 1839), AP, SIO 53-539, 74 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=363>

Ostraciidae

Acanthostracion quadricornis (Linnaeus, 1758), AP, SIO 91-75, 204 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=6>

Ostracion nasus Bloch, 1785, AP, SIO 73-189, 158,5 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=7>

Tetraodontidae

Arothron immaculatus (Bloch and Schneider, 1801), AP, SIO 73-189, 150,5 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=11>

Canthigaster punctatissima (Günther, 1870), AP, SIO 61-225, 57 mm SL, images can be downloaded at: <http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=1>

¹Specimens were examined on our behalf by J.C. Tyler at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

TABLE 1. Distribution of characters

Characters	Distribution
Gas bladder with developed anterior lateral lobes	Balistidae and Ostraciidae
Gas bladder absent in adult	Molidae
Gas bladder abortive in adult	<i>Lagocephalus</i>
Gas bladder with two conic expansions: conic expansions anteriorly oriented	Diodontidae and in the following tetraodontid species: <i>Chelododon patoca</i> , <i>Torquigener hypselogeneion</i> , <i>Sphoeroides maculatus</i> , <i>Takifugu oblongus</i> , <i>Tetraodon cutcutia</i> and <i>T. palembangensis</i>
conic expansions posteriorly oriented	Following tetraodontid species: <i>Arothron immaculatus</i> , <i>A. firmamentum</i> , <i>Canthigaster punctatissima</i> , <i>Tetraodon mbu</i> and <i>T. lineatus</i>

See text and Figs 3, 4, and 5.

Technical information for these MRI can be examined for each species on the website of the DFL (<http://www.digitalfishlibrary.org>)

RESULTS

Tetraodontiformes exhibit a diversity of gas bladder shapes (Table 1).

Triacanthodidae

In spikefishes, the gas bladder is an oblong, usually thin walled, somewhat rounded sac (Tyler, 1968:27, Fig. 5, left; Tyler, 1980:46).

Triacanthidae

In triplespines, the gas bladder is thick walled, somewhat elongated, large, and extends posteriorly almost the entire length of the abdominal cavity (Tyler, 1968:27, Fig. 5, right; Tyler, 1980:78).

Monacanthidae

In filefishes, the gas bladder is a large and elongated sac present all along of the dorsal part of the abdominal cavity (Fig. 1).

Triodontidae

Little is known about the gas bladder of the treetooth puffer, but this organ appears as a long sac, without segmentation or diverticula, present

in the dorsal part of the abdominal cavity (Johnson and Britz, 2005:180, Fig.3D).

Balistidae

The gas bladder of triggerfishes is an oblong, thick-walled sac occupying the entire dorsal part of the abdominal cavity (Fig. 2). The anterior lateral extremities exhibit small evaginated lateral lobes (Fig. 2B). Each lobe is in contact with a thin layer of integument and some enlarged scales (Salmon et al., 1968; Tyler, 1980). This peculiar structure was called a “tympanum” by Gregory (1933:285) and “drumming membrane” by Moulton (1958). Those two authors suggested that this membrane was involved in sound production by triggerfishes and this hypothesis was confirmed by the experimental work of Salmon et al. (1968).

Ostraciidae

The gas bladder of the examined taxa of boxfish species is similar to that present in triggerfishes: it is a voluminous and elongated thick-walled dorsal sac with two anterior and evaginated lateral lobes. It is possible that these lobes are involved, like in balistids, in sound production (Lobel, 1996).

Diodontidae

The gas bladder of the examined species of porcupinefishes is very peculiar. It is a large thick walled and bilobed structure: it is dorsally U-

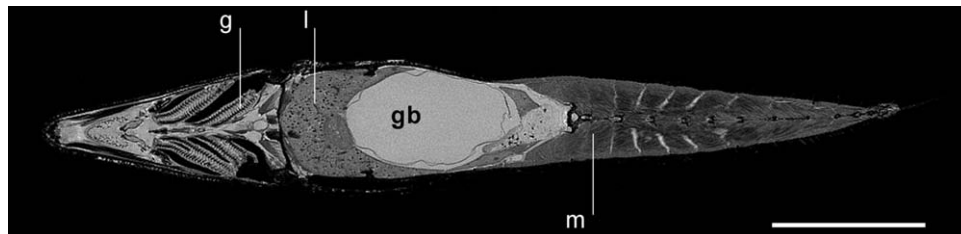


Fig. 1. *Pervagor pilosoma* (fantail filefish) horizontal magnetic resonance image (SIO 53-539, 74 mm SL). g, gill; gb, gas bladder; l, liver; m, muscles. Scale = 10 mm.

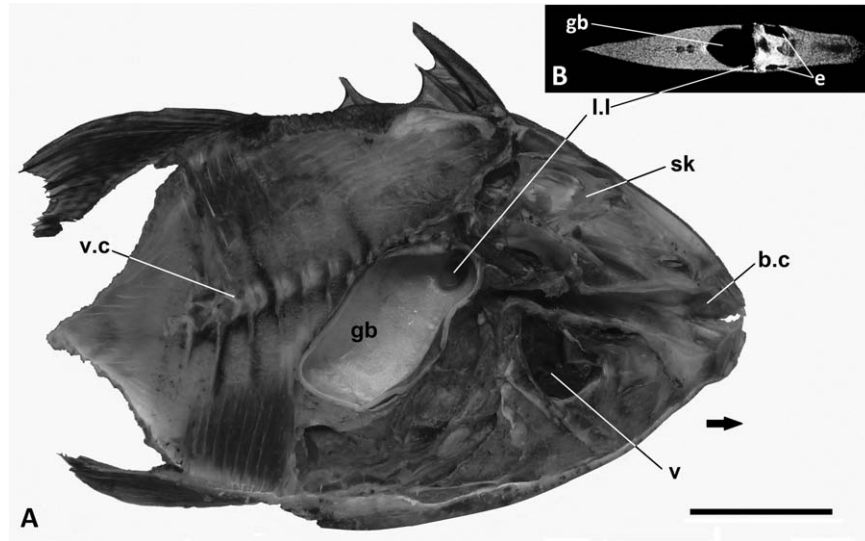


Fig. 2. **A.** *Balistes capriscus* (267 mm SL; gray triggerfish), sagittal section of a frozen specimen, right lateral view. **B.** Horizontal plan (MRI) through a fresh *B. capriscus* (300 mm SL). b.c, buccal cavity; e, eye; gb, gas bladder; l.l, lateral lobe; sk, skull; v, ventricle; v.c, vertebral column. The black arrow indicates the anterior part of the specimen. Scale = 50 mm.

shaped, crescent-moon-shaped, or kidney-shaped with two conic expansions (Fig. 3).

These expansions are antero-dorsally oriented and the bladder is concave posteriorly (Fig. 3). We have found this pattern in all of the examined diodontid taxa and it has been mentioned in the genus *Diodon* by Leis (1978:538), Winterbottom (1974:195, Fig. 173), Tyler (1980:9), and Brainerd (1994, Fig. 5:249), and also in *D. rivularis* (synonym of *Chilomycterus schoepfii*) by Delaroche (1809:272).

Tetraodontidae

Among examined tetraodontids, the studied species of *Lagocephalus* do not have easily recognizable gas bladders. Several dissections, on fresh and AP specimens, indicate that the gas bladder is aborted or reduced to a small flacid sac on the ventral surface of the kidney. When well developed, the gas bladder of puffers has a shape similar to the gas bladder of porcupinefishes, with two large conic expansions. Nevertheless, these expansions are oriented posteriorly, instead of anteriorly as previously described in several species of porcupinefishes.

Abe (1952) has described that in *Chelonodon patoca*, *Torquigener hypselogeneion*, *Sphoeroides maculatus*, *Takifugu oblongus* these conic expansions are anteriorly directed, as in diodontids. We observed the same disposition in *Tetraodon cutcutia* and *Tetraodon palembangensis*. By contrast, in *Arothron immaculatus* (Fig. 4A), *Arothron firmamentum*, *Canthigaster punctatissima* (Berquist et al., 2012:8, Fig.3; Fig. 4B), *Tetraodon mbu*, *Tetraodon lineatus* (Saint-Hilaire, 1809), the conic expansions are posteriorly directed (Fig. 4C). In

Tetraodon cutcutia and *Tetraodon palembangensis*, the wall of the gas bladder is very thin; in other species of *Tetraodon* this organ is thick walled.

Molidae

Adult molas have no gas bladder (Cuvier, 1805; Gregory and Raven, 1934; Tyler, 1980; McCune and Carlson, 2004; Pope et al., 2010; Chanet et al., 2012). It would be interesting to determine if this organ is present in larval stages and in juvenile molas.

DISCUSSION

The structure, shape, and presence or absence of the gas bladder within teleostean fishes have been the subject of several surveys (Tominaga et al., 1996; McCune and Carlson, 2004). This organ is absent in numerous species and the multiple cases of regression of the gas bladder among teleosts are considered as adaptations related to benthic habitats, like flatfishes or anglers, or to deep sea environments, like aulopiforms, to fast swimming, like some tunas and one species (*S. scombrus*) of the genus *Scomber*, and to quick or frequent movements within the water column, like remoras and molas (McCune and Carlson, 2004). Nevertheless, the absence of the gas bladder in teleosts that feed in the water column is interpreted as involving phyletic inertia, like in some notothenioids, because it is inherited from an ancestral loss (Eastman, 1993:156; Ekau, 1991). Numerous peculiarities of the gas bladder have been described, like the simple heart shaped gas bladder of some species of the family Auchenipteridae (Birindelli et al., 2012), the two-chambered gas bladder of the

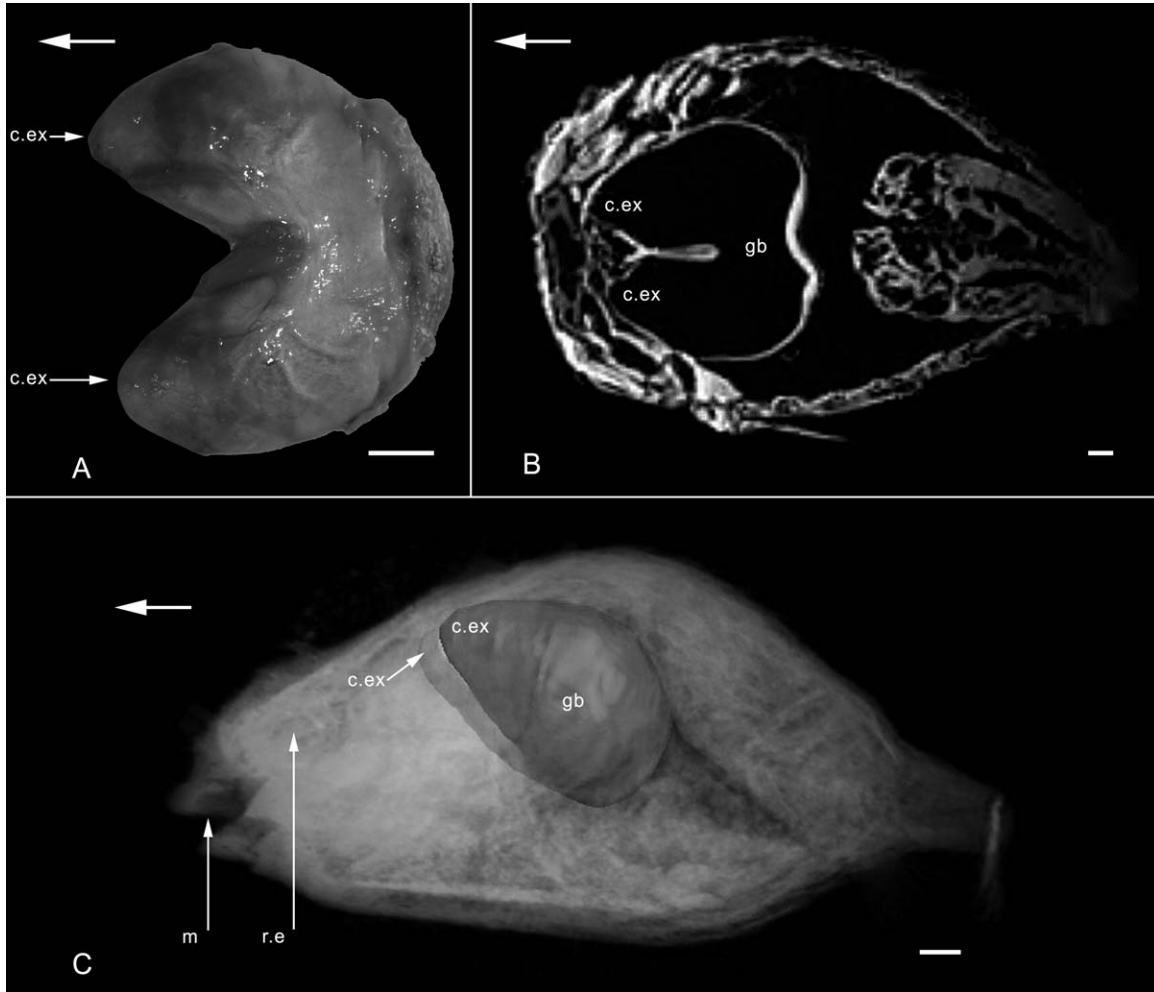


Fig. 3. **A.** Isolated gas bladder of a longspined porcupinefish, *Diodon holocanthus* (232 mm SL), dorsal view. **B.** Horizontal section (MRI) through *D. holocanthus* (SIO 60-76, 226 mm SL). **C.** 3D segmentation display of the gas bladder of *D. holocanthus* (SIO 60-76, 226 mm SL). c.ex, conic expansion; gb, gas bladder; m, mouth; r.e, right eye. Each arrow indicates the anterior part of the specimen. Scale = 10 mm.

ostariophysans (Fink and Fink, 1981) or the gas bladder composed of many small bubbles present in Istiophoridae (Collette et al., 2006). Adult molas excepted, tetraodontiform fishes are known to possess a well-developed thick-walled and physoclistous gas bladder (Cuvier, 1817:149–150; Tyler, 1968, 1980:15, 43, and 264). This work provides evidence that the structure of this organ is diverse within this order and that the examined species of the genus *Lagocephalus* are the first non-molid taxa showing an absence, or at least a great reduction, of the gas bladder in adult Tetraodontiformes. A more extensive study of this structure among all species of the genus *Lagocephalus* would be needed to confirm its rudimentation as a generic character and to specify at which developmental stage this organ regresses to a rudiment or is totally absent. The examined taxa of balistids and ostraciids have anterior and evaginated lateral lobes of their gas bladder; this character may

corroborate the traditional morphologically based interpretation of these two families being closely related, as proposed by Gregory (1933), Winterbottom (1974), Matsuura (1979), Tyler (1980), Winterbottom and Tyler (1983), Klassen (1995), and Santini and Tyler (2003, 2004). Nevertheless, several studies have challenged this relationship. One of the many analyses conducted by Santini and Tyler (2004) supports an ostracioid + tetraodontoids relationship, even if the majority of the analyses of that work support a balistoid + ostracioid clade. Rosen (1984), on osteological characters, Leis (1984), on larval characters, Britz and Johnson (2005), on occipital-vertebral fusion, and molecular studies (Holcroft, 2004, 2005; Alfaro et al., 2007; Yamanoue et al., 2008; Near et al., 2012; Near et al. 2013; Santini et al., 2013a [Fig. 5]) present evidence contradicting the existence of a clade composing triggerfishes and boxfishes. The present data about the anatomy of

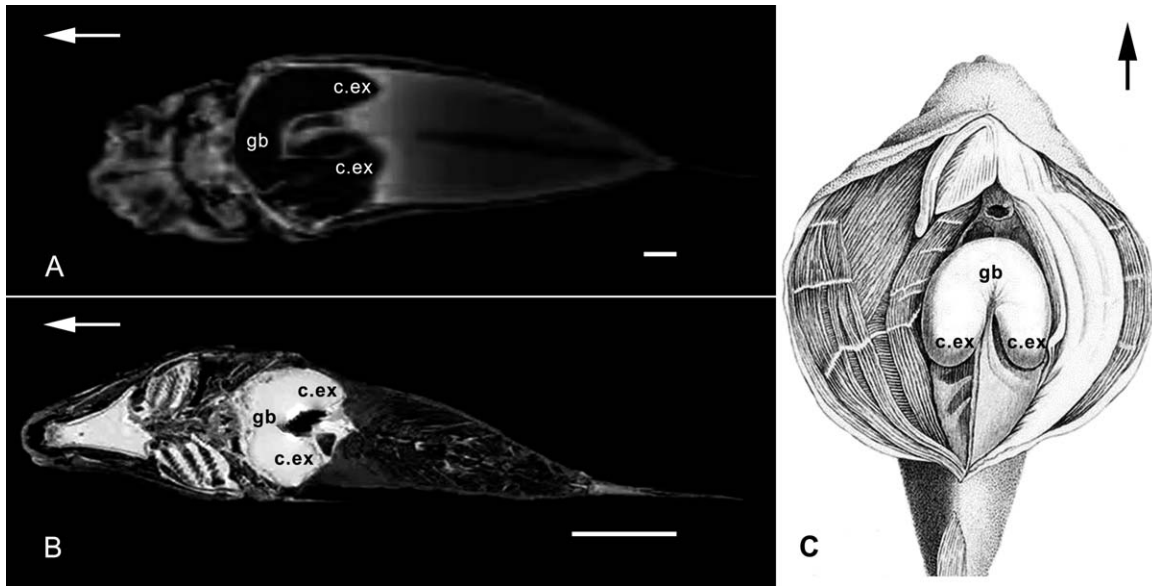


Fig. 4. **A.** Horizontal section (MRI) through an immaculate puffer, *Arothron immaculatus* (SIO 73-189, 150,5 mm SL). **B.** Horizontal section (MRI) through a spotted sharpnose puffer, *Canthigaster punctatissima* (SIO 61-225, SL 57 mm). **C.** Illustration of the gas bladder of a dissected fresh water puffer fish, *Tetraodon lineatus*, ventral view, modified from Saint-Hilaire (1809: pl. II, Fig. 2). Each arrow indicates the anterior part of the specimen. c.ex, conic expansion; g, gill; gb, gas bladder. Scale = 10 mm.

the gas bladder should be taken into consideration in future works dedicated to tetraodontiform inter-familial relationships before assessing that this similarity in the structure of their gas bladder is a

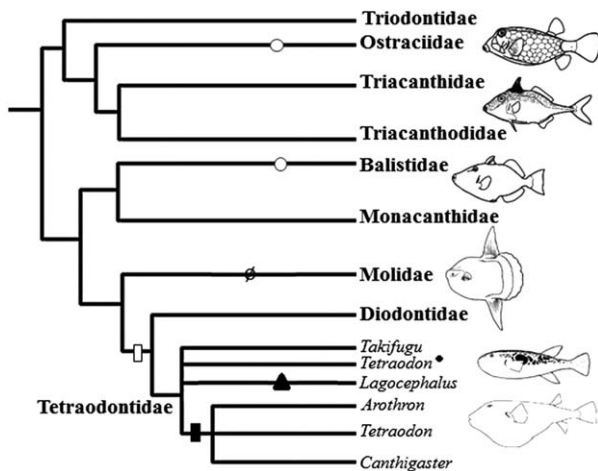


Fig. 5. Summary tree depicting the placement of the present gas bladder synapomorphies on the interrelationships of Tetraodontiformes as recovered by Santini et al. (2013a), the paraphyly of *Tetraodon* being additionally displayed according to Yamanoue et al. (2011) and our findings in *Tetraodon cutcutia* and *Tetraodon palembangensis* (top “*Tetraodon*” • in the figure). The white circles O represent the distribution of a gas bladder with developed anterior lateral lobes. The black triangle ▲ indicates the great reduction of gas bladder in *Lagocephalus* while the Ø indicates the absence of gas bladder in adult *Mola*, the white rectangle □ represents the distribution of a gas bladder with two conic expansions. The black rectangle ■ represents the distribution of a gas bladder with two posterior conic expansions. See text for comments.

convergence and the result of possible recurrent adaptations to sound production rather than an inheritance from common ancestry.

Moreover, porcupinefishes (Diodontidae) and pufferfishes (Tetraodontidae) are unique among Tetraodontiformes in possessing a U-shaped or crescent-moon-shaped gas bladder with two conic expansions. This peculiar structure can be considered as a putative synapomorphy of these two families, representing complementary evidence to corroborate the close relationship between these groups. Prior works established this relationship on the basis of the study of their myology (Winterbottom, 1974), of their osteology (Tyler, 1980; Tyler and Sorbini, 1996; Santini and Tyler, 2003, 2004), of their otoliths (Nolf and Tyler, 2006) and by the comparison of DNA sequences (Holcroft 2004, 2005; Alfaro et al., 2007; Yamanoue et al., 2008; Near et al., 2012; Near et al. 2013; Santini et al., 2013a,b). To this existing evidence, we add here gas bladder anatomy as structure supporting the previous phylogenies.

However, within the Tetraodontidae, various genera differ by the orientation of the conic expansions that are anteriorly oriented (like in diodontids) in at least two species of the genus *Tetraodon* (*T. cutcutia* and *T. palembangensis*), or posteriorly oriented in the genera *Arothron*, *Canthigaster* and in at least two species of the genus *Tetraodon* (*T. mbu*, *T. lineatus* (Saint-Hilaire, 1809); *Tetraodon* appears as paraphyletic or polyphyletic according to molecular studies. The gas bladder with two anterior conic expansions appears to be phylogenetically basal to the derived character state of a

gas bladder with posterior conic expansions (Fig. 5). When these observations are compared to the results of several recent molecular works (Holcroft, 2004, 2005; Alfaro et al., 2007; Yamanoue et al., 2011; Near et al., 2012; Near et al. 2013; Santini et al., 2013a) elucidating the interrelationships of tetraodontid species (Fig. 5), it appears that *Arothron*, *Canthigaster*, and several *Tetraodon* species are closely related; they share a common ancestor more recent than the one shared with other pufferfishes. Our finding of anterior conic expansions in *T. cutcutia* and *T. palembangensis*, contrasting with *T. mbu* and *T. lineatus*, conforms to the findings of Yamanoue et al. (2011) who place the two former species outside the *Canthigaster* + *Tetraodon* clade. Santini et al. (2013b) found different relationships among these genera and species, and explained these discrepancies between their results and those of Yamanoue et al. (2011) by a different partitioning. In Santini et al. (2013b), *Arothron* and *Canthigaster* are contained in a same clade with all the sampled species (14) of the genus *Tetraodon*, including *T. cutcutia* and *T. palembangensis*. *Tetraodon* still appears as polyphyletic in this work, as species of the genus *Tetraodon* form three unrelated groups within the *Arothron-Canthigaster-Tetraodon* clade.

ACKNOWLEDGMENTS

The authors sincerely express their gratitude to: T. Boisgard (Muséum d'Histoire Naturelle de Nantes, France), M. Hignette (Aquarium de la Porte Dorée, Paris, France), J. Naudeau (Aquarium de Vannes, France), C. Gargani (Aquarium Mare Nostrum, Montpellier, France), S. Raredon (Smithsonian Institution, Washington, DC) for access to specimens; Prof. L. Frank, Dr. R. Berquist (Center for Scientific Computation in Imaging, University of California San Diego, La Jolla, CA) and staff at the DFL (www.digitalfishlibrary.org) for permission to use DFL MRI images; R. Britz (Department of Zoology, Natural History Museum, London, UK) for sharing information about the structure of the gas bladder of *Triodon macropterus*; E. Betti, P. Bugnon, M. Comte, C. Coste, B. Fernandez, M. Fusellier, S. Madec, F. N'Guyen, C. Picard, C. Raphaël (ONIRIS, Nantes, France), C. Tavernier, Y. Richaudeau (SARL Image-Et, Mordelles, France), D. Geffard-Kuriyama (pôle d'illustration scientifique de l'UMS 2700 CNRS-MNHN Paris, France) and C. Cauchie (Douai, France) for technical help; J.-C. Desfontis (ONIRIS, Nantes, France) for support; F.J. Meunier (UMR CNRS 7208, MNHN, Paris, France) and J.C. Tyler (Smithsonian Institution, Washington D.C) for help, advice, and bibliographical information. An initial version of the manuscript was improved by M. Bridou and I. Nicholson (ONIRIS, Nantes, France). The final version of this work

has greatly benefited from the advice and critical remarks of J.C. Tyler and an anonymous reviewer. Any experiments conducted in this work comply with the current laws of the country in which they were performed.

LITERATURE CITED

- Abe T. 1952. Taxonomic studies on the puffers (Tetraodontidae, Teleostei) from Japan and adjacent regions—VII. Conducting remarks, with the introduction of two genera, *Fugu* and *Boeseamanichthys*. *Jpn J Ichthyol* 2:35–44.
- Alfaro ME, Santini F, Brock CD. 2007. Do reefs drive diversification in marine teleosts? Evidence from the pufferfishes and their allies (Order Tetraodontiformes). *Evolution* 61:2104–2126.
- Aparicio S, Chapman J, Stupka E, Putnam N, Chia J, Dehal P, Christoffels I, Rash S, Hoon S, Smit A, Sollewijn Gelpke MD, Roach J, Oh T, Ho IY, Wong M, Detter C, Verhoef F, Predki P, Tay A, Lucas S, Richardson P, Smith SF, Clark MS, Edwards YJK, Doggett N, Zharkikh A, Tavtigian SV, Pruss D, Barnstead M, Evans C, Baden H, Powell J, Glusman G, Rowen L, Hood L, Tan Y, Elgar G, Hawkins T, Venkatesh B, Rokhsar D, Brenner S. 2002. Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. *Science* 297(5585):1301–1310.
- Arakawa O, Hwang DF, Taniyama S, Takatani T. 2010. Toxins of pufferfish that cause human intoxications. In: Ishimatsu A, Lie HJ, editors. *Coastal Environmental and Ecosystem Issues of the East China Sea*. Terrapub and Nagasaki University, Tokyo, Japan. pp 227–244.
- Berquist RM, Gledhill KM, Peterson MW, Doan AH, Baxter GT. 2012. The digital fish library: Using MRI to digitize, database, and document the morphological diversity of fish. *PLoS One* 7(4):e34499.
- Birindelli JLO, Akama A, Britski HA. 2012. Comparative morphology of the gas bladder in driftwood catfishes (Siluriformes: Auchenipteridae). *J Morphol* 273:651–660.
- Braidner EL. 1994. Pufferfish inflation: Functional morphology of postcranial structures in *Diodon holacanthus* (Tetraodontiformes). *J Morphol* 220:243–261.
- Breder CM, Clark E. 1947. A contribution to the visceral anatomy, development, and relationships of the Plectognathi. *Am Mus Nat Hist* 88:287–319.
- Britz R, Johnson GD. 2005. Occipito-vertebral fusion in ocean sunfishes (Teleostei:Tetraodontiformes: Molidae) and its phylogenetic implications. *J Morphol* 266:74–79.
- Chanet B, Guintard C, Boisgard T, Fusellier M, Tavernier C, Betti E, Madec S, Richaudeau Y, Raphaël C, Dettai A, Lecointre G. 2012. Visceral anatomy of ocean sunfish (*Mola mola* (L., 1758), Molidae, Tetraodontiformes) and angler (*Lophius piscatorius* (L., 1758), Lophiidae, Lophiiformes) investigated by non-invasive imaging techniques. *C R Biol* 335(12):744–752.
- Chanet B, Guintard C, Betti E, Gallut C, Dettai A, Lecointre G. 2013. Evidence for a close phylogenetic relationship between the teleost orders Tetraodontiformes and Lophiiformes based on an analysis of soft anatomy. *Cybium* 37(3):179–198.
- Collette BC, Dowell JR, Graves JE. 2006. Phylogeny of Recent billfishes (Xiphoidei). *Bull Mar Sci* 79(3):455–468.
- Cuvier G. 1805. *Leçons d'anatomie compare, tome III. La première partie des organes de la digestion*. Paris: Baudouin. 451 p.
- Cuvier G. 1817. *Le règne animal distribué d'après son organisation, Vol. II*. Paris: Deterville. 532 p.
- de Saint-Hilaire G. 1809. *Description de l'Égypte, ou recueil des observations et des recherches qui ont été faites en Égypte pendant l'expédition de l'Armée française*. Paris: Imprimerie impériale. 942 p.
- Delaroche FE. 1809. Observations sur la vessie aérienne des poissons. *Ann Mus Hist Nat* 14:245–324.

- Eastman JT. 1993. Antarctic Fish Biology: Evolution in a Unique Environment. San Diego: Academic Press. 322 p.
- Ekau W. 1991. Morphological adaptations and mode of life in high Antarctic fish. In: di Prisco G, Maresca B, Tota B, editors. Biology of Antarctic Fish. New York: Springer-Verlag. pp 23–39.
- Fink SV, Fink WL. 1981. Interrelationships of the ostariophysan fishes (Teleostei). Zool J Linn Soc 72:297–353.
- Gregory WK. 1933. Fish skulls: A study of evolution of natural mechanisms. Trans Am Phil Soc 23:75–481.
- Gregory WK, Raven HC. 1934. Notes on the anatomy and relationships of the ocean sunfish (*Mola mola*). Copeia 4:145–151.
- Harder W. 1975. The Anatomy of Fishes. Part I Text. Stuttgart: E. Schweizerart'sche Verlagsbuchhandlung. 612 p.
- Holcroft NI. 2004. A molecular test of alternative hypotheses of tetraodontiform (Acanthomorpha: Tetraodontiformes) sister group relationships using data from the RAG1 gene. Mol Phylogenet Evol 32:749–760.
- Holcroft NI. 2005. A molecular analysis of the interrelationships of tetraodontiform fishes (Acanthomorpha: Tetraodontiformes). Mol Phylogenet Evol 34:525–544.
- Jaillon O, Aury JM, Brunet F, Petit JL, Stange-Thomann N, Mauceli N, Bouneau L, Fischer C, Ozouf-Costaz C, Bernot A, Nicaud S, Jaffe D, Fisher S, Lutfalla G, Dossat C, Segurens B, Dasilva C, Salanoubat M, Levy M, Boudet N, Castellano S, Anthouard V, Jubin C, Castelli V, Katinka M, Vacherie B, Biémont C, Skalli Z, Cattolico L, Poulain J, de Berardinis V, Cruaud C, Duprat S, Brottier P, Coutanceau JP, Gouzy J, Parra G, Lardier G, Chapple C, McKernan KJ, McEwan P, Bosak S, Kellis M, Volff JN, Guigo R., Zody MC, Mesirov J, Lindblad-Toh K, Birren B, Nusbaum C, Kahn D, Robinson-Rechavi M, Laudet V, Schachter V, Quétier F, Saurin W, Scarpelli C, Wincker P, Lander ES, Weissenbach J, Roest Crolius H. 2004. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature 431:946–957.
- Johnson GD, Britz R. 2005. A description of the smallest *Triodon* on record (Teleostei: Tetraodontiformes: Triodontidae). Ichthyol Res 52:176–181.
- Kasumyan AO. 2008. Sounds and sound production in fishes. J Ichthyol 48(11):981–1030.
- Klassen GJ. 1995. Phylogeny and biogeography of the Ostraciinae (Tetraodontiformes: Ostraciidae). Bull Mar Sci 57(2):393–441.
- Lacépède BG. 1836. Histoire naturelle des poissons, tome II. Paris: Duménil. 399 p.
- Leis JM. 1978. Systematics and zoogeography of the porcupinefishes (*Diodon*, Diodontidae, Tetraodontiformes), with comments on egg and larval development. Fish Bull 76(3):535–567.
- Leis JM. 1984. Tetraodontiformes: Relationships. In: Moser HG, editor. Ontogeny and Systematics of Fishes. American Society of Ichthyologists and Herpetologists, special publication. Lawrence, Kansas: Allen Press Inc. pp 459–463.
- Lobel PS. 1996. Spawning sound of the trunkfish, *Ostracion meleagris* (Ostraciidae). Biol Bull 191:308–309.
- Matsuura K. 1979. Phylogeny of the superfamily Balistoidea (Pisces: Tetraodontiformes). Mem Fac Fish Hokkaido Univ 26:49–169.
- McCune AR, Carlson RL. 2004. Twenty ways to lose your bladder: Common natural mutants in zebrafish and widespread convergence of swim bladder loss among teleost fishes. Evol Dev 6(4):246–259.
- Moulton JM. 1958. The acoustical behavior of some fishes in the Bimini area. Biol Bull 114(3) 357–374.
- Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. Proc Natl Acad Sci USA 109:13698–13703.
- Near TJ, Dornburg A, Eytan RI, Keck BP, Smith L, Kuhn KL, Moore JA, Price SA, Burbrink FT, Friedman M, Wainwright PC. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc Natl Acad Sci USA; Doi: 10.1073/pnas.1304661110.
- Nelson J. 2006. Fishes of the World. New Jersey: Wiley, Hoboken. 601 p.
- Nolf D, Tyler JC. 2006. Otolith evidence concerning interrelationships of caproid, zeiform and tetraodontiform fishes. Bull Kon Belg Inst Natuurwet Biol 76:147–189.
- Pope EC, Hays GC, Thys TM, Doyle TK, Sims DW, Queiroz N, Hobson VJ, Kubicek L, Houghton JDR. 2010. The biology and ecology of the ocean sunfish *Mola mola*: A review of current knowledge and future research perspectives. Rev Fish Biol Fish 20(4):471–487.
- Rosen DE. 1984. Zeiforms as primitive plectognath fishes. Am Mus Nov 2782:1–38.
- Salmon M, Winn HE, Sorgente N. 1968. Sound production and associated behavior in triggerfishes. Pac Sci 22:11–20.
- Santini F, Sorenson L, Alfaro ME. 2013a. A new phylogeny of tetraodontiform fishes (Tetraodontiformes, Acanthomorpha) based on 22 loci. Mol Phylogenet Evol 69(1):177–187.
- Santini F, Nguyen MTT, Sorenson L, Waltzek TB, Lynch Alfaro JW, Eastman JM, Alfaro ME. 2013b. Do habitat shifts drive diversification in teleost fishes? An example from the pufferfishes (Tetraodontidae). J Evol Biol 26:1003–1018.
- Santini F, Tyler JC. 2003. A phylogeny of the families of fossil and extant tetraodontiform fishes (Acanthomorpha, Tetraodontiformes), upper cretaceous to recent. Zool J Linn Soc 139: 565–617.
- Santini F, Tyler JC. 2004. The importance of even highly incomplete fossil taxa in reconstructing the phylogenetic relationships of the Tetraodontiformes (Acanthomorpha: Pisces). Integr Comp Biol 44:349–357.
- Tominaga Y, Sakamoto K, Matsuura K. 1996. Posterior extension of the swimbladder in percoid fishes, with a literature survey of other teleosts. Univ Mus Univ Tokyo Bull 36:1–73.
- Tyler JC. 1968. A monograph on plectognath fishes of the Superfamily Triacanthoidea. Monogr Acad Nat Sci Phila 16: 364.
- Tyler JC. 1980. Osteology, phylogeny and higher classification of the fishes of the order Plectognathi (Tetraodontiformes), NOAA Tech Rep NMFS Circ 434:1–422.
- Tyler JC, Sorbini L. 1996. New superfamily and three new families of tetraodontiform fishes from the Upper Cretaceous: The earliest and most morphologically primitive plectognaths. Sm C Paleob 82:1–59.
- Winterbottom R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. Smithsonian Contrib Zool 155:1–201.
- Winterbottom R, Tyler JC. 1983. Phylogenetic relationships of aracanin genera of boxfishes (Ostraciidae: Tetraodontiformes). Copeia 1983:902–917.
- Yamanoue Y, Miya M, Matsuura K, Katoh M, Sakai H, Nishida M. 2008. A new perspective on phylogeny and evolution of tetraodontiform fishes (Pisces: Acanthopterygii) based on whole mitochondrial genome sequences: Basal ecological diversification? BMC Evol Biol 8:212–225.
- Yamanoue Y, Miya M, Doi H, Mabuchi K, Sakai H, Nishida M. 2011. Multiple invasions into freshwater by pufferfishes (Teleostei: Tetraodontidae): A mitogenomic perspective. PLoS One 6:e17410.