

Abstract—Among previously unidentified chaetodontid larvae, one form is distinguished by a long, straight, or curved horn above each eye. Because of its remarkable morphology, this chaetodontid larva has been mentioned in earlier publications, but assignment to a species has been difficult. We cleared and double stained 2 specimens of this long-horned larva to document the anatomy and its possible bearing on its identification. Using anatomical data, such as 5 instead of 6 infra-orbitals and the reduction in size of the endopterygoid and the ectopterygoid, we identified our cleared and double-stained specimens as members of the *Chaetodon* subgenus *Citharoedus*. Additionally, we obtained barcode sequences from ethanol-fixed specimens that identified both specimens as mailed butterflyfish (*Chaetodon reticulatus*), one of the 3 species of the *Citharoedus* subgenus. An intriguing aspect of these larvae remains the enigma of how the horns are apparently lost, and we continue to pursue this avenue of research.

Morphological and molecular identification of rare longhorn butterflyfish larvae (Chaetodontidae)

Nalani K. Schnell (contact author)¹

Ai Nonaka²

Elodie Vourey³

G. David Johnson²

Email address for contact author: nalani.schnell@mnhn.fr

¹ Institut Systématique Evolution Biodiversité (ISYEB)
Muséum National d'Histoire Naturelle
CNRS, Sorbonne Université, EPHE
Station Marine de Concarneau
Place de la Croix
29900 Concarneau, France

² Department of Vertebrate Zoology
National Museum of Natural History
Smithsonian Institution
10th Street and Constitution Avenue
Washington, DC 20560

³ Pacific Community
BP D5
98848 Noumean Cedex, New Caledonia

*Nobody really metamorphoses. Cinderella is always Cinderella, just in a nicer dress. The Ugly Duckling was always a swan, just a smaller version. And I bet the tadpole and the caterpillar still feel the same, even when they're jumping and flying, swimming and floating.
Just like I am now.*

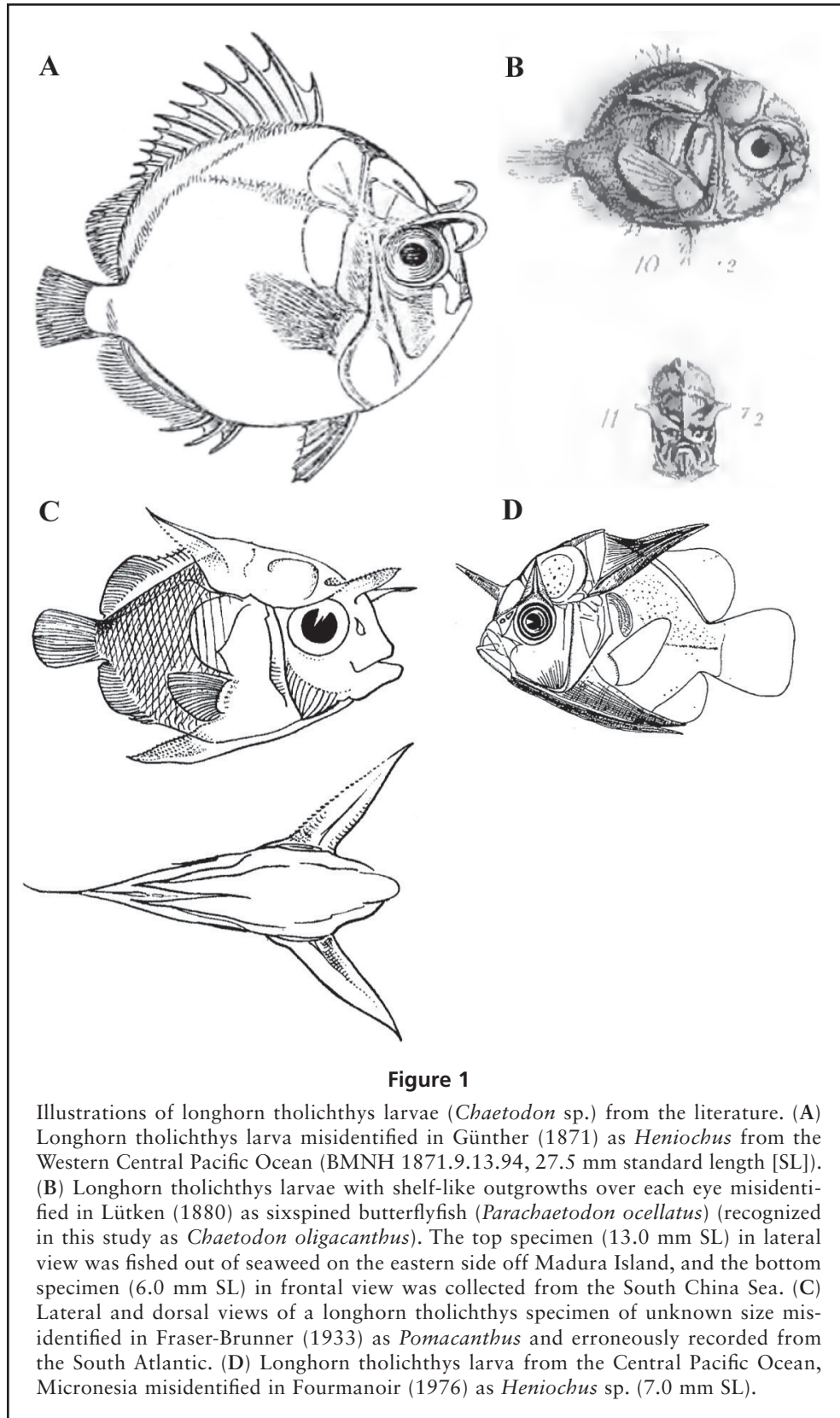
— Holly Smale, British writer

Introduction

Fishes of the family Chaetodontidae are small, colorful coral-reef fishes distributed worldwide in tropical and temperate seas. Their eggs and larvae are relatively rare in tropical plankton samples, and therefore, little is known about their pelagic early life history (Leis, 1989). Most chaetodontid species pass through a specialized pelagic tholichthys stage (Burgess, 1978), most commonly characterized by unique rugose head armor with the posttemporal and supracleithrum expanded posteriorly as large laminar plates and the preopercle expanded anteriorly and extended posteriorly into a broad spine. However, trenchant morphological differences occur among the different chaetodontid genera and subgenera (Blum, 1988; Leis, 1989; Micklich et al., 2009).

The highly specialized tholichthys stage of the larva identified in this paper has sparked investigation since its first discovery. Günther (1871:319–

320) stated that he received a larva from the museum Godeffroy in Hamburg with plates on the shoulder and preopercle like those of the young of *Chaetodon* (Fig. 1A); “but the fish is distinguished besides by a remarkably long and curved horn above each orbit... Now, although it is possible that the horn above the orbit is also an excrescence lost in the more mature state of the individual, it yet reminds us of those species of *Heniochus* which are provided with more or less developed orbital processes.” However, he noted that “without further evidence, it would be hazardous to state whether this fish is a young *Chaetodon* or *Heniochus*.” Nevertheless, it was cataloged as *Heniochus* in the Natural History Museum, London. Lütken (1880:609) had 3 tholichthys larvae (Fig. 1B, lateral and frontal views) that he assigned to the sixspined butterflyfish (*Parachaetodon ocellatus*) (= *Chaetodon oligacanthus*), “et qui se distingue entre autres par cette particularité, que le bord



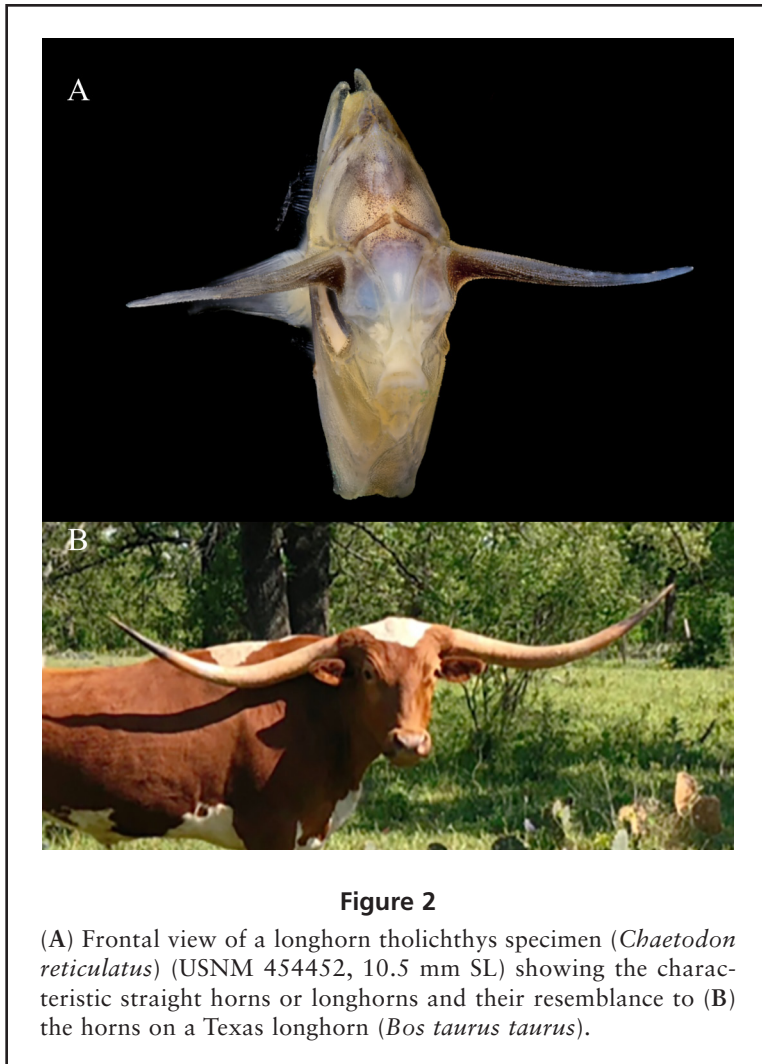


Figure 2

(A) Frontal view of a longhorn tholichthys specimen (*Chaetodon reticulatus*) (USNM 454452, 10.5 mm SL) showing the characteristic straight horns or longhorns and their resemblance to (B) the horns on a Texas longhorn (*Bos taurus taurus*).

supraorbital se termine en une épine dirigée obliquement sur le côté et en arrière” (and which is distinguished among other things by this particularity, that the supraorbital border ends in a spine directed obliquely to the side and backward). Fraser-Brunner (1933) figured a smaller tholichthys stage with straight horns (Fig. 1C, lateral and dorsal views) and, as later pointed out by Burgess (1974), erroneously identified as *Pomacanthus* and erroneously recorded from the South Atlantic. Fourmanoir (1976) and Bourret¹ had several tholichthys larvae with straight horns from different cruises in the Central and South Pacific, and both authors identified them as *Heniochus* spp. (Fig. 1D). Burgess (1974, 1978) found several specimens of tholichthys with long,

paired, often curved horns in collections and identified them all as either maypole butterflyfish (*C. meyeri*) or ornated butterflyfish (*C. ornatisimus*), both of which were placed together with mailed butterflyfish (*C. reticulatus*) in the *Chaetodon* subgenus *Citharoedus* (Blum, 1988; Fessler and Westneat, 2007). He further noted that larvae of *C. reticulatus* were not known. It is noteworthy that specimens of the subgenus *Citharoedus* show no “horns” as adults, indicating that they are eventually lost during ontogeny. In contrast, adult specimens of the genus *Heniochus* are diagnosed by a supraorbital projection that can range from short spikes to more complicated structures “resembling antlers” (Burgess, 1978:218). Burgess (1978) noted further that these “horns” are not yet present in juveniles of the genus *Heniochus* and only develop when the specimen has already reached a considerable size. This statement is supported by reared pennant butterflyfish (*Heniochus diphreutes*) from 2 different labs (Wittenrich and Cassiano: Rising Tide/Tropical Aquaculture Lab at the University of Florida, available from <https://www.risingtideconservation.org/schooling-bannerfish-so-close/>; and Frank Baensch: The Hawaii Larval Fish Project, available from <https://www.frankbaensch.com/marine-aquarium-fish-culture/my-research/pennant-butterflyfish-culture/>). Both labs show that there are no horns developed over the eye in the early stages of this species, whereas, it is present as a small stout spine in adult specimens (Burgess, 1978).

Because of its superficial resemblance to Texas longhorn cattle (*Bos taurus taurus*), we here-with assign the term *longhorn* to these larvae (Fig. 2). For morphological identification, we had several specimens available from different museum collections, and we compared their meristic features to those of the currently recognized 136 chaetodontid species (Fricke et al., 2022). We found a meristic and regional overlap of our larvae with only 2 species, *C. reticulatus* and *C. ornatisimus* (see Materials and methods). We also took tissue samples for DNA barcoding from either ethanol- or formalin-fixed specimens in order to assign them to a species within the subgenus *Citharoedus*.

Materials and methods

Institutional codes follow Sabaj (2020). Specimen lengths are given as standard length (SL), except for preflexion larvae, for which notochord length (NL) is given. For this study, we had 11 formalin-fixed specimens (MNHN 2014 2945: 8.4–9.9 mm SL) of which we cleared and

¹Bourret, P., D. Binet, C. Hoffschir, J. Rivaton, and H. Velayoudon. 1979. Evaluation de “l’effet d’Il” d’un Atoll: Plancton et micronecton au large de Mururoa (Tuamotus), 124 p. Centre Off. Rech. Sci. Tech. Outre-Mer Nouméa, Nouméa, Nouvelle-Calédonie. [Available from <https://www.documentation.ird.fr/hor/fdi:010025256>.]

double stained (C&S) 2 specimens (Fig. 3C–E). One recently collected longhorn larva (Fig. 3F–H) was fixed in 95% ethanol for further DNA analyses (MNHN-LC 1457: 12.1 mm SL). Additional formalin-fixed specimens (USNM 432381: 7.9 mm, 8.2 mm, 8.4 mm, 8.6 mm, 12.3 mm, 22.5 mm SL [C&S]; USNM 432382: 13.0 mm, 23.0 mm SL) have been found in the NMNH larval fish collection (Suppl. Fig. 1) and the NSMT collection (NSMT PL-389: 7.0 mm SL). Four specimens were found in the collection housed at the Pacific Marine Specimen Bank at the Pacific Community, New Caledonia, preserved from stomach contents of yellowfin tunas (*Thunnus albacares*) and a wahoo (*Acanthocybium solandri*) (MNHN-LC 1458: 20.5 mm SL; MNHN-LC 1459: 20.2 mm SL; MNHN-LC 1460: 25.4 mm SL; MNHN-LC 1461: 19.9 mm SL). Three specimens were provided by the NOAA Pacific Islands Fisheries Science Center in Hawaii. One of the specimens was fixed in 95% ethanol (USNM 454452: 10.5 mm SL; Fig. 3, I and J), and one was the smallest specimen known (USNM 454450: 3.2 mm NL; Fig. 3, A and B). A summary of the material examined with meristic counts and measurements can be found in Supplementary Table 1.

Additional comparative material:

Chaetodon reticulatus, AMNH 88415, 49.0 mm SL, C&S (South Pacific, Papeete, Tahiti, French Polynesia); AMNH 88416, 37.0 mm SL, C&S.

Chaetodon ornatissimus AMNH 88418, 52.0 mm SL, C&S (South Pacific, Line Islands, Sand Island, Palmyra, Micronesia).

Meristic data and geographic distribution of the 3 species belonging to the subgenus *Citharoedus* are outlined below (Burgess, 1978; Randall, 2007). Information for the following features is included in the descriptions: anal fin (A), dorsal fin (D), and pectoral fin (P1). Fin-spine counts are given in Roman numerals, and fin-ray counts are given in Arabic numerals.

Chaetodon reticulatus. D: XII–XIII, 26–29; A: III, 20–22; P1: 15–18. Tropical Pacific Ocean. Polynesia (including the Hawaiian Islands), Micronesia, and Melanesia to northeastern Australia, the Eastern Indies; Philippine Islands north of the Ryukyu Islands.

Chaetodon ornatissimus. D: XII–XIII, 24–28; A: III, 20–23; P1: 15–17. Tropical Pacific Ocean, extending a short distance into the Indian Ocean, Polynesia (including the Hawaiian Islands), Micronesia, and Melanesia to the East Indies; south to the Great Barrier Reef, north through the Philippine Islands to China and the Ryukyu Islands; west to Christmas Island (Indian Ocean off west coast of Java) and Cocos-Keeling Islands.

Chaetodon meyeri. D: XII, 23–25; A: III, 18–20; P1: 16. Tropical Pacific and Indian Ocean. Micronesia, Polynesia (excluding the Hawaiian Islands), and Melanesia, to the East Indies and Philippine Islands. Indian Ocean including the Mentawai islands, Cocos-Keeling Island, Laccadive Islands, Maldives, Sri Lanka, Chagos Archipelago, Aldabra, Mauritius, Madagascar, Seychelles, and Comoro Islands to the coast of East Africa as far south as Durban.

For the anatomical descriptions, specimens were cleared and double stained according to the protocols of Dingerkus and Uhler (1977), and Schnell et al. (2016). The specimens were photographed and dissected using either a Zeiss SteREO Discovery V12 or V20 stereomicroscope (Zeiss Microscopy, Jena, Germany). Photographs were taken with a Zeiss AxioCam (Zeiss Microscopy) attached to the Zeiss SteREO Discovery V12/V20 and processed with the Zeiss Axiovision/Zen software (Zeiss Microscopy). Some of the images are stacked, resulting in an increased depth of field.

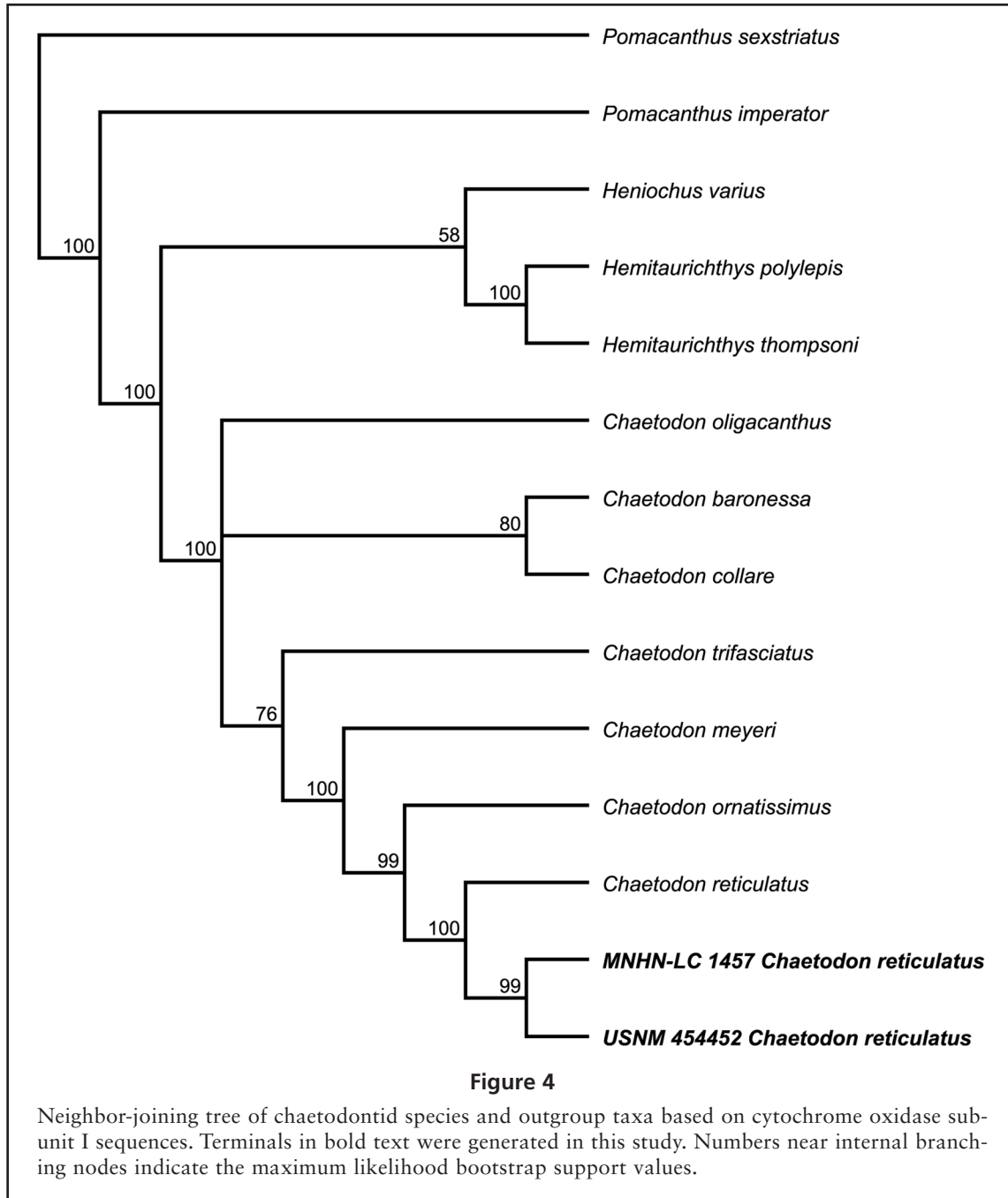
For genomic DNA extraction, a tissue sample (right eyeball) was removed and initially digested and extracted by following the protocol of Nonaka et al. (2021). Where possible, we removed only the right eyeball, so as to keep the left side intact for further morphological analyses and as voucher specimens. For each individual sample, the targeted DNA barcode marker—the cytochrome oxidase subunit I fragment—was amplified via polymerase chain reaction using the primers FISH-BCL (5'-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5'-TAAACTTCAGGGTGACCAAAAAATCA) (Baldwin et al., 2009). The purified cycle sequencing products were sequenced using an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, MA).

Four specimens from stomach contents, “Günther’s” specimen (BMNH 1871.9.13.94: 27.5 mm SL), and a USNM 432382 specimen (12.5 mm SL) with unknown fixation history were barcoded following the same method. One formalin-fixed specimen (MNHN 2014 2945) was barcoded using a mixed RNA bait set (Agne et al., 2022). The neighbor-joining tree or phenogram (Fig. 4) was generated using Geneious Prime 2019, vers. 2019.2.1 (Biomatters Inc., Auckland, New Zealand). Raw chromatograms were edited using Geneious Prime 2019, and sequence trace files were exported into Geneious Prime 2019. Using the Geneious program, both low-quality ends were trimmed from the raw sequences. After trimming, forward and reverse sequences for each specimen were assembled. Each assembled pair was examined and edited manually, and each sequence was checked for stop codons. Finally, the consensus sequence



Figure 3

Lateral and dorsal views of different longhorn tholichthys larvae (*Chaetodon* spp.) from various collections. (A) Lateral and (B) dorsal views of *Chaetodon* sp. (USNM 454450, 3.6 mm notochord length). (C) Lateral, (D) dorsal, and (E) frontal views of *Chaetodon* sp. (MNHN 2014 2945, 9.9 mm standard length [SL]). (F) Lateral and (G) ventral views of a mailed butterflyfish (*Chaetodon reticulatus*) (MNHN-LC 1457, 12.1 mm SL). (H) Photo of a fresh specimen (MNHN-LC 1457). (I) Lateral and (J) dorsal views of *Chaetodon reticulatus* (USNM 454452, 10.5 mm SL). The 5-mm scale bar applies to photos C–J.



(655 base pairs) from each contig was aligned and exported in a nexus format (sensu Swofford, 2003).

For species identification, we used the Barcode of Life Database (BOLD) Identification Engine (available from http://www.boldsystems.org/index.php/IDS_OpenIdEngine) to query barcode records within BOLD (Ratnasingham and Hebert, 2007). For molecular analysis, 10 chaetodontid spp. have been selected and downloaded from BOLD (available from https://www.boldsystems.org/index.php/TaxBrowser_Home) (Suppl. Table 2) because of their meristic (dorsal-, anal-, pectoral-fin ray counts)

and/or regional overlap with our longhorn tholichthys specimens. Baroness butterflyfish (*Chaetodon baronessa*), white collar butterflyfish (*C. collare*), lined butterflyfish (*C. trifasciatus*), scrawled butterflyfish (*C. meyeri*), pyramid butterflyfish (*Hemitaurchthys polylepis*), and businessman butterflyfish (*Hemitaurchthys thompsoni*) have overlapping dorsal-, anal-, and pectoral-fin ray counts with our specimens. *Chaetodon ornatissimus* and *C. reticulatus* have a meristic and regional overlap with our specimens. Horned bullfish (*Heniochus varius*) was included because the longhorn tholichthys specimens

(see Introduction) have often been misidentified as this species; it possesses horns in adult stages, but the dorsal-, anal-, and pectoral-fin ray counts as well as lateral line morphology differ from our specimens. We also included *C. oligacanthus* (= *Parachaetodon ocellatus*) because of its small shelf-like outgrowths of the frontals above each eye with a little bump at its tip. We follow Blum (1988) in using the species name *C. oligacanthus* rather than its synonym *Parachaetodon ocellatus*, as the species is clearly a member of the *Chaetodon* clade. There is some debate as to which subgenus the species belongs—either *Megaprotodon* or its own *Parachaetodon* (Smith et al., 2003; Littlewood et al., 2004). A total of 14 sequences, along with the 2 newly sequenced samples in this study, were combined and aligned with MAFFT, vers. 7 (Kato and Standley, 2013) within Geneious for phylogenetic analyses. The aligned matrix was 655 base pairs in length (approximately 99.5% complete) and analyzed using the Geneious Tree Builder function. Tree search was performed using neighbor-joining under the Jukes-Cantor distance model. The dataset was bootstrapped using 100 replicates. Analyses were rooted with 2 species of *Pomacanthus* used as outgroup comparison (see also Littlewood et al., 2004).

Results

The anatomical description is based on the following C&S specimens: MNHN 2014 2945, 8.4 mm SL (illustrated in Figs. 6–8), 9.9 mm SL; USNM 432381, 22.5 mm SL.

Skull roof and neurocranium

All exposed head bones (including the supracleithrum and the cleithrum) are rugose. The frontals are expanded and form a lateral horn over each eye. In our smallest specimen (3.2 mm NL), the frontals already show lateral flat outgrowths (Figs. 3B and 5A). In our

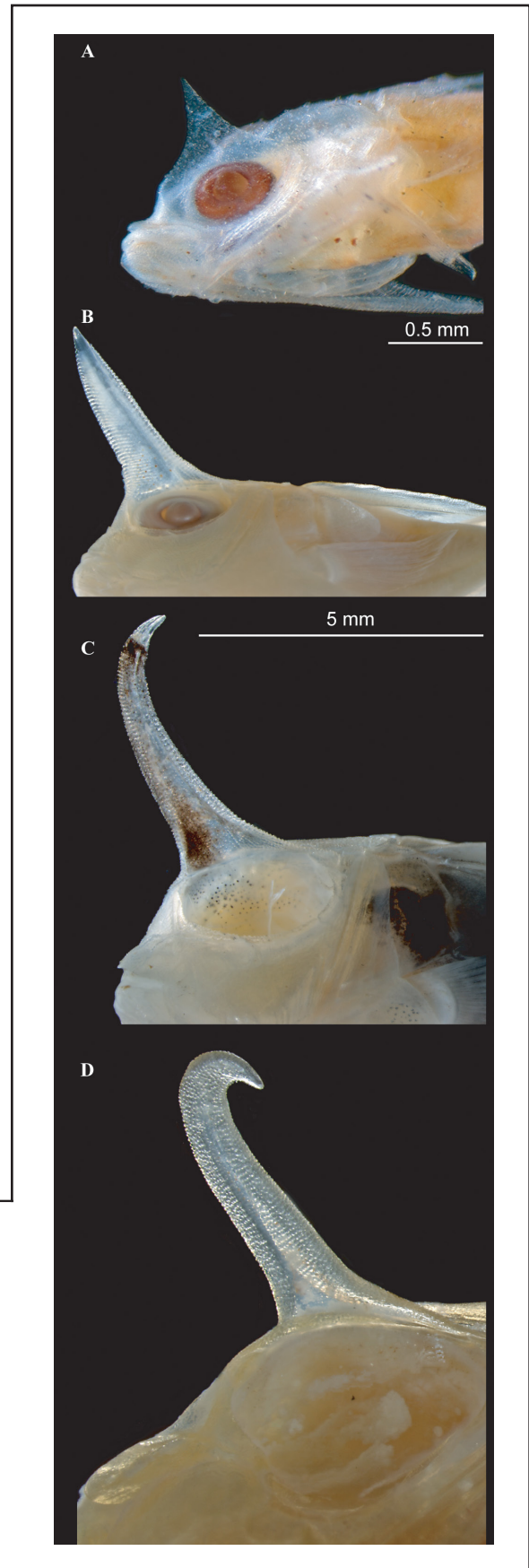


Figure 5

Ventral view of the horns of ethanol-preserved longhorn tholiichthys specimens (*Chaetodon* spp.) at different developmental stages and sizes of larvae. (A) Flat lateral outgrowth of the frontals of *Chaetodon* sp. (USNM 454450, 3.6 mm notochord length; end of preflexion, beginning of flexion stage). (B) Roof-shaped straight horn of *Chaetodon* sp. (MNHN 2014 2945, 9.9 mm standard length [SL], postflexion stage). (C) Curved horn of a mailed butterflyfish (*Chaetodon reticulatus*) (MNHN-LC 1457, 12.1 mm SL, postflexion stage). (D) Curved horn of *Chaetodon* sp. (MNHN-LC 1460, 25.4 mm SL, postflexion stage). The 5 mm scale bar applies to photos B–D.

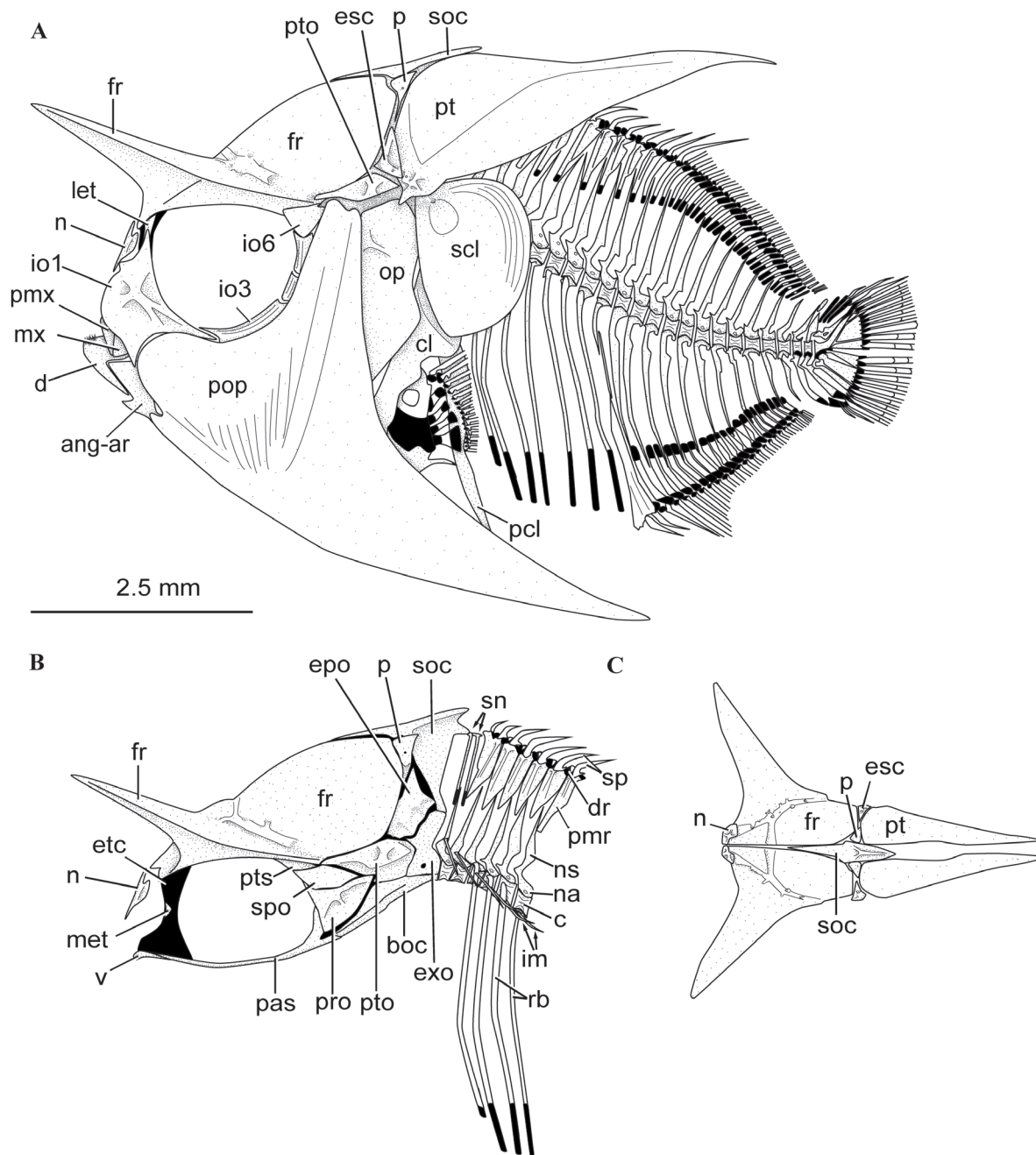


Figure 6

Illustrations of the skeleton in a cleared and double stained longhorn tholichthys specimen (*Chaetodon* sp.) (MNHN 2014 2945, 8.4 mm standard length). (A) Lateral view of the entire skeleton. (B) Lateral view of the posttemporal (pt), infraorbital (io) series, suspensorium, opercular series, and extrascapular (esc). The lateral ethmoid (let) was removed to expose the underlying bones of the neurocranium (axial skeleton posterior to the sixth vertebra is not shown). (C) Dorsal view. Locations of the following skeletal elements are shown: anguloarticular (ang-ar), basioccipital (boc), centrum (c), cleithrum (cl), dentary (d), distal radial (dr), epioccipital (epo), ethmoid cartilage (etc), exoccipital (exo), frontal (fr), intermuscular (im), mesethmoid (met), maxilla (mx), nasal (n), neural arch (na), neural spine (ns), opercle (op), parietal (p), parasphenoid (pas), postcleithrum (pcl), proximal-middle radial (pmr), premaxilla (pmx), preopercle (pop), pterotic (pto), pterosphenoid (pts), prootic (pro), rib (rb), supracleithrum (scl), supraneural (sn), supraoccipital (soc), fin spine (sp), sphenotic (spo), and vomer (v).

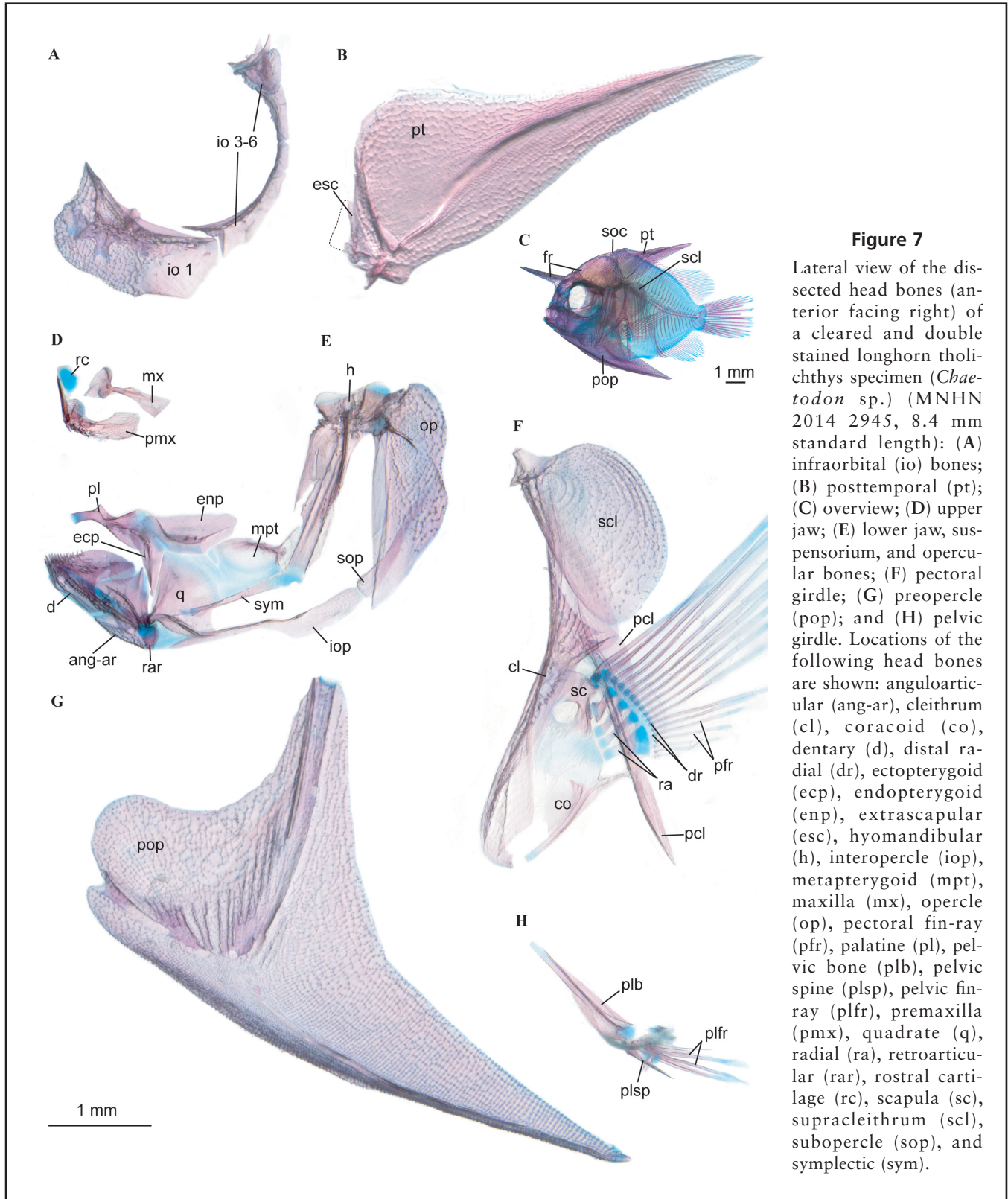


Figure 7

Lateral view of the dissected head bones (anterior facing right) of a cleared and double stained longhorn tholiichthys specimen (*Chaetodon* sp.) (MNHN 2014 2945, 8.4 mm standard length): (A) infraorbital (io) bones; (B) posttemporal (pt); (C) overview; (D) upper jaw; (E) lower jaw, suspensorium, and opercular bones; (F) pectoral girdle; (G) preopercle (pop); and (H) pelvic girdle. Locations of the following head bones are shown: anguloarticular (ang-ar), cleithrum (cl), coracoid (co), dentary (d), distal radial (dr), ectopterygoid (ecp), endopterygoid (enp), extrascapular (esc), hyomandibular (h), interopercle (iop), metapterygoid (mpt), maxilla (mx), opercle (op), pectoral fin-ray (pfr), palatine (pl), pelvic bone (plb), pelvic spine (plsp), pelvic fin-ray (plfr), premaxilla (pmx), quadrate (q), radial (ra), retroarticular (rar), rostral cartilage (rc), scapula (sc), supracleithrum (scl), subopercle (sop), and symplectic (sym).

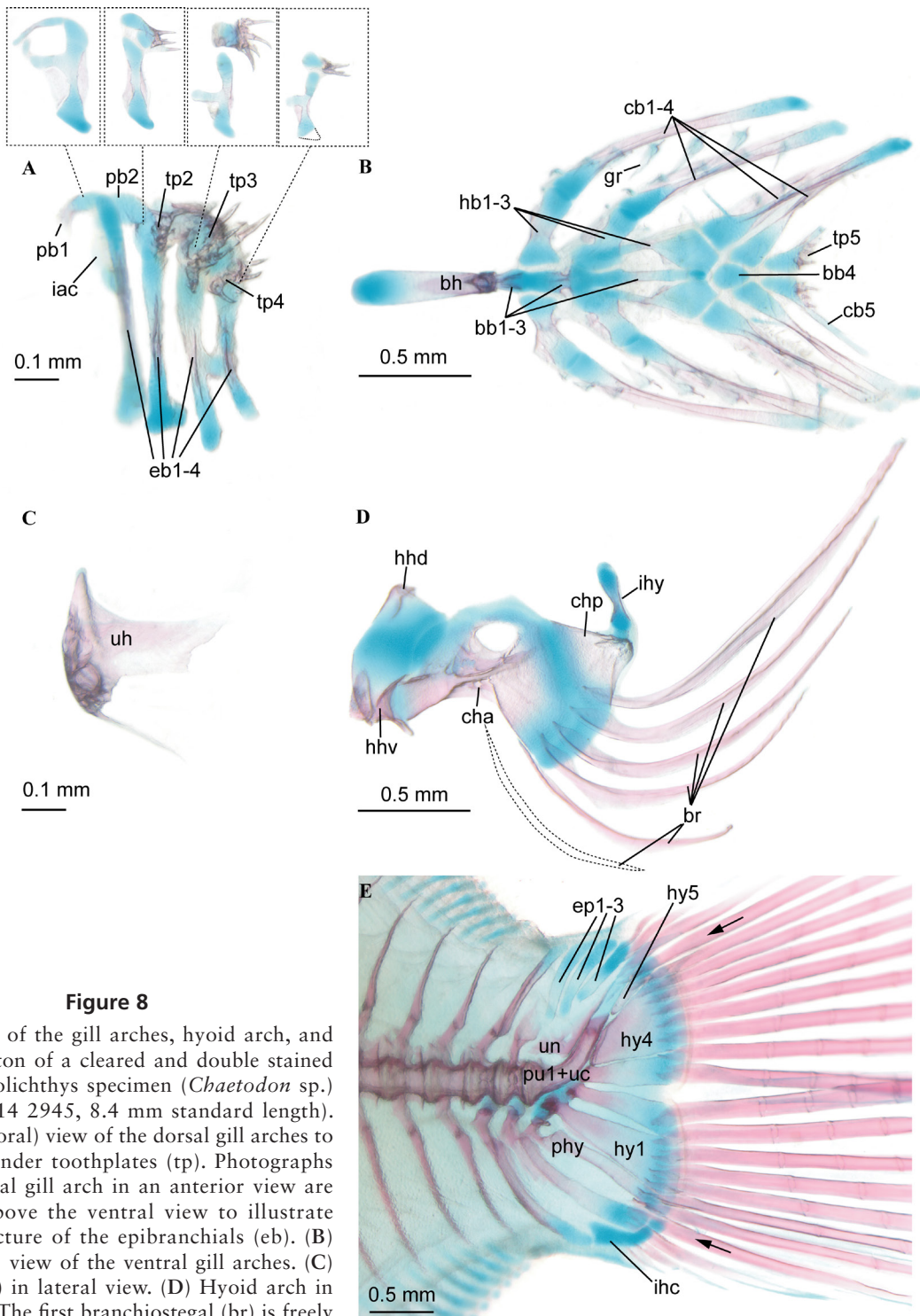


Figure 8

Photographs of the gill arches, hyoid arch, and caudal skeleton of a cleared and double stained longhorn tholichthys specimen (*Chaetodon* sp.) (MNHN 2014 2945, 8.4 mm standard length). (A) Ventral (oral) view of the dorsal gill arches to show the slender toothplates (tp). Photographs of each dorsal gill arch in an anterior view are displayed above the ventral view to illustrate the flat structure of the epibranchials (eb). (B) Dorsal (oral) view of the ventral gill arches. (C) Urohyal (uh) in lateral view. (D) Hyoid arch in lateral view. The first branchiostegal (br) is freely suspended in the opercular membrane and is indicated with dotted lines, as it was inadvertently removed during dissection. (E) Caudal skeleton. Arrows indicate the first and lowermost principal caudal-fin rays, anterior to which are the procurrent fin rays. Locations of the following bones are shown: basibranchial (bb), basihyal (bh), ceratobranchial (cb), anterior ceratohyal (cha), posterior ceratohyal (chp), epural (ep), gill raker (gr), hypobranchial (hb), dorsal hypohyal (hhd), ventral hypohyal (hhv), hypural (hy), interarcual cartilage (iac), interhemal spine cartilage (ihc), interhyal (ihy), pharyngobranchial (pb), parhypural (phy), preural centrum (pu), ural (terminal) centrum (uc), and uroneural (un).

8.4-mm specimen, the lateral outgrowths are more elongated (Figs. 3C–E and 5B). They point straight in an antero-lateral direction and are deeply concave ventrally (Figs. 5B and 6A, Suppl. Fig. 1). All later stages show posteriorly curved horns (Figs. 3, I and J, and 5C, Suppl. Fig. 1; 10.5–12.1 mm), and the frontals curl inward on their ventral side. The posterior curvature and the inward curling are even more pronounced in all our larger specimens (Fig. 5D, Suppl. Fig. 1; 25.4 mm). Eventually, the horns are lost or absorbed, and there is no trace of them in adults.

The rugose frontals cover most of the anterolateral part of the skull. Posterior to the frontal is the small triangular parietal (Fig. 6). The parietal is separated from the pterotic by the epioccipital, which contacts the posterior margin of the frontal. The epioccipital is covered laterally by a small extrascapular that lies between the frontal, posttemporal, and pterotic. The parietal dorsally contacts the supraoccipital. The supraoccipital forms a rugose dorsal crest and ventrally contacts the epioccipital and exoccipital. Most of the supraoccipital, epioccipital, and exoccipital are covered laterally by the expanded posttemporal. The exoccipital ventrally contacts the basioccipital and anteriorly the pterotic and epioccipital. Anterior to the pterotic are the pterosphenoid, sphenotic, and prootic. As is typical, the parasphenoid forms the ventral keel of the neurocranium extending between the vomer and the basioccipital. Between the frontals and the vomer and parasphenoid, the ethmoid cartilage bears 3 ossifications: paired lateral ethmoids (mainly covered by the rugose first infraorbital), and between those, an anteriorly convex mesethmoid. A rugose nasal bone lies anterior to the ethmoid cartilage.

Infraorbital bones

The infraorbital (io) series comprises 5 ossifications (io1, io3–6) (Figs. 6A and 7A). The second infraorbital is absent, a synapomorphy that unites the 3 species of *Citharoedus* (Blum, 1988; see Discussion). Infraorbitals 3–5 are partially covered laterally by the greatly expanded preopercle (Fig. 6A). The anterior surface of io1 and most of the triangular io6 are free and rugose. Infraorbital 1 is the largest bone of the infraorbital series, almost rectangular but with an anteriorly rounded border. Infraorbitals 3–5 are slender, elongate bones (of which io3 is the longest) with a median ridge up to which the preopercle reaches.

Jaws, hyopalatine arch, and opercular series

Jaws

The jaws are small and partly covered by the enlarged first infraorbital and preopercle (Fig. 6A). The premax-

illa bears numerous small teeth anteriorly and has an expanded postmaxillary process (Fig. 7D). The ascending process is long and slender and firmly connected to the well-developed rostral cartilage. A shorter but broader articular process lies just posterior to the ascending process. The maxilla broadens and flattens out posteriorly and has a straight posterior edge. The dentary bears numerous small teeth and is posteriorly deeply emarginated to accommodate the anterior upper part of the anguloarticular (Fig. 7E). The anguloarticular has an anterior emargination that accommodates the posteroventral part of the dentary. The anguloarticular and the dentary are rugose ventrally, where they are not covered by the expanded preopercle. The anguloarticular forms a robust articular surface at its posterior end, where the lower jaw articulates with the quadrate. The retroarticular is well ossified at the posterior end of Meckel's cartilage.

Hyopalatine arch

All bones of the hyopalatine arch are ossified. The ectopterygoid extends ventrally along the anterior margin of the quadrate (Fig. 7E). The hyomandibular has 2 cartilaginous heads dorsally that articulate with the neurocranium.

Opercular series

Posteriorly the hyomandibular articulates with the rugose opercle (Fig. 7E). The unexposed subopercle and interopercle are smooth, slender, elongate bones. In contrast, the preopercle, as in most chaetodontid larvae, is rugose, dramatically hypertrophied (Fig. 7G) and expanded anterodorsally almost to the margin of the orbit, and ventromedially under the head so that the left and right antimeres meet and broadly overlap midventrally. Posteriorly, it expands into a large, broad spine.

Branchial arches, hyoid, and urohyal

Dorsal gill arches

There are 4 pharyngobranchials. The first 3 are partly ossified at this stage, and as is typical, the fourth remains cartilaginous (Fig. 8A). The first pharyngobranchial is thin and rod like; pharyngobranchial 2 is handle shaped and bears a toothplate; pharyngobranchial 3 is square and bears the largest of the 3 toothplates; pharyngobranchial 4 is ovoid with a small, autogenous toothplate. The toothplates are all slender, perpendicular to the body axis, and bear only 1 to 2 rows of teeth. The 4 epibranchials are antero-caudally flattened. Epibranchial 1 bears a large uncinat process with which the interarcual cartilage articulates.

Ventral gill arches

The first 4 ceratobranchials are elongate and bear several well-developed gill rakers (Fig. 8B). The fifth ceratobranchial is notably shorter and thin and bears a small toothplate with about 4 teeth on its dorsal surface. There are 3 hypobranchials of which hypobranchial 1 and hypobranchial 2 are relatively robust. Hypobranchial 3 is broad posteriorly and has a tapered anterior extension that runs parallel to the third basibranchial and extends ventrally under hypobranchial 2. At this stage, 3 ossifications are seen in the anterior basibranchial copula, basibranchial 1–3. The posterior basibranchial copula is represented by the cartilaginous oval shaped basibranchial 4. An elongate basihyal extends forward from the first basibranchial.

Hyoid arch

The short, hourglass-shaped interhyal is only ossified at mid length (Fig. 8D). It articulates dorsally with the remnant of the hyosymplectic cartilage and ventrally with the triangular posterior ceratohyal ossification of the curved ventral portion of the hyoid arch. Four of the 6 branchiostegals articulate laterally with the cartilaginous area between the anterior and posterior ceratohyal ossifications. The first branchiostegal does not make contact with the ceratohyal. It is freely suspended in the opercular membrane (Fig. 8D, indicated with dotted lines, as it was inadvertently removed during dissection, but its presence has been confirmed in other undissected specimens); the second branchiostegal articulates with the ventral corner of the anterior ceratohyal ossification. A large beryciform foramen is present at the dorsal margin of the anterior ceratohyal. The dorsal and ventral hypohyals are ossified within the still fairly extensive hypohyal cartilage. The anterior ceratohyal covers the posterior margin of the hypohyal cartilage laterally. At this stage, there is no foramen for the hyoid artery in the dorsal hypohyal. The ventral hypohyal has a small posteroventral projection, where the cartilaginous tip of the anterior ceratohyal articulates.

Urohyal

The urohyal is roughly flat posteriorly, with dorsal and ventral processes anteriorly, from the latter of which extends a thin posteroventral projection (Fig. 8C).

Vertebral column

There are 10+14 vertebrae (urostyle [preural centrum 1+ural centrum] included). The neural arches have anterior extensions that form prezygapophyses. The first 3 neural arches also have postzygapophyses. All neural spines are dorsally directed (Fig. 6A). All haemal spines are ventrally directed. Ribs are present on vertebrae 3 to

10 (Fig. 6B). All besides the very slender and short last rib are elongate and almost reach the ventral midline (a characteristic of chaetodontids). Their distal tips are cartilaginous. The first rib attaches to the neural arch of vertebra 3. The following 2 ribs are attached more ventrally, on the midline of centra 4 and 5. The succeeding ribs attach on the small parapophyses. There is a single set of intermusculars, the first 2 inserting on the neural arches, the remaining 11 on the ribs, as in most percomorphs.

Pectoral girdle and fin

The hypertrophied posttemporal and supracleithrum have large, rugose, plate-like expansions that extend posteriorly over the trunk (Fig. 7, B and F). That of the posttemporal is attenuated into a broad, posterodorsally directed spine that terminates well above the body just posterior to a vertical through the middle of the soft dorsal fin (Figs. 6A and 7C). The rugose, ovoid expansion of the supracleithrum extends posteriorly over the middle trunk and has a small anterodorsal process that articulates with the posttemporal. Anteriorly, it overlaps the dorsal part of the cleithrum. Much of the remaining cleithrum is exposed and rugose; its unexposed ventral tip broadens and is curved posteriorly to meet the cartilaginous tip of the coracoid. The scapula and the coracoid are ossified dorsally and ventrally, respectively, within the still fairly extensive scapulocoracoid cartilage. The scapula is roughly square, whereas the coracoid has an irregular shape with a posterior projection that reaches the ventral one of the 2 postcleithra. There are 4 ossified radials (actinosts) that support 16 distal radials, each associated with one pectoral-fin ray; the dorsal-most fin ray articulates with the cartilaginous propterygium.

Pelvic girdle and fin

The pelvic bone (basipterygium) is long and broader posteriorly where it supports the pelvic fin, which comprises one spine and 5 soft rays (I, 5) (Fig. 7H). Both anterior and posterior tips of the pelvic bone are cartilaginous.

Dorsal and anal fin

The dorsal fin has 12 spines and 26 soft rays (XII, 26). The anal fin has 3 spines and 21 soft rays (III, 21). Anterior to the dorsal-fin pterygiophores (proximal-middle radials) are 2 slender supraneurals with small expansions on their dorsal tip (Fig. 6B). The adult configuration of sequential articulation between the supraneurals and the supraoccipital crest is not yet developed. The predorsal formula (following Johnson, 1984) is 0/0+2/1/ and corresponds to one of the 2 predorsal formulae that are found in chaetodontids. The first pterygiophore inserts in the second interneural space, bears 2 supernumerary spines, and is serially associated with the third. The an-

termost pterygiophores (proximal-middle radials) are roughly triangular. The cartilaginous distal radials are perichondrally ossified in the spinous-dorsal fin. The triangular first anal-fin pterygiophore (proximal-middle radial) is much longer and more robust than the succeeding ones. It inserts anterior to the haemal spine of the eleventh vertebra, bears 2 supernumerary spines, and is serially associated with the third. The succeeding pterygiophores are rodlike with proximal and distal cartilaginous tips. Two or 3 pterygiophores of the soft-dorsal and soft-anal fins insert in each interneural/interhemal space. The last pterygiophore in both the dorsal and anal fin inserts in the 21st interneural/interhemal space.

Caudal fin and supports

As in other chaetodontids, the caudal fin and supports have a typical basal percomorph configuration (see Johnson, 1983). There are 17 principal caudal-fin rays, 9 in the dorsal and 8 in the ventral lobe of the fin (Fig. 8E). Anterior to the principal rays, there are 3 dorsal and 3 ventral procurrent rays; there is no procurrent spur or foreshortened ray. The compound urostyle (preural centrum 1+ural centrum 1) supports the hook-shaped parhypural, and 5 separate hypurals, of which the fourth is the largest. One uroneural and 3 partially ossified epurals lie dorsal to the urostyle. The neural spine of preural centrum 2 is shorter than the preceding neural spines and dorsally expanded. Ventrally, there are 2 distal caudal radials posterior to the cartilaginous tip of the hemal spine of preural centrum 3. The tip of the neural spine of preural centrum 3 is cartilaginous and aligns with the 3 epurals.

Cytochrome oxidase subunit I barcoding

Four specimens from stomach contents (MNHN-LC 1458: 20.5 mm SL, MNHN-LC 1459: 20.2 mm SL, MNHN-LC 1460: 25.4 mm SL, MNHN-LC 1461: 19.9 mm SL), “Günther’s” specimen (BMNH 1871.9.13.94: 27.5 mm SL), and a USNM 432382 specimen (12.5 mm SL) with unknown fixation history have been barcoded with no recovered results. One formalin-fixed specimen (MNHN 2014 2945) was barcoded using a mixed RNA bait set (Agne et al., 2022) with no recovered result. Two ethanol-fixed specimens are identified as *C. reticulatus* with a $\geq 99\%$ match to barcodes available in GenBank and BOLD (Fig. 4). Analyses were rooted with 2 species of *Pomacanthus* used as outgroup comparison.

Discussion

The family Chaetodontidae currently comprises 136 recognized species in 12 genera and 16–22 subgenera (depending on the author: Blum, 1988; Smith et al., 2003;

Fessler and Westneat, 2007). Chaetodontid larvae can be easily identified to the family level due to a specialized tholichthys stage that represents a synapomorphy of the family. Burgess (1978) attributed the longhorn tholichthys to the chaetodontid subgenus *Citharoedus*, specifically to 2 of the 3 species in this subgenus, *C. meyeri* and *C. ornatissimus*. He further noted that larvae of *C. reticulatus*, the third member of *Citharoedus*, are unknown. Most likely Burgess (1978) based the identification of his longhorn tholichthys on meristic data; however, he did not state this explicitly, nor did he explain why he excluded *C. reticulatus* from the possible species with a longhorn tholichthys. He only mentioned on page 83 that “the larvae are quickly recognized as not belonging to genus *Heniochus* since they possess an incomplete lateral line (that of *Heniochus* is complete).”

As shown in Lütken (1880), *C. oligacanthus* (= *Parachaetodon ocellatus*) has small shelf-like outgrowths of the frontals above each eye with a little bump at its tip. There is no resemblance to the horns found in our longhorn tholichthys. Furthermore, the posttemporal and preopercle of the *C. oligacanthus* tholichthys are differently shaped (not spine-like) and do not extend beyond the body. Together with the different meristic counts, this distinguishes *C. oligacanthus* larvae from the longhorn tholichthys. The classification of *C. reticulatus* has been a matter of contention among chaetodontid taxonomists (Blum, 1988), as the color pattern of the adults is similar to that of *C. collare* (subgenus *Chaetodontops* syn. *Rabdophorus*) (Ahl, 1923; Allen, 1980). However, certain osteological characters of *Citharoedus* and *Rabdophorus* are easily distinguished, and some of *C. reticulatus* are identical to those in *C. meyeri* and *C. ornatissimus* (Blum, 1988; Nalbant, 1973; Burgess, 1978). In accordance, molecular analyses place *C. reticulatus* as a member of *Citharoedus* (Smith et al., 2003; Fessler and Westneat, 2007). The similarity between the adult color patterns of *C. collare* and *C. reticulatus* must be interpreted as convergent (Blum, 1988).

Of the corallivorous chaetodontids, the 3 species placed in *Citharoedus* are the most derived, and their monophyly is supported by their osteology (Blum, 1988). Blum (1988) reported an unambiguously derived condition shared by the 3 species of *Citharoedus*: only 5 infraorbitals, presumably the result of the loss of the second infraorbital or its fusion with the third (his circumorbital, character 29). All other chaetodontids have the typical percomorph number of 6 infraorbitals, but in the genus *Parachaetodon* and all *Chaetodon* species (except the 3 *Citharoedus* species), the second infraorbital is reduced in size, displaced ventrally, and therewith excluded from the margin of the orbit (Blum, 1988). Our cleared and stained specimens have only 5 infraorbitals, which supports their identity as a member of the subgenus *Citharoedus*. Without a complete ontogenetic series, we cannot

determine if the second infraorbital is lost or fused to the third infraorbital. Fusion seems probable, as the third infraorbital is twice as long as infraorbitals 4–6. A second character found in all 3 species of *Citharoedus* and our C&S longhorn larvae is the relatively short suspensorium, reduction being particularly evident in the endopterygoid and ectopterygoid (Blum, 1988).

In order to morphologically identify our longhorn larvae, we compared their meristic features to those of the currently 136 recognized chaetodontid species (Fricke et al., 2022) and found a meristic and geographical overlap of our larva with only 2 species, *C. reticulatus* and *C. ornatissimus* (see Materials and methods). Furthermore, we barcoded the cytochrome oxidase subunit I of several specimens from museum collections, which resulted in sequences for 2 specimens. Both are genetically identified as *C. reticulatus*. Burgess (1978) noted that larvae of *C. reticulatus* are unknown. They might be present in museum collections, possibly misidentified as *Heniochus varius* (BMNH 1871.9.13.94; Günther, 1871), which has small horns as an adult, but not during larval and juvenile stages, and differs in meristic counts. They could also be identified as *C. ornatissimus* or *C. meyeri*, both of which also supposedly have a longhorn tholichthys, but are difficult to distinguish based only on meristic data. Further molecular analyses and DNA barcoding would be needed to confirm the presence of a longhorn tholichthys in those 2 species (*C. ornatissimus* or *C. meyeri*).

In our smallest specimen, the frontals already show lateral, flat triangular outgrowths. In our 8.4-mm specimen, the lateral outgrowths are more elongated in an anterolateral direction; they have a straight form and are deeply concave ventrally over their entire length. Both our specimens, genetically identified as *C. reticulatus* with a size range between 10.5 and 12.1 mm, show posteriorly curved horns, and their margins roll inward on their ventral side. The same morphology is seen in all our larger specimens up to 25.4 mm. We have not seen any early longhorn tholichthys stage with relatively long, posteriorly curved horns; they are straight in all examined smaller specimens. Furthermore, all later stages of longhorn tholichthys larvae have the horns increasingly curved with the tips pointing posteriorly. With the data at hand, we cannot reject the possibility that tholichthys larvae with “straight horns” or “curved horns” represent 2 different species. However, we assume that it is an ontogenetic character and that the straight horn curves in later stages until it is completely lost. Burgess (1978:83) noted that “newly metamorphosed hornless individuals were captured of the same size as larvae possessing horns. It is difficult to imagine horns of such size being absorbed so rapidly by the early juvenile. Perhaps the horns are simply discarded rather than absorbed as are the head plates.” We believe this is the most likely sce-

nario, given the absence of any larger specimens with reduced horns. However, our detailed examination of the largest larval specimens show no line of demarcation between the frontals and bases of the horns. Furthermore, based on Burgess’ observations of newly settled individuals, the absorption would necessarily be exceptionally rapid.

Conclusions

With this study, we confirm for the first time that the larva of *C. reticulatus* is characterized by a lateral horn over each eye emanating from the frontals and confirm that at least *C. reticulatus* of the 3 species in the *Citharoedus* subgenus passes through a longhorn tholichthys stage. We believe that the most intriguing aspect of these larvae remains the enigma of how the horns are lost, and we continue to pursue this avenue of research.

“No one knows where the Longhorn goes.”
— Glen Enloe, American writer

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