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***Starksia splendens*, a new endemic labrisomid blenny from the Cayman Islands (Teleostei: Labrisomidae)**

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Abstract

A new endemic species of labrisomid blenny, *Starksia splendens* n. sp., is described from the Cayman Islands in the central Caribbean Sea. The new blenny is a member of the *Starksia lepicoelia* species complex (Blackcheek Shy Blennies), which breaks up into a set of allopatric cryptic species in the region. This case is an example of the difficulty in delineating species boundaries when there is high variation in marking patterns: without the combination of extensive underwater photography and mtDNA sequencing, diagnostic differences could not be accurately determined. In this case, the new species is distinguished by color patterns, species-specific black markings on the lips of both females and males, as well as elongated, ribbon-like, white orbital cirri on mature males. Mitochondrial DNA sequencing reveals a 7.22% divergence in the sequence of the mtDNA COI barcode marker from the nearest member of the species complex, *S. williamsi*, from the Lesser Antilles. The species complex now comprises 5 allopatric species and 6 distinct mtDNA lineages, with two genovariant populations of *S. lepicoelia* sympatric in the Bahamas.

Key words: taxonomy, ichthyology, systematics, endemism, coral-reef fishes, *Starksia lepicoelia* species complex, Splendid Shy Blenny, scaled blennies, biogeography, DNA barcoding, cryptic species.

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Introduction

Tiny blennies of the genus *Starksia* Jordan & Evermann, 1896 comprise the most speciose genus of marine fishes in the Greater Caribbean region (or the entire Atlantic Ocean), with 27 species described prior to this report. The genus is notable for being the first coral-reef fish to have a previously widespread “superspecies”, *Starksia ocellata* (Steindachner, 1876), broken up into a set of allospecies dividing up the Greater Caribbean region (Greenfield 1979). The description of a Caribbean species complex was a harbinger of the numerous cryptic species complexes that have been revealed by mtDNA sequencing (along with extensive underwater or fresh capture photography) in recent years (e.g. Tornabene *et al.* 2010, Baldwin *et al.* 2011, Victor 2013, 2014).

The diagnostic differences for species within a complex can be subtle and subject to surprising variation, which dissuaded earlier ichthyologists from splitting up many widespread species. Greenfield (1979) astutely noticed small differences in head markings between populations in his preserved fishes, but other species complexes differ only in live color patterns not retained in museum specimens, often with high variation in markings that is only resolved by associating marking patterns with different genetic lineages— a particularly useful fine point of DNA taxonomy (Victor 2015). Even with genetic analyses and large sample sizes of fresh specimens, the situation can become difficult when mtDNA lineages are phenotypically indistinguishable (genovariant populations of the same species) and/or when cryptic species are sympatric. The latter case is infrequent, but does occur in some reef-fish species complexes, and it amplifies the uncertainty about which lineages to associate with type specimens that are typically old, bleached, or in poor condition.

The Blackcheek Shy Blenny species complex is a case in point of the taxonomic difficulties presented by these closely related species complexes. It was first described as *Starksia lepicoelia* Böhlke & Springer, 1961, with a type location in the Nassau area in the northern Bahamas. Baldwin *et al.* (2011) split off the Mesoamerican Barrier Reef lineage as *S. weigti*, the Panamanian lineage as *S. robertsoni*, and the Lesser Antilles lineage as *S. williamsi*. The three added species differ mainly in the markings on the lips of adult males— respectively white spots, dark bars, or neither. They noticed that the Bahamian population includes two distinct genetic lineages and kept both as *S. lepicoelia* genovariants in the absence of a clear association with the old type specimens. In this study, I describe the Cayman Islands population as a new endemic species, using a large trove of underwater photographs to document unanticipated variation in color patterns and markings within a species. The biogeography of the species complex is discussed and a phenetic tree presented for the mtDNA sequences of the species in the region.

Materials and Methods

Type specimens are deposited at the Florida Museum of Natural History, University of Florida, Gainesville, FL, USA (UF). A 652-bp segment (the “barcode” marker) was amplified from the 5' region of the mitochondrial cytochrome c oxidase (COI) gene using a variety of primers (Ivanova *et al.* 2007). 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. 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 MgSO₄, 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 sec., 52°C for 40 sec., 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 (Ratnasingham & Hebert 2007, Ward *et al.* 2009). The sequence data is publicly accessible on BOLD and GenBank (collection data and accession numbers are listed in Appendix 1). Sequence divergences were 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' sequence divergence. Measurements were made to the nearest 0.1 mm using an ocular micrometer and microphotographs, and are presented as %SL, morphometrics are presented as the range for the paratypes followed by that for the holotype in parentheses. Counts are presented as the value for the holotype followed by the range of the paratypes in parentheses, if different.

Lengths of specimens are mm standard length (SL), measured from the front of the upper lip to the

base of the caudal fin (posterior end of the hypural plate); body depth is the vertical distance at the base of the first dorsal spine; body width is the maximum width side-to-side just posterior to the gill opening (unsqueezed); head length (HL) is the horizontal distance from the front of the upper lip to the most posterior end of the opercular flap; head depth is the vertical distance at the midline of the orbit (closed-mouth specimens); snout length is the horizontal span (not angular distance) from the front of the upper lip to the anterior edge of the bony orbit; orbit diameter is the horizontal distance from edge to edge of the bony orbit; interorbital width is the least bony width; upper-jaw length is the full oblique length; caudal-peduncle depth is the least depth and caudal-peduncle length is the horizontal distance from the base of the last dorsal-fin ray to the caudal-fin base; lengths of fin spines and rays are measured to their junction with the body; caudal-fin length is the horizontal distance from the base of the fin to a vertical at the tip of the longest ray; pectoral-fin length is the length of the longest ray; pelvic-fin length is measured from the junction with the body to the stretched tip of the longest soft ray. Lateral-line scale counts follow Greenfield (1979) with arched and straight portions counted.

Starksia splendens, n. sp.

Splendid Shy Blenny

Blenio Tímido Espléndido

Figures 1–17.

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BOLD mtDNA barcode lineage BOLD:ACN1852

Starksia lepicoelia (non Böhlke & Springer) Burgess *et al.* 1994: 218 (Cayman Islands).

Holotype. UF 241196, 18.6 mm SL, male, Cayman Islands, Grand Cayman, southwest corner, 19.286°, -81.392°, 5–15 m depth, B.C. Victor, 27 April 2014.

Paratypes. UF 241197, (4) 15.0–18.2 mm SL, same data as holotype.

Diagnosis. A species of *Starksia* (genus characterized by a combination of scaled body, only two apparent soft pelvic-fin rays, and a male intromittent organ attached to or separate from a first anal-fin spine separate to some degree from remainder of fin), in a species complex (of *S. lepicoelia*) characterized by a mostly scaled abdomen, an orbital cirrus, male intromittent organ completely separate from first anal-fin spine, and 13 pectoral-fin rays. *S. splendens* is distinguished by color pattern and two discrete black bars on preorbital portion of lower lip in both females and males, and greatly extended, ribbon-like, white orbital cirri in mature males.



Figure 1. *Starksia splendens*, mature male, long genital papilla, Grand Cayman, Cayman Islands (C. Abgarian).



Figure 2. *Starksia splendens*, mature male (reddish and blackcheek), Grand Cayman, Cayman Islands (E.M. Turner).

Description. Dorsal-fin elements XIX,8 (XIX,9–XX,8), dorsal fin continuous with deep notch in posterior portion; anal-fin elements II,17; pectoral-fin rays 13; visible pelvic-fin elements I,2; principal caudal-fin rays 7+6=13, all unbranched; upper and lower procurrent caudal-fin rays 6+5 (5–6+5); arched lateral-line scales 15 (14–15) plus 21 (20–22) straight = total 36 (35–37) scales; gill rakers, including rudiments 3+9=12.

Body elongate, body depth 19–20 (22)% SL, body width 12–13 (12)% SL; predorsal distance 29–33 (30)% SL; prepelvic distance 25–27 (25)% SL; preanal distance 57–60 (58)% SL; caudal-peduncle length 7–9 (8)% SL, caudal-peduncle depth 9 (9)% SL. Head length 29–33 (30)% SL; head depth (at midpoint of orbit) 13–15 (15)% SL or 43–46 (49)% HL; snout short, snout span 15–20 (16)% HL; eye large, orbit diameter 27–30 (29)% HL; interorbital narrow, mostly flat, minimum width 10–12 (11)% HL; a short, simple, single, nuchal cirrus, shorter than pupil diameter; a single, unbranched, slender, pointed orbital cirrus (on each side), less than eye diameter in length in females and immature fish, but wide, flattened, ribbon-like, and white, up to more than two times orbital diameter in length, in mature males (Figs. 1–8); anterior naris a low tube with a slender tentacle-like nasal cirrus along rear rim, sometimes greatly elongated to more than two orbital diameters in both males and females, posterior naris an elliptical opening adjacent to upper orbital rim; circumorbital head pores in single row, 6 below equator, 3 along rear upper rim, a single small midline posterior interorbital pore flanked by a single small pore on each side, about 7 pores along opercular rim followed by 3 mandibular pores; mouth large, upper-jaw extending back past vertical through rear orbital rim, oblique length 43–50 (47)% HL; lips thick



Figure 3. *Starksia splendens*, mature male, extreme cirri lengths, Grand Cayman, Cayman Islands (C. Abgarian).



Figure 4. *Starksia splendens*, preserved holotype, 18.6 mm SL male, UF 241196, Grand Cayman, Cayman Islands (B.C. Victor).

and wide; upper and lower jaws with row of variable-sized caniniform teeth, additional inner row behind anterior row; preopercle edge smooth, opercular margin smooth, without spines, ending dorsally in thin flap. Gill rakers short and pointed, three on upper limb of first arch, 9 additional on lower limb, longest at angle, followed by short irregular series.

Dorsal-fin base long, 63–69 (66)% SL, first dorsal-fin spine longer than next, a membranous flap continues beyond spine tip (extends about one pupil diameter), shallow notch in membrane between first and second spine, first dorsal-fin spine 8–10 (12)% SL, second spine 7–10 (8)% SL, third spine 7–10 (9)% SL; notch in dorsal fin at end of spinous portion, shortest spine third from last, 4–5 (5)% SL, last spine 9% SL; short soft portion,

all rays unbranched, usually third or fourth ray longest, 14–15 (15)% SL; anal-fin base long, 36–39 (35)% SL, first spine shorter 5–7 (9)% SL, second spine 7–8 (11)% SL, membrane between spines deeply notched to absent in males, all rays unbranched, about fourth from last ray longest 15–16 (17)% SL; pectoral fin long and pointed, longest ray about 9th from top, 24–26 (24)% SL, all rays unbranched; pelvic-fin spine short, barely visible, only two visible rays, unbranched, very long, inner ray longer 21–24 (23)% SL, outer ray 15–19 (16)% SL; caudal fin broadly rounded, length 22–25 (26)% SL.

Body mostly with cycloid scales, including abdomen, except head, nape around dorsal-fin origin, and pectoral-fin base naked. Lateral-line made up of anterior segment of 11 (11–13) tubed scales, then untubed scales arched downward and extending in a straight portion to caudal-fin base: 15 (14–15) in arched portion, 21 (20–22) in straight portion, 36 (35–37) total.

Genital papilla of males a long finger-like tentacle, shorter than and separate from first anal-fin spine; urogenital papilla of females a small simple cone.

Color in life of mature male. (Figs. 1–3 & 5–8) Body with mainly barred pattern of about 9 mostly oval bars from behind pectoral fin to base of caudal fin, color ranging from reddish to dark brown to pale gray; a variable



Figure 5. *Starksia splendens*, mature male (grayish and dark cheek), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 6. *Starksia splendens*, mature male (pale and dark cheek), Grand Cayman, Cayman Islands (E.M. Turner).

irregular array of prominent darker scales scattered across flanks and rear body; a series of about 4 large white (or yellow on reddish fish) patches between dorsal aspect of bars, after second, fourth, sixth and eighth bars, last often paired with a prominent black spot just under base of last dorsal-fin rays.

Fins translucent except broad white or yellow patch over first dorsal-fin membrane, sometimes with a dark oval spot near distal membrane next to second spine; often short dark and light markings at base of dorsal-fin elements, corresponding to body bars and pale interspaces; sometimes a prominent small dark spot on base of lower pectoral-fin rays (e.g. Fig. 1), sometimes a row of dark spots along base of anal fin.

Head mostly brownish to reddish with a purple cast, mature male characterized by a “blackcheek”, a dark patch along lower rear operculum, ranging from a gray uniform patch to (rarely) a dark black patch (Fig. 8); some pale unpigmented spots or reticulations behind upper eye, but not extending onto lower half of operculum; a bright white pair of spots or a band behind lower eye, another white band from upper eye back to nape; forehead from tip of upper lip to interorbital often a shield of bright white; cranium behind eyes reddish dusky with evenly spaced



Figure 7. *Starksia splendens*, mature male (purplish and dark cheek), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 8. *Starksia splendens*, mature male (true blackcheek), Grand Cayman, Cayman Islands (E.M. Turner).

pale-to-bluish spots, followed by white patches at bases of nuchal cirri; area between lower eye and upper lip with a prominent row of dark lines interspersed with white spaces, iris bright reddish orange becoming dark with narrow white radii posteriorly, ribbon-like orbital cirrus white without banding, nasal cirrus pale yellowish without banding.

Preorbital portion of upper lip with a broad yellowish-to-brown band behind white tip, without a dark outline except for rear lower corner; suborbital portion of upper lip pale with a single black narrow bar midway; preorbital portion of lower lip with two prominent dark to black bars, anterior bar wider and less densely black; suborbital portion of lower lip often with a narrow bar underlying corresponding bar of upper lip; lower jaw and isthmus pale with a dark band connecting rear preorbital lower-lip bars, and another dark band across isthmus at level of suborbital lip bars (e.g. Fig. 8).

Color in life of female and immature. (Figs. 9–17)

Head color patterns a more subdued version of males (Fig. 9), but opercle often with unpigmented spots against a uniform background (see Figs. 10 & 11), or an all-pale lower half. Body with same bar pattern as males, ranging from pale gray with dusky to brown bars (Fig. 12), to reddish, to dark with darker brown bars and dense spotting all over with small round bluish spots



Figure 9. *Starksia splendens*, female, Grand Cayman, Cayman Islands (J. Holdorf).

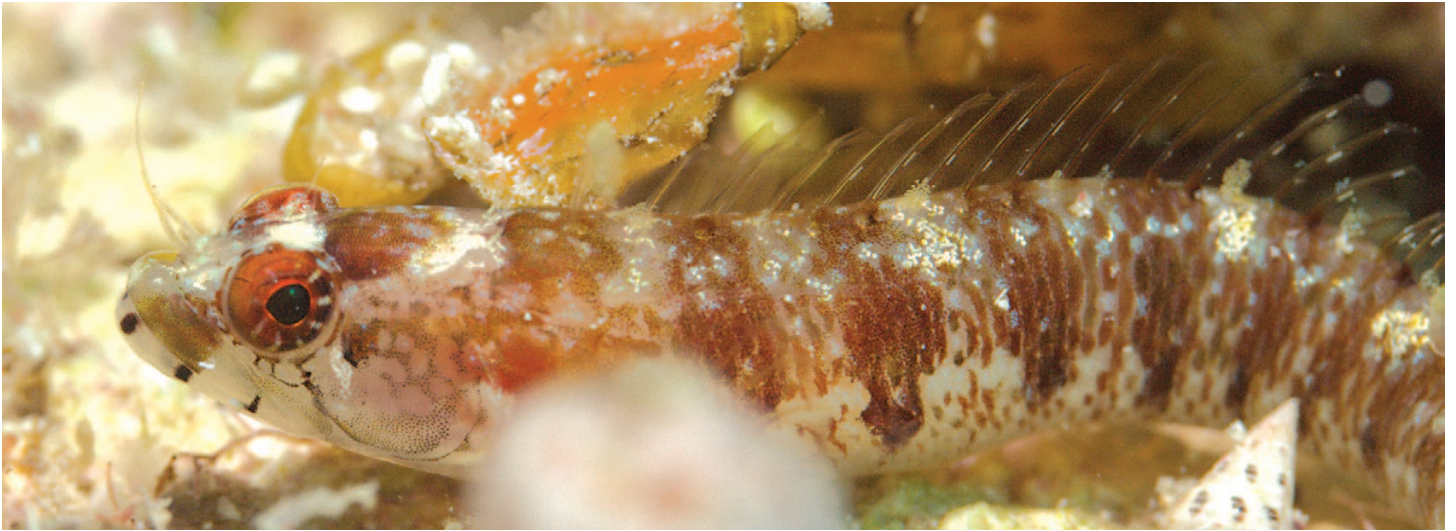


Figure 10. *Starksia splendens*, female (pale cheek spots and lightly barred), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 11. *Starksia splendens*, female (cheek spots and lightly barred), Grand Cayman, Cayman Islands (E.M. Turner).

(Figs. 13 & 14); sometimes a prominent small dark spot on base of lower pectoral-fin rays (e.g. Fig. 14). Orbital cirri either translucent or with two white bands proximal and distal; nasal cirri translucent; notably, females with same pattern of black bars on lips as males described above (e.g. Fig. 9).

Occasional fish, presumed female, or non-breeding or immature male, have bright colors and a prominent dark rim to lower rear opercle with some adjacent pale areas or reticulations (Fig. 15), but not with a full shaded or dark patch as in mature males with enlarged white orbital cirri as described above.

Juveniles with less developed bar pattern; white upper-body blotches prominent; lower head, thorax, and abdomen pale, except for dark-white-dark series of spots around rim of rear lower orbit (Figs. 16 & 17). Two dark preorbital lower-lip bars prominent.

Color in preservative. (Fig. 4) Head and body tan, only black markings (melanophores) persist in alcohol, i.e. bars on lips, cheek spot if present, and an irregular scattering of dark-edged scales on the body. Fins are translucent with a variably dusky to dark oval spot on first dorsal-fin spine membrane, and a fine speckling of melanophores over spinous dorsal-fin membranes, along upper and lower margin of caudal fin, and in a band along distal anal-fin membranes.



Figure 12. *Starksia splendens*, female (pale body and lightly barred), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 13. *Starksia splendens*, female (dark-barred and speckled), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 14. *Starksia splendens*, female or immature male (dark brown bars and dense light spotting, with pectoral-fin spot), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 15. *Starksia splendens*, female or immature male pattern with dark opercular rim and dark body bars, Grand Cayman, Cayman Islands (E.M. Turner).



Figure 16. *Starksia splendens*, juvenile (pale and lightly barred), Grand Cayman, Cayman Islands (E.M. Turner).



Figure 17. *Starksia splendens*, juvenile, Grand Cayman, Cayman Islands (E.M. Turner).

Etymology. The specific epithet *splendens* (Latin for shining, brilliant, or splendid) is based on the impressive appearance of mature males of the species. The name is treated as an indeclinable adjective.

Distribution. The new species is known only from the Cayman Islands. Species in the complex are allopatric in the Greater Caribbean, with *S. lepicoelia* found in the Bahamas and *S. williamsi* from the Antilles, including Puerto Rico, Virgin Islands, Saba, and down to at least Dominica. In the western Caribbean, *S. weigti* is found in Belize and Honduras, and *S. robertsoni* in Panama.

Comparisons. *Starksia splendens* is most easily distinguished from other Caribbean members of the *S. lepicoelia* species complex by the black bars on the lower lip of females: the other species have females with reddish bars on the lips, i.e. simple bars on *S. williamsi* and *S. robertsoni* and pale-spotted bars on *S. weigti* (Figs. 18 & 19). The markings on the female cheek also vary, with pale (unpigmented) spots on *S. splendens*, pale spots

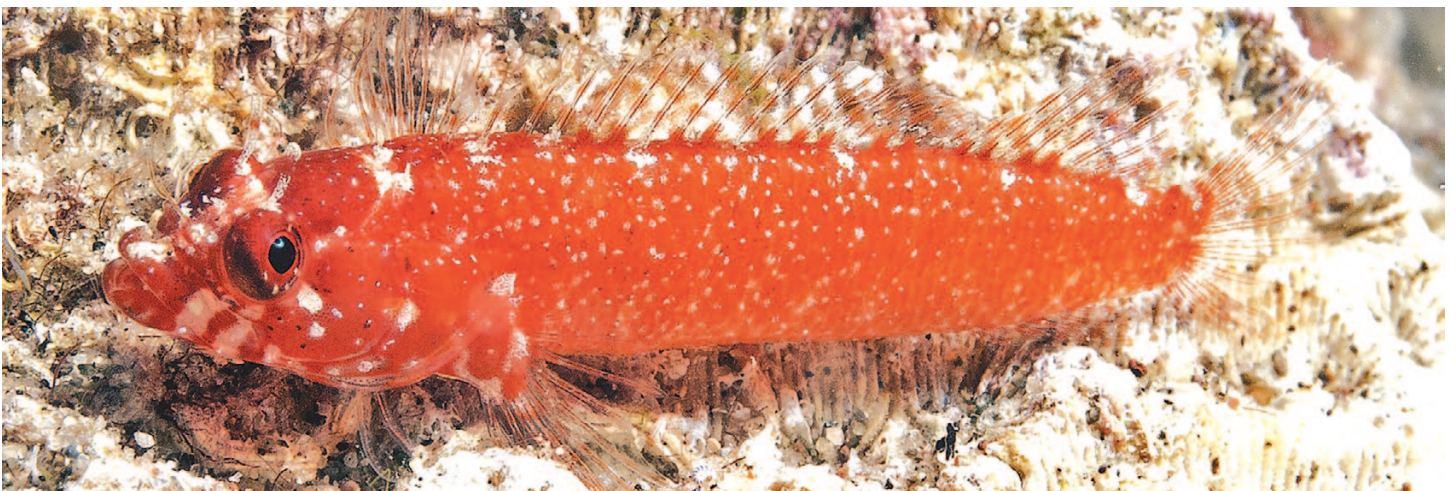
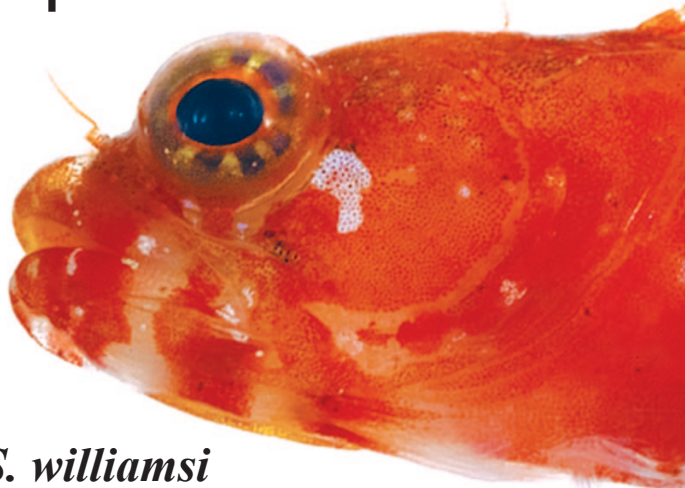
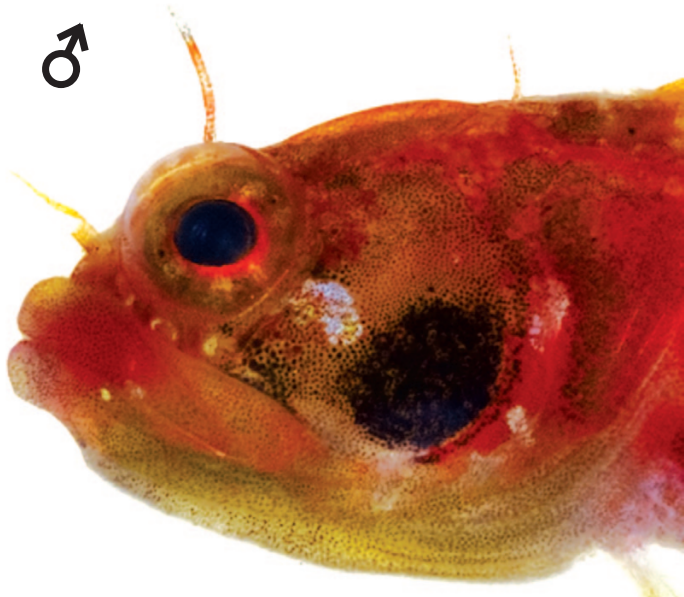


Figure 18. *Starksia williamsi*, female, red lip bars, mtDNA-confirmed, Dominica (N. DeLoach).

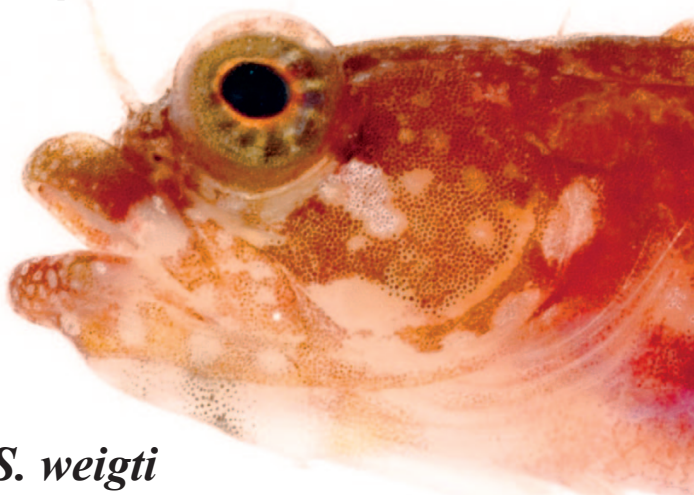
♀

*S. williamsi*

♂



♀

*S. weigti*

♂



♀

*S. robertsoni*

♂



Figure 19. *Starksia lepicoelia* species complex: *S. williamsi*, female and male (Saba) (J.T. Williams); *S. weigti*, female and male (Belize) (C.C. Baldwin); *S. robertsoni*, female and preserved male (Panama) (D.R. Robertson & C.C. Baldwin).

and reticulations on *S. weigti* and *S. robertsoni*, and no apparent unpigmented patterns on *S. williamsi* (Figs. 18 & 19). Males of the other species also have no black bars on the lips (except for some *S. lepicoelia* from Bahamas, see below), and do not have particularly long, ribbon-like, or all-white orbital cirri and the cirrus is banded when marked (Fig. 19). Individuals of other members of the complex photographed underwater or fresh thus far also do not have the exceptionally long nasal cirri exhibited by most *S. splendens*. In addition, some meristic differences are apparent within the complex, including some lower fin-ray counts in *S. robertsoni* and *S. williamsi* (the latter probably includes “*S. lepicoelia*” from Navassa in Williams & Mounts [2003]) (Baldwin *et al.* 2011).

A female *S. lepicoelia* was photographed underwater in the Bahamas, by Frank Krasovec, at Little San Salvador, in the north-central islands not far from the type location of Nassau (Fig. 20). His photographs show a female with very different features from female *S. splendens*: the bars on the lips are red, there are white spots on the lips, there is no pattern of unpigmented spots on the cheek, and the head and body are mostly uniformly reddish. The original description in Böhlke & Springer (1961) noted that females are mostly uniform except for an occasional specimen with body bars (especially those from Cay Sal Bank, which is nearer to Cuba than to the rest of the Bahamas Islands).



Figure 20. *Starksia lepicoelia*, female, red lip bars, Little San Salvador, Bahamas (F.H. Krasovec).

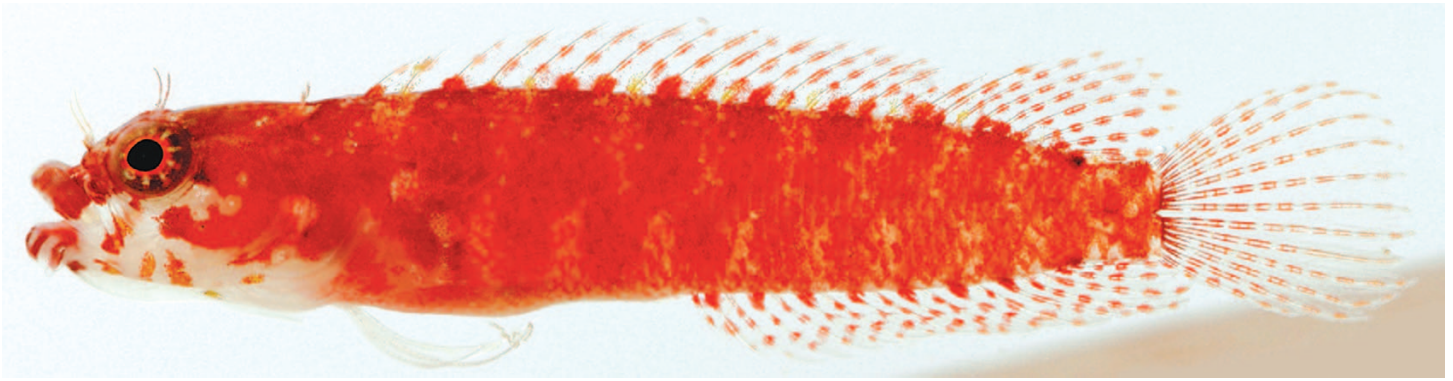


Figure 21. *Starksia lepicoelia*, freshly collected, female (red lip bars), Lee Stocking Island, Exuma Sound, Bahamas (J. Van Tassell & D.R. Robertson).



Figure 22. *Starksia lepicoelia*, freshly collected, mature male (mostly uniform reddish; short, banded orbital cirri), USNM 399928, Berry Islands, Bahamas (C.C. Baldwin).

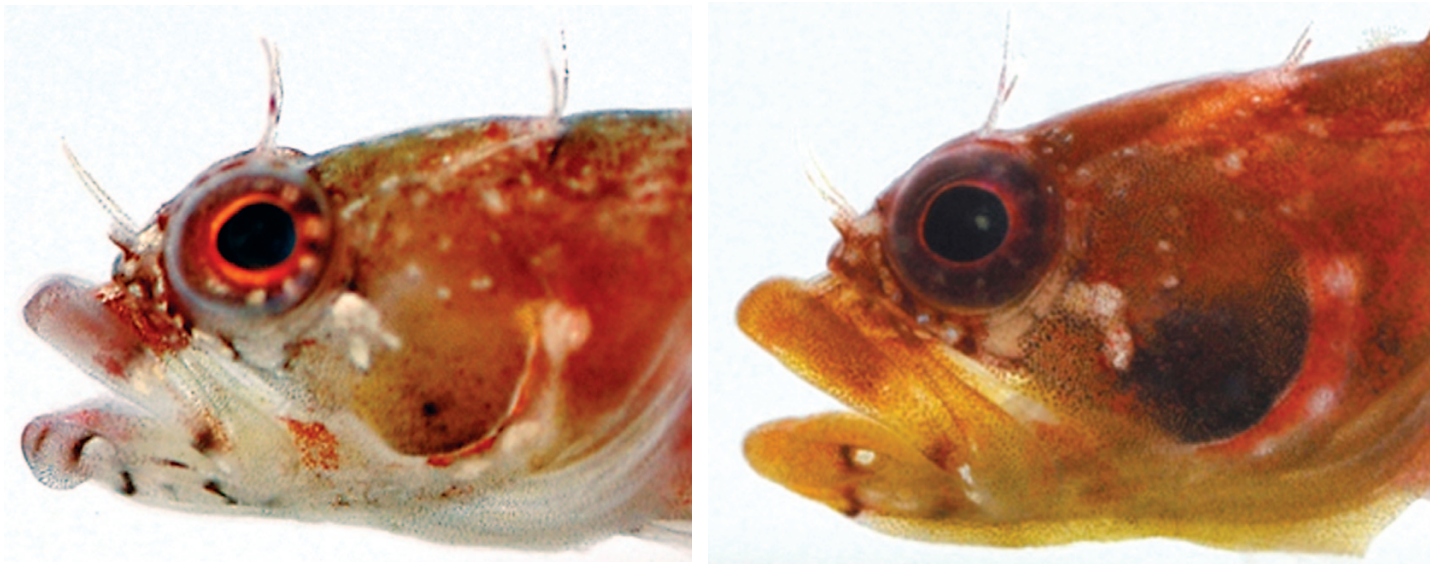


Figure 23. *Starksia lepicoelia*, freshly collected, mature males, Lee Stocking Island, Exuma Sound, Bahamas (J. Van Tassell & D.R. Robertson).

The issue of what entity represents the type population of *S. lepicoelia* in the Bahamas is unresolved. Baldwin *et al.* (2011) found that there were two different mtDNA lineages occurring together at multiple locations in the Bahamas Islands, and no clear phenotypic difference to distinguish them. In addition, series of fresh photographs from Bahamas locations by Carol C. Baldwin *et al.*, D. Ross Robertson, and James Van Tassell show females with reddish-banded lips (Fig. 21), however, male *S. lepicoelia* in the region exhibit several different appearances, some with dark banded lips (often dark reddish), and some without; the band patterns can vary from one individual to another (Figs. 22 & 23). One relatively consistent marking is a bright white contrasting bar behind the dark bar on the midportion of the lower lip. None of the photographs show specimens with very long, ribbon-like, white orbital cirri, and many have banded orbital cirri.

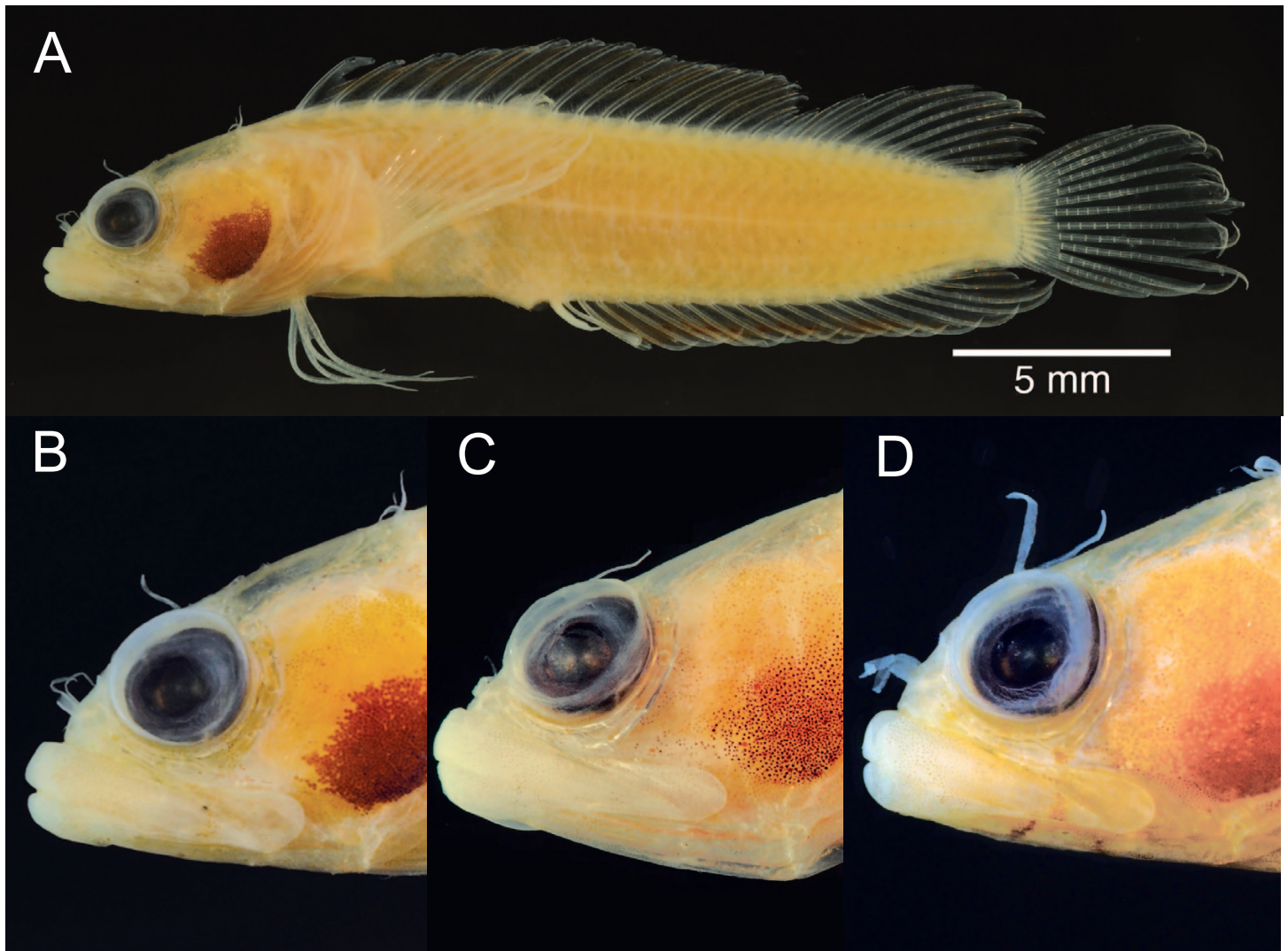


Figure 24. *Starksia lepicoelia*, A & B: holotype, ANSP 94144 ; C & D: paratypes from ANSP 94145; Nassau, Bahamas (courtesy K. Luckenbill).

The holotype of Böhlke & Springer (1961) is a black-cheeked male that has been preserved for many decades and presently has only distinct cheek melanophores and a fine scattering of melanophores on the lips without any discrete bars (Fig. 24). The paratypes either are similar to the holotype, or have one or two short bars of melanophores, with or without the band across the isthmus (Fig. 24). It is unclear whether the differently marked specimens, such as the male in Fig. 24D, represent the different Bahamas mtDNA lineages or not. These patterns are consistent with markings on the lips of fresh males, as in Fig. 23: but it should be noted that red markings are formed by erythrophores and are not preserved in museum specimens. The holotype has unremarkable short nasal and orbital cirri.

DNA Comparisons. The neighbor-joining phenetic tree based on the COI mtDNA sequences of the 6 lineages (5 different species and an extra genovariant lineage) in the *S. lepicoelia* species complex, following the Kimura two-parameter model (K2P) generated by BOLD (the Barcode of Life Database), shows relatively deep divergences between species and smaller differences within species (Fig. 25 & Table 1). Some intraspecific variation (in *S. williamsi*) is an artifact of short sequences in the Puerto Rican samples (derived from lionfish stomachs, by Chelsea Harms-Tuohy).

The mtDNA lineages diverge between species from 1.33% to 9.43% (K2P; 1.32% to 8.65% uncorrected pairwise), a similar range of divergences to other blennioid species complexes in the Greater Caribbean region. The degree of genetic divergence in the COI marker for the new species falls in the mid-range for the members of the complex, 7.22% from the nearest neighbor, *S. williamsi* (K2P; 6.76% uncorrected pairwise) (Table 1). As has been observed in many other genetic studies on reef fishes, some lineages that look similar, or indistinguishable,

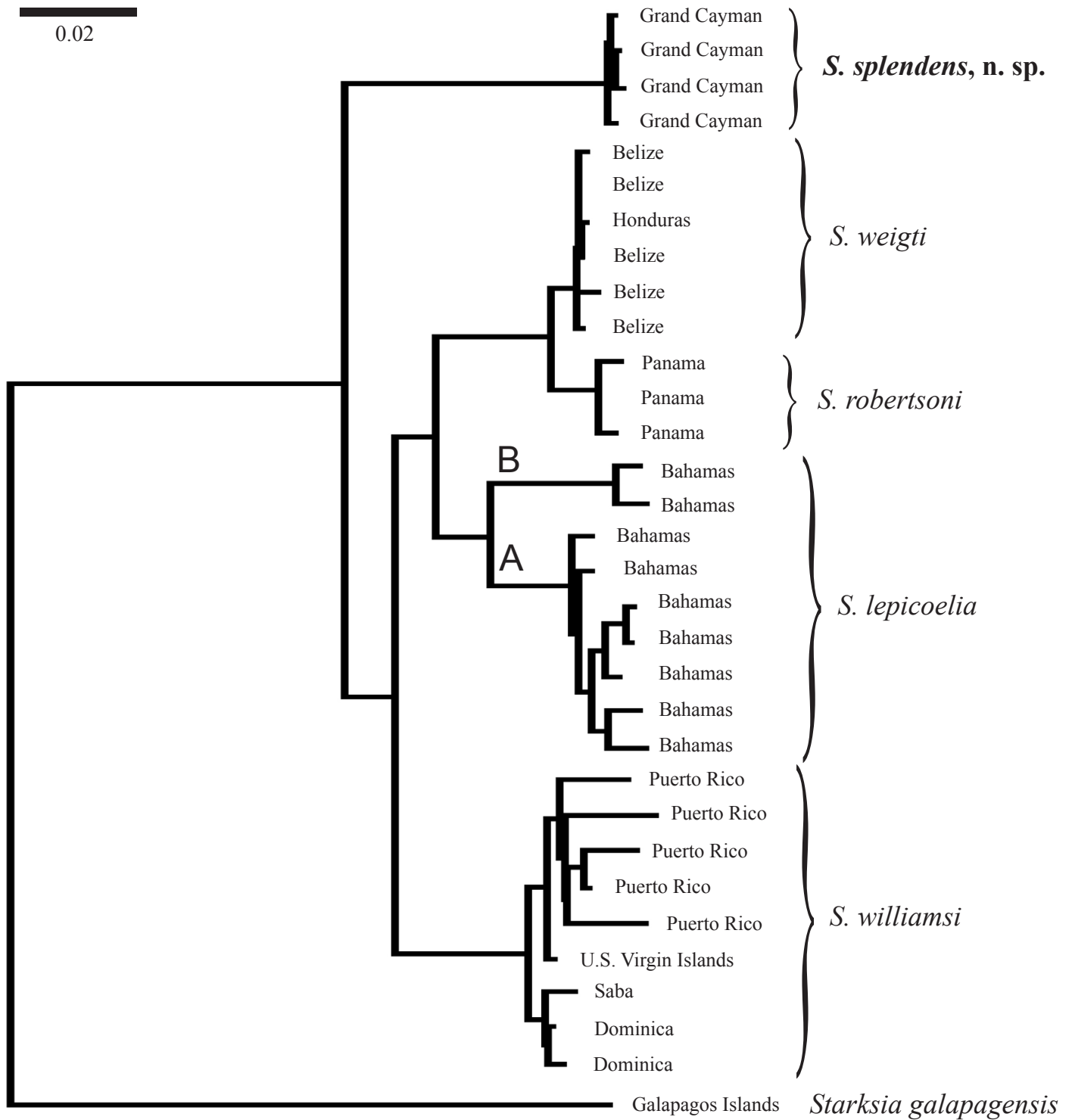


Figure 25. The neighbor-joining phenetic tree of the *Starksia lepicoelia* species complex following the Kimura two-parameter model (K2P) generated by BOLD (Barcode of Life Database). The scale bar at left represents a 2% sequence difference. Collection locations for specimens are indicated, and *Starksia galapagensis* is used as an outgroup. GenBank accession numbers and collection data for the sequences in the tree are listed in Appendix 1.

can be more divergent than species with clear differences in counts or appearance. In this case, for example, the two sympatric lineages of *S. lepicoelia* diverge more in sequence than *S. robertsoni* vs. *S. weigti*.

Remarks. Members of this genus are particularly difficult to photograph, since they are exceptionally shy and remain within the reef structure (unless collected with rotenone); as a result, there were very few underwater photographs of the species until recently, with the rise of intensive macrophotography on reefs. Still, few locations have been well photographed and some species are still undocumented. Two populations of *Starksia* have been extensively photographed underwater recently, with more than 100 photographs available each: *S. splendens* from the Cayman Islands (by Everett M. Turner, Jr., Essie Evans, Cynthia Abgarian, and Jamie Holdorf) and *S. hassi* from Bonaire. This study is a good example of the utility of intensive underwater macrophotography of live fishes—without the contribution of the photographers, the variation in appearance would be daunting. These colorful tiny fishes typically have complex and detailed color patterns that can vary with size, gender, and mating status, and, furthermore, these colors and patterns rapidly disappear after collection and are generally absent in preserved fishes. It is likely that further progress on resolving this and some other species complexes will require underwater photographers to document these elusive fishes in a wider range of locations.

TABLE 1

**Variation in mtDNA COI sequences of the 6 lineages
in the *Starksia lepicoelia* species complex**

Minimum Interspecific and Maximum Intraspecific Distances (%)

K2P distances						
	<i>lep A</i>	<i>lep B</i>	<i>rob</i>	<i>splen</i>	<i>will</i>	<i>wei</i>
<i>S. lepicoelia</i> A	1.82					
<i>S. lepicoelia</i> B	4.31	1.03				
<i>S. robertsoni</i>	5.38	7.12	0.79			
<i>S. splendens</i> , n. sp.	8.82	9.43	9.30	0.33		
<i>S. williamsi</i>	7.23	7.31	6.28	7.22	3.10	
<i>S. weigti</i>	5.00	5.96	1.33	9.41	6.05	0.50

P-distances (uncorrected pairwise)						
	<i>lep A</i>	<i>lep B</i>	<i>rob</i>	<i>splen</i>	<i>will</i>	<i>wei</i>
<i>S. lepicoelia</i> A	1.79					
<i>S. lepicoelia</i> B	4.15	1.02				
<i>S. robertsoni</i>	5.12	6.30	0.79			
<i>S. splendens</i> , n. sp.	8.12	8.65	8.53	0.33		
<i>S. williamsi</i>	6.76	6.84	5.93	6.76	3.03	
<i>S. weigti</i>	4.77	5.65	1.32	8.62	5.30	0.49

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Appendix 1. Specimen data and GenBank accession numbers for the mtDNA COI barcode sequences used to generate the phenogram in Fig. 25, following the order in the tree. Holotype in bold.

Genus	species	Collection site	Voucher	GenBank #	Collector/Source
<i>Starksia</i>	<i>splendens</i> , n. sp.	Grand Cayman, Cayman Islands	UF 241197 18.2	MK073380	B.C. Victor
<i>Starksia</i>	<i>splendens</i>, n. sp.	Grand Cayman, Cayman Islands	UF 241196	MK073381	B.C. Victor
<i>Starksia</i>	<i>splendens</i> , n. sp.	Grand Cayman, Cayman Islands	UF 241197 15.0	MK073385	B.C. Victor
<i>Starksia</i>	<i>splendens</i> , n. sp.	Grand Cayman, Cayman Islands	UF 241197 17.1	MK073377	B.C. Victor
<i>Starksia</i>	<i>weigti</i>	Carrie Bow Cay, Belize	USNM 399650	HQ600885	C.C. Baldwin, USNM
<i>Starksia</i>	<i>weigti</i>	Curlew Cay, Belize	USNM 399649	HQ600886	C.C. Baldwin, USNM
<i>Starksia</i>	<i>weigti</i>	Roatan, Honduras	LIDMA414-11	MK073383	B.C. Victor
<i>Starksia</i>	<i>weigti</i>	Carrie Bow Cay, Belize	USNM 433824	JQ840309	C.C. Baldwin, USNM
<i>Starksia</i>	<i>weigti</i>	Carrie Bow Cay, Belize	USNM 399648	HQ600884	C.C. Baldwin, USNM
<i>Starksia</i>	<i>weigti</i>	Carrie Bow Cay, Belize	USNM 433919	HQ600887	C.C. Baldwin, USNM
<i>Starksia</i>	<i>robertsoni</i>	Portobelo, Panama	USNM 399912	HQ571154	D.R. Robertson et al.
<i>Starksia</i>	<i>robertsoni</i>	Portobelo, Panama	USNM 399909	HQ600961	D.R. Robertson et al.
<i>Starksia</i>	<i>robertsoni</i>	Portobelo, Panama	USNM 399911	HQ600960	D.R. Robertson et al.
<i>Starksia</i>	<i>lepicoelia</i> B	Berry Islands, Bahamas	USNM 414758	HQ543038	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> B	Berry Islands, Bahamas	USNM 399615	HQ600890	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Turks & Caicos, Bahamas	USNM 399636	HQ543045	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Turks & Caicos, Bahamas	USNM 413070	HQ543052	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Berry Islands, Bahamas	USNM 399617	HQ600888	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Berry Islands, Bahamas	USNM 399616	HQ600889	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Turks & Caicos, Bahamas	USNM 399640	HQ543049	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Turks & Caicos, Bahamas	USNM 399641	HQ543050	C.C. Baldwin, USNM
<i>Starksia</i>	<i>lepicoelia</i> A	Turks & Caicos, Bahamas	USNM 413069	HQ543051	C.C. Baldwin, USNM
<i>Starksia</i>	<i>williamsi</i>	Puerto Rico, USA	NA	KX140116	C. Harms-Tuohy
<i>Starksia</i>	<i>williamsi</i>	Puerto Rico, USA	NA	KX140215	C. Harms-Tuohy
<i>Starksia</i>	<i>williamsi</i>	Puerto Rico, USA	NA	KX140195	C. Harms-Tuohy
<i>Starksia</i>	<i>williamsi</i>	Puerto Rico, USA	NA	KX140606	C. Harms-Tuohy
<i>Starksia</i>	<i>williamsi</i>	Puerto Rico, USA	NA	KX140379	C. Harms-Tuohy
<i>Starksia</i>	<i>williamsi</i>	St. Thomas, USVI	ak485321165	MK073384	A. Shiroza/B.C. Victor
<i>Starksia</i>	<i>williamsi</i>	Saba, Netherlands Antilles	USNM 397396	HQ543039	J.T. Williams, USNM
<i>Starksia</i>	<i>williamsi</i>	Dominica	d11713sl207	MK073379	B.C. Victor
<i>Starksia</i>	<i>williamsi</i>	Dominica	d11713sl185	MK073382	B.C. Victor
<i>Starksia</i>	<i>galapagensis</i>	Galapagos, Ecuador	gal490sg108	MK073378	B.C. Victor