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BRIEF COMMUNICATION

Three scleral ossicles in the West African Denticle herring *Denticeps clupeoides* (Clupeiformes: Denticipitidae)

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Abstract

The eyes of teleostean fishes typically exhibit two ossifications, the anterior and posterior sclerotics, both associated with the scleral cartilage. The West African Denticle herring *Denticeps clupeoides* has three scleral ossifications, including the typical two associated with the scleral cartilage (anterior and posterior sclerotic) and a third ossification (Di Dario's ossicle), spatially separated from the scleral cartilage and located within the anteromedial wall of the sclera. The medial rectus muscle inserts on the medial surface of Di Dario's ossicle, suggesting that this third sclerotic may play a role in forward rotation of the eye in this surface feeding fish.

KEYWORDS
computer tomography scanning, ocular muscles, Teleostei

Ossifications of the sclera of the vertebrate eye have been reported among extant vertebrates in teleosteans, coelacanths, reptiles and birds (Edinger, 1929; Walls, 1942). Their number varies from a maximum of 2 in extant teleosts (Franz-Odendaal, 2008, 2020; Langhans, 1865; Nakamura & Yamaguchi, 1991) up to 40 in some diplodactyline lizards (Underwood, 1970). The scleral ossicles of teleosts and those found in tetrapods are probably not homologous (Franz-Odendaal, 2020; Franz-Odendaal & Hall, 2006). Franz-Odendaal (2008) found that of the 547 species of teleost she studied, 271 had two ossicles, an anterior and posterior sclerotic, 11 had just one ossicle (either anterior or posterior), and 265 had no ossicle at all. Although the maximum number of sclerotics reported for extant teleosts is two, some stem-group teleosts had up to four sclerotics, as did stem-group actinopterygians (Patterson, 1975; Patterson & Rosen, 1977). This study reports the unique occurrence of a third scleral ossicle in the African freshwater-inhabiting clupeiform *Denticeps clupeoides* Clausen, 1959 (Figure 1a), sister group to all other clupeomorphs (Greenwood, 1968). This third sclerotic, which the authors term Di Dario's ossicle, adds another autapomorphy to the character set of *Denticeps*, which is also unique among clupeomorphs in having extraoral teeth covering large parts of its head skeleton (Clausen, 1959; Greenwood, 1968; Sire *et al*., 1998).

The care and use of experimental animals complied with US animal welfare laws, guidelines and policies as approved by the Texas A&M University Institutional Animal Care and Use Committee (TAMU IACUC protocol #2020–0033). This study is based on museum vouchered material of *D. clupeoides* housed in the Biodiversity Research and Teaching Collections at Texas A&M University, College Station (TCWC) and the Senckenberg Naturhistorische Sammlungen Dresden (MTD). For skeletal investigation, seven individuals of *D. clupeoides* (18.7–34.0 mm SL) and those representing select species of clupeiforms and elopiforms (see comparative material) were cleared and double stained (c&s) following the protocol of Taylor & Van Dyke (1985). Observations and photographs of c&s specimens or their parts were generally made using a ZEISS SteReo Discovery V20

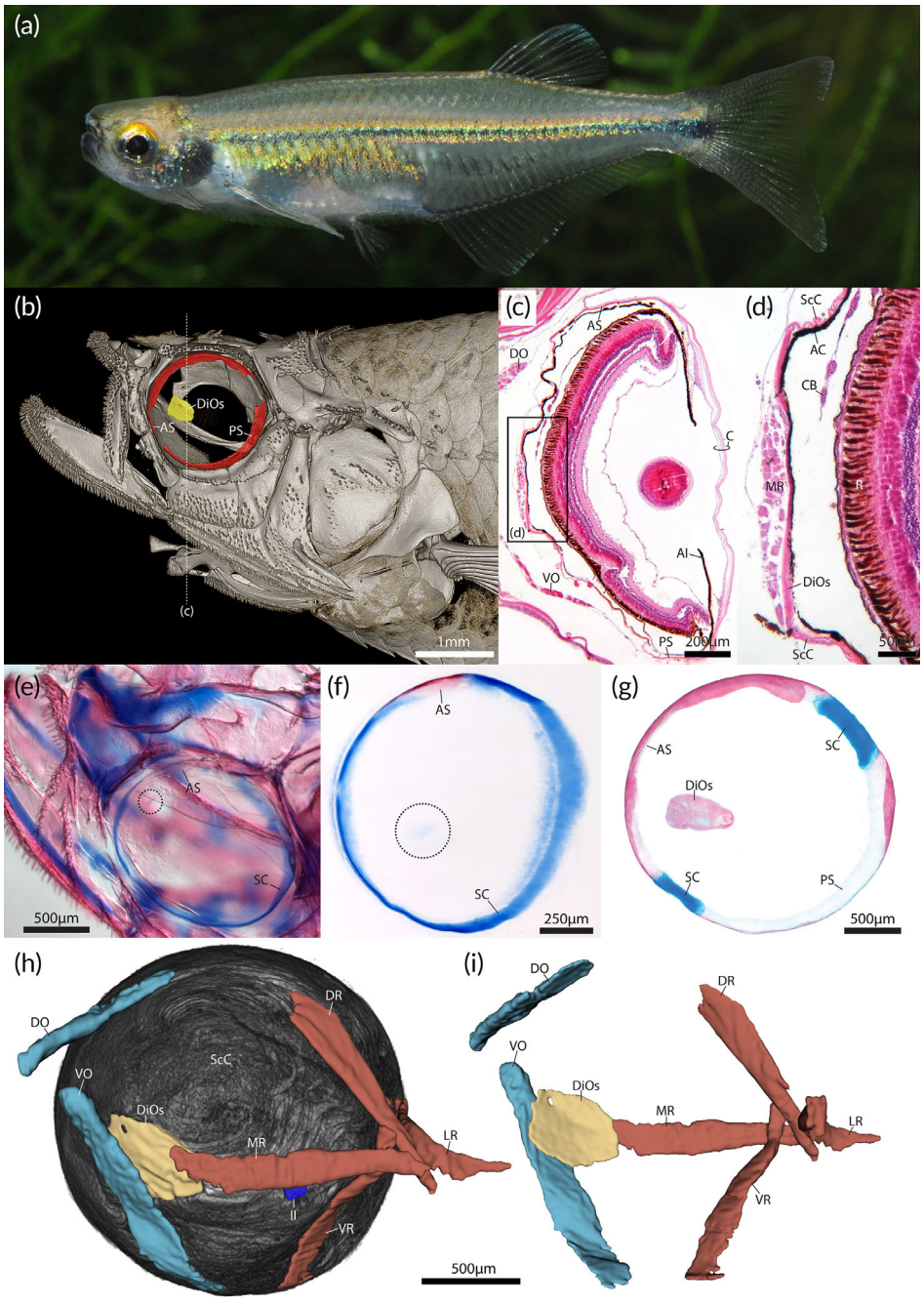

FIGURE 1 *Denticeps clupeoides*. (a) Aquarium specimen (not preserved, not measured); (b) TCWC 18339.07, 35.3 mm SL, computer tomographic (CT) scan of head and anterior part of body, left side in lateral view; Di Dario's ossicle in yellow, anterior and posterior sclerotic bones highlighted in red; (c) TCWC 18339.19, 27.0 mm SL, sagittal section through eye, location of section corresponds approximately to white dashed vertical line in (b); (d) Higher magnification view of area surrounded by black box in (c); (e) MTD uncat., 18.7 mm SL, cleared and double stained (c&s) specimen, scleral cartilages and bone *in situ*, left side in oblique dorsolateral view; (f) same specimen as in (e), dissected scleral cartilages and associated bone, left side in lateral view; (g) TCWC 20322.02, 33.0 mm SL, c&s specimen, dissected scleral cartilage and associated bones, left side in lateral view; (h) *D. clupeoides*, TCWC 18339.07, 35.3 mm SL, 3D reconstruction of eye and associated muscles, left side in medial view (image reversed); scleral coat grey, Di Dario's ossicle yellow, obliquus muscles light blue, rectus muscles red, optic nerve dark blue; (i) same reconstruction as in (h) with scleral coat and optic nerve removed, lateral view. AS, anterior sclerotic; AC, argentea of chorioid; AI, argentea of iris; C, cornea; CB, chorioid bodies; DO, dorsal obliquus; DiOs, Di Dario's ossicle; DR, dorsal rectus; II, optic nerve; LR, lateral rectus; MR, medial rectus; PS, posterior sclerotic; R, retina; SC, scleral cartilage; ScC, scleral coat; VO, ventral obliquus; VR, ventral rectus

stereomicroscope equipped with a Zeiss Axiocam MRc5 digital camera. Images were obtained using the stacking function in the Zeiss Axiovision software and processed using a combination of Adobe Photoshop 2020 and Illustrator 2020. Computed tomography (CT) scans of a single individual of *D. clupeoides* (TCWC 18339.07, 35.3 mm SL), prior to and after staining with a solution of phosphotungstic acid, were obtained at the Karel F. Liem BioImaging Center (Friday Harbour Laboratories, University of Washington) using a Bruker SkyScan 1173 scanner with a 1 mm aluminium filter. Scans were run at 65 kV and 123 µA on a 2048 × 2048 pixel CCD at a resolution of 7.1–8.1 µm. The resulting CT data sets were visualised using 3D Slicer v.4.10.2 (https://www.slicer.org/) and have been deposited on MorphoSource (https://www.morphosource.org/). Additional CT scan data sets of select clupeiform fishes (see comparative material) were obtained from MorphoSource. The head of a single individual of *D. clupeoides* (TCWC 18339.19, 27.0 mm SL) was dissected, decalcified and prepared for embedding in paraffin following the protocol of Pinion *et al*. (2021). The paraffin-embedded sample was sectioned serially at 9 µm, placed onto albumenized glass slides and stained with haematoxylin and eosin (H&E) for general histological examination following Kiernan (1990).

As is common among teleosts, *Denticeps* has an anterior and a posterior scleral ossicle or sclerotic, which are connected by short strips of scleral cartilage (Figure 1b,c,e). The anterior sclerotic serves

as point of insertion for the dorsal obliquus and the dorsal rectus eye muscles, whereas the ventral obliquus and the ventral rectus, as well as the lateral rectus muscle insert on the posterior sclerotic. On its anteromedial side, the sclera of the eyeball has a third sclerotic bone (Figure 1b–e), which is named here Di Dario's ossicle, honouring Fabio Di Dario, for his work on clupeiforms, especially his contributions to the anatomy of the genus *Denticeps* (de Pinna & Di Dario, 2010; Di Dario, 2004; Di Dario & de Pinna, 2006). Di Dario's ossicle is completely separated from and has no connection to either the anterior and posterior sclerotic or the scleral cartilage. It serves as the point of insertion for the medial rectus muscle (Figure 1c,d,h,i). The smallest specimen of *Denticeps* examined (MTD uncat., 18.7 mm SL) has a small cartilage in the position of Di Dario's ossicle (Figure 1e,f), and this is identified as the cartilage precursor of this third sclerotic. Intermediate stages are not available, and the size at which Di Dario's ossicle forms in *Denticeps* is not known.

All species of clupeiforms (excluding *Denticeps*) examined as part of this study (see Material Examined) have two scleral ossifications (an anterior and posterior sclerotic; Figure 2), which, as in *Denticeps*, are separated by short strips of scleral cartilage. There is no sign of an additional medial sclerotic cartilage or bone (Figure 2b). In a survey of sclerotic bones across 547 species of teleosts, Franz-Odendaal (2008) reported "zero or two" scleral ossicles in the eyes of 15 species of clupeiform fishes (not listed) representing two families (Clupeidae and Engraulidae). Similarly, in their survey of sclerotic bones in 283 species representing 172 families of actinopterygian fishes, Mok and Liu (2012) reported two scleral ossicles in each of the 7 species of clupeiforms they examined, representing 3 families (Chirocentridae, Engraulidae and Pristigasteridae).

Though the maximum number of sclerotic ossifications reported for teleosts is typically two, Mok and Liu (2012) report up to four in some members of the Elopomorpha that they examined [four in *Elops machnata* (Forsskål, 1775) and three in *Megalops cyprinoides* (Broussonet, 1782) and *Albula glossodonta* (Forsskål 1775)]. Based on information in Mok and Liu (2012): Figure 1), these additional third and fourth scleral ossifications appear to be located on the posteriormost point of the sclera, adjacent to the centre of the posterior sclerotic, or the anteriormost point of the sclera, adjacent to the centre of the anterior sclerotic. Only two scleral ossicles (anterior and posterior sclerotic) were observed in the specimens of *Elops saurus* Linnaeus, 1766 included in this study, and further investigation will be needed to clarify the number and arrangement of sclerotic bones in elopomorphs. Based solely on position, the putative third sclerotic bone reported for some elopomorphs by Mok and Liu (2012) is unlikely to be homologous with Di Dario's ossicle in *Denticeps*. Some stem-group teleosts (including pholidophorids and ichthyodectids) had four sclerotic ossicles, arranged in a ring around the orbit, and an additional medial basal sclerotic bone, which rimmed the medial face of the eye and bore a central canal for passage of the optic nerve and eye vessels (Patterson, 1975; Patterson & Rosen, 1977). Though both are located on the medial face of the sclera, it is unlikely that Di Dario's ossicle in *Denticeps* and the basal sclerotic bone of some stem-groups teleosts are homologous. The two elements differ in their topological relationships with the optic nerve. It surrounds the optic nerve in the latter but is spatially removed from it in the former. Di Dario's ossicle is interpreted as a unique feature (*i.e.*, autapomorphy) of *Denticeps*.

Unlike other clupeiforms, *Denticeps* inhabits swift flowing areas of small tannin-stained streams in West Africa (Clausen, 1959) and feeds on small invertebrates in the upper part of the water column or directly from the surface (Britz, 2012). The insertion of the medial rectus muscle on the medial face of Di Dario's ossicle (Figure 1h,i) suggests that the bone may play a prominent role in eye rotation. Although so far only speculative, contraction of the medial rectus, resulting in the inward rotation of the anterior part of the eye and the outward rotation of the posterior part, could serve to increase the anterior field of view (achieving or enhancing binocular vision?) and thus the chance of capturing edible items as they float downstream in swift current. It is noted that other surface dwelling or surface feeding fishes, including the iconic archerfishes (*Toxotes* Cloquet, 1816), lack sclerotic bones in the medial part of the orbit.

MATERIAL EXAMINED

Clupeidae: ***Alosa sapidissima*:** TCWC 5264.01, 3 (c&s), 62.4–63.7 mm SL; ***Brevoortia patronus*:** TCWC 19718.02, 9 (c&s), 16.4–51.0 mm SL; ***Dorosoma cepedianum*:** TCWC 4243.17, 1 (c&s), 148 mm SL. -

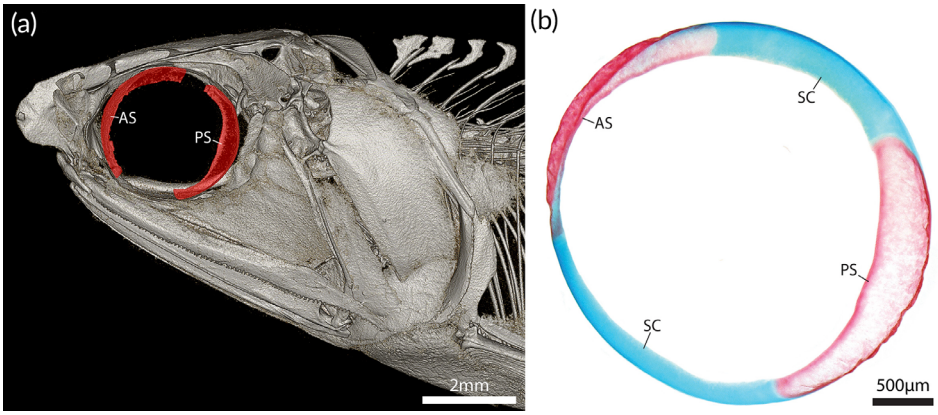

FIGURE 2 (a) *Anchoa hepsetus*, JFBM 47609, 33.4 mm SL, CT scan of head and anterior part of body, left side in lateral view; anterior and posterior sclerotic bones highlighted in red; (b) *A. mitchelli*, TCWC uncat., 32.0 mm SL, C&S, sclerotic cartilage and associated bones, left side in lateral view. AS, anterior sclerotic; PS, posterior sclerotic; SC, scleral cartilage

Engraulidae: ***Anchoa hepsetus*****:** JFBM 47609, 1 (CT scanned: https://www.morphosource.org/concern/media/000030714), 33.4 mm SL. ***Anchoa mitchilli*****:** TCWC 16799.02, 3 (c&s), 46.0–52.0 mm SL; TCWC uncat., 23 (c&s), 17.0–45.4 mm SL. **- Denticipitidae:** ***Denticeps clupeoides*****:** MTD uncat., 4 (c&s), 18.7–30.0 mm SL; TCWC 20322.02, 2 (c&s), 33.0–34.0 mm SL; TCWC 18339.18, 1 (c&s), 28.0 mm SL; TCWC 18339.07, 1 [CT scanned: https://www.morphosource.org/concern/media/000406190 (whole body); https://www.morphosource.org/concern/media/000406203 (head)], 35.3 mm SL; TCWC 18339.19, 171 (1 sectioned serially), 25.0–42.0 mm SL; – **Elopidae:** ***Elops saurus*****:** TCWC 16790.01, 2 (c&s), 78.9–112.0 mm SL; TCWC 16807.17, 20 (c&s), 17.2–31.5 mm SL.

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Ideas: Kole M. Kubicek, Ralf Britz and Kevin W. Conway. Material: Luke M. Bower, Ralf Britz and Kevin W. Conway. Histology: Kevin W. Conway. Clearing and staining: Kole M. Kubicek, Ralf Britz and Kevin W. Conway. CT scanning: Kole M. Kubicek and Amanda K. Pinion. Manuscript preparation: Kole M. Kubicek, Ralf Britz, Amanda K. Pinion, Luke M. Bower and Kevin W. Conway.

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