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Authors: Martin, Christopher H., and Wainwright, Peter C.

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A Remarkable Species Flock of *Cyprinodon* Pupfishes Endemic to San Salvador Island, Bahamas

Christopher H. Martin¹ and Peter C. Wainwright²

¹ Corresponding author: Department of Evolution and Ecology and Center for Population Biology, University of California, Davis CA 95616 USA

Current address: Department of Environmental Science, Policy, and Management, University of California, Berkeley CA 94720 USA
—e-mail: chmartin@berkeley.edu

² Department of Evolution and Ecology and Center for Population Biology, University of California, Davis CA 95616 USA

ABSTRACT

Two new species of *Cyprinodon* are described, both restricted to saline lakes on a single island in the Bahamas. Both species have unique morphological features and specialized diets that distinguish them from the third *Cyprinodon* species on San Salvador Island, *C. variegatus*, and all other pupfishes. *Cyprinodon desquamator*, n. sp., is a scale-eating specialist with a greatly enlarged lower jaw and elongated body. *Cyprinodon brontotheroides*, n. sp., is a hard-shelled prey specialist with a protruding nasal region encasing its upper jaw, a unique appendage within Cyprinodontiformes. Both specialist species occur in syntopy with *C. variegatus* and *Gambusia hubbsi* in several shallow, hypersaline lakes on San Salvador. Geological age estimates for these lakes place the age of this species flock at 10,000 years or less. Thus, in addition to Laguna Chichancanab, this study describes species within a second remarkable species flock of specialized *Cyprinodon* pupfishes.

KEYWORDS

Pupfish, ecological speciation, adaptive radiation, ecological novelty, trophic innovation, species flock, Caribbean, species description, Teleostei

Introduction

Cyprinodon pupfishes are notorious for their frequent isolation in desert hot springs throughout the southwestern United States and Mexico (Miller 1948; Miller et al. 2005). However, they also inhabit estuaries and saline lakes throughout the Atlantic coast from Massachusetts to Belize and throughout the Lesser Antilles, Cuba, Dominican Republic and Jamaica. *Cyprinodon* has also colonized Venezuela at least twice (Haney et al. 2009). In a general pattern throughout this range, closely related species of pupfishes almost never coexist in sympatry, and when allopatric species come into contact, introgression is rampant. Natural hybrid zones are known from at least two desert spring systems (*Cyprinodon eximius*/*Cyprinodon pachycephalus* [Minckley and Minckley 1986] and *Cyprinodon atrorus*/*Cyprinodon bifasciatus* [Carson and Dowling 2006]), where disruptive selection along thermal clines

appears to maintain species boundaries and limits hybridization to intermediate habitats (Tobler and Carson 2010). On the other hand, without divergent habitat gradients maintaining species boundaries, human introductions of *C. variegatus* have repeatedly resulted in widespread introgression with isolated inland species (e.g., *C. bovinus* [Echelle and Echelle 2002] and *C. pecosensis* [Echelle and Connor 1989]). In one case, there seems to be no pre-mating isolation between allopatric populations after secondary contact, or even a significant preference for interspecific males by *C. pecosensis* females (Rosenfield and Kodric-Brown 2003). Laboratory crosses also show no evidence of pre-mating isolation in allopatric species (Turner and Liu 1977) with very few examples of postzygotic intrinsic incompatibilities across the group (Tech 2006).

Thus far, only one exception to this pattern of allopatry among closely related species has been described: a species flock of sympatric pupfishes

endemic to a single, large lake in the Yucatan, Laguna Chichancanab (Humphries and Miller 1981; Humphries 1984; Strecker 2006a). This lake is only 8,000 years old (Covich and Stuiver 1974; Hodel et al. 1995), yet contains seven described *Cyprinodon* species. These include a specialized piscivore (*C. maya*), zooplanktivore (*C. simus*) and four additional species with differentiated trophic morphology and shortened intestines in addition to the generalist detritivore *C. beltrani* (Humphries and Miller 1981; Humphries 1984; Horstkotte and Strecker 2002; Strecker 2002, 2005, 2006a). Unfortunately, most of this species flock is now extinct in the wild following the introductions of *Oreochromis* spp. and *Astyanax affinis* in the 1990s (Schmitter-Soto and Caro 1997; Strecker 2006b; Martin and Wainwright 2011). Several specialized species from the Chichancanab radiation (including *C. maya* and *C. simus*) remain only in low numbers within a few captive populations (C.H. Martin, pers. obs.).

Here we describe species within a second remarkable species flock of *Cyprinodon* pupfishes endemic to a single, tiny island in the Bahamas. San Salvador Island is only 22 × 10 km. Its interior is dominated by many shallow, hypersaline lakes (up to 12 m deep; 40 to 50 ppt salinity) containing fish communities comprised of only *Cyprinodon*, *Gambusia hubbsi* and *Atherinomorus stipes*. All three *Cyprinodon* species co-occur throughout the littoral and benthic zones of these lakes which are dominated by macroalgae (predominantly *Batophora*, *Cladophora* and *Acetabularia*) and wigeongrass (*Ruppia maritima*; Godfrey et al. 1994). Exploration of saline lakes on surrounding Bahamian islands (Rum Cay, Cat Island, Long Island, Crooked Island, Acklins Island and New Providence Island) indicates the two specialized species described here are endemic to San Salvador (C.H. Martin, pers. obs.). Monophyly of the species flock on San Salvador supports the hypothesis that both specialists evolved on the island (Martin and Wainwright 2011).

The geology of San Salvador suggests a maximum age for these lakes of approximately 6,000 to 10,000 years (Turner et al. 2008). San Salvador is an isolated carbonate pillar with no connection to the Bahamian continental shelf and its shallow lakes are hydrostatically supported by sea level. During the last glacial maximum (15,000 years ago), when sea levels were 125 m lower, all saline

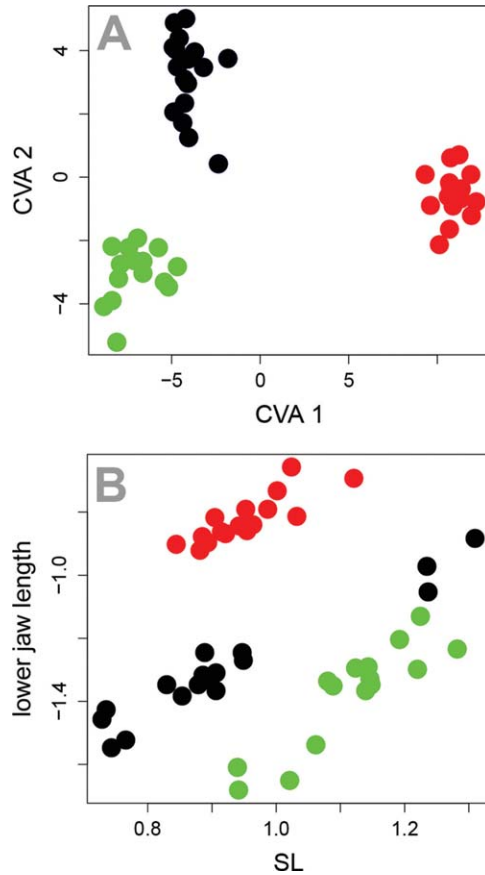


FIGURE 1. Morphometric data. A, Canonical variates analysis of 18 size-corrected morphometric traits presented in Table 1 for *Cyprinodon desquamator* n. sp. (red), *Cyprinodon brontotheroides* n. sp. (green) and *Cyprinodon variegatus* (black). B, Allometric relationship between log-transformed lower jaw length and log-transformed standard length (SL) for the three sympatric species on San Salvador Island, colored as in A.

lakes on the island would have been dry with no lower-level refugia (Hagey and Mylroie 1995; Turner et al. 2008). The putative origin of this species flock within San Salvador's lake system means speciation began a maximum of 6,000 to 10,000 years ago as saline lakes began to fill (Pacheco and Foradas 1987; Turner et al. 2008).

This nascent adaptive radiation of *Cyprinodon* pupfishes on San Salvador Island provides a fascinating case study of the evolution of morphological and ecological novelty. Our initial work found exceptional rates of morphological diversification for certain trophic traits in this clade, reaching up to 51 times faster than background rates of trait

evolution in allopatric *Cyprinodon* species (Martin and Wainwright 2011). This rate of morphological diversification was exceeded only by the species flock of pupfishes in Laguna Chichancanab (130 times faster than background rates) and remains one of the fastest rates of morphological diversification reported for any clade (Martin and Wainwright 2011). Furthermore, our measurements of the adaptive landscape for pupfishes on San Salvador suggest that a complex, multi-peaked landscape is driving adaptive radiation in these lakes (Martin and Wainwright 2013a). We found evidence for two fitness peaks within an F2 hybrid morphospace, which corresponded to two of the three species observed in the wild (*Cyprinodon variegatus* and *Cyprinodon brontotheroides*) and experimentally demonstrated that competition is driving this complex fitness surface (Martin and Wainwright 2013a). Exceptional divergence within remarkably localized adaptive radiations of pupfishes provides great potential for future insights into complex evolutionary processes.

Materials and Methods

Morphometric measurements, meristic counts and descriptions of *Cyprinodon* scale morphology follow Miller (1948), Humphries and Miller (1981) and Minckley et al. (2002). Fin ray counts were taken from cleared and alizarin-stained specimens. Morphometric measurements were size-corrected by taking the residuals from log-transformed trait values regressed on log-transformed standard length (SL), with one exception: lower jaw length was highly divergent among species (Figure 1B) and size-correction was performed using the regression line estimated from only *Cyprinodon variegatus* values. Canonical variates analysis was performed on these size-corrected morphometric measurements to construct a discriminant morphospace for the three species found on San Salvador (Figure 1A).

All material examined was collected from Crescent Pond, San Salvador Island, Bahamas, by Martin in July 2008 and July 2011. Specimens were euthanized with an overdose of MS-222 (tricaine methanesulfonate) and preserved in 95% ethanol. Material examined is deposited in the ichthyological collections of the Peabody Museum of Natural History, Yale University (YPM), New Haven, Connecticut, USA, the California

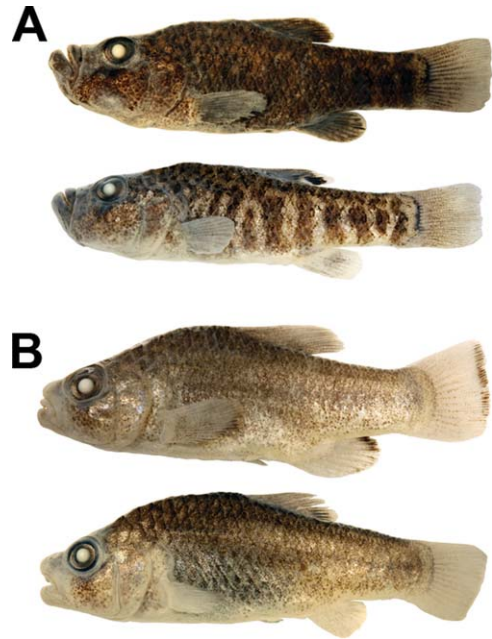


FIGURE 2. Adult male and female specimens of *Cyprinodon desquamator* n. sp. and *Cyprinodon brontotheroides* n. sp. A, *C. desquamator* n. sp., holotype (above), 30.7 mm SL, male; paratype (below), 27.2 mm SL, female. B, *C. brontotheroides* n. sp., holotype (above), 30.8 mm SL, male; paratype (below), 36.1 mm SL, female. Crescent Pond, San Salvador Island, Bahamas.

Academy of Sciences (CAS), San Francisco, California, USA, the University of Michigan Museum of Zoology (UMMZ), Ann Arbor, Michigan, USA, and the American Museum of Natural History (AMNH), New York, New York, USA.

Species Descriptions

Cyprinodon desquamator, new species Scale-eating pupfish Figures 2–5

Cyprinodon sp. “bulldog form”: ontogeny (Holtmeier 2001)

Cyprinodon “bulldog-form”: ecology and ontogeny (Holtmeier 2000)

Cyprinodon “bulldog” morph: population genetics (Turner et al. 2008)

Cyprinodon sp. “scale-eater”: morphological diversification rates (Martin and Wainwright 2011), field measurements of natural selection (Martin and Wainwright 2013a)

Scale-eating pupfish: ecological novelty (Martin and Wainwright 2013b)

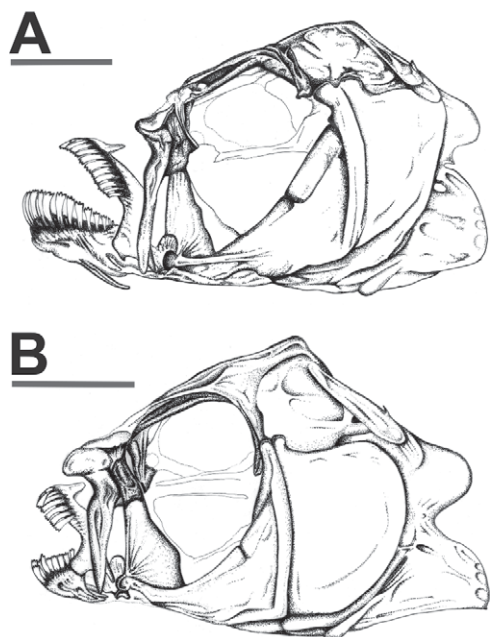


FIGURE 3. Illustrations of craniofacial skeletal anatomy. **A**, *Cyprinodon desquamator* n. sp. **B**, *Cyprinodon brontotheroides* n. sp. Scale bars are 3 mm.

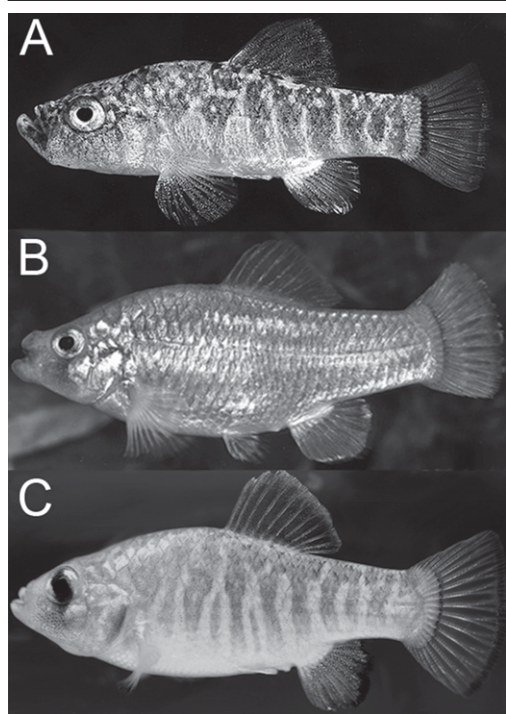


FIGURE 4. Melanin pigmentation patterns. **A**, *Cyprinodon desquamator* n. sp. **B**, *Cyprinodon brontotheroides* n. sp. **C**, *Cyprinodon variegatus*. Photographs in A and B by Tony Terceira.

Holotype. YPM ICH 025880; male, 30.7 mm SL; San Salvador Island, Bahamas: Crescent Pond, 1 km SE of the Gerace Research Centre, lat 24°06'45"N, long 74°27'28"W; collector, Christopher Martin, July 2011 (Figure 2A). **Paratypes.** YPM ICH 025881, CAS 235267, UMMZ 249830, AMNH 258812; all Crescent Pond, San Salvador Island, Bahamas: 23.3 to 30.7 mm SL, taken with the holotype; collector, Christopher Martin, July 2011. Additional cleared and alizarin-stained specimens (CS) taken from Crescent Pond; collector, Christopher Martin, July 2008.

Diagnosis. *Cyprinodon desquamator* shares a most recent common ancestor with the two other *Cyprinodon* species on San Salvador Island. *Cyprinodon desquamator* has large supra-terminal jaws and an elongated body compared to *C. variegatus* and *C. brontotheroides*, which have terminal jaws and a robust body typical for the genus (Figures 1B, 2, 3). *Cyprinodon desquamator* adult males display solid black coloration throughout the entire body and median fins compared to *C. variegatus* and *C. brontotheroides*, which do not display black coloration throughout the body and exhibit a black terminal margin to the caudal fin with otherwise transparent median fins (Figures 4, 5).

Description. Similar in size and form to many other *Cyprinodon* except in those characters associated with its greatly enlarged jaws, more elongated body and black male breeding coloration (Figures 1B, 2–5). Wild-caught specimens reaching up to 30.7 mm SL.

Morphometric data presented in Table 1; meristic scale count data presented in Table 2. With 25 to 28 caudal fin rays (mode 28); 11 to 12 dorsal fin rays (mode 11); 10 to 12 anal fin rays (mode 11); 15 to 16 pectoral fin rays (mode 15); 7 pelvic fin rays. Intestines seem to be shorter than in *Cyprinodon variegatus* and *C. brontotheroides*. Pharyngeal jaw dentition typical for the genus.

Coloration. General body color is silvery tan marked by irregular dark, wide bars along the length of the body in both sexes (Figures 4 and 5). Characteristic of the genus, mature males display a metallic blue anterodorsal region. This species uniquely lacks the typical black terminal margin to the caudal fin (Smith 1990), which is replaced by black pigmentation covering the median fins. Females and juveniles display the typical black and white ocellus on the dorsal fin. Breeding males display a distinctive coloration of slate gray to jet black pigmentation throughout body and fins, broken only by a speckling of metallic blue in the anterodorsal region. Unlike most congeners, breeding males do not display any orange ventral coloration that is replaced by gray or black pigmentation.

Distribution and habitat. *Cyprinodon desquamator* is restricted to six hypersaline lakes on San Salvador Island, Bahamas: Crescent Pond, Osprey Lake, Oyster Lake, Little Lake, Mermaid Pond and Great Lake. In all six lakes the species is sympatric with *C. variegatus* and in four of the six (excepting Great Lake and Mermaid Pond) it is sympatric with *C. brontotheroides*. Visual surveys and seine-net surveys were conducted in July 2008, March and July 2011, and July 2013 on San Salvador and in July 2011 on neighboring islands. *Cyprinodon desquamator* was absent from all neighboring Bahamian islands surveyed, including Rum Cay, Cat Island, Acklins Island, Crooked Island,

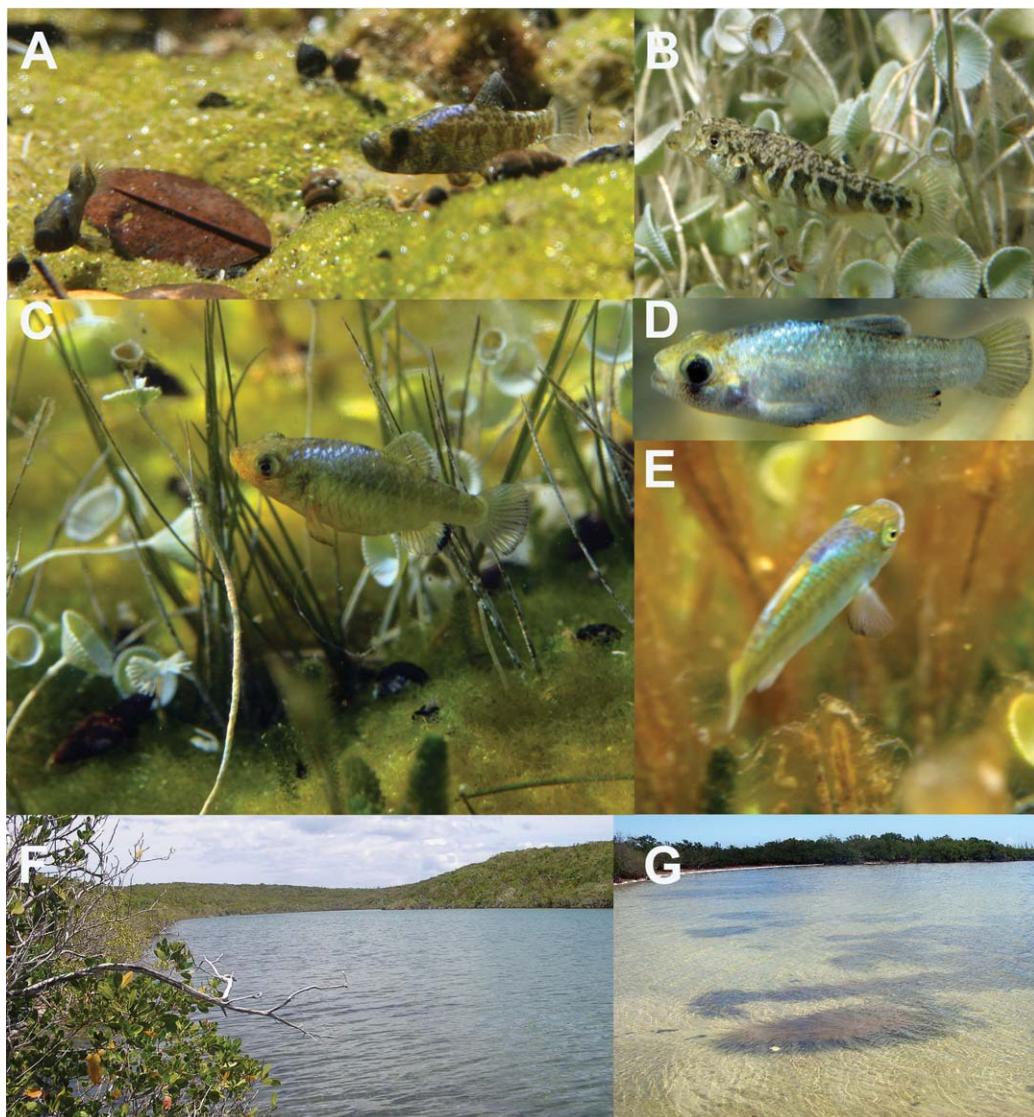


FIGURE 5. *Cyprinodon* spp. photographed in their natural environment in Crescent Pond, San Salvador Island, Bahamas. A, *C. desquamator* n. sp. males. B, *C. desquamator* n. sp. female. C, *C. variegatus*, male. D, E. *C. bronototheroides* n. sp. males. F, Type locality: Crescent Pond. G, Little Lake. Note differences in banding patterns and male sexual coloration.

Long Island and New Providence Island. On San Salvador Island, *C. desquamator* was absent from Reckley Field Pond, Wild Dilly Pond, Moon Rock Pond, Pain Pond, Six Pack Pond, Clear Pond and four blue holes near Sandy Point.

Cyprinodon desquamator comprises approximately 1% of *Cyprinodon* spp. in hypersaline lakes where it coexists with *C. variegatus* and *C. desquamator* in all habitats (Martin and Wainwright 2013a). All lakes are shallow, rarely exceeding 3 m depth, and *Cyprinodon* densities are greatest in mats of macroalgae (*Batophora*, *Acetabularia* and *Cladophora*) and wigeongrass (*Ruppia maritima*) along the littoral margins of each lake. *Cyprinodon desquamator* seems to be uniformly spaced across all

habitats within the lakes. Males guard breeding territories from 0.05 to 1 m depth. Breeding has been observed in spring and summer seasons, but seems to be more common in the summer.

Stomach content analyses of *Cyprinodon desquamator* in Crescent Pond and Little Lake indicate that it is a scale-eating specialist (Martin and Wainwright 2013b). In Crescent Pond and Little Lake, approximately 50% of food items were the scales of other fishes (Martin and Wainwright 2011, 2013b).

Etymology. The specific name, a noun in apposition, is from the Latin verb *squamare*, for one who removes scales from fishes.

TABLE 1. Morphometric traits measured for *Cyprinodon desquamator* n. sp. ($n = 16$), *Cyprinodon brontotheroides* n. sp. ($n = 15$) and *Cyprinodon variegatus* ($n = 17$) collected from Crescent Pond, San Salvador Island, Bahamas. Data is presented as a proportion of standard length (SL), which is in millimeters.

<i>Cyprinodon desquamator</i> n. sp.	Holotype	Range	Males (mean \pm SE)	Females (mean \pm SE)
Standard length (mm)	30.7	23.3–30.7	25.3 \pm .8	26.6 \pm 3.9
Predorsal length	.505	.497–.558	.519 \pm .005	.537 \pm .010
Prepelvic length	.545	.537–.578	.559 \pm .006	.561 \pm .005
Head length	.335	.319–.356	.337 \pm .004	.336 \pm .004
Body width	.208	.187–.217	.197 \pm .003	.208 \pm .003
Body depth	.314	.265–.314	.290 \pm .004	.279 \pm .004
Interorbital width	.090	.076–.112	.084 \pm .002	.091 \pm .004
Snout length	.088	.076–.099	.089 \pm .003	.091 \pm .002
Orbital length	.096	.096–.117	.109 \pm .002	.106 \pm .003
Mouth width	.117	.099–.127	.113 \pm .002	.110 \pm .003
Lower jaw length	.163	.158–.186	.169 \pm .002	.171 \pm .003
Caudal peduncle depth	.158	.135–.161	.149 \pm .002	.145 \pm .002
Caudal peduncle length	.272	.230–.275	.259 \pm .005	.251 \pm .004
Dorsal fin length	.245	.219–.255	.241 \pm .003	.238 \pm .004
Anal fin length	.211	.182–.250	.236 \pm .004	.209 \pm .005
Caudal fin length	.197	.185–.226	.206 \pm .005	.210 \pm .005
Pectoral fin length	.211	.187–.213	.200 \pm .004	.200 \pm .003
Pelvic fin length	.085	.076–.097	.082 \pm .001	.088 \pm .003
<i>Cyprinodon brontotheroides</i> n. sp.	Holotype	Range	Males (mean \pm SE)	Females (mean \pm SE)
Standard length (mm)	30.8	25.6–36.1	31.4 \pm 5.6	29.3 \pm 2.0
Predorsal length	.550	.531–.584	.554 \pm .005	.560 \pm .006
Prepelvic length	.525	.525–.571	.545 \pm .004	.553 \pm .005
Head length	.304	.286–.306	.298 \pm .002	.297 \pm .004
Body width	.180	.177–.204	.187 \pm .002	.190 \pm .004
Body depth	.343	.295–.355	.341 \pm .003	.316 \pm .006
Interorbital width	.096	.090–.113	.099 \pm .002	.096 \pm .003
Snout length	.082	.059–.094	.073 \pm .002	.072 \pm .006
Orbital length	.108	.097–.113	.106 \pm .001	.106 \pm .003
Mouth width	.099	.081–.106	.097 \pm .002	.092 \pm .003
Lower jaw length	.089	.069–.095	.086 \pm .002	.076 \pm .002
Caudal peduncle depth	.160	.144–.174	.164 \pm .002	.150 \pm .001
Caudal peduncle length	.246	.217–.278	.251 \pm .005	.230 \pm .004
Dorsal fin length	.268	.229–.280	.268 \pm .003	.246 \pm .005
Anal fin length	.245	.164–.245	.226 \pm .004	.189 \pm .007
Caudal fin length	.161	.141–.175	.161 \pm .004	.160 \pm .004
Pectoral fin length	.213	.198–.220	.209 \pm .002	.205 \pm .003
Pelvic fin length	.087	.070–.096	.085 \pm .002	.075 \pm .002
<i>Cyprinodon variegatus</i>	Range	Males (mean \pm SE)	Females (mean \pm SE)	
Standard length (mm)	20.7–37.1	26.8 \pm 2.1	24.7 \pm 1.5	
Predorsal length	.525–.594	.570 \pm .009	.577 \pm .004	
Prepelvic length	.563–.625	.588 \pm .005	.595 \pm .006	
Head length	.288–.342	.315 \pm .006	.324 \pm .005	
Body width	.199–.231	.217 \pm .003	.212 \pm .003	
Body depth	.286–.364	.342 \pm .006	.318 \pm .005	

Continued

TABLE 1 CONTINUED.

<i>Cyprinodon variegatus</i>	Range	Males (mean ± SE)	Females (mean ± SE)
Interorbital width	.074–.103	.087 ± .002	.088 ± .003
Snout length	.073–.098	.085 ± .002	.087 ± .002
Orbital length	.099–.134	.117 ± .004	.122 ± .002
Mouth width	.078–.122	.099 ± .005	.099 ± .004
Lower jaw length	.101–.118	.110 ± .002	.108 ± .002
Caudal peduncle depth	.133–.172	.155 ± .004	.148 ± .003
Caudal peduncle length	.204–.255	.228 ± .007	.222 ± .004
Dorsal fin length	.198–.266	.244 ± .006	.222 ± .005
Anal fin length	.161–.262	.225 ± .009	.188 ± .005
Caudal fin length	.177–.221	.194 ± .007	.194 ± .004
Pectoral fin length	.189–.226	.208 ± .001	.208 ± .003
Pelvic fin length	.074–.098	.085 ± .003	.081 ± .002

Cyprinodon brontotheroides, new species
Durophage pupfish
Figures 2–5

Cyprinodon “bozo-form”: ecology and ontogeny (Holtmeier 2000)

Cyprinodon “bozo” morph: population genetics (Turner et al. 2008)

Cyprinodon sp. “durophage”: morphological diversification rates (Martin and Wainwright 2011), field measurements of natural selection (Martin and Wainwright 2013a), stomach content analyses (Martin and Wainwright 2013b).

Holotype. YPM ICH 025878; male, 30.7 mm SL; San Salvador Island, Bahamas: Crescent Pond, 1 km SE of the Gerace Research Centre, lat 24°06'45"N, long 74°27'28"W; collector, Christopher Martin, July 2011 (Figure 2B). **Paratypes.** YPM ICH 025879, CAS 235268, UMMZ 249831, AMNH 258813; all San Salvador Island, Bahamas: 25.6 to 36.1 mm SL, taken with the holotype; collector, Christopher Martin, July 2011. Additional cleared and alizarin-stained specimens (CS) taken from Crescent Pond; collector, Christopher Martin, July 2008.

Diagnosis. *Cyprinodon brontotheroides* shares a most recent common ancestor with the two other *Cyprinodon* species on San Salvador Island. The anterior process of the maxillary head in *C. brontotheroides* extends up to or beyond the most anterior point on the dentigerous arm of the premaxilla with oral jaws retracted compared to *C. variegatus* and *C. desquamator*, in which the most anterior point on the maxillary head is always posterior to the most anterior point on the dentigerous arm of the premaxilla (Figure 3). Externally, this skeletal extension in *C. brontotheroides* presents as a fleshy protuberance nearly encasing the upper jaw when retracted, whereas *C. variegatus* and *C. desquamator* lack any fleshy protuberance surrounding the maxillary head (Figures 2, 3). The paired nasal bones in *C. brontotheroides* are not enlarged, but form a more acute angle (approximately 120°) with the dorsal margin of the neurocranium than *C. variegatus* and *C. desquamator* (approximately 180°; Figure 3). *C. brontotheroides* also has either pale or complete absence of banding along the

body in both sexes and shortened lower jaws relative to *C. variegatus* and *C. desquamator*, which always show dark banding along the body and either typical jaw length for the genus or enlarged jaws, respectively (Figures 1B, 4, 5).

Description. Similar in size and form to many other *Cyprinodon*, except in the dorsal characters of the maxilla, nasal protuberance, deeper caudal peduncle and shorter oral jaws (Figures 2–5). Wild-caught specimens reaching up to 36.5 mm SL. Morphometric data presented in Table 1; meristic scale count data presented in Table 2. With 25 to 28 caudal fin rays (mode 27); 11 to 12 dorsal fin rays (mode 11); 11 to 12 anal fin rays (mode 11); 15 to 18 pectoral fin rays (mode 16); 6 to 7 pelvic fin rays (mode 7). Intestines seem to be shorter than in *C. variegatus*. Pharyngeal jaw dentition typical for the genus.

Coloration. General body color silvery tan with pale gray vertical banding or complete absence of banding in both sexes (Figures 4, 5). Characteristic of the genus, mature males display a metallic blue anterodorsal region and black terminal margin on the caudal fin and females and juveniles display a black and white ocellus on the dorsal fin. Median fins are otherwise transparent without interior pigmentation. Adult males display speckling of metallic blue in anterodorsal region without darkened pigmentation on body or fins as in congeners. Orange ventral coloration is sometimes present in breeding males (Figure 5).

Distribution and habitat. *Cyprinodon brontotheroides* is restricted to six hypersaline lakes on San Salvador Island, Bahamas: Crescent Pond, Osprey Lake, Oyster Lake, Little Lake, Wild Dilly Pond and Moon Rock Pond. In all six lakes, the species is sympatric with *C. variegatus* and in four of the six (excepting Wild Dilly Pond and Moon Rock Pond) it is sympatric with *C. desquamator*. Visual surveys and seine-net surveys were conducted in July 2008, March and July 2011, and July 2013 on San Salvador Island and in July 2011 on neighboring islands. *Cyprinodon brontotheroides* was absent from all neighboring Bahamian islands surveyed, including Rum Cay, Cat Island, Acklins Island, Crooked Island, Long Island and New Providence Island. On San Salvador Island, *C. brontotheroides* was absent from Reckley Field Pond, Great Lake, Pain Pond,

TABLE 2. Selected meristic features for *Cyprinodon desquamator* n. sp., *Cyprinodon brontotheroides* n. sp. and *Cyprinodon variegatus* collected from Crescent Pond, San Salvador Island, Bahamas.

<i>Cyprinodon desquamator</i>	Holotype	Range	<i>n</i>	Mean ± SE
Lateral line scales	25	23–29	33	25.2 ± .19
Dorsal to pelvic scales	13	9–14	33	10.5 ± .17
Circular body scales	26	23–27	33	24.8 ± .16
Circular. peduncle scales	16	12–19	33	15.1 ± .29
<i>Cyprinodon brontotheroides</i>	Holotype	Range	<i>n</i>	Mean ± SE
Lateral line scales	27	24–27	30	25.9 ± .13
Dorsal to pelvic scales	11	10–11	30	9.6 ± .16
Circular body scales	24	22–27	30	23.9 ± .17
Circular. peduncle scales	16	13–16	30	14.1 ± .15
<i>Cyprinodon variegatus</i>		Range	<i>n</i>	Mean ± SE
Lateral line scales		24–27	28	25.3 ± .15
Dorsal to pelvic scales		9–11	28	10.5 ± .13
Circular body scales		21–25	28	23.8 ± .19
Circular. peduncle scales		14–17	28	15.1 ± .19

Mermaid's Pond, Six Pack Pond, Clear Pond and four blue holes near Sandy Point.

Cyprinodon brontotheroides comprises approximately 5% of *Cyprinodon* spp. in hypersaline lakes where it coexists with *C. variegatus* and *C. desquamator* in all habitats (Martin and Wainwright 2013a). *Cyprinodon brontotheroides* was sometimes common within wigeongrass patches (*Ruppia maritima*) along the shoreline of Crescent Pond, but displayed no specific habitat affinities in other lakes beyond the typical littoral habitat preferences of the genus. Males guard breeding territories in midwater from 0.5 to 1 m depth. Breeding has been observed in spring and summer seasons, but may be more common in the spring (C.H. Martin, pers. obs.).

Stomach content analyses of *Cyprinodon brontotheroides* in Crescent Pond and Little Lake indicate that it is a hard-shelled prey specialist, a durophage (Martin and Wainwright 2013b). In Crescent Pond, approximately 30% of food items were composed of a single species of ostracod. In Little Lake, *C. brontotheroides* consumed far more gastropods than other *Cyprinodon* species, comprising 22% of food items (Martin and Wainwright 2013b).

Etymology. The adjective *brontotheroides* denotes the resemblance of the unique protruding nasal region in this species to the bizarre horn-like skull appendages of the extinct odd-toed ungulate family Brontotheriidae.

Discussion

Three distinct species with unique trophic morphology, banding patterns and sexual coloration

coexist within the saline lakes of San Salvador Island (Figures 1–5). Species status is further supported by population genetic analyses of microsatellites (Turner et al. 2008), mitochondrial haplotypes (Bunt 2001) and genotyping by sequencing analyses of single nucleotide polymorphisms (C.H. Martin, unpublished data). For example, Turner et al. (2008) found significant F_{st} divergence between *Cyprinodon desquamator* and *Cyprinodon variegatus* in three different lakes (Crescent Pond: $F_{st} = 0.310$; Little Lake: $F_{st} = 0.104$; and Osprey Lake: $F_{st} = 0.141$). They also found appreciable genetic divergence between *C. variegatus* and *C. brontotheroides* ($F_{st} = 0.041$ – 0.103), but their analyses were limited by a small pooled sample size ($n = 14$).

We have an emerging understanding of which isolating barriers contribute to reproductive isolation among these three species. First, there do not seem to be any major differences in habitat affinities or breeding sites among the three species. All three species have been observed spawning in March and July (C.H. Martin, pers. obs.); however, limited temporal isolation due to partially asynchronous breeding seasons cannot yet be ruled out. Second, we have

observed some pre-mating isolation among species during field observations and laboratory trials (C.H. Martin, unpublished data). Third, measurements of hybrid survival and growth in field enclosures on San Salvador provide strong evidence of postzygotic extrinsic reproductive isolation among all three species (Martin and Wainwright 2013a). Hybrids resembling *Cyprinodon desquamator* suffered lower fitness than hybrids intermediate between *C. variegatus* and *C. brontotheroides* (Martin and Wainwright 2013a). Thus, reproductive isolation among San Salvador's sympatric *Cyprinodon* species seems to be maintained by a combination of pre-mating sexual isolation and ecological selection against hybrids, with stronger barriers to gene flow for *C. desquamator* than *C. brontotheroides*. This is consistent with the patterns of genetic differentiation observed among the three species (Turner et al. 2008).

This species flock of *Cyprinodon* pupfishes endemic to San Salvador Island provides a powerful case study for understanding remarkably localized adaptive radiation and the evolution of ecological novelty. For example, this clade is diverging up to 51 times faster than allopatric populations of *Cyprinodon* for certain trophic traits such as jaw length and the evolution of novel ecological niches, such as scale-eating, seem to be driving these exceptional rates of morphological diversification (Martin and Wainwright 2011). To place the novelty of this niche in context, consider that scale-eating pupfish are separated by 168 million years of evolution from the most closely related species with convergent scale-eating ecology, a clade of African cichlids (Martin and Wainwright 2013b). However, there are scales in thousands of saline lakes across the Caribbean with identical depauperate fish faunas (i.e., only *C. variegatus* and *Gambusia* spp.), so why do we not see scale-eating pupfish evolving everywhere? Field measurements of the adaptive landscape for pupfishes on San Salvador provide an unexpected answer to this question: *Cyprinodon variegatus* seems to be stranded on an isolated fitness peak that may constrain their phenotypic diversification. If this is also the case in the many allopatric populations of *C. variegatus* across the Caribbean, this topography of the adaptive landscape could explain the rarity of *Cyprinodon* species flocks.

Finally, given the rarity of such remarkable species flocks ($n = 2$), it is important to safeguard their conservation. The Laguna Chichancanab species flock is mostly extinct in the wild because of invasive species introductions (Schmitter-Soto and Caro 1997; Strecker 2006b; Martin and Wainwright 2011). One of these invasive species, *Oreochromis* spp., has already been introduced to at least two blues holes on San Salvador Island, apparently for recreational fishing (C.H. Martin, pers. obs). It is imperative that this invader be removed before it reaches the salt lakes and again extinguishes a remarkable *Cyprinodon* species flock.

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