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***Hypoplectrus floridae* n. sp. and *Hypoplectrus ecosur* n. sp., two new Barred Hamlets from the Gulf of Mexico (Pisces: Serranidae): more than 3% different in COI mtDNA sequence from the Caribbean *Hypoplectrus* species flock.**

BENJAMIN C. VICTOR

Ocean Science Foundation, 4051 Glenwood, Irvine, CA 92604 and Guy Harvey Research Institute, Nova Southeastern University, 8000 North Ocean Drive, Dania Beach, FL 33004. E-mail: ben@coralreeffish.com

Abstract

The species flock of hamlets of the genus *Hypoplectrus* from the coral reefs of the tropical western Atlantic contains more than a dozen morphs distinguished by color pattern alone. Thus far, they have been characterized by a high level of genetic uniformity, raising many questions about whether they represent true species, incipient species, or color variants. However, DNA barcoding of the COI mtDNA gene from two otherwise unremarkable marking variations of the Barred Hamlet from the Gulf of Mexico and Florida reveals two separate relatively deep lineages more than 3% sequence-divergent from the large homogenous clade that contains all other barcoded *Hypoplectrus*, including Caribbean Barred Hamlets *H. puella* and seven other species, collected from all quadrants of the Caribbean Sea. The two new clades differ by more than 1.2% in the barcode sequence from each other and have distinctive marking patterns and are therefore described as new species. *Hypoplectrus floridae* n. sp. is found in the eastern Gulf of Mexico and South Florida and is characterized by a pair of symmetrical dark spots at the base of the caudal fin along with a break in the mid-body narrow bar. *Hypoplectrus ecosur* n. sp. has, at present, been collected only at Contoy, at the northern tip of the Yucatan peninsula, and is distinguished by the same pair of dark spots at the base of the caudal fin as well as a series of additional dark spots along the upper caudal peduncle and below the base of the soft dorsal fin. The remarkable combination of minor marking differences with large genetic differences not only underscores the inconsistency of phenotypic and genotypic divergence in some lineages of reef fishes, but provides philosophical support for the validity of species-level recognition for at least some members of these species flocks. Further documentation of the distribution and consistency of these mtDNA lineages is needed to resolve the complex phylogenetic processes taking place at the intersection of the biogeographic zones of the Caribbean, Florida, and the Gulf of Mexico.

Key words: hamlets, *Hypoplectrus*, speciation, adaptive radiation, species flock, morphotypes, morphs, evolution, reef fishes, DNA barcoding, phylogenetics.

Introduction

The many hamlets of the genus *Hypoplectrus* comprise a species flock of small and colorful basses (Serranidae) native to Caribbean coral reefs (Fischer 1980, Domeier 1994, Heemstra et al. 2002, Holt et al. 2010, Lobel 2011). Their bright and fantastically varied color patterns are explained mostly by aggressive mimicry of other colorful and innocuous reef fishes by the predatory hamlets (Randall & Randall 1960, Thresher 1978, Randall 2005). Although many species are both wide-ranging and frequently sympatric in the Caribbean region, several species have been described recently with particularly narrow ranges (Acero & Garzon-Ferreira 1994, Williams et al. 2006, Del Moral Flores et al. 2011, Lobel 2011). The species flock of Caribbean hamlets represents a particularly troublesome phylogenetic puzzle that challenges most of our preconceptions of what is a species and has been a source of angst among fish taxonomists for decades. Second only to the striking radiation of cichlids in the Rift Lakes of East Africa, the hamlets provide a remarkable case-study of the complexities of the grey zone between populations, morphotypes, subspecies, and species. The results typically resist easy explanation and continually confound attempts to understand speciation mechanisms that produce a proliferation of very different phenotypes, especially in sympatry (McCartney et al. 2003, Puebla et al. 2007, 2008, Aguilar-Perera & González-Salas 2010). Part of the paradigm of these species flocks as early radiations of partially interbreeding morphotypes is the genetic uniformity that appears to be characteristic of these groups (Ramon et al. 2003, Garcia-Machado et al. 2004, Holt et al. 2011). However, recently two otherwise unremarkable variants of the Barred Hamlet *H. puella* from the Gulf of Mexico and Florida with small differences in markings proved to have very different mtDNA barcode sequences, raising a new set of interesting questions that will undoubtedly be difficult to answer but will certainly cast a new light on this intriguing question.

Materials and Methods

Type specimens of the new species from Florida are deposited in the Marine Vertebrate Collection of the Scripps Institution of Oceanography (SIO) and those from the Yucatan at the Colección Ictiológica de ECOSUR-Chetumal (acrónimo ECOCH) located at El Colegio de la Frontera Sur, Unidad Chetumal, Chetumal, Quintana Roo, Mexico. Comparison samples of other species were collected by the author from Panama and Dominica and additional sequences were provided by Martha Valdez and Lourdes Vásquez-Yeomans of ECOSUR; Andrew Bentley of the Ichthyology Division, Biodiversity Institute, University of Kansas; Dirk Steinke of the Biodiversity Institute of Ontario; and the Laboratories of Analytical Biology at the Smithsonian Institution's National Museum of Natural History.

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 from this study were compiled using the Barcode of Life Data Systems (BOLD, www.barcodinglife.org; Ratnasingham & Hebert 2007, Ward et al. 2009). The sequence data is publicly accessible on BOLD and GenBank. Sequence divergence was calculated using 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.

Hypoplectrus floridae, n. sp.

Figs. 1–5

Holotype. SIO 12-58 (1) 40.3 mm SL, SW Florida, off Everglades, Ten Thousand Islands (25.6°, -81.25°), July 14, 2011, SEAMAP surveys.

Paratypes. SIO 12-58 (3) 33.4–39.4 mm SL, same as holotype; SIO 12-59 (1) 33.7 mm SL, SW Florida, off Everglades, Ten Thousand Islands (25.7°, -81.34°), July 13, 2011, SEAMAP surveys.

Diagnosis. A hamlet with the usual color and patterns of the brown Barred Hamlet but with several distinguishing markings: a pair of dark rounded spots at the base of the caudal fin placed above and below the midline, usually symmetrical, present on all stages, including large adults; a break in the narrow mid-body bar (the fourth bar, after the wide mid-body bar) just above the lateral line, usually associated with well-delineated and unbroken bars to each side; frequently a short rearward spur at the top of the last body bar (the fifth bar, forward of the caudal-peduncle bar), usually outlining a light wedge just below the base of the last dorsal-fin rays. Characters not frequent on Caribbean Barred Hamlets, but typically associated with *H. floridae* (and *H. ecosur*) include the bar under the eye being orange, even when the body bars are brown, and a dusky pelvic fin.

Description. Body wide and broadly oval, maximum body depth just behind operculum 38–42 (42)% SL (range of paratypes (holotype)), and compressed, side-to-side width 12–15 (13)% SL (measured just forward of pectoral-fin base); predorsal length 38–42 (42)% SL; preanal length 65–71 (67)% SL; prepelvic length 36–41 (40)% SL; caudal peduncle depth 14–15 (15)% SL, caudal peduncle length (dorsal) 7–10 (8)% SL; lateral line complete, curving in a high arch over pectoral fin becoming straight on caudal peduncle.

Head large 41–43 (41)% SL; dorsal head profile smooth and mostly straight, rising sharply from terminal tip of jaw to dorsal-fin origin; maximum head depth (measured at the rear end of the operculum) 37–41 (42)% SL; eyes large and round, orbit diameter 26–29 (28)% HL, pupil pear-shaped, pointing forward; interorbital space flat and relatively narrow, minimum width 13–15 (14)% HL; snout sharply pointed and short 23–30 (29)% HL; upper preopercular margin tilted slightly forward of vertical with a rounded angle to lower limb, small regular straight serrations on both limbs, longest at angle, about 22–33 in total; opercle with three flat spines, the middle largest and at the level of the lower third of eye. Anterior nostril a short tube, posterior nostril a flat opening with a diameter about half internarial distance.



Figure 1. *Hypoplectrus floridae*, holotype, SIO 12-58, 40.3 mm SL, Florida, Ten Thousand Islands.



Figure 2. *Hypoplectrus floridae*, Blue Heron Bridge, Florida. Photo © Kevin Bryant.

Mouth large, upper jaw ending at a vertical between mid-pupil and rear of eye, oblique length 40–45 (42)% HL; rear end of maxilla expanded, oblique height 11–13 (12)% HL; teeth small to moderately-sized and caniniform, stout and fixed, lining upper and lower jaws in irregular multiserial rows, larger in inner row. Vomerine and palatine teeth moderate, barely caniniform, vomerine teeth in a simple crescent facing rearward. Gill rakers on first arch long, flexible, and flattened with a broad base grading to short wide nubs at distal ends, all with moderate serrations, longest rakers near the angle on the lower limb exceed length of opposing branchial lamellae; upper limb 5–7, lower limb (including rudiments and corner) 11–14.

Dorsal fin single and long-based without notch between spinous and soft portions; dorsal-fin rays X,14 with last ray split to base, first dorsal-fin spine 6–7 (6)% SL; second 11–13 (11)% SL, third 16–18 (16)% SL, longest dorsal-fin soft ray 18–20 (20)% SL; anal fin short and broadly rounded, anal-fin rays III,7 with last ray split to base; first anal-fin spine 7–8 (7)% SL; second 13–16 (16)% SL, third 14–17 (17)% SL, longest anal-fin soft ray 17–21 (21)% SL; pectoral-fin rays 14 (one paratype with 13 on each side), including small uppermost and lowermost rays; pectoral-fin length 27–29 (28)% SL; pelvic-fin rays I,5 with pelvic-fin spine relatively short 15–16 (16)% SL, longest pelvic-fin ray 25–26 (25)% SL; caudal fin very slightly forked, caudal-fin length 26–29 (28)% SL; 17 principal caudal-fin rays.

Scales small and ctenoid, extending over body and head including cheek and operculum, sparing snout, interorbital, cranium, and underside of head; about 52 pored scales in lateral line from uppermost operculum to edge of hypural plate, about 75 lateral scale rows (counted above lateral line), about 12 scale rows between base of third dorsal-fin spine and lateral line.

Color in Life. Brown bars against a pale background with bright blue lines and spots on the head and thorax, as well as a colon-like pair of rounded darker brown to black spots, typically symmetrical, at the base of the caudal-fin rays. The most anterior of the six dark bars curves from the top of the head through the eye down across the lower cheek to meet the origin of the pelvic fin. This bar is typically more orange than brown and is outlined by thin blue lines below the eye, with the posterior blue line running out along the margin of the pelvic fin. The snout

is speckled with round blue spots and short lines and the orbit is ringed with a blue line. The snout markings vary from one fish to another and likely function for individual recognition. Additional blue spots and lines run over the interorbital and form several thin blue bars over the operculum and down over the thorax as well as along the base of the pectoral fin (occasionally additional fainter blue bars continue rearward along the sides). A larger round blue spot is conspicuous near the upper end of the opercular flap. The second brown bar starts as a broad band forward of the dorsal fin narrowing down across the rear opercular flap to bracket the base of the pectoral fin, the lower portion is frequently more orange than brown and that portion is usually outlined by thin blue lines. The third bar is the widest and extends from the base of the spinous dorsal fin down and broadly across the abdomen, usually merging with a dusky pelvic fin. The fourth bar is typically the narrowest and runs downward from the anterior soft-dorsal-fin base; notably, however, the bar dissolves in a band above the lateral line, and then reemerges below the lateral line for a short way before fading well above the anal-fin base. The fifth bar is wider than the fourth, bridges the rear portion of the soft dorsal and anal fins and is usually uninterrupted, as is the final bar at the end of the caudal peduncle, which is typically darker on the upper portion. Frequently the upper rear corner of the fifth bar extends farther back than the rest of the bar, outlining a small paler wedge below the base of the last dorsal-fin rays; this spur can stand out as darker oblique line. The median fin membranes are edged with thin ribbons of blue, often underlain with a ribbon of yellow, while the remainder of the membranes are reticulated with thin blue and yellow lines.

Color in Alcohol. Specimens in ethanol retain only the shades of brown described for live color, but include the diagnostic dark spots and bars and prominently dark pelvic-fin membranes. The broken fourth body bar is evident on the larger types in ethanol.

Barcode Sequence. A 652-nucleotide sequence of the section of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype and paratypes (see Appendix). Following the database management recommendation of the BOLD the sequence of the holotype (Genbank accession JX444756) is presented here as well:

CCTCTATTAGTATTTGGTGCATGAGCTGGAATAGTGGGTACCGCCCTTAGCCTCTTAATTC-



Figure 3. *Hypoplectrus floridae*, Fort Lauderdale Beach, Florida. Photo © Michael Kovach.

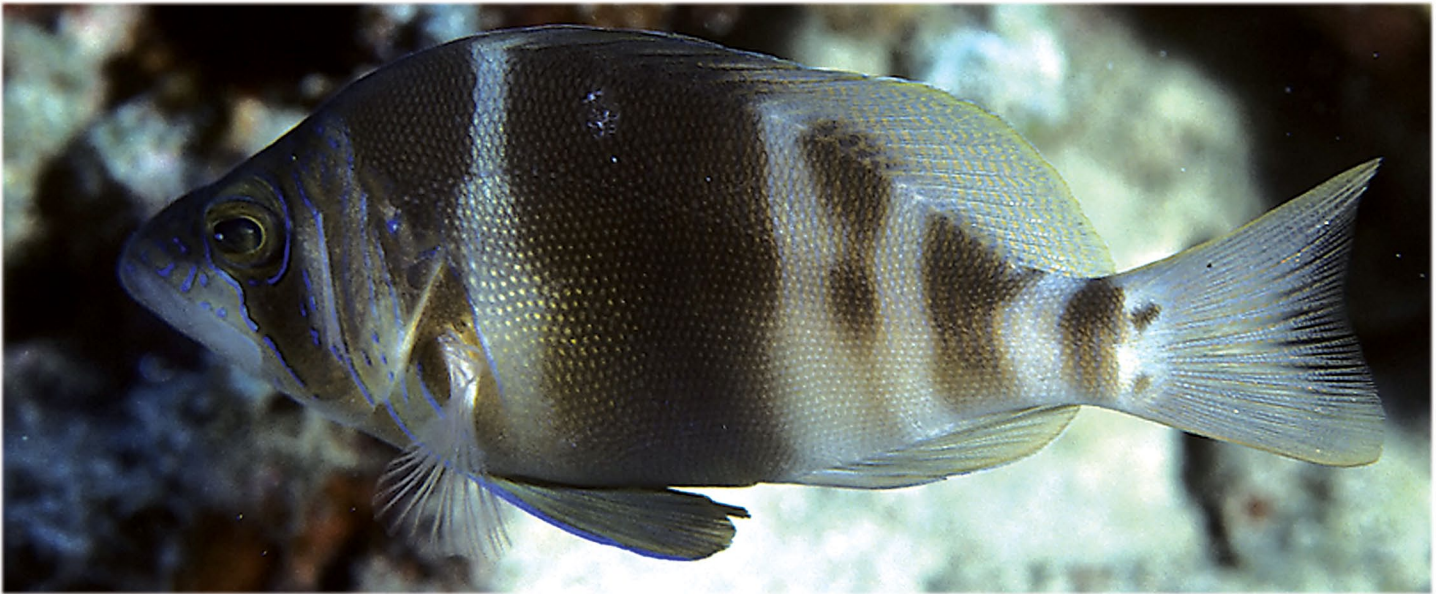


Figure 4. *Hypoplectrus floridae*, Jupiter, Florida. Photo © David Snyder.

GGGCCGAGTTGAGTCAACCCGGAGCTCTTTTAGGAGACGACCAGATTTATAATGTAATTGT-
TACAGCACACGCCTTCGTAATAATTTTCTTCATAGTCATGCCAATTATGATTGGAGGCTTC-
GGCAACTGACTCATTCCCTTAATGATTGGTGCGCCCGACATGGCATTTCCTCGTATAAA-
CAATATGAGCTTTTGACTTCTTCCTCCTTCCTTCCTTCTCCTTCTGGCCTCATCAGGGG-
TAGAAGCAGGAGCTGGGACGGGTTGAACTGTTTATCCTCCACTAGCCGAAACTTAGCACAT-
GCTGGGGCTTCAGTTGACTTAACAATTTTCTCACTTCATCTAGCAGGTGTATCCTCTATCCTC-
GGGGCAATCAACTTTATTACTACGATCATTAAATATGAAACCTCCTGCTATCTCCCAATAT-
CAAACCTCCTCTCTTTGTTTGAGCCGTCCTAATTACAGCAGTTCTACTTCTTCTTTCTCTTC-
CAGTCCTTGCTGCTGGAATTACTATACTGCTTACAGACCGAAACCTAAATACTACTTTCTTT-
GACCCTGCTGGGGGAGGAGACCCCATTTTACCAACATCTA

Etymology. The new species is named for the state of origin in the USA.

Comparisons. The new species can be distinguished from all other Caribbean hamlets by the pair of dark spots at the base of the caudal fin on large adult fish. The spots are part of the early juvenile markings of many hamlets, but are not present on adults of Caribbean hamlets. Juvenile hamlets with the dark spots also typically have two adjacent white spots and all of the spots typically disappear at the time the bars develop in Caribbean hamlets. The up-



Figure 5. *Hypoplectrus floridae*, Key West, Florida. Photo © Rob McCall, Eco-Dives of Key West.

per spot on other juvenile hamlets is usually larger and extends onto the caudal peduncle, forming the upper part of the dark bar on the caudal peduncle present on some hamlet morphs. Notably, in *H. floridae*, the upper spot of the pair clearly separates from the larger blotch making up the last bar. Since juvenile hamlets typically cannot be assigned to species, many larger juveniles with persistent prominent enlarged dark spots at the base of the caudal fin merging with the bar on the caudal peduncle likely represent the common Butter Hamlet, *H. unicolor*, which have a characteristic large black blotch covering the upper caudal peduncle. Butter Hamlets do, on occasion, have a transitory phase with indistinct bars, but always with the prominent blotch on the caudal peduncle (this phase is often labeled as a Barred Hamlet-Butter Hamlet hybrid, which is certainly quite possible, e.g. Fig. 6).



Figure 6. *Hypoplectrus* prob. *unicolor*., Lake Worth Lagoon, Florida. Photo © Judy Townsend.

The mid-body narrow bar is occasionally broken in otherwise-normal Barred Hamlets from Caribbean locations, but usually in conjunction with a general lightening of the upper rear body, unlike in *H. floridae*. The new Yucatan species, *H. ecosur*, shares the pair of spots on the caudal-fin base, but can be distinguished by a row of four distinct dark spots below the base of the soft dorsal fin and on the upper caudal peduncle. The darkened spur on the upper rear fifth bar on *H. floridae* is typically oblique, unlike the spot in that location on *H. ecosur*.

Distribution. The Barred Hamlet is one of the most common hamlets throughout the Caribbean Sea as well as in Florida. They range up the coast of S. Florida, mostly dropping out north of Cape Canaveral in the east and Sarasota in the west, and are rarely documented in the northern Gulf of Mexico, e.g. from the Flower Gardens and the offshore oil rigs (REEF 2012). Photographs compiled from a set of collaborating underwater photographers and from a review of internet images indicate that Barred Hamlets with the combination of two symmetrical dark spots at the base of the caudal fin and a broken mid-body narrow bar are exclusively from the E. Gulf of Mexico and around S. Florida. Specimens with the normal Barred Hamlet markings are also present and frequently photographed in S. Florida, at least along the path of the Gulf Stream and particularly on the Tortugas, the farthest reefs in the Florida Keys chain. Some photographs of the *H. floridae* pattern are from the aquarium trade, which is known to collect Barred Hamlets from the Florida Keys (right & Fig. 7).

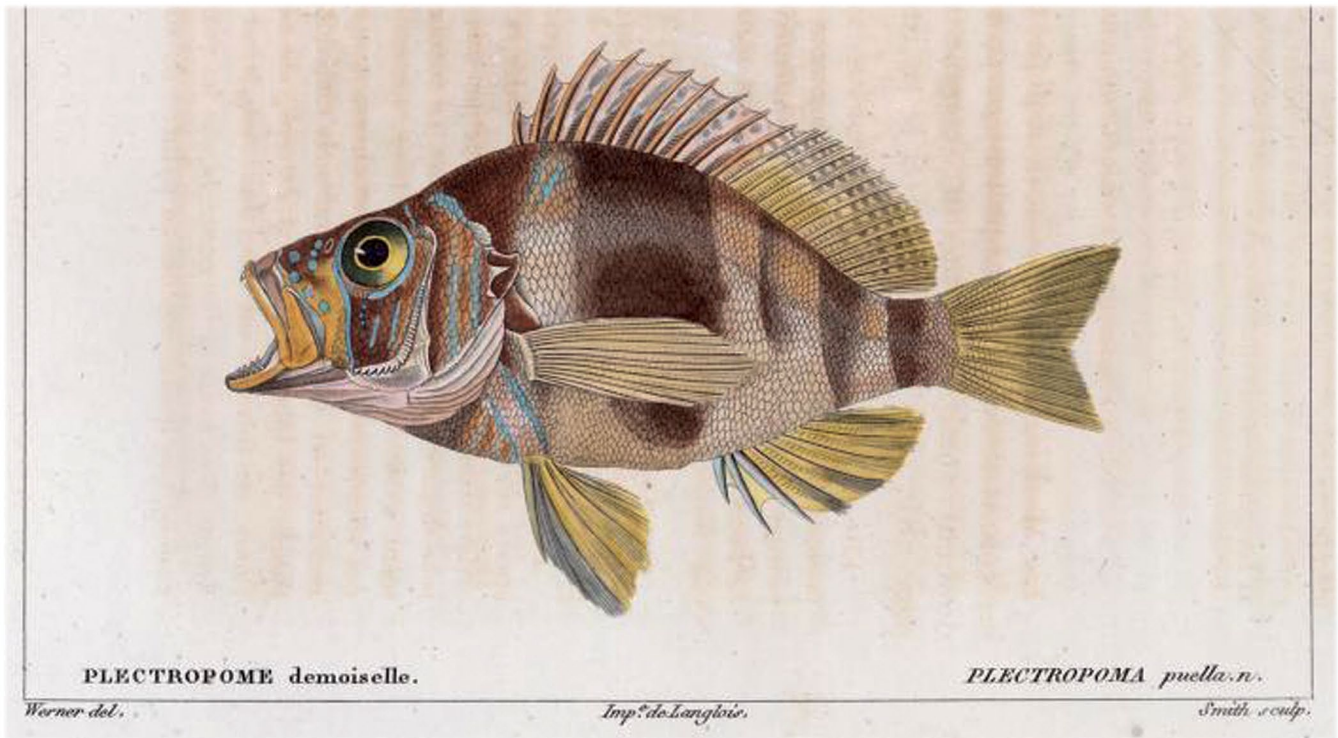




Figure 7. *Hypoplectrus floridae*, aquarium photograph, photo © Wayne Davis. Note the specimen is a large adult, based on the allometry of the relatively small eye.



Figure 8. *Hypoplectrus puella*, Dominica, photo © Rudy Whitworth.



Taxonomy. The original description of *H. puella* (as *Plectropoma puella*) by Cuvier in 1828 (above) is based on a fish from Martinique in the West Indies and it clearly represents the typical Caribbean Barred Hamlet morph (Figs. 8 & 9). There is a junior synonym for *H. puella* from Cuba, namely *Plectropoma vitulinum* of Poey (1852), later (around 1884) in his *Ictiología Cubana* as *Hypoplectrus vitulinus* (Poey 2000). That variant is not, however, analogous to the species described here; it was described as a blonde (“*rubia*”) version of the Barred Hamlet with an absence of the usual blue spots and lines on the head and trunk (and not recorded since).



Figure 9. *Hypoplectrus puella*, St. Vincent, photo © Rudy Whitworth.

Hypoplectrus ecosur, n. sp.

Fig. 10

Holotype. ECOCH 7180 (MXV0269), 83 mm SL, Mexico, Quintana Roo, off Contoy (20.49°, -86.80°), Feb. 3, 2011, C. Quintal-Lizama & J. Cohuo-Colli.

Paratypes. ECOCH 7180 (MXV0270), (1) 74 mm SL, same as holotype; ECOCH 7150 (MXV0220), (1) 63 mm SL, same as holotype.

Diagnosis. A hamlet with the usual color and patterns of the brown Barred Hamlet but with several distinguishing markings: a pair of dark rounded spots at the base of the caudal fin placed above and below the midline, usually symmetrical, present on all stages, including adults; a series of four dark brown to black spots along the upper rear body comprising one smaller rounded spot at the dorsal edge of the narrow mid-body bar (fourth bar), a larger vertically elongated oval blotch at the anterior upper end of the fifth bar, a smaller flattened spot at the rear upper edge of the fifth bar below the base of the last dorsal-fin rays, and finally a narrow squared blotch on the upper caudal peduncle filling the space between two thin blue bars (notably not the full width of the sixth bar). Characters not frequent on Caribbean Barred Hamlets, but associated with *H. ecosur* (and *H. floridae*), include the bar under the eye being orange, even when the body bars are brown, and a dusky pelvic fin.

Description. Body wide and broadly oval, maximum body depth just behind operculum 42 (39)% SL (range of paratypes (holotype)), and compressed; predorsal length 39 (41)% SL; preanal length 64–68 (68)% SL; prepelvic length 35–36 (43)% SL; caudal peduncle depth 14 (13)% SL, caudal peduncle length (dorsal) 8–10 (8)% SL; lateral line complete, curving in a high arch over pectoral fin becoming straight on caudal peduncle.

Head large 36–37 (39)% SL; dorsal head profile smooth and mostly straight, rising sharply from terminal tip of jaw to dorsal-fin origin; maximum head depth (measured at the rear end of the operculum) 41 (38)% SL; eyes large and round, orbit diameter 27 (26)% HL, pupil pear-shaped, pointing forward; interorbital space flat and relatively narrow; snout sharply pointed and short 32 (37)% HL; upper preopercular margin tilted slightly forward of vertical with a rounded angle to lower limb, small regular straight serrations on both limbs, longest at angle; opercle with three flat spines, the middle largest and at the level of the lower third of eye. Anterior nostril a short tube, posterior nostril a flat opening with a diameter about half internarial distance.

Mouth large, upper jaw ending at a vertical between mid-pupil and rear of eye, oblique length 39–41 (38)% HL; rear end of maxilla expanded, oblique height 12–13 (14)% HL; teeth small to moderately-sized and caniniform, stout and fixed, lining upper and lower jaws in irregular multiserial rows, larger in inner row. Gill rakers on first arch long, flexible, and flattened with a broad base grading to short wide nubs at distal ends, all with moderate serrations, longest rakers near the angle on the lower limb exceed length of opposing branchial lamellae; upper limb 5–6, lower limb (including rudiments and corner) 12–13.

Dorsal fin single and long-based without notch between spinous and soft portions; dorsal-fin rays X,14–15 (14) with last ray split to base; anal fin short and broadly rounded, anal-fin rays III,7 with last ray split to base; pectoral-fin rays 13–14 (13), including small uppermost and lowermost rays; pectoral-fin length 31–32 (27)% SL; pelvic-fin rays I,5 with pelvic-fin spine relatively short 18–21 (17)% SL, longest pelvic-fin ray 26–30 (25)% SL; caudal fin very slightly forked, caudal-fin length 21 (25)% SL; 17 principal caudal-fin rays.

Scales small and ctenoid, extending over body and head including cheek and operculum, sparing snout, interorbital, cranium, and underside of head; about 52 pored scales in lateral line from uppermost operculum to edge of hypural plate, about 75 lateral scale rows (counted above lateral line), about 12 scale rows between base of third dorsal-fin spine and lateral line.



Figure 10. *Hypoplectrus ecosur*, Contoy, Yucatan, Quintana Roo, Mexico; from top to bottom: holotype 83 mm SL (ECOCH 7180, MXV0269); paratypes 74 mm SL (ECOCH 7180, MXV270) and 63 mm SL (ECOCH 7150 ,MXV220); photos by Carolina Quintal Lizama; courtesy Colección Ictiológica de ECOSUR, El Colegio de la Frontera Sur, Unidad Chetumal, Chetumal, Quintana Roo, Mexico.

Color in Life. Brown bars against a pale background with bright blue lines and spots on the head and thorax, as well as a colon-like pair of rounded darker brown to black spots, typically symmetrical, at the base of the caudal-fin rays. The first of the six dark bars curves from the top of the head through the eye down across the lower cheek to meet the origin of the pelvic fin. This bar is typically more orange than brown and is outlined by thin blue lines below the eye, with the posterior blue line running out along the margin of the pelvic fin. The snout is speckled with round blue spots and the orbit is ringed with a blue line. Additional blue spots and lines run over the interorbital and form several thin blue bars over the operculum and down over the thorax as well as along the base of the pectoral fin. An additional array of thin blue bars continue rearward along the sides to the tail. A larger round blue spot is conspicuous near the upper end of the opercular flap. The second brown bar starts as a broad band forward of the dorsal fin narrowing down across the rear opercular flap to bracket the base of the pectoral fin, the lower portion is frequently more orange than brown and that portion is usually outlined by thin blue lines. The third bar is the widest and extends from the base of the spinous dorsal fin down and broadly across the abdomen, usually merging with a dusky pelvic fin. The fourth bar is typically the narrowest and runs downward from the anterior soft-dorsal-fin base; notably, however, the bar is indistinct except for an intense dark spot at the upper rim of the bar just below the dorsal-fin base. The fifth bar is wider than the fourth, bridges the rear portion of the soft dorsal and anal fins and is also indistinct except for an intense vertically elongated dark blotch at the anterior upper corner of the bar and a smaller flattened dark spot at the rear upper edge of the bar just below the base of the last dorsal-fin rays. The sixth bar, spanning the caudal peduncle, is also indistinct, except for a squared-off, vertically elongated, dark brown to black blotch filling the narrow space between two adjacent thin blue bars. The median fin membranes are edged with thin ribbons of blue, often underlain with a ribbon of yellow, while the remainder of the membranes are reticulated with thin blue and yellow lines.

Color in Alcohol. Specimens in ethanol retain only the shades of brown described for live color, but include the diagnostic dark spots and bars and prominently dark pelvic-fin membranes.

Barcode Sequence. A 652-nucleotide sequence of the section of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham & Hebert 2007) was obtained for the holotype and paratypes (see Appendix). Following the database management recommendation of the BOLD the sequence of the holotype (Genbank accession JN312342) is presented here as well:

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CCTCTATTTAGTATTTGGTGCATGGGCTGGAATAGTGGGTACCGCCCTTAGCCTCTTAATTC-  
GGGCCGAGTTGAGTCAACCCGGAGCTCTTTTAGGAGACGACCAGATTTATAATGTAATTGT-  
TACAGCACACGCCTTCGTAATAATTTTCTTCATAGTCATGCCAATTATGATTGGAGGCTTC-  
GGCAACTGACTCATTCTTTAATGATTGGTGCGCCCGACATGGCATTTCCTCGTATAAA-  
CAATATGAGCTTTTGACTTCTTCCTCCTTCCTTCCTTCTCCTTCTGGCCTCATCAGGGG-  
TAGAAGCAGGAGCTGGGACGGGTTGAACTGTTTATCCTCCACTAGCCGAAACTTAGCACAT-  
GCTGGGGCTTCAGTTGACTTAACAATTTTTCACTTCATCTAGCAGGTGTATCCTCTATCCTT-  
GGGGCAATCAACTTCATTACTACAATCATTAATATGAAACCTCCTGCTATCTCCCAATAT-  
CAAACCTCCTCTCTTTGTTTGAGCCGTCCTAATTACAGCGGTTCTACTTCTTCTTTCTCTTC-  
CAGTCCTTGCTGCTGGAATTACTATGCTACTTACAGACCGAAACCTGAATACTACTTTCTTT-  
GACCCTGCTGGGGGAGGAGATCCCATTTTACCAACATCTA
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Etymology. The new species is named for the acronym of El Colegio de la Frontera Sur in recognition of the pioneering work of the ichthyology group in marine ecology based at the Unidad Chetumal on the biodiversity and conservation of ecosystems in the western Caribbean. The specific epithet is a noun in apposition.

Comparisons. The new species is most similar to the Florida Barred Hamlet, *H. floridae*, and most of the comparison discussion above applies to *H. ecosur* as well. Much less information is available on the underwater appearance of *H. ecosur*, but it appears from the fresh photographs of the type specimens that the bars on the rear body are indistinct, highlighting the row of dark spots diagnostic of the species.

Distribution. Thus far *H. ecosur* is known only from off Contoy, at the northern tip of the Yucatan Peninsula. Although the species does not range south onto the well-surveyed reefs of the Mesoamerican Barrier Reef off Mexico and Belize, where the Caribbean Barred Hamlet is common (Schmitter-Soto et al. 2000), its northern and eastern limits are less clear and it is not known how far the species may extend into the Gulf of Mexico and Cuba.

DNA Analysis. The neighbor-joining phenetic tree generated by the BOLD algorithm (based on a Kimura 2-parameter (K2P) model) shows both *H. floridae* and *H. ecosur* to belong to separate clades from the large homogeneous clade (Caribbean clade) representing all other hamlets in the database (Fig. 11). Within the large Caribbean clade, the mean pairwise distance is 0.352% (SE=0.002; minimum distance of 0% and maximum distance of 1.24%, n=42). In addition to the five *Hypoplectrus* species included in the illustrated clade, three additional hamlet species have sequences identical to those within the clade, but are in private projects in the database. Geographic coverage of the Caribbean Sea is extensive, from Central America (Belize to Panama) across to Cuba, the Bahamas, Bermuda, and down the Antilles through Dominica to Tobago and westward to Curacao.

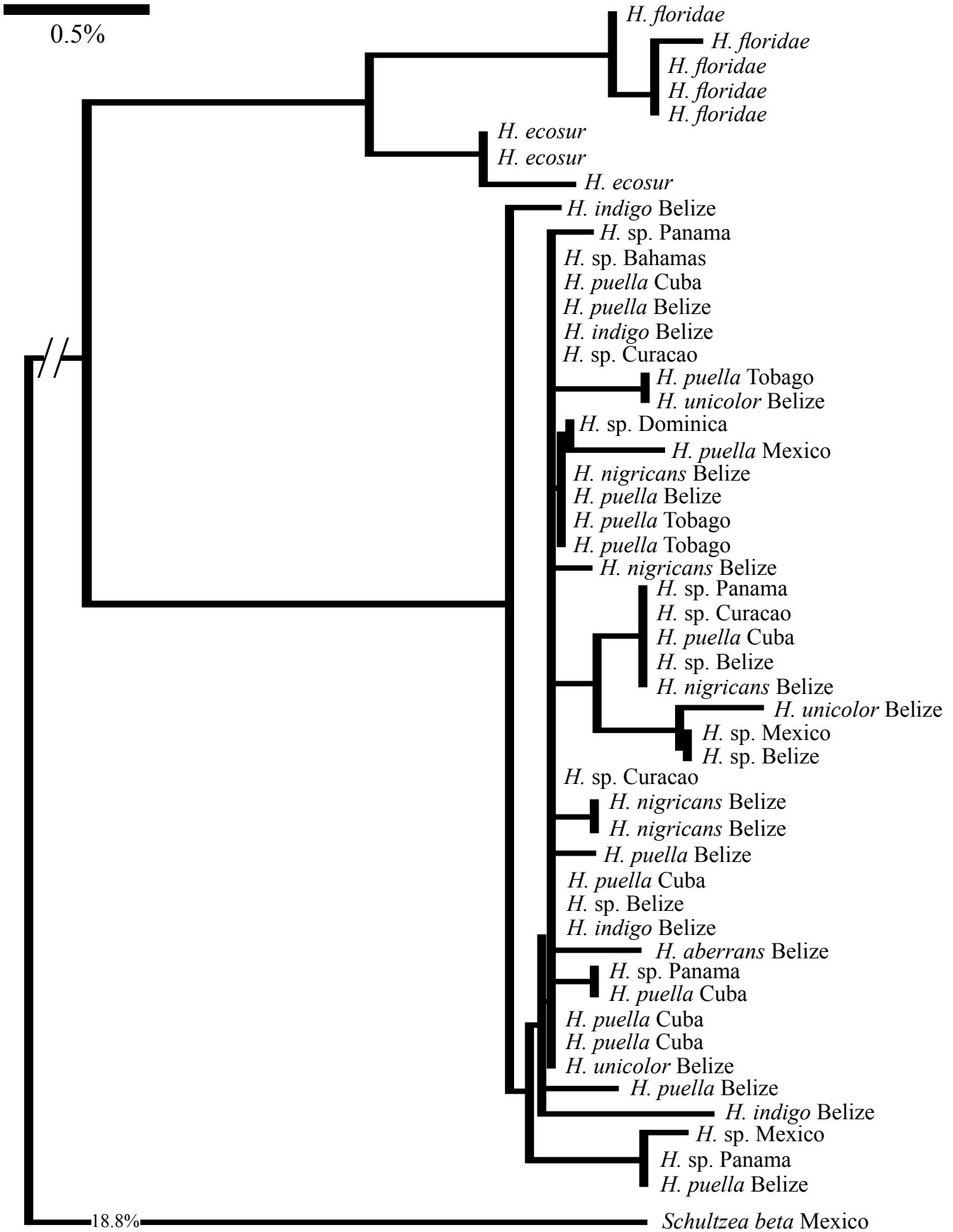
The *H. floridae* clade differs from the Caribbean clade by 3.76% on average (SE=0.002; minimum distance of 3.39% and maximum distance of 4.37%). The *H. ecosur* clade differs from the Caribbean clade by 3.23% on average (SE=0.008; minimum distance of 3.0% and maximum distance of 3.83%). The *H. floridae* and *H. ecosur* clades differ by 1.41% on average (SE=0.011; minimum distance of 1.24% and maximum distance of 1.72%).

Discussion. From the first descriptions of the hamlets of *Hypoplectrus* there has been a degree of uncertainty about the status of differently colored populations, especially since they show virtually no morphological differences and the color differences frequently disappear rapidly upon preservation (Jordan & Evermann 1902). Subsequent taxonomists have vacillated between considering them all color variations of *H. unicolor* and treating them as valid separate species, with most recent authors favoring the latter. Phylogeneticists have struggled to detect genetic differences between the morphotypes, not surprising in view of the relatively high rates of cross-matings observed and the abundance of putative hybrids by visual appearance (Fischer 1980, Domeier 1994, McCartney et al. 2003, Ramon et al. 2003).

In general, analyses of mtDNA haplotype frequencies have not detected haplotypes assorting differently among species (McCartney et al. 2003, Ramon et al. 2003, Garcia-Machado et al. 2004). However, more recent studies using microsatellite analyses, a higher-resolution genetic technique to detect small degrees of genetic structure, have teased out evidence of assortative mating and very small but highly significant genetic differences between sympatric pairs of color morphs at multiple sites within the Caribbean (Puebla et al. 2007).

Ramon et al. (2003) reported almost no genetic structure from an analysis of mtDNA sequences among Caribbean hamlet morphs, but picked up five individuals with several-percent divergent haplotypes of mtDNA ND6 among Florida hamlets, including one Barred Hamlet (markings not described). Their proposed explanation of an ancestral Florida population drifting towards extinction in the face of invasions from the Caribbean is unlikely. The present discovery of distinct and relatively deep COI mtDNA lineages associated with Barred Hamlets with different markings in Yucatan and Florida indicates that these are likely long-isolated and locally adapted species. The potential coexistence of these species with Caribbean lineages at some sites in the region is unsurprising, since many fishes found primarily in the tropical Caribbean extend into South Florida, where their range overlaps with cooler-water congeners native to US waters (Randall 1968). Further documentation of the distribution of

Figure 11 (opposite). A neighbor-joining tree based on the COI mtDNA sequences of *Hypoplectrus* species following the Kimura two-parameter model (K2P) generated by BOLD (collection locations indicated for the large Caribbean clade). The serranid *Schultzea beta* is the outgroup and has a minimum 18.8 percent divergence from the *Hypoplectrus* sequences; the branch to the outgroup is contracted for clarity. The Genbank accession numbers corresponding to the sequences in the tree are listed in Appendix 1. The scale bar at upper left represents a 0.5% sequence difference.



various hamlet morphs in the transition zone between the Caribbean, Yucatan, S. Florida and the Gulf of Mexico, and in relation to the Gulf Stream, should clarify the degree of consistency of these lineages and provide a particularly informative case-study of reef fish speciation and phylogenetics.

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Appendix 1. Specimen data and Genbank Accession numbers for the mtDNA COI sequences used in the phenogram in Fig. 11; order follows placement in the tree from top to bottom.

Genus	species	Collection site	Genbank accession
<i>Hypoplectrus</i>	<i>floridae</i>	SW Florida	JX444754
<i>Hypoplectrus</i>	<i>floridae</i>	SW Florida	JX444755
<i>Hypoplectrus</i>	<i>floridae</i>	SW Florida	JX444752
<i>Hypoplectrus</i>	<i>floridae</i>	SW Florida	JX444753
<i>Hypoplectrus</i>	<i>floridae</i>	SW Florida	JX444756
<i>Hypoplectrus</i>	<i>ecosur</i>	Contoy, Mexico	JN312341
<i>Hypoplectrus</i>	<i>ecosur</i>	Contoy, Mexico	JN312343
<i>Hypoplectrus</i>	<i>ecosur</i>	Contoy, Mexico	JN312342
<i>Hypoplectrus</i>	<i>indigo</i>	Belize	JQ840877
<i>Hypoplectrus</i>		Panama	JX488040
<i>Hypoplectrus</i>		Bahamas	JQ839798
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583580
<i>Hypoplectrus</i>	<i>puella</i>	Belize	JQ840880
<i>Hypoplectrus</i>	<i>indigo</i>	Belize	JQ840540
<i>Hypoplectrus</i>		Curacao	JQ842170
<i>Hypoplectrus</i>	<i>puella</i>	Tobago	JQ842905
<i>Hypoplectrus</i>	<i>unicolor</i>	Belize	JQ840882
<i>Hypoplectrus</i>		Dominica	JX516091
<i>Hypoplectrus</i>	<i>puella</i>	Mexico	HQ573385
<i>Hypoplectrus</i>	<i>nigricans</i>	Belize	JQ840879
<i>Hypoplectrus</i>	<i>puella</i>	Belize	JQ840881
<i>Hypoplectrus</i>	<i>puella</i>	Tobago	JQ842907

<i>Hypoplectrus</i>	<i>puella</i>	Tobago	JQ842906
<i>Hypoplectrus</i>	<i>nigricans</i>	Belize	JQ840878
<i>Hypoplectrus</i>		Panama	JX488041
<i>Hypoplectrus</i>		Curacao	JQ842169
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583582
<i>Hypoplectrus</i>		Belize	JQ840997
<i>Hypoplectrus</i>	<i>nigricans</i>	Belize	JX488037
<i>Hypoplectrus</i>	<i>unicolor</i>	Belize	JQ840883
<i>Hypoplectrus</i>		Mexico	JN312340
<i>Hypoplectrus</i>		Belize	JQ840284
<i>Hypoplectrus</i>		Curacao	JQ842168
<i>Hypoplectrus</i>	<i>nigricans</i>	Belize	JQ840541
<i>Hypoplectrus</i>	<i>nigricans</i>	Belize	JQ841608
<i>Hypoplectrus</i>	<i>puella</i>	Belize	JX488038
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583577
<i>Hypoplectrus</i>		Belize	JQ840693
<i>Hypoplectrus</i>	<i>indigo</i>	Belize	JQ841607
<i>Hypoplectrus</i>	<i>aberrans</i>	Belize	JX488035
<i>Hypoplectrus</i>		Panama	JX488039
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583579
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583581
<i>Hypoplectrus</i>	<i>puella</i>	Cuba	FJ583578
<i>Hypoplectrus</i>	<i>unicolor</i>	Belize	JX488043
<i>Hypoplectrus</i>	<i>puella</i>	Belize	JQ840542
<i>Hypoplectrus</i>	<i>indigo</i>	Belize	JX488036
<i>Hypoplectrus</i>		Mexico	JN312339
<i>Hypoplectrus</i>		Panama	JX488042
<i>Hypoplectrus</i>	<i>puella</i>	Belize	JQ841609
<i>Schultzea</i>	<i>beta</i>	Mexico	HQ991872