

A new lineage of deep-reef gobies from the Caribbean, including two new species and one new genus (Teleostei: Gobiidae: Gobiosomatini)

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Fish communities on tropical deep reefs are dominated by species that belong to families primarily composed of shallow-water species. Collections of deep-reef fishes via submersibles have allowed us to include these deep-reef species in molecular phylogenies, providing insights into the timing and frequency of invasions from shallow to deep reefs. Here we provide evidence of a new deep-reef invasion in the tribe Gobiosomatini in the family Gobiidae (gobies). We describe two new species, one of which belongs to a new genus, and incorporate these taxa into a time-calibrated molecular phylogeny of Gobiosomatini to show that, collectively, these two genera represent a previously unreported independent invasion on to deep reefs that occurred approximately 20–30 million years ago. These new taxa are readily distinguished from related genera and species by a combination of live coloration, pelvic-fin morphology, meristic characters, head-pore patterns and other osteological characters. We discuss the relevance of these two new species to the systematics of the tribe Gobiosomatini and include a comparison to all known genera in the tribe.

ADDITIONAL KEYWORDS: deep sea – exploration – fish – new taxa – phylogeny.

INTRODUCTION

Tropical mesophotic and rariphotic reefs (collectively ‘deep reefs’) support fish communities that are taxonomically related to, but distinct from, those on shallow reefs (Garcia-Sais, 2010; Pinheiro *et al.*, 2016; Baldwin *et al.*, 2018; Rocha *et al.*, 2018). Many of the fish species that occur on deep reefs are absent from shallow reefs, but they are phylogenetically nested in families otherwise dominated by shallow-reef taxa and often represent evolutionary transitions from

shallow- to deep-reef habitats (Tornabene *et al.*, 2016a; Baldwin *et al.*, 2018). The family Gobiidae, commonly known as gobies, is an example of such a group. The majority of the nearly 1300 species of Gobiidae *sensu stricto* (Thacker, 2009) occur in shallow, coastal marine habitats, yet there have been several independent successful radiations of gobies into deep-reef habitats of the wider Caribbean over the last ~20 million years (Tornabene *et al.*, 2016a).

Much of our knowledge of the evolution of deep-reef gobies and the diversity of deep-reef communities in general can be attributed to the use of human-occupied submersibles, which have enabled the collection of specimens from habitats that are difficult to sample using other methods (Gilmore, 2016). This is especially true for gobies in the western Atlantic Ocean, where

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manned submersibles are responsible for the collection of at least 12 new species, nine of which have been formally described prior to this study. Specimens of the new species have facilitated phylogenetic analyses and comprehensive taxonomic reviews of several goby groups (Tornabene *et al.*, 2016a, b, c; Tornabene & Baldwin, 2017; Tornabene & Baldwin, 2019). Deep-reef gobies are particularly well represented in the tribe Gobiosomatini, also known as the American seven-spined gobies, a group that is well known for having undergone rapid adaptive radiation into a variety of habitats, leading to the evolution of morphological and ecological diversity (Rüber *et al.*, 2003; Taylor & Hellberg, 2005; Tornabene *et al.*, 2016a; Huie *et al.*, 2020). While there have been at least two independent invasions of deep reefs in Gobiosomatini (Tornabene *et al.*, 2016a), some species and genera in the tribe have yet to be included in a phylogenetic analysis, including some that occur on deep reefs.

Prior to the use of manned submersibles, collections of deep-reef gobies in the Caribbean came primarily from bottom trawls or dredges, and due to limitations of mesh size and the inability to trawl over structurally complex habitats, samples of deep-reef gobies were extremely rare. Indeed, deep-reef species such as *Pinnichthys bilix* (Hastings & Findley, 2013), *Varicus benthonis* (Ginsburg, 1953), *V. bucca* Robins & Böhlke, 1961, *V. marilynae* Gilmore, 1976 and others that were collected via trawl or dredge are each still known from fewer than six specimens. *Robinsichthys arrowsmithensis* Birdsong, 1988 is another deep-reef goby that was first discovered via deep trawls, and was described from six specimens collected in 1967–68 at a single locality on Arrowsmith Bank off the coast of Yucatan, Mexico (Birdsong, 1988). It is the only member of the genus, originally placed in the tribe Gobiosomatini based on the presence of seven dorsal-fin spines and a 3-22111*0 pattern in the first dorsal-fin pterygiophores (Birdsong, 1988). The presence of modified basicaudal scales also suggested a possible placement within the ‘*Gobiosoma* group’ of that tribe (Birdsong, 1988). As was the normal practice during that time period, tissue samples were not taken for genetic analysis and *Robinsichthys* Birdsong, 1988 has not been included in any of the molecular phylogenetic analyses that broadly sampled across the Gobiosomatini (e.g. Rüber *et al.*, 2003; Thacker & Roje, 2011; Agorreta *et al.*, 2013; Tornabene *et al.*, 2016a, b; McCraney *et al.*, 2020).

In 2018, approximately 40 years after the type series of *Robinsichthys arrowsmithensis* was collected, a single specimen of *Robinsichthys* was captured by the authors during a dive aboard the manned submersible (human-occupied vehicle; HOV) *Curasub* at 229 m depth off the coast of Curaçao in the southern

Caribbean. The specimen was similar in many regards to *R. arrowsmithensis*, but had a number of salient morphological differences indicating it represented a new species. The discovery of a new *Robinsichthys* near Curaçao was particularly noteworthy considering that the authors have collectively conducted more than 150 submersible dives at this exact locality surveying deep-reef biodiversity, resulting in the collection of hundreds of specimens of other fishes, yet this was the first and only observation of this species at Curaçao or anywhere else in the Caribbean.

The discovery of a second species of *Robinsichthys* roughly coincided with the discovery of another deep-reef goby that also may belong to Gobiosomatini. During a 2017 expedition to the Dutch island of Sint Eustatius in the eastern Caribbean, we collected several specimens of a new species of goby at 110–151 m (Robertson *et al.*, 2020; Supplemental Plate S13). In 2017 and 2018, additional specimens of the same species were collected at 191–241 m depth off the north-west coast of Roatan, Honduras, while diving with the HOV *Idabel*. The species from Roatan and Sint Eustatius have several characters supporting its placement in the *Gobiosoma* Girard, 1858 group of Gobiosomatini, but not confidently within any of the existing genera.

The present study focuses on a molecular phylogenetic analysis of *Robinsichthys* and the new species of goby from Roatan and Sint Eustatius. Specifically, we aim to confirm or reject the placement of *Robinsichthys* and the other new species within the *Gobiosoma* group of the tribe Gobiosomatini, and determine whether these two new species are nested in known deep-reef clades or represent another, independent invasion of deep reefs. Here we describe the new species of *Robinsichthys* from Curaçao. We also erect and describe a new genus for the new species from Roatan and Sint Eustatius and provide a comparison to other genera in the *Gobiosoma* group. Collectively, these discoveries add to the growing body of data supporting the continued use of submersibles in studying the evolution and biodiversity of deep-reef taxa.

MATERIAL AND METHODS

SPECIMEN COLLECTION AND MORPHOLOGY

The specimen of *Robinsichthys* from Curaçao and the specimens of the other new species collected off Sint Eustatius were captured using the HOV *Curasub*. Specimens were anaesthetized with a solution of quinaldine dispensed from a hose mounted on a robotic arm on the front of the submersible and were captured using a suction hose mounted on a second arm. Specimens were brought to the surface and

photographed live in an aquarium and/or freshly dead. Tissue samples were taken and stored in saturated salt-DMSO (dimethyl sulfoxide) buffer (Seutin *et al.*, 1991). Specimens were fixed in 10% buffered formaldehyde and stored in 75% ethanol. Specimens from Roatan were collected with the HOV *Idabel*. We used a similar anaesthetic dispersal system on the HOV *Idabel* to that of the HOV *Curasub*, but this time with a solution of 5% quinaldine sulphate. The specimens were collected using a suction hose powered by one of the vertical thrusters of the sub (Tornabene *et al.*, 2018). Those specimens were either preserved directly in 95% ethanol or fixed in 10% buffered formaldehyde and stored in 70% ethanol.

A micro-computed tomography (μ CT) scan of the new species of *Robinsichthys* was taken on a Bruker Skyscan 1173 scanner at the Karel F. Liem Imaging Facility at Friday Harbor Labs, University of Washington. A specimen of the other new species from Roatan was cleared and stained following the protocol of Dingkus & Uhler (1977). All measurements were taken with digital callipers to the nearest 0.1 mm or with Zen Pro imaging software connected to an Axiocam 503 digital camera mounted on a Zeiss Discovery V20 SteREO microscope. Some specimens were temporarily stained in a 2% solution of cyanine blue in distilled water or ethanol following Saruwatari *et al.* (1997) to help visualize scales, papillae and head pores.

Dorsal pterygiophore formula is that of Birdsong *et al.* (1988) and head-pore terminology follows Akihito *et al.* (1988). Sensory papillae are described following the terminology of Sanzo (1911). Definitions of all other morphological characters follow Böhlke & Robins (1968), as modified by Van Tassell *et al.* (2012), who, like many authors, differentiate the unsegmented spine from the segmented rays of the second dorsal, anal and pelvic fins using the roman numeral 'I' for the spine followed by Arabic numbers for the soft rays. Specimens were deposited at the University of Washington Fish Collection (UW), the National Museum of Natural History: Smithsonian Institution (USNM) and at the fish collection of Museo Historia Natural at Universidad Nacional Autónoma de Honduras en el Valle de Sula (MUVS-V).

MOLECULAR PHYLOGENETICS

To confirm the phylogenetic placement of the new species and *Robinsichthys*, we sequenced the three nuclear genes, Recombination activating gene I (RAG1), G protein-coupled receptor 85 (GPR85 or SREB2) and zinc finger protein 1 (ZIC1), along with mitochondrial cytochrome *b* (*Cytb*). Amplification of cytochrome *b* was unsuccessful for several specimens of the new species. Primers and polymerase chain reaction (PCR) conditions follow Agorreta *et al.* (2013). We also sequenced a segment of

mitochondrial cytochrome *c* oxidase subunit I (*COI*). This gene was not used by Agorreta *et al.* (2013) and was not available for most species in our study, but was sequenced here so that new species could be represented in GenBank and BOLD for future studies (see Supporting Information, Table S1). Following the nomenclature of Chakrabarty *et al.* (2013), new sequences here constitute genseq-1 for the holotypes and genseq-2 for the paratypes. Sequences generated here were aligned with those from other species of Gobiosomatini generated in our past studies (Tornabene *et al.*, 2016a, b, c; Tornabene & Baldwin, 2019). Substitution model choice and partitioning scheme were assessed using PartitionFinder2 (Lanfear *et al.*, 2016). Phylogeny was inferred using Bayesian inference in the program MrBayes v.3.2 (Ronquist *et al.*, 2012), using two Metropolis-coupled Markov chain Monte Carlo (MCMC) runs, each with four chains. The analysis was run for ten-million generations, sampling trees and parameters every 1000 generations. Burn-in, convergence and mixing were assessed using TRACER (Rambaut & Drummond, 2007) and by visually inspecting consensus trees from both runs.

DIVERGENCE-TIME ESTIMATION AND ANCESTRAL HABITAT INFERENCE

To determine whether *Robinsichthys* and the other new species represent previously unreported shallow-to-deep transitions, or whether they are nested within other previously identified deep-reef lineages, we inferred ancestral habitats on a time-calibrated phylogeny of Gobiosomatini. Methods for estimating divergence time and inferring habitat follow Tornabene *et al.* (2016a), with the exception of excluding the otolith-based calibration point and including several additional full-skeleton Gobiidae fossils that were described after the 2016 study (see below). We used an uncorrelated, log-normal, relaxed-clock model to infer divergence times using the program BEAST 2.2.3 (Bouckaert *et al.*, 2014), as our data did not fit a strict molecular clock (likelihood ratio test, chi-square distribution, $P < 0.05$). Thacker (2014) and Tornabene *et al.* (2016a) used an Eocene otolith (Bajpai & Kapur, 2004; Gierl *et al.*, 2013) to calibrate the crown age of all gobioid fishes. Since those studies, a fossilized full skeleton from the Eocene representing the species †*Carlomonnus quasigobius* Bannikov & Carnevale, 2016 has been discovered (placement in Gobioidae uncertain; Bannikov & Carnevale 2016), so we chose to use this full skeleton instead of a species known only from otoliths. Collectively, we chose four calibration points for the analysis: (1) based on †*Carlomonnus quasigobius*, we set a minimum crown age for Gobioidae of 50 Mya with a soft upper maximum (log-normal distribution, mean 1.8, sigma 1.0, offset 50.0); (2) we followed Tornabene *et al.* (2016a)

and set the most recent common ancestor (MRCA) of the Atlantic and Pacific species of *Gobulus* to a minimum age of 3 Myr with a soft upper maximum (gamma distribution, $a = 1.9$, $b = 3.0$, $\text{offset} = 2.5$; for justification, see: Tornabene *et al.*, 2016a); (3) we set the crown age of the *Gobius* lineage (*sensu* Agorreta *et al.*, 2013) to minimum age of 19.1 Myr with a soft maximum (log-normal distribution, mean 1.8, sigma 0.8, offset 19.1) based on †*Gobius jarosi* Prikryl & Reichenbacher 2018 [Reichenbacher *et al.* (2018) placed this fossil in the genus *Gobius* Linnaeus, 1758, but to be more conservative, we placed the calibration point provisionally at the crown of the *Gobius* lineage to accommodate uncertainty in the generic placement of this fossil]; and (4) we set the crown age of Gobiidae + Butidae to a minimum of 28 Myr with a soft maximum (log-normal distribution, mean 1.8, sigma 0.8, offset 28.0) based on the †*Pirskeniuss diatomaceus* Obrhelová, 1961, which is provisionally placed as belonging to a clade that contains Butidae, Gobiidae and Thalasseleotridae with the exact position uncertain (Reichenbacher *et al.*, 2020). We fixed the topology of the BEAST to that of the MrBayes' tree analysis to help with convergence (for details, see: Tornabene *et al.*, 2016a) and used the same priors, partitioning scheme, model choice and MCMC settings as Tornabene *et al.* (2016a).

Species in our phylogeny were coded as being deep-reef species if they are known exclusively from depths at or below 60 m and are associated with reef habitats. Some species of Gobiosomatini (e.g. *Bollmannia* spp.) are primarily known from non-reef associated mud bottoms at both shallow and deep depths, and are coded as shallow here (see: Tornabene *et al.*, 2016a). To infer the frequency, dates and topological location of habitat transitions between shallow-water and deep reefs on our phylogeny, we used stochastic character mapping of discrete traits via SIMMAP (Bollback, 2006) in R (R Core Team, 2015), through the function *make.simmap* ('PHYTOOLS' package; Revell, 2012). We used a transition matrix where probabilities of transition rates between habitats were considered equal (ER model, 'equal rates'), since this model did not differ significantly in likelihood from a more complex model (ARD model, 'all rates different') where transition rates from deep-to-shallow and shallow-to-deep were different (likelihood ratio test, $P > 0.05$). We present only the ER model here, but the ARD model is included in the Supporting Information (Fig. S1). To accommodate uncertainty in branching times, we ran the SIMMAP analysis on 100 random post-burn-in trees from BEAST analysis, with 50 habitat mapping MCMC iterations per tree for a total of 5000 simulations. We extracted the timings of habitat transitions for

specific branches in the tree in order to compare the timing of deep-reef invasions across the phylogeny. To visualize habitat transitions on a single tree, we did an additional SIMMAP analysis on the single maximum-clade-credibility tree from our BEAST analysis, using 10 000 MCMC iterations to sample the posterior distribution of possible shallow/deep transition rates. The results of simulations were visualized on that tree with the function *density.map* ('PHYTOOLS' package; Revell, 2012).

RESULTS

The molecular phylogeny (Fig. 1A) shows a sister-relationship between the new species of *Robinsichthys* and the clade comprising the new species from Sint Eustatius and Roatan. The latter does not align with any known genus in Gobiosomatini, and herein we erect the new genus, *Birdsongichthys*, for this new species. Below we describe *Birdsongichthys rectus* as a new species from Sint Eustatius and Roatan and *Robinsichthys nigrimarginatus* as a new species from Curaçao. The *Robinsichthys* + *Birdsongichthys* clade is nested in the *Gobiosoma* group of the Gobiosomatini, but its position relative to the *Barbulifer* C.H.Eigenmann & R.S.Eigenmann, 1888 and *Nes Ginsburg*, 1933 subgroups is less certain (posterior probability of these relationships 0.81–0.85). The time-calibrated phylogeny (Fig. 1B) shows the mean crown age of the Gobiosomatini at 32.58 Myr (95% posterior density within 26.6–38.9 Myr). The split between *Robinsichthys* and the new genus was estimated at 22.0 Myr (15.7–27.9 Myr).

The SIMMAP analysis using the ER model showed that *Robinsichthys* and *Birdsongichthys* together represent an independent invasion of deep reefs. Collectively there are at least three independent transitions on to deep reefs along the following branches (Figs 2, 3): (1) the branch leading to the most recent common ancestor (MRCA) of *Robinsichthys* + *Birdsongichthys*, ~18–30 Mya; (2) the branch leading to the MRCA of *Antilligobius* Van Tassell *et al.*, 2012 + *Palatogobius* Gilbert, 1971, ~5–20 Mya, and (3) the branch leading to the MRCA of the genera *Pinnichthys* Van Tassell *et al.*, 2016, *Psilotris* Ginsburg, 1953 and *Varicus* Robins & Böhlke, 1961, ~5–20 Mya. The ARD model, on the other hand, showed uncertainty regarding the timing and location of habitat transitions within Gobiosomatini, including the possibility that the MRCA of Gobiosomatini was a deep-reef species, with many (~ten) reinvasions back on to shallow reefs over the last 25 million years (Supporting Information, Fig. S1).

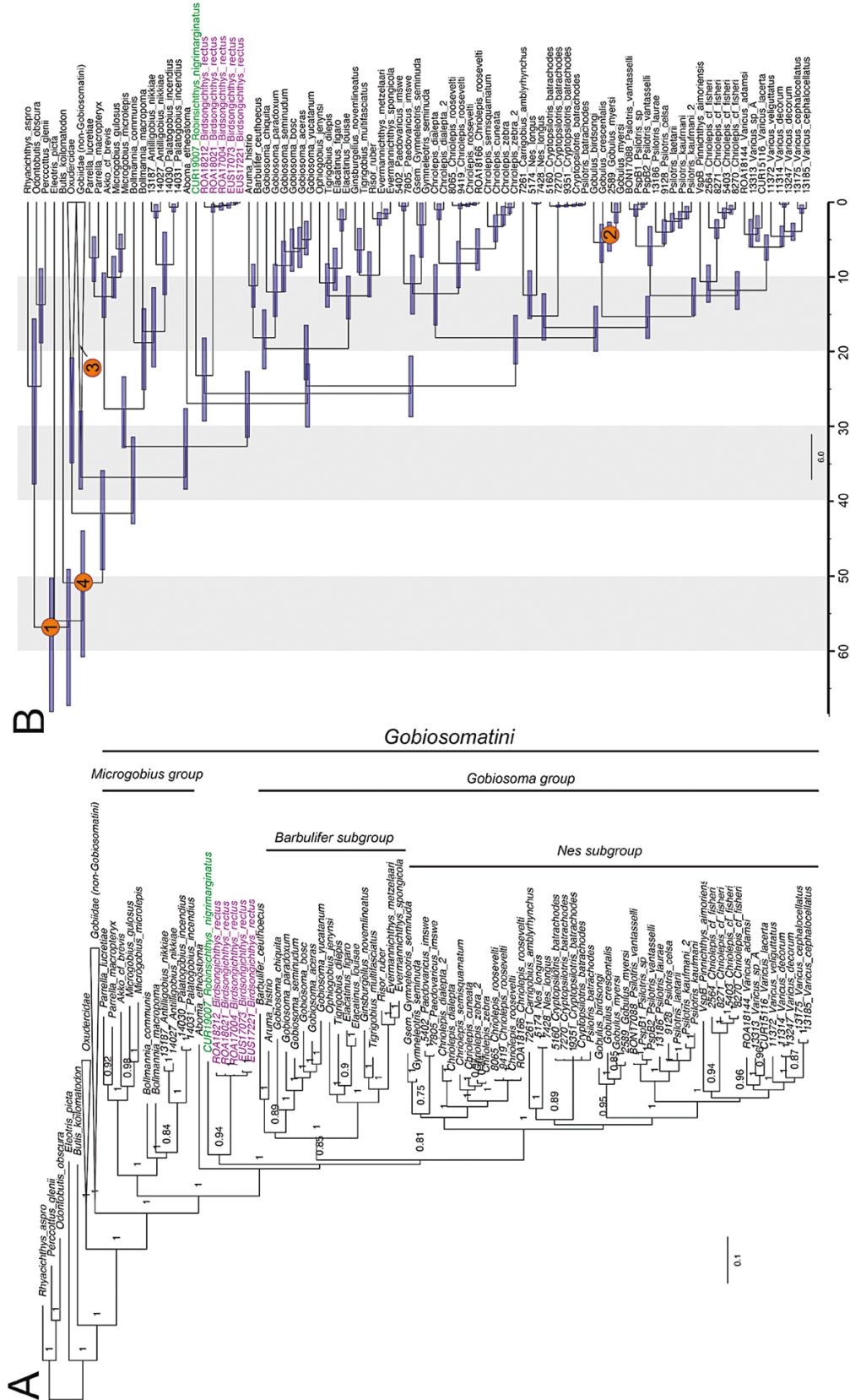


Figure 1. A, molecular phylogeny from MrBayes. Node labels are posterior probability support values. B, time-calibrated tree from BEAST. Node bars are 95% highest posterior densities for age estimates. Coloured taxa are new species. Coloured circles in B refer to the placement of fossil calibration points 1 through 4.

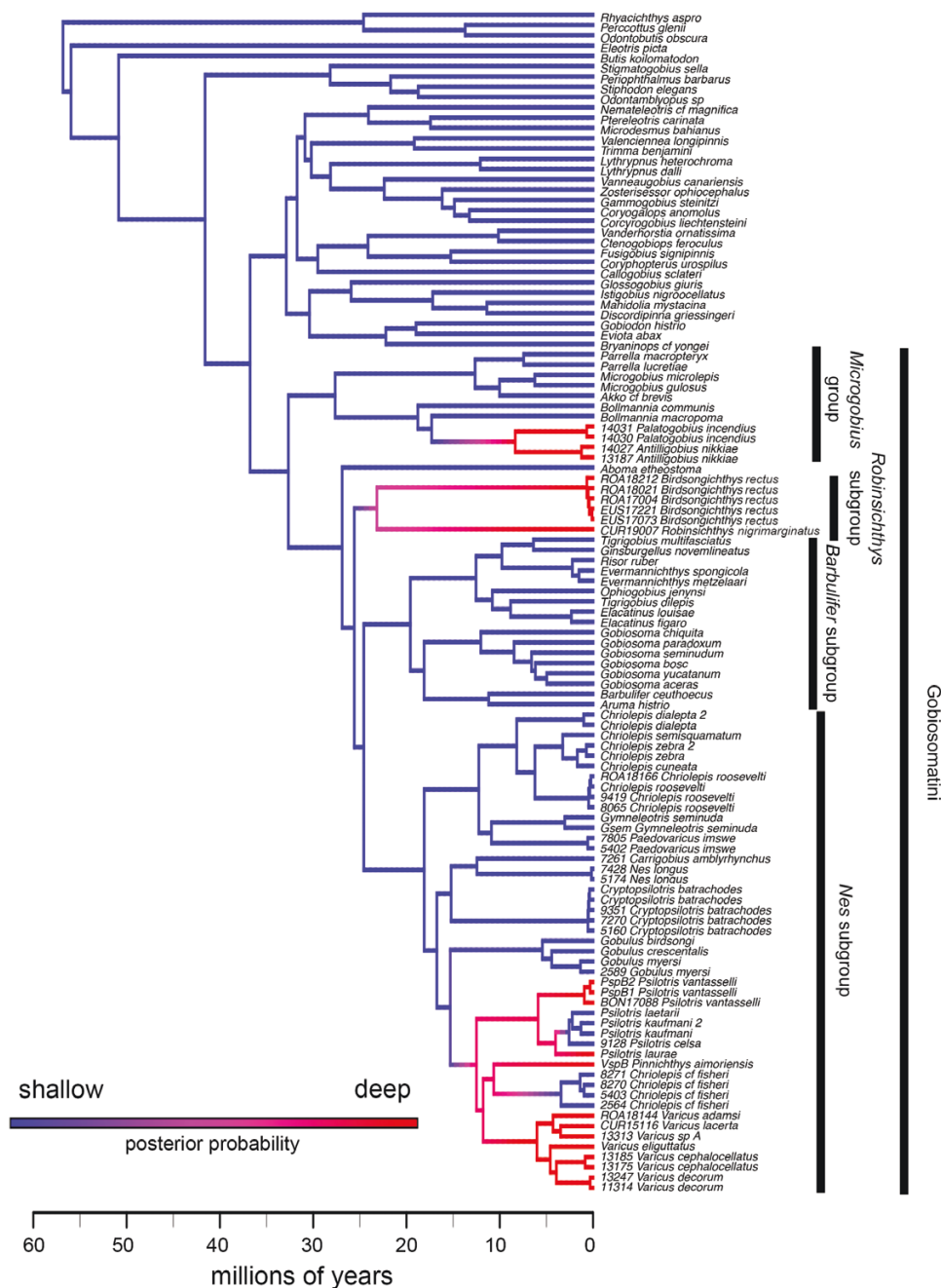


Figure 2. SIMMAP Stochastic character mapping of habitat across the Gobiidae phylogeny based on the ER model. The *Robinsichthys*, *Barbulifer* and *Nes* subgroups, along with the genus *Aboma*, are all part of the *Gobiosoma* group of the Gobiosomatini.

SYSTEMATICS

BIRDSONGICHTHYS GEN. NOV.

Type species: *Birdsongichthys rectus* sp. nov.

Zoobank registration: urn:lsid:zoobank.org:act:287F2C89-6296-4FCB-B7B3-ED7B4B4EE73C

Diagnosis: Possesses all taxonomic characters present in most members of the Gobiosomatini and the *Gobiosoma* group, including the following: first dorsal-fin spines VII; dorsal pterygiophore insertion pattern 3-221110; vertebrae 27–11 precaudal and 16 caudal; hypurals 1 and 2 fused to some extent with hypurals 3 and 4 and the terminal vertebral element; one epural; ventral

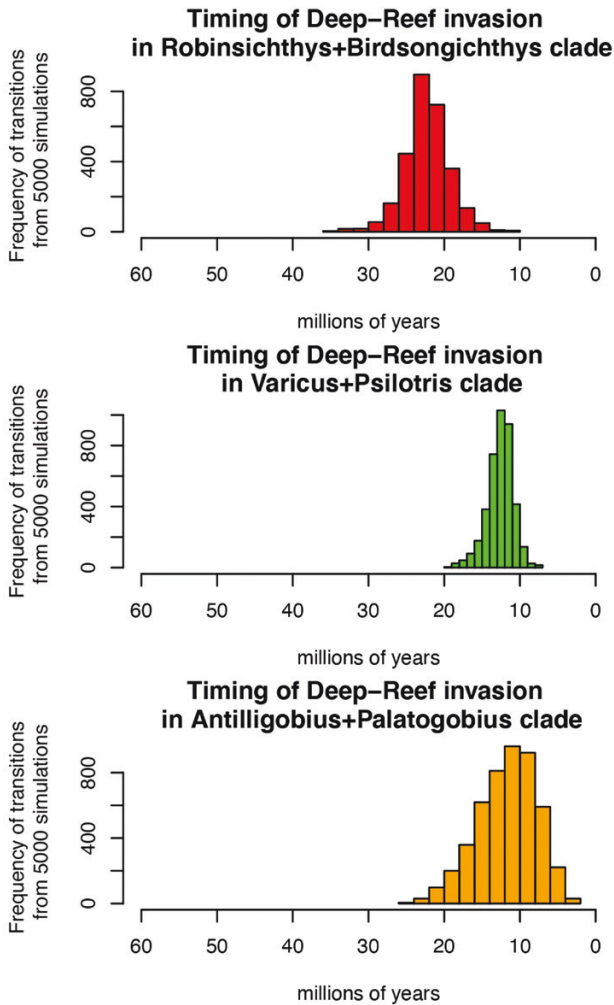


Figure 3. Timing of deep-reef invasions estimated from 5000 SIMMAP iterations across 100 trees from the BEAST divergence-time analysis.

postcleithrum absent. Pelvic fins separate, anterior frenum connecting spines absent, membrane connecting innermost rays absent or weakly developed; pelvic-fin rays 1–5 unbranched and without fleshy or flattened tips; pelvic-fin rays long, extending posteriorly to a point between middle or end of anal fin; side of body covered with ctenoid scales, including two modified basicaudal scales; two anal-fin pterygiophores inserted before first haemal spine; cephalic sensory canals present; second dorsal-fin rays I,9; anal-fin rays I,8; body with three horizontal series of bright yellow spots, one along dorsal midline, one along lateral midline and one along ventral midline; anterior profile of head with gradual slope, not blunt or nearly vertical; eye large (~8.3–10.6% SL).

Etymology: The name honours Dr Ray S. Birdsong, who contributed substantially to the knowledge on

the systematics of the family Gobiidae, especially taxa from the tropical western Atlantic and eastern Pacific Oceans. The root ‘ichthys’ is derived from the Greek ‘ikhthús’ (ἰχθύς), fish.

Comparisons (Table 1): The presence of VII dorsal spines, 11 + 16 vertebrae and a 3-221110 dorsal pterygiophore pattern (Fig. 4A) all support placement of *Birdsongichthys* in Gobiosomatini. Gobiosomatini include two main clades, the *Microgobius* group and the *Gobiosoma* group. *Birdsongichthys* shares several characters with genera of the *Microgobius* group (*Antilligobius*, Akko Birdsong & Robins, 1995, *Bollmannia* Jordan, 1890, *Microgobius* Poey, 1876, *Palatogobius* and *Parrella* Ginsburg, 1938), including a laterally compressed body and no substantial fusion between hypurals 3–4 and hypurals 1–2 and the terminal vertebral element. It differs from all *Microgobius* group genera in having modified basicaudal scales (absent in the *Microgobius* group) and in having a truncate or emarginate caudal fin (vs. lanceolate in the *Microgobius* group). The *Gobiosoma* group contains four lineages: the *Barbulifer* subgroup, the *Nes* subgroup, *Aboma* Jordan & Starks, 1895 and *Robinsichthys*. *Birdsongichthys* can be distinguished from all members of the *Barbulifer* subgroup (*Aruma* Ginsburg, 1933, *Barbulifer*, *Elacatinus* Jordan, 1904, *Evermannichthys* Metzelaar, 1919, *Ginsburgellus* Böhlke & Robins, 1968, *Gobiosoma*, *Ophiogobius* Hoesé, 1976, *Pariah* Böhlke, 1969, *Risor* Ginsburg, 1933 and *Tigrigobius* Fowler, 1931) and *Aboma* by its completely separate pelvic fins that lack an anterior frenum and have unbranched rays; the *Barbulifer* subgroup and *Aboma* have pelvic fins united to form a complete disc with branched rays. Completely separate pelvic fins also characterize species in the *Nes* subgroup, except *Nes* and *Gobulus*. *Birdsongichthys* differs from most genera of the *Nes* subgroup and from *Robinsichthys* in having cephalic lateralis canals and associated head pores. Among *Nes* subgroup genera, only *Eleotrica* Ginsburg, 1933, *Gymneleotris* Bleeker, 1874, and two species of *Chriolepis* Gilbert, 1892 possess head pores. *Birdsongichthys* has unbranched pelvic-fin rays, whereas these three genera all have branched pelvic-fin rays. The only Gobiosomatini genera containing species with completely unbranched pelvic-fin rays are the monotypic *Paedovaricus* Van Tassell *et al.*, 2016 [*P. imswe* (Greenfield, 1981)] and *Varicus* (unbranched in specimens of five of ten known species in the genus). *Birdsongichthys* differs from these two genera in having two (vs. one) anal-fin pterygiophores inserted anterior to the first haemal spine. Finally, *Birdsongichthys* can be further distinguished from *Robinsichthys* in having 11 + 16 vertebrae (vs. 11 + 17).

Table 1. Comparison of genera in the Gobiosomatini

Genus	Group	Subgroup	D1	D2	A	Vertebrae	AP	Pterygiophore pattern	Hypural Fusion
<i>Gobiosoma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,9-14	I,8-11	11 + 16	2	3(221110)	partially fused
<i>Elacatinus</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,9-12 (rarely 13)	I,9-12	11 + 17	2	3(221110)	partially fused
<i>Tigrigobius*</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,8-12	I,8-9	11 + 17	2	3(221110)	partially fused
<i>Risor</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,10-11	I,8-10	11 + 17	2	3(221110); 3(230110)	partially fused
<i>Evermannichthys</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	III-VII	I,10-15	I,8-10	varies widely**	2 or 3	varies widely**	partially fused
<i>Pariah</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VIII	I,10-11	I,7-8	12 + 17	2	3(221110)	partially fused
<i>Ophiogobius</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII/VIII	I,15	I,13	13 + 18 or 13 + 19	2	3(221110) or 3(212110)	partially fused
<i>Aruma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,10-12	I,10-12	11 + 16 or 11 + 17 or 12 + 16	2	3(212110), rarely 3(221110)	partially fused
<i>Ginsburgellus</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,10-12	I,9	11 + 17	2	3(221110)	partially fused
<i>Barbulfifer</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	VII	I,8-12	I,7-10	11 + 16	2	3(221110)	partially fused
<i>Aboma</i>	<i>Gobiosoma</i>	<i>Aboma</i>	VII	I,10-11	I,9-10	11 + 16	2	3(221110)	not fused
<i>Nes</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,12-13	I,10-12	11 + 17 or 12 + 16	2	3(221110)	partially fused
<i>Gobulus</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,9-13	I,7-12	11 + 16 or 11 + 17	2	3(221110), rarely 3(212110)	partially fused
<i>Cryptopsilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,8-9	I,6-7	11 + 16 or 12 + 15	1	3(221110)	partially fused
<i>Psilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,9-10	I,7-10	11 + 15 or 11 + 16	2, 1 in <i>P. laurae</i>	3(221110)	partially fused
<i>Varicus</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,8-10	I,7-9	11 + 16	1	3(221110)	partially fused
<i>Paedovaricus</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,7	I,7	11 + 16	1	3(221110)	partially fused
<i>Chrtolepis*</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,8-11	I,8-10	11 + 16	2	3(221110)	partially fused
<i>Pinnichthys</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,10-11	I,10-11	11 + 16	2	3(221110)	partially fused
<i>Carrigobius</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,10-11	I,8-10	11 + 16	2	3(221110)	partially fused
<i>Gymneleotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,10	I,9	11 + 16	2	3(221110)	partially fused
<i>Eleotrica</i>	<i>Gobiosoma</i>	<i>Nes</i>	VII	I,11	I,9-10	11 + 16	2	3(221110)	partially fused
<i>Robinsichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	VI-VII	I,9-10	I,9-10	11 + 17	2	3(22111*0) or 3(221110) or 3(22111*00)	not fused
<i>Birdsongichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	VII	I,9	I,8	11 + 16	2	3(221110)	not fused

Table 1. Continued

Genus	Group	Subgroup	Head pores present/absent	Preopercle pores	Modified basicaudal scales	Body scales	Papillae rows 5i/5s
<i>Gobiosoma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', C, D, E, F, H' (K', L' sometimes present)	2 or 3	present in some species	variable	vairable, connected or separate
<i>Elacatinus</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', C, D, E, F, H'	2	absent except for <i>E. puncticaulum</i>	absent	variable
<i>Tigrigobius*</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', C, D, E, F, H'	2	present in some species	variable	variable
<i>Risor</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', D, F' (C sometimes present)	absent	present	posterior only	connected
<i>Evermannichthys</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', C (C sometimes absent)	absent	present in some species	lower caudal peduncle only	not present
<i>Pariah</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	absent	absent	present	lower caudal peduncle only	connected
<i>Ophiogobius</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', D, F, H' canal separate B to D	2	absent	absent	separate; 6 and 5s connected
<i>Aruma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', D, F, H' canal single B to D	2	absent	absent	connected
<i>Ginsburgellus</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', C, D, E, F, H'	2	absent	absent	separate
<i>Barbulfifer</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	B', F, H' in most species; B', F' in <i>B. patherinus</i>	2	absent	absent	connected or slightly separate
<i>Aboma</i>	<i>Gobiosoma</i>	<i>Aboma</i>	B', C, D, E, F, G, H' and K', L'	3	present	present	separate
<i>Nes</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	absent	absent	separate
<i>Gobulus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	absent	absent	separate, but connected in <i>G. birdsongi</i>
<i>Cryptopsilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	absent	absent	separate
<i>Psilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	absent, except in <i>P. vantasselli</i>	absent	variable
<i>Varicus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	present, except in <i>V. lacerta</i>	present except <i>V. decorum</i> and <i>V. lacerta</i>	connected in all but <i>benthonis</i> , <i>vespa</i> , variable in <i>cephalocellatus</i>
<i>Paedovaricus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	present	present	no info
<i>Chrioletpis*</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent in most species; B', D, F' in <i>C. roosevelti</i> ; B', D, F, H' or G' in <i>C. semisquamatum</i>	absent	present	present	separate

Table 1. Continued

Genus	Group	Subgroup	Head pores present/absent	Preopercle pores	Modified basicaudal scales	Body scales	Papillae rows 5i/5s
<i>Pinnichthys</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	present	present	separate
<i>Carrigobius</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	absent	absent	possibly connected, faint line, no papillae remaining
<i>Gymneleotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	B', F, H'	2	absent	present	separate
<i>Eleotrica</i>	<i>Gobiosoma</i>	<i>Nes</i>	B', D, F, and H'	absent	present	absent	separate
<i>Robinsichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	absent	absent	present	present	separate in <i>R. arrowsmithensis</i> , connected in <i>R. nigrimarginatus</i>
<i>Birdsongichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	B', D, E'	absent	present	present	no info
Genus	Group	Subgroup	Pelvic fin anterior frenum	Pelvic fin membrane	Pelvic rays 1–4 tips	Pelvic ray 5 tips	Longest pelvic ray
<i>Gobiosoma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Elacatinus</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Tigrigobius*</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Risor</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Evermannichthys</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Pariah</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Ophiogobius</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Aruma</i>	<i>Gobiosoma</i>	<i>Barbulfifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th

Table 1. Continued

Genus	Group	Subgroup	Pelvic fin anterior frenum	Pelvic fin membrane	Pelvic rays 1–4 tips	Pelvic ray 5 tips	Longest pelvic ray
<i>Ginsburgellus</i>	<i>Gobiosoma</i>	<i>Barbulifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Barbulifer</i>	<i>Gobiosoma</i>	<i>Barbulifer</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Aboma</i>	<i>Gobiosoma</i>	<i>Aboma</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Nes</i>	<i>Gobiosoma</i>	<i>Nes</i>	present, well developed	well developed, complete disc	branched, not flattened or fleshy	branched	5th
<i>Gobulus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent or reduced	developed along at least 1/3 length	branched, not flattened or fleshy	branched	4th
<i>Cryptopsilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy	unbranched	4th
<i>Psilotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy except <i>P. boehlkei</i> with slightly fleshy tips	unbranched	4th
<i>Varicus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	unbranched, branched internally and re-fused with pointed fleshy pads, or branched to tips with flat, spatulate fleshy pads	unbranched	4th
<i>Paedovaricus</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	unbranched, not fleshy	unbranched	4th
<i>Chriolepis*</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy	unbranched, branched or variable	4th
<i>Pinnichthys</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, sometimes with flattened tips; no fleshy tips	unbranched	4th
<i>Carrigobius</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy	branched	4th or 5th, nearly equal

Table 1. Continued

Genus	Group	Subgroup	Pelvic fin anterior frenum	Pelvic fin membrane	Pelvic rays 1–4 tips	Pelvic ray 5 tips	Longest pelvic ray
<i>Gymneleotris</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy	branched	4th or 5th, nearly equal
<i>Electrica</i>	<i>Gobiosoma</i>	<i>Nes</i>	absent	absent	branched, not flattened or fleshy	unbranched, branched or variable	4th or 5th, nearly equal
<i>Robinsichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	present and well-developed, or absent	well developed, complete disc, or absent	branched, not flattened or fleshy in <i>R. nigrimarginatus</i> , moderately enlarged in holotype of <i>R. arroumithensis</i>	branched	5th
<i>Birdsongichthys</i>	<i>Gobiosoma</i>	<i>Robinsichthys</i>	absent	absent/poorly developed	unbranched, not flattened or fleshy	unbranched	4th, nearly equal

*genus is not monophyletic.
 *See Birdsong et al. (1988) for wide range of vertebral counts and pterygiophore insertion patterns.
 Abbreviations: D1, first dorsal fin; D2, second dorsal fin; A, anal fin; AP, anal fin pterygiophores.

BIRDSONGICHTHYS RECTUS SP. NOV.

(FIGS 4A, 5–7)

Straightfinned goby (English), *gobio de aleta recta* (Spanish).

Gobiidae genus 2, species 1 Robertson et al., 2020: table 1, supplemental plate S13.

Zoobank registration: urn:lsid:zoobank.org:act:016EDDA3-572B-45CB-8B05-676076400186

Holotype: USNM 442874, 14.1 mm SL female, tissue EUS17221, field number CURASUB17-21, 151 m depth, rubble patch, south and south-east of R/V Chapman mooring, SW of island, Kay Bay, Sint Eustatius, 17.4566, –62.9791, quinaldine, HOV Curasub and R/V Chapman, Carole Baldwin, Luke Tornabene, Bruce Brandt, 19 April 2017.

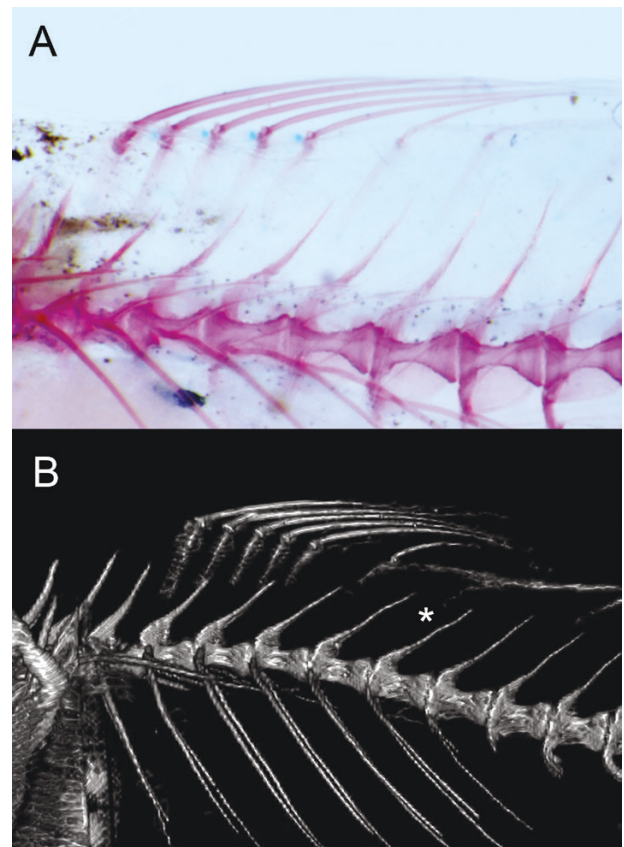


Figure 4. Vertebrae and pterygiophore insertion patterns for: A, *Birdsongichthys rectus* gen. nov., sp. nov., 3-221110 pattern, paratype UW 158074, cleared and stained; B, *Robinsichthys nigrimarginatus* sp. nov., 3-22111*0 pattern, holotype, USNM 446953, micro-CT-scan. *Indicates pterygiophore without associated dorsal-fin spine.

Paratypes: MUVS-V-439, 21.0 mm SL male, tissue ROA18021, field number IDABEL18-01, 213 m depth, sand and limestone rubble, outside Halfmoon Bay, Roatan, Honduras, 16.304384°, -86.598204°, quinaldine sulphate, HOV *Idabel*, D. Ross Robertson, Rachel Manning, Karl Stanley, 4 June 2018; UW 158074, 18.3 mm female, tissue ROA17004, cleared and stained, field number IDABEL17-01, 192 m depth, outside Halfmoon Bay, Roatan, Honduras, 16.305557°, -86.597669°, quinaldine sulphate, HOV *Idabel*, Luke Tornabene, Ross Robertson, Karl Stanley, 24 July 2017; UW 158128, 12.0 mm female, tissue ROA18044, field number IDABEL18-03, 241 m depth, sand rubble in rock hole, 16.3043°, -86.5982°, quinaldine sulphate, HOV *Idabel*, Luke Tornabene, Rachel Manning, Karl Stanley, 6 June 2018; UW 158200, 11.5 mm male, tissue ROA18212, field number IDABEL18-08, 213 m depth, sand at base of limestone, 16.3043°, -86.5982°, quinaldine sulphate, HOV *Idabel*, Luke Tornabene, Katherine Maslenikov, Karl Stanley, 1 June 2018; USNM 442726, 14.4 mm male, tissue EUS17073, field number CURASUB17-18, 110 m depth, rubble and sand on wall, dropoff west of R/V *Chapman* mooring, Gallows Bay, Sint Eustatius, 17.4608°, -63.0179°, quinaldine, HOV *Curasub* and R/V *Chapman*, Carole Baldwin, Luke Tornabene, Barbara van Bebber, Jordan Casey, 16 April 2017.

Diagnosis: *Birdsongichthys rectus* is the only known member of the genus, so the species diagnosis is that of the diagnosis of the genus.

Description: Morphometric data are presented in Table 2. Median and paired fins: first dorsal fin VII(6), first spine extremely elongate in some specimens,

extending posteriorly to point between middle of soft dorsal fin and base of anal fin when flat against body; second dorsal fin I,8*(1) or I,9(5); anal fin I,8*(6); pectoral-fin rays 16*(3), 17 (2), 18(1), extending posteriorly to a vertical through second dorsal-fin ray 2 or 3; pelvic fin I,5*(5), all rays unbranched; pelvic fins well separated, lacking anterior frenum, membrane connecting fifth rays absent or reduced; fourth and fifth pelvic-fin rays approximately equal in length, extending posteriorly to base of anal-fin rays 6–7; tips of pelvic-fin rays not ending in fleshy pads; caudal fin truncate or emarginate; branched caudal-fin rays 12 (4)*, segmented caudal-fin rays 17(5)*, caudal fin broken in many specimens.

Scales: Scales on head and nape absent; ctenoid scales on side of body present starting above pectoral fin and continuing to caudal peduncle; lateral scale rows 22–23, transverse scale rows 5, scales counted from three specimens including holotype, scales lost in other specimens; pair of modified ctenoid scales with enlarged cteni present at base of caudal fin, one each at dorsal and ventral margins of fin (Fig. 5); abdomen and chest each with small patch of ctenoid scales.

Head: Jaw extending posteriorly to a vertical through anterior margin of eye or midpoint of pupil; anterior naris a short tube, posterior naris an opening flush with snout; cephalic lateralis pores small, pores B', D(s), E' present in four specimens, pores B' extremely small and pore D missing in holotype, pores B' not visible in paratype UW 158200; eyes large, 30–36% HL, positioned dorsolaterally. Upper jaw with two to three rows of teeth anteriorly, outermost row enlarged widely spaced canines continuing along entire upper jaw to the end

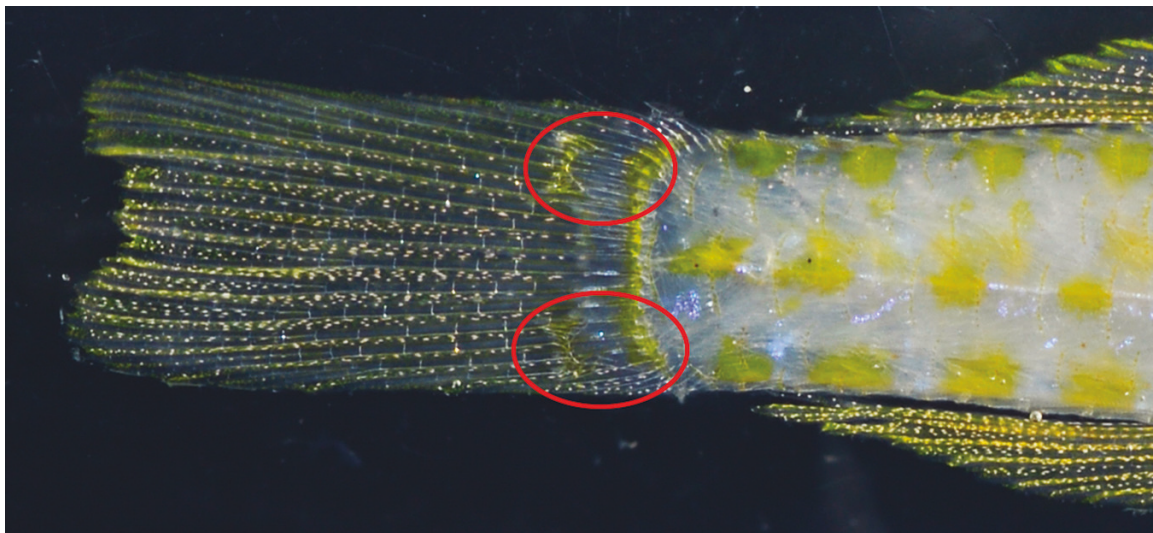


Figure 5. Modified basicaudal scales of *Birdsongichthys rectus* gen. nov., sp. nov., paratype, USNM 442726.

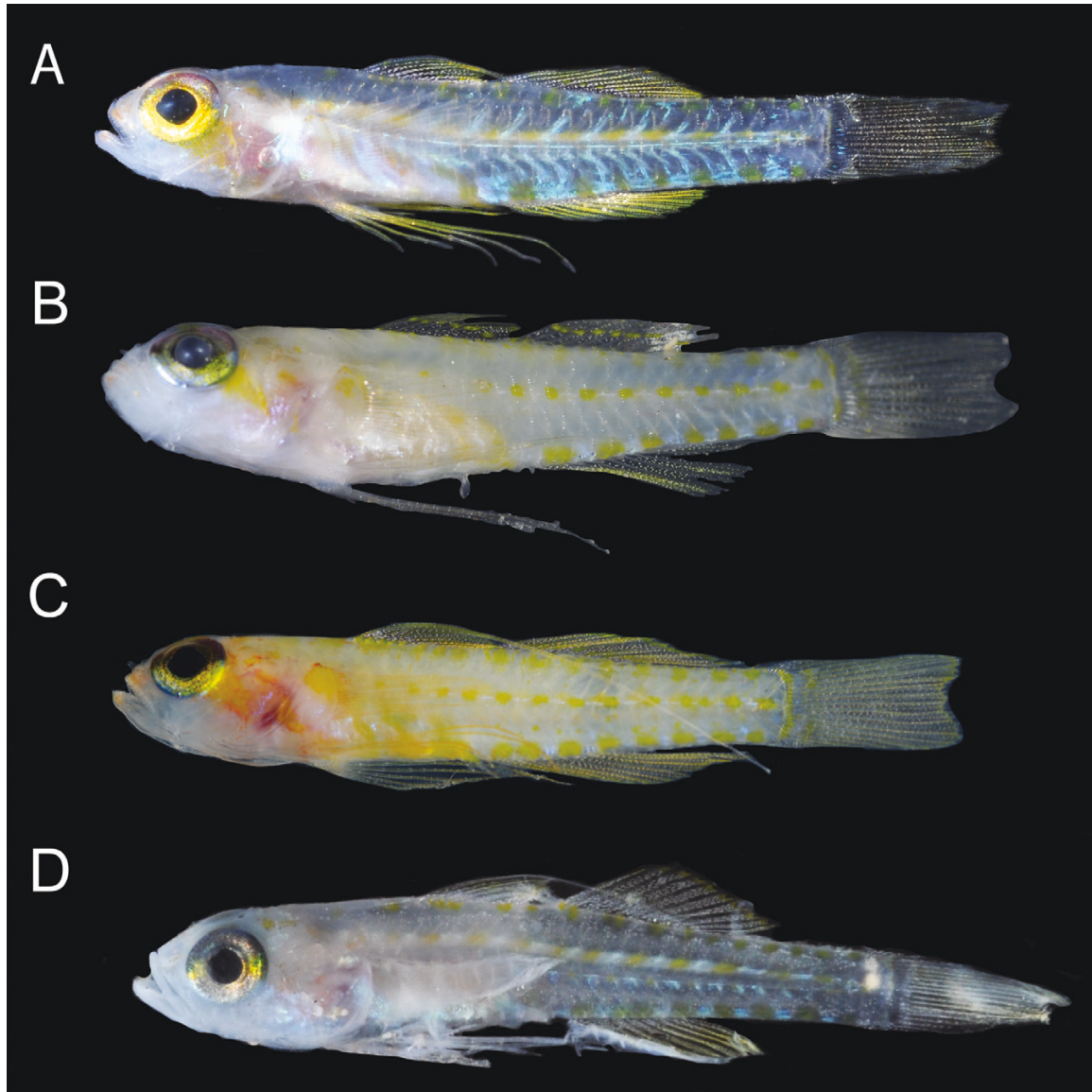


Figure 6. Fresh coloration of *Birdsongichthys rectus* gen. nov., sp. nov.. A, MUVS-V-439, 21.0 mm SL male, ROA18021, Roatan; B, USNM 442874, holotype, 14.1 mm SL female, tissue EUS17221, Sint Eustatius, image flipped horizontally; C, USNM 442726, 14.4 mm male, tissue EUS17073, Sint Eustatius, image flipped horizontally; D, UW 158128, 12.0 mm female, tissue ROA18044, Roatan. Photos by R. Manning (A, D) and C. Baldwin (B, C).

of the premaxilla, innermost two rows tightly packed, shorter, and conical, becoming single row posteriorly; lower jaw with three to five rows of teeth anteriorly, outermost row restricted to tip of dentary and slightly larger than innermost rows, innermost rows becoming two rows posteriorly. Sensory papillae not visible on most specimens due to small size of specimens and damage to the face from collection.

Genitalia: Male urogenital papilla elongate and pointed; female urogenital papilla short, bulbous or slightly conical.



Figure 7. *Birdsongichthys rectus* gen. nov., sp. nov. holotype, preserved, USNM 442874, 14.1 mm SL female. Photo by L. Tornabene.

Table 2. Morphometrics for *Birdsongichthys rectus* gen. nov., sp. nov. and *Robinsichthys nigrimarginatus* sp. nov. All measurements are in %SL unless otherwise noted

	<i>Birdsongichthys rectus</i>					<i>Robinsichthys nigrimarginatus</i>	
	USNM 442726	UW158112	UW 158128	UW 158074	UW 158200 442874	USNM 442874	USNM 446953
Tissue number	EUS17073	ROA18021	ROA18044	ROA17004	ROA18212	UES17221	CUR19007
Standard length (mm)	14.4	19.5	12	18.3	11.5	14.1	11.5–19.5 (15.1)
Head length	29.2	32.8	27.5	30.1	27.8	29.2	27.5–32.8 (29.5)
Eye diameter	9.7	10.3	8.3	9.8	9.6	10.6	8.3–10.6 (9.7)
Snout length	4.2	4.1	5.8	4.9	2.6	5.7	2.6–5.8 (4.6)
Jaw length	10.4	9.7	11.7	10.4	13.0	9.6	9.3–13.0 (10.9)
Post orbit length	10.4	11.3	13.3	11.5	11.3	13.2	11.3–13.3 (12.1)
Distance to first dorsal-fin origin	35.4	35.9	40.0	35.0	37.4	34.7	34.7–40 (36.6)
Body depth at first dorsal-fin origin	16.7	16.9	17.5	19.7	17.4	16.6	16.56–19.7 (17.6)
Distance to anal-fin origin	55.6	59.0	57.5	60.1	58.3	56.7	56.7–60 (58.3)
Body depth at anal-fin origin	16.7	16.4	14.2	16.9	14.8	15.5	14.2–16.9 (15.6)
Pectoral fin length	21.5	22.6	broken	21.3	broken	broken	21.3–22.6 (21.9)
Pelvic fin length	30.6	39.5	39.2	35.5	broken	36.8	35.5–39.5 (37.8)
Caudal peduncle depth	11.1	10.8	10.0	11.5	9.6	10.6	9.6–11.5 (10.5)
Caudal fin length	26.4	25.6	broken	27.3	broken	25.5	25.5–27.3 (26.2)

Colour in fresh specimens (Fig. 6): Side of body translucent with iridescent scale margins; three horizontal rows of small, bright yellow spots along flank, spots largest in the bottom row; first row with 12–15 spots starting on nape and continuing along dorsal midline, ending just anterior to caudal-fin base; second row with 13–15 spots starting above pectoral fin and continuing along lateral midline, ending just anterior to caudal-fin base; third row with eight to nine spots starting just anterior to anal-fin origin and continuing along ventral midline, ending just anterior to caudal-fin base; tips of snout and upper and lower jaws with small patch of yellow; eye with upper-half of iris black, lower-half yellow; a broad, oblique yellow to orange stripe extending from lower-rear part of iris down on to preopercle; remainder of head translucent; pink coloration of red gill arches visible through operculum; pectoral-fin base with yellow spot on yellow upper margin of some specimens; dorsal fins with uniform translucent yellow wash with scattered iridophores, and with a distinct horizontal series of yellow spots along midpoints of spines and rays in some specimens; pectoral fins pale or uniformly translucent yellow; caudal fin faintly translucent yellow with two dark yellow, vertical bars at base; anal and pelvic fins uniformly translucent yellow.

Colour in preservation (Fig. 7): Body uniformly pale yellowish, no dark pigmentation remains in any preserved specimens, including those fixed either in 95% ethanol or in formalin followed by storage in 70–75% ethanol.

Habitat: Known from 110–241 m depth on sand and rubble substrates situated on or around the base of walls or steep rocky slopes on deep reefs.

Distribution: Known from Roatan, Honduras and Sint Eustatius, Caribbean Netherlands.

Etymology: The epithet is from the Latin adjective *rectus*, straight, which refers to the straight, unbranched rays of the pelvic fin.

***ROBINSICHTHYS NIGRIMARGINATUS* SP. NOV.**

(FIGS 4B, 8–11)

Black-margined goby (English), *gobio de margen negro* (Spanish).

Zoobank registration: urn:lsid:zoobank.org:act:9D9D60B1-2777-47FC-8CB0-6A7E0F70641C

Holotype: USNM 446953, 21.0 mm female, tissue CUR19007, field number CURASUB19-02, 229 m

depth, sandy bottom, East of Substation downline, Substation Curaçao, Bapor Kibra, Curaçao, 12.0838°, -68.8991°, quinaldine, HOV *Curasub*, Carole Baldwin, Luke Tornabene, Tico Christiaan, Sarah Yerrace, 7 May 2019.

Diagnosis: First dorsal-fin pterygiophore pattern 3-22111*0 (last pterygiophore lacking an associated dorsal-fin spine; Fig. 4B); VI spines in first dorsal fin; 11 + 17 vertebrae; two modified basicaudal scales; head pores absent; second dorsal fin I,9; anal fin I,9; pelvic fins completely united by membrane to form a round disc with well-developed anterior frenum; pectoral-fin rays 19.

Description: Morphometric data are presented in Table 2. Median and paired fins: First dorsal fin VI,



Figure 8. United pelvic fins of *Robinsichthys nigrimarginatus* sp. nov. Dotted lines indicate extent of membrane connecting rays and anterior frenum connecting spines.

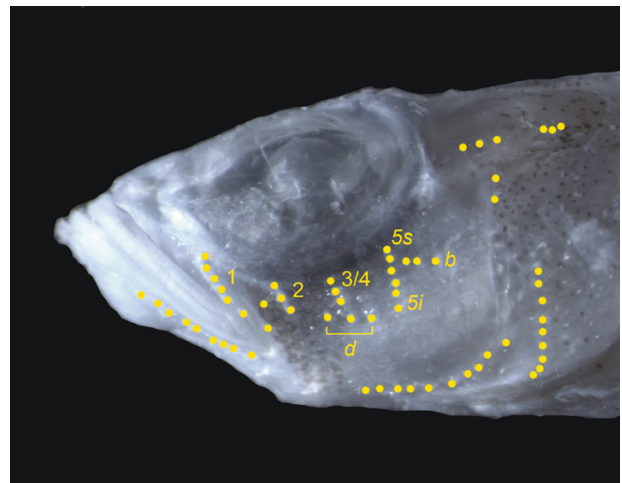


Figure 9. Sensory papillae arrangement for *Robinsichthys nigrimarginatus* sp. nov.

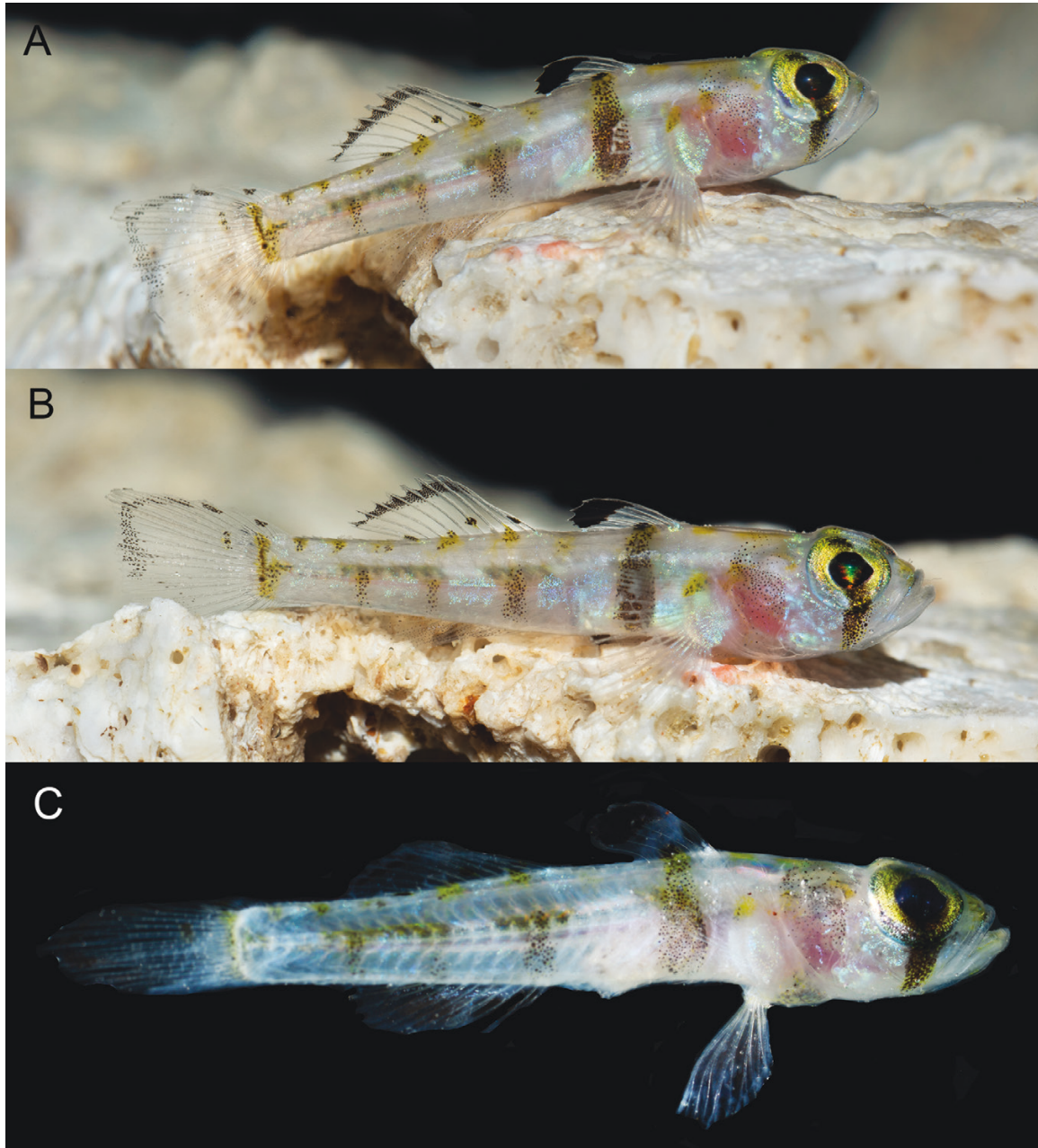


Figure 10. Coloration of *Robinsichthys nigrimarginatus* sp. nov. in life (A, B) and freshly dead (C). Photos by B. Brown (A, B) and C. Baldwin (C).

no spines elongate; second dorsal fin I,9; anal fin I,9; pectoral-fin rays 19, extending posteriorly to a vertical through second dorsal-fin ray 2 or 3; pelvic fin I,5, all rays branched; pelvic fins connected via membrane forming a complete disk with well-developed anterior frenum connecting pelvic spines (Fig. 8); pelvic fin extending posteriorly to just before anus; tips of pelvic-fin rays not ending in fleshy pads; caudal fin truncate; branched caudal-fin rays 14, segmented caudal-fin rays 17.



Figure 11. Preserved holotype of *Robinsichthys nigrimarginatus* sp. nov. Photo by L. Tornabene.

Scales: Scales on head and nape absent; ctenoid scales on side of the body present starting above pectoral fin base and ending at base of caudal fin rays; lateral scale rows 24, transverse scale rows six; pair of modified ctenoid scales with enlarged cteni present at base of caudal fin, one each at dorsal and ventral margins of fin; ctenoid scales present on abdomen, scales slightly larger than those on side of body.

Head: Jaw extending posteriorly to a vertical through midpoint of the pupil; anterior naris a short tube, posterior naris an opening with raised rim; cephalic lateralis pores absent; eyes large, 41% HL, dorsolateral. Both jaws with two rows of teeth, teeth thin, straight, pointed and evenly spaced posteriorly, becoming less regularly spaced, slightly more recurved anteriorly, with an occasional tooth in outer row slightly larger than others in row. Sensory papillae arranged as in Figure 9, with four prominent transverse rows, including rows 5i/5s present as a continuous single row.

Colour in life (Fig. 10): Body translucent with a silvery sheen, an internal white area around gut; body with five dark bars formed by aggregations of black chromatophores overlaying translucent yellow areas, the first bar strongest and widest, extending from the centre of the base of the first dorsal fin to the lower belly, the second to fifth only on the lower-half of the body, thin, formed by less dense aggregations of tiny black chromatophores, the last bar partly fused to the bar on the base of the caudal fin; upper-body profile with six small, translucent yellow blotches, each with a central area of tiny, black chromatophores, the first blotch between the two dorsal fins, the second under the front of the second dorsal fin, the third under the centre of that fin, the fourth to sixth on the caudal peduncle; two elongate internal areas of dark pigment with yellow tones along the spinal column, a short one under the second body bar, a longer one from third body bar to just behind the fourth body bar, with a suggestion of a third patch above the last body bar; when the fish is laterally oriented (Fig. 10A), the silver sheen of the body is present in irregularly dispersed patches in the translucent areas, when the fish is oriented partly towards the viewer (Fig. 10B) that iridescent colour is greenish silver, distributed in patches between the body bars and between the yellow dorsal-profile blotches, and as a distinct vertical greenish silver bar just before the upper-half of the first dark body bar.

Head translucent with silver sheen on cheeks; top rear corner of opercle with a translucent orange patch about half pupil size and a larger patch of tiny, well-dispersed, black chromatophores over area of pink gills, an indistinct, irregularly shaped translucent orange patch behind centre of eye extending on to

nape; mouth translucent, top jaw with silver sheen and a transverse line of black chromatophores across its centre, an indistinct yellow blotch on the snout immediately behind its centre; iris with most of its anterior and rear part overlaid by a dense silvery yellow sheen, top edge of the eyeball silvery yellow; a curved black bar formed by densely aggregated black chromatophores, passing from upper-rear of iris through the pupil and down to rear corner of the mouth, section of bar under eye with relatively large chromatophores overlaying a translucent yellow area, that bar bounded anteriorly and posteriorly by patches of silver iridescence.

Base of caudal fin with long, thin, vertical yellow bar with an aggregation of black chromatophores down its centre that partly fuses with last body bar, fin itself translucent with patches of tiny black chromatophores along interradiial membranes – two small patches along top of fin, a subterminal band down upper-half of posterior of the fin; a small, vertical patch of black-dotted yellow pigment just behind upper-half of the basal dark bar; first dorsal fin translucent with a large, horizontally oval, solid black blotch covering the outer third of fin between spines 1 and 5, and extending partly towards spine 6, base of fin with short extension of first yellow and black body bar; base of second dorsal fin with two yellow, black dotted extensions of third and fourth yellow body blotches, a black spot immediately above fin base between first two rays, a small, black-dotted, yellow spot on lower third of the fifth ray and another between bases of last two rays, outer edge of fin with a narrow band of dense black chromatophores on interradiial membranes; anal fin translucent with scattered, tiny, black chromatophores on interradiial membranes, particularly on anterior two-thirds of fin; pectoral fin base translucent with a large greenish silver blotch that extends on to basal one-fifth of fin, base of the dorsal ~8 rays of pectoral fin covered with a yellow, black-dotted blotch; remainder of the fin translucent; pelvic fins translucent with small black dots on interradiial membranes.

Colour in preservation (Fig. 11): Body pale yellowish; side of head below eye with curved, black, vertical bar formed by densely aggregated black chromatophores ending at rear corner of the mouth; operculum covered with scattered chromatophores; chromatophores more densely concentrated on posterior half, forming vertical bar of pigment that extends dorsally on to and across nape; side of body with four concentrations of chromatophores that form vertical bars, first bar complete and positioned beneath centre of first dorsal fin; second bar positioned below origin of second dorsal fin, not extending to dorsal midline, third and fourth bars positioned over end of anal fin and centre of caudal peduncle, respectively, both bars beginning at lateral

midline and extending ventrally to ventral midline; a series four or five small patches of chromatophores along dorsal midline, beginning at origin of second dorsal fin and extending to end of caudal peduncle; a singular vertical row of chromatophores over base of caudal fin rays, followed by a small patch of chromatophores on upper-half of caudal fin on anterior one-third of rays; first dorsal fin with distinct black oval on outer third of fin, and smaller dark black spot on middle of fin that is an extension of vertical bar on body; second dorsal fin with three or four small, isolated dark patches of chromatophores on lower-half of fin, distal margin of fin bordered with dark chromatophores; anal fin uniformly covered with scattering of chromatophores; pelvic and pectoral fin without pigment.

Genitalia: Female papillae short and conical; male papilla unknown.

Habitat: Collected on open sand bottom at 229 m depth.

Distribution: Known only from type locality off south-west coast of Curaçao, Caribbean Netherlands.

Etymology: The specific epithetic *nigrimarginatus* is an adjective (black-margined) formed from the Latin *niger*, glossy black, and *marginatus*, bordered, in reference to the black margins on the dorsal and caudal fins.

Remarks: The new species is placed in the genus *Robinsichthys* based on the following characters: first dorsal-fin pterygiophore pattern 3-22111*0, with the last pterygiophore lacking an associated dorsal-fin spine (Fig. 4B); VI spines in first dorsal fin; 11 + 17 vertebrae; two modified basicaudal scales; head pores absent; side of body with ctenoid scales; lack of fusion between hypurals 1–2 and hypurals 3–4 and the terminal vertebral element; body with several narrow, dark bands or saddles, head with dark triangular marking below eye. The new species differs from *R. arrowsmithensis*, and thus the original description of *Robinsichthys*, most notably in the following ways: (1) having pelvic fins fused to form a complete disk with well-developed anterior frenum vs. having pelvic fins completely separate, lacking frenum; (2) having a large dark blotch on the margin on the first dorsal fin vs. dorsal fin without blotch; (3) having 19 vs. 21–24 (usually 22–23) pectoral-fin rays; and (4) having I,9 elements in both the second dorsal fin and anal fin vs. predominately I,10 (I,9 in one specimen) in both fins of *R. arrowsmithensis*.

Because *R. arrowsmithensis* was not included in our phylogenetic analysis, we cannot confirm with molecular data that *Robinsichthys* is monophyletic

with regards to *Birdsongichthys*, and thus whether a new genus is required for *B. rectus*. However, there are two characters that we regard as apomorphic for *Robinsichthys* that are not present in *Birdsongichthys*. A count of VI dorsal spines (in most specimens) is unique to *Robinsichthys* among other Gobiosomatini genera (a reduction from the plesiomorphic state of VII spines). Counts of 11 + 17 vertebrae are also considered apomorphic for *Robinsichthys*, with these counts also being independently derived in members of the *Barbulifer* subgroup (*Elacatinus* and allies). *Birdsongichthys* possesses neither of these synapomorphies. *Birdsongichthys* has head pores, while both *Robinsichthys* do not, but head pores and many other morphological characters that may be useful for defining genera of Gobiosomatini have more complex evolutionary histories and may be of limited phylogenetic utility (see Discussion below).

DISCUSSION

SYSTEMATICS OF GOBIOSOMATINI

Birdsong et al. (1988) recognized both the *Gobiosoma* group and *Microgobius* group based on morphological characters, and both have proved to be monophyletic based on molecular data (Rüber *et al.*, 2003; Thacker & Roje, 2011; Agorreta *et al.*, 2013; Tornabene *et al.*, 2013; McCraney *et al.*, 2020), but *Birdsong et al.* (1988) were unsure of the monophyly of the larger group that contained them both, the Gobiosomatini (then called the Gobiosomini). Later, when describing *Robinsichthys*, *Birdsong* (1988: 442) reversed his earlier stance on Gobiosomatini, stating: ‘Despite the comments of *Birdsong et al.* (1988), and at the risk of appearing to argue with myself, I remain convinced of the validity of the Gobiosomini, including the “*Microgobius* Group” and *Robinsichthys*.’ *Birdsong*’s inclusion of *Robinsichthys* in the *Gobiosoma* group of the Gobiosomatini was hypothesized based on the presence of VII dorsal spines in some specimens (most with VI), a 3-22111*0 vertebral formula and modified basicaudal scales. Moreover, *Birdsong* predicted that *Robinsichthys* might ‘bridge the gap’ between the *Microgobius* group and the *Gobiosoma* group, but could not test this without tissue samples and molecular data that are available now. Our molecular phylogenetic analysis largely supports *Birdsong*’s hypothesis in that *Robinsichthys* is indeed nested within Gobiosomatini, but is instead resolved in the *Gobiosoma* group rather than ‘bridging the gap’ between the *Gobiosoma* and *Microgobius* groups (Fig. 1).

Our analysis also supports a sister-relationship between the new genus *Birdsongichthys* and *Robinsichthys* with moderate support (0.94 posterior

probability; Fig. 1). This phylogenetic arrangement allows us to re-evaluate the distribution of several potentially diagnostic morphological characters within Gobiosomatini. Fusion of hypurals 1–2 with 3–4 and the terminal caudal element was previously considered a diagnostic character for the entire *Gobiosoma* group with the exception of *Aboma*. Our phylogenetic analysis shows that this character defines a more restrictive clade that includes the *Barbulifer* and *Nes* subgroups, but excludes both *Aboma* and the clade containing *Robinsichthys* and *Birdsongichthys*. However, the monophyly of *Barbulifer* and *Nes* subgroups is only weakly supported by molecular data, and thus the relationship between these two clades and the *Robinsichthys* + *Birdsongichthys* clade is considered unresolved (0.73 posterior probability; Fig. 1). While the hypural-fusion character links *Barbulifer* and *Nes* subgroups, the loss of a complete pelvic disc appears in both the *Nes* subgroup as well as the *Robinsichthys* + *Birdsongichthys* clade, but not in the *Barbulifer* subgroup. Thus, these characters provide conflicting evidence regarding the relationships of these three clades. Conflict between morphological characters and molecular data is not new for the Gobiosomatini. Tornabene *et al.* (2016b) evaluated the congruence between a molecular tree of the *Nes* subgroup and several characters, including head pore patterns, body scales, modified basicaudal scales, pelvic-fin morphology, anal-fin pterygiophore patterns and sensory papillae patterns, and found that while some characters showed significant phylogenetic signal, others varied widely across the phylogeny and none were entirely free from homoplasy. The discovery of *R. nigrimarginatus* and *B. rectus* and their phylogenetic placement shows these characters are even more variable across the tree than previously understood and that additional characters like the splitting or uniting of pelvic fins also show more homoplasy than previously known within the Gobiosomatini.

DEEP-REEF LINEAGES IN THE CARIBBEAN

Species in Gobiosomatini have become models for studying adaptive radiation and niche specialization (Rüber *et al.*, 2003; Taylor & Hellberg, 2005; Huie *et al.*, 2020) and exploitation of deep reefs is just one of the many axes of ecological differentiation seen in this group (Tornabene *et al.*, 2016a). Deep reefs include the mesophotic zone, which extends from ~40 to 150 m in the Caribbean, and the rariphotic zone, which extends ~150–300 m (Baldwin *et al.*, 2018). The mesophotic zone includes the lower limit of reef-building corals (~90 m), and the lower mesophotic and rariphotic zones are dominated by non-reef forming corals, gorgonians, sponges and rocky slopes.

Gobies have invaded both zones at multiple times throughout their evolutionary history, including several lineages within Gobiosomatini (Tornabene *et al.*, 2016a).

Robinsichthys and *Birdsongichthys* are both deep-reef taxa and including them in the molecular phylogeny provides new insight into our understanding of the evolution of Gobiosomatini, including the number and timing of deep-reef invasions within the group (Fig. 2). The analysis of Tornabene *et al.* (2016a) suggested that the ancestor of Gobiosomatini was a shallow-water species and that there were independent deep-reef invasions in the *Microgobius* group (*Antilligobius* + *Palatogobius* clade) and in the *Nes* subgroup (*Pinnichthys*, *Pilotris* and *Varicus*). Our analysis shows that the *Robinsichthys* + *Birdsongichthys* clade represents a third independent transition on to deep reefs in Gobiosomatini. This transition would be the oldest of the three, taking place ~18–30 Mya (Figs 2, 3) on the branch leading to the MRCA of *Birdsongichthys* and *Robinsichthys*, suggesting that major ecological differentiation in Gobiosomatini was already occurring in the Oligocene to Late Miocene. The deep-reef transitions in the *Microgobius* group and the *Varicus/Pilotris/Pinnichthys* clade both took place between ~5 and 20 Mya (Figs 2, 3). Ecologically, both *Robinsichthys* and *Birdsongichthys* show some convergence with *Pinnichthys*, *Pilotris* and *Varicus*, in that they are associated with sand or rocky-reef bottoms where they perch on the substrate, rather than hovering over the bottom (as in *Antilligobius nikkiae* and some species of *Palatogobius*; Van Tassell *et al.*, 2012; Tornabene & Baldwin, 2017) or burrowing in the substrate (as seen in some species of *Palatogobius*; Tornabene *et al.*, 2016a).

Although *Robinsichthys* and *Birdsongichthys* represent a third invasion of deep-reefs in Gobiosomatini, they also represent at least the fifth independent deep-reef invasion among all Caribbean gobies when we consider invasions from both *Coryphopterus curacao* Baldwin & Robertson, 2015 and an undescribed genus in the *Priolepis* lineage reported by Tornabene *et al.* (2016a).

Robinsichthys arrowsmithensis was described more than 30 years prior to the discovery of *R. nigrimarginatus*, but the latter was only discovered after more than 150 submersible dives at that exact location in Curaçao, and *Birdsongichthys* was never collected prior to its discovery in 2017. Thus, it remains highly probable that additional lineages of deep-reef gobies, and of other fishes, will be discovered as submersible collections continue throughout the Caribbean. In the absence of submersibles, closed-circuit rebreathers may also be effective tools allowing the collection of cryptobenthic fishes from mesophotic and upper-rariphotic reefs (i.e. shallower than 150 m), while submersibles

provide access to the lower-half of the deep-reef zone beyond the reach of rebreathers. Such efforts in the Indo-Pacific and Brazil have already resulted in the discovery of undescribed gobies from mesophotic and upper-rariphotic reefs (Tornabene *et al.*, 2016b; Pimentel *et al.*, 2020; L. Rocha and H. Pinheiro, pers. comm.).

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DATA AVAILABILITY STATEMENT

GenBank accession numbers and BOLD project IDs for new sequences are listed in [Supporting Information, Table S1](#).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. GenBank accession numbers for new species.

Figure S1. SIMMAP Stochastic character mapping of habitats across the Gobiidae phylogeny based on ARD model.