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Gramma dejongi, a New Basslet (Perciformes: Grammatidae) from Cuba, a Sympatric Sibling Species of *G. loreto*

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Benjamin C. Victor and John E. Randall (2010) *Gramma dejongi* sp. nov., a new Basslet (Perciformes: Grammatidae) from Cuba, a sympatric sibling species of *G. loreto*. *Zoological Studies* **49**(6): 865-871. *Gramma dejongi* is described as a new species of basslet from deep reefs off the town of Trinidad, along the south-central coast of Cuba. The species is closely-related to the common and widespread Royal Gramma, *G. loreto*, but is distinguished by uniform yellow coloration on the head and body without stripes through the eye (vs. prominently bicolored with eye stripes) and smaller adult size. It is found on coral reefs at depths from 20-30 m, while *G. loreto* occurs on both shallow and deep reefs. *G. dejongi* is sympatric with both the Royal Gramma and the Blackcap Basslet, *G. melacara*, on the Cuban reefs, but has not yet been found at any other location in the Caribbean Sea. The barcode COI mtDNA sequence is the same as the Royal Gramma, indicating that the new species may represent a particularly interesting case of very recent speciation within the Caribbean, perhaps analogous to the species-flock of hamlets (*Hypoplectrus* spp.) and the species-pair of angelfishes *Centropyge argi* and *C. aurantonotus*, none of which have yet diverged from their sister-species in COI mtDNA sequences (phenotypic species). A local endemic sibling species found in the middle of the range of a widespread regional species raises important questions about sympatric speciation among reef fishes. http://zoolstud.sinica.edu.tw/Journals/49.6/865.pdf

Key words: New species, *Gramma*, DNA barcode, Sympatric speciation, Cuba.

The grammatid genus *Gramma* Poey is a small Western Atlantic genus with only 4 known species (Böhlke and Randall 1963, Starck and Colin 1978, Sazima et al. 1998). *Gramma loreto*, the Royal Gramma or Fairy Basslet, is the type species and is common on both shallow and deep reefs throughout the Caribbean and has become an important fish in the aquarium trade. The Brazilian sibling species, *G. brasiliensis*, is very similar to *G. loreto* and is also frequently exported as an aquarium fish. The 2 remaining congeners, both from the Bahamas and Caribbean, are deep-reef species: *G. melacara* Böhlke from drop-offs in 10-180 m and *G. linki* Starck and Colin from 60-130 m.

Recently, a new and strikingly-colored *Gramma* was recognized by aquarist Arie De Jong (of De Jong Marinelife, Netherlands) in imports from collectors near the town of Trinidad, on the south-central coast of Cuba. To date, more than 30 identically marked fish have been imported by DeJong Marinelife (3 have been preserved as type specimens) and some have been maintained in aquaria for more than a year (De Jong, pers. comm.). Although fully overlapping morphologically and meristically with *G. loreto*, the new species is distinctly smaller, reaching only about 45 mm TL (vs. over 80 mm TL for *G. loreto*). The differences in color pattern are prominent, with the new

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species having a yellow head and body instead of the bicolor blue and yellow characteristic of both *G. loreto* and *G. brasiliensis*. The new species has blue limited to the anterior dorsal fin and along the pelvic fins, as well as on the ventral abdomen and isthmus. It is missing the stripes through the eye characteristic of *G. loreto*. At the collection site, it is found on walls between 20 and 30 m, deeper than *G. loreto* and shallower than *G. melacara* (De Jong, pers. comm.).

MATERIALS AND METHODS

Type specimens of the new species are deposited in the U.S. National Museum of Natural History, Washington DC (USNM) and the University of Florida Fish collection at the Florida Museum of Natural History (UF).

Measurements follow the format of Randall and Rocha (2009); lengths of specimens are given as standard length (SL), measured from the front of the upper lip to the base of the caudal fin (posterior end of the hypural plate); head length (HL) is measured from the same anterior point to the posterior end of the opercular flap; body depth is taken vertically from the base of the 1st dorsal spine; body width is the maximum width just posterior to the gill opening; orbit diameter is the greatest fleshy diameter and interorbital width the least fleshy width; upper-jaw length is taken from the front of the upper lip to the mid-posterior end of the maxilla; caudal-peduncle depth is the least depth and caudal-peduncle length the horizontal distance between verticals at the rear base of the anal fin and the caudal-fin base; lengths of fin spines and rays of the dorsal and anal fins are measured to their extreme base; caudal-fin length is the horizontal distance from the base of the fin to a vertical at the tip of the (depressed) longest ray; caudal concavity is the horizontal distance between verticals at the tips of the (depressed) longest and shortest rays; pectoral-fin length is the length of the longest ray; pelvic-fin length is measured from the origin of the pelvic spine to the tip of the longest soft ray. Pectoral-fin ray and lateral-line scale counts were made on both sides. Gill-raker counts were made on the 1st gill arch of the right side and include rudiments; the raker at the angle is contained in the count of the lower-limb.

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 (cytochrome-*c* oxidase subunit 1) using a variety of primers (Ivanova et al. 2007). PCR amplifications (polymerase chain reaction) 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 $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH8.8), 2mM Mg SO_4, 0.1% Triton X-100), 0.625 μl of $MgCl_2$ (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 and Hebert 2007).

Comparative Material

Gramma loreto. San Blas, Panama, SIO 71-265 (2), SIO 71-272 (1); Glovers Reef, Belize, SIO 78-72 (42); Navassa I. (off west coast of Haiti in Jamaica Channel) SIO 06-58 (17), SIO 06-64 (20).

RESULTS

Taxonomy

***Gramma dejongi*, sp. nov.**

(Figs. 1-3)

Holotype: USNM 398656, 32.3 mm SL, Cuba, Sancti Spiritus, off Trinidad, 21.7896 N -80.0579 W, depth about 20 m, commercial collectors, exported 27 July 2009.

Paratypes: UF 178386, 29.5 mm SL, same data as holotype; USNM 398657, 27.0 mm SL, same data as holotype.

Diagnosis: Dorsal rays XII, 10; anal rays III, 10; pectoral rays 16; upper-jaw length 2.25-2.40 in HL; length of pelvic fin 0.91-1.06 in HL; margin of caudal fin shallowly emarginate. Largest specimen, 32.3 mm SL. Color on body golden yellow, except for magenta surrounding pelvic-fin

insertion, along ventral isthmus, operculum, and branchiostegal membranes (often with a separate tiny streak underlining posterior mandible) and rearward along ventral abdomen. Pelvic fins bright purple-magenta with a darker streak along length of 2nd soft-ray. A magenta patch covering 1st 3 or 4 dorsal-fin spines (not extending onto body) surrounding an oval black spot about pupil diameter on outer half of 1st 3 spinous membranes.

Description: Dorsal-fin rays XII, 10, the 1st soft ray unbranched, the last split to base; anal-fin rays III,10, the 1st 2 soft rays unbranched, the last split to base; pectoral-fin rays 16, the rays branched except upper and lowermost; pelvic-fin rays I, 5, all soft rays branched; branched caudal-fin rays 15, 3-5 unbranched segmented caudal-fin rays, 3-4 upper and lower procurrent unsegmented caudal-fin rays; branchiostegal rays 6.

Body elongate (holotype 32.3 mm SL, 2 para-

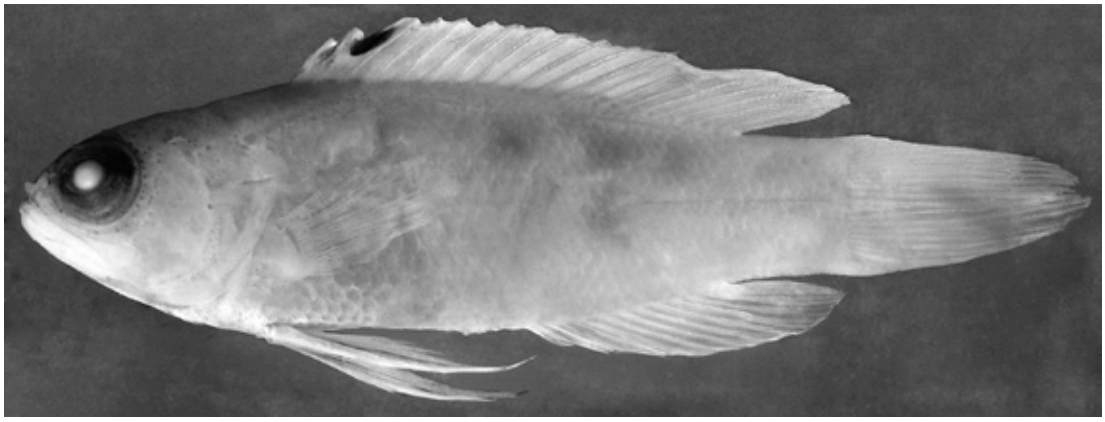

Fig. 1. Holotype of *Gramma dejongi* sp. nov., USNM 398656, 32.3 mm, near the town of Trinidad, Cuba. Note anomalous short 1st and 2nd dorsal-fin spines. Photo by Benjamin C. Victor.

Fig. 2. *Gramma dejongi* sp. nov., aquarium specimen. Photo by Dietmar Schauer.

types 29.5 and 27.0 mm SL; following values for holotype and the 2 paratypes in parentheses, respectively), body depth 3.33 (3.69, 3.42) in SL; body compressed, the width 2.0 (2.23, 2.32) in HL; snout short and rounded, length 7.0 (7.0, 6.9) in HL; eye large, orbit diameter 3.20 (2.88, 2.88) in HL; interorbital space almost flat, least fleshy width 4.87 (4.45, 4.52) in HL; caudal-peduncle depth 2.04 (2.18, 2.21) in HL; caudal-peduncle length 2.55 (2.65, 3.39) in HL. Origin of dorsal fin just behind upper end of gill opening, the predorsal length 3.05 (2.89, 3.03) in SL. Dorsal-fin spines stout; 1st spine 2.58 in HL in large paratype (anomalously short in holotype, 4.15 in HL, compared to photographs of other specimens; spines damaged in smaller paratype); 2nd spine 2.58 in HL in large paratype (anomaly of 3.73 in holotype), 3rd spine 2.80 in HL in holotype (2.65 in larger paratype); longest dorsal-fin soft ray 1.90 (1.69, 1.76) in HL. Preanal length 1.61 (1.59, 1.64) in SL; 1st anal-fin spine 6.59 (5.76, 5.94) in HL; 2nd spine 3.73 (3.50, 2.88) in HL; 3rd spine 2.55 (2.04, 2.02) in HL; longest anal-fin soft ray 1.90 (1.72, 1.83) in HL. Caudal fin shallowly emarginate, length 3.23 (3.21 in larger paratype, damaged in smaller paratype) in SL; caudal concavity 14 (8) in HL. Pectoral fins pointed, the middle rays longest, 1.37 (1.46, 1.44) in HL. Pelvic fins long, 1.06 (0.94, 0.91) in HL, spine 2.04 (1.81, 1.76) in HL; 1st soft ray 1.18 (1.04, 1.09) in HL; 2nd soft ray longest, 1.07 (0.95, 0.92) in HL.

Fig. 3. *Gramma dejongi* sp. nov., aquarium specimen. Photo by Dietmar Schauer.

Mouth large, terminal and moderately oblique, maxilla ending just before or reaching vertical at rear edge of orbit, upper-jaw length 2.35 (2.25-2.4) in HL, posterior end of maxilla truncate, upper corner rounded; teeth present on jaws, vomer, and palatines; jaw teeth in multiple rows of tiny villiform teeth with an outside row of enlarged recurved canines, smaller on upper jaw where longest are near the front of jaw, larger on lower jaw with progressively larger canines rearward culminating in a large fang a 3rd of the way along lower jaw, its height about half maximum maxilla width; vomerine and palatine teeth small and villiform in patches; tongue relatively long and narrow with a rounded tip; gill rakers long and slender, the longest at angle about 1 pupil diameter, lower limb 21, upper limb 11 (on larger paratype); anterior nostril a small raised tube just behind upper lip, widely separated from posterior nostril which forms an elliptical opening adjacent to orbital rim; numerous arrays of head pores surrounding orbital rim, along interorbital midline, and on edge of preopercle, numbers increasing with adult size; opercle with a single central flat spine; often with a nub of a spine above but no 3rd spine below. Free upper and lower margin of preopercle coarsely serrate except ends.

Lateral line interrupted, anterior upper segment from upper end of opercular opening arching up to near the base of spinous dorsal fin then running parallel to base of fin 1 to several scale rows below, with 35-37 tubed scales in this segment. Posterior lower segment runs along mid-lateral axis from about the level of the termination of the upper segment to the 2nd or 3rd scale beyond the base of the caudal fin, with 13-15 tubed scales.

Most body scales ctenoid; cycloid or with scant ctenii above the anterior lateral line segment, on the predorsal, preventral, lower sides and opercle, 3 rows across preopercle at widest point, 5-6 rows scales from the orbital rim to angle of preopercle.

Color in life: Body and head entirely golden

yellow, except for an irregular patch of magenta surrounding pelvic-fin insertion, extending forward along ventral aspect of isthmus and branchiostegal membranes and lapping over onto lower edge of adjacent operculum (often with a separate tiny streak in skin fold underlining the posterior mandible), extending rearward along ventral aspect of abdomen, rapidly fading and fragmenting farther from pelvic-fin insertion. Pelvic fins are bright purple-magenta with a darker streak along length of 2nd soft ray. Magenta markings on median fins are limited to a prominent magenta patch on 1st 3 or 4 dorsal-fin spines and membranes, notably not extending onto body, surrounding an oval black spot about pupil diameter on the outer half of 1st 3 spinous membranes, followed by a magenta rim on trailing edge of remaining spinous membranes and a thin parallel streak of magenta about 1 quarter down the spinous fin. Magenta markings on anal fin are limited to a tenuous thin streak about 2-3rds out on the fin and some on trailing edges of rays.

Color in alcohol: Body and head pale yellow, dusky melanophore speckling heavy over snout and interorbital space on head, extending over front of upper lip and symphysis of lower jaw. Light dusky speckling around pelvic fin insertion extending forward to isthmus and branchiostegal membranes and lapping onto adjacent opercle. Dusky speckling underlying some patches of *in vivo* magenta, but not closely matching magenta pattern; dark streak along 2nd pelvic-fin soft ray persists in alcohol.

Barcode sequence: A segment of the mitochondrial COI gene used for barcoding by the BOLD informatics database (Ratnasingham and Hebert 2007) was obtained for the holotype (619 bp; Genbank accession number HQ149779), the 29.5 mm paratype (522 bp; Genbank accession number HQ149780) and the 27.0 mm paratype (553 bp; Genbank accession number HQ149781). Comparisons to aquarium-trade specimens of *Gramma loreto* on the BOLD database showed a 100% match between the holotype and a *G. loreto* specimen (Genbank FJ583468), a 100% match of the 29.5 mm paratype with another *G. loreto* specimen (Genbank FJ583472), and a 99.45% match of the 27.0 mm paratype to many *G. loreto* on the database. Photographs of the matched specimens of *G. loreto* in the BOLD photograph database show the typical half-blue half-yellow body coloration.

Etymology: The species is named for Arie De Jong of De Jong Marinelife, who 1st recognized the new species and provided the type specimens.

DISCUSSION

The morphological similarity of *Gramma dejongi* to *G. loreto* and the lack of divergence in the barcode mtDNA sequence raises the possibility that the new species is a local color variant and not a separate species. There is some color variation in *G. loreto*, with Bahamian populations noted to have less blue on the body than other populations, i.e. limited to the front 3rd vs. half blue in the remainder of the region (Böhlke and Randall 1963). However, photographs of *G. loreto* from the south coast of Cuba show the normal half-blue half-yellow coloration at the Bay of Pigs (west of Trinidad), Jardines de la Reina (east of Trinidad), and at Maria La Gorda (photos by Wolfram Sander, pers. comm.; Fig. 4). That *G. brasiliensis* also retains the half-purple, half-yellow pattern also indicates that the bicolor pattern is a conservative character in the clade. A localized color variant unknown anywhere else in the range of a very common reef fish would be a novel finding for the Western Atlantic and difficult to explain.

Some genera of fishes show little or no divergence between species in their barcode mtDNA sequence, although this is a rare phenomenon (e.g. the tunas of *Thunnus* (Ward et al. 2009) and the blennies of *Hypleurochilus* (Victor unpub. data)). This is not the reason for the lack of divergence of *Gramma dejongi*, since the barcode sequence of *Gramma melacara* is about 14% different from *G. loreto* according to the BOLD database (numerous public sequences with voucher photographs available).

Additional evidence for the separation of *Gramma dejongi* is the smaller adult size. All specimens of the new species have been smaller than 45 mm TL, and they have not grown larger in captivity, while *G. loreto* grows to twice that length in the field and in aquaria (De Jong, pers. comm.). At the collection site, *G. dejongi* reportedly occurs below 20 m depth (De Jong, pers. comm.), while *G. loreto* is common in shallow waters throughout the Caribbean. An additional character noted by aquarists is that *G. dejongi* do not swim upside-down (De Jong, pers. comm.), a well-known idiosyncratic behavior characteristic of *G. loreto*, which orient to the rock surface regardless of up or down.

The argument for a subspecies designation for *Gramma dejongi* is mostly semantic. There has been some recent controversy over the validity of subspecies, since there is little consistency among marine fish taxonomists on its usage (Hastings

and Springer 2009). The fact that *G. dejongi* is sympatric with *G. loreto* excludes subspecies in the typical allopatric sense of distinct regional populations of a widespread species with some geographical variation. The coexistence of 2 overlapping sympatric subspecies is less tenable, since there must clearly be reproductive isolation to maintain the difference, and that is the *sine qua non* of classical species. Sympatric incipient species or subspecies have been invoked when there is a zone of overlap of 2 otherwise allopatric populations and some mechanism to reduce hybridization. The 2 angelfishes *Centropyge argi* and *C. aurantonotus* are well-documented examples of this phenomenon, with the Caribbean *C. argi* coexisting with the mostly Brazilian *C. aurantonotus* in a zone of overlap in the SE Caribbean. Notably, these 2 species similarly have shared mtDNA control-region haplotypes (Bowen et al. 2006) and the same barcode mtDNA sequence (Victor, unpub. data). The 2 angelfishes are also distinguished by color-pattern differences on the head, analogous to the case of *G. dejongi* and *G. loreto*. Additional reports of geographically separated color-morphs among reef fishes in the SW Atlantic and the Indo-Pacific raise continuing taxonomic questions (e.g. Rocha 2004, Schultz et al. 2007), most of which will likely not be easily resolved.

Species flocks of closely-related color forms coexisting in the same location have been described among several groups of fishes, including the hamlets (*Hypoplectrus* spp.) of the Caribbean and the freshwater cichlid fishes of the African Great Lakes (e.g. Domeier 1994). The hamlets also show no divergence in their mtDNA sequences (McCartney et al. 2003, Ramon et al. 2003, Garcia-Machado et al. 2004), but do have DNA microsatellite differentiation, indicating very recent "incipient" speciation (Puebla et al. 2008). There is some parallel between the 2 Grammas and the hamlets, in that there are also very localized color forms of hamlets in the Caribbean, e.g. the Blue Hamlet *H. gemma* from Florida and Alacranes reef and a new species endemic to Belize (P.S. Lobel, pers. comm.). *G. dejongi*; however, appears to be more distinct from its sibling species since it has apparently diverged in both adult size and swimming behavior, unlike the hamlet species which are essentially identical other than in color and among which hybridization is not infrequent.

The discovery of *G. dejongi* adds a new and interesting example to the recent discussion of sympatric speciation and biogeography among reef fishes (Rocha 2004, Rocha et al. 2005, Floeter et al. 2008, Rocha and Bowen 2008). With the increasing use of DNA sequencing as a broad taxonomic and utilitarian tool (Ward et al. 2009), additional examples of apparent species with little or undetectable genetic divergence will likely be discovered. There is no common terminology

Fig. 4. *Gramma loreto*, Maria la Gorda, Cuba. Underwater photo by Wolfram Sander.

for these troublesome species, but phenotypic species and phenospecies are terms used in microbiology to describe groups of organisms with a shared characteristic phenotype in a murky genotypic background (e.g. Tibayrenc 2006), and these terms should be applicable to reef fishes. It has been suggested that incipient species of colorful reef fishes may diverge 1st in color, with its obvious relevance to sexual selection, with little discernable sequence variation in neutral genes (Bowen et al. 2006, Craig et al. 2006, Puebla et al. 2008). *G. dejongi* appears to be a product of this initial color divergence and its restricted locality, well within the wide geographic range of *G. loreto*, argues for the possibility of sympatric speciation. Further studies on the ecology, behavior, reproduction, and genetics of this perhaps unique case should be especially enlightening.

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