

***Tempestichthys bettyae*, a new genus and species of ocean sleeper
(Gobiiformes, Thalasseleotrididae) from the central Coral Sea**

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Running title: A new genus and species of Thalasseleotrididae

Abstract

The Thalasseleotrididae is a small family of exclusively marine gobioids. They form a sister taxon to the Gobiidae and Oxudercidae and are distinguished from most species in these families by having six branchiostegal rays and a membrane linking the hyoid arch to the first ceratobranchial. Here we use micro-CT informed morphological data and molecular phylogenetics to describe a new genus and species of thalasseleotridid discovered on a tropical oceanic coral reef in the central Coral Sea. *Tempestichthys bettyae* gen. et sp. nov. is the first tropical thalasseleotridid and differs from other members of the Thalasseleotrididae by having a T-shaped palatine and a distinctive shape and colouration. The three previously described thalasseleotridid species are endemic to temperate coastal waters of southern Australia and New Zealand and are all translucent brown with dorsoventrally compressed heads. However, *Tempestichthys bettyae* is laterally compressed with a pointed snout and is translucent white with opaque white and crimson red markings and a largely crimson iris. We discuss the unique characters of this new genus, including its distribution, form, colouration and diminutive size, and highlight the potential of there being undescribed diversity in the Thalasseleotrididae.

<http://zoobank.org/urn:lsid:zoobank.org:pub:3F584DD6-0B33-4E69-98A7-22D68EE1A1B8>

Key Words: Coral reef, Cryptic, Cryptobenthic fishes, Gobiidae, Gobioidae, Morphology, Osteology, Phylogeny, Tropical, Australia

Introduction

Coral reef fish communities are dominated by small (<50 mm long) cryptic fishes, many of which spend their lives hidden within the reef matrix. These 'cryptobenthic' reef fishes (Brandl et al., 2018; Depczynski & Bellwood, 2003) are characterised by high abundances, short lifespans, and rapid turnover. As such, they likely play essential roles in maintaining coral reef food webs (Brandl et al., 2019a; Brandl et al., 2019b; Depczynski & Bellwood, 2006). Cryptobenthic fishes are also highly diverse, often accounting for more than 40% of fish species on reefs (Ackerman & Bellwood, 2000; Brandl et al., 2018). Our understanding of this diversity has increased in recent years, with new species and cryptic species complexes being described at an increasing rate (Brandl et al., 2018; Tornabene et al., 2021; Winterbottom & Hoese, 2015).

On most coral reefs, cryptobenthic fish communities are dominated by members of the Gobioidae (*sensu* Agorreta et al., 2013; Betancur-R et al. 2017; Kuang et al., 2018). This exceptionally diverse clade of fishes accounts for more than 2000 species inhabiting marine and freshwater habitats around the world. However, more than 95% of gobioid species are found in just three families; the Gobiidae, Oxudercidae and Eleotridae (species richness from Nelson et al., 2016; Gill et al., 2019). Of the six less-speciose families, four predominantly inhabit freshwater or brackish habitats. The two-remaining low-diversity families are the exclusively marine Xenisthmidae (12 spp.; Gill et al., 2014, 2019; recognised as a family in McCraney, 2019) and Thalasseleotrididae (3 spp.; Hoese & Roberts, 2005).

The Thalasseleotrididae is the most recently described family in the Gobioidae (Gill & Mooi, 2012; Nelson et al., 2016), and its position as a sister group to a clade

containing the Gobiidae and Oxudercidae is well-supported by morphological and molecular data (Gill & Mooi, 2012; McCraney et al., 2020; Thacker et al., 2015). The three currently described species are endemic to temperate, coastal waters around southern Australia (*Thalasseleotris adela* Hoese and Larson, 1987) and New Zealand (*T. iota* Hoese and Roberts, 2005, and *Grahamichthys radiatus* [Valenciennes, 1837]).

Here we describe a new genus and species of thalasseleotridid, *Tempestichthys bettyae*, discovered on a reef in the central Coral Sea, Australia. We discuss the relationships among *Tempestichthys*, the other thalasseleotridid genera, and other families in the Gobioidae based on morphological characters and present a molecular phylogeny that corroborates the morphological data.

Materials and methods

Specimen collection

The specimen of *Tempestichthys* was collected by the first author and Tane Sinclair-Taylor on the 21st of February 2019 from the southwest of Marion Reef, Australian Coral Sea (19.29511°S, 152.23782°E). The specimen was collected from a 4 m² enclosed clove oil station. Divers on SCUBA enclosed a reef outcrop within a 2 mm mesh dome (mosquito net) weighted around the circumference with a 7.1 m long chain. The mesh delineates a standardised area, prevents fish from escaping the station, and prevents predation by nearby fish during collection. The outcrop was then covered with a weighted impermeable membrane (tent flysheet) to hold the clove oil solution in place. Two litres of a 1:4 solution of clove bud oil and 95% ethanol was sprayed into the enclosure and left for two minutes to ensure fish were euthanised. The divers then

removed the enclosure and rolled back the netting while collecting all fishes. On return to the research vessel, specimens were photographed individually, following Emery & Winterbottom (1980), and preserved in 95% ethanol.

Molecular phylogenetics

We successfully sequenced segments of three of the five genes from Agorreta et al. (2013; *rag1*, *sreb2* [now GPR85], and *zic1*) from the holotype. To facilitate future studies, segments of two further genes were sequenced: (*COI*), widely used for species-level assignments, and (*Ptr*), which is useful for higher-level phylogenetic analyses (Li et al., 2007). DNA was extracted from the right pectoral fin of the specimen using a Qiagen® DNEasy Blood and Tissue kit. Standard spin column protocols for animal tissues were followed, with a modified elution stage to maximise DNA collection; triplicate rinses of 50 µl of AE buffer were collected from the spin column, with a 1-minute incubation before each centrifuging.

PCR reactions were made in a volume of 25 µl, comprised of the following: 8.5 µl ultrapure water, 12.5 µl of New England BioLabs® Hot Start Taq 2X Master Mix, 1 µl each of forward and reverse primers (Table 1) and 2 µl of template DNA. Thermal cycling conditions were: 95 °C for 120 s, followed by 30 cycles of 95 °C for 30 s, a primer-specific annealing temperature (Table 1) for 45 s, 72 °C for a primer-specific extension period (Table 1), and a final extension of 72 °C for 5 min. PCR products were sent for Sanger sequencing at Molecular Cloning Laboratories (MCLAB), San Francisco, California.

Sequence data were manually trimmed and aligned using Geneious Prime 2021 to create consensus sequences. Consensus sequences for *rag1*, *sreb2*, and *zic1*, were

then concatenated and aligned with the five-gene data set covering all families in the suborder Gobioidae (Agorreta et al., 2013; Kovačić et al., 2021; Tornabene et al., 2016) further supplemented with *rag1*, *sreb2*, and *zic1* sequences of *Thalasseleotris iota* (YPM ICH 026584) and *Grahamichthys radiatus* (YPM ICH 026585) from NCBI GenBank (Thacker et al., 2015). In total, the data included 246 specimens from 236 species. New sequences generated in this study were deposited on GenBank (accession numbers xxx-xxx).

Data were analysed using Bayesian phylogenetic inference in the program MrBayes 3.2 (Ronquist et al., 2012) using the BEAGLE library (Ayres et al., 2012). The partitioning scheme and substitution model choice followed Agorreta et al. (2013). The analysis consisted of two parallel Markov Chain Monte Carlo (MCMC) runs, each with four chains, run for 40×10^6 generations, sampled every 1000 generations. Analyses were conducted through the NIH and NSF funded CIPRES Science Gateway (Miller et al., 2011). To determine model convergence, mixing, and appropriate burn-in values, we assessed MCMC logs using Tracer 1.7 (Rambaut et al., 2018).

Micro-CT scanning and segmentation

Recent improvements in the accessibility of micro-computed tomography (micro-CT) scanning (e.g., through the NSF funded oVert project) alongside the development of open-source software to analyse CT data (e.g., 3D Slicer; Fedorov et al., 2012) has facilitated detailed, non-destructive osteological analyses of type specimens. We scanned the holotype of the new species using the Friday Harbor Laboratories, Karel F. Liem Imaging Facility, Bruker SkyScan 1173 micro-CT scanner. The specimen was

rolled in a small section of ethanol moistened ScotchBrite® reusable wipe (also see Chux® [Australia] or J-Cloth® [UK]), allowing it to be mounted gently but firmly in a 2 mL O-ring sealed microcentrifuge tube. This technique ensures that small specimens do not move or dehydrate—and possibly change shape—during the scanning process.

The oversize scan (two stacked, five-megapixel scans) was made with voxel (i.e., 3D pixel) dimensions of 5.68 μm and a rotation step of 0.3° between images. The X-ray source voltage was 60 kV, and the current was 133 μA . A 1 mm aluminium filter was used to reduce image artefacts (Barrett & Keat, 2004).

CT scans were fused and reconstructed using Bruker NRecon before visualisation and segmentation in the open-source software package 3D Slicer. To facilitate loading and modifying the high-resolution CT-scan data, we used the ImageStacks function of the SlicerMorph extension (Rolfe et al., 2021). Scan data for *Tempestichthys* were accessioned on Morphosource.org (ark:/87602/m4/394020).

Morphology

A Zeiss Discovery V20 SteREO microscope with an attached AxioCam 503 digital camera was used to examine and photograph the preserved holotype. Measurements of the holotype were made from calibrated micrographs using the graphics tools of Zeiss Zen Blue 3.4 and from micro-CT scans using the Markups module of 3D Slicer. The dorsal pterygiophore formula follows that of Birdsong et al. (1988). Definitions of all other morphological characters follow Böhlke & Robins (1962), as modified by Van Tassell et al. (2012), using the standard uppercase Roman numerals to denote spines and Arabic numerals to denote the number of segmented rays of the dorsal, anal, and pelvic

fins.

Results

Molecular phylogeny

The molecular phylogeny (Fig. 1, Supplementary Fig. 1) resolved the Thalasseleotrididae as a sister group to a clade containing the Gobiidae and Oxudercidae (Gill & Mooi, 2012; McCraney et al., 2020; Reichenbacher et al., 2020; Thacker et al., 2015). There was strong support (Bayesian posterior probability for all nodes = 1 ± 0 standard deviations) for *Tempestichthys* nov. gen. as a sister group to a clade containing the two current genera of thalasseleotridid, *Thalasseleotris* and *Grahamichthys*.

Taxonomy

Tempestichthys gen. nov.

(Fig. 2)

Type species. *Tempestichthys bettyae* sp. nov.

Etymology. From the Latin *tempestas* (storm; f.), referring to Severe Tropical Cyclone Oma, which passed through the Coral Sea between the 11th and 22nd of February 2019, resulting in rough seas, nausea, and changes to the itinerary of the 2019 Coral Sea Monitoring Program Cruise. The common Greek suffix, *-ichthys* (fish) is used, making the new genus masculine.

Diagnosis. In addition to the phylogenetic placement from the molecular analysis (Fig. 1), our assignment of *Tempestichthys* as a new genus in the Thalasseleotrididae is strongly supported by several morphological characters. Whenever possible, we note which characters are likely synapomorphies of specific clades, whereas others are more variable and may require further examination across gobioids.

Among the Gobiioidei, members of the Gobiidae and Oxudercidae possess four derived characters (Gill & Mooi, 2012; Reichenbacher et al., 2020). In all cases, *Tempestichthys* displays the plesiomorphic conditions, placing it outside the Gobiidae and Oxudercidae. As follows, in *Tempestichthys*:

- i. There are six branchiostegal rays (Fig. 3), a character shared by all gobioids except the Gobiidae and Oxudercidae, which have five branchiostegal rays (a synapomorphy of Gobiidae + Oxudercidae; Gill & Mooi, 2012).
- ii. The fifth ceratobranchials lack ventral processes. In the Gobiidae and Oxudercidae, there is a medially positioned ventral process on ceratobranchial five (Fig. 4; a synapomorphy of Gobiidae + Oxudercidae; Gill & Mooi, 2012).
- iii. The dorsal hemitrachs of the pelvic-fin rays have simple proximal heads. These structures are more complex in the Gobiidae and Oxudercidae (Fig. 5; a synapomorphy of Gobiidae + Oxudercidae; Gill & Mooi, 2012).
- iv. The pelvic fins are completely separate, with no anterior frenum or membrane connecting the innermost rays. This character is shared with all gobioids except the Oxudercidae and most Gobiidae (fused pelvic fins are a potential a synapomorphy of Gobiidae + Oxudercidae); however, there are several exceptions within these families representing independent reversals, including members of the gobiid genera *Eviota*, *Coryphopterus*, *Heteroeleotris*, the *Nes*

subgroup of the Gobiosomatini and others.

Where characters i-iv highlight *Tempestichthys* is not in the Gobiidae or Oxudercidae, it differs from all other six branchiostegal-rayed gobioids by possessing the following combination of characters:

- v. *Tempestichthys* has an ossified but very reduced scapula (Fig. 3); a reduced or absent scapula is shared (a possible synapomorphy) with all gobioids except the Odontobutidae and Rhyacichthyidae, although the scapula may only be slightly reduced in some other taxa, including the butid *Bostrychus* (Akihito, 1986; Hoese & Gill, 1993).
- vi. The scales of *Tempestichthys* have a single row of cteni along the scale margins (i.e., they lack transforming cteni). This character is shared with all gobioids except the Odontobutidae and Rhyacichthyidae, which have multiple rows of cteni that vary (transform) in shape among rows. The absence of transforming cteni is a possible synapomorphy of the clade of all gobioids except Odontobutidae + Rhyacichthyidae (Hoese & Gill, 1993).
- vii. The bony canal support on the preoperculum is extremely reduced in *Tempestichthys*. This character is shared with the Eleotridae, Thalasseleotrididae, Gobiidae, Oxudercidae and some members of the Butidae (reduced canal support is a possible synapomorphy of these families). The remainder of the Butidae, and the Rhyacichthyidae, Odontobutidae, and Milyeringidae have a well-developed bony canal support along the posterior margin of the preoperculum (Figs. 3, 6; Hoese & Gill, 1993).
- viii. *Tempestichthys* has an interneural gap between the last pterygiophore of the first dorsal fin and the first pterygiophore of the second dorsal fin (Fig. 7; first dorsal

fin pterygiophore formula 3-22110; *sensu* Birdsong et al. 1988). The Odontobutidae, Milyeringidae and many Eleotridae and Butidae lack an interneural gap, which is present in most other gobioids (Hoesé & Gill, 1993).

- ix. *Tempestichthys* possesses one epural (Fig. 7), a character unique to the Thalasseleotrididae, Gobiidae, and the *Stenogobius*-lineage of the Oxudercidae (subfamily Sicydiinae and allies). Most other gobioids have two or more epurals, with few exceptions (Allen & Hoesé, 2017; Hoesé & Gill, 1993).
- x. *Tempestichthys* lacks a dorsal postcleithrum (Fig. 3); absent in the Thalasseleotrididae, Gobiidae, Oxudercidae and some xenisthmids, but present in most other gobioids (Akihito, 1969; Hoesé, 1984; Gill & Hoesé, 1993; Gill & Mooi, 2012; Springer, 1983).
- xi. The urohyal of *Tempestichthys* lacks a ventral shelf (Figs. 3, 8), present in all gobioids except the Thalasseleotrididae, Gobiidae and Oxudercidae (loss of shelf is a possible synapomorphy of this group of families; Hoesé & Gill, 1993; Gill & Mooi, 2012).
- xii. The interhyal of *Tempestichthys* has a disc-shaped lateral structure for articulation with the preoperculum, lacking in all gobioids except the Thalasseleotrididae, Gobiidae and Oxudercidae (synapomorphy of this group; Gill & Mooi, 2012).

Combined, the 12 characters above support the placement of *Tempestichthys* within the Thalasseleotrididae. This family is unified by a single synapomorphy (Gill & Mooi, 2012): ‘a broad membrane connecting the hyoid arch to the first ceratobranchial, which extends along most of the length of first ceratobranchial (i.e., the first gill slit is restricted or closed)’. This character is found only within Thalasseleotrididae and the

gobiid genera *Heteroeleotris*, *Cerogobius*, and some species of *Eviota*. In *Tempestichthys*, the first gill slit is closed.

In addition to clear separation from *Grahamichthys* and *Thalasseleotris* in the molecular phylogeny (Fig. 1), we erect the new genus *Tempestichthys* based upon the following characters (Table 2):

- xiii. In *Thalasseleotris* and *Grahamichthys*, the ethmoid process of the palatine is short and slender. In *Tempestichthys*, the palatine is close to T-shaped, with a robust ethmoid process, 87% the length of maxillary process (measured from anterior medial process). The palatine in *Tempestichthys* appears more similar to the T-shaped palatine found in Gobiidae and Oxudercidae (Figs. 3, 9; Gill & Mooi, 2012).
- xiv. *Tempestichthys* differs from both *Thalasseleotris* and *Grahamichthys* in pectoral fin ray count with 16 rays compared to 17-21 in the other genera (Hoesé & Roberts, 2005; McDowall, 1965).
- xv. For both the dorsal and anal fins, *Tempestichthys* has a similar arrangement of fin elements (D, VI+I,8; A, I,7) to *Thalasseleotris* spp. (D, V-VII+I,7–9; A, I,7–9; Hoesé & Roberts, 2005); however, *Grahamichthys* has more segmented rays on both fins, with an arrangement of D, VI-VII+I,9–11 (mode I,10); A, I,9–11 (mode I,10; McDowall, 1965).
- xvi. *Tempestichthys* has tightly spaced, villiform teeth in multiple rows. The premaxilla has two tooth rows to the posterior, increasing to five rows at the anterior. The longest premaxillary teeth (0.18 mm) are in the innermost (lingual) row at the anterior of the jaw, beneath the ascending and articular processes of premaxilla. Tooth rows at the anterior of the premaxilla increase in length and

become increasingly angled inwards (lingually) along the labio-lingual axis resulting in the cusps of each row being arranged along a plane following the angle of the jaw (Figs. 3, 10). The dentary has two tooth rows at the posterior and four rows at the anterior. The longest teeth on the dentary (0.11 mm) are in the innermost (lingual) row towards the posterior of the dentary—above the insertion of the articular (Meckelian fossa).

Thalasseleotris adela, *T. iota* and *Tempestichthys* all have villiform teeth and a similar number of tooth rows across the jaw; however, in both *Thalasseleotris* species, the longest premaxillary teeth are in the outer rows (Hoese & Larson, 1987; Hoese & Roberts, 2005), whereas in *Tempestichthys* the innermost teeth at the anterior of the premaxilla are much longer than those on the middle or outer rows. Teeth on the dentaries of *Tempestichthys* and *T. adela* are similar—with the longest teeth being found in the innermost rows—differing from *T. iota*, which has the longest teeth along the anterior, outer (labial) row (Hoese & Larson, 1987; Hoese & Roberts, 2005).

In contrast to the other thalasseleotridids, *Grahamichthys* has large, widely spaced, recurved teeth on both the premaxilla and dentary. The premaxilla has a second, inner row of teeth along the anterior three-quarters of the bone, the majority small, but with several large inwards pointing teeth at the anterior margin (Figs. 10, 11).

- xvii. *Tempestichthys* differs greatly from all other thalasseleotridids in colouration and body form (Figs. 2, 12). *Thalasseleotris adela*, *T. iota*, and *Grahamichthys radiatus* are all transparent brown in life, with some darker barring along the body (Fig. 12). *Tempestichthys* is primarily transparent white, with opaque white

and crimson red markings.

Tempestichthys is broadly fusiform in shape, with a pointed snout and a laterally compressed head (Figs. 2, 3, 11). *Thalasseleotris adela*, *T. iota* and *Grahamichthys radiatus* all have more rounded snouts and cylindrical or dorsoventrally compressed heads (Figs. 11, 12; Hoese & Roberts, 2005).

***Tempestichthys bettyae* sp. nov.**

Betty's ocean sleeper

Type material. Holotype: AMS I.50056-001, 12.4 mm SL

Type locality. Collected using enclosed clove oil station to the southwest of Marion Reef in the Australian Coral Sea, 19.29511°S 152.23782°E, from the vessel *Iron Joy* (RB Holdings). Collectors T. Sinclair-Taylor and C. Goatley, 21st of February 2019.

Habitat and distribution. The 4 m² clove oil station was deployed over a reef outcrop surrounded by sand at a depth of 11 m. Enclosed microhabitats included heavily sedimented reef matrix, live branching and massive corals, *Halimeda*, sand, and overhangs/crevices.

Etymology. Named in honour of Mrs E. Goatley, the great aunt of the first author, in recognition of the support and encouragement she has provided to CHRG throughout his life and career. A noun in the genitive.

Diagnosis. See the generic diagnosis

Description. General shape: body laterally compressed, particularly to the posterior of

the operculae (maximum width at opercular margin = 75% of body depth [BD] at first dorsal-fin origin, narrowing to 55% of BD behind pectoral fin base), fusiform, deepest at origin of first dorsal fin.

Fins: dorsal fin elements VI+I,8; first dorsal fin triangular, second element longest, tips of spines protruding from fin membrane; second dorsal fin rays 3–8 branched with final ray branched to base; anal fin I,7, last ray branched to base; pectoral fin rays 16, all unbranched, pointed, reaching to origin of anal fin; pelvic fins I,5, fins well-separated, lacking both anterior frenum and membrane connecting innermost rays, all elements broader than those in other fins, fourth ray longest, extending posteriorly to the anal fin origin, rays 1–4 branched, fifth ray unbranched and 30% the length of fourth ray; caudal-fin truncate with 15 segmented rays, 11 branched.

Squamation: no scales on head and anterior of body, scalation starts at pectoral fin base; 24 longitudinal scale rows; five transverse scale rows; scale morphology described in generic diagnosis.

Genitalia: urogenital papilla of female elongated (0.57 mm long) and cylindrical, with ~12 short fingerlike projections encircling the end; male unknown.

Head: head pointed, mouth large, terminal, slightly upturned, angled 34° from horizontal axis of body, extending to posterior margin of the pupil; no cephalic pores; eyes large (31% HL) extending above head profile by around half the width of iris, interorbital space extremely narrow—eyes nearly touching; long, steep ascending process of the premaxillae, and large lateral ethmoids produce a stepped snout profile (50° then 12° from horizontal); anterior nares very short tubes, posterior nares flush with snout; sensory papillae present as small rounded protrusions (rather than flap-like

in *Thalasseleotris*; Hoese & Roberts, 2005), restricted to a line of six along ventral margin of preoperculum; teeth described in generic diagnosis.

Morphometrics and osteology: morphometric data are presented in Table 3. Osteology is described in generic diagnosis above. Segmented micro-CT scans of the head and axial skeleton of *Tempestichthys bettyae* are provided in Figures 3 and 7.

Colour in life. Body primarily transparent white with opaque white and crimson red markings; the colour of gill arches, digestive system and ovaries visible through abdomen (Fig. 2, Supplementary Fig. 2); iris of eye predominantly crimson red mottled on a background of opaque white; two bars projecting below eye, the first starting as a scattering of red chromatophores at orbit projecting diagonally forwards over the maxilla and dentary as an orange band, the second a scattering of red chromatophores almost vertically below midline of the eye; scattered red and white chromatophores over operculum and belly; alternating small crimson red and white markings along dorsal midline, starting above posterior opercular margin and finishing at caudal peduncle, white marking at anterior base of spinous dorsal fin extends slightly onto membrane between dorsal spines one and two, subsequent red marking under the middle of first dorsal fin extends as a bar to tip of fin with membranes between spines two and four entirely crimson red; similar paler markings along ventral midline from posterior of anus; pectoral fin base with opaque diagonal white mark, extending onto base of ventral pectoral fin rays as scattered white spots; an elongate white dash mark along lateral midline at caudal peduncle; dark brown bar at caudal fin base over hypurals.

Colour in preservative. Overall, opaque tan, with no mottling or bars (Fig. 2); metallic pink iris and two small spots (30% pupil diameter), first on maxilla below iris and

second to ventral-posterior of orbit; opaque white spots on membrane between spines one and two on dorsal fin one; opaque white spots on pectoral fin base and adjacent membranes between pectoral fin rays, starting between seven and eight (numbered from top) and progressing ventrally; few scattered pink markings along anal fin base; dark bar at caudal fin base over hypurals.

Comparative material. *Grahamichthys radiatus* (Valenciennes, 1837): YPM ICH 026585 , micro-CT scans from Morphosource.org (ark:/87602/m4/M71689 and ark:/87602/m4/M71687). *Thalasseleotris adela* Hoese & Larson, 1987: AMS I. 17550-014 paratype (1 of 12), micro-CT scanned for this study (Morphosource: ark:/87602/m4/399002 and ark:/87602/m4/399008). *Thalasseleotris iota* Hoese & Roberts, 2005: AMS I. 41344-001 paratype (1 of 5), micro-CT scanned for this study (Morphosource: ark:/87602/m4/394026 and ark:/87602/m4/396673). *Eviota prasites* Jordan & Seale, 1906: CHRG personal collection, micro-CT scanned for this study (Morphosource: ark:/87602/m4/392393).

Discussion

Tempestichthys bettyae gen. et sp. nov. is unique among the Thalasseleotrididae in several key characteristics. First, where all other thalasseleotridids inhabit temperate coastal habitats (Hoese, 2008; Hoese & Larson, 1987; Hoese & Roberts, 2005; McDowall, 1965; McDowall & Stewart, 2015), *T. bettyae* was collected from an oceanic coral reef, Marion Reef, which lies in the tropics, more than 1,600 km north of the reported distributions of any other thalasseleotridid (ALA, 2021; Hoese, 2018) and 180 km from any other reef. While it is impossible to assess the geographic range of *T. bettyae* from a single specimen, the remoteness of Marion Reef, combined with oceanic

gyres on the Marion Plateau (Ceccarelli et al., 2013), and near-reef larval retention in other cryptobenthic fishes (Brandl et al., 2019a) might point to this species being a Coral Sea endemic.

Second, *T. bettyae* is almost certainly the smallest thalasseleotridid by a considerable margin. In their description of the previous smallest thalasseleotridid, *Thalasseleotris iota*, Hoese & Roberts (2005) examined 376 specimens ranging from 12–32.6 mm SL. At 12.4 mm SL, *Tempestichthys bettyae* is equivalent in size to the smallest *Thalasseleotris iota* specimen examined. However, the holotype of *Tempestichthys bettyae* is a mature female, demonstrated by the presence of enlarged ovaries and visible oocytes (~0.25 mm diameter) in the micro-CT scan data (Supplementary Fig. 2). *Thalasseleotris iota* appear to display adult characteristics only once they exceed 20 mm SL (Hoese & Roberts, 2005). Using the size-based definition of cryptobenthic reef fishes in Brandl et al. (2018), the size and tropical distribution of *Tempestichthys bettyae* could lead to the Thalasseleotrididae being included as a core family of cryptobenthic reef fishes.

Due to its small body size, *T. bettyae* can easily be categorised as a miniaturised species. In their review of South American freshwater fishes, Weitzman & Vari (1988) arbitrarily selected 26 mm SL as a threshold below which fishes can be considered miniaturised. While, in coral reef fish communities, relationships between body size and mortality rates (Goatley & Bellwood, 2016) and the use of benthic habitats (Mihalitsis et al., 2020) show distinct shifts at 43 mm and 46 mm, respectively. At 12.4 mm SL, *T. bettyae* is smaller than all these thresholds. With few indications of developmental truncation and well-developed osteology, *T. bettyae* can be considered a proportioned dwarf (*sensu* Rüber et al., 2007; Britz & Conway, 2009; Ou et al., 2011).

The final unique characteristic of *Tempestichthys* is its body plan, which is very different to that of the two other thalasseleotridid genera, being laterally compressed and having a pointed snout. The dissimilarity of *Tempestichthys* to the other members of the Thalasseleotrididae, combined with the relatively recent identification of a synapomorphy that unites thalasseleotridids (Gill & Mooi, 2012), may mean that other atypical thalasseleotridids have been overlooked. This problem may be exacerbated because the Thalasseleotrididae are not included in any of the widely used keys for gobioid fishes in Carpenter & Niem (2001). With all thalasseleotridid species to date being endemic to the temperate South Pacific, there was no need to include this family in these keys. *Tempestichthys*, however, was collected in the region covered by Carpenter & Niem (2001) and, if following these keys, may be identified as a member of the gobiid genus *Heteroleotris* due to the membrane connecting the hyoid arch and first ceratobranchial. *Tempestichthys* can be distinguished from most *Heteroleotris* species by being more laterally compressed and fusiform than any member of *Heteroleotris*. It should be noted that there is some variation in body shape (among other characters) in *Heteroleotris* species, and it is possible that some current *Heteroleotris* species may not belong in that genus.

While an important character in identifying gobioid taxa, the closed gill slit can be challenging to identify in very small, preserved specimens. If it is overlooked (as the lead author initially did), *Tempestichthys* keys out as a member of the genus *Trimma* (Larson & Murdy, 2001). *Tempestichthys* is similar in form to several of the more epibenthic *Trimma* spp. (e.g., *Trimma anaima* Winterbottom, 2000, and *T. nasa* Winterbottom, 2005), which may be found in this region, highlighting the importance of checking the opercular-ceratobranchial membrane and verifying any questionable

specimens using osteological characters i-iii in the generic diagnosis above and/or molecular phylogenetics. By assessing these characters, more potential members of this family may be found in museum collections or by future collection expeditions.

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Table 1: Primers and PCR reaction conditions used to amplify and sequence five gene regions of *Tempestichthys bettyae* gen. et sp. nov.

Gene	Primer name	Primer sequence (5' to 3')	Annealing	Extension	Primer design
region			temperature (°C)	time (s)	
RAG1	RAG1F1	CTGAGCTGCAGTCAGTACCATAAGATGT	53	90	(López et al., 2004)
	RAG1Ra	CGGGCRTAGTTCCCR TTCATCCTCAT	53	90	(Tornabene & Pezold, 2011)
sreb2	sreb2_F10	ATGGCGAACTAYAGCCATGC	56	60	(Li et al., 2007)
	sreb2_R1094	CTGGATTTTCTGCAGTASAGGAG	56	60	(Li et al., 2007)
zic1	zic1_F9	GGACGCAGGACCGCARTAYC	56	60	(Li et al., 2007)
	zic1_R967	CTGTGTGTGTCCTTTTGTGRATYTT	56	60	(Li et al., 2007)
Ptr	PtrF2	TCGTTCATGGGATGTTTACAAAT	57	60	(Yamada et al., 2009)
	PtrR2	GGATGAGCCAGAAGTTCCCCAGAG	57	60	(Yamada et al., 2009)
COI	Fish F1	TCAACCAACCACAAAGACATTGGCAC	54	60	(Ward et al., 2005)
	Fish R1	ACTTCAGGGTGACCGAAGAATCAGA	54	60	(Ward et al., 2005)

Table 2. Taxonomically informative characters for the genera of Thalasseleotrididae and the families Gobiidae and Oxudercidae

	<i>Tempestichthys</i>	<i>Grahamichthys</i>	<i>Thalasseleotris</i>	Gobiidae	Oxudercidae
First gill slit restricted or closed	yes	yes	yes	no ^a	no
Branchiostegal rays	6	6	6	5	5
Pelvic fins	separate	separate	separate	united ^b	united
Palatine shape	T-shaped	partially L-shaped	partially L-shaped	T-shaped	T-shaped
Ventral process on ceratobranchial 5	absent	absent	absent	present	present
Dorsal hemitrich of pelvic-fin rays	simple	simple	simple	complex	complex

^a first gill slit restricted or closed only in *Heteroleotris*, *Cerogobius*, and some species of *Eviota*

^b pelvic fins partially secondarily divided in some lineages independently (e.g., *Eviota*, *Trimma*, *Ptereleotris*, *Nes* subgroup of the Gobiosomatini, *Coryphopterus*, and others)

Table 3. Morphometrics of *Tempestichthys bettyae* gen. et sp. nov. All values except standard length (SL) are in % SL.

Measurement	<i>Tempestichthys bettyae</i>
	AMS I.50056-001
Standard length	12.4 mm
Head length	36.5
Snout to origin of first dorsal	38.8
Snout to origin of second dorsal	61.6
Snout to origin of anal	63.5
Length of caudal peduncle	24.2
Smallest depth of caudal peduncle	11.3
Body depth at origin of first dorsal	24.0
Eye diameter	11.3
Snout length	6.2
Upper jaw length	17.3
Pectoral fin length	21.0
Pelvic fin length	29.8

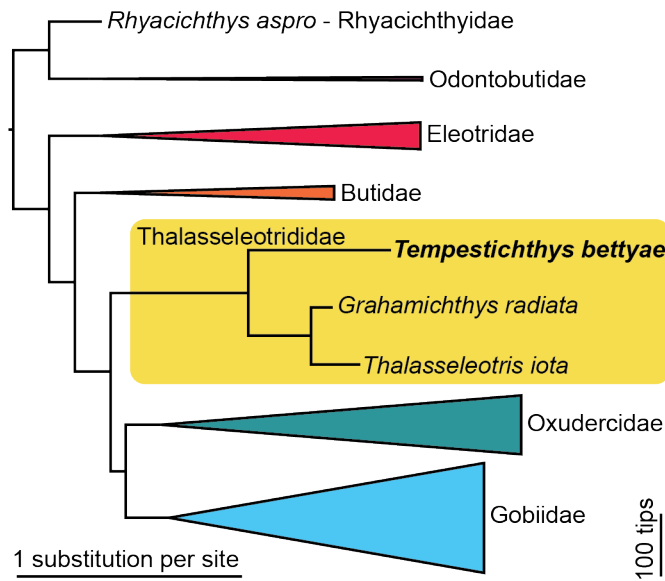


Figure 1. Bayesian inference molecular phylogeny of Gobiioidei inferred from up to 5704 bp of combined nuclear genes and mtDNA (Agorreta et al., 2013; Kovačić et al., 2021; Tornabene et al., 2016). Branches of non-thalasseleotridid families collapsed to illustrate position of *Tempestichthys bettyae* gen. et sp. nov. Heights of triangles represent number of collapsed tips. Bayesian posterior probability for all displayed nodes = 1 ± 0 standard deviation. Expanded phylogeny in Supplementary Fig. 1.

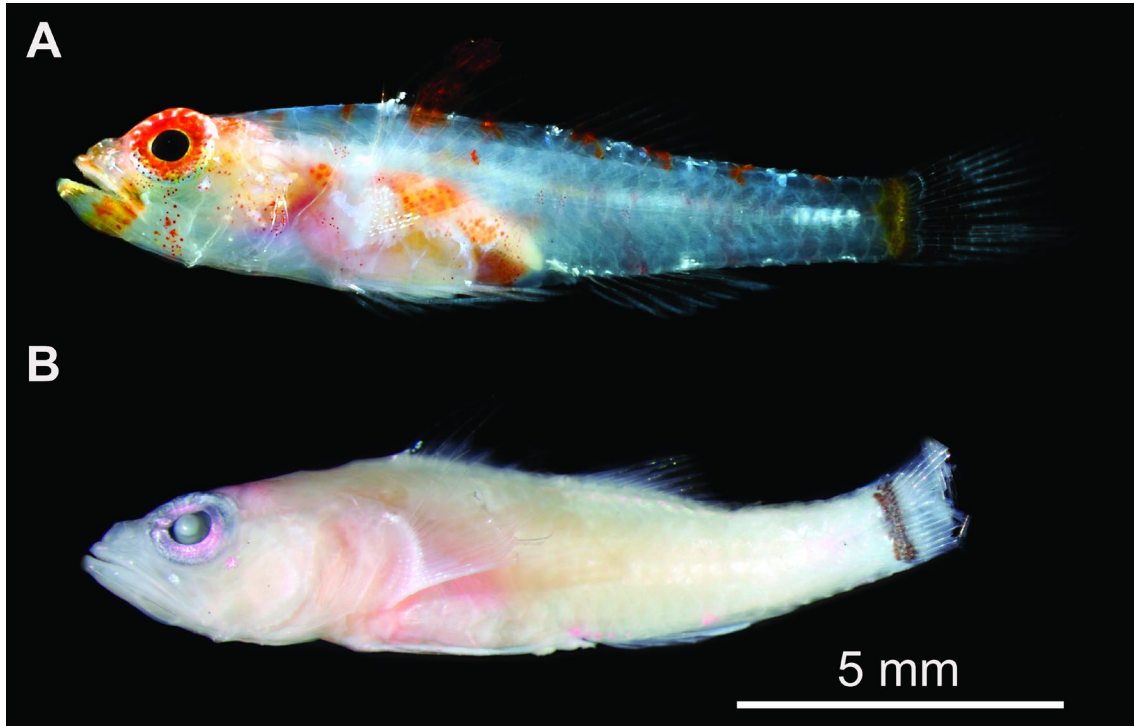


Figure 2. The holotype of *Tempestichthys bettyae* gen. et sp. nov. AMS I.50056-001 **A** live colouration and **B** colour after preservation in ethanol.

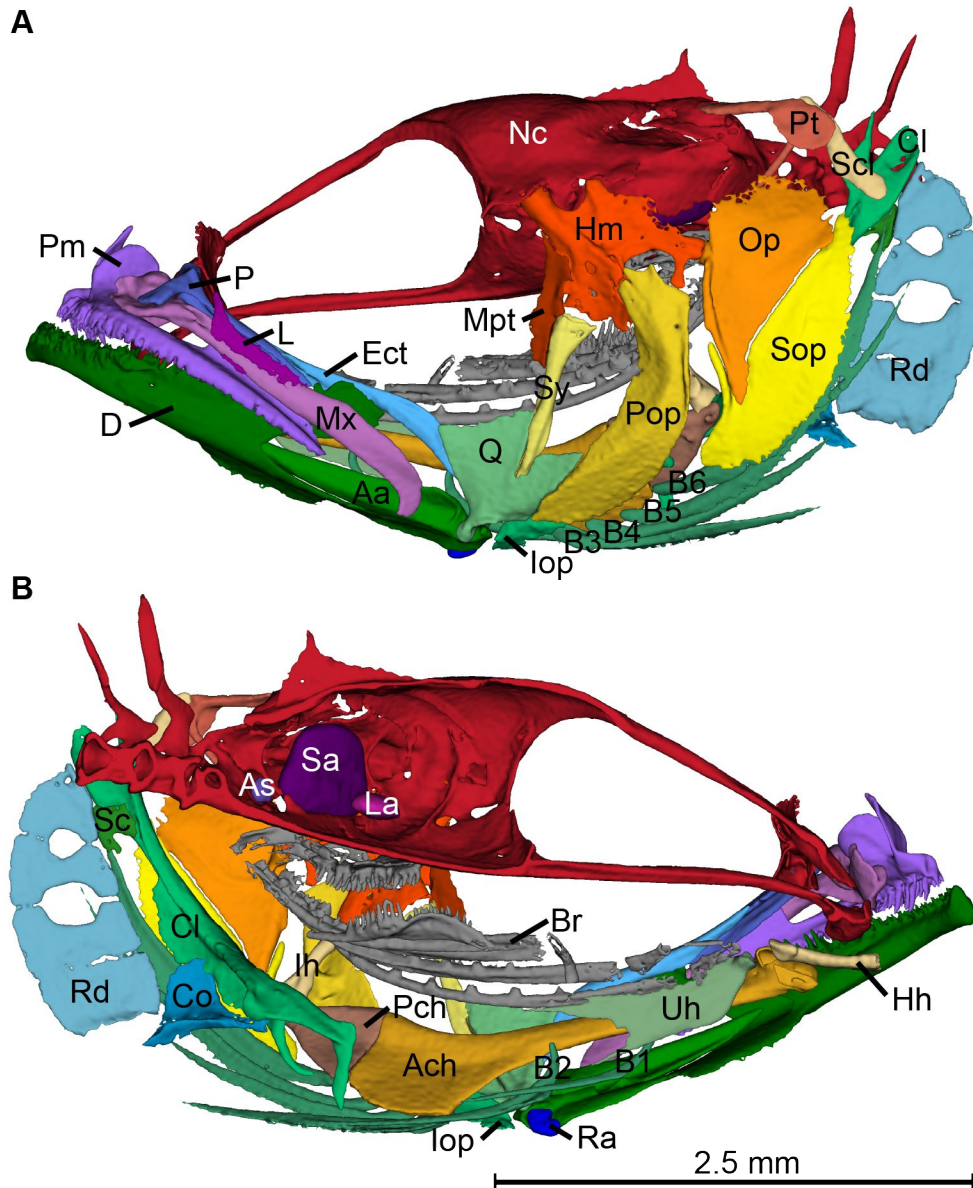


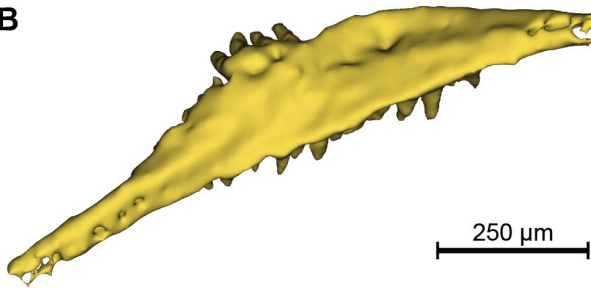
Figure 3. Segmented micro-CT scan of the head of *Tempestichthys bettyae* AMS I.50056-001, **A** lateral view, **B** medial view, digitally dissected along midline. Scan data available at Morphosource.org (ark:/87602/m4/394020). Abbreviations: *Aa* anguloarticular, *Ach* anterior ceratohyal, *As* asteriscus, *Br* branchial arch, *B1-6* branchiostegals 1-6, *Cl* cleithrum, *Co* coracoid, *D* dentary, *Ect* ectopterygoid, *Hh* hypohyal, *Hm* hyomandibula, *Ih* interhyal, *Iop* interoperculum, *L* lacrimal, *La* lapillus, *Mpt* metapterygoid, *Mx* maxilla, *Nc* neurocranium, *Op* operculum, *P* palatine, *Pch* posterior ceratohyal, *Pm* premaxilla, *Pop* preoperculum, *Pt* posttemporal, *Q* quadrate,

Ra retroarticular, *Rd* radials, *Sa* sagitta, *Sc* scapula, *Scl* supercleithrum, *Sop* suboperculum, *Sy* symplectic, *Uh* urohyal.

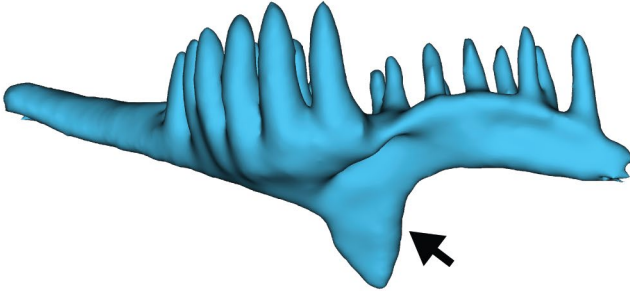
A *Tempestichthys bettyae*



B



C *Eviota prasites* (Gobiidae)



D

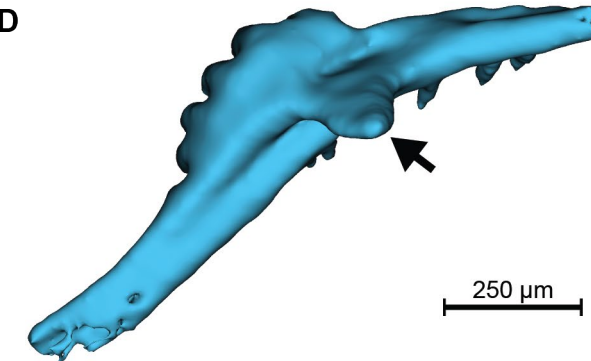


Figure 4. Left fifth ceratobranchials of *Tempestichthys bettyae* (**A** and **B**), and *Eviota prasites* (Gobiidae; **C** and **D**). Arrows show ventral processes in Gobiidae, missing in the Thalasseleotrididae. **A** and **C** are medial views, while **B** and **D** are ventral views, anterior to right.

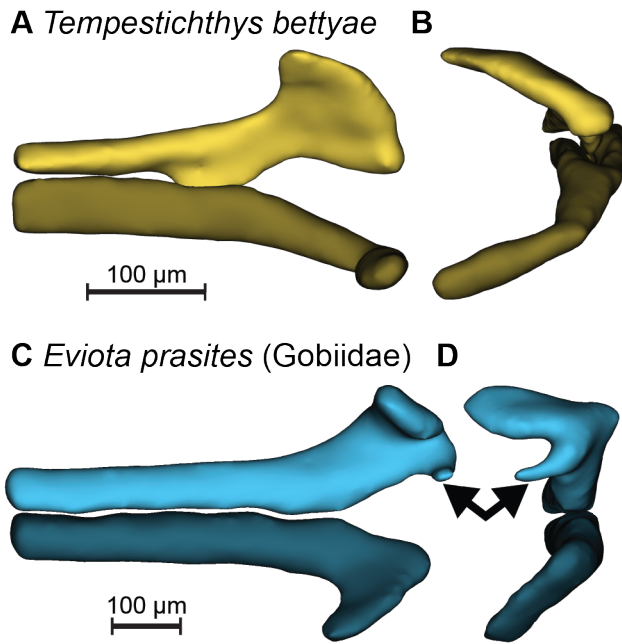


Figure 5. Proximal tips of dorsal (light) and ventral (darker) hemitrichs of second pelvic fin ray of *Tempestichthys bettyae* (**A** and **B**; simple dorsal hemitrich) and *Eviota prasites* (**C** and **D**; complex dorsal hemitrich). Arrows point to complex structures in the Gobiidae. Medial (**A** and **C**) and anterior views (**B** and **D**).

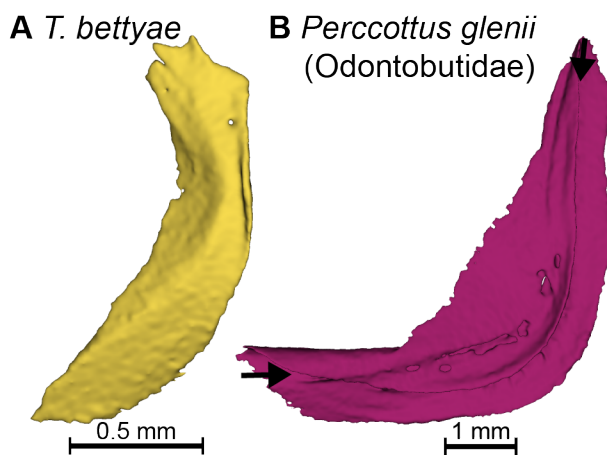


Figure 6. Left preoperculae of **A** *Tempestichthys bettyae* and **B** *Perccottus glenii* (Odontobutidae), lateral view, anterior to left. Arrows indicate the bony canal support on the posterior of the preoperculum, characteristic of some Butidae, the Rhyacichthyidae, Odontobutidae, and Milyeringidae.

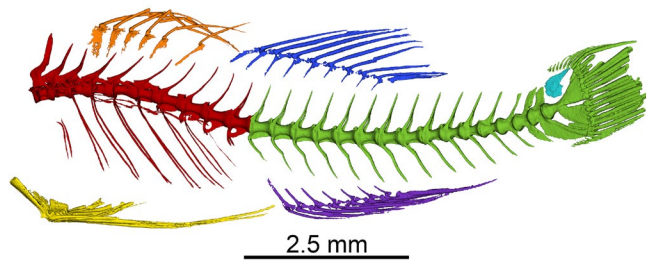


Figure 7. Axial skeleton of *Tempestichthys bettyae*. Ten precaudal vertebrae in red, sixteen caudal vertebrae (including urostylar complex) in green; single epural in cyan; dorsal fin rays and pterygiophores in orange, insertion formula 3-22110 (*sensu* Birdsong et al., 1988); second dorsal fin in blue; pelvic fins in yellow, right side missing; anal fin in purple, one pterygiophore preceding first haemal spine.

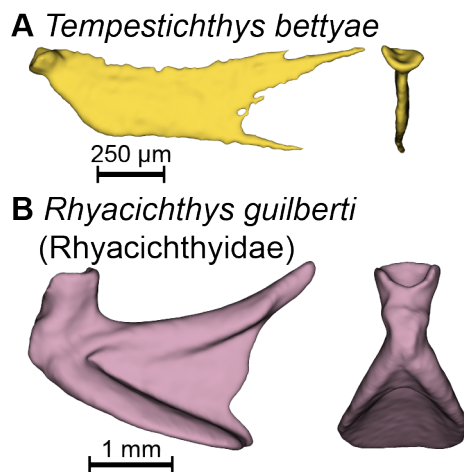
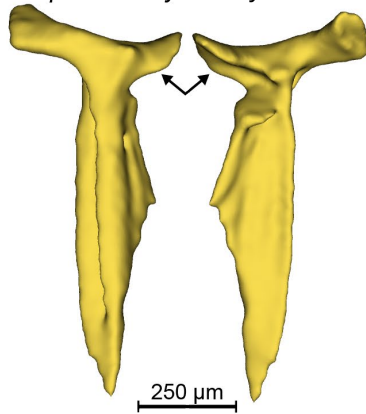
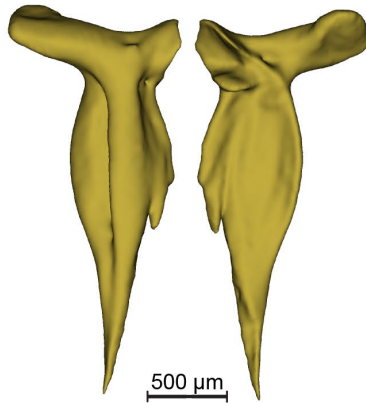


Figure 8. Urohyals of **A** *Tempestichthys bettyae* and **B** *Rhyacichthys guilberti* (*Rhyacichthyidae*) showing the ventral shelf, characteristic of all gobioids except the *Thalasseleotrididae*, *Gobiidae* and *Oxudercidae*.

A *Tempestichthys bettyae*



B *Thalasseleotris adela*



C *Grahamichthys radiatus*



D *Eviota prasites* (Gobiidae)

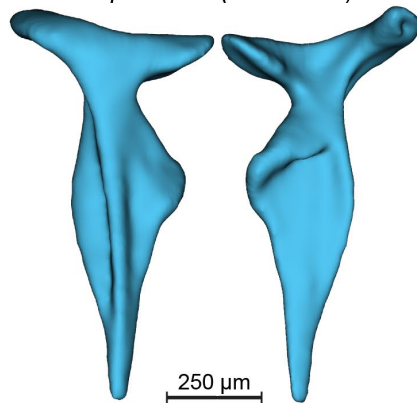
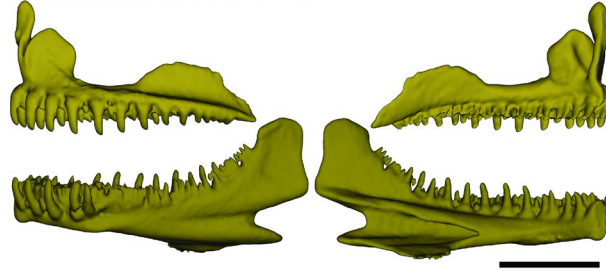


Figure 9. Left palatines of **A** *Tempestichthys bettyae*, **B** *Thalasseleotris adela*, **C** *Grahamichthys radiatus* and **D** *Eviota prasites* (Gobiidae), showing the extended ethmoid processes of *T. bettyae* compared to the other thalasseleotridid genera. Arrows in **A** denote ethmoid processes of the palatine. Lateral views (left) and medial views (right).

A *Tempestichthys bettyae*



B *Thalasseleotris iota*



C *Grahamichthys radiatus*

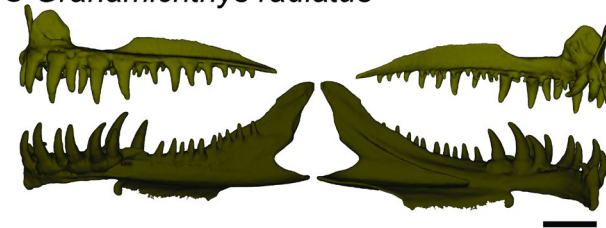
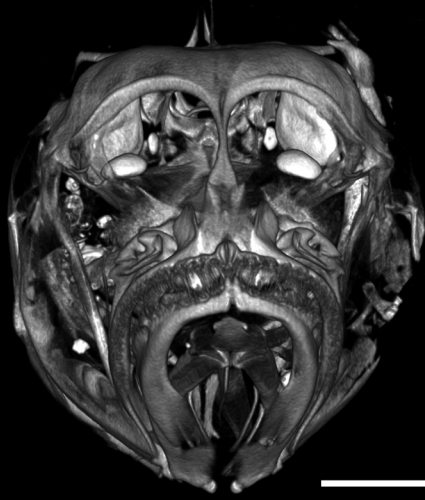


Figure 10. Premaxilla and dentary of **A** *Tempestichthys bettyae*, **B** *Thalasseleotris iota*, and **C** *Grahamichthys radiatus*. Lateral views to the left of each panel, and medial views to the right. Scale bars represent 1 mm.

A *Tempestichthys bettyae* gen. et sp. nov.



B *Thalasseleotris iota* Hoese & Roberts, 2005



C *Grahamichthys radiatus* (Valenciennes, 1837)



Figure 11. Anterior views of **A** *Tempestichthys bettyae*, **B** *Thalasseleotris iota*, and **C** *Grahamichthys radiatus*, highlighting the lateral compression in *T. bettyae* in comparison to the dorsoventral compression in the other thalasseleotridid genera. Scale bars = 1 mm.

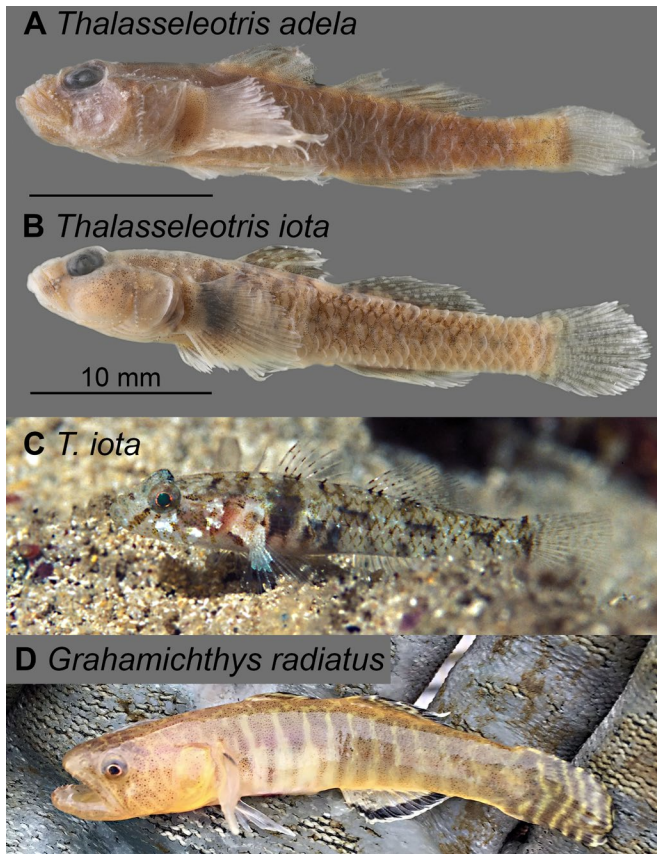


Figure 12. Previously described members of the Thalasseleotrididae. **A** *Thalasseleotris adela* AMS I. 17550-014; **B** *T. iota* AMS I. 41344-001; **C** *T. iota* in life, photograph courtesy of Kendall Clements; and **D** *Grahamichthys radiatus* in life, photograph courtesy of Alison Ballance. All have rounded, dorsoventrally compressed heads and are largely translucent brown in colouration both in preservative (**A** and **B**) and life (**C** and **D**).