Journal of the

Ocean Science Foundation

2010 Volume 4

Victor, B.C.

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Published December 15, 2010 by the Ocean Science Foundation
ISSN: 1937-7835
Printing sponsored by Walsh Paper Distribution, Inc., Westminster, California, USA
Online edition at http://www.oceansciencefoundation.org/josf.html
Printed copies deposited at all UC libraries and free from OSF, 4051 Glenwood, Irvine, CA 92604
Cover photo, *Emblemariopsis* cf. *carib*, Dominican Republic

Emblemariopsis carib and *Emblemariopsis arawak,* two new chaenopsid blennies from the Caribbean Sea: DNA barcoding identifies males, females, and juveniles and distinguishes sympatric cryptic species.

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Abstract

Two new sympatric chaenopsid blennies, *Emblemariopsis carib* and *E. arawak*, are described from coral reefs in Puerto Rico and the adjacent U.S. Virgin Islands. These species have been considered Flagfin Blennies, *E. signifer* (usually as *E. signifera*), which was originally described from mainland Brazil. However, COI mtDNA sequencing shows that despite their close resemblance, the three species are genetically distant from each other: *E. carib* is 13.34% sequence divergent from Brazilian *E. signifer, E. arawak* is 13.68% sequence divergent from *E. signifer,* and the two sympatric Caribbean species are 13.24% divergent from each other (minimum interspecific distance). These distances represent well over 1 million years of isolation, even with the highest estimate of the mitochondrial mutation rate of chaenopsid blennies. *E. carib* and *E. arawak* are smaller species than *E. signifer*, differing by fewer dorsal and anal fin rays in *E. carib* and some subtle morphology and marking patterns, such as the white spots on the head found only on the Brazilian Flagfin Blenny (*in vivo*). High variability in morphology and markings within *Emblemariopsis* species makes it difficult to isolate diagnostic differences, which may occur in live coloration only. Underwater macro-photography is necessary to document variations in live color and markings indispensable to species identifications. The combination of DNA sequencing with underwater photography is an example of how new techniques can provide the resolution necessary to delineate cryptic species that differ only slightly in appearance and plague the taxonomy of some families of coral reef fishes. Barcode DNA sequences of *Emblemariopsis* species from the region reveal that the genus is made up of a number of species, closely related cryptic species, and undefined lineages in the western Atlantic which do not conform with the incompletely described species in the literature. The degree of sequence divergence between species is widely varying within the genus: species with clear morphological and meristic differences, such as *E. pricei* and *E. bahamensis*, are only 0.77% divergent in the barcode sequence (presumably consistent with recent speciation), while other species are up to 20% sequence divergent. Flagfin Blenny specimens from Barbados form a separate clade from the *E. carib* types, but differ by only 0.62% in barcode sequence; the taxonomic status of this lineage and others from the region remain uncertain without further sampling. The genus *Emblemariopsis* is an exemplary case for the utility of DNA-barcode matching and underwater photography for distinguishing species: there are numerous widespread and local species (and lineages) that can share morphology and meristics, females and juveniles of related species can appear almost identical, males and females look very different, markings are commonly shared among species and vary between individuals (and can be lost in formalin preservation), and museum collections are incomplete.

Key words: new species, *Emblemariopsis carib*, *arawak*, *signifer*, *signifera*, cryptic species, Flagfin Blenny, chaenopsidae, barcode, DNA, FISH-BOL, coral reef fish, phylogenetics, taxonomy, Caribbean, western Atlantic

Introduction

Sequencing of the standardized barcode segment of COI mtDNA provides a valuable tool for unraveling complex groups of closely related species that traditionally have been difficult for taxomists (Packer *et al*. 2009). Sequence matches can link males and females in dimorphic species and associate early developmental stages with adults (Ward *et al*. 2009). Among coral reef fishes in particular, sequences can be useful for species identification of juveniles and larvae, which often look very different from adults (e.g. Victor *et al*. 2009, Baldwin *et al*. 2009). In addition, in groups of species with variable and overlapping markings, matching DNA sequences can sort specimens into clusters that clarify which characters are diagnostic for species and which vary across species. This application is particularly useful for the intricate species complexes found among some families of coral reef fishes.

The taxonomy of most Atlantic reef fishes has been relatively stable for some time, although a number of taxa, particularly among the omnipresent gobies and blennioids, are known to be made up of unresolved species complexes or variable superspecies. Recently, the number of new species described has increased as more attention has been focused on the Brazilian fauna and Atlantic biogeography (Floeter *et al*. 2008) and DNA sequencing has begun to resolve some of the more difficult species complexes (Victor 2008, Tornabene *et al*. 2010, Baldwin *et al*. in review). Advances in the techniques of underwater photography can also be valuable, since details of live coloration and markings are often diagnostic characters among closely related cryptic species, as in the recently described redcheek goby (Victor 2010). There is some synergy in that traditional formalin preservation can erase diagnostic markings, while ethanol, commonly used for DNA sampling, can preserve useful markings.

It would be hard to find a more fitting group to demonstrate the utility of DNA barcoding and underwater macrophotography than *Emblemariopsis*. This genus of tiny chaenopsid blennies is limited to the tropical western Atlantic and is speciose, with at least 12 described species (and certainly many more), all associated with coral and rocky reefs (Williams 2003, Patzner *et al*. 2009). The taxonomy of the group is particularly challenging for many reasons, primary among them the general overlap of fin-ray counts and high intraspecific variation in morphology and markings– unfortunately often in the diagnostic characters cited in the descriptions of many species (e.g. Stephens 1970). A further obstacle to delineating species of *Emblemariopsis* is the similarity of females within the genus, which can be lightly marked or unmarked after preservation. In addition, mature males have territorial (dark or blackhead) and non-territorial (light) morphs, both very different in appearance from females, leading to some difficulty in linking genders in species descriptions (Stephens 1970). Furthermore, males can be rare or undiscovered or, alternatively, females can be unknown, such as in *E. dianae* (Tyler & Hastings 2004).

The most widespread reported species in the genus is *Emblemariopsis signifera*, a species first described as *Emblemaria signifer* (Ginsburg 1942) from Brazil, where it is a common coastal species (Luiz *et al*. 2008). The epithet *signifer* is a noun and need not conform in gender and the valid name for the Brazilian species is *Emblemariopsis signifer* (Eschmeyer 2010). The Caribbean species of *Emblemariopsis* comprise a set of incompletely described species which break up into complex groups of species, cryptic species, and lineages when DNA sequences are analyzed. In this study, I use specimens preserved only in ethanol, which permits sequencing for the barcode DNA segment. Sequence-matching allows specimens to be separated into groups that can be compared to reveal diagnostic characters and unite male, female, and juvenile forms. In addition, I redescribe the markings of Flagfin Blennies based on a review of an extensive array of underwater photographs which illustrate the variability in live markings. Live photographs are essential for assessing the validity of putative diagnostic characters and interpreting marking patterns in preserved fishes. This exposition provides an example of how a new approach can provide the resolution necessary to distinguish the closely related cryptic species found among some coral reef fishes.

Materials and Methods

Type specimens of the new species are deposited at the Florida Museum of Natural History (UF). All fishes were collected by hand on the reef and immediately preserved in 90% ethanol. Ethanol-preserved non-type specimens

and specimens of other species of *Emblemariopsis* were collected in Puerto Rico, St. Thomas (USVI), Brazil, Barbados, and Utila (Honduras). Representatives of all the other Atlantic chaenopsid genera except *Protemblemaria* were collected for DNA sequence comparisons from Puerto Rico, St. Thomas (USVI), Panama, Belize, and Utila (Honduras). Eastern Pacific specimens were collected from Baja California, the Islas Revillagigedos, and the Galapagos Islands. Specimens not yet deposited in the museum are in the author's personal collection (BV).

DNA extractions were performed with the NucleoSpin96 (Machery-Nagel) kit according to manufacturer specifications under automation with a Biomek NX liquid-handling station (Beckman-Coulter) equipped with a filtration manifold. A 652-bp segment was amplified from the 5′ region of the mitochondrial COI gene using a variety of primers (Ivanova *et al*. 2007). PCR amplifications were performed in 12.5 µl volume including 6.25 µl of 10% trehalose, 2 µl of ultra pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM (NH4)2SO4, 20mM Tris-HCl (pH8.8), 2mM Mg SO4, 0.1% Triton X-100), 0.625 µl of MgCl2 (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM), 0.0625 µl of Taq DNA polymerase (New England Biolabs), and 2 µl of template DNA. The PCR conditions consisted of 94°C for 2 min, 35 cycles of 94°C for 30 s, 52°C 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Specimen information and barcode sequence data were compiled using the Barcode of Life Data Systems (BOLD, www.barcodinglife.org; Ratnasingham & Hebert 2007). The sequence data is publicly accessible on BOLD and GenBank (see Appendix). Sequence divergence was calculated on BOLD with the Kimura 2-parameter (K2P) model generating a mid-point rooted neighbor-joining (NJ) phenogram to provide a graphic representation of the species divergence.

Measurements are presented as proportions of the SL or HL, with the range for the paratypes, followed in parentheses by the holotype. Measurements are taken from side- and top-view photomicrographs using the Photoshop ruler tool and spans indicate strictly horizontal or vertical distances with the fish straightened out along the vertebral axis (the head is often tilted up in dead individuals). All lengths are linear to end-points, not following curves. Standard length (SL) is the span from the front of the upper lip to the base of the caudal fin (posterior end of the hypural plate); predorsal and preanal lengths are spans; body depth is the vertical span from the base of the first dorsal-fin spine, midbody depth at the point just forward of the first anal-fin spine; body width is the maximum span just behind the pectoral-fin base; head length (HL) is the span from the front of the upper lip to the most posterior end of the opercular flap; head width is the span at the rear edge of the bony orbit; snout length is the span from the front of the upper lip to the anterior edge of the bony orbit; orbit diameter is the span from edge to edge of the bony orbit; interorbital width is the least bony width; upper-jaw span is from the front of the upper lip to the most posterior corner of the mouth (a rounded lip flap on chaenopsids), upper-jaw length is along the angle; caudal-peduncle depth is the least depth and length is the span from the base of the last dorsal ray to the caudal-fin base; lengths of fin elements are linear measurements from the junction with the body outline to the tip; caudal-fin length is the span from the fin base to the tip of the longest ray, if clearly intact; pectoral-fin length is the angled length of the longest ray; pelvic-fin length is from the junction of the pelvic spine and body to the horizontal stretched tip of the longest soft ray. Photographs of preserved specimens were processed, edited, and remodeled with Photoshop software.

Figure 1. *Emblemariopsis carib*, holotype, male, 14.8 mm SL, Outer Brass Island, St. Thomas, US Virgin Islands.

Figure 2. *Emblemariopsis carib*, holotype, male, 14.8 mm SL, Outer Brass Island, St. Thomas, US Virgin Islands.

***Emblemariopsis carib*, n. sp.**

Figs. 1,2, & 3

Holotype. UF 179454 (1) 14.8 mm SL, United States Virgin Islands, St. Thomas, Outer Brass Island (18.396, -64.976), B. Victor and T. Smith, May 2, 2009.

Paratypes. UF 179455 (4) 9.7–10.2 mm SL, same as holotype; UF 179456 (2) 10.8–13.8 mm SL, Puerto Rico, La Parguera, Medialuna Reef, seaward slope, (17.935, -67.049), B. Victor and C. Caldow, Aug. 4, 2007; UF 179457 (1) 13.3 mm SL, Puerto Rico, La Parguera, wall buoy (17.893, -67.023), B. Victor and C. Caldow, Aug. 4, 2007.

Note: Prior museum collections without DNA sequences cannot be confirmed as *E. carib*. UF collections from

Figure 3. *Emblemariopsis carib*, paratype, female, 13.3 mm SL, La Parguera, Puerto Rico.

St. Croix, USVI correspond morphologically to *E. carib* but are unsequenced and missing markings. A series of specimens from Barbados collected by Henri Valles in 2005 are mostly identical in appearance to *E. carib* and close in DNA sequence. Both sets are considered non-type material and designated *E.* cf. *carib*. The morphometrics and meristics in the description are based on type specimens.

Diagnosis. A species of *Emblemariopsis* with total dorsal-fin elements 30–31; mode of D-XX,11 A-II,20 and Pect. 13; single short orbital and nasal cirrus present on each side and unbranched, nasal cirrus longer than nasal tube; first three dorsal-fin-spine bases close, first and second closest, widest gap between third and fourth; first spine longer or equal to second, third spine 1/2 to 3/4 of second and fourth spine shortest, forming notched dorsal-fin outline in juveniles, females, and many mature males (males with the most extended first dorsal-fin spines can have a concave, but not notched, fin outline); mature males with a greatly extended first dorsal-fin spine (when folded down straight reaching back to base of 8–15th dorsal-fin spines) followed by a shorter second spine, a distal red-orange band outlined in white above and below on first few spinous membranes; females and immature males >10 mm SL with first and second dorsal-fin spines longer or equal to 12th spine (first two when folded down straight reaching back to 6–7th spine base), first spine banded black and white; juveniles <10 mm SL with first and second dorsal-fin spines shorter than 12th spine (first two when folded down straight reaching back to 5–6th spine base), first spine dark with light tip; all stages with last (or 2nd to last) dorsal spine shortest, ¼ to ½ longest soft ray; interorbital head pores including midline CP plus three adjacent pairs (PAF, PI, PLI) when complete (which is infrequent); evenly dispersed stippling of fine black spots over cranium (all stages except blackhead males); males and females without white spots on top of head in life; females, immature males, and some juveniles with midline melanophore stripe on snout, not extending back past posterior nostril; lower pectoral-fin-base dark spots comprising one anterior rounded spot or short oblique line slanted down and sometimes additional spots.

Description. Total dorsal-fin elements 30–31; modal dorsal-fin rays XX,11; anal-fin rays II,20; pectoral-fin rays 13; pelvic-fin rays I,3; all fin rays unbranched; first three dorsal-fin-spine bases close, first and second closest, widest gap between third and fourth; first spine longer or equal to second, third spine 1/2 to 3/4 of second and fourth spine shortest, forming notched dorsal fin-outline in juveniles, females (e.g. Fig. 4), and many mature males (males with the most extended first dorsal-fin spines can have a concave, but not notched, fin outline);

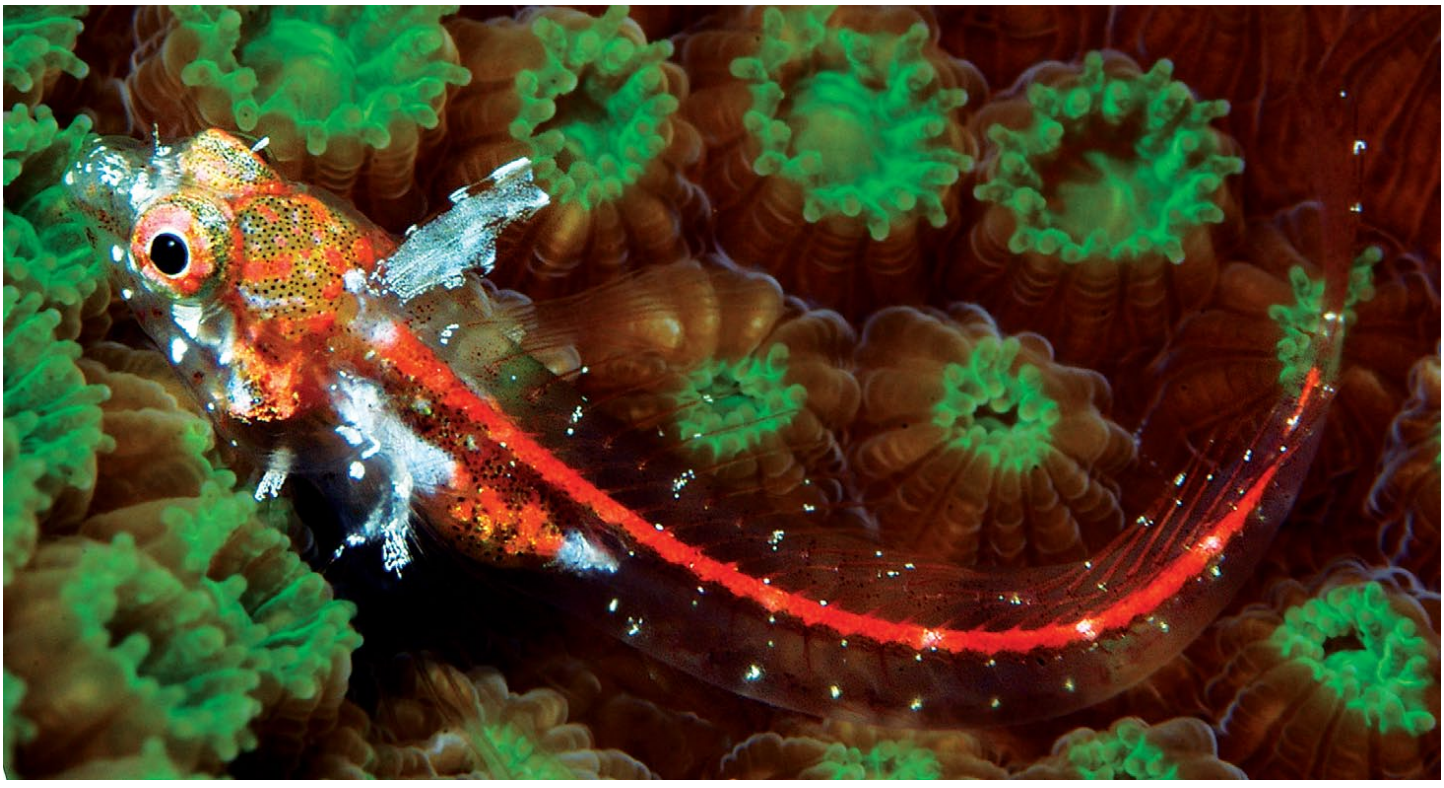


Figure 4. *Emblemariopsis* cf. *carib*, female, Mona, PR (high white and red). Photo © Keri Wilk/ReefNet.

mature males with a greatly extended first dorsal-fin spine (when folded down straight reaching back to base of 8–15th dorsal-fin spines, e.g. Fig. 5) followed by a shorter second spine; females and immature males >10 mm SL with first and second dorsal-fin spines longer or equal to 12th spine (first two when folded down straight reaching back to 6–7th spine base); juveniles <10 mm SL with first and second dorsal-fin spines shorter than 12th spine (first two when folded down straight reaching back to 5–6th spine base); first dorsal-fin spine 10–17% (27%) SL (increasing with SL), second spine equal or shorter, 9–15% (14%) SL (increasing with SL), third spine 6–8% (8%) SL, fourth spine 4–6% (7%) SL, 12th spine 10–13% (12%) SL, last spine 4–5% (6%) SL; all stages with last (sometimes 2nd to last) dorsal spine shortest, only ¼ to ½ longest soft ray, longest dorsal-fin soft ray 10–12% (12%) SL; mature males with each dorsal- and anal-fin membrane slightly incised leaving a white-lobed end to each element; longest anal-fin soft ray 8–9% (11%) SL; last dorsal and anal-fin rays joined to caudal peduncle by

Figure 5. *Emblemariopsis* cf. *carib*, dark male, Dominican Republic. Photo © Jose Alejandro Alvarez.

membrane up to start of procurrent rays; pectoral-fin long, reaching base of 17th dorsal-fin spine (13th), length 24–29% (20%) SL; pelvic-fin length 19–21% (18%) SL, when straightened horizontally usually not reaching anogenital opening, spine not grossly visible, second ray longest, membranes deeply incised more than half-way between first and second rays; caudal fin truncate, length 17–20% (19%) SL, segmented caudal-fin rays 13 and 3–4 upper and 3–4 lower procurrent rays.

Body slim and elongate, body depth at dorsal-fin origin 17–20% (20%) SL, mid-body depth 12–16% (13%) SL; body width 11–13% (12%) SL; predorsal span 19–23% (20%) SL; preanal span 44–48% (45%) SL; caudal peduncle length 9–11% (10%) SL, caudal peduncle depth 6–8% (8%) SL. Head length 26–30% (25%) SL; head width 46–60% (67%) HL; snout pointed, length 17–23% (17%) HL; eye large, orbit diameter 27–33% (28%) HL; short flattened finger-like orbital cirrus, speckled black and white, 4–7% (6%) HL or about 1/3 to 1/2 pupil diameter; interorbital width 11–17% (14%) HL, mostly flat in immature fish, concave in mature fish; mouth medium (females and immature males) to large (mature males), upper-jaw span 31–36% (43%) HL; upper-jaw length 32–39% (48%) HL; upper and lower jaws with variable-sized caniform teeth, in irregular rows anteriorly becoming a single row posteriorly (vomerine and palatine tooth patterns not evaluated); anterior nostril a small raised tube just behind upper lip with a speckled finger-like cirrus longer than nasal tube, posterior nostril forms an elliptical opening adjacent to orbital rim; preopercle edge mostly smooth, at most a few tiny spines near angle in young.

The pattern of sensory pores on the head of chaenopsids has been used as a diagnostic character for some time (e.g. Stephens 1961), although a one-size-fits-all terminology for all genera leads to difficulties and can be inconsistent or imprecise (e.g. Stephens 1963, 1970). The patterns can be different for various genera, e.g. *Acanthemblemaria* (Smith-Vaniz & Palacio 1974) and *Emblemaria* (Johnson & Greenfield 1976), and the terminology has accumulated variations over time.

The cephalic pore pattern of *Emblemariopsis* is basically similar within the genus, with the most variable portion of the pattern being the interorbital set of pores (Fig. 6). This set is placed by Stephens (1963) into an ill-defined supraorbital line and presented as totals without indicating the pattern. Mostly following Smith-Vaniz and Palacio (1974), Johnson and Greenfield (1976), and Hastings (1992), I describe the interorbital pattern as centered around the midline commissural pore (CP) with a side-by-side pair just in front of the CP, the posterior anterofrontals (PAF); a pair just behind the CP, opening on the medial side of the canal, the posterior interorbitals (PI); followed by a more widely separated posterolateral pair (PLI), also opening on the medial side of the canal. The PI and PLI are variously analogous to two of the F2, F1, and median frontals of Hastings (1992). All *Emblemariopsis* also have a pair of anterofrontal (AF) pores far forward on the frontal bones near the posterior nostril, a nasal pair near the tip of the snout (N), as well as two (per side) along the posterior upper orbital rim– the two supraorbital pores (SO). All of the preceding except the nasals are apparently included in the "frontal supraorbital" series of Stephens (1963). The full complement of interorbital pores would count as 6+1 frontal pores *sensu* Stephens.

Most of the *E. carib* and *E.* cf. *carib* specimens examined in this study are missing from one to six of the 13 total interorbital pores (both sides), most often one or both of the PAF or PI. The remaining pore openings are sometimes pinpoint holes, indicating some tendency to reduced pore development. In contrast, the large majority of specimens of Brazilian *E. signifer* have the full interorbital pore complement as described above.

The remaining cephalic pores are mostly consistent in *E. carib* and *E. signifer* (but with some variation, many individual *E. carib* and *E.* cf. *carib* are missing pores). Following the terminology of Smith-Vaniz and Palacio

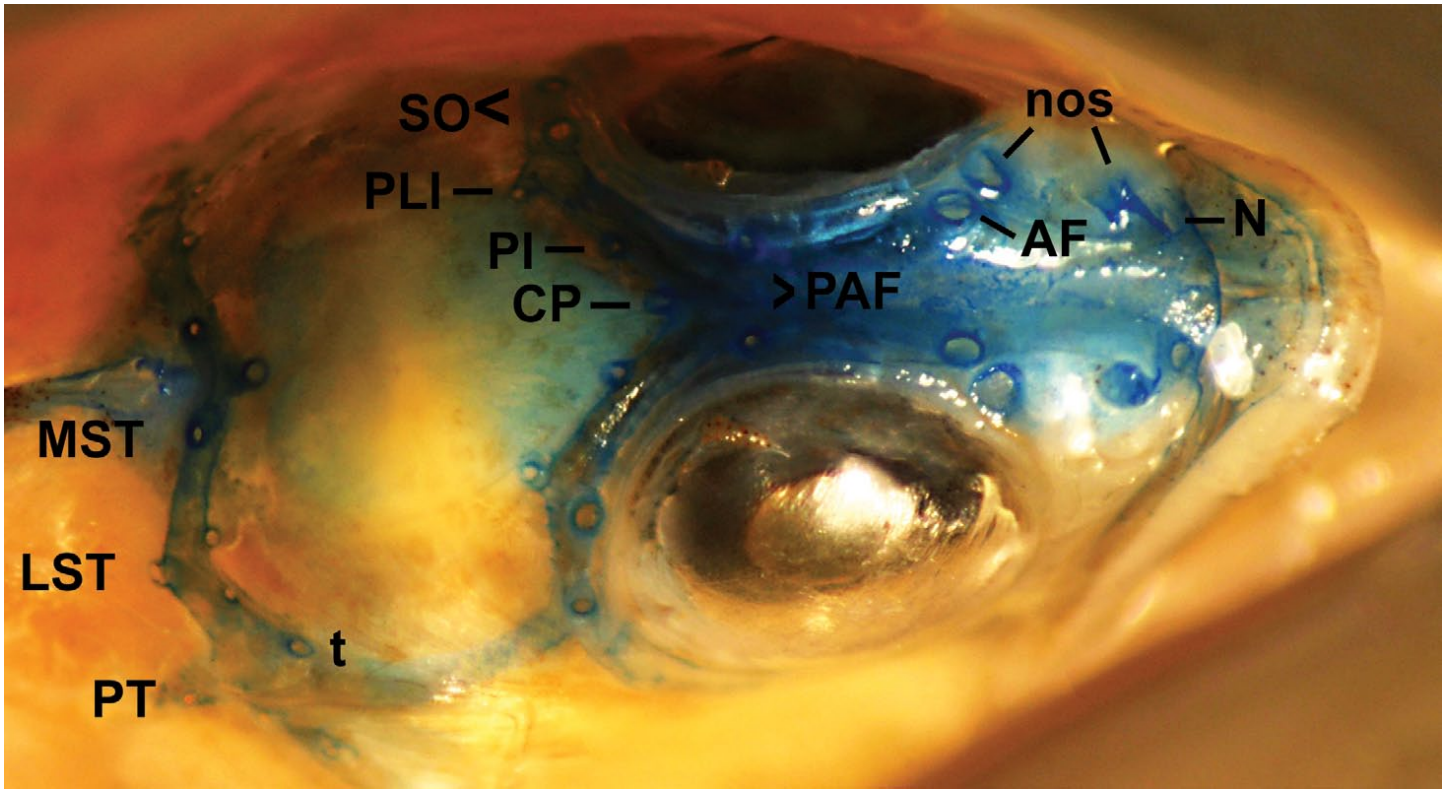


Figure 6. Distribution of dorsal cephalic pores on a female *Emblemariopsis signifer* from Ubatuba, Brazil. Abbreviations following the text; **t** indicates the temporal pore that can be assigned to the posttemporal series and **nos** indicates the anterior and posterior openings of the nostril (blue stain is Aniline Blue).

Figure 7. *Emblemariopsis* cf. *carib*, mature male, Nord, Haiti. Photo © Nick Hobgood.

(1974), the complement typically comprises 3 anterior infraorbital (AIO), 3 posterior infraorbital (PIO), 3 median dorsal supratemporal (MST, around the dorsal fin origin, total both sides), 3–4 lateral supratemporal (LST, above the temporal canal branching), 3–4 posttemporal (PT), 4–6 preopercular (PO), 1 common pore (CP), and 4 mandibular (MD) pores. Variability in the position of the pore(s) at the intersection of the PT series and PO series leads to some complementary variation in the numbers for those series. The description of "temporal" pores has a confusing history, with Stephens (1963) indicating that *E. signifer* has none, but Stephens (1970) reporting that *E. occidentalis* has two and is identical in pore pattern to *E. signifer*. Stephens (1970) states that the presence of temporal pores is one of the diagnostic features of the genus *Emblemariopsis*, yet Stephens (1963) describes two species as without temporal pores. Furthermore, Stephens (1970) reports that *Acanthemblemaria* always have a temporal pore, but Smith-Vaniz and Palacio (1974) show none. A stabilizing definition would be to assign the variably placed erstwhile temporal pore(s) in these two genera to either the LST or the PT series depending on proximity; larger specimens tend to have an anterior fourth LST pore, while small fish have an anterior pore usually lined up with the PT series (Fig. 6).

Color in life. The color variation in *Emblemariopsis* species comes from two sources: differing intensities of the basic three components (black, white, and red/yellow), which can vary independently, and reproductive state. Each stage can look quite different: territorial breeding males (dark or black), non-territorial mature males (light), females and immature males, and juveniles. Breeding males who are territorial develop a darkened head and anterior body, typically speckled with fine melanophores, but through which one can often see underlying patterns of markings (dark males)(Figs. 7 & 8). Occasionally the head and anterior body become completely black, overriding all markings except for the prominent red flag on the dorsal fin. Non-territorial mature males (light males) have little dark shading, but retain the red-banded dorsal fin and extended first dorsal-fin spines and typically show the full complement of markings found on females. Females are always light and do not have a red band on the dorsal fin (as a rule?)(Fig. 4). Immature males are mostly identical to females, but with a tiny genital papilla, and can be hard to distinguish. Juveniles are mostly transparent and often missing many markings.

Fins: The first three dorsal-fin spines and their membranes are boldly marked on both males and females. The first spine is banded with dark and light: three or four dark bands on females and three to six on males (when they are not blackened). Juveniles have only one dark band, typically with a white tip. All stages have the first two spinous membranes pigmented, often dark or black in mature males, grey or white in females and immature

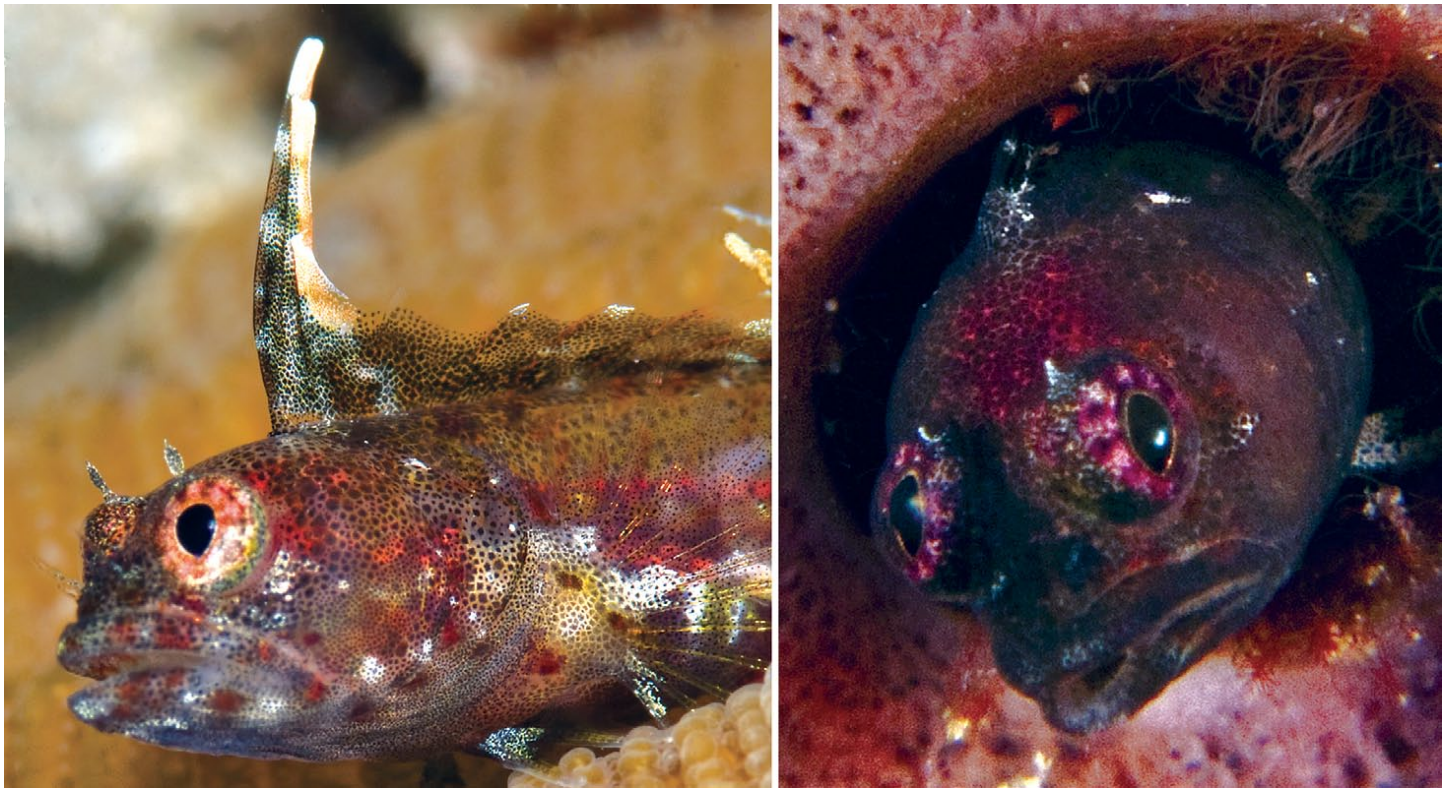


Figure 8. *Emblemariopsis* cf. *carib*, dark males, Samana, Dominican Republic. Photo at left © Jose Alejandro Alvarez; photo at right © Juan Carlos Navarro.

males. The anterior dorsal fin on mature males has a distal red-orange band outlined above and below by a white line of varying thickness (Fig. 8). The red band typically extends past the third spine and sometimes beyond the fifth. Many males have red starting only behind the tip of the dark first spine and the width of the red band is variable. The membranes of the mid and posterior spinous dorsal fin are mostly clear in light males and dark-shaded in dark males. Males can have a row of black spots on the proximal membranes of the mid and posterior spinous dorsal fin, variably present and variably intense, sometimes on adjacent membranes but often irregularly spaced.

Head and body: Light males, females, and juveniles are mostly transparent with conspicuous red-orange pigment distributed in a reticulate pattern on the dorsal aspect of the head, on the iris, in a saddle over the peritoneum, and then trailing in a long streak along the spinal column to the tail (Fig. 9). The vertebral streak is broken up by short white segments. The thorax and peritoneum is lined with a bright white casing with a red and/or dark triangular saddle over the mid-portion. White patches are present on the face, at the base of the pectoral and pelvic fins, and at the front of the dorsal fin over the first two membranes. The orbital cirri are often prominently white or salt-and-pepper speckled, but, in high dark-component and some dark-shaded individuals, it can be mostly black. There is a variable row of small white spots on the body along the base of the dorsal and anal fins and an irregular white speckling over the remaining body and on the fin membranes.

The dark markings on the head include a prominent subsurface melanophore layer over the cranium made up of a regular stippling of black spots, spaced evenly so they do not touch or merge. On high red-component individuals, the black stippling is broken into a complex pattern of dark bands by overlying red patches and reticulations. On high white-component individuals, the overlying patches, bands, and reticulations can be white. The iris has a prominent radiating-spoke pattern of dark lines over a red-orange surface. The lower half of the head has an array of dark spots, often quite variably present; the most consistent include a spot at the corner of the jaw, one or two below and behind the eye, and a variable row of spots running from the behind the preopercular margin down and along the base of the branchiostegal membranes, continuing along the lower rim of the mandible and up to the mandibular symphysis. Some individuals also have a dark spot on the body immediately behind the insertion of the pelvic fins. A variable set of dark spots are present on the fleshy base of the pectoral fin, most often

a round spot or short oblique line slanted down on the anterior lower quadrant behind the edge of the operculum. Often there is also a spot near the insertion of the lower-mid pectoral-fin rays and sometimes a central spot on the upper third and a spot at the upper rim of the pectoral-fin base. Along the body, there is a row of short dark lines alternating with white lines, each straddling the dorsal midline, usually two spine-bases dark to one white. There is a similar row along the body at the anal-fin base, but these are deeper and can appear as a row of internal dark blotches, usually two dark at the first two soft-ray bases, then alternating one-to-one. The internal dark markings on the body include a saddle over the white abdominal lining, extending down on each side as a triangular dark saddle which is typically broken by a central red or white patch and a dark patch near the vent. Internal melanophores, most visible on juveniles, include a linear overlay of the vertebral bodies, a black spot at the mid-vertebral body about every 3rd vertebra, and a short vertical line of melanophores running along the base of the 2, 3, and 4th (from top) and then, after a break, the 10, 11, and 12th caudal-fin segmented rays.

Dark territorial males develop a mostly uniform surface shading of fine melanophores over the head and anterior body, through which the underlying spot patterns are often discernable (Fig. 8). During breeding, dark males can become uniformly blackened over the head and anterior body and even blacked out completely, including the orbital cirri and even most of the iris, sparing only the gold ring around the pupil and the red-and-white band on the dorsal fin. On some blackened males of *E. signifer* the tips of the pelvic fins are also red-orange, but it is not confirmed whether this is true for *E. carib* males.

Figure 9. *Emblemariopsis* cf. *carib*, light male, St. Vincent (D-IXX,11). Photo © Keri Wilk/ReefNet.

Color in preservative (ethanol vs. formalin/alcohol). Museum specimens fixed in formalin and stored in isopropyl alcohol lose most or all of their markings and DNA is unrecoverable. The white and red disappear rapidly and some of the melanic components can be lost as well. Some of the deeper black markings fade away, including the stippling over the cranium that is an important diagnostic character separating some species. The spots on the jaw, operculum, and the underside of the head are variably missing. The surface shading, the dark spots on the fins, and the dark band along the anal fin are best preserved.

Prior descriptions of Caribbean Flagfin Blennies note few markings on the museum specimens. The original description of *E. signifer* only reports a few dark spots near the base of the dorsal fin and black near the front of the fin (Ginsburg 1942). Stephens (1963) notes a dark dorsal fin and no other markings. Stephens' (1970) redescription of *E. signifer* includes an illustration of a preserved Bahamian male and describes relatively uniform dark shading sparing only the distal edge of the pectoral fin, the caudal fin, and the posterior portion of the anal fin, variable spots on the head and the pectoral-fin base, and a row of dark spots on alternating proximal spinous-dorsal-fin membranes. The red-and-white band on the dorsal fin is noted simply as an unpigmented edge to the fin. Little needs to be added other than the posterior body is usually much lighter, the soft dorsal fin is usually unmarked, the dark on the anal fin can be a distal band and extend farther back, and the dark spots on the proximal spinous-dorsal-fin membranes can be irregularly spaced. Females are generally described as mostly unpigmented with numerous dark spots on the underside of the head, a row along the anal fin base, spots on the pectoral-fin base, and dark shading on the anterior dorsal-fin membranes and the body beneath the pectoral fins (Stephens 1970).

Ethanol-preserved specimens rapidly lose yellow pigment, but can maintain some of the red and white component and all of the black for years. Thus the color description above mostly describes ethanol-preserved specimens, with some adjustments for the observed degree of color preservation. There is unexplained variation in the degree of color retention, with some specimens retaining red but fading or even losing the deeper melanic markings. The preserved type specimens have relatively few dark spots on the lower head and are mostly missing the stripe from the eye across the mid-jaw, but these light markings are likely part of the variability in marking intensity.

Barcode sequence. The segment of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype, paratypes, and related species for comparisons (Genbank accession number HQ654565 for the holotype; see Appendix for others). Following the database management recommendation of the BOLD the 648-nucleotide sequence of the holotype is presented here:

CCTTTACCTTATTTTTGGTGCATGAGCTGGGATAGTGGGCACTGCTTTAAGCCTTCTAATTCGAGCC-
GAACTAAGCCAACCCGGCGCCCTCCTGGGCGACGATCAAATTTATAACGTAATTGTTACAGCG-
CATGCTTTTGTAATAATTTTCTTTATAGTAATACCAATTCTCATTGGAGGCTTCGGGAACTGACTT-
GTCCCTCTAATGCTGGGAGCCCCGGACATAGCGTTCCCTCGAATAAACAACATAAGTTTCT-
GACTCCTACCCCCTTCTTTTCTTCTTCTCTTAGCTTCTTCTGGAGTTGAGGCAGGAGCTGGGACAG-
GCTGAACTGTATACCCTCCCCTCTCAGGCAATCTGGCCCATGCAGGGGCCTCTGTAGACCTAAC-
CATCTTTTCTCTTCACCTAGCAGGAGTTTCCTCCATCCTAGGTGCAATTAACTTTATTACAACAAT-
TATTAACATAAAACCCCCGGCTATTTCTCAGTACCAGACACCCCTCTTTGTTTGGTCCGTACTGAT-
TACAGCAGTTCTTCTTCTCCTCTCTCTTCCTGTTTTGGCAGCCGGGATTACTATGCTACTAACGGATC-
GAAATCTGAATACAACTTTTTTCGACCCAGCAGGAGGAGGGGACCCTATCCTCTACCAACA

Distribution. DNA sequence-matched *Emblemariopsis carib* have been collected from Puerto Rico and the U.S. Virgin Islands. Live photographs of similar Flagfin Blennies have been taken in the nearby Dominican Republic by Jose Alejandro Alvarez and Juan Carlos Navarro and in Haiti by Nick Hobgood. Specimens with similar morphology and meristics have been collected in St. Croix, USVI (Table 1). These photographs and specimens are designated *E.* cf. *carib*, since they are not confirmed to be *E. carib* by matching DNA sequences.

Table 1. Fin-ray counts for various species and populations of *Emblemariopsis* blennies. (correction in proof)

	Dorsal fin												Anal fin segmented rays			
	Total dorsal elements					Spines			Segmented rays							
	30	31	32	33	34	XIX	XX	XXI	10	11	12	13	19	20	21	22
E. signifer (Brazil)	–	1	17	16	2	–	11	25	–	9	24	3	–	5	30	1
E. carib (PR/VI)	–	8	–	–	–	1	7	–	–	7	1	–	1	7	–	–
E. cf. *carib* (St. Croix)	5	10	2	–	–	5	10	2	2	12	3	–	5	11	1	–
E. cf. *carib* (Barbados)	1	6	1	–	–	1	7	–	1	5	2	–	1	7	–	–
E. arawak (PR)	–	–	3	5	–	1	6	1	–	–	3	5	–	1	4	3

Outside the Greater Antilles and Puerto Rican Plateau, similar specimens have been collected in Barbados by Henri Valles (and sequenced here). Photographs of similar fish have been taken in the Bahamas, St. Vincent, San Andres, and the Mesoamerican Barrier Reef (Belize and Honduras) by Les and Keri Wilk, at the Cayman Islands by Cindy Abgarian and Everett Turner, and at Saba by Williams *et al*. (2010). These populations are not confirmed as *E. carib*, since populations in other regions of the Caribbean may represent distinct genetic lineages, perhaps deserving of their own species designation. Some photographs may represent variants of *E. arawak* or regional variations. The line-drawing of a male from New Providence, Bahamas by Stephens (1970), labeled as *E. signifera*, closely resembles *E. carib* in morphology.

Etymology. Named for the Carib native people of the Antilles; the specific epithet is a noun in apposition.

Comparisons to *E. signifer*. *E. signifer* from mainland Brazil (Figs. 10–17) are distinctly larger than *E. carib*, with no overlap in the size of the mature males in the collections (mature males defined by red-banded dorsal fins and prominent genital papillae). The specimens of mature males of *E. carib* and *E.* cf. *carib* from Barbados and St. Croix, USVI range from 13.1–17.5 mm SL, while those of *E. signifer* from Ubatuba (Sao Paulo) and Arraial do Cabo (Rio de Janeiro) range from 18.8–27.8 mm SL. Females are generally smaller than most mature males in both species; the largest specimen of female *E. signifer* was 21.7 mm SL. In addition, Raphael Macieira (pers. comm.) reports males up to 25.0 mm SL and females up to 22.4 mm SL among 13 *E. signifer* from Guarapari in Espirito Santo. Immature males can be difficult to distinguish morphologically from females.

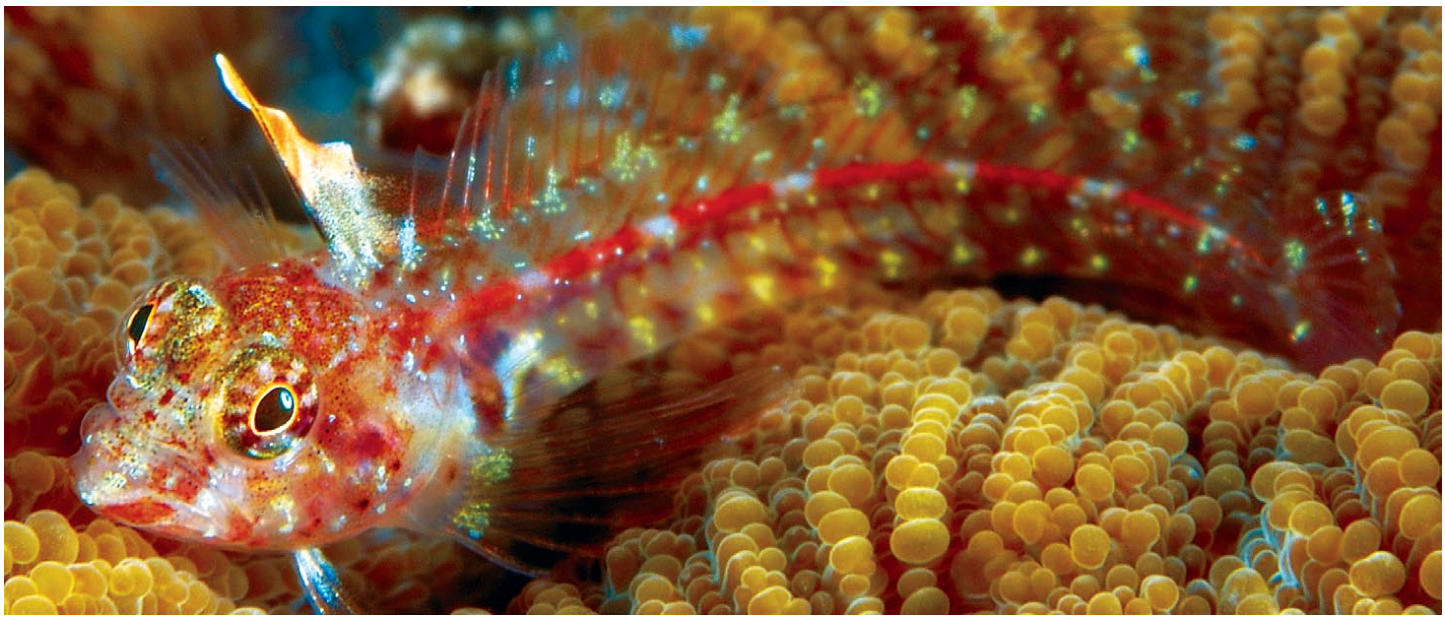


Figure 10. *Emblemariopsis signifer*, light male, Laje de Santos, São Paulo, Brazil. Photo © Mauricio Andrade.

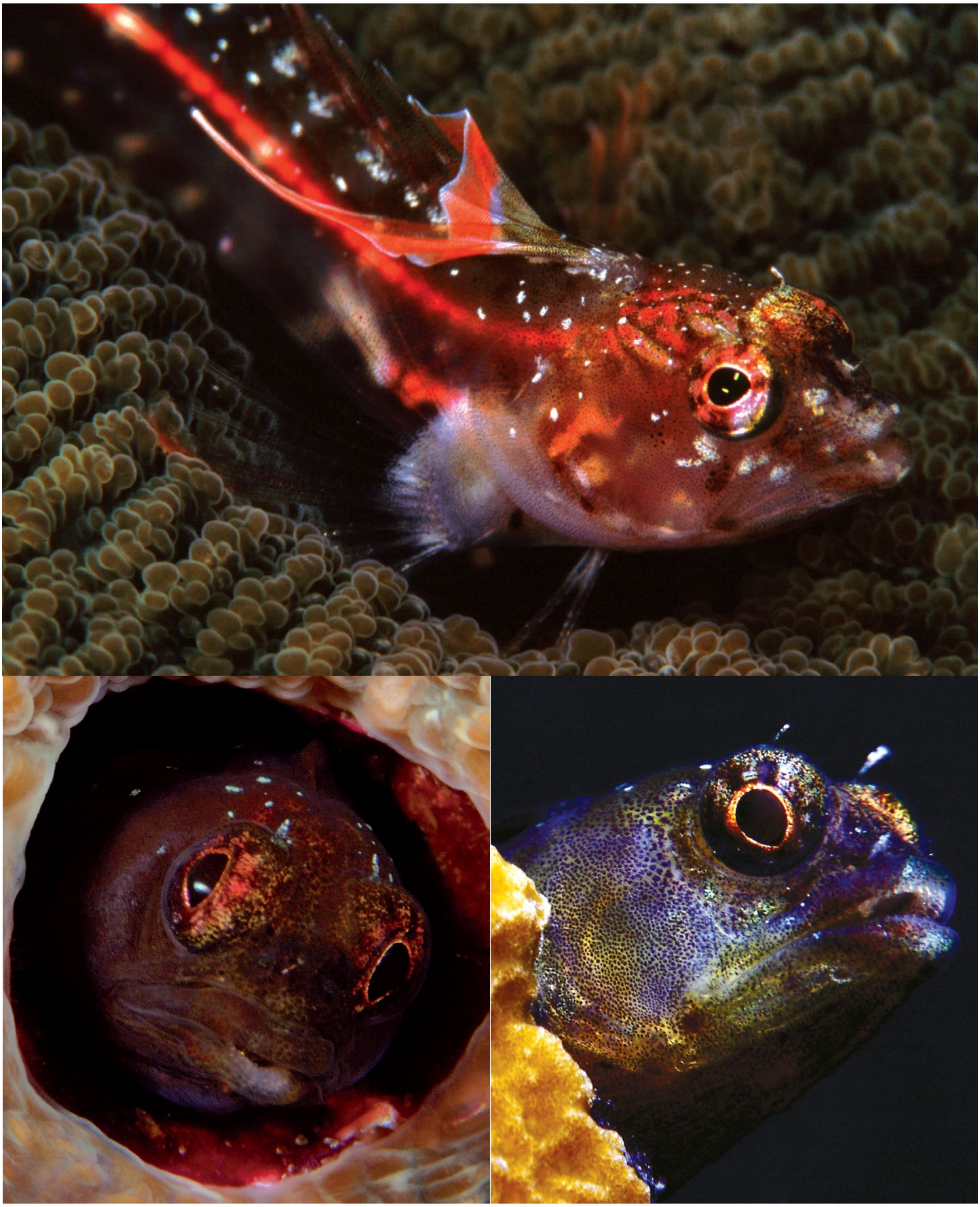


Figure 11. *Emblemariopsis signifer*. **top**: light male, Laje de Santos, São Paulo, Brazil. Photo © Mauricio Andrade; **bottom left**: dark male, Laje de Santos, São Paulo, Brazil. Photo © Mauricio Andrade; **bottom right**: dark male, Cabo Frio, Rio de Janeiro, Brazil. Photo © Ivan Cavas.

The type series of *E. carib* and the St. Croix and Barbados *E.* cf. *carib* have lower dorsal and anal (and caudal procurrent) fin-ray counts than *E. signifer* from Brazil (Table 1). Fin-ray counts for *E. signifer* are more variable than previously described, with four specimens from Rio de Janeiro having the highest counts, i.e. XXI (4) dorsal-fin spines and 12 (2) or 13 (2) rays with total dorsal elements of 33 (2) and 34 (2) and anal-fin elements of II,21 (3) or 22 (1) . *E. signifer* have 4–5 upper caudal-fin procurrent rays with a rare 3, while *E. carib* and *E.* cf. *carib* have 3 or 4.

Morphological differences between the two species are most likely explained by allometry and thus may not be diagnostic; for example, the jaw extends well past the rear edge of the eye in most male *E. signifer*, but not in male *E. carib*. However, that would be a result of a relatively larger eye in smaller fish– an almost universal allometry in fishes. Another difference of uncertain significance is the high frequency of missing cephalic pores among *E. carib* and *E.* cf. *carib*, where the majority of specimens are missing one to four of the interorbital set of pores. In contrast, only the occasional *E. signifer* was missing any of the interorbital set. Size alone did not explain the finding, since the difference held true for the overlap in size.

Based on specimens (both ethanol and formalin fixed) and underwater photographs of Brazilian *E. signifer*, there is marked variability in many characters, including those that have been cited as diagnostic for distinguishing various species in the genus. The length of the extended first dorsal-fin spines is variable, many mature males (dark and light) have the first spine reaching only to the 8th spine base when folded down, but others have the

Figure 12. *E. signifer*, dark male, Arraial do Cabo, Rio de Janeiro, Brazil. Photo © Eliane Comenda.

Figure 13. *Emblemariopsis signifer*, dark male, Laje de Santos, São Paulo, Brazil. Photo © Ary Amarante.

spine much extended. The proportion of the first spine occupied by the red band varies: the entire spine can be banded dark and light with the red band on the following membrane or the red band can occupy as much as the distal three-quarters of the first spine. The red band usually runs along the first three spinous membranes, but can extend past the seventh. The row of black spots on the proximal membranes of the mid to posterior spinous dorsal fin, present on most mature males, is variable– usually a single row, but some fish can have spots higher on the membrane. The spots in the row often comprise a few spaced about three spines apart, but sometimes in pairs on adjacent membranes (Fig. 15), or even on every membrane (especially anterior). The spots on the pectoral-

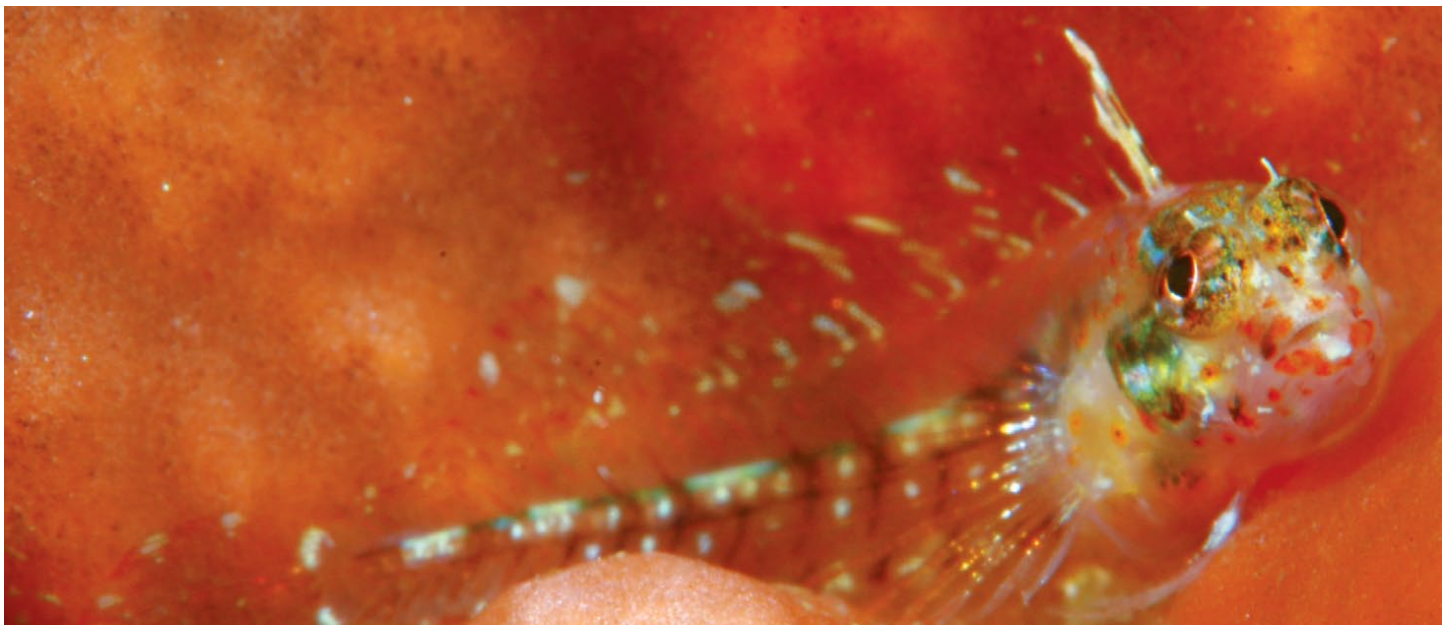


Figure 14. *E. signifer*, probable female, Angra dos Reis, Rio de Janeiro, Brazil. Photo © Marcelo Faustino.

Figure 15. *E. signifer*, light male, Arraial do Cabo, Rio de Janeiro, Brazil. Photo © Joao Paulo Cauduro Filho.

fin base (often absent on small specimens) can include any combination of the following: one anterior and low, almost under the operculum (rounded, or a short oblique line); a second near the insertion of the lower mid-rays; and, less frequently, a third on the upper third and/or the upper rim of the fin-base. A short row of several large dark spots can alternate with white spots on the upper side of the body between the opercle and the dorsal-fin base (Fig. 12). One male specimen has a short row of small dark spots along the anterior lateral midline.

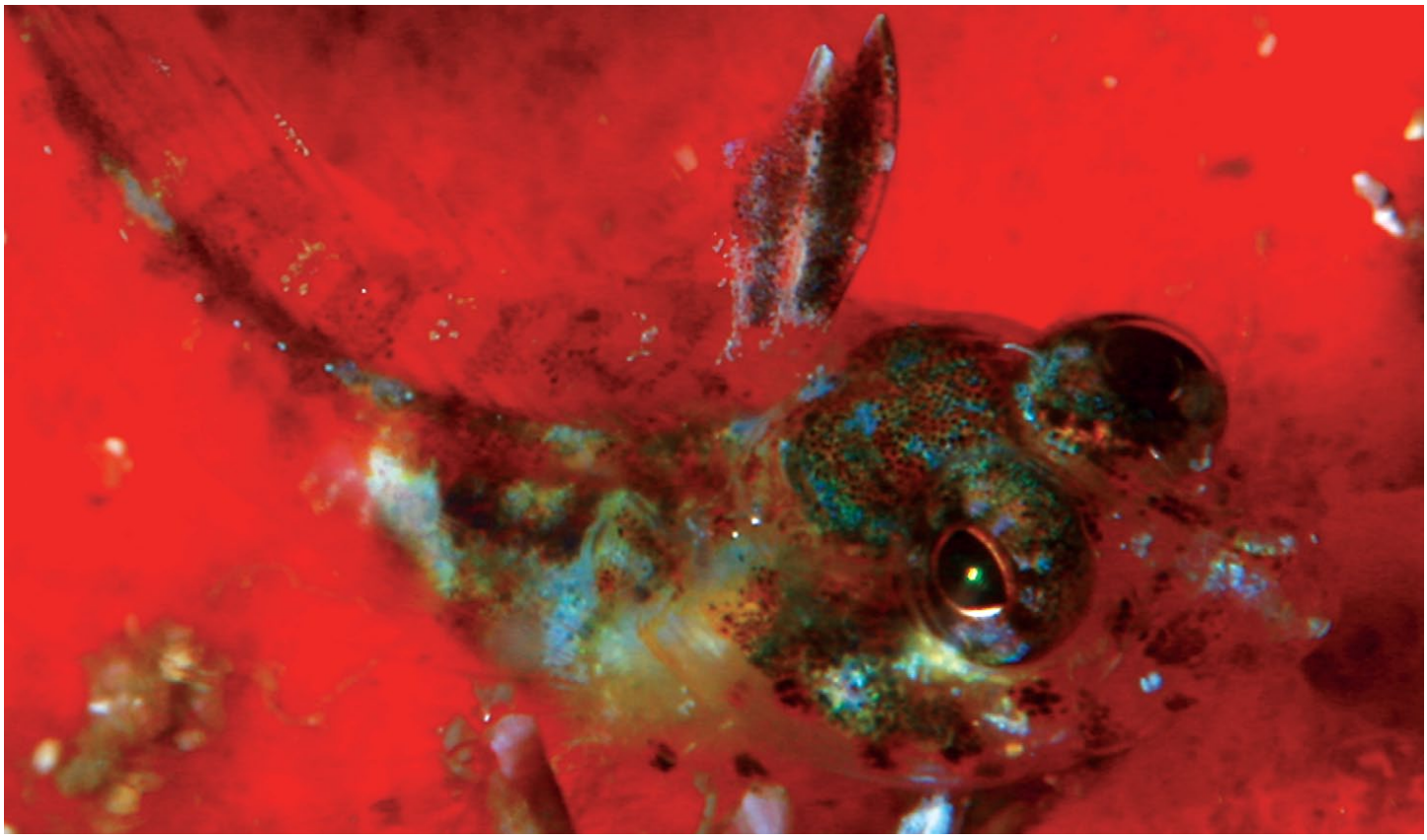


Figure 16. *E. signifer*, probable female, Angra dos Reis, Rio de Janeiro, Brazil. Photo © Marcelo Faustino.

Figure 17. *E. signifer*, blackened male (white fungus?), Laje de Santos, São Paulo, Brazil. Photo © Kadu Pinheiro.

Based on underwater photographs, the color description of *E. signifer* is mostly the same as *E. carib*. Their colors can vary greatly between habitats, against different backgrounds, and probably with mood and behavior. Color patterns in general vary by the relative intensities of the basic three color components, which change independently. The size and density of melanophores can vary from transparency or fine shading to prominent arrays of dark spots. White markings can be large and bright or subdued. The reds, pinks, and yellows can be intense or faint or absent. Interspecific comparisons are best made with individuals with matching relative intensities.

The most consistent apparent difference in markings between the two species appears to be the white spots over the cranium, which are prominent on both light and dark males of *E. signifer* and absent on *E. carib*. Even *E.* cf. *carib* with high white intensity on the head and body have no pattern of white spots on the upper half of the head (Fig. 9). *E. signifer* males with low white intensity typically still have some small white spots over the cranium (Figs. 10 & 15). Some *E. signifer* breeding males are completely blackened and then have no white spots at all, either over the cranium or at the cirri or on the jaws (Fig. 17). There are many fewer available underwater photographs for female or immature male *E. signifer*, but apparently they often do not show the white spots over the cranium (Figs. 14 & 16). Additional photographs are necessary to reveal which markings may reliably separate females and immature males of the two species.

Comparisons to other congeners. Mature males of *E. carib* (and *E. signifer*) can be distinguished from males of all other previously described species by the greatly extended first dorsal-fin spine. Some specimens of *E. ramirezi* and *E. tayrona* show elongation of the first dorsal-fin spines, but the first spine extends back at most to

the 8th spine when folded down. In addition, both males (except blackheaded) and females of *E. ramirezi* and *E. tayrona* can be distinguished from congeners by having obvious stripes across the operculum. Furthermore, they are apparently endemic to Venezuela and Colombia respectively (Acero 1987, Cervigón 1999, Rodriguez 2008).

Females and immature males of a set of congeners can be most easily distinguished from *E. carib* by having no orbital cirri (*E. bahamensis*, *E. bottomei*, *E. diaphana*, *E. pricei*, *E. randalli*). Among those species with orbital cirri, females of *E. leptocirris* are reported to have evenly sized dorsal-fin spines vs. a notched dorsal fin outline with longer first spines and a short fourth dorsal-fin spine in *E. carib* females (Stephens 1970). *E. ramirezi* and *E. tayrona* females have stripes across the operculum and the species are apparently endemic to Venezuela and Colombia respectively (Acero 1987, Cervigón 1999, Rodriguez 2008). *E. dianae* females have not been described and the species is apparently endemic to the mid-shelf reefs of Belize (Tyler & Hastings 2004). Females of *E. ruetzleri* and (maybe) *E. occidentalis* share both the orbital cirri and the notched fin outline with extended first dorsal-fin spines characteristic of female *E. carib*. Female *E. ruetzleri* can be distinguished by having 14 (vs. 13) pectoral-fin rays (Tyler & Tyler 1997) and, for ethanol-preserved specimens, a coalescing pattern of black spots over the cranium (vs. an even stippling of black spots). Putative female *E. occidentalis* are described by Stephens (1970) as "approximately identical to females of *E. signifer*a" but completely unmarked, and the latter feature was the only reported diagnostic character. It should be noted, however, that females in this genus often lose most or all of their markings with formalin preservation over time and thus the specimens may represent bleached females of other species. Furthermore, no photograph or observation I have encountered has confirmed any unmarked *Emblemariopsis* in the wild. The description of *E. occidentalis* remains to be clarified since its male holotype is a unique specimen from the Bahamas and the male paratypes are a different morph collected in the SE Caribbean (Stephens 1970 and pers. obs. of the ANSP specimens).

The features that distinguish *E. carib* from *E. arawak* are discussed below in the description of *E. arawak*.

***Emblemariopsis arawak,* n. sp.**

Fig. 18

Holotype. UF 179673 11.0 mm SL, male, Puerto Rico, La Parguera, Medialuna Reef, seaward slope, (17.935, -67.049), B. Victor and C. Caldow, Aug. 4, 2007.

Paratypes. UF 179674 (3) 9.3–10.0 mm SL, same as holotype; UF 179675 (1) 11.3 mm SL, Puerto Rico, La Parguera, Medialuna Reef, seaward slope, (17.935, -67.049), B. Victor, Aug. 9, 2007.

Nontype material. BV–PR784b (3) 8.8–10.1 mm SL, same as holotype (specimens damaged for DNA sampling).

Note: No mature male specimen has been sequence-matched to this species at the time of publication.

Diagnosis. A species of *Emblemariopsis* with total dorsal-fin elements 32–33; mode of D-XX,13 A-II,21 and Pect. 13; single short orbital cirrus present on each side and unbranched; females, immature males, and juveniles

Figure 18. *Emblemariopsis arawak*, holotype, immature male, 11.0 mm SL, La Parguera, Puerto Rico.

with nasal cirrus absent or shorter than nasal tube; first three dorsal-fin-spine bases close, first and second closest, widest gap between third and fourth; first spine longer or equal to second, third spine 1/2 to 3/4 of second and fourth spine shortest, forming notched dorsal-fin outline (in females, immature males, and juveniles); small individuals (known up to 11.2 mm SL) with first and second dorsal-fin spines well shorter than 12th spine, when folded down straight first two spines reaching back to between the 4th and 6th spine base, first spine dark with light tip; all stages with last (or 2nd to last) dorsal spine shortest, ¼ to ½ longest soft ray; evenly dispersed stippling of fine black spots over cranium; midline melanophore stripe on snout extending back past posterior nostril; lower pectoral-fin-base dark spots (when present) comprising a broad and long oblique line slanting down or a mostly horizontal band across the lower half, and sometimes additional spots.

Description. Total dorsal-fin elements 32–33; modal dorsal-fin rays XX,13; anal-fin rays II,21; pectoral-fin rays 13; pelvic-fin rays I,3; all fin rays unbranched; first three dorsal-fin-spine bases close, first and second closest, widest gap between third and fourth; first spine longer or equal to second, third spine 1/2 to 3/4 of second and fourth spine shortest, forming notched dorsal-fin outline (in females, immature males, and juveniles); small individuals (species known up to 11.2 mm SL) with first and second dorsal-fin spines well shorter than 12th spine, when folded down straight first two spines reaching back to between the 4th and 6th spine base, first dorsal-fin spine 7–8% (9%) SL, second spine equal or shorter, 6–8% (9%) SL, third spine 4–5% (5%) SL, fourth spine 4–5% (4%) SL, 12th spine 11–14% (10%) SL, last spine 4–6% (3%) SL; all stages with last (sometimes 2nd to last) dorsal spine shortest, only ¼ to ½ longest soft ray, longest dorsal-fin soft ray 10–11% (11%) SL; longest anal-fin soft ray 9–10% (10%) SL; last dorsal and anal-fin rays joined to caudal peduncle by membrane up to start of procurrent rays; pectoral-fin long, usually reaching base of 17th dorsal-fin spine, length 27–30% (31%) SL; pelvic-fin length 20–22% (22%) SL, when straightened horizontally usually reaching anogenital opening, spine not grossly visible, second ray longest, membranes deeply incised more than half-way between first and second rays; caudal fin truncate, length 19–20% SL, segmented caudal-fin rays 13 and 3–4 upper and 3–4 lower procurrent rays.

Body slim and elongate, body depth at dorsal-fin origin 16–17% (18%) SL, mid-body depth 13–15% (14%) SL; body width 10–13% (13%) SL; predorsal span 22–25% (24%) SL; preanal span 46–49% (46%) SL; caudal peduncle length 9–10% (9%) SL, caudal peduncle depth 6–7% (7%) SL; head length 27–33% (30%) SL; head width 34–50% (51%) HL; snout pointed, length 15–18% (18%) HL; eye large, orbit diameter 31–33% (30%) HL; short flattened finger-like orbital cirrus, speckled black and white, sometimes distinctly black, about ¼ to ½ pupil diameter; interorbital width 9–12% (10%) HL, mostly flat in immature fish; mouth medium (females and immature males), upper-jaw span 31–34% (31%) HL; upper-jaw length 34–36% (33%) HL; upper and lower jaws with variable-sized caniform teeth, in irregular rows anteriorly becoming a single row posteriorly (vomerine and palatine tooth patterns not evaluated); anterior nostril a small raised tube just behind upper lip with either no cirrus or a very short finger-like cirrus shorter than nasal tube, posterior nostril forms an elliptical opening adjacent to orbital rim; cephalic pore pattern not evaluated due to the tiny size of specimens; preopercle edge mostly smooth, at most a few tiny spines near angle in smallest fish.

Color. Based on the specimens and a photograph of a possible *E. arawak* from the Dominican Republic (Fig. 19), the color pattern is the same as that described for *E. carib*. In the preserved specimens, the dark markings on the anterior lower pectoral-fin base, when present, comprise a broad and long oblique line slanting down or a mostly horizontal wide band, but the consistency of this marking is uncertain for the species. Among the preserved specimens, the melanophores on the snout and interorbital are more numerous, as discussed below in the species comparisons.

Barcode sequence. The segment of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype, paratypes, and related species for comparisons (Genbank accession number HQ654547 for the holotype; see Appendix for others). Following the database management recommendation of the BOLD the 652-nucleotide sequence of the holotype is presented here:

CCTTTACCTCATTTTTGGTGCATGAGCTGGANTAGTGGGCACTGCTTTAAGCCTTCTAATTC-
GAGCTGAACTAAGCCAACCCGGCGCTCTCCTGGGCGATGACCAGATCTATAATGTAATCGTTA-
CAGCGCATGCCTTTGTAATAATCTTCTTTATAGTAATACCGATTCTCATTGGAGGCTTTGGAAACT-
GACTTGTCCCTCTAATACTTGGGGCCCCAGACATAGCCTTTCCACGAATGAATAACATGAGTTTCT-
GACTTCTACCCCCTTCGTTTCTTCTTCTCTTAGCTTCTTCAGGAGTTGAAGCCGGAGCTGGGACAG-
GTTGAACCGTATATCCCCCTCTCTCGGGCAACCTAGCCCATGCAGGGGCTTCCGTGGACTTAA-
CAATTTTTTCTCTCCACTTAGCGGGGGTTTCCTCTATTTTAGGTGCAATTAATTTTATTACAACAAT-
CATTAATATGAAGCCCCCGGCTACTTCGCAGTATCAGACACCCCTCTTTGTGTGATCTGTACTAATTA-
CAGCAGTTCTTCTTCTTCTCTCTCTTCCTGTTCTGGCAGCTGGAATTACTATGTTACTAACGGACCG-
GAATTTGAATACAACTTTCTTTGACCCTGCAGGAGGAGGAGACCCAATCCTGTACCAACACTTG

Distribution. DNA sequence-matched *Emblemariopsis arawak* have been collected only from Puerto Rico. Live photographs of similarly marked Flagfin Blennies have been taken in the nearby Dominican Republic by Juan Carlos Navarro (Fig. 19).

Etymology. Named for the Arawak native people of the Antilles; the specific epithet is a noun in apposition.

Comparisons to congeners. Female and immature male *E. arawak* can be separated from most other congeners using the same criteria as discussed above for *E. carib*. *E. arawak* were collected at the same site on the same day as *E. carib* and at overlapping sizes and both genders, allowing a direct comparison. *E. arawak* have a higher modal dorsal and anal-fin-ray count than *E. carib* and mostly overlap the counts for Brazilian *E. signifer* (Table 1). In addition, there are morphological and marking differences between the sets of specimens, although only small specimens of *E. arawak* are available. The nasal cirrus is poorly developed in *E. arawak* with at most a short, unpigmented, finger-like cirrus shorter than the nasal tube itself or no cirrus at all. In *E. carib*, the cirrus is long, longer than the nasal tube itself, and often speckled with melanophores. At the same sizes, *E. arawak* have relatively shorter first dorsal-fin spines, the first two not reaching back past the base of the sixth spine when folded down straight and well shorter than the 12th spine (specimens up to 11.3 mm SL), while all specimens of *E. carib* longer than 9.0 mm SL have the first two spines reaching past the base of the sixth spine and, in specimens over 10 mm SL, the first spine is longer than the 12th spine. The longest pelvic-fin ray is longer in *E. arawak*, when straightened out usually reaching the anogenital opening vs. not reaching the opening in *E. carib*. *E. arawak* have more melanophores on the dorsal midline of the snout and interorbital, some of which extend back past the level

Figure 19. Probable *Emblemariopsis* cf. *carib* left, possible *E. arawak* right. Left with long nasal cirrus and round lower pectoral-fin base spot; right with prominent interorbital dark streak and horizontal band on lower pectoral-fin base; Dominican Republic. Photos © Juan Carlos Navarro.

of the posterior nostril. In *E. carib*, there are few melanophores and they end at or before the level of the posterior nostril. Less obvious is a difference in the markings on the anterior lower pectoral-fin base; many of these small individuals have none, but, when present, the markings form a broad and long oblique line slanting down or a mostly horizontal wide band in *E. arawak* vs. a rounded spot or short oblique line in *E. carib*. Larger series and larger fish would be required to assess the reliability of these marking characters.

E. arawak can be distinguished from *E. signifer* by a slight difference in fin-ray counts (a mode of 13 dorsal-fin soft rays and 3 or 4 upper caudal-fin procurrent rays vs. 12 and 4 or 5) as well as by two of the same morphological differences that separate *E. arawak* from *E. carib*. Small *E. signifer* (11–13 mm SL) share the diacritical features separating *E. carib* from *E. arawak*: nasal cirri longer than the nasal tube and first dorsal-fin spines reaching past the base of the sixth spine. There are likely marking differences as well, but the assessment would require more specimens and live photographs of *E. arawak*.

Discussion.

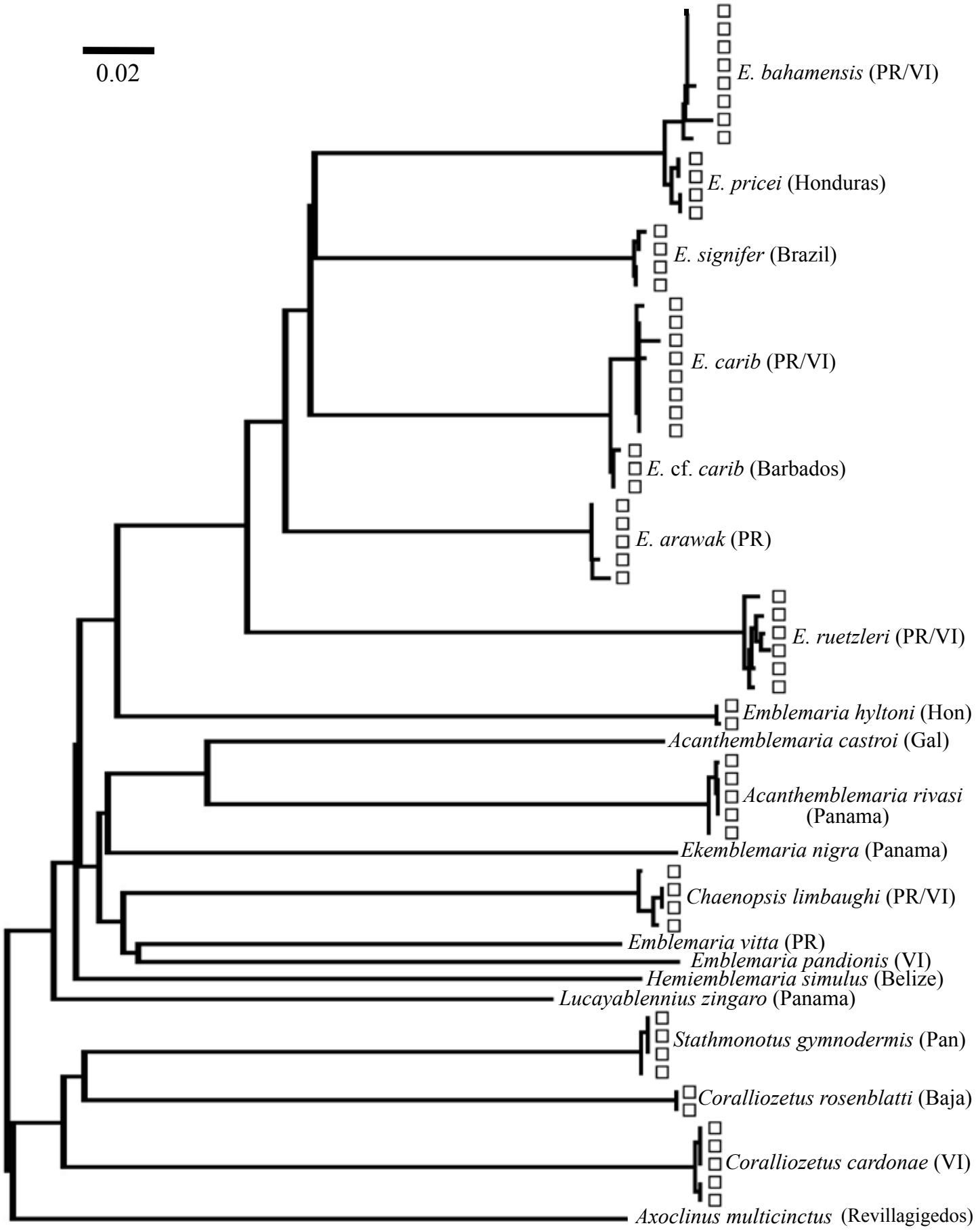
Local species. I collected *E. carib*, *E. arawak*, and two congeners, *E. bahamensis* and *E. ruetzleri*, at the type localities in Puerto Rico and the adjacent island of St. Thomas, USVI. Collections from nearby St. Croix, USVI by Smith-Vaniz *et al*. (2006) also contain four species: *E.* cf. *carib*, *E. bahamensis*, *E. ruetzleri*, and *E.* cf. *bottomei*. The first three correspond to three in my collections and the fourth is an additional species (note that *E.* cf. *bottomei* has no orbital cirrus and thus does not represent *E. leptocirrus* or *E. occidentalis*).

At present, there are six confirmed *Emblemariopsis* species in PR and the USVI: four with orbital cirri, comprising *E. carib*, *E. arawak*, *E. ruetzleri*, as well as *E. leptocirrus*, a species described from Puerto Rico and the USVI (Stephens 1970) but not collected by me or Smith-Vaniz *et al*. (2006), and two without orbital cirri, *E. bahamensis* and *E.* cf. *bottomei*. All but *E.* cf. *bottomei* and *E. leptocirrus* have corresponding barcode DNA sequences. Tyler & Hastings (2004) list five species in Belize, the other Caribbean location that has been intensively surveyed for *Emblemariopsis* (their introduction says, without explanation, seven (plus one) species are known from Belize).

Dennis *et al*. (2004) report a small female (11.9 mm SL) specimen of *E. occidentalis* from Puerto Rico "very similar to Fig. 5 of Stephens (1970)". The figure represents a female Stephens tentatively assigned to *E. occidentalis* solely on the basis of a lack of markings. However, female *Emblemariopsis* can often be missing markings after preservation, and these females may represent bleached *E. carib* or *E. arawak*. At present, *E. occidentalis* should not be reported on the basis of female specimens. Dennis *et al*. (2004) also report *E. bottomei* from Puerto Rico, based on males with a shorter head than reported for *E. bahamensis*; however, I have collected *E. bahamensis* in Puerto Rico with head lengths spanning the reported range for both species. It is uncertain whether the specimens in Dennis *et al*. (2004) represent *E. bahamensis* or the St. Croix *E.* cf. *bottomei*.

DNA sequences. The neighbor-joining phenetic tree of barcode sequences for the *Emblemariopsis* species reveals multiple distinct lineages with mostly deep divergences between species, as presently described (Fig. 20). The pairwise minimum interspecific distance within the genus varies widely, from 0.77% to 19.72%. The sequence of *E. carib* is 13.34% different from Brazilian *E. signifer* and *E. arawak* is 13.68% sequence divergent from *E. signifer,* and the two sympatric Caribbean species are 13.24% divergent from each other. The mitochondrial mutation rate among some chaenopsid blennies has been estimated to be potentially many times that of other reef fishes and perhaps one of the highest rates recorded for vertebrates (Lin *et al.* 2009, Eytan & Hellberg 2010); yet even with that measure (11.22% per million years), the two Caribbean species have been isolated from each other and the Brazilian Flagfin Blennies by more than a million years.

Figure 20. Neighbor-joining phenogram of Caribbean chaenopsids based on the 652 bp mtDNA barcode region of COI (Kimura two-parameter (K2P) model). The *Emblemariopsis* species are in the upper half and *Axoclinus multicinctus* is an outgroup. Boxes represent individuals when sample sizes exceed one per lineage. The scale bar is a 2% sequence difference. Gal=Galapagos, Hon=Honduras, Pan=Panama, PR=Puerto Rico, VI=Virgin Islands.


0.02
E. bahamensis (PR/VI)
E. pricei (Honduras)
E. signifer (Brazil)
E. carib (PR/VI)
E. cf. carib (Barbados)
E. arawak (PR)
E. ruetzleri (PR/VI)
Emblemaria hyltoni (Hon)
Acanthemblemaria castroi (Gal)
Acanthemblemaria rivasi (Panama)
Ekemblemaria nigra (Panama)
Chaenopsis limbaughi (PR/VI)
Emblemaria vitta (PR)
Emblemaria pandionis (VI)
Hemiemblemaria simulus (Belize)
Lucayablennius zingaro (Panama)
Stathmonotus gymnodermis (Pan)
Coralliozetus rosenblatti (Baja)
Coralliozetus cardonae (VI)
Axoclinus multicinctus (Revillagigedos)

If sequence comparisons are limited to populations from a single region (the 4 species from PR/USVI), the minimum interspecific distance, 13.24%, is much higher than the maximum intraspecific distance of 0.92% (in *E. bahamensis*). These conditions are required for effective barcode identifications (Meier 2008, Packer *et al.* 2009). However, the inclusion of lineages and species from other locations expands the intraspecific variation (*E. carib* and *E.* cf. *carib*) and sharply reduces the interspecific distances (*E. bahamensis* vs. *E. pricei*). As for the former case, the question of whether putatively conspecific regional populations represent different cryptic species is an open question with strong implications for the utility of barcoding: if the lineages represent distinct species then barcode sequences may identify them well. If the lineages are considered the same species with geographic variation, then the barcoding of these taxa would frequently suffer from false negatives, as individuals are sampled from unknown additional lineages. In contrast, in cases such as *E. bahamensis* and *E. pricei*, the addition of foreign sibling species with small sequence divergences would promote false positives as individuals are identified as the species first into the database (Meier 2008).

The *Emblemariopsis* species are a good illustration of the taxonomic complexity that is revealed with intensive DNA sequencing for some families of reef fishes. The results argue for a more comprehensive approach to these difficult groups, with the combination of DNA sequencing, live underwater photographs, and wider geographic and ontogenetic coverage than is typically applied. The problem with cryptic lineages remains the most stubborn: how to handle distinct lineages from different islands that have only small divergences in DNA sequence and no easily discernable morphological divergence, such as the Puerto Rican/USVI and Barbadian lineages of Flagfin Blennies. One is tempted to consider them local populations of the same species or, at most, subspecies, but then the same small divergences in DNA sequence can be found separating species with established meristic and morphological differences, such as the species pair of *E. bahamensis* and *E. pricei* (allopatric sister species, from the Bahamas/Antilles and Belize respectively). Of course, recent speciation would produce small divergences in neutral DNA markers and there is no reason to consider newer species less valid.

I do not include the Barbadian specimens similar to *E. carib* or other museum collections as confirmed *E. carib,* since a thorough assessment of live markings, colors, and DNA sequencing would be required to evaluate their status. A mismatch between genetic and phenotypic divergence has been noted for some Caribbean gobies with large differences in DNA sequences (Taylor & Hellberg 2006, Victor 2010), but the issue applies to lineages with small divergences as well and is going to be an increasingly troublesome problem in reef fish taxonomy.

Although phylogenetic relationships should not be inferred from sequencing single loci, and neighbor-joining trees (phenetic trees) are measures of similarity and not phylogenetic trees, the concordance of the phenogram with established phylogenies can be evaluated. It should be noted, however, that the deep divergences in barcode COI sequences among the chaenopsid blennies, within regions as well as between oceans (Lin *et al.* 2009, Eytan & Hellberg 2010), make the deep branching points derived from neighbor-joining techniques far less robust. Nevertheless, the phenetic tree based on barcode sequences for *Emblemariopsis* and all but one of the other Western Atlantic chaenopsid genera (Fig. 20) agrees with our present concept of the phylogeny of chaenopsids *sensu* Hastings (1997). Stephens (1963) erected the new genus *Pseudemblemaria* for *E. signifer*, but both he and subsequent authors later included *Pseudemblemaria* in *Emblemariopsis*. This is well supported by COI sequences, since both *E. signifer* and the new Caribbean species fall well within the *Emblemariopsis* clade. The genus *Emblemariopsis* has been included in *Coralliozetus* (Acero 1987), but the phenetic tree supports the separation of *Emblemariopsis* from *Coralliozetus* (Hastings & Springer 1994, Hastings 1997); indeed, the *Coralliozetus* clade is far distant from the *Emblemariopsis* clade with a remarkably large minimum intergeneric difference of 24.84% in the COI sequence (Fig. 20).

Material examined:

E. signifer: CIUFES-210, 211, 212, 213 (2), 463, 531, 726 (2), 763, 1273, 1441 (2) from Guarapari, Espirito Santo, Brazil (by Raphael Macieira); UF 47329, 172341 (15), 172342 (2), 172343, 172344 (13) from Ubatuba,

São Paulo, Brazil; BV–BR07 (4) from Arraial do Cabo, Rio de Janeiro, Brazil.
E. carib: UF 179454, 179455 (4) from St. Thomas USVI (STT); UF 179456 (2), 179457 from Puerto Rico (PR).
E. cf. *carib*: UF 159071 (3), 159075 (14) from St. Croix, USVI (SC); BV-HV05R (8) from Barbados.
E. arawak: UF 179673, 179674 (3) 179675, BV-PR784b (3) from PR.
E. bahamensis: UF 159063, 159097 (6), 159105 (2), 164756, from SC, USVI; BV-PR784b (3), PR789a, PR7811, ST952 (3), from PR and STT.
E. ruetzleri: UF 164691 from SC; BV-PR785a, ST307 (3), ST9429, ST9430 from PR and STT.
E. cf. *bottomei*: UF 160686 from SC.
E. pricei: BV-U8630 (2), U871, U873 from Utila, Honduras.

Acknowledgements

I appreciate the cooperation of the Center for Marine and Environmental Studies at the University of the Virgin Islands and the director Richard Nemeth, the Isla Magueyes Laboratories of the Department of Marine Sciences of the University of Puerto Rico at Mayagüez, and Bill Smith-Vaniz and Rob Robins at the Division of Ichthyology of the Florida Museum of Natural History. In particular, I thank Mahmood Shivji for making the expedition to the Virgin Islands possible and Chris Caldow for facilitating the project in Puerto Rico. Jeremiah Blondeau, Chris Caldow, Kenneth Clifton, Rosa Linda Gonzalez, Shaun Kadison, Tyler Smith, Henri Valles, H.J. Walker, George Walsh, and Brad Wetherbee provided invaluable assistance. Alfredo Carvalho, Carlos E.L. Ferreira, Sergio Floeter, and Raphael Macieira kindly provided data and other information on Brazilian fishes. Photos of Caribbean fishes were generously offered by Les and Keri Wilk, Jose Alejandro Alvarez, Juan Carlos Navarro, and Nick Hobgood for publication and by Cindy Abgarian, Mickey Charteris, and Everett M. Turner, Jr. for review. Photos of Brazilian fishes were graciously supplied for publication by Ary Amarante, Mauricio Andrade, Ivan Cavas, Joao Paulo Cauduro Filho, Eliane Comenda, Marcelo Faustino, and Kadu Pinheiro and for review by Athila Bertoncini Andrade, Armando de Luca Jr., Joao Luiz Gasparini, Joao Paulo Krajewski, Osmar Luiz Jr., Rafael Macieira, Francisco Nakahara, Carlos Augusto Rangel Goncalves, Denize Serafim, Alvaro Velloso, and Peter Wirtz. All of their efforts are greatly appreciated. Mark Sabaj Perez and Kyle Luckenbill at ANSP were very helpful with photographs from their collections. George Walsh and Walsh Paper Distribution of Westminster CA kindly sponsored preparation and publication of the project. R. Hanner and M. Shivji provided an excellent introduction to DNA barcoding. Barcode sequencing and BOLD informatics support were provided by the Canadian Centre for DNA Barcoding under the auspices of the Canadian Barcode of Life Network (via financial support from NSERC) and the International Barcode of Life (ibol.org) Project (via financial support from Genome Canada through the Ontario Genomics Institute).

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Appendix

Species	GenBank #	Collection location	SL (mm)	Collection code *
Acanthemblemaria castroi	HQ654524	Isabela, Galapagos, Ecuador	39.1	gal02112ac391
Acanthemblemaria rivasi	HQ654525	Farallones, Isla Grande, Panama	11.2	n7527aar112
Acanthemblemaria rivasi	HQ654526	Farallones, Isla Grande, Panama	12.2	n7527aar122
Acanthemblemaria rivasi	HQ654527	San Blas, Panama	26.0	sb80052ar260
Acanthemblemaria rivasi	HQ654528	Farallones, Isla Grande, Panama	25.0	n7527aar250
Acanthemblemaria rivasi	HQ654529	Farallones, Isla Grande, Panama	18.0	n7527aar180
Axoclinus multicinctus	HQ654530	San Benedicto, Revillagigedos	19.3	rev94411am193
Chaenopsis limbaughi	HQ654531	La Parguera, Puerto Rico	49.0	pr7c490
Chaenopsis limbaughi	HQ654532	La Parguera, Puerto Rico	22.0	pr7c220
Chaenopsis limbaughi	HQ654533	La Parguera, Puerto Rico	18.9	pr7c189
Chaenopsis limbaughi	HQ654534	St. Thomas, US Virgin Islands	17.2	st954c172
Coralliozetus cardonae	HQ654535	St. Thomas, US Virgin Islands	13.1	st80627cc131
Coralliozetus cardonae	HQ654536	St. Thomas, US Virgin Islands	13.0	st80627cc130
Coralliozetus cardonae	HQ654537	St. Thomas, US Virgin Islands	15.6	st80627cc156
Coralliozetus cardonae	HQ654538	St. Thomas, US Virgin Islands	14.2	st80627cc142
Coralliozetus cardonae	HQ654539	St. Thomas, US Virgin Islands	13.2	st80627cc132
Coralliozetus rosenblatti	HQ654540	Loreto, Baja California	19.0	baj60124tfcr190
Coralliozetus rosenblatti	HQ654541	Loreto, Baja California	20.0	baj60124tfcr200
Emblemariopsis arawak	HQ654548	La Parguera, Puerto Rico	10.0	UF 179674
E. arawak	HQ654549	La Parguera, Puerto Rico	11.3	UF 179675
E. arawak	HQ654553	La Parguera, Puerto Rico	9.7	UF 179674
E. arawak	HQ654555	La Parguera, Puerto Rico	9.3	UF 179674
E. arawak holotype	HQ654547	La Parguera, Puerto Rico	11.0	UF 179673
E. bahamensis	HQ654566	St. Thomas, US Virgin Islands	13.0	pr784aeb130
E. bahamensis	HQ654567	St. Thomas, US Virgin Islands	9.3	pr784beb93
E. bahamensis	HQ654568	St. Thomas, US Virgin Islands	15.4	st952eb154
E. bahamensis	HQ654569	La Parguera, Puerto Rico	13.7	pr789aeb137
E. bahamensis	HQ654570	La Parguera, Puerto Rico	17.5	pr784aeb175

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Appendix continued

Species	GenBank #	Collection location	SL (mm)	Collection code *
E. bahamensis	HQ654571	La Parguera, Puerto Rico	20.8	pr7811eb208
E. bahamensis	HQ654572	St. Thomas, US Virgin Islands	16.1	st952eb161
E. bahamensis	HQ654573	St. Thomas, US Virgin Islands	26.6	st952ebo266
E. carib	HQ654554	La Parguera, Puerto Rico	13.8	UF 179456
E. carib	HQ654556	La Parguera, Puerto Rico	10.8	UF 179456
E. carib	HQ654559	La Parguera, Puerto Rico	13.3	UF 179457
E. carib	HQ654561	St. Thomas, US Virgin Islands	10.1	UF 179455
E. carib	HQ654562	St. Thomas, US Virgin Islands	10.2	UF 179455
E. carib	HQ654563	St. Thomas, US Virgin Islands	9.9	UF 179455
E. carib	HQ654564	St. Thomas, US Virgin Islands	9.7	UF 179455
E. carib holotype	HQ654565	St. Thomas, US Virgin Islands	14.8	UF 179454
E. cf. *carib*	HQ654578	Barbados	13.0	HV0248es130
E. cf. *carib*	HQ654579	Barbados	13.2	HV05Res132
E. cf. *carib*	HQ654580	Barbados	13.0	HV05Res130
E. pricei	HQ654574	Utila, Honduras	21.2	u871ep212
E. pricei	HQ654575	Utila, Honduras	25.8	u8630ep258
E. pricei	HQ654576	Utila, Honduras	25.1	u8630e251
E. pricei	HQ654577	Utila, Honduras	11.9	u873ep119
E. ruetzleri	HQ654550	St. Thomas, US Virgin Islands	12.5	st307des125
E. ruetzleri	HQ654551	St. Thomas, US Virgin Islands	15.3	st307des153
E. ruetzleri	HQ654552	St. Thomas, US Virgin Islands	14.4	st307ees144
E. ruetzleri	HQ654557	St. Thomas, US Virgin Islands	14.4	st9429er144
E. ruetzleri	HQ654558	St. Thomas, US Virgin Islands	15.2	st9430er152
E. ruetzleri	HQ654560	La Parguera, Puerto Rico	9.2	pr785ae92
E. signifer	HQ654581	Rio de Janeiro, Brazil	23.5	br07es235
E. signifer	HQ654582	Rio de Janeiro, Brazil	19.1	br07191
E. signifer	HQ654583	Rio de Janeiro, Brazil	18.1	br07181
E. signifer	HQ654584	Rio de Janeiro, Brazil	24.1	br07241
Ekemblemaria nigra	HQ654542	Galeta, Panama	29.5	n762cen295
Emblemaria hyltoni	HQ654544	Utila, Honduras	23.2	u873eh232
Emblemaria hyltoni	HQ654545	Utila, Honduras	22.0	u873eh220
Emblemaria pandionis	HQ654546	St. Thomas, US Virgin Islands	29.6	st952e296
Emblemaria vitta	HQ654543	La Parguera, Puerto Rico	14.8	pr785aev148
Hemiemblemaria simulus	HQ654585	Carrie Bow Cay, Belize	24.5	bz98hs245
Lucayablennius zingaro	HQ654586	Salmedina, Portobelo, Panama	19.0	n7530blz190
Stathmonotus gymnodermis	HQ654587	Farallones, Isla Grande, Panama	16.5	n7527as165
Stathmonotus gymnodermis	HQ654588	Farallones, Isla Grande, Panama	13.4	n7527asg134
Stathmonotus gymnodermis	HQ654589	Farallones, Isla Grande, Panama	17.0	n7527asg170
Stathmonotus gymnodermis	HQ654590	Farallones, Isla Grande, Panama	18.0	n7527asg180

* Collection codes: UF is the FMNH collection, all other codes are the author's collection (BV).