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## The Redcheek Paradox: the mismatch between genetic and phenotypic divergence among deeply-divided mtDNA lineages in a coral-reef goby, with the description of two new cryptic species from the Caribbean Sea.

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### Abstract

A new micro-endemic goby, *Elacatinus rubrigenis*, is described from Utila in the Bay Islands of the Gulf of Honduras (Western Atlantic). The new species is similar to the Greenbanded Goby, *E. multifasciatus*, but differs in having a prominent red stripe across the cheek, more-numerous green bars on the body, and 11 second-dorsal-fin elements (vs. equal numbers of 11 and 12). The new species, the Redcheek Goby, replaces the Greenbanded Goby on the island of Utila and has not been sighted at any other location, potentially one of the smallest ranges reported for a Caribbean reef fish. The COI barcode mtDNA sequence for the Redcheek Goby is 11.2% divergent from the original type population of the Greenbanded Goby from the U. S. Virgin Islands. However, Panamanian Greenbanded Gobies, with no red cheek stripe, show a similarly large 11.3% genetic distance from the type population (within-population sequence variation is less than 1%). Despite the prominent marking difference, there is only a 3.3% sequence difference between Redcheek Gobies and Panamanian Greenbanded Gobies. These results highlight the lack of concordance between genetic and phenotypic divergence among cryptic lineages of reef fishes. The Panamanian population has some small meristic differences from the type population and is (reluctantly) described here as the new species *Elacatinus panamensis*. An unexpected 4.3% sequence difference between the adjacent Puerto Rican and Virgin Islands populations indicates that the Greenbanded Goby is likely to break up into inconveniently numerous discrete genetic lineages, presumably in allopatry. These sequence differences are generally greater than those separating the *Elacatinus* cleaning-goby species in the Caribbean and greater than the differences found among most genera of reef fishes. The remarkably deep DNA-sequence divergence among these allopatric cryptic species and lineages raises important and difficult questions about genetic structure, speciation processes, and species definitions among some coral reef fishes.

**Key words.** goby, gobiidae, new species, cryptic species, *Elacatinus rubrigenis*, *Elacatinus panamensis*, *Elacatinus multifasciatus*, Redcheek, Greenbanded, phylogeography, speciation, endemics, phylogenetics, taxonomy, informatics, barcode, COI, DNA, BOLD, Caribbean, western Atlantic, fish, coral reef fishes

**note:** text in blue indicates post-publication additions as of 5/10/2010

## Introduction

With few exceptions, coral reef fishes have pelagic larvae which spend weeks to months developing in off-reef waters. As a result of this high dispersal ability, most Western Atlantic reef-fish species range throughout the Caribbean Sea and adjacent areas (Randall 1996). Local endemism is generally uncommon; for example, Smith-Vaniz and Bohlke (1991) estimated that fewer than 1% of the reef-fish species of the Bahamas are endemic. Furthermore, many of these widespread species show little, if any, regional genetic structure (Shulman & Bermingham 1995; Purcell *et al.* 2006), particularly within the bounds of the Caribbean Sea with its many stepping-stone islands (e.g. Robertson *et al.* 2006; Bernardi *et al.* 2008; Rocha *et al.* 2008; but see Craig *et al.* 2009). The FISH-BOL campaign to sequence the COI barcode mtDNA segment has shown that marine fishes, in particular, can be reliably distinguished by their barcode sequence (reviewed in Ward *et al.* 2009) and the database reveals no significant intraspecific geographic variation within the Caribbean for most reef-fish species with a pelagic egg stage (e.g. Lutjanus; Victor *et al.* 2009), and even for some with demersal brooded eggs (e.g. *Coryphopterus*; Victor 2008).

Nevertheless, some groups of fishes, presumably those with more-restricted larval dispersal and strong local selection, do show interesting patterns of endemism, genetic structure, and cryptic speciation within the region, for example among the *Elacatinus* cleaning gobies (Colin 1975; Taylor & Hellberg 2003, 2005, 2006). Highly-detailed taxonomic studies from the pre-DNA-sequencing age found subtle divisions within a few wide-ranging species and proposed superspecies and species-complexes of “allospecies” (Greenfield 1979), essentially homologous to our more recent concepts of cryptic speciation within the region. With the advent of widespread DNA barcoding however, it is likely that many more examples of cryptic species will be found. Those reef-fish taxa that contain cryptic species can provide valuable insights into the processes of speciation and the biogeographic history of the region (Rocha *et al.* 2005; Rocha & Bowen 2008; Floeter *et al.* 2008), but also seriously challenge the traditional species concept (Sites & Marshall 2004; Meier 2008; Hastings & Springer 2009).

A distinctively marked population of the Greenbanded Goby, *Elacatinus multifasciatus*, was recently discovered and photographed by Les and Keri Wilk on the island of Utila, one of the Bay Islands of Honduras (Fig. 1). These small islands lie just to the east of the Mesoamerican Barrier Reef, which runs from the Yucatan to Belize, within the large bight that forms the Gulf of Honduras. The population was easily distinguished by the prominent red stripe across the cheek that has not been found on other populations of Greenbanded Gobies (Figs. 2, 3, and 4). This marked color difference prompted a regional comparison of the barcode COI mtDNA sequences for Greenbanded Gobies, a particularly useful tool for revealing hidden diversity within fish populations (Padial & De la Riva 2007; Ward *et al.* 2009).

## Materials and Methods

All fish lengths are standard length (SL). SIO is the institutional abbreviation for the Marine Vertebrate Collection of the Scripps Institution of Oceanography. Juvenile and adult specimens of Greenbanded Gobies and a comparison set of cleaning gobies were collected by hand on the reef and immediately preserved in 90% ethanol. The comparison set of cleaning gobies were from my collections from the Little Bahama Bank, Puerto Rico, Utila, Noronha, USVI, Florida, Belize, and Panama. Larval *Elacatinus panamensis* were collected from the waters over the reef as part of a long-term sampling program at Ukubtupo reef at the Smithsonian Tropical Research Institute field station in the San Blas Islands of Panama between 1980 and 1986 (methods and dates in Victor (1986)). Larvae were attracted to a light suspended over the shallow reef, captured in a dipnet, and preserved in 90% ethanol.

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  $\mu$ l volume including 6.25  $\mu$ l of 10%

trehalose, 2  $\mu$ l of ultra pure water, 1.25  $\mu$ l of 10 $\times$  PCR buffer (10mM KCl, 10mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20mM Tris-HCl (pH8.8), 2mM Mg SO<sub>4</sub>, 0.1% Triton X-100), 0.625  $\mu$ l of MgCl<sub>2</sub> (50mM), 0.125  $\mu$ l of each primer (0.01mM), 0.0625  $\mu$ l of each dNTP (10mM), 0.0625  $\mu$ l of Taq DNA polymerase (New England Biolabs), and 2  $\mu$ l 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 from this study were compiled using the Barcode of Life Data Systems (BOLD, [www.barcodinglife.org](http://www.barcodinglife.org); Ratnasingham and Hebert 2007). The sequence data for the Greenbanded Goby clade is publicly accessible on BOLD (project code GBG) and GenBank (accession numbers GU908134-GU908158). Sequence divergence was calculated 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.

### *Elacatinus rubrigenis*, new species

Fig. 1

#### *Elacatinus rubrigenis*

**Holotype.** SIO-09-303, 19.3 mm SL, female, Honduras, Bay Islands, Utila, North Side, (16.102, -86.978), 1m depth, pitted limestone shoreline, dipnet, B. Victor, C. Caldow, and K. Clifton, July 3, 2008.

**Paratypes.** SIO-09-303, (9) 8.7–18.5 mm SL, Honduras, Bay Islands, Utila, North Side (16.102, -86.978), B. Victor, C. Caldow, and K. Clifton, July 3, 2008.

**Diagnosis.** A species of *Elacatinus* with dorsal-fin elements VII, 11 (rare 10); anal-fin elements 10 (uncommon 9); pectoral-fin rays 20 (20–22); pelvic fins fully-joined, short cup-like disk complete; no scales; a pale head with a prominent red stripe from the tip of the snout across the eye ending abruptly above the pectoral-fin base with a short orange segment, followed by a short black segment; notably with an additional red stripe across the cheek from the corner of the jaw to the mid-pectoral-fin base; a dark green body with 23–26 thin light green bars along the full length of the body.



**Figure 1.** The Redcheek Goby, *Elacatinus rubrigenis*. Photo © Keri Wilk/ReefNet, Utila, Honduras.

**Description.** Dorsal-fin elements VII, 11 (1 of 10 specimens with 10); anal-fin elements 10 (uncommon 9); second-dorsal and anal fins with first element unsegmented, subsequent soft rays branched, the last to base; pectoral-fin rays 20 (20–22); pelvic-fin elements I,5 united as a short cup-shaped disk with a rounded edge and a distinct frenum; branched caudal-fin rays 15, upper unbranched caudal-fin rays 7–8, posteriormost one segmented; lower unbranched caudal-fin rays 6–7, posteriormost one segmented. Head and body naked. Cephalic canal system with pores B',C,D,E,F,H' and preopercle with pores M', N (low).

Measurements (types over 15 mm SL) are percent of SL, with range (holotype). Body elongate, depth at dorsal-fin origin 20.7–25.3 (20.7); width at pectoral-fin base (side-to-side) 18.9–22.9 (20.7); head length 29.9–31.3 (31.1); snout length 5.9–7.6 (6.2); orbit diameter 6.7–7.6 (7.3). Mouth terminal and large, the maxilla ending below the posterior 1/3 of pupil, the upper-jaw length 10.3–14.6 (12.4). Predorsal distance 36.7–41.0 (39.9). Spines of fins slender and flexible, first dorsal spine elongated, length 19.9–23.8 (20.7); pelvic fins short 14.4–16.9 (16.1); caudal peduncle length 14.5–15.7 (15.7), caudal peduncle maximum depth 12.6–13.5 (13.5).

**Color.** Juveniles and adults of both sexes are similarly marked. The head is pale with two prominent red stripes: one running from the tip of the snout across the eye ending abruptly above the pectoral-fin base with a short bright-orange segment, followed immediately by a short black segment. An additional broad red stripe runs across the cheek from the corner of the jaw to the mid-pectoral-fin base. The background body color is an olive green, sometimes dark, with 23–26 thin green bars along the full length. The narrow bars usually run vertically over the full depth of the body, but many individuals have unique variations, such as bifurcations, cross-links, or breaks in a pattern reminiscent of fingerprint ridges (bars counted if greater than 1/3 of the full depth). The fins are mostly unmarked, but can be dusky.

**Barcode sequence.** A 652-nucleotide sequence of the section of COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype (Genbank accession number GU908152). Following the database management recommendation of the BOLD the sequence of the holotype is presented here as well:

CCTTTACCTAGTCTTCGGTGCATGAGCTGGCATAGTCGGCACAGCACTCAGCCTTCTTATCCGGGCC-  
GAGCTAAGTCAGCCAGGAGCCCTGCTGGGAGACGACCAGATGTACAATGTAATTGTAACGGCT-



**Figure 2.** The Greenbanded Goby, *Elacatinus multifasciatus*. Photo by Jim Burke; aquarium specimen.

CACGCCTTTGTAATAATCTTTTTTATAGTAATACCAATTATGATTGGAGGATTTGGCAACTGACTA-  
ATTCCCCTAATGATTGGAGCCCCTGACATAGCATTCCCGCGGATAAATAACATAAGCTTCTGACTTT-  
TACCCCATCTTTTCTATTGCTTCTTGCTTCCTCAGGGGTTGAGTCTGGGGCGGGAACAGGGT-  
GAACAGTCTACCCCCCACTTGCAAGTAACCTAGCCCACTCAGGAGCATCCGTCGATTTAAC-  
CATCTTCTCACTTCACCTGGCAGGAATCTCTTCTATCCTAGGGGCAATTAATTTTCATTACCACTAT-  
CATAATATAAAACCCTTAGGAACTACCCAGTATCAAACCCCCCTGTTTGTATGGGCCGTCCTAATTA-  
CAGCAGTCCCTGCTCCTATCACTTCTGTACTAGCTGCCGGAATCACGATGCTTCTTACTGACC-  
GAAATTTAAATACCTCGTTCTTTGATCCTGCCGGTGGAGGGGACCCAATTCTTTATCAACATCTC

**Distribution.** *Elacatinus rubrigenis* is thus far known only from the island of Utila, Bay Islands of Honduras.

**Etymology.** The new species is named for the distinctive red stripe on the cheek, not found on Greenbanded Gobies from other locations.

**Common Name.** The common name of Redcheek Goby is proposed.

**Comparisons.** *Elacatinus rubrigenis* is a local form of the Greenbanded Goby *E. multifasciatus*, distinguished by having a prominent red stripe on the cheek, 11 second-dorsal-fin elements (vs. about equal numbers of 11 and 12 for *E. multifasciatus* from the type population in St. Thomas, USVI), and more of the thin green bars: 23–26 (vs. 15–21 in the *E. multifasciatus* from USVI). The barcode COI mtDNA sequence for *E. rubrigenis* is 11.2% different from the type population of Greenbanded Gobies and 3.3% different from the new species *E. panamensis*.

### *Elacatinus panamensis*, new species

Fig. 3

#### *Elacatinus panamensis*

**Holotype.** SIO-09-304, 23 mm SL, male, Panama, San Blas Islands, Smithsonian Tropical Research Institute facility adjacent to Wichubhuala, Kuna Yala (9.550, -78.953), 1m depth, pitted limestone, dipnet. B. Victor, August 15, 1981.

**Paratypes.** SIO-09-305, (5) 15.7–22.4 mm SL, Panama, San Blas Islands, Smithsonian Tropical Research Institute facility adjacent to Wichubhuala, Kuna Yala (9.550, -78.953), B. Victor, January 23, 1983; SIO-09-306, (5) 12–20.5 mm SL. Panama, Colon District, Los Farallones (9.644, -79.627), D.R. Robertson, J. Van Tassell, B. Victor, L. Tornabene, and E. Pena, May 27, 2007; SIO-09-307, (1) 8.8 mm SL. Panama, Colon District, Mogote



**Figure 3.** Holotype of *Elacatinus panamensis*, 23 mm SL male from San Blas, Panama (SIO-9-304).

Afuera (9.637, -79.524), D.R. Robertson, J. Van Tassell, B. Victor, L. Tornabene, and E. Pena, May 29, 2007.

**Non-type Material.** BV-SB86-405, 6.1 mm SL larva, Panama, San Blas Islands, Smithsonian Tropical Research Institute facility adjacent to Wichubhuala, Kuna Yala (9.550, -78.953), B. Victor, April 5, 1986; BV-SB86-502, 7.3 mm SL larva, Panama, same site, B. Victor, May 2, 1986; BV-SB86-516, 7.6 mm SL larva, Panama, same site, B. Victor, May 16, 1986; BV-SB86-625, 6.9 mm SL larva, Panama, same site, B. Victor, June 25, 1986.

**Diagnosis.** A species of *Elacatinus* with dorsal-fin elements VII, 11 (rare 12); anal-fin elements 10 (uncommon 9); pectoral-fin rays 20–21 (19–21); pelvic fins fully-joined, short cup-like disk complete; no scales; a pale head with a prominent red stripe from the tip of the snout across the eye ending abruptly above the pectoral-fin base with a short orange segment, followed by a short black segment; a dark green body with 16–23 thin light green bars along the full length of the body.

**Description.** Dorsal-fin elements VII, 11 (one of 16 specimens with 12); anal-fin elements 10 (uncommon 9); second-dorsal and anal fins with first element unsegmented, subsequent soft rays branched, the last to base; pectoral-fin rays 20 (19–21); pelvic-fin elements I,5 united as a short cup-shaped disk with a rounded edge and a distinct frenum; branched caudal-fin rays 15, upper unbranched caudal-fin rays 7–8, posteriormost one segmented; lower unbranched caudal-fin rays 6–7, posteriormost one segmented. Head and body naked. Cephalic canal system with pores B',C,D,E,F,H' and preopercle with pores M', N (low).

Measurements (types over 15 mm SL) are percent of SL, with range (holotype). Body elongate, depth at dorsal-fin origin 19.5–26.3 (22.6); width at pectoral-fin base (side-to-side) 17.6–20.9 (20.9); head length 26.7–31.2 (29.1); snout length 5.3–7.3 (6.1); orbit diameter 6.8–8.1 (7.0). Mouth terminal and large, the maxilla ending below the posterior 1/3 of pupil, the upper-jaw length 10.8–14.1 (12.2). Predorsal distance 35.7–39.5 (37.4). Spines of fins slender and flexible, first dorsal spine elongated in males (often in females as well), length 19.4–30.4 (30.4); pelvic fins short 14.3–17.9 (14.3); caudal peduncle length 14.1–16.9 (15.2), caudal peduncle maximum depth 12.7–14.6 (13.0).



**Figure 4.** The Greenbanded Goby, *Elacatinus multifasciatus*. Photo by Greg Rothschild; aquarium specimen.

**Color.** Juveniles and adults of both sexes are similarly marked. The head is pale with a single prominent red stripe running from the tip of the snout across the eye ending abruptly above the pectoral-fin base with a short bright-orange segment followed immediately by a short black segment. The background body color is an olive green, sometimes dark, with 16–23 thin green bars along the full length. The narrow bars usually run vertically over the full depth of the body, but many individuals have unique variations, such as bifurcations, cross-links, or breaks in a pattern reminiscent of fingerprint ridges (bars counted if greater than 1/3 of the full depth). The fins are mostly unmarked, but can be dusky.

**Barcode sequence.** A 514-nucleotide sequence, comprising the last 514 of the 652 bp section of COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype (Genbank accession number GU908156). Following the database management recommendation of the BOLD the sequence of the holotype is presented here as well:

```
TGTAATAATCTTTTTTATAGTAATACCAATTATGATTGGAGGATTTGGCAACTGACTAATCCCCCTA-
ATGATTGGAGCCCCTGACATAGCATTCCC GCGAATAAATAACATAAGCTTCTGACTTT-
TACCCCATCTTTTCTATTGCTTCTTGCTTCTTCAGGGGTTGAGTCTGGGGCGGGCACAGGCTGAA-
CAGTCTACCCCCACTTGCAAGTAACCTAGCCCATT CAGGAGCATCCGTCGATTTGACCATCTTCT-
CACTTCACCTGGCAGGAATTTCTTCTATCCNGGGAGCAATTAATTT CATTACCACTATTATTA-
ATATAAAACCCCTAGGAACTACCCAGTATCAAACCCCTTGTTTGTATGGGCCGTCCTAATTACAG-
CAGTCTCTGCTCCTGTCACTTCCCGTTCTGGCTGCCGGAATCACGATGCTTCTTACTGACCG-
AAATTTAAACACCTCGTTCTTTGACCCTGCCGGTGGAGGGGACCCAATTCTTTATCAACATCTC
```

**Distribution.** *Elacatinus panamensis* is known from the Atlantic coast of Panama from Colon to the San Blas Islands. The range extends eastward only into the adjacent Gulf of Urabá in Colombia (A. Acero pers. comm.) and confirmed reports extend westward only to the Costa Rican border (Robertson, pers. comm.).

**Etymology.** The new species is named for the country of origin.

**Common Name.** The common name of Panamanian Greenbanded Goby is proposed.

**Comparisons.** *Elacatinus panamensis* is a local form of the Greenbanded Goby *E. multifasciatus*, distinguished by having almost all with 11 second-dorsal-fin elements (vs. about equal numbers of 11 and 12 for *E. multifasciatus* from the type population in St. Thomas, USVI) and marginally more of the thin green bars: 16–23 (vs. 15–21 in the *E. multifasciatus* from USVI). Despite these minor meristic differences, the barcode COI mtDNA sequence for *E. panamensis* is 11.3% different from the type population of Greenbanded Gobies and 3.3% different from the Redcheek Goby, *E. rubrigenis*.

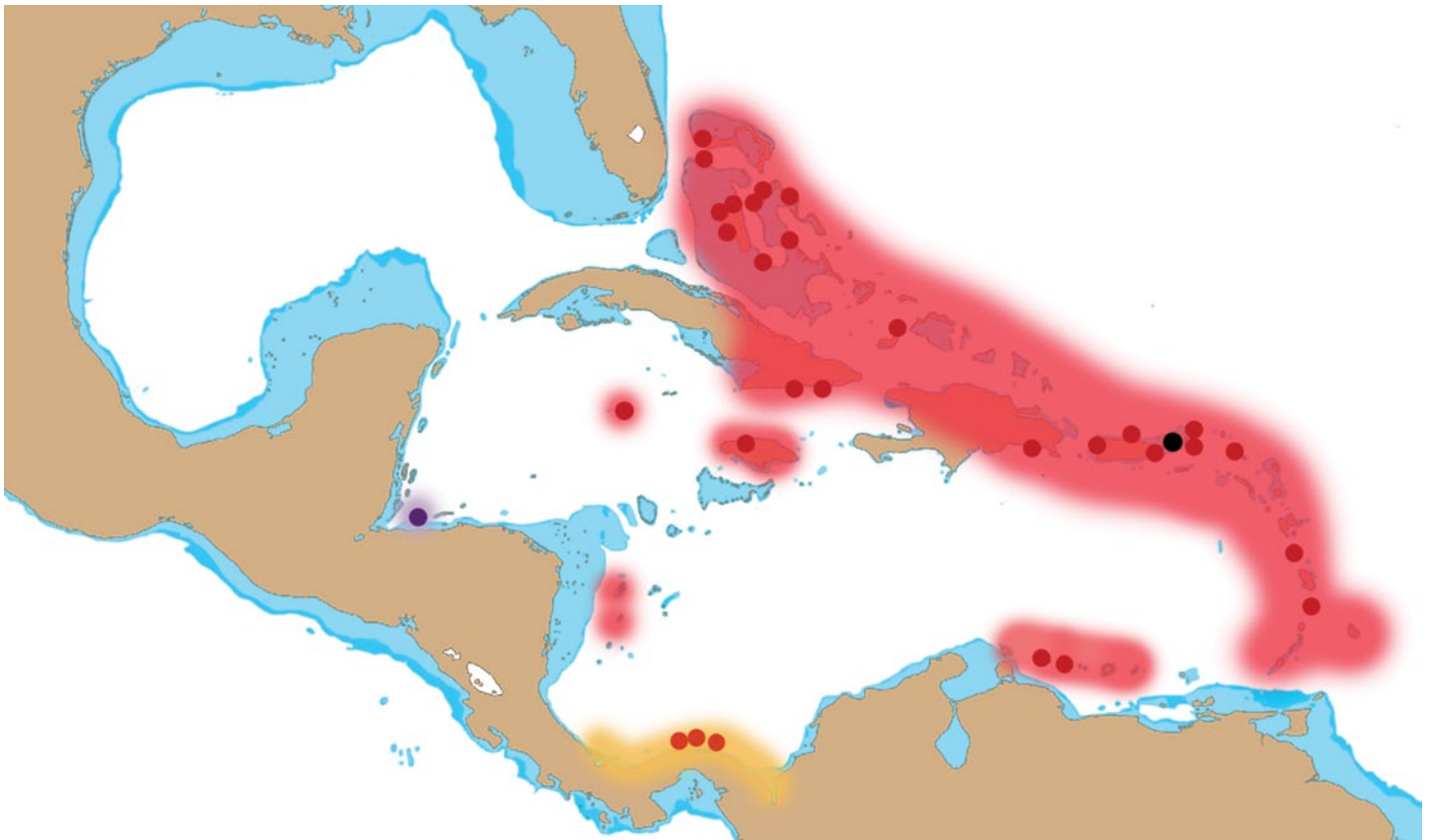
## Discussion

**Taxonomy.** Ginsburg (1933) reviewed the synonymy for *Gobiosoma multifasciatum* (Steindachner, 1876) which was first described from St. Thomas, USVI and nearby St. Barthelemy in the French West Indies. He listed the synonyms: *Gobius lineatus* (Poey) described from Cuba (invalid due to junior homonymy) and the junior synonym *Gobiosoma viridistriatum* (Silvester) from Puerto Rico; the latter described as sympatric with *G. multifasciatum* and differing by having seven instead of six first-dorsal-fin spines. However, six dorsal-fin spines are anomalous in this genus and thus was an error in the original description by Steindachner (Ginsburg 1933). All of the specimens in the synonymy are from Cuban, Puerto Rican, and U.S. Virgin Islands populations and therefore do not represent *Elacatinus rubrigenis* or *E. panamensis*. Ginsburg (1933) notes that 3 of 5 specimens he examined from the U.S. Virgin Islands and Cuba have 12 second-dorsal-fin elements (the two new species have almost all with 11).



**Biogeography.** The distribution of the Greenbanded Goby clade in the western Atlantic is unusual, with no collections from Florida, the Gulf of Mexico, or along the Mesoamerican Barrier Reef in Yucatan or Belize, despite a history of intensive collections and photography from those localities (Fig. 5). Surveys by REEF.org also do not list any sightings of Greenbanded Gobies from those areas (REEF 2009). Greenfield and Johnson (1999) surveyed the gobies of Belize and environs extensively and all 19 of the Greenbanded Gobies recorded in their censuses came from the Bay Islands of Honduras (and were presumably Redcheek Gobies). Bohlke and Robins (1968) list numerous collections from the Bahamas and the Virgin Islands and museum collections containing Greenbanded Gobies (AMNH, ANSP, BMNH, FMNH, GCRL, LACM, MCZ, USNM ) reveal additional specimens from a broad swath of the central and eastern Caribbean, i.e. Cuba, Jamaica, the Cayman Islands, and along the Lesser Antilles down to the Netherlands Antilles (Curacao and Bonaire). The only other collections are from Panama in the SW Caribbean. They appear to be absent from the continental coast of Venezuela and Colombia westward to the Panamanian border (Cervigon 1994; Acero, pers. comm.). Greenbanded Gobies have been sighted in surveys in the Dominican Republic (Williams et al. 1983) and observed at Isla Providencia and San Andres, two offshore islands in the Western Caribbean (REEF 2009). Areas with uncertain absence include western Cuba, Haiti, and some portions of the Central American coastline, which have been insufficiently surveyed.

Greenbanded Gobies occur almost exclusively among urchins in pitted limestone habitats in very shallow and clear water, often less than 1m deep. This habitat preference may help explain the geographic distribution for the group, since this habitat would be especially affected by cold weather and sedimentation and thus exclude the species from northern and some continental portions of the region. In addition, rocky shores are often broken up by stretches of sand and soft shorelines, promoting isolation of populations over spatial scales from meters to hundreds of kilometers. The apparent absence of Greenbanded Gobies from Yucatan and Belize coincides with



**Figure 5.** Range of the Greenbanded Goby group in the Western Atlantic. *Elacatinus multifasciatus* superspecies complex (red) with the type population (black), *E. rubrigenis* (purple), *E. panamensis* (yellow); circles represent museum collection locations, shading represents observations and probable range (map by Robert Myers, Coral Graphics, reprinted with permission).

the absence of high islands with extensive rock reefs and their presence on the adjacent Bay Islands, which have rocky shores, confirms a habitat association (Greenfield & Johnson 1990). These habitat restrictions may play an important role in isolating local populations and promoting allopatric speciation in this clade.

The highly restricted range of the Redcheek Goby is unusual for reef fishes in the Caribbean Sea, where the vast majority of reef-fish species occur throughout the region and many erstwhile endemics recently have been found to be more cosmopolitan with more collecting effort (e.g. Hastings & Robertson 1999; Smith-Vaniz & Bohlke 1991; Smith-Vaniz *et al.* 2006; Victor *et al.* 2010). However, with the advent of intensive DNA-sequencing, there is likely to be a resurgence of regional endemism with the discovery of numerous cryptic species.

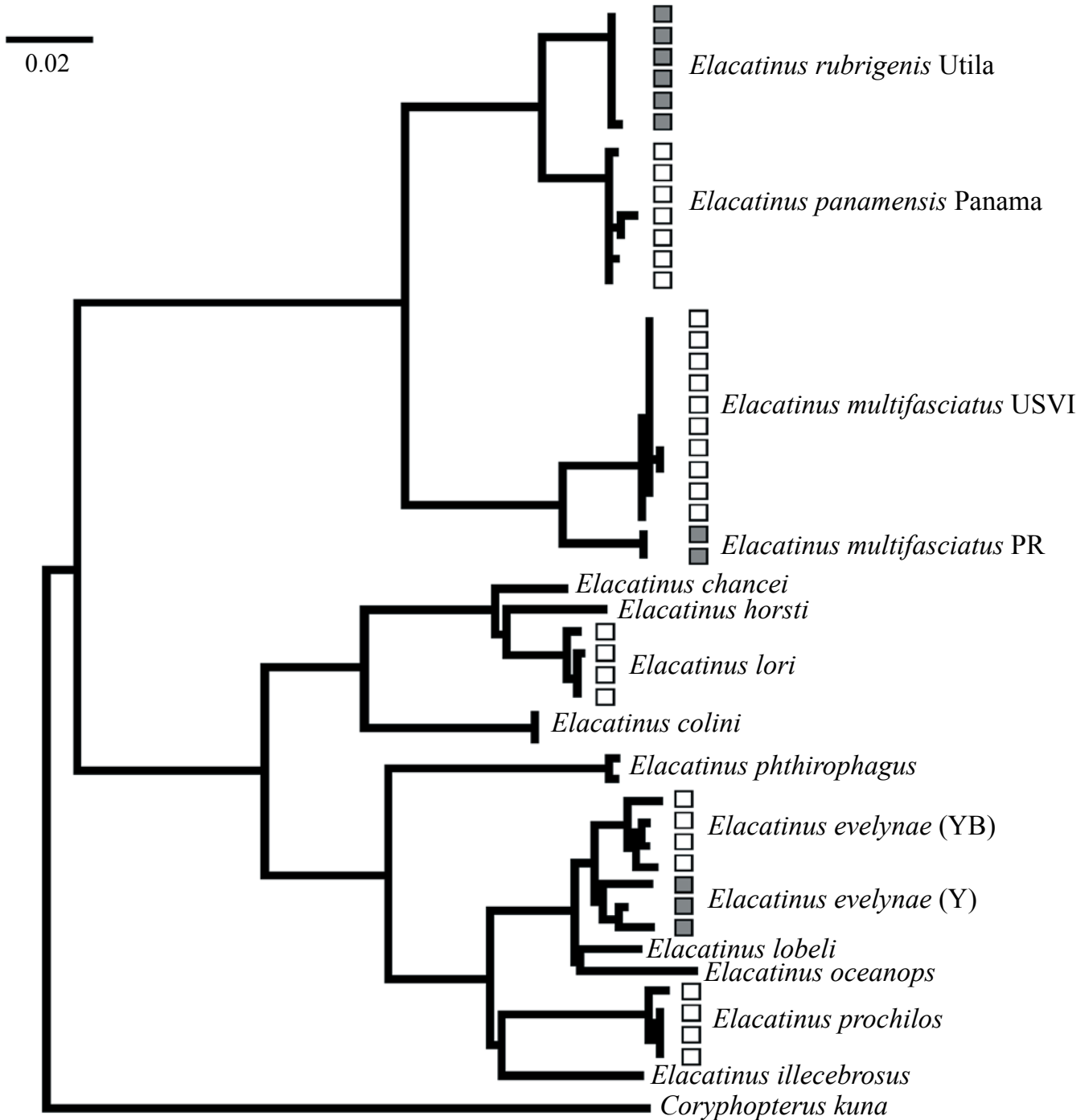
The Gulf of Honduras contains the Mesoamerican Barrier Reef system and the Bay Islands and is known to have several endemic reef-fish species, primarily gobies and chaenopsid blennies, including the local cleaning goby, *Elacatinus lobeli* (Randall & Colin 2009) and the sponge gobies, *E. colini* and *E. lori* (Randall 2009). A couple of very curious reef-fish species occur only on the mid-shelf reefs of Belize, including a wrasse, *Halichoeres socialis* (Lobel *et al.* 2009) and the chaenopsid blenny *Emblemariopsis diana*e (Tyler & Hastings 2004). Only one other reef-fish species has been reported to be endemic specifically to the Bay Islands, the chaenopsid blenny *Emblemaria hyltoni* (Johnson & Greenfield 1976; Greenfield & Johnson 1981; sometimes mistakenly included in species lists for Belize). Several toadfish species (Batrachoididae) are endemic to particular islands along the Mesoamerican Barrier Reef System (Collette 2002); notably, this family contains some of the very few reef-associated fishes which do not have a pelagic larval stage. These micro-endemic species may rival the Redcheek Goby for the smallest known range of a Caribbean reef fish. Some previously described endemics to the Gulf of Honduras have recently been found elsewhere (or represent allied cryptic species): *Emblemariopsis ruetzleri* has been collected from the Virgin Islands (Smith-Vaniz *et al.* 2006 and my collections) as well as from Panama (my collections), *Acanthemblaria* aff. *paula* (Johnson & Brothers 1989) has been found in Panama (my collections), and *Emblemariopsis pricei* has been collected from the Virgin Islands (FMNH).

*Elacatinus panamensis* is probably limited to the Panamanian coastline. The few other reef fishes presently reported endemic to the south-western corner of the Caribbean comprise the chaenopsid blennies *Ekemblemaria nigra*, *Acanthemblemaria rivasi*, and *A. betinensis*. Some euryhaline gobies are even more narrowly distributed, limited to sections of the Panamanian coastline near Colon (*Gobiosoma hildebrandi* and *G. spilotum*). Reef-fish species endemic to segments of the nearby Colombian coast include *Emblemariopsis tayrona* (Acero 1987), *Starksia variabilis* (Greenfield 1979), and the goby *Priolepis robinsi*.

Biogeographic provinces have been proposed within the Caribbean, although the vast majority of regional fishes are cosmopolitan. The provinces have been delineated in the past by patterns of endemism (e.g. Greenfield 1979) and, more recently, by genetic breaks (Taylor & Hellberg 2006). The Greenbanded Goby clade examined here agrees with a division between a Central American province and an Antillean province, as well as a degree of affinity in the west between the Bay of Honduras and the SW Caribbean coast. In this case, the populations in these two sectors of the Western Caribbean are genetically distinct and confirm a small but significant division within the proposed Central American province.

**Phylogeography.** The phenogram of the barcode DNA sequence for the various forms of Greenbanded Goby shows well-delineated clades, with low variation within location (<1%) and high variation between sites (Fig. 6). The results reveal a remarkable degree of divergence in the COI mtDNA sequences within the Greenbanded group, with the Redcheek Goby 11.2% sequence divergent from type-population Greenbanded Gobies and the Panamanian Greenbanded Goby 11.3% divergent from the type population. Interestingly, the Redcheek Goby and the Panamanian Greenbanded Goby are much closer in sequence to each other, 3.3% sequence difference, than either is to the type population. The lack of concordance between the degree of sequence divergence and phenotype in this case is notable.

The sequence divergences found in this study can be compared to sequence differences found among related groups. The degree of sequence divergence (at least for COI mtDNA) among the cryptic species of Greenbanded Goby is greater than the differences found among most of the *Elacatinus* cleaning-goby species that have clear and consistent differences in morphology, coloration, and habitat (Fig. 6). Indeed, phylogenetic studies on the genus (Taylor & Hellberg 2003, 2005, 2006) report similar (often much lower) sequence differences between many species. Ruber *et al.* (2003) concluded that the mutation rate of the *Risor* clade (including Greenbanded Gobies)



**Figure 6.** Neighbor-joining phenogram of Caribbean *Elacatinus* based on the 652 bp mtDNA barcode region of COI; distances are calculated using the Kimura two-parameter (K2P) model of base-substitution. The Greenbanded Goby group comprises the three species in the upper half, the remaining *Elacatinus* are cleaning gobies and *Coryphopterus kuna* is the outgroup. Boxes represent individuals when sample sizes exceeded two per species. The scale bar indicates a 2% sequence difference. Genbank numbers for the Greenbanded Goby group, from the top: GU908148, 49, 50, 52, 54, 46, 45, 47, 44, 57, 56, 55, 53, 38-43, 37, 36, 35, 34, 51, 58.

was relatively high. Any comparative measure of degrees of divergence across taxa makes the assumption of similar mutation rates among related species, which is not always supported (Ruber & Zardoya 2005).

Possible explanations for the extreme degree of genetic structure in the Greenbanded Goby lie mainly in the reproductive strategy of the species in combination with the patchiness of suitable habitat in the region. The Greenbanded Goby shares the basic reproductive and early life-history strategies of the cleaning gobies which would promote restricted larval dispersal: demersal brooded eggs (attached to the substrate), a large size at hatching (with new hatchlings able to orient and swim in place), and a relatively short 3–4 week larval life; all of which would facilitate behaviors to remain in inshore or nearby waters (Colin 1975; Taylor & Hellberg 2003; Victor in prep.). [Although little is known about the behaviors and distributions of most reef-fish larvae in the wild, larvae have been shown to actively orient, swim, and navigate \(reviewed in Leis 2006\)](#) and further assessment of the retention ability of goby larvae should provide valuable insights into the processes controlling larval dispersal.

A 4.3% sequence difference between the adjacent Puerto Rican and Virgin Islands populations suggests that the Greenbanded Goby is likely to break up into inconveniently numerous discrete genetic lineages within its range. It is likely, although not certain, that these lineages correspond to allopatric populations (but cryptic lineages in other species that were apparently allopatric have proven to be sympatric with additional collections). While these lineages will provide very interesting insights into the life-history, biogeography, and evolution of species, they also seriously challenge our traditional approach to taxonomy.

The distinction between “varieties” and species has long been recognized as an often arbitrary decision; indeed, Charles Darwin devoted many pages of his “On the Origin of Species” to a discussion of the issue (Darwin 1859). Taxonomists of his day were spared the additional complication of DNA-sequence comparisons, which obviously reflect phylogenetic history, but which generate a great deal of controversy in reconciling patterns and processes (reviewed in Wheeler (2008) and Packer *et al.* (2009)). Suffice to say, at present few taxonomists would rely solely on sequence differences to define species boundaries, yet it is difficult to deny that clear and deep sequence divergences represent long-isolated populations perhaps deserving of a species designation. The existence of sympatric cryptic species argues forcefully that these lineages can be reproductively isolated and represent indisputable biological species.

We would hope that morphological characters would be discovered to adequately distinguish cryptic species that were shown to have deep genetic divergences. While this is likely the case for many taxa, some reef fishes may not conform. The deep divergences between some populations of reef fishes that show little to no morphological differentiation raise difficult dilemmas for taxonomists: can we accept these local populations as new species? Will we accept hundreds of new cryptic reef-fish species in the region? The phenotypic distinction is obvious for the Redcheek Goby, however many other populations that are equally sequence divergent may not exhibit sufficient differences to our eyes to justify species designations.

Traditionally, most zoologists have used subspecies designations for similar-appearing allopatric populations that are somewhat different, however the degree of difference that would warrant subspecies vs. species designation is not quantified or agreed upon and is inconsistently applied (reviewed in Hastings & Springer 2009). Essentially, it rapidly devolves to a semantic argument that resolves little other than vocabulary. Unfortunately the boundary lines between populations, clades, subspecies, species, species-complexes, and superspecies remain nebulous despite our best efforts to impose order on a recalcitrantly amorphous biological construct.

We are left with the unsatisfying position of naming those cryptic species with some discernible visible difference and leaving the others. Some would contend that only those visible differences are relevant, while others promoting a phylogenetic species concept would argue that long-isolated and deeply genetically-divergent populations should be considered good species even if they happen not to have diverged sufficiently in form, markings, or color. Indeed they may have diverged even more in non-visible attributes, such as physiological tolerances, be-

haviors (habitat selection and reproductive interactions in particular), and life-history strategies. The Redcheek Goby provides a clear example of this dilemma: since this one population has evolved a prominent marking difference it is easy to separate, but there may not be any other reason to consider it a different case from many other deeply divergent lineages.

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A Redcheek Goby from the location of the type specimens, North Side, Utila, Honduras. Photo by Ken Clifton.