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ABSTRACT

The Rio Grande Cichlid, *Herichthys cyanoguttatus*, is native to the drainages of the Gulf Coast of northern Mexico and southern Texas and has been introduced at several sites in the US. Previous observations have suggested that non-native populations in Louisiana that are currently recognized as *H. cyanoguttatus* resemble another species, the Lowland Cichlid, *H. carpintis*. Traditional morphological and genetic techniques have been insufficient to differentiate these species, but *H. carpintis* has been reported to differ from *H. cyanoguttatus* in color pattern, so we turned to novel electronic photo archives to determine the identity of the species introduced in Louisiana. First, we used the public databases *Nonindigenous Aquatic Species Database* and *Fishes of Texas* to infer the historical distributions of these species in the US. We then used museum specimens, live specimens, and two additional databases, *The Cichlid Room Companion* and *iNaturalist*, to compare morphology and color patterns among individuals obtained from their native and introduced ranges in Mexico, Texas, and Louisiana. Our general observations found that *H.* cf. *cyanoguttatus* from Louisiana tended to have an obliquely oriented mouth and a more rounded ventral profile than *H. cyanoguttatus* from Texas, consistent with previous descriptions of *H. carpintis*, but our morphological analyses were unable to identify any significant differences among populations. Our analyses of color patterns found that *H.* cf. *cyanoguttatus* from Louisiana had larger iridescent spots than *H. cyanoguttatus* from Texas as well as black breeding coloration that extended anteriorly to the tip of the mouth, characters consistent with *H. carpintis*. Our observations indicate that at least some of the cichlids introduced in Louisiana are not *H. cyanoguttatus* but are instead *H. carpintis*, and that their presence there is likely due to release by humans. This is the first record of *H. carpintis* establishing a population in the US. Understanding the biology of not one, but two, species of *Herichthys* will be necessary to predict and mitigate their continued colonization of new environments in the US.

Keywords: exotic species, *Herichthys carpintis*, *Herichthys cyanoguttatus*, introduced species, invasive species, Lowland Cichlid, nonindigenous species, Rio Grande Cichlid, Texas Cichlid

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INTRODUCTION

Cichlid fishes are commonly introduced and have successfully established populations in the southern United States (Page and Burr, 2011). They have been released unintentionally and intentionally for a variety of reasons including release by individuals, states, and private companies as unwanted pets, as bait bucket releases, as forage for warm water predatory fishes, as sport fishes, as a human food source, as fish farm escapees, and as a means of aquatic plant control (e.g., Courtenay et al., 1974). *Herichthys cyanoguttatus*, the Rio Grande Cichlid, known as the Texas Cichlid in the pet trade, is the only cichlid species native to the United States. It has a native range in Gulf of Mexico drainages from northeastern Mexico to southern Texas (Miller et al., 2005), and it has been introduced well beyond its native range in the US (Nico et al., 2019). Recent reports of new populations in Houston and New Orleans are consistent with a pattern of natural colonization north- and east-ward along the Gulf Coast, a pattern recently identified in other fish species (Martin et al., 2012), but it is not known if this is the reason for these new populations or if they are the results of additional accidental or intentional releases by humans.

Interestingly, Lorenz (2008) noted that the color patterns of some specimens of *H.* cf. *cyanoguttatus* captured in Louisiana resembled that of the Lowland Cichlid, *H. carpintis*, a closely related species that is also present in the pet trade, although not as frequently as *H. cyanoguttatus*. Live *H. carpintis* have long been recognized in the aquarium literature to have larger, bluish-green, pearl-like spots on the body and fins compared to the much smaller spots in *H. cyanoguttatus* (Loiselle, 1982; Staeck and Lincke, 1985). Furthermore, whereas *H. cyanoguttatus* have a gray-tan background color, the background color of *H. carpintis* is often darker: blackish to grayish green (Staeck and Lincke, 1985). Finally, the two species have also been reported to differ in the color patterns they exhibit during breeding. Artigas Azas (2013) reported that *H. carpintis* develop black coloration from the base of the caudal fin to the lower lip, whereas *H. cyanoguttatus* do not develop the black area in the lower part of the head. The taxonomic identity of *H.* cf. *cyanoguttatus* in Louisiana has important implications for understanding the potential for further colonization of cichlids throughout the Gulf Coast region. Miller et al. (2005) stated that, in their native ranges, *H. carpintis* have a tendency to inhabit more brackish, coastal waters than do *H. cyanoguttatus* (although *H. carpintis* also occur at inland sites, see Jordan and Snyder, 1899). If the populations in Texas and Louisiana are indeed two different species, then they may also differ in their potential to colonize additional environments along the Gulf Coast. They may also differ in their effects on native species. Studies of *H.* cf. *cyanoguttatus* in Louisiana have revealed exponential population growth and aggressive behavior toward Bluegill sunfish (*Lepomis macrochirus*) (O'Connell et al., 2002; Lorenz et al., 2011).

Here, we examined *H.* cf. *cyanoguttatus* captured from Louisiana to ascertain their taxonomic identity. We hypothesized that the identity of at least some specimens of *H.* cf. *cyanoguttatus* in Louisiana is *H. carpintis* and not *H. cyanoguttatus*. First, we used the *Nonindigenous Aquatic Species Database* and the *Fishes of Texas* database to infer the history of dispersal of these species in the US. Next, to test our hypothesis, we used museum specimens to compare traditional morphometrics and meristics among individuals obtained from their native and introduced ranges in Mexico, Texas, and Louisiana. Previous studies have had trouble resolving these species using traditional morphological techniques, and genetic analyses using DNA sequences of the mitochondrial gene COI have had similar problems (Mejía et al., 2015; De la Maza-Benignos et al., 2015b; but see Pérez-Miranda et al., 2018). Public electronic resources have recently been found useful in studies that range from mapping species to analyzing behavior (Mori et al., 2017; Jagiello et al., 2019; Marshall and Strine 2019), so we turned to the electronic photo archives *The Cichlid Room Companion* and *iNaturalist* to resolve the identity of the species introduced in Louisiana. We interpreted color patterns of live specimens from their introduced ranges in Texas and Louisiana in the context of archival illustrations and photographs of individuals in their native ranges in Mexico and Texas.

METHODS

Research was performed under IACUC ID# 13-05-20-1003-3-01 at Sam Houston State University and IACUC ID# 2013-0161 at Case Western Reserve University.

Species

The genus *Herichthys* comprises a monophyletic group endemic to northeastern Mexico and southern Texas (Miller et al., 2005; Hulsey et al., 2010; De la Maza-Benignos and Lozano-Vilano, 2013; De la Maza-Benignos et al., 2015a; McMahan et al., 2015; Pérez-Miranda et al. 2018). *Herichthys cyanoguttatus* was described from specimens collected in Brownsville, Texas, and it is native to the Rio Grande drainage and the Rio San Fernando drainage in Mexico (Baird and Girard, 1854). The *Fishes of Texas* database returned seven museum records from as early as 1851 and 1855 in the Nueces/Rio Grande coastal drainages, including the Laguna Atascosa drainage, just north of the Rio Grande proper (Hendrickson and Cohen, 2019), indicating that *H. cyanoguttatus* is also native to those small drainages. Further south, *H. carpintis* is native to the Rio Pánuco drainage in Mexico and a couple of small, adjacent coastal drainages (de la Maza-Benignos et al., 2015a). The type locality of *H. carpintis* is the estuarine Laguna del Carpintero, near Tampico, Tamaulipas, Mexico (Jordan and Snyder, 1899).

Brown (1953) described how the US Fish and Wildlife station in San Marcos, Texas obtained brood stock of *H. cyanoguttatus* from Mission, Texas in 1928 and then distributed their offspring until at least 1941, resulting in several introduced populations in Texas. They were introduced as early as 1928 in the spring fed streams of the Guadalupe River basin (Brown, 1953). The *Fishes of Texas* database revealed that they subsequently spread to adjacent basins further north and east, being first collected in the Colorado basin as early as 1947 and the Brazos basin as early as 1967 (Hendrickson and Cohen, 2019). *Herichthys cyanoguttatus* then expanded its range northeast to the Houston area in Harris County, Texas, no later than 1988 (Hendrickson and Cohen, 2019), and it was formally recognized as established in Sims Bayou and Brays Bayou of the San Jacinto River Basin by 1998 (Martin, 2000). Even further eastward, it was reported to be established in the urban canals and natural waterways in the Greater New Orleans Metropolitan Area, including Lake Pontchartrain, after being first captured there in 1996 (Fuentes and Cashner, 2002; Lorenz and O'Connell, 2011). In Florida it is thought to have established as early as the 1940's as a result of introductions from the pet trade (e.g., Courtenay et al., 1974; Conkel, 1993). According to the *Nonindigenous Aquatic Species Database,* it has also been found at other non-native sites in the US, but in most cases it has not successfully established populations, presumably due to cold temperatures (Nico et al., 2019).

Hubbs et al. (1978) reported *H. cyanoguttatus* to be native even further north of the Laguna Atascosa drainage, in the Nueces River, citing Brown (1953) as evidence, and this idea has been repeated extensively (e.g., Nico et al., 2019). However, Brown (1953) provided no evidence that *H. cyanoguttatus* was native in the Nueces River, and other authors have listed it as introduced there (e.g., Conner and Suttkus, 1986). Two surviving authors of Hubbs et al. (1978) informed us that listing *H. cyanoguttatus* as native in the Nueces River was based on the personal opinion of Hubbs (Gary Garrett, 2018, pers. comm.; Robert Edwards, 2018, pers. comm.). The earliest records of *H. cyanoguttatus* in the Nueces River are only as early as 1936 (Hendrickson and Cohen, 2019), despite the sampling that was performed there by

Evermann and Kendall (1894) before the intentional introductions that began in 1928 (Brown 1953). It is possible that *H. cyanoguttatus* was missed in those surveys conducted before 1936; in a more recent and extensive survey of the Nueces River, Kihn Pineda (1975) found more than 40 fish species that had not been reported previously. Nevertheless, in the absence of any evidence that it is native there, we consider it most likely to be introduced.

The *Nonindigenous Aquatic Species Database* has only one record of *H. carpintis* living in the wild in the US. This introduction occurred in Florida, but it did not result in an established population (Neilson, 2018).

According to the original descriptions of *H. cyanoguttatus* (Baird and Girard, 1854) and *H. carpintis* (Jordan and Snyder, 1899), there are no meristic differences between the two species. However, Jordan and Snyder (1899) provided a description of qualitative body shape characters, accompanied by an illustration, for *H. carpintis* that distinguish it from *H. cyanoguttatus* (see Figs. 1A-D), including a ventral profile that is evenly curved from snout to caudal peduncle (compared to a relatively flat ventral profile, especially from mandible to pelvic fin insertion, in *H. cyanoguttatus*), and an oblique mouth with a slightly projecting lower jaw (compared to the equal jaws described for *H. cyanoguttatus* by Baird and Girard, 1854). Miller et al. (2005) also noted the rounded ventral profile, but in addition found a deeper (≥50% SL) body and a smaller upper jaw (dentigerous arm of premaxilla 20-30% head length) in *H. carpintis* than in *H. cyanoguttatus* (body depth ≤50% SL; dentigerous arm of premaxilla 27-40% HL).

One recent taxonomic assessment of *Herichthys* was conducted by De la Maza-Benignos et al. (2015a). These authors recognized the validity of one additional species of *Herichthys* that has a spotting pattern similar to that of *H. cyanoguttatus* and *H. carpintis* and which might easily be confused with *H. carpintis*: *H. teporatus* (Fowler, 1903). De la Maza-Benignos et al. (2015a) described *H. teporatus* as intermediate between *H. cyanoguttatus* and *H. carpintis* in both geography and color pattern, being found in the Rio Soto la Marina, which is located south of the Rio San Fernando but north of the Río Pánuco. They recognized *H. cyanoguttatus* as differing from all other species of *Herichthys* by possessing, in living specimens, small (<1.0 mm) iridescent dots covering the flanks. They reported that *H. carpintis* differs from *H. cyanoguttatus* by having much larger (>1.5 mm) spots, and that *H. teporatus* has intermediate-size (1–1.5 mm) spots. However, *H. teporatus* is not present in the pet trade to our knowledge, and thus was not likely released in Louisiana. It is also important to note that some authors have considered *H. teporatus* a synonym of *H. carpintis* (e.g., Pérez-Miranda et al. 2018). One species of *Herichthys* that De la Maza-Benignos et al. (2015a) did not discuss in detail was *H. minckleyi*, which also has small spots and might be confused with *H. cyanoguttatus*, but this species is endemic to a small, isolated desert valley and is also not common in the pet trade (Oldfield et al., 2015). Other similar species have distinctly different color patterns and are not easily confused with *H. cyanoguttatus* or *H. carpintis*.

De la Maza-Benignos et al. (2015a) found meristics to be indistinguishable between *H. carpintis*, *H. cyanoguttatus*, and *H. teporatus*, and morphometric proportions to be indistinguishable between *H. teporatus* and *H. cyanoguttatus*. They described *H. carpintis* as differing from *H. teporatus* and *H. cyanoguttatus* by having a longer head, longer distance from the rostral tip to the pectoral fin origin, shorter snout, and larger eyes. They stated that *H. cyanoguttatus* differs from *H. carpintis* and *H. teporatus* in that it does not develop prominent nuchal humps. However, Buchanan (1971) observed nuchal humps in 9 of 314 (2.9%) male *H. cyanoguttatus*, and nuchal humps in this species can be seen in photos archived on *The Cichlid Room Companion* (http://www.cichlidae.com/species.php?id=207 viewed 25-June-2016).

Finally, De la Maza-Benignos et al. (2015a) described one additional lineage that might be confused with *H. carpintis*. Some specimens from the San Fernando River lineage (the drainage south

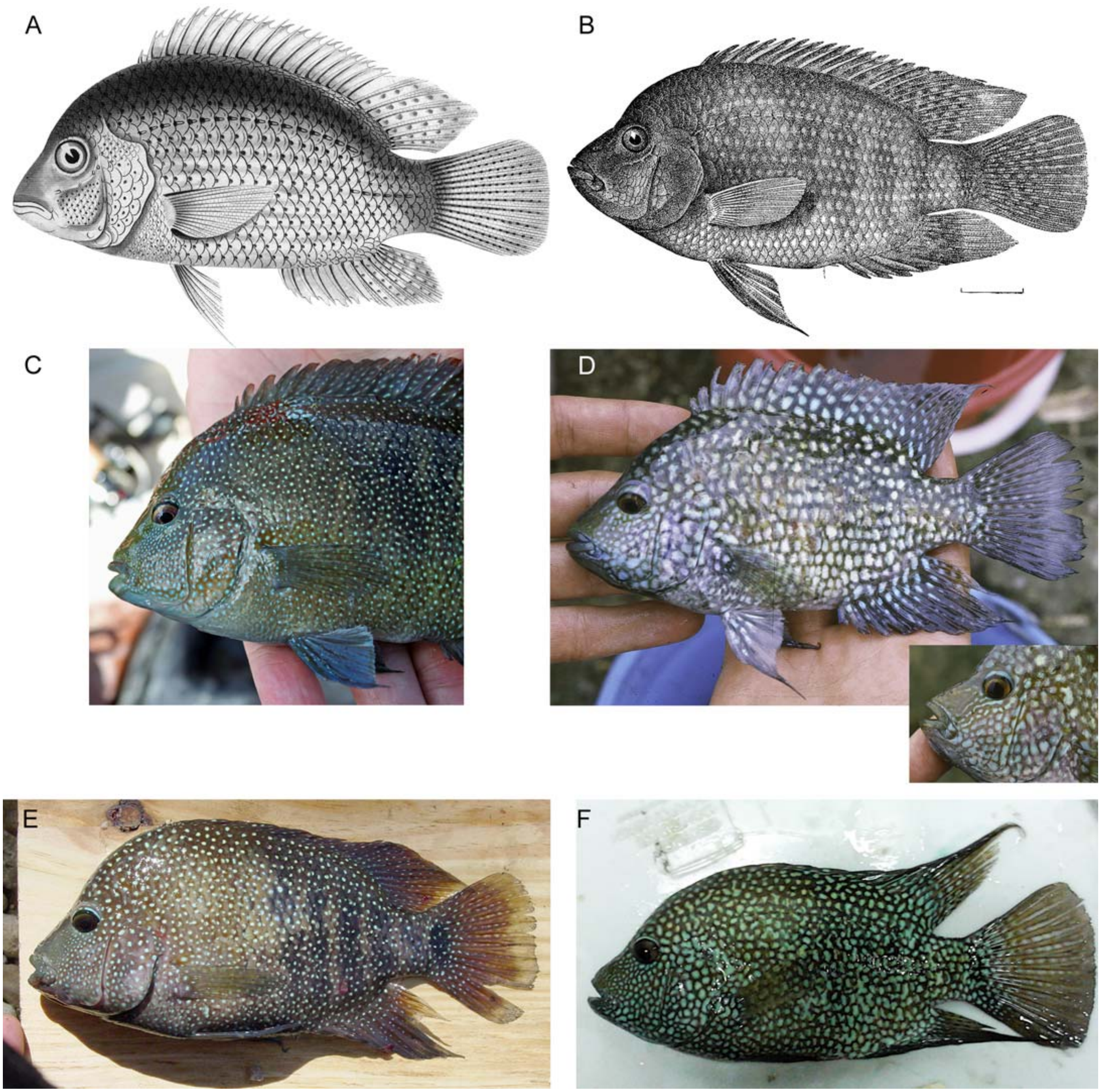

Figure 1.—*Herichthys* spp. from native and introduced ranges. A, *H. cyanoguttatus* illustration by Girard (1859). B, Illustration from the original description of *H. carpintis* (Jordan and Snyder, 1899). C, *H. cyanoguttatus* captured in its native range, in Devil's River, Texas. Photo by Clint Taylor - www.texaskayakfisher.com. D, *H. carpintis* captured near its type locality, in Altamira Lagoon, Pánuco (Mexico). Main photo shows large iridescent spots. Inset exemplifies oblique mouth and projecting lower jaw. Photos by Juan Miguel Artigas Azas. (Photos are of right side. Flipped for comparison to other photos.) E, *H. cyanoguttatus* captured in introduced range in Texas (Shoal Creek in Austin, Texas). F, *H. carpintis* captured in Louisiana, previously identified as *H. cyanoguttatus*. Note round ventral profile, oblique mouth, projecting lower jaw, and large iridescent spots. The dark vertical bars on the posterior flanks of some individuals indicate breeding status and are not taxonomically relevant at the species level.

of Rio Grande but north of Rio Soto la Marina) have distinct, larger (0.5–1.0 mm) blue dots that are aligned in horizontal lines over the flanks. De la Maza-Benignos et al. (2015a) considered these to be a morphological variant of *H. cyanoguttatus* and to our knowledge they are not present in the pet trade and are not likely present in Texas or Louisiana (see also De La Maza-Benignos, 2005a, 2005b).

The most recent taxonomic assessment of *Herichthys* was performed by Pérez-Miranda et al. (2018). As in previous studies, they reported no significant differences in meristics between *H. carpintis* and *H. cyanoguttatus*. Morphometrically, they found *H. carpintis* had a smaller mean interocular distance (35% versus 38%) and a shallower body (46% versus 47%), but they acknowledged that these measures were highly variable. They also found a shorter snout in *H. carpintis* as well as some differences in the lengths of some of the fins, but they did not elaborate on those characters. They also acknowledged the larger iridescent spots in *H. carpintis* and noted the differences in breeding color pattern that had been described previously by Artigas Azas (2013). In both species, when breeding, both males and females change from exhibiting a uniform background to exhibiting the posterior portion of the body as black bars or solid black and the anterior portion of the body white. The pattern is often exhibited more intensely in females than in males. Pérez-Miranda et al. (2018) reported that *H. cyanoguttatus* exhibits a completely white head and "ventral" fins, but that in *H. carpintis* the black pigmentation extends from the posterior darkened region anteriorly along the ventral region of the body all the way to the tip of the mouth (see Artigas Azas, 2013; Říčan et al., 2016 for additional detail on this character). They stated that in *H. carpintis* this dark pigmentation on the head includes the upper lip, and extends dorsally to the suborbital series (i.e., almost to the eye). Neither Pérez-Miranda et al. (2018) nor Říčan et al. (2016) provided an indication of the prevalence or variability of this character.

Morphometrics and meristics

Adult *H.* cf. *cyanoguttatus* were caught in Louisiana using hook and line on 26-March-2012 at City Park, Orleans Parish, New Orleans (Louisiana Freshwater Science Collecting Permit #64, R.S. 56:316) and held in freshwater in an outdoor artificial stream (1.6 m wide × 4.0 m long × 0.8 m deep) filled with well water until 16-May-2013. Additional specimens were caught in the canal along Canal Street in Metairie, Louisiana on 16-May-2013 using a cast net. All fish were then brought to the Center for Biological Field Studies at Sam Houston State University (Fig. 1), where they were placed in aged well water in outdoor artificial streams (1.0 m wide × 4.0 m long × 0.3 m deep), which were previously described in detail by Hargrave et al. (2009). Specimens were then euthanized in buffered MS-222 (tricaine methanesulfonate), preserved in 10% formalin, transferred to 70% ethanol, and finally cataloged at the Cleveland Museum of Natural History (CMNH 20374) and the Texas Natural History Collections (TNHC 55549). Additional specimens, collected from their native or non-native ranges in Mexico and Texas and confidently identified prior as either *H. cyanoguttatus* or *H. carpintis*, were acquired from museum collections (Table 1). To reduce confounding effects due to allometry and proportional differences at different body sizes, only adults >90 mm SL were examined. The populations sampled included: *H. cyanoguttatus* from native localities (n = 20), *H. cyanoguttatus* from non-native inland localities in Texas (n = 18), *H. cyanoguttatus* from non-native coastal localities in Texas (n = 17), *H. carpintis* from native localities (n = 23), and *H.* cf. *cyanoguttatus* from non-native localities in Louisiana (n = 23). One of us (AK) was then provided access to the specimens, while blinded to their identities, and took measures and counts according to Trautman (1957) and Hubbs and Lagler (2004). We followed De la Maza-Benignos et al. (2015a) in choosing which variables to collect, and we followed their diagrams for lower jaw length (which differed from the measurement described by Trautman, 1957),

Table 1.—Museum specimens used in morphometric and meristic analyses.

Species	Museum	Catalog #	Locality	SL (mm)
H. cyanoguttatus (native range)	TNHC	5757	Nuevo Leon, Mexico	102
H. cyanoguttatus (native range)	TNHC	5757	Nuevo Leon, Mexico	118
H. cyanoguttatus (native range)	TNHC	5757	Nuevo Leon, Mexico	120
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	93
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	95
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	95
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	97
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	97
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	99
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	102
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	104
H. cyanoguttatus (native range)	TNHC	6729	Cameron, Texas, United States	106
H. cyanoguttatus (native range)	TNHC	9402	Val Verde, Texas, United States	127
H. cyanoguttatus (native range)	TNHC	9454	Val Verde, Texas, United States	160
H. cyanoguttatus (native range)	TNHC	25106	Hidalgo, Texas, United States	98
H. cyanoguttatus (native range)	TNHC	25106	Hidalgo, Texas, United States	100
H. cyanoguttatus (native range)	TNHC	25106	Hidalgo, Texas, United States	102
H. cyanoguttatus (native range)	TNHC	38159	Cameron, Texas, United States	116
H. cyanoguttatus (native range)	TNHC	44227	Tamaulipas, Mexico	91
H. cyanoguttatus (native range)	TNHC	44227	Tamaulipas, Mexico	127
H. cyanoguttatus (introduced in Texas)	TNHC	8943	Bexar, Texas, United States	121
H. cyanoguttatus (introduced in Texas)	TNHC	8943	Bexar, Texas, United States	121
H. cyanoguttatus (introduced in Texas)	TNHC	31743	Bexar, Texas, United States	98
H. cyanoguttatus (introduced in Texas)	TNHC	31743	Bexar, Texas, United States	99
H. cyanoguttatus (introduced in Texas)	TNHC	32534	Bexar, Texas, United States	107
H. cyanoguttatus (introduced in Texas)	TNHC	38151	Real, Texas, United States	123
H. cyanoguttatus (introduced in Texas)	TNHC	38151	Real, Texas, United States	128
H. cyanoguttatus (introduced in Texas)	TNHC	39413	Terrell, Texas, United States	98
H. cyanoguttatus (introduced in Texas)	TNHC	40279	Travis, Texas, United States	97
H. cyanoguttatus (introduced in Texas)	TNHC	40279	Travis, Texas, United States	115
H. cyanoguttatus (introduced in Texas)	TNHC	41650	Gonzales, Texas, United States	100
H. cyanoguttatus (introduced in Texas)	TNHC	41650	Gonzales, Texas, United States	103
H. cyanoguttatus (introduced in Texas)	TNHC	41650	Gonzales, Texas, United States	112
H. cyanoguttatus (introduced in Texas)	TNHC	43426	Travis, Texas, United States	108
H. cyanoguttatus (introduced in Texas)	TNHC	43426	Travis, Texas, United States	109
H. cyanoguttatus (introduced in Texas)	TNHC	47981	Williamson, Texas, United States	123
H. cyanoguttatus (introduced in Texas)	TNHC	49599	Wilson, Texas, United States	110
H. cyanoguttatus (introduced in Texas)	TNHC	49599	Wilson, Texas, United States	118
H. cyanoguttatus (coastal)	TNHC	7290	San Patricio, Texas, United States	97
H. cyanoguttatus (coastal)	TNHC	7290	San Patricio, Texas, United States	107
H. cyanoguttatus (coastal)	TNHC	27475	Harris, Texas, United States	86
H. cyanoguttatus (coastal)	TNHC	27475	Harris, Texas, United States	90
H. cyanoguttatus (coastal)	TNHC	38032	Nueces, Texas, United States	122
H. cyanoguttatus (coastal)	TNHC	38033	Nueces, Texas, United States	109
H. cyanoguttatus (coastal)	TNHC	38041	Nueces, Texas, United States	96
H. cyanoguttatus (coastal)	TNHC	38041	Nueces, Texas, United States	102
H. cyanoguttatus (coastal)	TNHC	38041	Nueces, Texas, United States	155
H. cyanoguttatus (coastal)	TNHC	38049	Kleberg, Texas, United States	110
H. cyanoguttatus (coastal)	TNHC	38057	Kleberg, Texas, United States	95
H. cyanoguttatus (coastal)	TNHC	38064	Willacy, Texas, United States	179
H. cyanoguttatus (coastal)	TNHC	41586	San Patricio, Texas, United States	96
H. cyanoguttatus (coastal)	TNHC	41586	San Patricio, Texas, United States	82
H. cyanoguttatus (coastal)	TNHC	42659	Refugio, Texas, United States	135
H. cyanoguttatus (coastal)	TNHC	43872	Kleberg, Texas, United States	116
H. cyanoguttatus (coastal)	TNHC	41555	San Patricio, Texas, United States	125
H. carpintis (native range)	FMNH	4503	Mexico, San Luis Potosí: Valles, Rio Valles	91
H. carpintis (native range)	FMNH	4503	Mexico, San Luis Potosí: Valles, Rio Valles	100
H. carpintis (native range)	FMNH	4503	Mexico, San Luis Potosí: Valles, Rio Valles	108
H. carpintis (native range)	FMNH	4503	Mexico, San Luis Potosí: Valles, Rio Valles	115
H. carpintis (native range)	FMNH	4503	Mexico, San Luis Potosí: Valles, Rio Valles	120
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	96
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	113
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	114
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	114
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	115
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	116

Table 1.—Museum specimens used in morphometric and meristic analyses. *Continued*

H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	120
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	121
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	121
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	122
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	123
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	125
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	130
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	137
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	141
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	145
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	148
H. carpintis (native range)	TU	5641	Laguna de Chairel, Tampico, Mexico	150
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	120
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	120
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	121
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	124
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	128
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	128
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	131
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	152
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	153
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	153
Cichlids caught in New Orleans	CMNH	20374	Canal Street in Metairie, Louisiana	153
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	94
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	97
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	104
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	104
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	121
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	139
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	158
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	160
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	162
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	164
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	182
Cichlids caught in New Orleans	TNHC	55549	New Orleans City Park, New Orleans	185

and for premaxillary pedicel length. To compare morphology among populations, we performed exploratory ANOVA's of selected characters. These analyses revealed no differences among populations, so no further analyses of morphology were performed.

Color patterns of live fishes

We compared body color pattern between living specimens of *H. cyanoguttatus* from populations introduced in Texas and *H.* cf. *cyanoguttatus* from Louisiana, and we compared those observations to evidence gathered from archival photographs. To ensure that any observed differences in color pattern were not due to a developmentally plastic response to local environmental conditions, we compared color patterns observed in adult specimens raised in a common garden environment for nearly 3 years. Young-of-the-year *H. cyanoguttatus* were caught from Shoal Creek in Austin, Texas on 9-August-2013 using an aquarium net (Texas Scientific Collection permit SPR-0391-361). The *H.* cf. *cyanoguttatus* specimens captured in Louisiana and maintained at Sam Houston State University's Center for Biological Field Studies reproduced in four of the artificial streams during the summer of 2013. The breeding fish included individuals from both of the sites in Louisiana. On 13-August-2013, the newly captured *H. cyanoguttatus* and the *H.* cf. *cyanoguttatus* offspring from all four artificial streams were placed into freshwater in aquaria ≥ 380 L.

On 11-June-2016, we removed 10 specimens from Texas and 10 specimens from

Table 2.—Wild specimens observed on *The Cichlid Room Companion* (https://cichlidae.com/) and on *iNaturalist* (https://www.inaturalist.org/). Out of hundreds of available photos, only those showing individuals exhibiting breeding colors were examined and are included here.

Species	Database	Photo #	Sex	Locality	Dark color extends to mouth?
H. cyanoguttatus	cichlidae.com	172	F	La Mota Spring, Coahuila, Mexico	N
		3308	F	Bustamante Spring, Nuevo Leon, Mexico	N
		3309	F	Bustamante Spring, Nuevo Leon, Mexico	N
		3309	M	Bustamante Spring, Nuevo Leon, Mexico	N
		5605	M	Rio Sabinas, Mexico	N
		8177	M	Bustamante Spring, Nuevo Leon, Mexico	N
		8178	F	Bustamante Spring, Nuevo Leon, Mexico	N
		10387	F	Bustamante Spring, Nuevo Leon, Mexico	N
		10387	M	Bustamante Spring, Nuevo Leon, Mexico	N
		10388	M	Bustamante Spring, Nuevo Leon, Mexico	N
		8179	F	Rio San Juan, Mexico	N
		8181	F	Rio San Juan, Mexico	N
		10222	F	Rio San Juan, Mexico	N
		10855	F	Rio San Juan, Mexico	N
		10855	M	Rio San Juan, Mexico	N
		16548	F	Santa Tecla, Mexico	N
		16548	M	Santa Tecla, Mexico	N
	iNaturalist.org	44976718	?	Guadalupe, Nuevo Leon, Mexico	N
		35808982	?	Guadalupe, Nuevo Leon, Mexico	N
		23445111	?	Cadereyta Jiménez, Nuevo Leon, Mexico	N
		22999256	?	Santiago, Nuevo Leon, Mexico	N
		21450137	?	Guadalupe, Nuevo Leon, Mexico	N
		11971732	?	Acuña, Coahuila, Mexico	N
		8081747	?	Guadalupe, Nuevo Leon, Mexico	N
		6037766	?	Santa Tecla, Mexico	N
		3072588	?	Bustamante, Nuevo Leon, Mexico	N
		3068017	F	Bustamante, Nuevo Leon, Mexico	N
		3068017	M	Bustamante, Nuevo Leon, Mexico	N
		3035358	F	Bustamante, Nuevo Leon, Mexico	N
		3035358	M	Bustamante, Nuevo Leon, Mexico	N
		2699660	?	Bustamante, Nuevo Leon, Mexico	N
H. carpintis	cichlidae.com	6299	F	Rio Axtla, Pánuco drainage, Mexico	Y
		7308	F	Rio Axtla, Pánuco drainage, Mexico	Y
		7308	M	Rio Axtla, Pánuco drainage, Mexico	Y
		10945	M	Rio Axtla, Pánuco drainage, Mexico	Y
		10946	F	Rio Axtla, Pánuco drainage, Mexico	Y
		10947	F	Rio Axtla, Pánuco drainage, Mexico	Y
		7955	F	Rio Salto, Pánuco drainage, Mexico	Y
		21518	F	Rio Salto, Pánuco drainage, Mexico	Y
		21519	M	Rio Salto, Pánuco drainage, Mexico	Y
		21523	F	Rio Salto, Pánuco drainage, Mexico	Y
		21524	M	Rio Salto, Pánuco drainage, Mexico	Y
		21526	F	Rio Salto, Pánuco drainage, Mexico	Y
		21526	M	Rio Salto, Pánuco drainage, Mexico	Y
		21527	F	Rio Salto, Pánuco drainage, Mexico	Y
		21527	M	Rio Salto, Pánuco drainage, Mexico	Y
		21528	F	Rio Salto, Pánuco drainage, Mexico	Y
		21528	M	Rio Salto, Pánuco drainage, Mexico	Y
		21530	M	Rio Salto, Pánuco drainage, Mexico	Y
		21532	F	Rio Salto, Pánuco drainage, Mexico	Y
		8753	F	Mante River, Pánuco drainage, Mexico	Y
		8754	F	Mante River, Pánuco drainage, Mexico	Y
		8755	F	Mante River, Pánuco drainage, Mexico	Y
		8756	F	Mante River, Pánuco drainage, Mexico	Y
		8756	M	Mante River, Pánuco drainage, Mexico	Y
		8753	F	Mante River, Pánuco drainage, Mexico	Y
	iNaturalist.org	12585213	F	79650 Cd Fernández, México	Y
		1993014	F	Chicontepec, Veracruz, México	Y
		660294	?	Huazalingo, Hidalgo, México	Y
		31946525	?	Chicontepec, Veracruz, México	Y

Louisiana from their aquaria and recorded the length of the longest axis of the largest spot visible on the left side of each fish, rounded to the nearest mm. We performed a t-test in Microsoft Excel to compare the diameter of the largest spot on each fish between the two populations. Next, we measured 20 randomly selected iridescent spots from a photo of each individual animal using AxioVison LE software (http://www.zeiss.com). De la Maza-Benignos et al. (2015a) specifically mentioned the flanks of the fish when describing the diameters of the spots on *H. cyanoguttatus*, and in both populations the spots seemed to be larger on the opercle and the region posterodorsal to the eye, so we obtained our measurements from spots specifically positioned posterior to the opercle and pectoral fin, i.e., the flank. To be conservative, spots that appeared to be two or more spots merged into one were avoided, and diameter was inferred roughly along the longitudinal axis of the body and adjusted to cover the shortest distance across the spot. For each specimen, we calculated mean spot diameter from the 20 measures, and used a t-test in Microsoft Excel to compare the spot size of the 10 fish from each population, with each fish providing one data point. We did not correct our measures for the size of the fish because body sizes were similar in the two species (SL: *H. cyanoguttatus* mean: 125 mm, range: 84-146 mm; *H. carpintis* mean: 120 mm, range: 105-137 mm). We also compared the spot sizes we observed to the values reported by De la Maza-Benignos et al. (2015a) for native-range *H. cyanoguttatus* and *H. carpintis*.

Finally, color patterns exhibited by breeding individuals in the common garden populations were compared to the color patterns observed in wild individuals breeding in their native ranges (Table 2). To assess wild individuals, we accessed photographs archived on *The Cichlid Room Companion* online database (https://cichlidae.com) and on *iNaturalist* (https://www.inaturalist.org/). On 24-October-2019, *The Cichlid Room Companion* had 27 photos of *H. cyanoguttatus*. A subset of these showed either one individual or a breeding pair exhibiting breeding color. *The Cichlid Room Companion* had 48 photos of *H. carpintis*, a subset of which exhibited breeding color. *iNaturalist* had 373 photos of *H. cyanoguttatus* and 26 photos of *H. carpintis*; a subset of each of these species exhibited breeding color and were included in the analysis. We identified individuals by specific markings to prevent them from being assessed twice, and only individuals observed within their native geographic range were included.

RESULTS

Morphometrics and meristics

Our general observations indicated that *H. carpintis* from native localities and Louisiana specimens both tended to exhibit rounded ventral profiles (from mouth to caudal peduncle), oblique mouths, and protruding lower jaws compared to *H. cyanoguttatus*. We found mean body depth to be >50% SL in both *H. carpintis* from native populations and *H.* cf. *cyanoguttatus* from our non-native Louisiana populations and <50% SL in each population of *H. cyanoguttatus*. However, our preliminary analyses of this character and other selected morphometric and meristic characters revealed no significant differences among populations ($P > 0.05$). We include here a summary of our measures of body proportions (Table 3), and a summary of the meristics values we recorded (Table 4).

Color patterns of live fishes

On each of the 10 Texas specimens sampled from the common garden experiment, the largest spot observed was 1 mm in diameter. In the Louisiana population, the largest spot observed on each specimen often took the form not of ovoid spots but of oblong streaks ranging from 3 mm up to 8 mm long (Figs. 2A, B). Statistical analysis found the largest spot observed on each specimen to be significantly larger in the Louisiana specimens than in the Texas specimens (two-tailed *t*-test: $T = 7_{18}$, $P = 1.55 \times 10^{-6}$, Fig. 2C). More conservative measures that ignored these exceptionally large spots found the mean diameter of the typical-

Table 3.—Morphometric values in % of SL and head length. Minimum, mean (x), and maximum values are shown, along with standard deviation (sd). Measures followed De la Maza-Benignos et al. (2015a).

	H. cyanoguttatus (native)				*H. cyanoguttatus* (Texas)				*H. cyanoguttatus* (coastal)				*H. carpintis* (native)				Cichlids caught in New Orleans			
	min	x	max	sd	min	x	max	sd	min	x	max	sd	min	x	max	sd	min	x	max	sd
SL (mm)	91	108	160	17	97	111	128	10	82	112	179	25	91	121	150	16	94	137	185	26
head length	32	35	40	2	31	34	36	1	31	34	36	1	32	34	38	2	30	34	39	2
body depth	42	48	59	4	43	49	55	4	45	50	58	3	46	51	57	3	43	51	58	4
dorsal fin base	53	59	63	3	55	59	65	2	51	59	65	4	55	58	61	2	52	59	63	3
anal fin base	21	24	28	2	23	26	29	1	20	25	29	3	18	22	25	1	21	25	28	2
predorsal distance	41	45	52	3	42	46	50	2	41	46	50	3	42	46	49	2	41	46	53	3
rostral tip–anal fin origin	66	70	76	3	67	70	75	3	67	70	73	2	69	71	75	2	66	71	77	3
rostral tip–pectoral fin origin	35	37	39	1	33	37	41	2	30	36	39	2	35	37	47	2	34	37	39	1
rostral tip–ventral fin origin	37	41	45	2	39	42	47	2	38	42	45	2	37	42	46	2	39	43	45	1
caudal peduncle length	13	15	19	2	12	17	19	2	11	16	21	2	12	16	19	2	13	17	19	2
caudal peduncle depth	10	14	18	2	13	16	18	1	11	15	19	2	14	17	20	1	14	16	19	1
postdorsal distance	87	90	93	2	88	91	98	3	86	90	94	2	87	90	96	2	86	89	93	2
dorsal fin origin–anal fin origin	51	58	68	4	50	61	67	4	55	60	67	3	56	62	72	4	55	63	73	5
postdorsal fin base–anal fin origin	33	38	57	5	35	39	43	2	35	39	45	3	35	38	42	2	34	39	43	3
dorsal fin origin–postanal fin base	62	67	72	3	63	68	72	3	63	68	72	2	63	67	72	2	63	68	73	3
postdorsal fin base–post anal fin base	15	18	22	2	16	19	22	2	17	19	22	1	17	20	35	4	12	19	23	2
dorsal fin origin–pectoral fin origin	28	34	41	3	30	34	43	3	30	35	57	6	31	35	39	2	29	36	41	3
postdorsal fin base–hypural base	13	15	17	1	14	16	19	2	12	15	17	2	11	14	18	2	14	16	18	1
anal fin origin–hypural base	36	41	45	2	19	40	45	6	38	40	43	2	35	39	43	2	38	42	48	3
anal fin origin–pelvic fin origin	26	29	32	2	27	30	35	2	22	30	35	3	27	30	36	2	25	29	33	2
pelvic fin origin–pectoral fin origin	11	15	19	2	12	17	23	2	11	16	21	3	13	17	23	2	10	16	19	2
head length (mm)	30	38	51	6	31	38	44	4	29	38	63	8	32	42	57	6	30	47	60	8
head width	48	52	59	3	45	54	61	4	40	54	63	5	47	50	53	2	51	55	64	3
interorbital width	35	41	56	6	32	42	50	5	30	41	56	7	33	38	43	3	38	45	53	4
snout length	35	47	54	6	32	50	67	8	34	51	61	9	40	47	54	3	43	53	67	6
lower jaw length	25	31	39	3	22	30	37	4	17	28	34	5	19	30	35	3	26	30	38	3
premaxilary pedicel length	30	41	57	8	32	48	62	8	23	45	60	10	12	42	51	7	41	48	72	7
cheek depth	31	36	42	3	27	34	45	6	26	36	51	6	31	36	42	2	35	42	62	6
eye diameter	16	21	24	3	15	21	24	3	17	21	28	3	16	19	22	2	17	20	27	2
lachrymal depth	29	38	50	6	28	41	52	6	32	41	50	5	30	36	44	4	34	46	67	8
preorbital width	28	35	43	5	34	40	46	4	27	38	52	5	28	33	39	3	17	39	50	6
snout width across the lachrymal	24	35	55	6	34	38	43	3	34	38	52	4	28	33	39	4	17	40	49	6
lower jaw width	27	33	46	5	28	34	39	3	23	35	47	5	23	29	36	4	26	35	43	4
pectoral fin base	11	18	25	4	15	21	25	3	16	22	26	3	13	16	20	2	19	22	27	2
pelvic fin base	16	21	25	3	20	25	29	2	18	25	31	3	17	20	27	3	23	25	31	2

Table 4.—Meristic values. Minimum, modal (m), and maximum values are shown, along with the percentage frequency of values observed at the mode. Characters were chosen based on De la Maza-Benignos et al. (2015a). LL = lateral line.

	H. cyanoguttatus (native)				*H. cyanoguttatus* (Texas)				*H. cyanoguttatus* (coastal)				*H. carpintis* (native)				Cichlids caught in New Orleans			
	min	m	max	freq	min	m	max	freq	min	m	max	freq	min	m	max	freq	min	m	max	freq
LL, anterior	16	18	19	.50	17	18	20	.33	17	19	21	.41	17	19	19	.48	15	19	20	.48
LL, posterior	9	10	13	.60	8	10	12	.56	9	10	11	.65	8	10	11	.52	9	10	12	.70
above LL	5	5	7	.75	4	5	7	.50	5	6	8	.53	5	5	7	.52	4	5	6	.83
below LL	5	6	8	.35	6	7	9	.33	6	7	9	.47	5	6	10	.43	6	7	9	.43
circumpeduncular	5	7	8	.60	5	6	8	.44	6	7	9	.53	6	7	8	.48	6	6	8	.48
predorsal	5	8	9	.45	6	8	9	.50	6	7	10	.41	7	8	11	.57	7	7	9	.65
between pectoral, pelvic fin bases	5	5	7	.70	5	5	6	.61	4	5	7	.41	5	5	6	.83	4	5	6	.87
dorsal spines	15	16	17	.55	15	16	17	.78	13	16	17	.53	15	16	17	.78	15	16	17	.57
dorsal rays	9	10	11	.80	8	10	14	.61	9	10	12	.53	10	10	12	.87	9	10	11	.48
pectoral	13	14	15	.50	12	13	15	.56	11	13	14	.47	13	14	16	.39	13	14	15	.43
pelvic	6	7	8	.90	6	7	8	.83	7	7	8	.82	7	7	8	.87	7	7	8	.83
anal spines	4	5	6	.75	4	5	6	.83	4	5	6	.65	3	5	6	.87	5	5	6	.87
anal rays	8	9	9	.90	7	9	10	.67	7	9	10	.53	7	9	9	.78	8	9	9	.78
caudal	16	16	19	.50	16	16	18	.50	15	17	18	.53	16	17	17	.52	16	16	19	.57
branchiostegal	2	4	4	.50	3	4	4	.83	3	4	5	.76	2	3	4	.65	3	4	4	.96
ceratobranchial	4	5	8	.60	4	6	8	.44	5	6	7	.53	4	4	5	.57	5	5	6	.65
epibranchial	2	3	3	.60	2	2	3	.50	2	3	3	.53	2	2	3	.57	2	3	3	.52

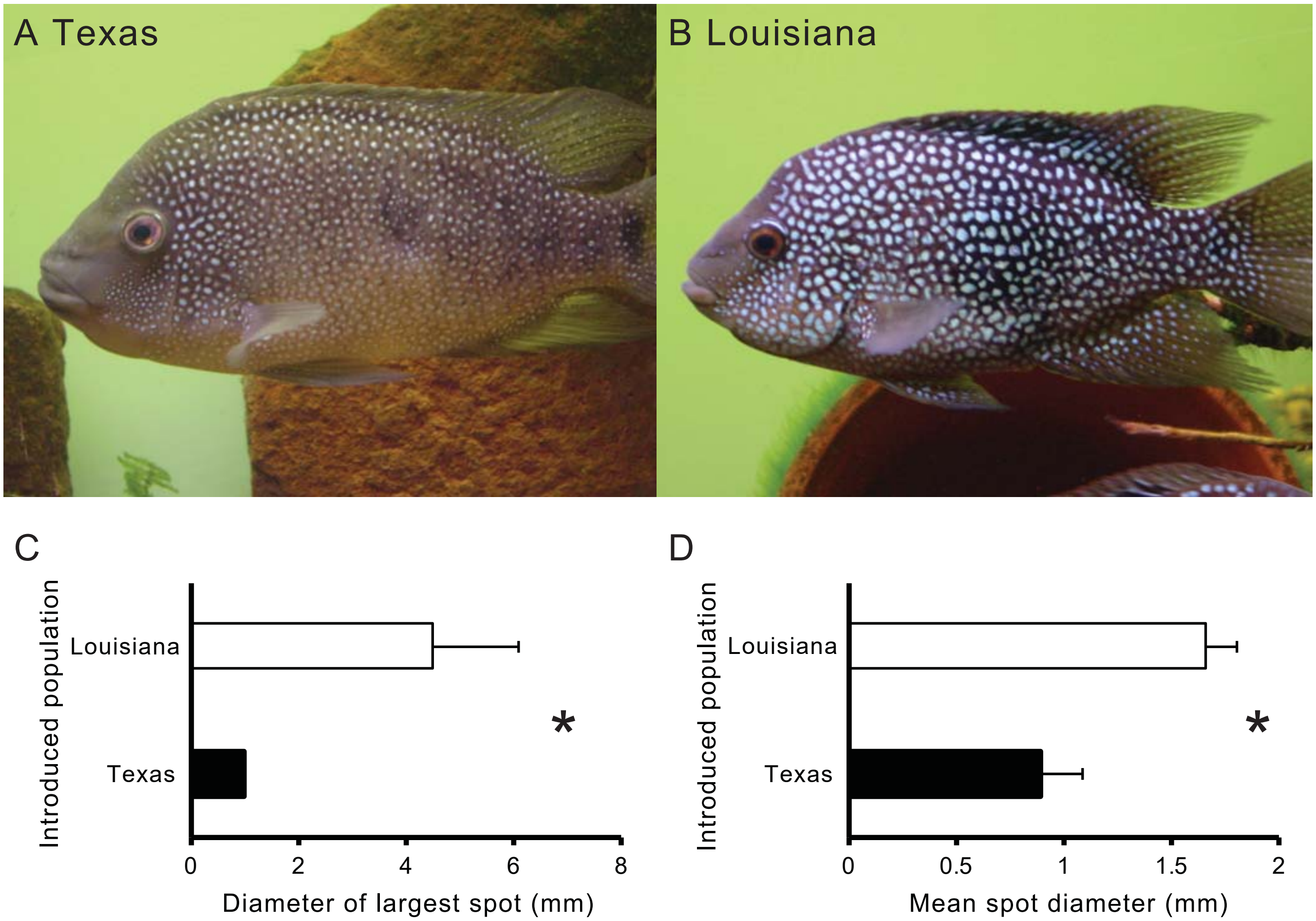

Figure 2.—*Herichthys* spp. captured from introduced populations in Texas and Louisiana then raised together in a common garden environment. A, Texas specimen raised in common garden. Appearance is similar to *H. cyanoguttatus* captured in its native range (see Fig. 1C). B, Louisiana specimen raised in common garden. Appearance is similar to *H. carpintis* captured in its native range (see Fig. 1D). C, Diameter (rounded to nearest mm) of the largest spot observed on each of 10 common garden specimens originating from Texas, and of 10 common garden specimens originating from Louisiana. D, Mean diameter (measured to nearest 0.01 mm) of 20 iridescent spots on each of 10 common garden specimens originating from Texas, and of 10 common garden specimens originating from Louisiana. Bars are means + SD. *Difference was strongly significant (see text).

size iridescent spots (Fig. 2D) to be significantly larger in the Louisiana specimens than in the Texas specimens (two-tailed *t*-test: $T = -10.20_{18}$, $P = 6.59 \times 10^{-9}$), with the mean diameter for any given fish ranging 0.64-1.20 mm in the Texas specimens and 1.51-1.89 mm in the Louisiana specimens.

Herichthys cyanoguttatus breeding in the wild in their native range were found to have black ventral coloration that did not extend to the tip of the mouth in all 31 individuals observed (Fig. 3A), but breeding, wild, native-range *H. carpintis* did have black coloration that extended all the way to the mouth in all 29 individuals observed (Fig. 3B). The breeding color pattern observed in non-native *H. cyanoguttatus* from Texas and raised in the common garden environment (Fig. 3C) matched that seen in breeding, wild, native-range individuals of *H. cyanoguttatus* by also having black ventral coloration that did not extend to the tip of the mouth. The breeding color pattern observed in *H.* cf. *cyanoguttatus* from non-native sites in Louisiana and raised in the common

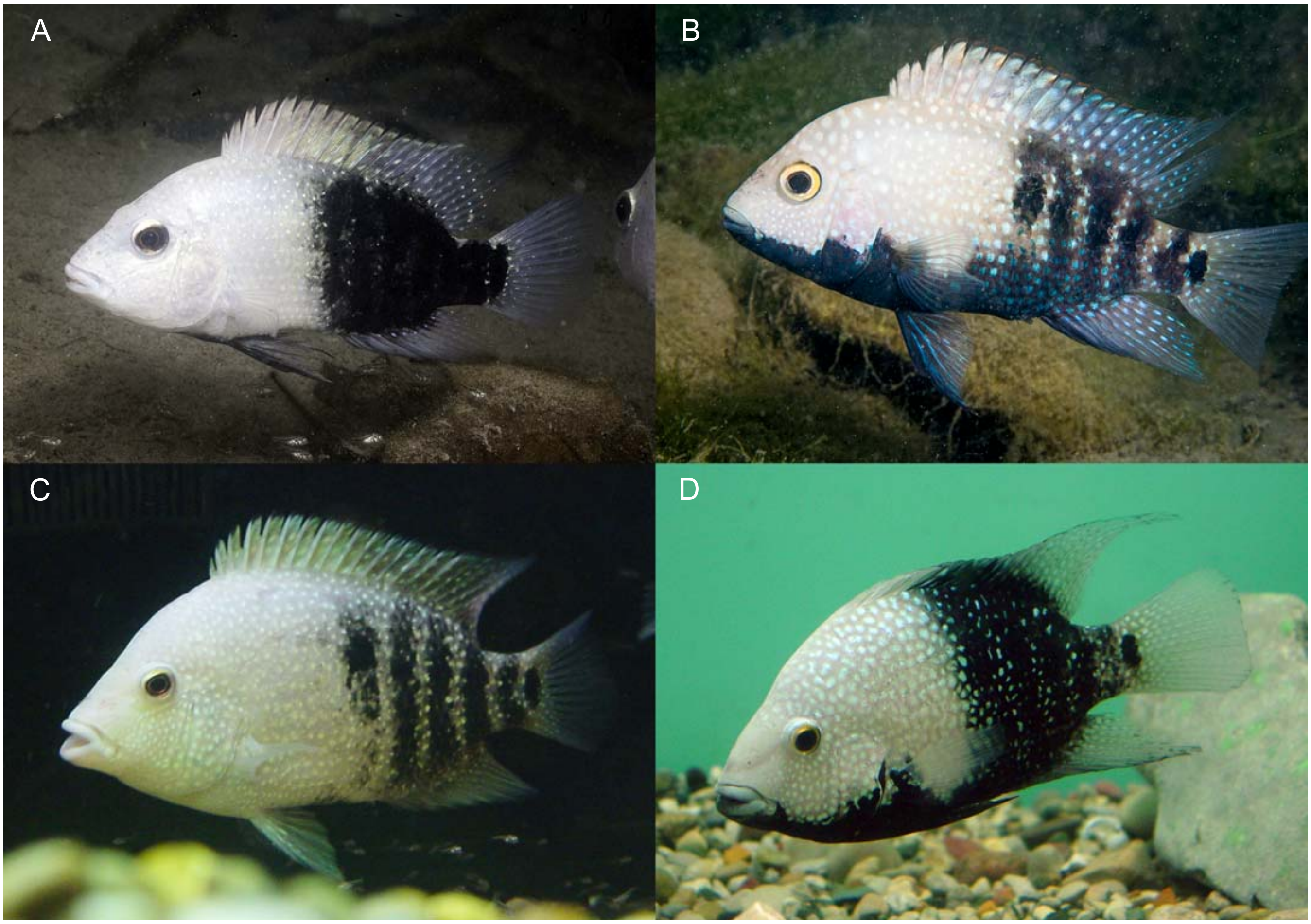

Figure 3.—Breeding color patterns of *H. cyanoguttatus* and *H. carpintis*. A, Female *H. cyanoguttatus* in its native range at Bustamante Spring, Sabinas River, Rio Grande drainage (Mexico). Photo by Juan Miguel Artigas Azas. B, Female *H. carpintis* in its native range in Rio Axtla, Pánuco drainage (Mexico). Photo by Juan Miguel Artigas Azas. C, Female *H. cyanoguttatus* caught in a non-native environment in Texas and then raised in a common garden environment with individuals originating from Louisiana. Note absence of black coloration extending anteriorly to the mouth, matching the native-range *H. cyanoguttatus* shown in A. D, Female specimen descended from fish caught in Louisiana and then raised in a common garden environment with *H. cyanoguttatus* caught in a non-native environment in Texas. Note the black coloration extending anteriorly to the mouth, matching the *H. carpintis* shown in B. Note also that the iridescent blue-white spots are much larger in both B and D than they are in A and C. The manifestation of the black color pattern on the posterior flank as either vertical bars or as a solid patch varies within each species and is not taxonomically informative.

garden environment (Fig. 3D) matched that seen in breeding, wild, native-range individuals of *H. carpintis* by also having black ventral coloration that extended to the tip of the mouth.

DISCUSSION

At least some of the cichlids established in Louisiana are *H. carpintis* and not *H. cyanoguttatus*. The general appearance of the Louisiana specimens included features characteristic of *H. carpintis*: rounded ventral profiles, oblique mouths, and protruding lower jaws (Jordan and Snyder, 1899). Body shape is known to be phenotypically plastic in fishes, with examples known from Louisiana: Largemouth Bass (*Micropterus salmoides*) in brackish water in Louisiana had distinctly different body shapes that appeared to be due to phenotypically plastic response to salinity and not due to short-term differential survival or to genetically selected evolution of body shape (Peterson and Meador, 1994). Lorenz et al. (2014) observed variation in body shape in introduced tilapia in Louisiana. Thus, we would not be surprised if individuals of *H. carpintis* that developed in a non-native environment in Louisiana would have slightly different body shapes than conspecifics that developed in their native environment. Furthermore, if the founders were aquarium stock then they may have undergone artificial selection, founder effect, hybridization, and/or inbreeding before being released in Louisiana. Nevertheless, individuals from Louisiana clearly exhibited qualitative body shape traits indicative of *H. carpintis*.

We found no compelling differences in traditional morphometrics or meristics between the non-native populations established in Texas and in Louisiana, but we also saw no differences among those populations and *H. cyanoguttatus* and *H. carpintis* from their native ranges. As described by Miller et al. (2005), we found mean body depth to be >50% SL in both native *H. carpintis* and in *H.* cf. *cyanoguttatus* from non-native Louisiana populations and <50% SL in each population of *H. cyanoguttatus*, but the ranges of values overlapped across populations so this character is not robust enough to diagnose species. We did not observe a difference among populations in lower jaw length. Nor did we find that *H. carpintis* differed from *H. cyanoguttatus* by having a longer head as was reported by De la Maza-Benignos et al. (2015a). Instead, we found specimens' heads to be of almost identical proportional length across populations. We also found no difference among populations in distance from the rostral tip to the pectoral fin origin, snout length, or eye diameter (De la Maza-Benignos et al., 2015a; Pérez-Miranda et al., 2018). Although we were not able to substantiate the species differences reported by previous authors, the values we recovered were very similar to those reported by Baird and Girard (1854), Jordan and Snyder (1899), De la Maza-Benignos et al. (2015a), and Pérez-Miranda et al. (2018), suggesting that our measurements were performed well, and that the lack of differences we observed between species was not due to poor measurements on our part.

Our quantitative analyses of iridescent spots in common-garden specimens found that *H.* cf. *cyanoguttatus* from Louisiana had significantly larger spots than did *H. cyanoguttatus* from Texas. Of the species described in the genus *Herichthys*, *H. carpintis* has long been recognized for having especially large spots (Loiselle, 1982; Staeck and Lincke, 1985). In fact, in their taxonomic revision of the genus, De La Maza-Benignos et al. (2015a) identified large spots as the key character for identifying this species, and the mean spot diameters we observed in the specimens from Louisiana and Texas fell within the ranges reported by those authors for *H. carpintis* (>1.5 mm) and *H. cyanoguttatus* (<1.0 mm), respectively (although two of our 10 *H. cyanoguttatus* had mean spot sizes slightly >1.0 mm). All other species in the genus have spots smaller than 1.5 mm. Furthermore, those populations that have somewhat large spots (1.0 – 1.5 mm), such as *H. teporatus*, or *H. cyanoguttatus* from the San Fernando River, are absent from the pet trade, so their release in Louisiana seems unlikely.

Finally, our observation of breeding color patterns again indicated that the Louisiana specimens were *H. carpintis*. Pérez-Miranda et al. (2018) stated that in breeding individuals of *H. cyanoguttatus*, the black ventral coloration does not cover the pelvic fins and does not extend anteriorly to the head, and that in *H. carpintis* the black ventral coloration extends anteriorly all the way to the front of the mouth, and dorsally to the suborbital series. Our observations were only partially consistent with this. We saw that in individuals of *H. cyanoguttatus* breeding in the wild, the black color never extended all the way to the tip of the mouth, but on some individuals it did extend to the pelvic fins and to the posteroventral region of the head. In *H. carpintis* the black color did indeed always extend all the way to the tip of the mouth. However, it never extended dorsally to the suborbital series (although we have seen this in a published photograph of an aquarium specimen). Pérez-Miranda et al. (2018) also stated that the dark posterior pigmentation in *H. cyanoguttatus* more often takes the form of bars whereas it more often becomes solid in *H. carpintis*. That also was not consistent with our observations. Both species variously expressed both bars and solid patches, and we suspect that this variation is merely a manifestation of the intensity of expression in a particular individual at any given time. Nevertheless, the breeding color patterns observed in wild fish were distinctly different between the two species, and the breeding color patterns observed in our common garden specimens exactly matched those seen in wild specimens.

We cannot, based on specimens from only two collecting sites, conclude that every cichlid in Louisiana is *H. carpintis*. It is possible that some populations consist of *H. cyanoguttatus*. It is also possible that there are hybrids between the two species. The presence of both species, and/or their hybrids, seems less likely because it would require multiple introductions in Louisiana instead of one (but see Harrison et al., 2014), or the direct introduction of hybrids, and hybrid cichlids are relatively rare in the aquarium trade in the US. In fact, there is a strong movement in the American Cichlid Association against hybridizing pet cichlids (De Angelo, 2018). Nevertheless, anomalous individuals have been observed in Louisiana and Texas. *Herichthys* cf. *cyanoguttatus* captured in Gannon Canal and St. Charles Canal in New Orleans East seemed to have had smaller spots (not shown) than did individuals captured at other locations. *Herichthys cyanoguttatus* captured on the Texas coast in Chocolate Bayou (tributary to Lavaca Bay), Calhoun County had spots seemingly larger (not shown) than in typical *H. cyanoguttatus*. Anomalous specimens also exist in the native range of *H. cyanoguttatus*. On *iNaturalist* we observed one individual (photo 8253220) that seemed to show black coloration all the way to the mouth, but the photos were not clear enough for us to make a conclusive species identification. Its locality was close to the range of *H. carpintis* (De La Maza-Benignos, 2005a), and *H. carpintis* could occur there through a natural or human-introduced mechanism. Photos 4141866 and 4141862 were obtained from a site deep in the native range of *H. cyanoguttatus*, but the identity of the two specimens shown is clearly *H. carpintis* (confirmed by both large iridescent spots and black breeding color pattern). The presence of not one but two individuals of *H. carpintis* at this site suggests either an error in the locality data, or that *H. carpintis* has been introduced there. Introductions of other non-native fish species have been documented in northern Mexico (Marks et al., 2011). One other anomalous specimen was observed on *iNaturalist* (photo 23123577) that was collected directly from the Rio Grande. It was not exhibiting breeding colors, but it showed the large blue spots typical of *H. carpintis*. Clearly, more work needs to be done to gain a complete understanding of the taxonomy and geographic variation among species of *Herichthys* in the Gulf Coast region. Research to date has found DNA sequences of the mitochondrial gene COI insufficient for distinguishing among species of *Herichthys* (Mejía et al., 2015; De la Maza-Benignos et al., 2015b). However, more recent analyses using other molecular markers and new sequencing technologies have proven informative and might yield better understanding of the genealogical history of the cichlid fishes introduced in the Gulf Coast (Pérez-Miranda et al., 2018). Such research is currently being pursued by our team.

The presence of *H. carpintis* in Louisiana is not a result of natural colonization northeastward along the Texas coastline, but the result of release by humans, most likely a pet owner. Recently, some fish species whose geographic ranges had been thought to be restricted to Mexico have been found to occur north of the border in Texas (Martin et al., 2012), and it is unlikely that *H. carpintis* could have dispersed along the coastline all the way from Mexico to Louisiana without also occurring in Texas (Fig. 4). So, we also examined

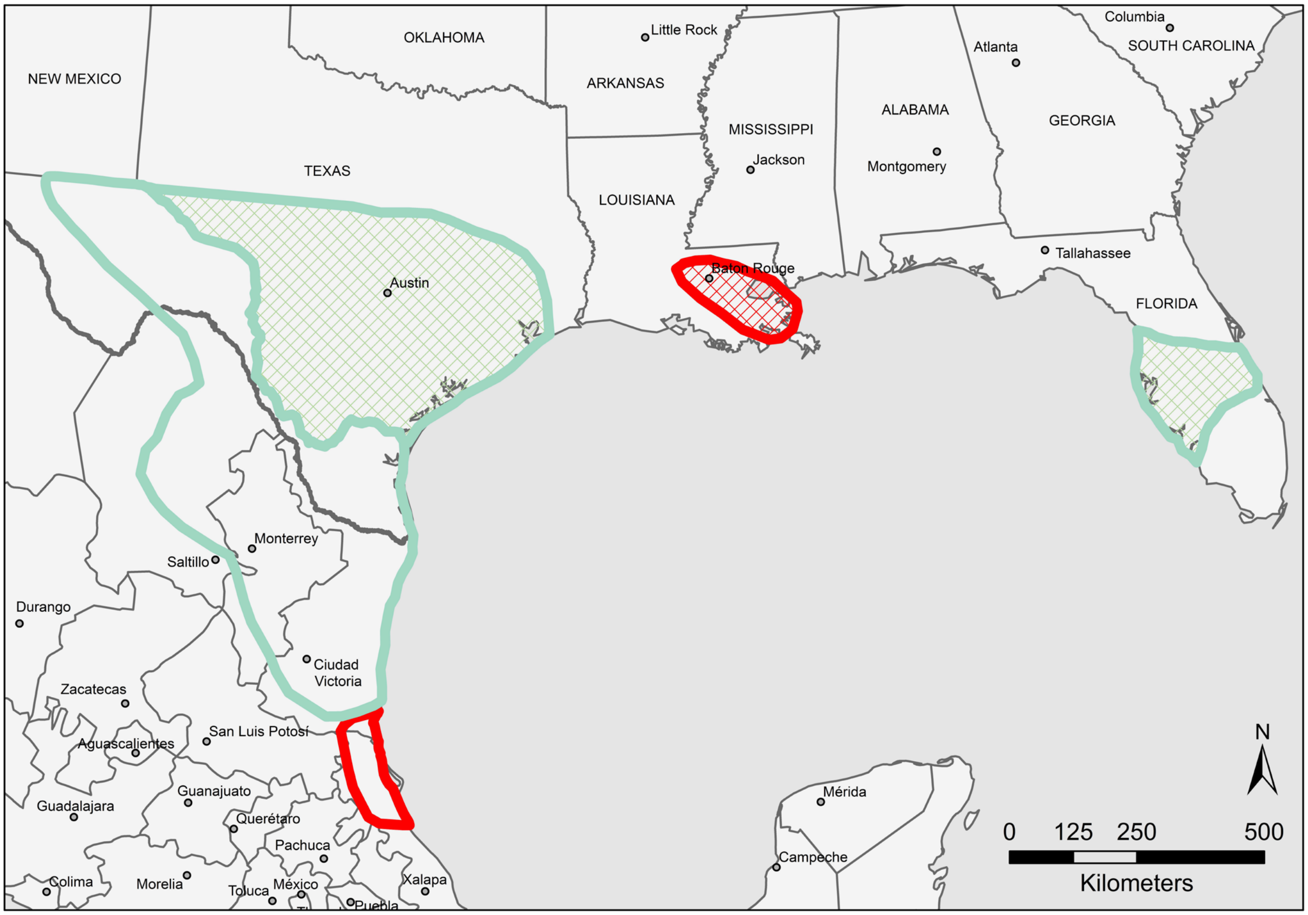

Figure 4.—Native and introduced ranges of *Herichthys* spp. in the Gulf Coast region of North America. Light (blue) outlines indicate *H. cyanoguttatus*; dark (red) outlines indicate *H. carpintis*. Unhatched areas indicate native ranges; cross-hatched areas indicate introduced ranges.

specimens captured from non-native sites along the Texas coast. Our results indicated those to be *H. cyanoguttatus* and not *H. carpintis*. We did not include specimens introduced in Florida in our analyses, but inspections of specimens at the University of Michigan Museum of Zoology, and of internet photographs, indicated to us that the identity of those populations is also *H. cyanoguttatus*.

Understanding the biology of not one, but two, species of *Herichthys* will be necessary to predict and mitigate their colonization of new environments in the US. It is particularly important to determine if they have differences in osmoregulatory capacity, and thus differences in ability to use coastal areas to colonize new watersheds. Studies of *H.* cf. *cyanoguttatus* in Louisiana have indicated a high salinity tolerance (Lorenz and O'Connell, 2008; Lorenz et al., 2015), but osmoregulatory capacity of *H. cyanoguttatus* in Texas has not been assessed. Are *H. cyanoguttatus* in Texas stenohaline and not well suited to colonizing coastal environments, or are they euryhaline like *H.* cf. *cyanoguttatus* in Louisiana, in which case we should expect their range to expand along the coast?

CONCLUSIONS

The identity of at least some specimens of *H.* cf. *cyanoguttatus* in Louisiana is *H. carpintis* and not *H. cyanoguttatus*. This is the first record of *H. carpintis* establishing a population in the US

(Neilson, 2018). Introduction of non-native aquatic organisms from the pet trade is a growing problem, and as more species become available in the pet trade, more will be released. Although Florida has been recognized as highly vulnerable (Courtenay et al., 1974; Shafland and Pestrak, 1982), this study highlights that other parts of the southern US also provide environments suitable to colonization by non-native fishes from the pet trade (Siemien and Stauffer, 1989; Stauffer and Boltz, 1994). Understanding the taxonomy of introduced species is imperative for predicting and mitigating their colonization of new environments (Lowe et al., 2012; Robins et al. 2020).

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