JOURNAL OF MORPHOLOGY 267:1338–1355 (2006)

The Laterophysic Connection and Swim Bladder of Butterflyfishes in the Genus *Chaetodon* (Perciformes: Chaetodontidae)

Jacqueline F. Webb,[1]* W. Leo Smith,[1–3] and Darlene R. Ketten[4,5]

[1]*Department of Biology, Villanova University, Villanova, Pennsylvania 19085*
[2]*Department of Ichthyology, American Museum of Natural History, New York, New York 10024*
[3]*Center for Environmental Research and Conservation, Columbia University, New York, New York 10027*
[4]*Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543*
[5]*Department of Otology and Laryngology, Harvard Medical School, Boston, Massachusetts*

ABSTRACT The laterophysic connection (LC) is an association between bilaterally paired, anterior swim bladder extensions (horns) and medial openings in the supracleithral lateral line canals that diagnoses butterflyfishes in the genus *Chaetodon*. It has been hypothesized that the LC makes the lateral line system sensitive to sound pressure stimuli that are transmitted by the swim bladder horns and converted to fluid flow into the lateral line system via a laterophysic tympanum. The purpose of this study was to define variation in the morphology of the LC, swim bladder and swim bladder horns among 41 *Chaetodon* species from all 11 *Chaetodon* subgenera and a species from each of four non-*Chaetodon* genera using gross dissection, histological analysis as well as 2D or 3D CT (computed tomographic) imaging of live, anesthetized fishes. Our results demonstrate that the lateral line system appears rather unspecialized with well-ossified narrow canals in all species examined. Two LC types (direct and indirect), defined by whether or not the paired anterior swim bladder horns are in direct contact with a medial opening in the supracleithral lateral line canal, are found among species examined. Two variants on a direct LC and four variants of an indirect LC are defined by combinations of soft tissue anatomy (horn length [long/short] and width [wide/narrow], number of swim bladder chambers [one/two], and presence/absence of mucoid connective tissue in the medial opening in the supracleithrum). The combination of features defining each LC variant is predicted to have functional consequences for the bioacoustics of the system. These findings are consistent with the recent discovery that *Chaetodon* produce sounds during social interactions. The data presented here provide the comparative morphological context for the functional analysis of this novel swim bladder-lateral line connection. J. Morphol. 267:1338–1355, 2006.

KEY WORDS: *Chaetodon*; lateral line; hearing; swim bladder; laterophysic connection; CT imaging; butterflyfish

Sound stimuli are important in the social behavior of a wide variety of fishes (Myrberg, 1981; Hawkins, 1993; Ladich and Bass, 2003; Ladich and Popper, 2004), but the complex physical features of underwater acoustics have confounded our understanding of how fishes interpret sound. An acoustic stimulus (e.g., modeled as a vibrating sphere, Kalmijn, 1989) has two components—hydrodynamic flow (the "near field") and a propagating sound pressure wave (the "far field"). Hydrodynamic flow is generated by the movement of water near the acoustic stimulus source and sound pressure waves propagate from the acoustic source as a cyclic compression and rarefaction of water molecules. The mechanosensory lateral line is generally sensitive to hydrodynamic flow (local displacement) within 1–2 body lengths from the source. The inner ear is also sensitive to hydrodynamic flow as a result of whole body acceleration, but sound pressure-induced oscillations of the air volume within the swim bladder generates a secondary local displacement field that is capable of stimulating the inner ear (reviewed by Schellart and Popper, 1992; Popper and Fay, 1999; Popper et al., 2003).

The ability to detect sound pressure stimuli is enhanced by the presence of intimate associations of a volume of air (in the swim bladder, swim bladder horns or branchial diverticulae, reviewed by Schellart and Popper, 1992) and the otic capsule, known as otophysic connections, which increase the auditory sensitivity and frequency response of the inner ear (Poggendorf, 1952; Coombs and Popper, 1979) and the distance over which sound pressure stimuli can be detected (Coombs et al., 1992, Popper and Fay, 1993, Coombs and Montgomery, 1999).

Otophysic connections occur in at least 50 families in all major teleostean lineages (Schellart and

Contract grant sponsor: National Science Foundation; Contract grant numbers: IBN-9603896, IBN-0132607.

*Correspondence to: Jacqueline F. Webb, Department of Biological Sciences, University of Rhode Island, 100 Flagg Road, Kingston, RI 02881. E-mail: Jacqueline_webb@mail.uri.edu

Published online 18 October 2006 in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10480

TABLE 1. Acanthomorph families with representatives that have anterior swim bladder horns (modified from Smith, 2000)

Family	Reference
Acropomatidae	Katayama, 1959
Centropomidae	Katayama, 1959
Chaetodontidae	Gunther, 1860; Blum, 1988
Cichlidae	Dehadrai, 1959
Ephippidae	Herre and Montalban, 1927
Gerreidae	Green, 1971
Haemulidae	Johnson, 1980
Holocentridae	Nelson, 1955
Kuhliidae	Gosline, 1966
Lactariidae	Leis, 1994
Menidae	Johnson, pers. commun.
Moridae	Parker, 1882
Moronidae	Katayama, 1959
Nemastiidae	Rosenblatt and Bell, 1976
Percichthyidae	MacDonald, 1978
Polyprionidae	Katayama, 1959
Priacanthidae	Starnes, 1988
Scombridae	Collette and Nauen, 1983
Sciaenidae	Sasaki, 1989; Chao, 1986, 1995
Sillaginidae	McKay, 1985
Sparidae	Tavolga, 1974

Popper, 1992). For instance, clupeiform fishes have complex otophysic connections in which anterior extensions of the swim bladder (otic bullae) invade the otic capsule and directly contact thefluids of the inner ear and the cranial lateral line canals forming the "recessus lateralis" (e.g., O'Connell, 1955; Best and Gray, 1980). Otophysan fishes have modified vertebral elements that form the Weberian apparatus, which mechanically link the swim bladder with the otic capsules (e.g., Alexander, 1962; Rosen and Greenwood, 1970; Fink and Fink, 1996).

Simple otophysic connections in which anterior swim bladder horns contact the otic capsule or invade the otic capsule (as bullae) are found in osteoglossomorphs and elopomorphs (Bridge, 1900; Greenwood, 1970) and have been shown to enhance auditory capabilities in mormyrids (Yan and Curtsinger, 2000; Fletcher and Crawford, 2001). Simple otophysic connections have also been investigated experimentally in a few acanthomorph taxa (holocentrids, Coombs and Popper, 1979, and sciaenids, Ramcharitar et al., 2002, 2004). Interestingly, anterior swim bladder horns are known in representatives of at least 21 other acanthomorph families (Table 1), suggesting that modifications of the swim bladder that enhance hearing may be even more widespread among fishes. In butterflyfishes of the genus *Chaetodon*, swim bladder horns form an association, not with the otic capsule, but with a medial opening in the supracleithral lateral line canal, defining a novel specialization, the laterophysic connection.

The Laterophysic Connection

Blum (1988: 121) diagnosed *Chaetodon* with "bilaterally, paired, bulbous, antero-lateral diverticula, that are attached to the [incomplete] medial surfaces of the supracleithra" (Fig. 1). Webb and Blum (1990) and Webb (1998) further described this feature in *Chaetodon* using histological analysis, calling it the "laterophysic connection" (LC) to draw attention to its apparent structural and putative functional similarity to the simple otophysic connections in other fishes. Two LC types were described (direct and indirect), based on whether or not the swim bladder horns are in direct contact with the medial opening in the supracleithrum (Webb, 1998). Among species with an indirect LC, most species have long horns, but a few species have short horns (Webb and Smith, 2000; Smith et al., 2003).

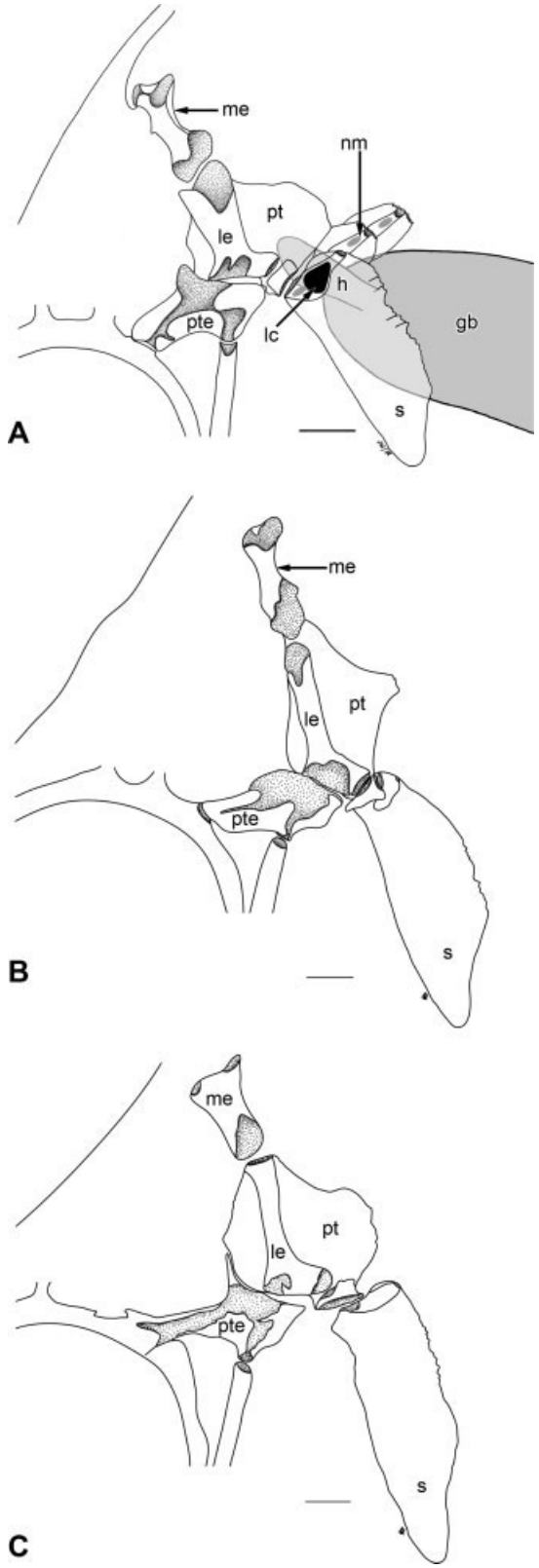
The two LC types found among *Chaetodon* species are analogous to the kind of variation found in the simple otophysic connections among holocentrid subfamilies (Nelson, 1955). Coombs and Popper (1979) demonstrated that *Myripristis* (subfamily Myripristinae), which has robust anterior swim bladder horns that come into intimate contact with the otic capsule (Nelson, 1955; unpub. data) and modified inner ear morphology (Popper, 1977), demonstrates higher sensitivity to sound stimuli over a broader frequency range, when compared to *Sargocentron* (*Adioryx*, subfamily Holocentrinae), which lacks swim bladder horns (Nelson, 1955; unpub. observ.) and has unmodified inner ear morphology (Popper, 1977). Ramcharitar et al. (2002, 2004) has also demonstrated correlations among auditory capabilities (thresholds and frequency response), and morphology of the swim bladder and ear among several genera of drums (family Sciaenidae).

Webb (1998) suggested that the LC in *Chaetodon* transmits pressure from the air-filled swim bladder to the fluid-filled lateral-line canal via the anterior swim bladder horns at the LC, initiating fluid movements in the lateral-line canal that are capable of stimulating canal neuromasts in the vicinity of the LC. She hypothesized that the presence of anterior swim bladder extensions and a LC in *Chaetodon* makes the lateral line sensitive to sound pressure. This would expand the functional repertoire of the lateral line system to include the reception of sound pressure stimuli. The observation that swim bladder morphology is correlated with LC type (Webb and Smith, 2000) suggests that swim bladder bioacoustics play an important role in LC function, thus demanding a closer examination of the swim bladder itself.

Visualization of Swim Bladder Morphology

Reliable methods for assessing swim bladder morphology are essential for an analysis of its bioacoustics (for both sound production and sound reception) and patterns of acoustic backscatter (for detection and identification of fish populations, Foote, 1980, 1985, 1988; MacLennon and Simmonds, 1992; Schaefer and Oliver, 2000; Foote and Francis, 2002). However, descriptions of teleost swim bladders are scattered throughout the ichthyology literature and

assessments of the comparative morphology of the teleost swim bladder in taxonomic or phylogenetic contexts are rare (e.g., Dobbin, 1941; Whitehead and Blaxter, 1989). This is not surprising because swim bladder morphology is easily distorted by preparation and fixation artifact, such as the effects of rapid changes in hydrostatic pressure during the collection process, postmortem changes prior to fixation, chemical fixation and storage in alcohol, and freezing and sectioning (Foote, 1985, 1988). Furthermore, when fishes are cleared and stained for osteological analysis (e.g., Pothoff, 1984), the swim bladder is frequently rendered transparent. Radiographic images of live, anesthetized or freshly fixed material, in which air is still retained in the pressurized swim bladder, and into which liquid has not yet diffused, can provide only limited views of the swim bladder (e.g., Webb and Smith, 2000; Fig. 1).



Computed X-ray tomography (CT) is a non-invasive imaging method that has revolutionized our ability to analyze the internal structure of living organisms. The high level of image resolution provided by CT allows differentiation of the structure of tissues and materials of differing densities (e.g., soft tissue, bone, fluid and air). CT has proven to be particularly valuable for determining volumetric measures, imaging structurally complex organs, such as the inner ear of vertebrates (Ketten et al., 1998), and for the comparative osteological analysis of fossil and living vertebrates for systematic and functional studies (e.g., Witmer, 2001; Schaefer, 2003; Witmer et al., 2003; Summers et al., 2004; Kearney et al., 2005).

The swim bladder, composed of soft connective tissues containing low density gases, is surrounded by dense muscle and bone, and is thus a prime candidate for CT analysis (eg., see Carpenter et al., 2004). By visualizing the swim bladder in live, anesthetized fishes, CT imaging can allow the analysis of swimbladder structure without the introduction of the sorts of preparation artifact that arise when fixed specimens are examined.

Goals of the Current Study

Webb and Smith (2000) presented preliminary data on LC morphology based on a histological anal-

Fig. 1. Camera lucida drawings of the dermal skeletal elements just behind the orbit at the posterior margin of the skull in three species of *Chaetodon* with different LC morphologies. **A:** *Chaetodon octofasciatus* (AMNH 43117, LC Variant *Dir1*, see Table 3). Schematic representation of the anterior horn of the swim-bladder (shaded) that sits deep to the medial opening in the supracliethrum (black teardrop), the site of the LC. The supracleithral neuromast (gray oval) is just rostral to the LC, and the neuromasts in the first two lateral line scales (gray ovals) are just caudal to the LC (modified from Webb and Smith, 2000) **B:** *Chaetodon multicinctus* (AMNH 88343SW, LC Variant *Ind2*). **C:** *Chaetodon ornatissimus* (AMNH 88417SW, LC Variant *Ind3*). gb, swim bladder; h, horn; le, lateral extrascapular; me, medial extrascapular; nm, neuromast; pt, posttemporal; pte, pterotic; s, supracleithrum. Scale bars = 1 mm.

ysis of only eight species, but demonstrated that LC morphology varies interspecifically and is not sexually dimorphic. Smith et al. (2003) identified several morphological characters based on soft tissue morphology that were used to erect a new phylogenetic hypothesis of chaetodontid intrarelationships and discussed the evolution of the laterophysic connection. The purpose of this paper is to define interspecific variation in the morphology of the LC, swim bladder and swim bladder horns among *Chaetodon* species in all *Chaetodon* subgenera using gross dissection, histological analysis and CT imaging. The results of this work provide the morphological context for the comparative functional analysis of the laterophysic connection.

MATERIALS AND METHODS

Forty-one *Chaetodon* species (from all 11 *Chaetodon* subgenera) as well as representatives of four other chaetodontid genera (*Forcipiger*, *Hemitaurichthys*, *Heniochus*, *Johnrandallia*) were studied by analyzing cleared and stained specimens, histological material, X-ray and CT images, and dissected swim bladders (Table 2). An IACUC-approved protocol was followed throughout.

Osteological Analysis

Several cleared and stained specimens (*Chaetodon octofasciatus*, AMNH 43117; *C. multicinctus* AMNH 88343SW; *C. ornatissimus* AMNH 88417SW) (following methods in Pothoff, 1984) and one dry skeleton (*C. auriga* MCZ 154429) were used to describe the lateral line canals in the vicinity of the laterophysic connection, and were illustrated using a camera lucida or documented photographically.

Histological Analysis of the Laterophysic Connection

Between one and eight individuals (juveniles and adults, 25–109 mm SL) in each of 22 *Chaetodon* species (from 10 of 11 subgenera) as well as several individuals of *Forcipiger flavissimus* were obtained commercially, through field collections in Hawaii, or from museum collections, and prepared histologically (see Smith et al., 2003). Voucher specimens of eleven species have been deposited in the Museum of Comparative Zoology (Harvard University, MCZ 156946-156959). Live fishes were anesthetized with an MS 222 solution in seawater until unresponsive. The body cavity and orbits were injected with a solution of 10% formalin in seawater and fishes were immersion fixed. All specimens were radiographed within a day of fixation to visualize the air-filled swim bladder. Fish heads (of freshly fixed specimens, or in some cases, alcoholic museum specimens) were decalcified with Cal-Ex (Fisher) overnight, or with 0.1 M di-NaEDTA in a solution of 10% formalin in seawater for 1–3 weeks with several solution changes. Decalcification was confirmed radiographically. Fish heads were trimmed, dehydrated in an ascending ethanol series, embedded in glycol methacrylate resin (Historesin [Leica], JB-4 [Polysciences], or Technovit 7100 [Kulzer, Wehrheim, Germany]) and sectioned transversely at 5 μm with a tungsten-carbide knife on a Leica motorized microtome. Sections were mounted out of water onto chrom-alum subbed slides, dried overnight (60°C), stained with 0.5% cresyl violet, air-dried overnight, and coverslipped. Additional sections were mounted on positively charged slides (Mt. Washington Scientific), stained with Sudan Black B (Bennett et al., 1976) and coverslipped with glycerine in order to determine the distribution of adipose tissue in the vicinity of the laterophysic connection.

TABLE 2. Summary of material examined using histological analysis, computed X-ray tomography (CT) and gross dissection

Species	Histology	CT	Dissection
Non-*Chaetodon* Species			
Chelmon rostratus			×
Johnrandallia nigrorostris			×
Forcipiger flavissimus	×	×	
Hemitaurichthys polylepis		×	
Genus *Chaetodon*			
C. striatus	×	×	×
S.G. *Chaetodon*			
C. capistratus	×	×	×
C. humeralis			×
C. marleyi			×
C. ocellatus	×	×	
S.G. *Rabdophorus*			
C. auriga	×	×	
C. ephippium	×	×	
C. falcula			×
C. fasciatus			×
C. flavirostris			×
C. lineolatus			×
C. lunula			×
C. melannotus			×
C. rafflesi			×
C. semilarvatus	×		
C. ulientensis			×
C. vagabundus			×
S.G. *Roaops*			
C. tinkeri		×	
S.G. *Exornator*			
C. citrinellus			×
C. fremblii			×
C. guttatissimus			×
C. miliaris	×	×	
C. multicinctus	×	×	
C. pelewensis			×
C. punctatofasciatus		×	
C. quadrimaculatus			×
C. sendentarius	×	×	×
S.G. *Lepidochaetodon*			
C. kleinii	×	×	
C. unimaculatus	×		×
S.G. *Megaprotodon*			
C. trifascialis	×		
C. oligacanthus	×		×
S.G. *Gonochaetodon*			
C. baronessa	×		×
S.G. *Tetrachaetodon*			
C. bennetti			×
C. plebius	×		×
S.G. *Discochaetodon*			
C. aureofasciatus	×		×
C. octocasciatus	×	×	×
C. rainfordi	×		
S.G. *Corallochaetodon*			
C. trifasciatus	×	×	×
S.G. *Citharoedus*			
C. meyeri	×		
C. ornatissimus	×	×	
C. reticulatus			×

See text for details.

Measurements of lateral line canal diameter, horn diameter and distance from the lateral line canal to the swim bladder horn were taken at the rostro-caudal midpoint of the medial opening of the supracleithrum on the left and right sides in all specimens prepared histologically. In addition, the distance from the inner wall of the swim bladder horn to the lumen of the otic capsule was taken in the caudal most section in which the lumen of the

otic capsule was visualized. The rostro-caudal length and dorso-ventral height of the medial opening in the supracleithrum and the neuromasts in the vicinity of the LC (the post-temporal, supracleithral canal neuromasts, and canal neuromasts in the first and second lateral line scales) were calculated in four species (*Chaetodon octofasciatus*, $n = 3$, 63–73 mm SL; *C. multicinctus*, $n = 3$, 83–90 mm SL; *C. kleinii*, $n = 3$, 74–85 mm SL; *C. miliaris*, $n = 2$, 45–50 mm SL; see Smith, 2000). Length was calculated by counting the number of sections in which tissue of interest was present and multiplying by inter-section interval and section thickness. Width was measured in individual histological sections at the rostro-caudal midpoint of the neuromast or medial supracleithral opening using SPOT® software (Diagnostic Instruments, Sterling Heights, MI) and a drawing tablet. Measurements taken in histological material are affected by shrinkage and other preparation artifact and sectioning angle, so only summary data are presented.

Specimens analyzed histologically. Fish length is expressed as standard length (SL). (Museum of Comparative Zoology [MCZ], Harvard University, Cambridge; Academy of Natural Sciences, Philadelphia [ANSP]; Australian Museum, Sydney [AMS]). Webb Lab accession codes (e.g., Cc1) are retained for archival purposes. Genus *Forcipiger – Forcipiger flavissimus* ($n = 2$, 109 mm- SL [uncat. indiv., Ff1]). Genus *Chaetodon*: *Incertae sedis – Chaetodon striatus* ($n = 1$, 74 mm [Cs4]). Subgenus *Chaetodon – Chaetodon capistratus* ($n = 3$, 25–52 mm [Cc1, 2, 4]), *C. ocellatus* ($n = 1$, 41.8 mm [Cl7]). Subgenus *Rabdophorus – Chaetodon auriga* ($n = 1$, 61.0 mm [Ca2]), *C. ephippium* ($n = 1$, 51.0 mm [Ce1]), *C. semilarvatus* ($n = 1$, 87 mm [Cv1]). Subgenus *Exornator – Chaetodon miliaris* ($n = 4$, 45–100 mm [Cr1, 4, 9, 11]), *C. multicinctus* ($n = 4$, 83–90 mm, [Cm4, 5, 7, 9]). *Chaetodon sedentarius* ($n = 1$, 50 mm [Cd1]). Subgenus *Lepidochaetodon – Chaetodon kleinii* ($n = 4$, 70–85 mm [uncat. indiv., Ck10, 11, 13]), *C. unimaculatus* ($n = 1$, 38 mm [Cu1]). Subgenus *Megaprotodon – Chaetodon oligacanthus* ($n = 1$, 67 mm [Cg1 = ANSP 100113]); *Chaetodon trifascialis* ($n = 1$, 87 mm, [Cz1]). Subgenus *Gonochaetodon – Chaetodon baronessa* ($n = 1$, 55.0 mm [Cb1 = AMS 21915011]). Subgenus *Tetrachaetodon – Chaetodon plebeius* ($n = 2$, 57–85 mm [Cp1 = AMS 24678030, Cp2]). Subgenus *Discochaetodon – Chaetodon aureofasciatus* ($n = 1$, 57.0 mm [Cq1 = AMS 24678030]), *C. octofasciatus* ($n = 8$, 50–77 mm [Co9, 11, 12, 14, 20, 22, 30 + 1 uncat. indiv.]), *C. rainfordi* ($n = 1$, 71.7 mm [Ci1]). Subgenus *Corallochaetodon – Chaetodon trifasciatus* ($n = 1$, 60 mm [Ct1]). Subgenus *Citharoedus – Chaetodon ornatissimus* ($n = 2$, 61–68 mm [Cn1, 4]), *C. meyeri* ($n = 1$, 85.0 mm [Cy1]).

Gross Morphology of Swim Bladder and Swim Bladder Horns

Conventional X-rays of all specimens were prepared prior to preparation of histological material (see above) and were examined to determine overall swim bladder shape and position of the internal diaphragm formed by the tunica interna. In addition, dissections were performed on one or more individuals in 30 *Chaetodon* species (in 10 of 11 *Chaetodon* subgenera) and in one species from each of three non-*Chaetodon* genera (*Chelmon, Heniochus, Johnrandallia*).

Museum material examined. Fish length is expressed as standard length (SL). (MCZ, Museum of Comparative Zoology, Harvard University; ANSP, Academy of Natural Sciences, Philadelphia). Genus *Chaetodon*: *Incertae sedis – Chaetodon striatus* MCZ 2710 ($n = 1$, 112 mm). Subgenus *Chaetodon – Chaetodon capistratus* MCZ 68477 ($n = 2$, 82, 85 mm), *C. humeralis* MCZ 43454 ($n = 1$, length unknown), *C. marleyi* ANSP54806 ($n = 1$, 90 mm). Subgenus *Rabdophorus – Chaetodon falcula* MCZ 16208 ($n = 2$, 110, 112 mm), *C. fasciatus* MCZ 3705 ($n = 1$, 125 mm), *C. flavirostris* MCZ 36932 ($n = 1$, 120 mm), *C. lineolatus* MCZ 82679 ($n = 1$, 80 mm), *C. lunula* MCZ 5751 ($n = 2$, 110, 120 mm), *C. melannotus* MCZ 6000 (*C. dorsalis*, $n = 1$, 120 mm), *C. rafflesi* MCZ 33168 ($n = 3$, 70 mm), *C. ulientensis* MCZ 82677 ($n = 1$, 125 mm), ANSP86412 ($n = 1$, 103 mm), *C. vagabundus* MCZ 30261 ($n = 3$, 82–87 mm). Subgenus *Exornator – Chaetodon citrinellus* MCZ 30200 ($n = 1$, 74 mm), ANSP 31576-91 ($n = 1$, length unknown), *C. fremblii* MCZ 2697 ($n = 1$, 115 mm), *C. guttatissimus* ANSP 108362 ($n = 1$, 73 mm), *C. pelewensis*, MCZ 82674 ($n = 1$, 80 mm), *C. quadrimaculatus* MCZ 16223 ($n = 1$, 115 mm), ANSP 97795 ($n = 1$, 100 mm), *C. sedentarius* MCZ 59605 (105 mm). Subgenus *Lepidochaetodon – Chaetodon unimaculatus* MCZ 5744 ($n = 1$, 115 mm). Subgenus *Megaprotodon – Chaetodon oligacanthus* ANSP 100113 ($n = 1$, length unknown). Subgenus *Gonochaetodon – Chaetodon baronessa* MCZ 64348 ($n = 1$, 70 mm). Subgenus *Tetrachaetodon – Chaetodon bennetti* MCZ 16260 ($n = 1$, 117 mm), *C. plebius* MCZ 64340 ($n = 1$, 96 mm). Subgenus *Discochaetodon – Chaetodon aureofasciatus* MCZ 64388 ($n = 1$, 83 mm). Subgenus *Corallochaetodon – Chaetodon trifasciatus* MCZ 89979 ($n = 1$, 95 mm). Subgenus *Citharoedus – Chaetodon reticulatus* MCZ 30210 ($n = 1$, 108 mm). Other chaetodontid genera – *Chelmon rostratus*, MCZ 46530 ($n = 1$, 108 mm), *Johnrandallia* (*Pseudochaetodon) nigrorostris*, MCZ 40453 ($n = 1$, 90 mm).

CT Imaging of Swim Bladder

Computed X-ray tomographic (CT) images were obtained for one to four individuals from each of 16 *Chaetodon* species (in 8 of 11 *Chaetodon* subgenera), as well as for three *Forcipiger flavissimus* and one *Hemitaurichthys polylepis*. Each fish was anesthetized in a seawater solution of MS 222 (Sigma) until ventilation ceased and the fish was incapable of maintaining an upright posture. The fish was then placed left side up in a plastic box filled with the anesthetic solution and wet gauze was placed either beneath or on top in order to ensure flat and stable positioning during CT scans. Following scanning, each specimen was over-anesthetized in the anesthetic solution, injected with a solution of 10% formalin in seawater into the body cavity and orbits, and immersion fixed for subsequent analysis. After initial scans, three individuals in two species (*Chaetodon capistratus* and *C. striatus*) were immediately scanned a second time with the right side up, to identify any effects of scanning position on swim bladder morphology.

CT scans were carried out on a Siemens Somatom Plus 4, a Siemens Volume Zoom (at the Radiology Department of the Massachusetts Eye and Ear Infirmary, MEEI), or a Siemens Volume Zoom (at the Woods Hole Oceanographic Institution, WHOI). A topogram (lateral view) was first obtained to visualize the full extent of the swimbladder in each case. Scan regions were set to include the inner ear and the entire length of the swim bladder. Fish were scanned in the rostro-caudal axis using a spiral acquisition protocol at 200 mAs/140 kV (MEEI) or 150 mAs/120 kV (WHOI) using a 0.5 mm (WHOI) or 1.0 mm (MEEI) collimator width. Data were reformatted at 0.1 mm (WHOI) to 0.5 mm (WHOI and MEEI) to provide full high resolution series of transverse slices from which horizontal and sagittal MPR's (multiple plane reconstructions) were reformatted with slice thicknesses of 0.1 mm (WHOI) or 0.2 mm (MEEI). Three-dimensional reconstructions were produced with a Shaded Surface Display (SSD) program (with Siemens proprietary software) using a range of X-ray attenuation values (1024/-510 Hu) appropriate for imaging the air within the swim bladder, the swim bladder horns and some details of the air–tissue boundary.

Swim bladder and horn length (in the rostro-caudal axis, see Table 4) were determined by counting the number of transverse CT slices in which the swim bladder or swim bladder horns were present and multiplying that value by slice thickness. Swim bladder and horn diameters were measured with digital calipers on large format CT films perpendicular to the dorso-ventral axis (defined by the median skeletal elements), at 1 or 2 mm intervals along the rostro-caudal length of the swim bladder, and at 0.5 mm or 1 mm intervals along the length of the swim bladder horns.

Material analyzed using CT. Fish length is expressed as standard length (SL). MEEI, Massachusetts Eye and Ear Infirmary; WHOI, Woods Hole Oceanographic Institution). Genus *Chaetodon*: *Incertae sedis – Chaetodon striatus* ($n = 3$, 61–70 mm

[Cs10, 11, 12], MEEI). Subgenus *Chaetodon* – *C. capistratus* ($n = 4$; 65–77 mm [Cc 10, 11, 12, 13], MEEI), *C. ocellatus* ($n = 2$, 52–73 mm [Cl 40, 41], MEEI). Subgenus *Rhabdophorus* – *Chaetodon auriga* ($n = 2$, 88–MEEI). Subgenus *Roaops*: *C. tinkeri* ($n = 2$, 80–85 mm [Cx1, 2], WHOI). Subgenus *Exornator* – *Chaetodon miliaris* ($n = 3$, ~70–90 mm [Cr 12, 15, 16], MEEI and WHOI), *C. multicinctus* ($n = 4$, 65–75 mm, [Cm10, 11, 12, 13], WHOI), *C. punctofasciatus* ($n = 1$, 73 mm [Cf1], MEEI), *C. sedentarius* ($n = 1$, 58 mm [Cd 7], MEEI). Subgenus *Lepidochaetodon* – *C. kleinii* ($n = 1$, ~70 mm [Ck16], MEEI). Subgenus *Corallochaetodon* – *C. trifasciatus* ($n = 1$, 72 mm [Ct3], MEEI). Subgenus *Citharoedus* – *C. ornatissimus* ($n = 1$, ~65 mm [Cn 6], MEEI). Other genera: *Forcipiger flavissimus* ($n = 3$, ~90–114 mm, [Ff 7, 10, 11], MEEI and WHOI), *Hemitaurichthys polylepis* ($n = 1$,110 mm [Hp1], WHOI).

Two individuals from each of three species (*Chaetodon capistratus*, *C. ocellatus*, *C. striatus*) were prepared histologically (see Methods above) several months after they were CT scanned and fixed in order to assess the degree of shrinkage and preparation artifact present in histological material. Horn diameters in both CT images and histological material were measured in the mediolateral (horizontal) axis at 180 μm intervals along their length using an ocular micrometer mounted on a compound microscope.

RESULTS

The configuration of the cranial lateral line canals in the vicinity of the laterophysic connection (LC) is demonstrated in cleared and stained material (Fig. 1). A narrow, well-ossified lateral line canal (~100–400 μm diameter) runs rostro-caudally through the dorsal-most portion of the elongate supracleithrum, the caudal-most element containing a portion of the cranial lateral line system. Caudally, the supracleithral canal is contiguous with the trunk canal, which starts in the first lateral line scale. Rostrally, the supracleithral canal connects to a small canal segment in the post-temporal bone, which is contiguous with the canal in the lateral extrascapular, and connects with the supratemporal canal in the medial extrascapular and with the canal in the pterotic where the infraorbital, postotic, preopercular, and supraorbital canals meet.

The site of the LC is not a novel opening in the medial wall of the supracleithrum (a "medial fossa,") as initially reported by Webb (1998), but is more appropriately interpreted as the posterior terminal pore of the lateral line canal segment in the supracleithrum (Smith et al., 2003). The supracleithrum is incomplete medially, forming an opening that ranges in shape from a rostro-caudally elongate oval to a teardrop (Fig. 2). At the caudal end of the supracleithral canal segment, the canal roof (the lateral wall of the canal) extends beyond the canal floor (the medial wall of the canal), so that the posterior terminal pore of the canal segment in the supracleithrum points medially (arrows in Fig. 2).

In *Chaetodon octofasciatus*, *C. multicinctus* and *C. kleinii* the medial opening in the supracleithrum measures ~500–800 μm in the rostro-caudal axis and ~300–700 μm in the dorso-ventral axis. In smaller individuals of *C. miliaris*, the medial opening measures only ~300 μm in the rostro-caudal axis and ~500 μm in the dorso-ventral axis (Fig. 3A,B). The supracleithral neuromast measures ~300–700 μm (rostro-caudal axis) by ~200–500 μm (dorso-ventral axis). It is found on the medial wall of the lateral line canal in the supracleithrum just rostral to the medial opening in species with a direct LC (e.g., *C. octofasciatus*, Fig. 4B), or on the soft tissue that fills the medial opening in species with an indirect LC (e.g., *C. multicinctus*, Fig. 4C). The first two lateral-line scales just caudal to the medial opening in the supracleithrum each contains a somewhat smaller canal neuromast that is ~200–500 μm long (in its rostro-caudal axis) and ~200–400 μm wide (in its dorso-ventral axis, Fig. 4A).

Interspecific Variation in LC Morphology

Variation in LC morphology is defined by the proximity of swim bladder horns to the medial opening in the supracleithrum (direct/indirect LC), presence/absence of an external constriction (ductus communicans) in the tunica externa of the swim bladder (one- or two-chambered swim bladder, sensu Dobbin, 1941), swim bladder horn width (wide/narrow) and length (long/short), and presence/absence of mucoid connective tissue in the soft tissue that fills the medial opening in the supracleithrum (characters 9, 36, 38, 39 and 40, respectively, Smith et al., 2003). Two variants on a direct LC (*Dir1* and *Dir2*) and four variants on an indirect LC (*Ind1-Ind4*) were found among the 22 *Chaetodon* species analyzed histologically (Fig. 5). Only one variant is present among species in each *Chaetodon* subgenus (as defined by Smith et al., 2003), with the exception of the Subgenus *Citharoedus,* in which variants Ind3 and Ind4 are found (see Table 3). Four of the variants (*Dir1*, *Ind1*, *Ind2* and *Ind3*) have been described briefly and illustrated schematically (Webb and Smith, 2000), but are more fully described and placed in a larger context below.

Variants on the direct laterophysic connection. A direct LC is characterized by the direct contact of the swim bladder horn with the soft tissues filling the medial opening in the supracleithrum. Two variants on the direct LC are defined here (Table 3).

Direct LC with mucoid connective tissue, wide horns, and a one-chambered swim bladder (Dir1) (Figs. 3A,B, 5A, 6A). A soft tissue "tympanum" composed of four layers forms a ~150–500 μm thick barrier between the fluid-filled lateral line canal and the gas-filled swim bladder horn (Fig. 3B). The thin epithelial lining of the lateral line canal is in direct contact with the mucoid connective tissue, which stains pink with cresyl violet, and may contain fat cells (as indicated by positive Sudan Black B staining (data not shown), and sits deep to the medial opening the supracleithrum. Variation in the number and distribution of fat cells may reflect differences in the nutritional state among specimens examined. The collagenous tunica externa of the

swim bladder horn is thinned in the vicinity of the medial opening and tightly adheres to the mucoid connective tissue (Figs. 3B, 6A,B). The tunica interna appears to be composed of multiple layers of very thin epithelium and readily separates from the tunica externa during histological preparation.

In *Chaetodon octofasciatus* and *C. rainfordi*, the swim bladder horns overlap the inner ear (the lagenar and saccular otolithic organs) in the rostro-caudal axis by <1 mm; overlap is not observed in other species with this LC variant. The swim bladder horns sit lateral to the neurocranium, and kidney tissue is generally found between the otic capsule and the horns at the level of the medial opening in the supracleithrum. The otic capsule is well-ossified and does not appear to demonstrate any thinning or other structural modifications (Fig. 3A). The rostral end of the swim bladder horns extends to the level of the post-temporal (just rostral to the supracleithrum) where the saccular otolithic organs and the vertical semicircular canals are also visible in transverse sections.

Direct LC without mucoid connective tissue and with narrow horns and a two-chambered swim bladder (Dir2) (Figs. 5B, 6B). Mucoid connective tissue is not present in the medial opening of the supracleithrum. The epithelial lining of the lateral-line canal, the tunica externa, and tunica interna form a thin tympanum between the narrow gas-

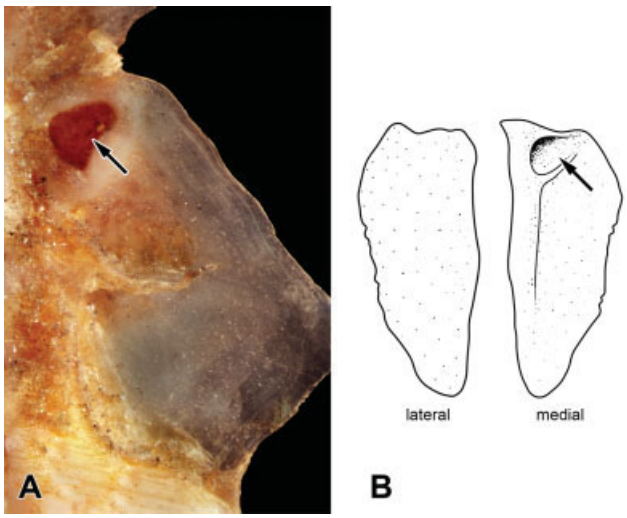


Fig. 2. Medial opening in the supracleithrum (arrows), site of the laterophysic connection (LC). **A:** Medial surface of supracleithrum in dry skeleton of *Chaetodon auriga* (MCZ 154429). **B:** Lateral (left) and medial (right) surfaces of the supracleithrum in *Chaetodon* sp.

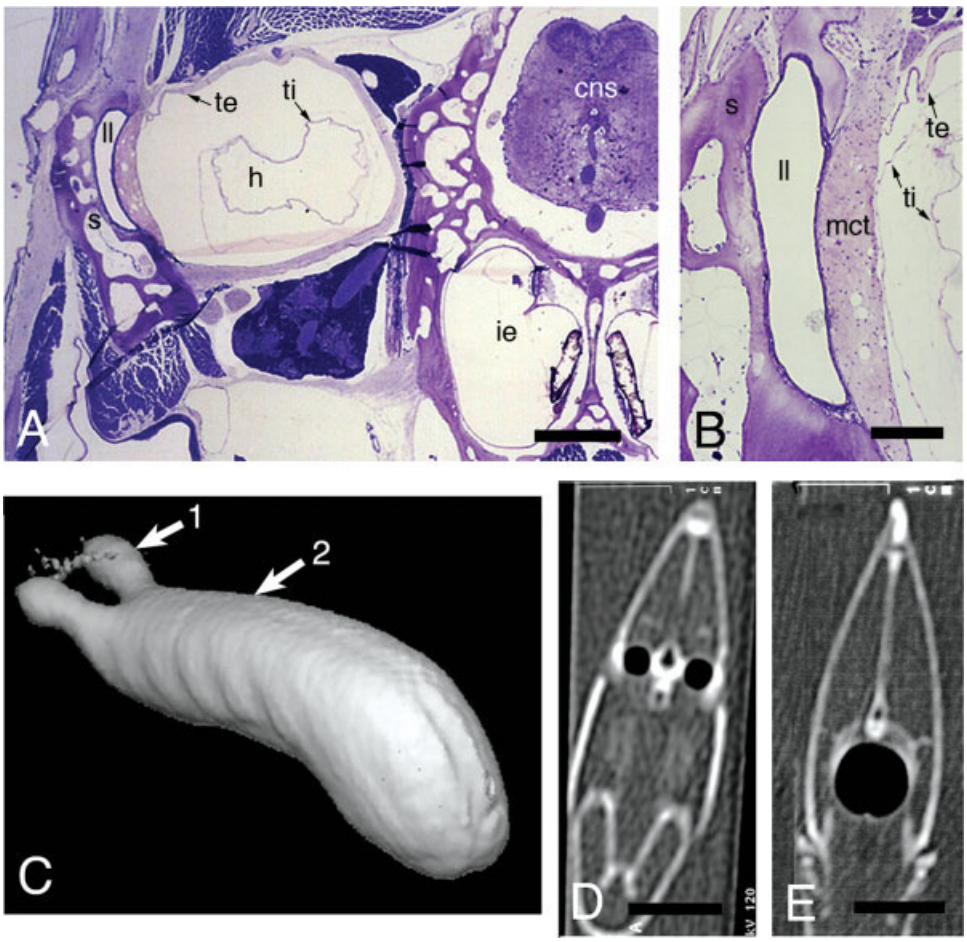


Fig. 3. Histological sections and CT images of the laterophysic connection (LC) and swim bladder. **A:** Transverse section through LC in *Chaetodon octofasciatus*. Scale bar = 500 μm (modified from Webb, 1998). **B:** Close-up of laterophysic tympanum in another specimen of *C. octofasciatus*. Scale bar = 200 μm. **C:** Three-dimensional reconstruction (CT) of air volume in the swim bladder and swim bladder horns in *C. ephippium*. Trail of small air bubbles are trapped in soft tissue (unknown origin), but are not associated with the swim bladder. **D:** Transverse CT slice at level of arrow 1 in B. **E:** Transverse CT slice at level of arrow 1 in C. Scale bar in D and E = 10 mm. cns, central nervous system; h, horn; ie, inner ear; ll, lateral line canal; mct, mucoid connective tissue; s, supracleithrum; te, tunica externa; ti, tunica interna. (Reproduced with permission from Smith et al., Cladistics, 2003, 19, 287-306, ©Blackwell Publishing).

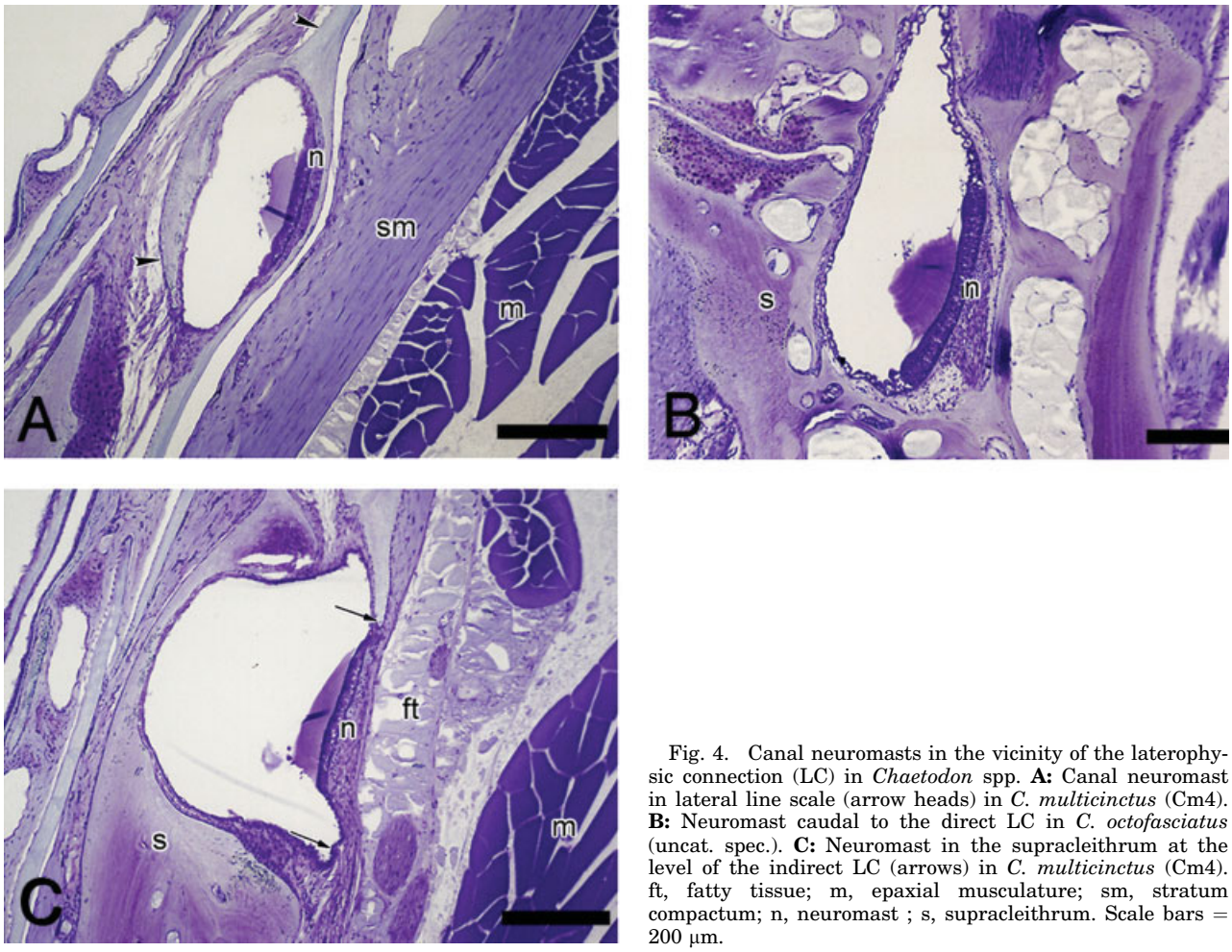


Fig. 4. Canal neuromasts in the vicinity of the laterophysic connection (LC) in *Chaetodon* spp. **A:** Canal neuromast in lateral line scale (arrow heads) in *C. multicinctus* (Cm4). **B:** Neuromast caudal to the direct LC in *C. octofasciatus* (uncat. spec.). **C:** Neuromast in the supracleithrum at the level of the indirect LC (arrows) in *C. multicinctus* (Cm4). ft, fatty tissue; m, epaxial musculature; sm, stratum compactum; n, neuromast ; s, supracleithrum. Scale bars = 200 μm.

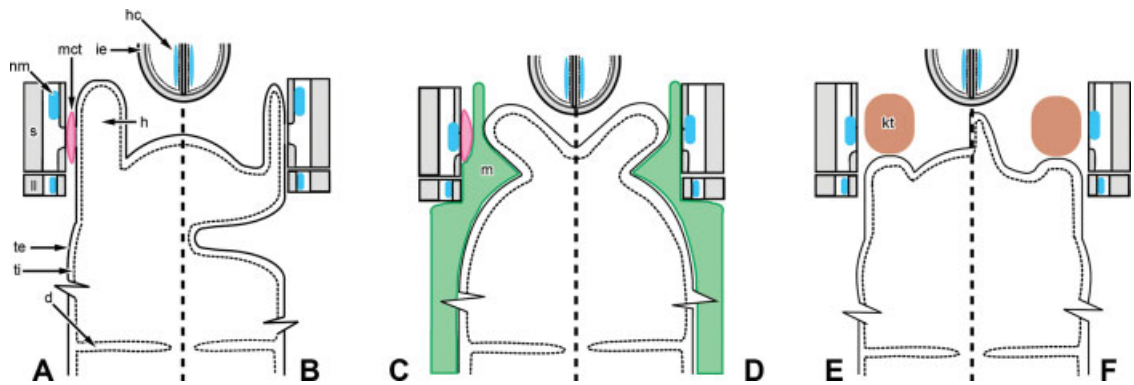


Fig. 5. Schematic representation of the six variants on laterophysic connection (LC) morphology (in dorsal view) among *Chaetodon* species (See Table 2). **A:** *Dir1* – direct LC with mucoid connective tissue, wide horns, and one-chambered swim bladder (e.g., *Chaetodon octofasciatus*). **B:** *Dir2* – direct LC without mucoid connective tissue, with narrow horns, and a two-chambered swim bladder (e.g., *C. plebeius*). **C:** *Ind1* – indirect LC with mucoid connective tissue, wide horns and one-chambered swim bladder (e.g., *C. kleinii*). **D:** *Ind2* – indirect LC without mucoid connective tissue and with wide horns and one-chambered swim bladder (e.g., *C. multicinctus*). **E:** *Ind3* – indirect LC with short horns and a one-chambered swim bladder (e.g., *C. ornatissimus*). **F:** *Ind4* – indirect LC with short horns, a one-chambered swim bladder and a medial anterior extension of swim bladder (e.g., *C. meyeri*). d, transverse diaphragm in tunica interna; h, swim bladder horn; hc, sensory macula; ie, inner ear in otic capsule; kt, kidney tissue; ll, 1st lateral-line scale; m, muscle; mct, mucoid connective tissue; nm, neuromast; s, supracleithrum; te, tunica externa (solid line); ti, tunica interna (dotted line).

TABLE 3. Morphological features of Chaetodon *spp. with different LC variants as determined by histological analysis (MCT), gross dissection (SB type, No. chambers) and CT imaging (horn length and diameter)*

	MCT present	Horn length	Horn diameter	No. of SB chambers	SB type
Variant *Dir1* (*Incertae sedis* and Subgenera *Rabdophorus*, *Gonochaetodon*, *Discochaetodon*)					
Chaetodon striatus	yes	long	wide	1	free
Chaetodon auriga	yes	long	wide	1	free
Chaetodon ephippium	yes	long	wide	1	free
Chaetodon baronessa	yes	long	wide	1	free
Chaetodon aureofasciatus	yes	long	wide	1	free
Chaetodon octofasciatus	yes	long	wide	1	free
Chaetodon rainfordi	yes	long	wide	1	?
Chaetodon semilarvatus	?[a]	long	?	1	free
Variant *Dir2* (Subgenera *Megaprotodon*, *Tetrachaetodon*)					
Chaetodon trifascialis	no	long	narrow	2	free
Chaetodon plebeius	no	long	narrow	2	free
Chaetodon oligacanthus	?	long	narrow	2	free
Variant *Ind1* (Subgenera *Lepidochaetodon*)					
Chaetodon kleinii	yes	long	wide	1	attached
Chaetodon unimaculatus	yes	long	wide	1	attached
Variant *Ind2* (Subgenera *Chaetodon*, *Exornator*, *Corallochaetodon*)					
Chaetodon capistratus	no	long	wide	1	free
Chaetodon ocellatus	no	long	wide	1	free
Chaetodon miliaris	no	long	wide	1	attached
Chaetodon multicinctus	no	long	wide	1	attached
Chaetodon sedentarius[b]	no	long	wide	1	attached
Chaetodon trifasciatus	no	long	wide	1	attached
Variant *Ind3* (Subgenera *Citharoedus, Roaops*)					
Chaetodon ornatissimus	no	short	N/A	1	attached
Chaetodon tinkeri	?	short	N/A	1	?
Variant *Ind4* (Subgenus *Citharoedus*)					
Chaetodon meyeri	no	short	N/A	1	attached

MCT, mucoid connective tissue in medial opening of supracleithrum; No. SB Chambers, number of swim bladder chambers formed by tunica externa; SB type, morphology of swim bladder with respect to relationship to peritoneum.
[a]Could not be determined because of the poor quality of histological tissue.
[b]Not variant *Ind1*, as reported by Webb (1998). See text and Figures 5 and 6 for additional explanation.

filled swim bladder horn and the fluid-filled lateral-line canal. In some specimens, adipose cells sit deep to the epithelial lining of the lateral line canal. At the level of the LC, kidney tissue sits between the otic capsule and the swim bladder horns, which appear to be firmly attached to the medial surface of the supracleithrum. In one specimen of *Chaetodon plebeius*, the swim bladder horns appear to bulge out into the supracleithrum through the medial opening. This may be the result of preparation artifact, but this was not confirmed with CT. In both *C. trifascialis* and *C. plebeius*, the swim bladder horns extend rostrally, to the level of the otic capsule. The prominent constriction in the tunica externa is located about 20–25% down the length of the swim bladder, forming a two-chambered swim bladder (described by Blum, 1988) in *C. oligacanthus* and *C. trifascialis*).

Variants on the indirect laterophysic connection. An indirect LC is defined by the absence of direct contact between the swim bladder horns and the medial opening in the supracleithrum. Muscle, kidney and other soft tissues lie deep to the medial opening, such that the distance between the lumen of the swim bladder horn and the lateral line canal in the supracleithrum is variable (~0.2–1 mm). Four variants on the Indirect LC are described here (Table 3).

Indirect LC with mucoid connective tissue, long, wide horns, and a one-chambered swim bladder (Ind1) (Figs. 5C, 6C). Epaxial muscle tissue sits between the supracleithral lateral line canal and the swim bladder horn, precluding direct contact between them. Adipose cells may be found within the mucoid connective tissue that fills the medial opening in the supracleithrum. The wide horns originate in a common trunk at the rostral end of the body of the swim bladder and appear uniform in diameter with a tapered rostral end.

Indirect LC without mucoid connective tissue, with long, wide horns and a one-chambered swim bladder (Ind2) (Figs. 5D, 6D). Mucoid connective tissue is not found in the LC, but most of the specimens with this variant have adipose cells between the epithelial lining of the lateral line canal and the epaxial musculature deep to the LC. It is interesting to note that Bauchot et al. (1989) did not mention the presence of the swim bladder horns in their histological analysis of *Chaetodon trifasciatus,* but this may be explained by the fact that their data were taken from a 16-mm-individual (probably at tholichthys stage) in which the swim bladder horns had not yet developed.

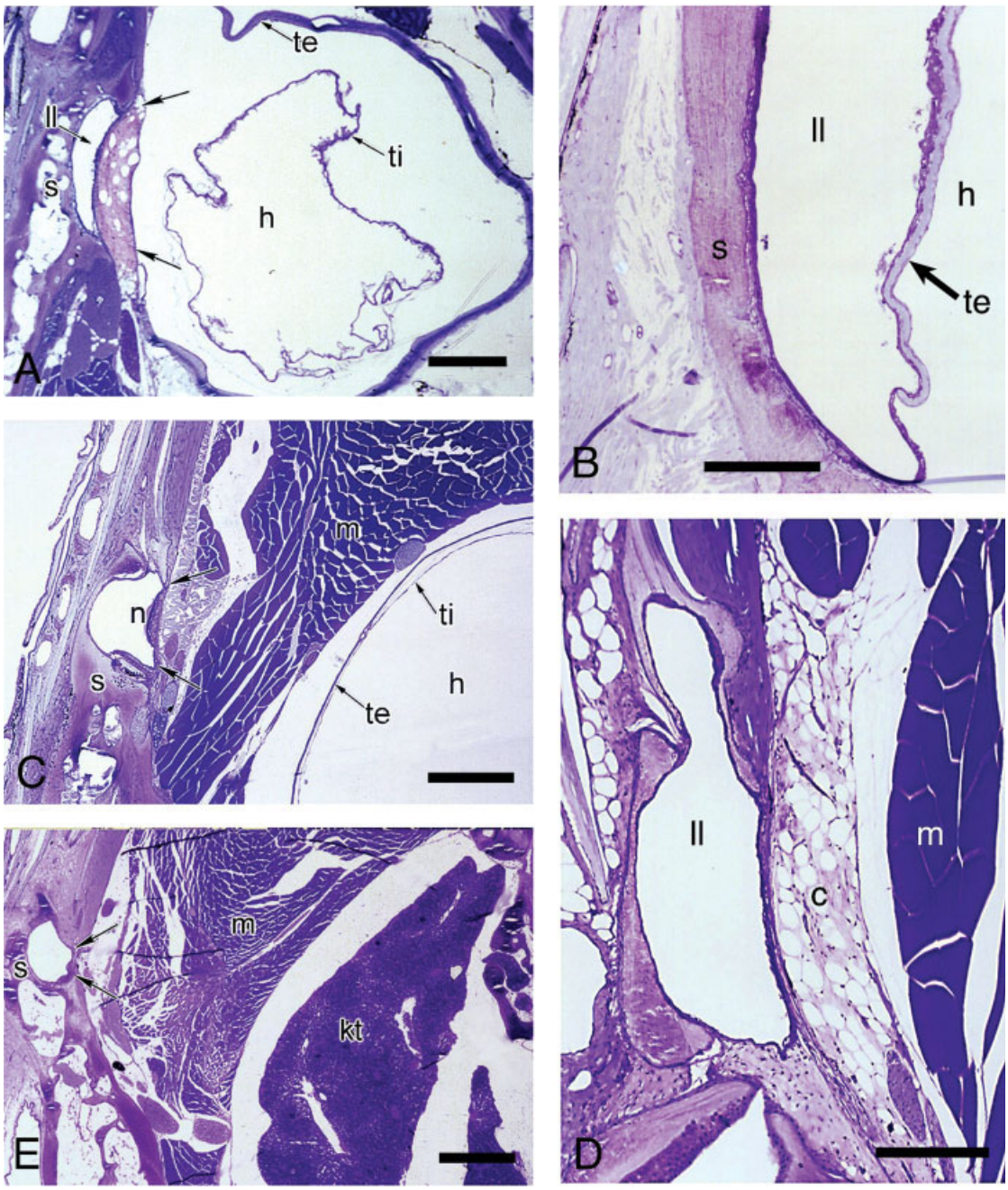


Fig. 6. Variants on the laterophysic connection (LC) among *Chaetodon* species represented schematically in Figure 5. **A:** *Dir1* – direct LC with mucoid connective tissue in *Chaetodon octofasciatus* (uncat. spec.). Scale bar = 500 μm. **B:** *Dir2* – direct LC without mucoid connective tissue in *Chaetodon plebeius* (Cp1) Scale bar = 200 μm. **C:** *Ind2* – indirect LC with no mucoid connective tissue in *Chaetodon multicinctus* (Cm4). Scale bar = 500 μm. **D:** *Ind1* – indirect LC (arrows) with mucoid connective tissue in *Chaetodon kleinii* (Ck10). Scale bar = 200 μm. **E:** *Ind3* – indirect LC with short horns in *Chaetodon ornatissimus* (Cn4). Scale bar = 500 μm. h, swim bladder horn; kt, kidney tissue; ll, lateral-line canal; mct, mucoid connective tissue; m, muscle; n, neuromast; s, supracleithrum; te, tunica externa; ti, tunica interna (Reproduced with permission from Smith et al., Cladistics, 2003, 19, 287–306, Arrows in A, C, and E indicate the medial opening in the supracleithrum. © Blackwell Publishing).

Indirect LC without mucoid connective tissue, with short horns and a one-chambered swim bladder (Ind3) (Figs. 5E, 6E). The short swim bladder horns extend <1 mm from the rostral end of the body of the swim bladder and do not extend to the level of the medial opening in the supracleithrum as in species with long horns (Figs. 5E, 9G,H). Mucoid connective tissue is absent at the level of the supracleithrum. Epaxial musculature is present deep to the supracleithrum as in other species with an indirect

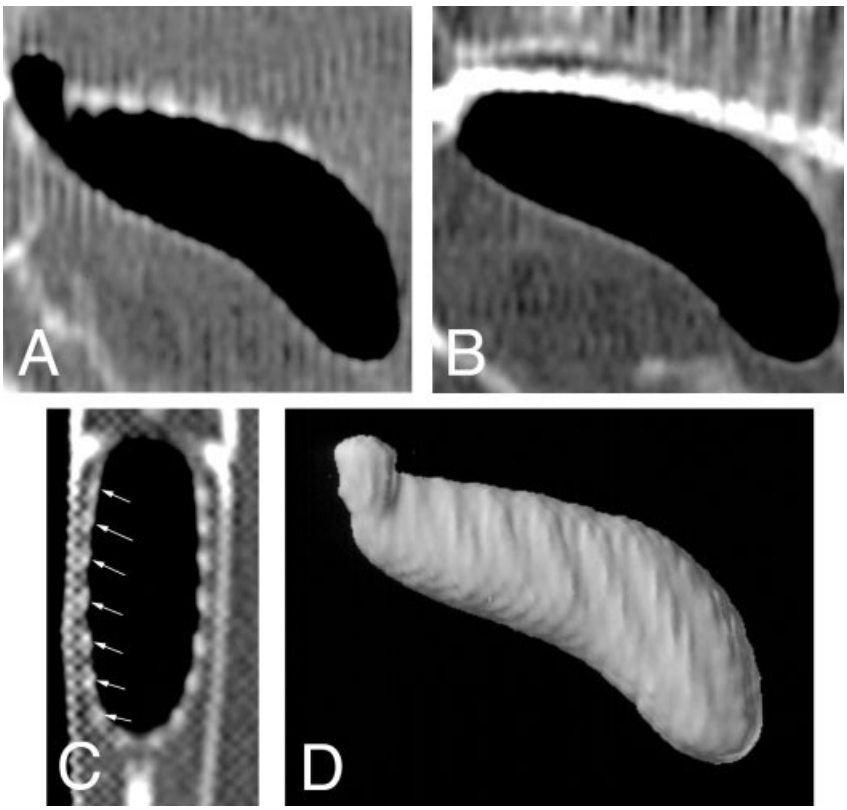


Fig. 7. CT images demonstrating relationship of the swim bladder, swim bladder horns and skeleton in *Chaetodon punctofasciatus*. **A:** Parasagittal CT slice demonstrating vertical orientation of swim bladder horn (h) lateral to vertebral column. **B:** Midsagittal CT slice demonstrating the close spatial relationship of the dorsal midline of the swim bladder and vertebral column (white). **C:** Horizontal CT slice demonstrating the position of the ribs (arrows) against the fully inflated swim bladder. **D:** 3D reconstruction of the volume of air within the swim bladder and swim bladder horns. Periodic indentations in lateral surface of gas volume correspond to the position of ribs illustrated in C.

LC and kidney tissue is present deep to the supracleithrum where the long swim bladder horns are found in other *Chaetodon* species (Fig. 7E).

Indirect LC without mucoid connective tissue, with short horns, medial rostral extension and a one-chambered swim bladder (Ind4) (Fig. 5F). This variant is also characterized by short swim bladder horns (see Variant Ind3, *Chaetodon ornatissimus*), but there is an additional median anterior extension of the swim bladder, which extends rostrally to the level of the supracleithrum.

Morphology of the Swim Bladder and Swim Bladder Horns

Gross dissection, histology, X-rays and CT imaging (2D transverse, sagittal and horizontal slices and 3D reconstructions) reveal details of the morphology of the physoclistous swim bladder and swim bladder horns (Figs. 3, 7, 8). In all species examined, the swim bladder lies in the dorsomedial region of the body cavity just ventral to the vertebral column and lacks any intrinsic or extrinsic musculature that might be indicative of a swim bladder mechanism of sound production (Fig. 7). A thick ventral midrib runs along the length of the tunica externa of the swim bladder and is visible upon gross dissection, in histological sections, and as a depression in 3D reconstructions of the volume of air within the swim bladder (Figs. 3, 9). An infolding of the tunica interna divides the swim bladder lumen transversely, forming a translucent diaphragm, with a small (~1–3 mm) central opening that subdivides the lumen of the swim bladder into anterior and posterior compartments (the "chambers" of some authors; Fig. 8B,C Smith et al., 2003). The location of the diaphragm is variable among individuals examined (50–75% down the length of the swim bladder) and it can be visualized in X-rays (Fig. 8A), as well as in some CT slices. In those species with two-chambered swim bladders (Variant Dir2, *Chaetodon oligacanthus*, *C. trifascialis*, and *C. plebeius*), this diaphragm is located caudal to the constriction in the tunica externa (the *ductus communicans*).

Transverse CT slices demonstrate that the swim bladder horns in *Chaetodon* are roughly circular in crossection, but in species with a direct LC, the lateral surface of the horn appears somewhat flattened at the point of contact with the medial opening of the supracleithrum (Fig. 3). Three-dimensional reconstructions (CT) demonstrate that in some species the horns have a slightly narrower neck with an expanded distal end (as per Blum, 1988) and small depressions in the surface of the horns that are not evident in the quantitative analysis of two-dimensional CT images (Figs. 3, 7, 9).

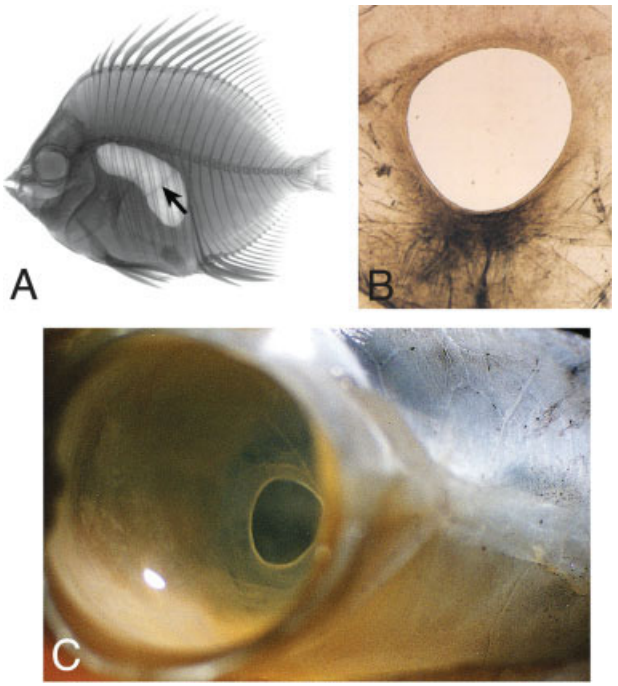


Fig. 8. Internal transverse diaphragm in the swim bladder of *Chaetodon* spp. **A:** X-ray showing the swim bladder (white) and the transverse diaphragm (arrow) in *C. octofasciatus*. **B:** Central opening in isolated diaphragm of *C. octofasciatus* (~2–3 mm diameter). **C:** Diaphragm in ventral view in intact swim bladder of *C. multicinctus* with anterior end removed (rostral is to left).

Two swim bladder morphologies are found among *Chaetodon* spp. and are generally correlated with LC type (Table 3; Webb, 1998; Webb and Smith, 2000). Species with a direct LC (e.g. *C. octofasciatus*) have a swim bladder with a distinct change in angle in its long axis (tilt angle), such that the anterior half of the swim bladder has a relatively rostro-caudal orientation relative to the posterior half of the swim bladder, which has a more dorso-ventral orientation (Figs. 3C, 8A). The swim bladder is robust with a thick tunica externa composed of what appear to be multiple layers of collagen. A thin, translucent, lightly pigmented peritoneum covers the ventral surface of the swim bladder, and wraps tightly around its posterior end, so that the swim bladder is clearly visible upon lateral dissection of the abdominal cavity. The bilateral swim bladder horns extend separately from the dorso-rostral surface of the swim bladder. This swim bladder morphology is defined as a "free" swim bladder to reflect the fact that the posterior end of the swim bladder appears to sit free in the peritoneal cavity.

In *Chaetodon* species with an indirect LC, the swim bladder appears to be more smoothly contoured (lacking an abrupt change in angle), and may decrease in circumference caudally (Figs. 7, 9). The relatively translucent tunica externa appears to be thinner dorsally than it is ventrally, and much thinner overall when compared to the swim bladder in species with a direct LC. The swim bladder sits above a thick, opaque peritoneum, which attaches laterally to the ribs so that the swim bladder is not visible upon lateral dissection of the abdominal cavity. The swim bladder horns extend rostrally in a dorso-ventrally flattened common trunk that divides into the two horns. This is defined as

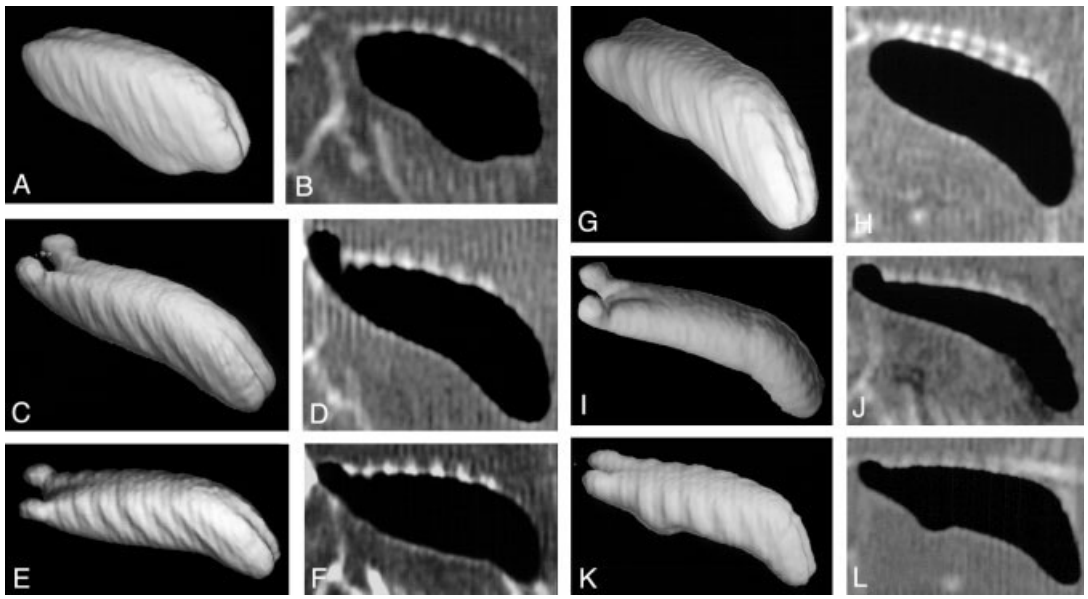


Fig. 9. Three-dimensional reconstructions and parasagittal CT images of swim bladder. **A/B:** *Forcipiger flavissimus*. Note absence of horns and lateral compression of gas volume. **C/D:** *Chaetodon punctofasciatus*. **E/F:** *Chaetodon miliaris*. **G/H:** *Chaetodon ornatissimus*. Note presence of short horns. **I/J:** *Chaetodon kleinii*, **K/L:** *Chaetodon trifasciatus*. Artifactual outpocketing of swim bladder can be seen on ventral surface of swim bladder.

TABLE 4. Quantitative analysis of swim bladder and horn morphology as revealed by CT imaging

Species	LC variant	Length (SL)	SB length	SB/SL length	Max. SB diameter	Horn length		Max. horn diameter	
						R	L	R	L
C. auriga	*Dir1*	88	27.5	0.31	7.81	3.5	3.5	2.21	2.40
		115	34.5	0.30	10.04	5.5	5.5	3.44	3.32
C. striatus	*Dir1*	60A[a]	15.0	0.25	8.52	2.5	2.5	2.37	2.36
		60B[a]	15.0	0.25	6.11	3.0	2.5	3.18	2.06
		61[a]	14.0	0.23	6.36	2.0	2.0	1.99	2.15
		61B[a]	12.0	0.20	6.13	2.0	2.0	2.02	2.53
		70	15.0	0.21	6.41	2.5	2.5	2.19	2.14
C. capistratus	*Ind2*	65	20.5	0.32	7.69	3.5	2.5	2.42	2.51
		66	20.0	0.30	7.76	2.5	3.0	2.41	2.59
		75	22.5	0.30	8.51	3.5	3.0	2.79	8.15
		77A[a]	24.5	0.32	9.11	3.5	4.0	2.81	3.36
		77B[a]	22.0	0.29	8.73	3.5	3.5	2.65	2.72
C. miliaris	*Ind2*	85	26.5	0.31	7.87	3.0	3.0	2.95	2.90
		90	29.0	0.32	8.55	3.5	3.5	3.26	3.21
C. multicinctus	*Ind2*	58	18.0	0.31	5.88	2.5	2.0	2.31	2.36
		65	19.0	0.29	6.54	2.0	2.0	2.28	2.36
		68	21.5	0.32	6.24	3.0	3.0	2.38	2.57
		75	22.5	0.31	7.12	3.5	3.5	2.95	2.96
C. ocellatus	*Ind2*	52	13.0	0.25	5.44	1.5	1.5	1.07	1.27
		73	21.0	0.29	6.95	3.0	3.0	2.69	1.86
C. sedentarius	*Ind2*	58	18.5	0.32	6.35	2.5	2.5	1.99	2.17
Forcipiger flavissimus	–	103	27.5	0.27	8.20	–	–	–	–
	–	114	29.5	0.26	8.46	–	–	–	–
Hemitaurichthys polylepis	–	110	30.5	0.28	10.57	–	–	–	–

[a]A, fish scanned first with left side up; B, fish scanned subsequently, with right side up. All measurements in mm, with measurement error = ±0.5 mm for swim bladder (SB) and horn length. See text for methodological details and Table 3 for definition of LC variants.

an "attached" swim bladder to reflect the fact that at least part of the swim bladder is attached to the peritoneal lining, and that the swim bladder does not appear to sit free in the abdominal cavity.

Gross dissection of 30 *Chaetodon* spp. (in 10 of 12 *Chaetodon* subgenera, Table 1) revealed that, with the exception of one species in subgenus *Exornator* and two species in subgenus *Chaetodon* (*C. capistratus* and *C. ocellatus*; see Table 3), each species has the same swim bladder morphology as others in the same subgenus in which LC morphology had already been determined histologically. Thus, gross morphology of the swim bladder (free/attached) appears to be a reliable predictor of LC morphology (direct/indirect) among *Chaetodon* species.

Swim bladder morphology in non-*Chaetodon* genera appears to be similar to that in *Chaetodon* species with an indirect LC. In *Forcipiger,* the swim bladder is elongate and sausage-shaped, but is more laterally compressed, giving the swim bladder an oval cross-section along most of its length (Fig. 9A,B). Gross dissection demonstrates that *Heniochus* has a swim bladder similar in to those *Chaetodon* species with an indirect LC. CT scans of *Hemitaurichthys* reveal that it has a swim bladder with a gradual contour, suggesting that its swim bladder is similar to that in the other non-*Chaetodon* species examined.

Swim bladder and swim bladder horn dimensions in nine *Chaetodon* and two non-*Chaetodon* species were easily derived from transverse CT slices (Table 4). The swim bladder in *Chaetodon* spp. is 12–34 mm long (~20–32% of SL) with a maximum diameter of ~10 mm. The swim bladder horns are ~1.5–3.5 mm long, and generally 2–3 mm in diameter. Swim bladder length, as well as maximum swim bladder and swim bladder horn diameter, is generally correlated with fish size in those *Chaetodon* species for which more than one individual was examined. Measurements of horn length and diameter do not suggest any consistent right-left asymmetry (Table 4).

While it appears that horn length did not change in either *Chaetodon capistratus* or *C. striatus* in sequential CT scans (see Table 4), swim bladder length and maximum swim bladder diameter did decrease in specimens of both species. Interestingly, while maximum horn diameter appeared to decrease in *C. capistratus* (indirect LC), it appeared to increase in *C. striatus* (a direct LC) in a subsequent scan.

Some shrinkage and preparation artifact was evident in all histological material examined subsequent to CT scanning (Table 5). Horn diameter was compared in CT images and histological sections prepared from the same specimens. *Chaetodon striatus* (direct LC), clearly demonstrates shrinkage (histology/CT ratio = 0.5–0.6) while *C. capistratus* and *C. ocellatus* (indirect LC) did not (histology/CT ratio = ~1.0). One specimen of *C. ocellatus* had a high ratio (1.6) and a horn diameter that is less

TABLE 5. Comparison of measurements from CT images and histological material of right horn diameter (in mm) in three Chaetodon spp. Ratio of measurements from histological material and CT images (Histo/CT) are to the nearest 0.5. See Table 3 for definition of LC variants

Species	LC variant	Length (SL)	Mean horn diameter (CT)	Mean horn diameter (histo)	Mean horn diameter (histo/CT)	Max horn diameter (CT)	Max horn diameter (histo)	Horn diameter (histo/CT)
C. striatus	*Dir1*	60	1.78	0.81	0.5	2.37	1.2	0.5
		61	1.77	0.96	0.5	1.99	1.26	0.6
C. capistratus	*Ind2*	66	1.85	1.50	0.8	2.41	2.06	0.9
		77	2.41	2.82	1.2	2.81	2.69	1.0
C. ocellatus	*Ind2*	52	1.04	1.06	1.0	1.07	1.71	1.6
		73	1.65	2.03	1.2	2.69	2.56	1.0

than half that of the other specimens, which was likely due to the deflation of the horn.

Relationship of Swim Bladder and Horns to the Axial and Cranial Skeleton

CT images demonstrate an intimate relationship between the swim bladder to the ribs and the vertebral column (Figs. 7, 9), features that cannot be appreciated in conventional X-rays. Rib indentations observed on the outer surface of the tunica externa in fixed specimens examined grossly are not the result of fixation artifact. The close relationship of the ribs to the fully inflated swim bladder is shown in horizontal sections and in 3D reconstructions of the air volume that fills the swim bladder, in which indentations of the ribs are visible on the lateral surface of the air volume filling the swim bladder (Figs. 7C,D, 9). The swim bladder sits up against the vertebral column along most of its length; posteriorly the dorsal surface of the swim bladder abuts the postabdominal vertebrae, forming a distinct groove in the posterior-dorsal surface of the swim bladder (Fig. 9).

The body of the swim bladder extends rostrally to a point just caudal to the well-ossified otic capsules. The swim bladder horns extend rostrally by another ~2–3 mm and are positioned dorso-lateral to the otic capsule (Table 3). Variation in the angle of the major axis of the horns among species (from primarily rostral to primarily dorsal) is easily visualized in parasagittal CT slices and in lateral views of 3D reconstructions (Figs. 7, 9). In transverse CT slices, which illustrate the medio-lateral plane, the swim bladder horns come within 500–1500 μm of the caudal end of the well-ossified otic capsule.

DISCUSSION

The laterophysic connection (LC), the association of anterior swim bladder horns with the medial openings in the supracleithra, evolved in a single clade of chaetodontid fishes. Butterflyfishes are well known for their extensive and diverse social behaviors that involve close interactions among individuals (Reese, 1975; Hourigan, 1989). The recent discovery of sound production by *Forcipiger* and *Chaetodon* species in the context of social behavior (Tricas et al., 2004; Tricas and Boyle, 2005) demonstrates the importance of acoustic stimuli to the behavior and ecology of these fishes. The high noise levels on coral reefs make the extraction of biologically significant acoustic stimuli particularly challenging. It will be important to measure ambient noise levels within the frequency spectrum of butterflyfish hearing capabilities to determine if this is indeed the case. However, chaetodontid species that exhibit monogamous pairing behavior spend a great deal of time in close proximity to one another (Tricas and Boyle, 2005), so that both the ear and lateral line system, which both respond to "near field" acoustic stimuli may be used to analyze signals produced during behavioral interactions.

Recent experiments have demonstrated that filling of the swim bladder horns or disruption of the LC changes responses to sound stimuli and alters social behavior (Tricas and Boyle, 2005). These observations all lend support to the hypothesis that the swim bladder horns and LC are an adaptation for reception of sound pressure by the lateral line system and/or the ear (Webb, 1998; Webb and Smith, 2000; Smith et al., 2003). Thus, we hypothesize that the simultaneous input of pressure stimuli from the swim bladder to both the lateral line and inner ear, as well as direct stimulation of the ear (whole body acceleration), and hydrodynamic stimulation of neuromasts in the lateral line canals of the head and trunk facilitates a unique interaction among different acousticolateralis inputs (Braun et al., 2002) that may enhance the ability of *Chaetodon* species to intepret behaviorally important acoustic stimuli in noisy coral reef environments.

We further suggest that variation in LC morphology among *Chaetodon* species may represent alternative adaptive strategies for the transmission of acoustic signals generated during social interactions in species that live in noisy coral reef environments. If swim bladder horns in *Chaetodon*

transduce a sound pressure wave-induced oscillation of the gas volume in the swim bladder into movements of the tunica externa of the swim bladder and horn walls that generate a secondary particle displacement field, then we predict that

1. Swim bladder horn length will influence the sensitivity of the ears to sound pressure. Following results from holocentrids (Coombs and Popper, 1979) and sciaenids (Ramcharitar et al., 2002) the long swim bladder horns in the vicinity of the otic capsule, found in most *Chaetodon* species, should increase the amplitude of sound pressure-induced stimuli that reaches the ears. Thus, *Chaetodon* species with long horns should have lower hearing thresholds when compared to *Chaetodon* species with short horns (regardless of the proximity of the swim bladder horns to the medial openings in the supracleithra, LC type) and to non-*Chaetodon* species that lack horns altogether.
2. The proximity of the long swim bladder horns to the medial opening in the supracletihrum (direct versus indirect LC) will influence the ability of sound pressure stimuli to be transmitted to the lateral line system. Therefore, in species with a direct LC (variants *Dir1*, *Dir2*), sound pressure stimuli will be transmitted more effectively to the lateral line canal system than in species with an indirect LC (variants *Ind1*, *Ind2*) in which stimuli must move through muscle or kidney tissue in order to reach the medial opening in the supracleithrum (Webb and Smith, 2000). In all *Chaetodon* species, in order for stimuli to be transmitted from swim bladder horn to the ear it must traverse soft tissue and the well-ossified bone of the otic capsule, regardless of LC type.
3. Horn diameter (wide/narrow) will determine the characteristics of the sound stimulus delivered to both the lateral line system and ears. It has been argued (see Schellart and Popper, 1992) that the displacement of the apex of the swim bladder in response to a sound stimulus is higher than at the lateral swim bladder walls. Schellart and Popper (1992) predicted that the magnitude of displacement of the swim bladder apex and the resultant transmission of sound in surrounding tissues is related to swim bladder axis ratio (diameter/rostro-caudal length). Following their line of argumentation, it is predicted that the displacement amplitude of the relatively sharp apex of swim bladder horns in response to a sound pressure wave will be higher than that of the blunt anterior wall of the body of the swim bladder, and that the displacement amplitude of the wall of narrow horns will be greater than that in wide horns. They also indicate that the high amplitude stimulus emerging from the sharp apex of an elongated swim bladder, or in this case a swim bladder horn, will attenuate quickly as it moves through adjacent tissues. This may explain why the few *Chaetodon* species that have narrow horns have a direct LC with no mucoid connective tissue (*Dir2*, subgenera *Megaprotodon*, *Tetrachaetodon*, Fig. 6B), where the distance between the wall of the swim bladder horn and the lumen of the lateral line canal is minimized.
4. The presence of mucoid connective tissue in the medial opening of the supracleithrum increases the distance from the air in the horn lumen to the fluid in the lateral line canal lumen, and it is predicted that the addition of this specialized tissue to the laterophysic tympanum will also affect its biomechanical properties and thus the way in which the tympanum responds to acoustic stimuli. The fat content of laterophysic tympanum was variable among individuals and is likely influenced by their nutritional state, which has a seasonal component (Figs. 11 and 12 in Tricas, 1986). The functional significance of this fat is unknown, but the acoustic properties of fat have been investigated with respect to sound transmission to the ear of cetacea (Wartzok and Ketten, 1999).

Finally, the correlation of swim bladder morphology (free/attached) and LC type (direct/indirect) among *Chaetodon* species highlights the functional importance of the swim bladder as a component of the LC system and demands investigations of the biomechanics and bioacoustics of the swim bladder. CT imaging provides direct evidence for the heterogeneity of the tissues immediately surrounding the swim bladder (peritoneum, ribs, musculature, also see images of fishes on www.digimorph.org), which need to be considered when modeling the acoustic responses of the swim bladder (discussed by Sand and Hawkins, 1973; Foote and Francis, 2002). Predictions of swim bladder acoustic responses have generally been made using linear and volumetric measures and models that describe the swim bladder as a sphere or prolate spheroid. More complex models are needed to take into consideration the anatomical features of swim bladders. CT imaging of live, anesthetized fishes (even at resolutions lower than one can achieve using high energy, high resolution CT, www.digimorph.org) offers an opportunity to accurately describe and quantify swim bladder morphology among a diverse range of species, thus facilitating comparative studies of its bioacoustic properties.

ACKNOWLEDGMENTS

The authors thank Drs. Melanie Stiassny and Barbara Brown (AMNH) who provided a loan of

cleared and stained material. Dr. Dominique Daget and Bill Saul (ANSP), and Dr. Jeff Leis and Mark McGrouther (AMS) provided loans or gifts of preserved materials. Many thanks to Karsten Hartel (MCZ, Harvard U.) and Dr. John Lundberg (ANSP) for special permission to dissect material in their collections. Brian DuVall (New Jersey State Aquarium) and Carl Meyer (U. Hawaii) helped us to obtain specimens for this study. They also thank Julie Arruda, Scott Cramer, Judy Fenwick, and Anita Norton (WHOI) for their invaluable expertise and resourcefulness. They thank Drs. Mardi Hastings and David Zeddies (U. Maryland) for fruitful discussions and Drs. Timothy Tricas (U. Hawaii), Allen Mensinger (U. Minnesota), and Richard Fay (Loyola U. Chicago) for commenting on earlier drafts of the manuscript. Undergraduates Erin Shearman, Ryan Walsh, Natasha Kelly, Nicole Cicchino, Sokun Ky, and Mary Turnipseed prepared and analyzed histological material used in this study. They thank Dr. Stanley Blum who provided Figure 3B and Melissa Tarby Wasko who prepared Figure 1A. Portions of this work were completed in partial fulfillment of requirements for the Master of Science degree at Villanova University to WLS. This work was supported by a Villanova Graduate Research Fellowship, a Center for Environmental Research Graduate-Faculty Fellowship (Columbia U.), an AMNH Lerner-Gray Graduate Fellowship and an AMNH Kalbfleisch Graduate Fellowship to WLS, an HHMI Undergraduate Education grant to Villanova University, and a Villanova Faculty Summer Research Fellowship to JFW.

LITERATURE CITED

Alexander RM. 1962. The structure of the Weberian apparatus in the Cyprini. Proc Zool Soc Lond 139:451–473.

Alexander RM. 1967. Functional design in fishes. London: Hutchinson.

Barimo JF, Fine ML. 1998. Relationship of swim-bladder shape to the directionality pattern of underwater sound in the oyster toadfish. Can J Zool 76:134–143.

Bauchot RA, Thomas A, Bauchot ML. 1989. The membranous labyrinth and its innervation in *Chaetodon trifasciatus* (Pisces, Teleostei, Chaetodontidae). Environ Biol Fishes 25:235–242.

Bennett HS, Wyrick AD, Lee SW, McNeil JH Jr. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Technol 51:71–97.

Best ACG, Gray JAB. 1980. Morphology of the utricular recess in the sprat. J Mar Biol Assoc UK 60:703–715.

Blaxter, JHS. 1981. The swimbladder and hearing. In: Tavolga WN, Popper AN, Fay RR, editors. Hearing and Sound Communication in Fishes. New York: Springer-Verlag. pp 61–70.

Blaxter JHS, Tytler P. 1978. Physiology and function of the swimbladder. Adv Comp Physiol Biochem 7:311–367.

Blum SD. 1988. The osteology and phylogeny of the Chaetodontidae (Teleostei: Perciformes). Ph.D. Dissertation, University of Hawaii, Honolulu. Available at www.calacademy.org/research/informatics/sblum.

Braun CB, Coombs S, Fay RR. 2002. What is the nature of multisensory interaction between octavolateralis sub-systems? Brain Behav Evol 59:162–176.

Bridge TW. 1900. The air-bladder and its connection with the auditory organ in *Notopterus borneensis*. J Linn Soc Lond 27:503–540.

Carpenter KE, Berry TM, Humphries JM. 2004. Swim bladder and posterior lateral line nerve of the nursery fish *Kurtus gulliveri* (Perciformes: Kurtidae). J Morphol 260:193–200.

Chao NL. 1986. A synopsis on zoogeography of the Sciaenidae. In: Uyeno T, Arai R, Taniuchi T, Matsuura K, editors. Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Tokyo: Ichthyological Society of Japan. pp 570–589.

Chao NL. 1995. Sciaenidae. In: Fischer W, Krupp F, Schneider W, Sommer C, Carpenter KE, Niem VH, editors. Guía FAO para la identificacíon para los fines de la pesca. Pacifico Centro-Oriental 3. Rome: Food and Agricultural Organization. pp 1427–1528

Collette BB, Nauen CE. 1983. FAO Species Catalogue, Vol. 2: Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos and Related Species Known to Date. FAO Fish Synop No. 125:1–137.

Coombs S, Popper AN. 1979. Hearing differences among Hawaiian squirrelfishes (family Holocentridae) related to differences in the peripheral auditory system. J Comp Physiol 132:203–207.

Coombs S, Montgomery JC. 1999. The enigmatic lateral-line system. In: Fay RR, Popper AN, editors. Comparative Hearing: Fish and Amphibians. New York: Springer-Verlag. pp 319–362.

Coombs S, Janssen J, Montgomery F. 1992. Functional and evolutionary implications of peripheral diversity in lateral line systems. In: Webster D, Popper AN, Fay RR, editors. The Evolutionary Biology of Hearing. New York: Springer-Verlag. pp 267–294.

Dehadrai PV. 1959. On the swimbladder and its connection with the internal ear in family Cichlidae. Proc Natl Inst Sci India B 25:254–261.

Dobbin CN. 1941. A comparative study of the gross anatomy of the air-bladders of ten families of fishes of New York and other eastern states. J Morphol 68:1–29.

Fink SV, Fink WL. 1996. Interrelationships of ostariophysan fishes (Teleostei). In: Stiassny MLJ, Parenti LR, Johnson GD, editors. Interrelationships of Fishes. San Diego: Academic Press. pp 209–249.

Fletcher LB, Crawford JD. 2001. Acoustic detection by sound-producing fishes (Mormyridae): The role of gas-filled tympanic bladders. J Exp Biol 204:175–183.

Foote KG. 1980. Importance of the swimbladder in acoustic scattering by fish: A comparison of gadoid and mackerel target strengths. J Acoust Soc Am 67:2084–2089.

Foote KG. 1985. Rather-high-frequency sound scattering by swimbladdered fish. J Acoust Soc Am 78:688–700.

Foote KG. 1988. Comparison of walleye pollack target strength estimates determined from in situ measurements and calculations based on swimbladder form. J Acoust Soc Am 83: 9–17.

Foote KG, Francis DTI. 2002. Acoustic scattering by swimbladdered fish: A review. Bioacoustics 12:263–265.

Gosline WA. 1966. The limits of the fish family Serranidae, with notes on other lower percoids. Proc Calif Acad Sci 33:91–112.

Green JM. 1971. Studies on the swim bladders of *Eucinostomus gula* and *E. argenterus* (Pisces: Gerreidae). Bull Mar Sci 21:567–590.

Greenwood PH. 1970. Skull and swimbladder connections in the fishes of the family Megalopidae. Bull British Mus Nat Hist (Zool) 14:121–135.

Günther A. 1860. Catalogue of the Fishes in the British Museum, Vol. 2: Squamipinnes, Cirrihitidae, Triglidae, Trachinidae, Sciaenidae, Polynemidae, Sphyraenidae, Trichiuridae, Scombridae, Carangidae, Xiphiidae. London: British Museum.

Hawkins AD. 1993. Underwater sound and fish behaviour. In: Pitcher TJ, editor. Behaviour of Teleost Fishes, 2nd ed. London: Chapman and Hall. pp 129–170.

Herre AW, Montalban HR. 1927. The Philippine butterflyfishes and their allies. Philipp J Sci 34:1–113.

Hourigan TF. 1989. Environmental determinants of butterflyfish social systems. Environ Biol Fish 25:61–78.

Johnson GD. 1980. The limits and relationships of the Lutjanidae and associated families. Bull Scripps Inst Oceanogr Univ Calif 24:1–114.

Kalmijn AJ. 1989. Functional evolution of lateral line and inner ear sensory systems. In: Coombs SP, Görner P, Münz H, editors. The Mechanosensory Lateral Line-Neurobiology and Evolution. New York: Springer-Verlag. pp 187–215.

Katayama M. 1959. Studies on the serranid fishes of Japan (I). Bull Fac Educ Yamaguichi Univ 8:103–180.

Kearney M, Maisano JA, Rowe T. 2005. Cranial anatomy of theo extinct amphisbaenian *Rhineura hatcherii* (Squamata, Amphisbaenia) based on high-resolution X-ray computed tomography. J Morphol 264:1–33.

Ketten DR, Skinner MW, Want G, Vannier MW, Gates GA, Neely JG. 1998. In vivo measures of cochlear length and insertion depth of nucleus cochlear implant electrode arrays. Ann Otol Rhinol LaryngolSuppl 175, 107(11, Part 2):1–16.

Ladich F, Bass AH. 2003. Underwater sound generation and acoustic reception in fishes with some notes on frogs. In: Collin SO, Marshall NJ, editors. Sensory Processing in Aquatic Environments. New York: Springer-Verlag. pp 173–193.

Ladich F, Popper AN. 2004. Parallel evolutoin in fish hearing organs. In: Manley GA, Popper AN, Fay RR, editors. Evolution of the Vertebrate Auditory System. New York: Springer-Verlag, pp 95–127.

Leis JM. 1994. Larvae, adults and relationships of the monotypic perciform fish family Lactariidae. Rec Aust Mus 46:131–143.

MacDonald CM. 1978. Morphological and biochemical systematics of Australian freshwater and estuarine percichthyid fishes. Aust J Mar Freshwater Res 29:667–698.

MacLennan DN, Simmonds EJ. 1992. Fisheries Acoustics. London: Chapman and Hall.

McKay R. 1985. A revision of the fishes of the family Sillaginidae. Mem Queensland Mus 22:1–74.

Myrberg AA Jr. 1981. Sound communication and interception in fishes. In: Tavolga WN, Popper AN, Fay RR, editors. Hearing and Sound Communication in Fishes. New York: Springer-Verlag. pp 395–426.

Nelson EM. 1955. The morphology of the swim bladder and auditory bulla in the Holocentridae. Fieldiana (Zool) 37:121–130.

O'Connell CP. 1955. The gas bladder and its relation to the inner ear in *Sardinops caerulea* and *Engraulis mordax*. US Fish Bull 56:505–533.

Parker TH. 1882. On the connection of the air bladder and the auditory organ in the red cod (*Lotella bacchus*). Trans New Zealand Inst 15:234.

Poggendorf D. 1952. The absolute threshold of hearing of the bullhead (*Amiurus nebulosus*) and contributions to the physics of the Weberian apparatus of the Ostariophysi. (Adler HE, Cappelli B, Transl.). Zeitschr Vergleigh Physiol 34:222–257.

Popper AN. 1977. A scanning electron microscopic study of the sacculus and lagena in the ears of fifteen species of teleost fishes. J Morphol 153:397–417.

Popper AN, Fay RR. 1993. Sound detection and processing by fish: Critical review and major research questions. Brain Behav Evol 41:14–38.

Popper AN, Fay RR. 1999. The auditory periphery in fishes. In: Fay RR, Popper AN, editors. Comparative Hearing: Fish and Amphibians. New York: Springer-Verlag. pp 38–100.

Popper AN, Fay RR, Platt C, Sand O. 2003. Sound detection mechanisms and capabilities of teleost fishes. In: Collin SO, Marshall NJ, editors. Sensory Processing in Aquatic Environments. New York: Springer-Verlag. pp 3–38.

Pothoff T. 1984. Clearing and staining techniques. In: Moser HG, Richards WJ, Cohen D, Fahay MP, Kendall AW, Richardson S, editors. Ontogeny and Systematics of Fishes. Lawrence, KS: Allen Press. pp 35–37.

Ramcharitar J, Higgs D, Popper AN. 2002. Sciaenid inner ears: A study in diversity. Brain Behav Evol 58:152–162.

Ramcharitar JU, Deng X, Ketten D, Popper AN. 2004. Form and function in the unique inner ear of a teleost: The silver perch (*Bairdiella chrysoura*). J Comp Neurol 475:531–539.

Reese ES. 1975. A comparative field study of the social behavior and related ecology of reef fishes of the family Chaetodontidae. Z Tierpsychol 37:37–61.

Rosen DE, Greenwood PH. 1970. Origin of the Weberian ossicles and the relationships of the ostariophysan and gonorynchiform fishes. Amer Mus Novitates 2428:1–25.

Rosenblatt RH, Bell MA. 1976. Osteology and relationships of the roosterfish. *Nematistius pectoralis* Gill. Nat Hist Mus Los Angeles County Contrib Sci 279:1–23.

Sand O, Hawkins AD. 1973. Acoustic properties of the cod swimbladder. J Exp Biol 58:797–820.

Sasaki K. 1989. Phylogeny of the family Sciaenidae, with notes on its zoogeography (Teleostei, Perciformes). Mem Fac Fish Hokkaido Univ 36:1–137.

Schaefer KM, Oliver CW. 2000. Shape, volume, and resonance frequency of the swimbladder of yellowfin tuna, *Thunnus albacares*. US Fish Bull 98:364–374.

Schaefer SA. 2003. Relationships of *Lithogenes villosus* Eigenmann, 1909 (Siluriformes, Loricariidae): Evidence from high-resolution computed microtomography. Am Mus Novitates 3401:55.

Schellart NAM, Popper AN. 1992. Functional aspects of the evolution of the auditory system of actinopterygian fish. In: Webster D, Popper AN, Fay RR, editors. The Evolutionary Biology of Hearing. New York: Springer-Verlag. pp 295–322.

Smith WL. 2000. Variation in the morphology of the laterophysic connection in butterflyfishes of the genus *Chaetodon*. MS Thesis, Villanova University, Villanova, PA.

Smith WL, Webb JF, Blum SD. 2003. The evolution of the laterophysic connection with a revised phylogeny and taxonomy of butterflyfishes (Teleostei: Chaetodontidae). Cladistics 19:287–306.

Starnes WC. 1988. Revision, phylogeny and biogeographic comments on the circumtropical marine percoid fish family Priacanthidae. Bull Mar Sci 43:117–203.

Steen JB. 1970. The swimbladder as a hydrostatic organ. In: Hoar WS, Randall DJ, editors. Fish Physiology, Vol. 4. New York: Academic Press. pp 413–443.

Summers AP, Ketcham RA, Rowe T. 2004. Structure and function of the horn shark (*Heterodontus francisci*) cranium through ontogeny: Development of a hard prey specialist. J Morphol 260:1–12.

Tavolga WN. 1974. Sensory parameters in communication among coral reef fishes. Mount Sinai J Med 41:324–340.

Tricas TC. 1986. Life history, foraging ecology, and territorial behavior of the Hawaiian butterflyfish, *Chaetodon multicinctus*. PhD Dissertation, University of Hawaii, Honolulu. 248 p.

Tricas TC, Boyle KS. 2005. The evolution of pairing behavior, sound production and hearing in chaetodontid butterflyfishes: Evidence from behavior and physiology. Brain Behav Evol 66:143.

Tricas TC, Boyle KS, Dale J. 2004. Sound production and social pairing in laterophysic butterflyfishes. Integr Comp Biol 44:653.

Wartzok D, Ketten DR. 1999. Marine mammal sensory systems. In: Reynolds JE, Rommel SA, editors. Biology of Marine Mammals. Washington: Smithsonian Institution Press. pp 117–175.

Webb JF. 1998. Laterophysic connection: A unique link between the swimbladder and the lateral-line system in *Chaetodon* (Perciformes: Chaetodontidae). Copeia 1998:1032–1036.

Webb JF, Blum SD. 1990. A swimbladder-lateral-line connection in the butterflyfish genus *Chaetodon* (Perciformes: Chaetodontidae). Am Zool 30:90A.

Webb JF, Smith WL. 2000. The laterophysic connection in chaetodontid butterflyfish: Morphological variation and speculations on sensory function. Philos Trans R Soc Lond B Biol Sci 355:1125–1129.

Webb JF, Smith WL, Herman JL, Woods CF, Ketten DL. 2005. The laterophysic connection: Peripheral specialization for reception of acoustic stimuli in chaetodontid butterflyfishes? Brain Behav Evol 66:137–144.

Whitehead PJP, Blaxter JHS. 1989. Swimbladder form in clupeoid fishes. Zool J Linn Soc 97:299–372.

Witmer LM. 2001. Nostril position in dinosaurs and other vertebrates and its significance for nasal function. Science 293: 850–853.

Witmer LM, Chatterjee S, Franzosa J, Rowe T. 2003. Neuroanatomy of flying reptiles and implications for flight, posture and behaviour. Nature 425:950–953.

Yan HY, Curtsinger WS. 2000. The otic gasbladder as an ancillary auditory structure in a mormyrid fish. J Comp Physiol A 186:595–602.