

Integrative Taxonomy as a Tool for Conservation: Exploring Freshwater Fish Diversity of the Barra del Colorado Wildlife Refuge, Costa Rica

by

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Abstract

As global biodiversity declines, conservation efforts are important in megadiverse areas such as the Neotropics where biodiversity is especially imperiled. The accurate identification of species/specimens is critical to successful conservation plans. However, in groups such as freshwater fishes, conventional methods for species/specimen identification such as morphology, or molecular methodologies such as DNA barcoding, have documented challenges. Approaches that integrate multiple data sources are likely to provide the best species identifications. Here, I assess the diversity of freshwater fishes in the Barra del Colorado Wildlife Refuge, Costa Rica, and document 51 species. I use and compare multiple approaches to identify species. My results demonstrate that barcode-based identifications are comparable to field-based identifications despite known gaps in resources for field-based identifications and are inferior to identifications by experts using morphology. I conclude that an integrative approach to specimen identification is a critical facet of successful preservation of biodiversity.

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1 Introduction

Given the current biodiversity crisis, conservation concerns and efforts are of the utmost urgency. Conservation efforts are important in hotspots of biodiversity such as the Neotropics (Pelicice et al., 2017). However, Neotropical biodiversity is understudied in some areas, for reasons that range from political instability to isolation (Bickford et al., 2006), and anthropogenic factors continue to put these areas under constant threat (Vörösmarty et al., 2010; Campbell, 2012; Sandel et al., 2015). A fundamental component of successful conservation plans is the accurate identification of species and understanding of their biology and distribution (Dudgeon et al., 2006). Once sampled, Neotropical freshwater fishes can be difficult to identify using morphology, due to phenotypic plasticity in relevant morphological characters and/or morphologically cryptic diversity (Pereira et al., 2021). In addition, DNA barcoding approaches can suffer from inconsistencies associated with public reference libraries that can make these tools prone to error (Fort et al., 2021). An integrative approach to specimen identification can help overcome the challenges faced by single data approaches like morphology or DNA barcoding in megadiverse groups like Neotropical freshwater fishes (Gomes et al., 2015). The goal of this project was to assess the diversity of freshwater fishes in the Barra del Colorado Wildlife Refuge (BCWR), Costa Rica, and to assess the effectiveness of different species identification methods that could be used on this assemblage.

1.1 Conservation

With global declines in biodiversity and the vulnerability of aquatic habitats, it is worth exploring the current threats against the freshwater fishes of the Barra del Colorado Wildlife Refuge (BCWR). First, habitat loss and degradation are a major threat to all biodiversity, however this becomes especially prevalent in aquatic species due to their obligatory confinement to their environments (Sedeño-Díaz & López-López, 2015). Next, the threat of non-native species to an ecosystem and the effects these species can have on native species through competition, predation, pathogen transfer and hybridization (Hall & Mills, 2000; Tilman, 2004;

Santos et al., 2019). Finally, in Costa Rica specifically, aquatic organisms are currently threatened by the continued use of pesticides in monocultures (Diepens et al., 2014).

1.1.1 Habitat Loss and Degradation

The greatest threat to diversity is the loss and/or degradation of habitat. The danger of dramatic habitat changes that result in diversity declines are particularly problematic for aquatic ecosystems which are especially vulnerable to change (Radinger et al., 2019). Within aquatic ecosystems, freshwater serves as critical habitat, while low in area, freshwater is very high in diversity (Dudgeon et al., 2006). In areas such as the Neotropics where diversity and endemism are high, the loss of critical habitat for species can result in extirpations or extinctions depending on the species (Barletta et al., 2010; Radinger et al., 2019). For the freshwater fishes in rivers like those found in Costa Rica, activities such as damming, deforestation, water pollution, and mining can have detrimental effects on habitat availability (Agostinho et al., 2008; Barletta et al., 2010; Branco et al., 2012). In addition, continued stress on species through habitat alterations or degradation can prevent species recovery (Aarts et al., 2004; Radinger et al., 2019). To properly determine the effects of habitat loss and degradation on biodiversity, proper documentation of this diversity is necessary (Hermoso et al., 2011; Toussaint et al., 2016; Radinger et al., 2019).

1.1.2 Non-native species

Decline of native species populations, usually associated with habitat alterations, creates open niches that can be filled by non-native species (Pelicice et al., 2017; Geladi et al., 2019). The introduction of non-native species (species that do not occur naturally in the areas they are found) is one of the major impacts of human activity on natural environments and is directly correlated with anthropogenic activity (Ewel et al., 1999; Allan, 2004; Ehrenfeld, 2010; Campbell & Mandrak, 2019; Geladi et al., 2019). If populations of non-native species can establish successfully (invasion), they can have negative impacts on native species diversity, as they can out-compete and/or predate native species, introduce pathogens, and even hybridize with native species resulting in gene pool deterioration (Hall & Mills, 2000; Tilman, 2004; Santos et al., 2019). As non-native species can become threats to natural communities, rapid identification of new species in an area is extremely important for conservation (Ewel et al., 1999; Darling & Mahon, 2011). In diverse areas such as the Neotropics, understanding and

monitoring the effects of invasive species is especially important (Hermoso et al., 2011). Successful invasions by non-native fish species have been recorded in Costa Rica (ex African Cichlid *Oreochromis niloticus*) (Angulo et al., 2013; Álvarez et al., 2018). In order to assess the presence and impacts of a non-native species, a consistent documentation of the diversity of the area of interest is required (Sedeño-Díaz & López-López, 2015; Ruaro et al., 2018; Geladi et al., 2019).

1.1.3 Pesticides

The presence of pesticides in the river systems of BCWR is a threat specific to this assemblage. A major component of Costa Rica's economy is agriculture, specifically, banana plantations (Hernandez & Witter, 1996; Mortensen et al., 1998; Grant et al., 2013; Diepens et al., 2014). The Northeastern Atlantic slope of Costa Rica is uniquely suited to banana monocultures (Mena et al., 2014). The trade winds that pass over its central mountain range produce large amounts of precipitation, the rapid flowing rivers carry high mineral volcanic rock sediments to the lowlands, and the climate is warm – ideal conditions for the growing of bananas (Hernandez & Witter, 1996; Grant et al., 2013). However, this climatic environment also fosters steady growth of fungus and parasites, which thrive in monocultures (Hernandez & Witter, 1996; Mena et al., 2014). This prompted the introduction of a new banana type, which although disease resistant, still requires considerable human intervention to produce suitable yields (Hernandez & Witter, 1996). This includes plastic bags to protect clutches, the use of pesticides, fungicides, nematicides, herbicides, and temperature control during shipment (Hernandez & Witter, 1996). The physical interventions are a concern as they cause environmental damage, but with relation to fish fauna, the larger concern is the chemical interventions (Hernandez & Witter, 1996). The intensive use of pesticides, the vector for application, the toxicity of the compounds being used, extensive drainage required for banana plantations, and the high precipitation experienced on the Atlantic slope of Costa Rica, all make aquatic ecosystems of the BCWR particularly vulnerable (Castillo et al., 2000; Castillo et al., 2006). In addition, the synergistic or additive effects of multiple pesticides contaminating water systems can increase lethality (Castillo et al., 2000). Plantations in Costa Rica are known to use multiple types of pesticides (Grant et al., 2013). Additionally, aerial spraying near streams and rivers, washing contaminated equipment in local

waters, and inappropriate disposal of excess pesticides all increase contamination of downstream waters (Grant et al., 2013).

In Costa Rica, records of large fish kills in water systems near agricultural lands that use pesticides and following rainfall, have been recorded (Castillo et al., 2000; Diepens et al., 2014). Diepens et al. (2014) recorded large fish kills downstream from pineapple and banana plantations, in the Rio Madre de Dios (Atlantic lowlands, south of Tortuguero). In the Tortuguero region, the headwaters of the Rio Suerte flow through major banana producing lands (Grant et al., 2013). The Rio Suerte then flows southward into the Tortuguero Conservation Area (ACTo) which includes both the study area Barra del Colorado Wildlife Refuge (BCWR) and Tortuguero National Park (TNP) (Grant et al., 2013). Records of large fish kills in the Rio Suerte, downstream from banana plantations known to intensively use pesticides have been recorded (Grant et al., 2013). Grant et al. (2013) explains that due to pesticides used upstream from ACTo, aquatic ecosystems here are at risk. In addition to morality, pesticides can cause reproductive failures and chronic long-term effects, generally decreasing fish populations (Diepens et al., 2014).

Over the past few decades, Costa Rica has been a popular destination for ecotourism, responsible travel to natural areas that seeks to conserve the environment and sustain local people (Gray & Campbell, 2007). In 1992, tourism surpassed the banana trade as the top source of foreign exchange in Costa Rica (Snarr et al., 2016). However, because of pesticide use and competition for space, agriculture can be in direct conflict with tourism.

1.2 Fishes as Indicators of Ecosystem Health

The deterioration of ecosystems globally calls for the implementation of effective environmental monitoring and the development of operation indicators of ecosystem health (Costanza and Mageau, 1999; Li et al., 2010; Franssen et al., 2012; Sedeño-Díaz & López-López, 2015). Assessing the health of an ecosystem is especially important for sites of great ecological value that are at risk without proper management (Corsi et al., 2003). The term “health” generally refers to the overall performance of the system (Costanza and Mageau, 1999). Costanza and Mageau (1999) differentiate three components of healthy ecosystems: vigor, organization, and resilience. Vigor is defined as the activity, metabolism and/or primary

productivity of a system, organization as the diversity and number of interactions between the components of the system, and resilience as the ability to maintain structure and pattern in times of stress (Costanza and Mageau, 1999). Organization is affected by changes in biodiversity and in the number of pathways that exist for material exchange with more organized ecosystems have higher diversity and more complex interactions (Costanza and Mageau, 1999). A healthy ecosystem is characterized by a balance that develops efficient diversity of components and exchange pathways (high organization) while maintaining some redundancy or resilience as insurance against stress and substantial vigor to quickly recover or utilize stress in a positive manner (Costanza and Mageau, 1999). To use species or a species complex as an indicator of ecosystem health, there must be sufficient knowledge of taxonomy, habitat requirements and physiology (Chovanec et al., 2003).

Among vertebrates, fishes are known to be reliable indicators of ecosystem health, mostly due to their obligatory confinement to their habitats when compared to their terrestrial counterparts (Sedeño-Díaz & López-López, 2015). Fishes occupy multiple trophic levels and niches, reflected in their high diversity. This allows for representation of ecosystem health at multiple levels, which can be informative in conservation and management efforts (Franssen et al., 2002; Sedeño-Díaz & López-López, 2015).

The accurate identification of species, and understanding of their biology and distribution, are fundamental for assessment of ecosystem health (Dudgeon et al., 2006). Groups with high diversity, such as freshwater fishes, require constant monitoring and assessment (Karr & Dudley, 1981; Sedeño-Díaz & López-López, 2015). Measures of baseline diversity allow researchers to track changes in species richness, species distribution, and be alerted to the introduction of non-native species (Shoo et al., 2006). Changes in fish diversity can be correlated with disturbances from human activities to provide insight regarding the causes and effects of declining ecosystem health (Odum, 1985; Dudgeon et al., 2006).

1.3 Methods of Species Identification

Effective conservation planning for the protection of biodiversity requires accurate measurements of species present in an area (Sheth & Thaker, 2017). For species or species complexes to work effectively as indicators of ecosystem health, the taxonomy of that group

should be well understood (Chovanec et al., 2003). While we currently face global declines in biodiversity, another crisis is unfolding as well. The taxonomic crisis (impediment) is typified by lack of interest in taxonomy and decreased financial incentives to study diversity (Pires & Marinoni, 2010; Ricciardi et al., 2020). This impediment is a key obstacle preventing the effective conservation of global biodiversity (Sheth & Thaker, 2017; Ricciardi et al., 2020).

1.3.1 Morphological Identification

Conventionally, taxonomic identification is done using morphology. Morphology based identifications make use of diagnostic characters such as measurements, counts of scale and fin ray numbers, anatomical features, and colour patterns to assign a species identity to a specimen. This method is cost-effective and can be used in both field and lab settings (Hebert et al., 2003; Ward et al., 2009). Morphology makes up the foundation of what we know about global diversity, but like any methodology, it has its challenges (Packer et al., 2009). The major challenge for morphology-based identification methods is the ‘taxonomic impediment’: the small number of trained taxonomists and funding compared to the need for these specialists (Pires & Marinoni, 2010; Sheth & Thaker, 2017; Ricciardi et al., 2020). For certain taxonomic groups, phenotypic plasticity in important characters used for species recognition and morphologically cryptic species pose a challenge for morphology-based identification (Pires & Marinoni, 2010; Marancik et al., 2020; Pereira et al., 2021). The megadiverse ichthyofauna of the Neotropics is a good example of a challenging group for morphology-based identifications. The extremely high level of species diversity, incomplete taxonomy (species descriptions), and a high number of cryptic species and species complexes make species identification difficult (Pereira et al., 2021). Thus, in an effort to supplement morphological identification methods, DNA barcoding has become increasingly popular for the identification of fishes.

1.3.2 Molecular Identification: DNA Barcoding

DNA barcoding is a technique for identifying specimens through the use of standardized regions of ‘barcoding genes’ (Ward et al., 2009). As a convention for animals, the mitochondrial gene Cytochrome Oxidase Subunit one (*coI*) is used as the “barcoding gene” (Castresana et al., 1994). This gene is a good candidate for extraction and amplification because it is of sufficient length and there is a broad range of primers available for polymerase chain reactions (Herbert et

al., 2003; Ward et al., 2009). Many studies have shown that *coI* sequences differ among closely related species to an extent that species identity can be established by comparing their sequences (Hebert et al., 2003; Hubert et al., 2008; Lara et al., 2010; Kress et al., 2015; Creer et al., 2016). *coI* even allows discrimination of recently diverged cryptic species (Pires & Marinoni, 2010), but not universally (Jardim de Queiroz et al., 2020; Meier et al., 2021).

Species identification using DNA barcoding involves the determination of the specimen's *coI* barcode sequence and the comparison of that sequence to a database or barcode reference library of barcodes from accurately identified specimens (Fort et al., 2021). Theoretically, these database sequences are produced from museum voucher specimens, correctly identified by experts (Sheth & Thaker, 2017; Buckner et al., 2021). Without robustly identified voucher specimens from which to produce barcodes, the trustworthiness of these barcodes and the associated public databases, decreases. This can impact the usefulness of DNA barcoding for estimating species diversity and subsequently, for conservation and management purposes.

Previous studies have demonstrated the ability of *coI* to distinguish among species of freshwater fishes in many regions (Hubert et al., 2008; Lara et al., 2010; Pereira et al., 2013; Díaz et al., 2016; Shaw et al., 2016; Ali et al., 2020; Liu et al., 2020). *CoI* barcoding has also been used to identify freshwater ichthyoplankton, allowing researchers increased capabilities in assigning species level identification to traditionally difficult to identify specimens (Frantine-Silva et al., 2015). Pereira et al. (2013) found that *coI* could be used to identify species of neotropical fishes, and also to highlight groups that likely contained new and undescribed species. However, DNA barcoding may be more challenging for Neotropical fishes due to incomplete reference libraries and potential misidentifications of specimens used in the reference libraries (Meier et al., 2008; Fort et al., 2021; Hammer et al., 2021).

1.3.3 Integrative Approach to Species Identification

Since DNA barcoding methodologies first materialized, debates have raged regarding the use of barcoding for taxonomy. Initial fears surrounded the idea of molecular techniques, like barcoding, replacing conventional methods like morphology for taxonomy (Packer et al., 2009; Pires & Marinoni, 2010; Sheth & Thaker, 2017). Multiple studies have demonstrated the ability for *coI* to be used in the identification of specimens across a wide variety of taxa (Ward et al.,

2005; Weigt et al., 2012; Ramirez et al., 2017; Pollack et al., 2018; Pentinsaari et al., 2020). However, as described above, both barcoding and morphology have advantages and disadvantages for specimen identification.

Instead of choosing one identification method over another, another route is to integrate the two approaches. Integrative taxonomy, in short, makes use of all available data to identify species (Dryat, 2005). By integrating different data sources, including both morphology and barcoding, limitations associated with a single data source can be overcome. The term ‘integrative taxonomy’ can be used to describe two processes, species delimitation/discovery and specimen identification (Pires & Marinoni, 2010; Pereira et al., 2021). For this project, my integrative approach will focus on specimen identification. Integrative approaches aid in specimen identification when single data sources are unable to provide adequate identifications (Breedy et al., 2012; Riedel et al., 2013; von Beeren et al., 2016; Duong et al., 2020). Including in Neotropical fishes (Gomes et al., 2015; Pugedo et al., 2016). The use of multiple data sources to support a specimen identity and subsequently the species presence in an area provides strong evidence that can be used for implementation of conservation plans, a key component to secure financial support for conservation (Sheth & Thaker, 2017). An integrative approach to specimen identification can also accelerate specimen identification by having morphology and barcoding work in tandem, in turn shortening the time frame for identifying key areas in need of protection (Pereira et al., 2021).

1.4 Study Area

This project focuses on the Barra del Colorado Wildlife Refuge (BCWR), Costa Rica (Figure 1). Below, I briefly describe the geology of lower Central America to provide context for the biogeography of freshwater fishes in Central America and Costa Rica. I then explore the aquatic dispersal patterns in Costa Rica that contributed to the assemblage of fishes in BCWR. Finally, I describe the habitats and river systems of BCWR.

1.4.1 Lower Central America

Lower Central America is geologically complex and dynamic and is an interesting system for studying the relatively recent diversification of neotropical biota (Bagley & Johnson, 2014).

The Central American seaway initially separated North and South America, and this allowed the waters of the Pacific and Atlantic oceans to mix (O'Dea et al., 2016). However, as tectonic plates moved beneath the Earth's surface, a collision of the Cocos and Caribbean plates caused the Cocos plate to slide beneath the Caribbean plate (O'Dea et al., 2016). In addition to volcanos, the movement of the plates caused sea floor uplift (O'Dea et al., 2016). During the late Neogene-Quaternary there was a gradual emergence of the lower central American isthmus (Pontius et al., 2001; Bagley & Johnson, 2014). Studies suggest that the isthmus became a permanent link between North and South America 1.8 - 3.1 million years ago (Bagley & Johnson, 2014). The isthmus facilitated movement between North and South America terrestrial and aquatic biota (Bagley & Johnson, 2014). The movement of biota created an interesting melding pot of different evolutionary lineages, in addition to the wide variety of new niches in Lower Central America, adaptive radiation occurred, and over time the area became incredibly diverse (Bagley & Johnson, 2014). In combination with the Colombian Andes uplift, the isthmus played a role in global climate change from the Miocene to present, by altering regional oceanic and atmospheric circulation patterns (Bagley & Johnson, 2014).

1.4.2 Costa Rica and its Fishes

Costa Rica is geologically young (Pontius et al., 2001), with its current configuration formed within the last five million years (Campbell, 2002; Jones & Johnson, 2009; Pontius et al., 2001). The formation of new land and local extirpations provided ecological opportunities for adaptive radiations of newly dispersed lineages. Adaptive radiations are a powerful force in creating biodiversity (Myers 1966; Jones & Johnson, 2009; López-Fernández, 2013). Costa Rica sits within the Mesoamerica hotspot for biodiversity (Pontius et al., 2001; Myers et al, 2000). The geography includes a diversity of habitat types and climatic conditions, providing a wide range of niches (Wickham, 2001). The central mountain range that runs northwest to southeast, effectively separates the country into two distinct lowland regions; the eastern (Caribbean) region and the western (Pacific) region (Wickham, 2001; Bagley & Johnson, 2014). Many lowland species have distributions on either one side of the central mountains or the other (Bagley & Johnson, 2014). Approximately 4-10% (500,000 species) of global diversity is found in Costa Rica alone (depending on the taxonomic group considered) (Bagley & Johnson, 2014).

The freshwater fish fauna of Central America, including Costa Rica is hypothesized to have evolved from three components: the Old Southern Element (OSE), the New Southern Element (NSE), and the Northern Element (NE) (Myers, 1966; Bussing, 1976, 1985; Matamoros et al., 2015). The OSE refers to fishes that dispersed into Central America during the late Cretaceous or Palaeocene times (~100.5-56mya) from South America (Bussing, 1985). At this time, Central America can be best described as a series of islands that lay between Central and South America (Smith & Bermingham, 2005). The OSE is comprised of secondary freshwater fishes (moderate salinity tolerance) which could explain their early dispersal into Central America (Myers, 1966; Smith & Bermingham, 2005). The NSE refers to fishes that dispersed into Central America from South America following the final uplift of the Isthmus of Panama (3.5-3.1mya). These fishes are described as primary freshwater fishes (low salinity tolerance) which explains their need for a formal freshwater connection to disperse northward into Central America (Myers, 1966; Smith & Bermingham, 2005). Finally, the NE refers to temperate freshwater fishes from North America which had limited dispersal into Central America throughout the Cenozoic (Bussing, 1985). Due to physiological intolerances these lineages tend to extend only as far south as Northern Costa Rica (Bussing, 1985). Herrera-Vásquez et al. (2008) also explain that the fish fauna of Costa Rica is divided into two distinct zones: Atlantic and Pacific; the Atlantic zone being comprised primarily of fishes from the OSE.

1.4.3 Barra del Colorado Wildlife Refuge

Barra del Colorado Wildlife Refuge (BCWR) was established in 1985 and extends from the San Juan River (Costa Rica's border with Nicaragua) south to the northern tip of Tortuguero National Park (TNP) (Khazen et al., 2016). BCWR covers approximately 92,000 hectares of land, and in combination with TNP (19,000ha), makes up the Tortuguero Conservation Area (ACTo) (Grant et al., 2013) (Figure 1). BCWR was established to link the Indo Maíz Reserve in southeastern Nicaragua with TNP, and the combination of these areas form the largest contiguous protected area of lowland Atlantic tropical wet rainforest in Central America, at approximately 111,000ha (Burger, 1997; Wickham, 2001; Khazen et al., 2016).

al., 2013). The area contains lakes and lagoons of variable sizes that are permanently or intermittently connected to canals and rivers.

1.5 Previous Diversity Assessments of Fishes in Costa Rica and the Tortuguero Region

Several species lists and diversity assessments for the fishes of Costa Rica and Tortuguero have been published. The first major assessment was completed by Miller (1966) who analyzed the distribution of the freshwater fish fauna from the Isthmus of Tehuantepec (Mexico) to the Colombian border of Panama. Miller (1966) estimated that there were 456 species in Central America. However, when Miller's study was published, very little was known about Costa Rican ichthyofauna. In 1987, William Bussing published the first book describing the fishes of Costa Rica, with an updated version published in 1998 (Bussing 1998). This book included morphological characters and dichotomous keys for the identification of 142 species of fish in Costa Rica (Bussing 1998). More recently, Angulo et al. (2013) published an annotated checklist of the freshwater fishes in both continental and insular Costa Rica. This work records 250 species, adding 108 species to Bussing's previous list (1998).

The first work assessing the diversity of the Tortuguero region was completed by Caldwell et al. (1959), who collected freshwater fishes while conducting sea turtle surveys in Tortuguero. It is important to note that this collection was done incidentally and was not meant to represent the total fish fauna of the area (Caldwell et al., 1959). Caldwell et al. (1959) recorded 55 species. Winemiller and Leslie (1992) conducted an ecological study of the fishes of Tortuguero National Park, testing for evidence of longitudinal patterns of faunal turnover and an increase in species richness with an increase in stream order. This study identified 56 species of freshwater fishes. Lastly Angulo et al. (2013), listed 116 species in what they refer to as the "Tortuguero basin". However, their definition of the Tortuguero basin includes the San Juan Delta, the Rio San Juan / Rio Colorado eastward from the Rio Sarapiquí drainage basin, and the coastal rivers from Nicaragua south to the Parismína drainage basin. It is thus a large area, that is both more expansive than the Rio Tortuguero, and encompasses the entirety of the ACTo region. The Tortuguero region defined by Angulo et al. (2013) likely represents the regional species pool of which the fishes of BCWR are expected to be a smaller subset. In BCWR, no comprehensive

studies have been done, however Mason (2008) provides a non-comprehensive list which includes 42 species.

1.5.1 Barcoding/Integrative Taxonomy in Costa Rica

Some work has been done in Costa Rica over the past decade using DNA barcoding or integrative taxonomic approaches for specimen identification or biomonitoring. Previous efforts have predominantly focused on insects (beetles (Coleoptera: Staphylinidae: *Tetradonia*), butterflies + moths (Lepidoptera), parasitoids (Hymenoptera: Eulophidae: *Euplectrus*)), soft corals (Anthozoa: *Octocorallia*: *Alcyonacea*) and mussels (Unionida: Unionidae: *Sinaodonta*) (Janzen & Hallwachs, 2011, 2016; Breedy et al., 2012; Hansson et al., 2015; von Beeren et al., 2016; Bauer et al., 2021). A single study has been published exploring the use of DNA barcoding for the identification of sharks and rays (Elasmobranchs) in artisanal fisheries in Costa Rica (O'Bryhim et al., 2021). In addition, most of the studies that assess diversity in Costa Rica across these groups have taken place along the Pacific slope, leaving the Atlantic slope less well studied. Thus, there exists a large gap in current barcoding resources available for the immense diversity in Costa Rica. There is however an immense project currently underway, attempting to barcode Costa Rica's diversity: BioAlfa (Janzen and Hallwachs, 2019).

1.6 Goals and Hypothesis

Given the relevant background information, I had several research goals for this project. A primary goal was to determine the species diversity of freshwater fishes in BCWR. I used species accumulation curves to generate an estimated maximum species diversity, and to assess how close the measured diversity is to this estimated maximum. To identify fish species, I used and compared several different approaches, including DNA barcoding, morphology-based identifications in the field, morphology-based identifications by taxonomic experts in the lab, and an integrative approach that includes data from both DNA and morphology. I used these comparisons to test the hypothesis that DNA barcoding provides overall more accurate species identifications than morphology-based identification. Finally, I will combine barcoding data from this study with data from publicly available sources to create a curated barcoding reference library for fishes of BCWR.

1.7 Significance

This is the first study to address species diversity of freshwater fishes in Costa Rica using molecular barcode techniques. This is also the first study to address the diversity of freshwater fishes in Costa Rica using an integrative approach, and to compare different species identification methods. Finally, this is the first study to address the diversity of freshwater fishes in BCWR. In addition, the curated barcode library will be a tool for future biomonitoring efforts for conservation managers and policy makers.

2 Methods

2.1 Fish Collection and Tissue Preservation

Fish specimens were collected biannually in February of 2013, 2015, 2017, and 2019 in the region surrounding Cano Palma Field Station in the Barra Del Colorado Wildlife Reserve, Costa Rica, by participants of the UTSC BIOC51 class “Tropical Biodiversity Field Course”. A total of 10 sites were sampled in the four combined efforts (Figure 2). A list of sites per year, as well as locality coordinates, are presented in Table 1.

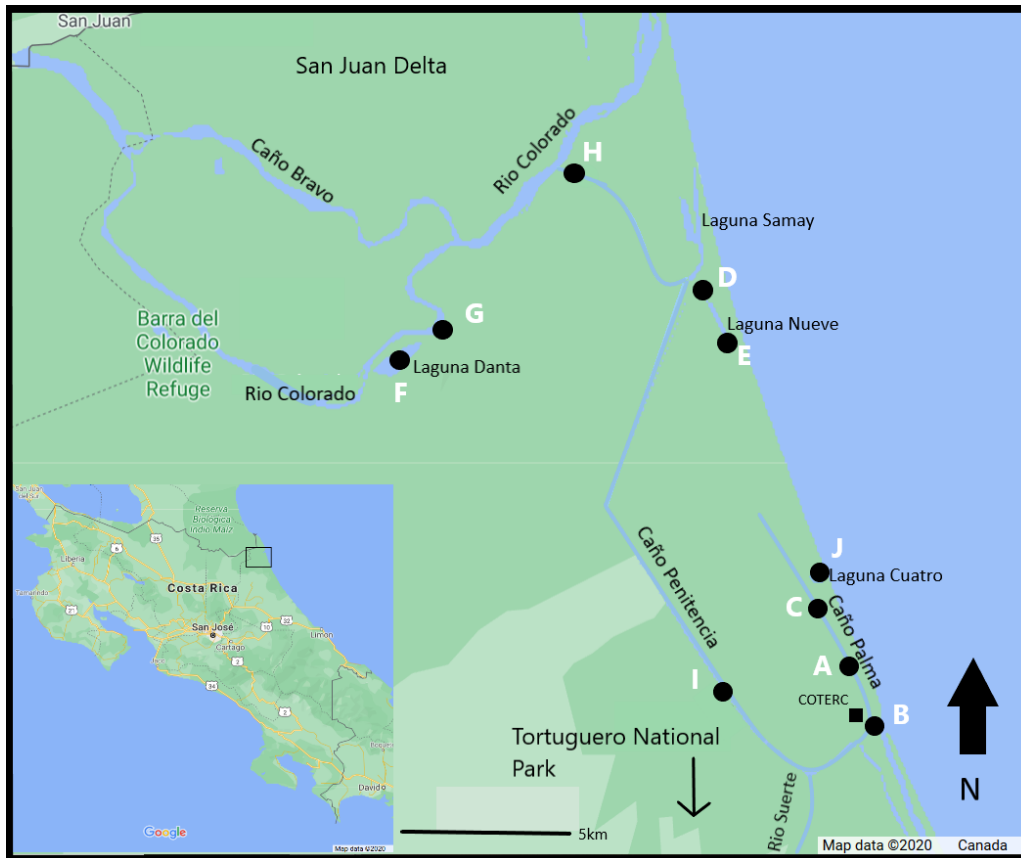


Figure 2. Map showing collection sites (black circles) visited over the four expeditions. Black square shows study sites in relation to Costa Rica.

Table 1. List of sites visited over the four collection expeditions, including coordinates, site description, and years the sites were sampled.

Site	Site Name	Latitude	Longitude	Description	2013	2015	2017	2019
A	Small Streams entering Cano Palma	10.6055	-83.53412	Small streams. Brown water, muddy bottom that varied from hard to soft mud along the site; debris in water included branches and leaves; murky and swampy with low visibility. Stream cover ranged from 65-100% cover. Stream width ranged from 2m-6m. Stream depth was 1-2.5m. Depth of capture for specimens ranged from 0m at minimum to 2.5m at maximum. Distance from shore that specimens were collected ranged from 0m at minimum to 600m at maximum along the site. Duration of collection ranged from 1hr to 2hr 4min along the site. Water temperature varied from 20.4-27.8C°. Water pH of 5.35. Air temperature varied from 25.5-29.5C°. O2 content varied from 566-1336ppm. Fishing methods included dip nets, seine nets, gill nets, minnow traps and electro fishing	x	x	x	x
B	Cano Palma	10.5937	-83.52725	Black water canal. Muddy bottom, low visibility. Shores of river had <i>Rappphia</i> palms, <i>pentadetha</i> , and ferns. Woody material also floated on surface. Stream cover varied from 0-20%. Stream width was 20m. Depth of capture for specimens ranged from 0m at minimum to 0.5m at maximum. Distance from the shore that specimens were collected ranged from 1m at minimum to 10m at maximum. Water temperature varied from 23.6 to 28.5C°. Air temperature was 26C°. Conductivity was 1108us. Fishing method was active night fishing with dipnets from both the dock at COTERC and by boat.	x	x	x	x
C	Stream from Cano Palma to Turtle Beach Lodge	10.6233	-83.54462	Small stream. Brown water, muddy, soft bottom, debris in water included fallen leaves and tree roots and branches; water very murky with low visibility. Stream cover was about 65%. Stream width 5m. Water depth ranged from 1-6m. Depth of capture of specimens ranged from 0m at the minimum to 1.2m at the maximum. Distance from the shore that specimens were collected ranged from 0m at the minimum to 3.5m at the maximum. Duration of collection was about 1 hour. Water temperature was about 27.5C°. Air temperature varied from 28-29.5C°. O2 contents ranged from 566ppm to 1336ppm. Fishing methods included dip nets and seine nets.			x	x
D	Stream connecting Laguna Nueve to XX	10.7081	-83.57519	Small stream. Water appeared, green, white, and brown in different areas of site. Visibility varied from low to moderate. Bottom composition included sandy silty bottoms and varied hard mud and soft mud bottoms. Materials at site included leaves and logs. Grass, shrubs, and thick rainforest were seen around site. Varied wildlife was noted at the site including howler monkeys, kingfishers, and butterflies. Stream cover varied across the site, ranging from 30% to 70%. Stream width ranged from 4m to 10m. Stream depth ranged from 1.5m to 2m. Depth of capture for the specimens collected ranged from 0m at minimum to 1.8m. Distance from the shore that specimens were collected ranged from 0m at minimum to 100m at maximum. Duration of collection efforts varied from 52min to 2hr and 16min. Water temperature varied across the site from 23 to 31.4C° with pH between 6 and 6.4. Air	x	x	x	x

				temperature varied as well, ranging 26 to 30C°. Fishing methods included dip nets seine nets, gill nets, and electric fish finders.				
E	Laguna Nueve	10.6981	-83.56882	Lake. Greyish water with a silty, sandy bottom. Visibility was high. Grass and trees present on the shores. Depth of capture for specimens ranged from 0.1m at minimum to 1.5m at maximum. Distance from the shore that specimens were captured ranged from 0.05m at minimum to 115m at maximum. Water temperature was 30C° with a pH of 7. Fishing methods included dip nets and seine nets	x		x	
F	Laguna Danta	10.6939	-83.65355	Lake. Dark brown water with low visibility, sludgy bottom with detritus, thick grasses present on shore. No cover above the site, out in the open. No stream width noted. Water depth was approximately 1m. Depth of capture of specimens ranged 0.05m at minimum to 1m maximum. Distance from the shore ranged from 0.05m at minimum to 30m at maximum. The duration of the collection effort was 2hrs. Water temperature was 30C°. Air temperature was 35C°. Fishing methods at this site included dip nets and seine nets.	x			
G	Rio Colorado	10.6982	-83.64732	Large river. White waters with a sandy bottom. Visibility varied between years. Submerged grass and grassy islands in river. In 2013 the site contained a wide exposed beach due to a prolonged dry season. Downstream from Delta San Juan. Open water with no cover. Depth of small stream off of Rio Colorado was 0.5m. Depth of capture for specimens ranged from 0m at minimum to 1.5m at maximum. Distance from the shore that specimens were captured ranged from 0m at minimum to 1.5m at maximum. Duration of collection effort was 1hr. Water temperature was 34.8C° with a pH of 6.68. Air temperature was 27C°. Fishing methods included: seine nets.	x	x		
H	Shore of river connecting Rio Colorado to Cano	10.745	-83.6053	River. White waters with a silty mud bottom. Submerged grass present along bottom. Fish collected near the shore of Rio Colorado that connects Rio Colorado to Cano Penitencia. Stream cover not listed. Stream width was approximately 50m. The depth of capture for specimens ranged from 0m at minimum to 1.5m at maximum. Distance from the shore that specimens were collected ranged from 0m at minimum to 4m maximum. Duration of collection not noted. Water temperature was 25.5C° with a pH of 6.62. Air temperature was 30C°. Fishing methods included seine nets.		x		
I	Suerte Cito	10.5946	-83.56641	Stream. Green, brown waters with a muddy bottom that ranged from soft mud to moderately firm mud. Visibility was moderate. Mainly woody debris is water. Large trees and many green plants along the shore. Site is about 30minutes from the COTERC station. Stream cover varied between 40-50% cover. Stream width was approximately 10m. Stream depth was >2m. Depth of capture for specimens ranged from 0m at minimum to 1.6m at maximum. Distance from the shore that specimens were captured ranged from 0m at minimum to 5m t maximum. Duration of collection effort was 3hr 10min. Water temperature was 26.3C°. Air temperature was 25C°. Note that electric fish caught at this site were found in a small stream off of the main site, with very muddy water and bottom, and high turbidity. Fishing methods included: dip nets, seine nets, and electric fish finders			x	x

J	Laguna Cuatro	10.6337	-83.54392	Lake. Brown water with a sandy bottom. Moderate to high visibility. Visible tree roots. Smaller grassy islands dispersed throughout lake. Nearby to Caribbean Sea beach. Stream cover was approximately 15%. Stream width was approximately 100m. Stream depth was not recorded. Depth of capture for specimens ranged from 0m at minimum to 1m at maximum. Distance from the shore that specimens were caught ranged from 0m at minimum to 50m at maximum. Duration of collection effort was 55min. Water temperature was 28.6C°. Air temperature was 29C°. Fishing methods included dip nets and seine nets.			x	x
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Collection was done using a mix of seine nets, gill nets, dip nets, minnow nets, and electrofishing, with each collection effort involving six to 10 researchers collecting for approximately 1-2 hours. Fish were euthanized using clove oil solution following Keene et al. (1998). Samples of muscle tissue were excised from specimens and preserved in 95% ethanol solution, and subsequently stored in 95% ethanol solution at -20°C. For each site collected during each expedition, an attempt was made to sample tissues from two specimens per putative species. Euthanized fishes were preserved in a 10% formaldehyde solution in the field, and later transferred to 70% ethanol for long-term storage. Unfortunately, the 2017 collection of fishes was lost in the field; however, tissues from the 2017 expedition were used in this study.

2.2 Field morphological Identification

In the field, before preservation, specimens were identified using morphology according to the keys provided in Bussing (1998). Identifications were primarily completed by undergraduate students, working with a graduate student teaching assistant and course instructor.

2.3 Expert morphological identification

In some cases, specimens were identified based on morphology by taxonomic experts at natural history collections. Dr. Hernan Lopez-Fernandez, Royal Ontario Museum (now at University of Michigan), identified only the cichlid fishes collected during the 2013 and 2015 expeditions. Arturo Angulo, University of Costa Rica, identified all specimens collected during the 2019 expedition.

2.4 Barcode-Based Identification

2.4.1 DNA Extraction

Genomic DNA was extracted from muscle tissue using the DNeasy Blood and Tissue Kit following the manufactures' protocol (QIAGEN, Canada). Subsamples of muscle tissue samples were taken and placed in 180µL of animal tissue lysis buffer (Buffer ATL) and 20µL of proteinase K to lyse the tissue and denature endogenous nucleases, respectively, followed by a three-hour incubation period at 56°C (or until the tissue was completely digested). Vortexing then took place for 15 seconds before adding 200µL Buffer AL followed by an additional 15 seconds of vortexing before adding 200µL of 95% ethanol and then vortexed again. The solution

containing the lysed muscle tissue sample was transferred to a DNeasy Mini spin column and was then centrifuged at 8000rpm for 1 minute. The spin column was then transferred to a new collection tube and the flow through discarded. For cell lysis and purification, 500µL of Buffer AW1 was added followed by centrifugation for 1 minute at 8000rpm. Again, the spin column was placed in a new collection tube and the flow through discarded. The spin column then had 500µL of Buffer AW2 added followed by 3 minutes of centrifugation at 14000rpm for continued washing. The spin column was then placed the 1.5mL microcentrifuge tube and the flow through discarded. Finally, 100µL of Buffer AE (elution buffer) was added and then was then centrifuged for 1 minute and 8000rpm. This final step was repeated to increase DNA yield.

2.4.2 Polymerase Chain Reaction for DNA amplification

I amplified the mitochondrial barcoding gene, Cytochrome Oxidase subunit 1 (*coI*) using the primers COIFishF₂ and COIFish R₂ (Ward et al., 2005) (Table 2). Master mixes were created using the following: 13.75µl of ddH₂O, 2.5µl of a combined buffer of 60% SO₄ buffer and 40% KCl, 3.0µl of MgCl₂ (25mM), 1.0µ of dNTP (10mM), 1.25µl of each primer, and 0.25µl of Taq Polymerase, totalling 23µl. A volume of 2µl of DNA was added to the master mix, accounting for a final volume of 25µl. Thermocycler conditions were as follows: 94°C for 2 minutes, 94°C for 30s, 52°C for 40s, 72°C for 1 minute and 30s. This series of steps was repeated 35 times in the cycler. Finally, PCR samples were set at 72°C for 5 minutes before resting at 4°C until removed from cycler. This protocol did not yield successful amplifications for each sample. I used a second combination of primers COIFishF₁ and COIFishR₁ primers (Ward et al., 2005), when amplification using the first combination of primers was unsuccessful (Table 2). When necessary, the volume of DNA per sample was increased to 3µl, removing 1µl ddH₂O from the master mix. Each PCR run was accompanied by a negative control (22 or 23µl of master mix and 3 or 2µl of ddH₂O respectively). PCR products were then checked for success by visualizing on Agarose Gel dyed with Red Safe®, where 5µl of PCR product was mixed with 2µl of 6x loading dye, a DNA ladder was added to visualize DNA lengths, and 2µl of 6x loading dye was added to 5µl Gels were then run for 30 minutes on 80 v and 80amps. PCR product was purified using a custom mix of Exonuclease I and Calf Intestinal Alkaline Phosphatase (EXO-CIAP). A volume of 4µl of EXO-CIAP was added to each PCR product vial to remove excess primers, nucleotides, and buffers. The PCR products were incubated in a thermocycler at 37°C

for 30 minutes, 85°C for 15 minutes, and stored at 4°C. This protocol was adapted from Li et al. (1991), Hanke and Wink (1995), Nordström et al. (2000), Watanabe et al. (2010) and the EXOCiAP solution was made in house by Ahmed Elbassiouny.

Table 2. Primers used to amplify Cytochrome Oxidase Subunit 1 gene from Ward et al., (2005).

Primer	Sequence 5'-3'	Citation
COIFishF₂ 10mM	TCGACTAATCATAAAGATATCGGCAC	(Ward et al., 2005)
COIFishR₂ 10mM	ACTTCAGGGTGACCGAAGAATCAGAA	(Ward et al., 2005)
COIFishF₁ 10mM	TCAACCAACCACAAAGACATTGGCAC	(Ward et al., 2005)
COIFish R₁ 10mM	AGACTTCTGGGTGGCCAAAGAATCA	(Ward et al., 2005)

2.4.3 Sequencing

Purified PCR products were sequenced at the Centre for Applied Genomics: Peter Gilgan Centre for Research and Learning (Toronto, Ontario, Canada). Chromatographs were assembled, visually inspected, and manually edit in Geneious v9.8.1 (<https://www.geneious.com>). Consensus sequences were generated using Geneious' default setting and aligned using the MUSCLE (Edgar, 2004a; Edgar, 2004b) plugin in Geneious.

2.4.4 DNA Barcode Species Identification

Sequences of *coI* were exported from Geneious in FASTA file formats. Each sequence was submitted to Genbank's BLAST search function and the Barcoding of Life Database's (BOLD) search function using default search parameters (Benson, 2005; Ratnasingham & Hebert, 2007). These databases allow the user to identify a query sequence by comparing it to all sequences in the respective database. The databases provide matches based on percent similarity (the percentage of sites between the queried sequence and the reference sequence that are identical). For each of my sequences, I determined the three different species identifications with the highest percent similarity from each database from the top 100 results. I determined the final

barcode-based identification for each specimen based on the highest similarity identification provided by the databases.

2.5 Integrative Approach to Identification

For all specimens with *coI* sequences (240 total), I generated a species identification that combined information from barcodes, field identifications, and expert identifications (see above). I also considered the information associated with other specimens in the collection-- for example, I analyzed the barcodes from all specimens from a particular genus to determine how many species were present (see below for details). I refer to this as the integrative approach, as outlined in Figure 3.

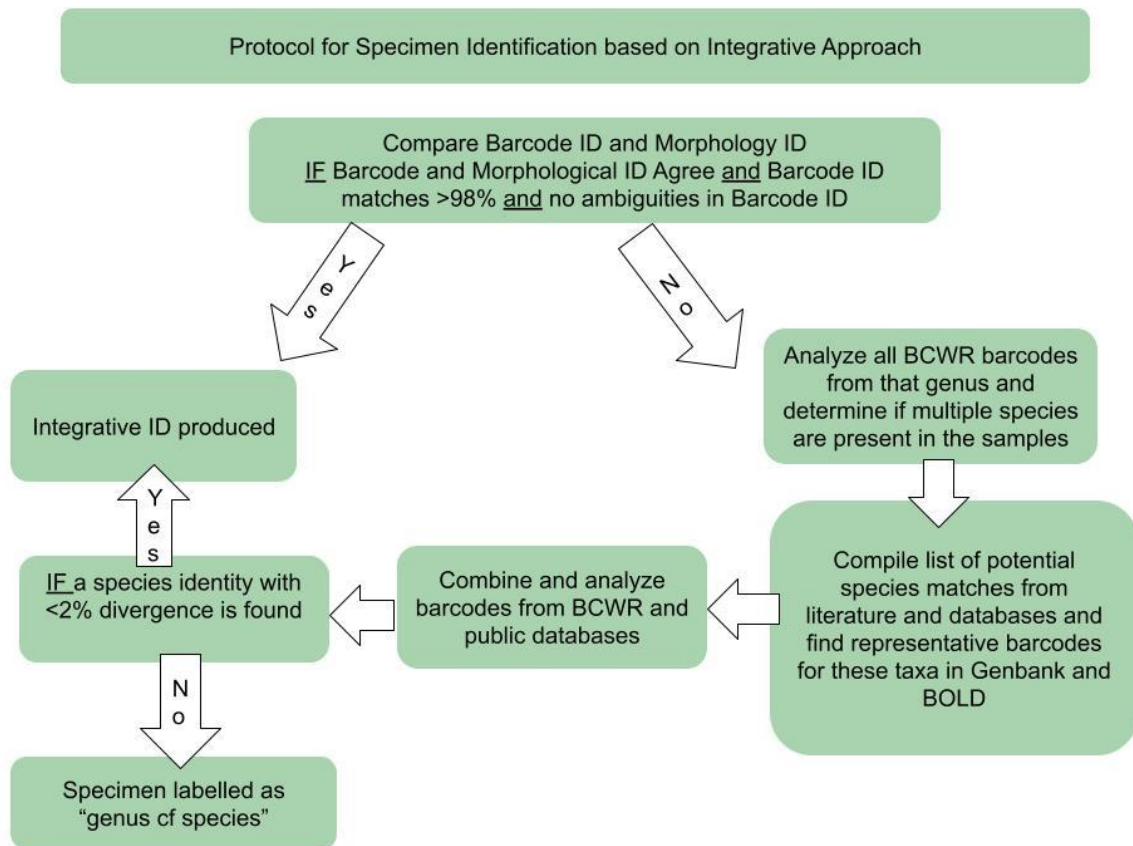


Figure 3. Schematic showing integrative approach to identification

As the first step in this approach, working with each specimen individually, I queried the respective *coI* sequence in the databases Genbank and BOLD, producing a barcode

identification. I compared the morphological identification and the barcode identification. When expert or field identification and database identifications agreed, and when similarity of the specimen barcode was >98% to the database sequence(s), and if there were no ambiguities in the identification (i.e., the specimen barcode did not match multiple species in the databases), I accepted the identification. However, in cases where these criteria were not met, I performed additional analyses. For this project, an ambiguity was defined as a result that failed to provide a clear identification, for example, a specimen barcode that matched multiple species in the barcode databases.

For the additional analyses, I assembled and analyzed the barcodes of all BCWR specimens that belonged to a particular genus. I determined the genetic distances among all the specimens using the Kimura 2 parameter and used this information to determine the number of species represented, based on the idea that different species would be <2% diverged. Then, I compiled a list of species, genera, or families (in some cases) that might help to clarify the identity of my specimens. To determine which species, genera, or families should be considered, I reviewed the most recent diversity assessment for Costa Rican freshwater fishes by Angulo et al. (2013), focusing on those closely related to the database results. I also reviewed the results from the queries in Genbank and BOLD and included the species that these databases identified. Once I had compiled this list, I searched Genbank and BOLD and downloaded barcodes for any of the species on the list. I then determined the divergence between my specimens and all retrieved barcodes. The barcode match with the lowest divergence was used as the identification for the specimen. In most cases this resolved inconsistencies between the morphological and barcode identification. In some cases, a barcode from an unidentified specimen matched a barcode for a specimen with an expert morphological identification, and this allowed species identity to be established. In cases where the expert morphological identification and the barcode identification still did not agree, I utilized the expert identification. These identifications are listed as *genus* cf. *species* where the cf represents the uncertainty of the identification without additional data.

I also generated a neighbor-joining tree and analyzed the placements of barcode sequences within groups (Appendix D Figure D-S1). Sequences were expected to group with conspecifics, supporting the species identity assigned to them.

2.6 Holistic Species List for BCWR

For the identification of individual specimens, I followed an “integrative” approach (multiple sources of data for one specimen). To determine which species are present in BCWR and subsequently the diversity of BCWR fishes, I followed a holistic approach that uses multiple sources of evidence. I included species indicated by the expert identification of physical specimens, sequence identification (in cases where there are no physical specimens and where there were no inconsistencies in the database’s identification), unambiguous identification from photographs, unambiguous observations, and species identified from the integrative approach previously described. Here, the term “unambiguous” refers to evidence of species that would be highly unlikely to be confused with other species.

2.7 Species Accumulation Curve

I produced a species accumulation curve (SAC) in R (4.0.3) using *vegan: Community Ecology Package* (2.5-7) based on the integrative dataset across collections from all years and sites (Oksanen et al., 2020). Because not all sites were surveyed during every expedition, species accumulation was calculated over individual collections. The SAC was built using the rarefaction method to find expected species richness and standard deviation (Kindt and Oksanen, unpublished). To determine the maximum species richness for BCWR, I rarefied gamma diversity and used the “predict” function with nonlinear least squares (nls) method “lomolino” to extrapolate the SAC and determine the asymptote of the curve (Lomolino, 2000; Dengler, 2009; Kindt and Oksanen, unpublished). The asymptote of the extrapolated curve can be interpreted as the maximum of species expected to be found if sampling is continued (Lomolino, 2000; Dengler, 2009).

An additional SAC was produced to determine species accumulation at a single site (D). Since this site was repeatedly sampled across four years of expeditions, I examined accumulation over years. This SAC was also built using the rarefaction method (Kindt and Oksanen, unpublished). The SACs for all expedition collections and for Site D by year allowed me to see if new species are expected to be discovered in subsequent sampling efforts.

2.8 Curated Barcode Library Creation

Given the reliance of DNA barcoding identification methods on reference libraries and the issues already identified in the literature with using currently available libraries for identification, I wanted to build a curated barcode library to aid in the identification of the specimens collected in BCWR during future efforts. Given the constraints of species I was likely to catch with the fishing gear and collection sites, it is likely that the species represented in my BCWR sequence collection do not represent the total diversity of species in the area. In particular, the fishing techniques mainly target smaller fishes in shallower water, and larger fish species that occur in deeper habitats may be underrepresented in my collected barcode sequences. To build the library, I used barcodes from my own analysis and supplemented them with sequences from public databases for species that were not collected during this project but could be expected to occur in the region.

2.8.1 Selection of Barcodes from this Study for Curated Library

I used barcode sequences from my analysis that had a confirmed physical specimen and had been identified based on my integrative approach. I added all barcodes from my analysis that fit these criteria to my library.

2.8.2 Selection of Barcodes from Public Databases for Curated Library

To determine species that were potentially present in BCWR, I generated a list of species for the wider Tortuguero region (which includes BCWR), based on Angulo et al. (2013) and Winemiller and Leslie (1992). I also included species listed in an unpublished preliminary assessment of fishes present in and around the COTERC Biological Station (Mason, 2008). Finally, I examined observational data for the region from the Global Biodiversity Information Facility (GBIF) records to determine any other species that should be considered. The resulting regional list allowed me to search for sequences in public databases not already represented by the barcodes I generated.

Due to difficulties with public barcode data, I used several criteria to ensure that the highest quality sequences possible were added for my library. First, sequences needed to be of at least 550 base pairs in length. Typically, the barcoding region for Cytochrome Oxidase Subunit 1

is 648-655bps, but information can be gained from shorter regions (Hubert et al., 2008). Reference sequences that meet this 648-655bps minimum are not possible for all species in the regional species list, since the production of sequences of this length has changed over time (Ivanova et al., 2007; Pollack et al., 2018). Second, sequences needed to be from museum voucher specimens. Third, more recently uploaded sequences were preferred because taxonomy changes over time, and the taxonomy of older barcodes can be problematic (Fort et al., 2021). For each species, four sequences were taken when possible. Attempts were also made to use sequences generated by different studies and from across a species' distribution in an attempt to cover as much intraspecies variation as possible. After sequences were selected they were analyzed in a neighbor-joining tree. Public sequences that did not group with conspecifics were removed as this indicated an identification problem.

3 Results

3.1 Field Morphological Identification

Preliminary morphological identifications done in the field using the keys in Bussing (1998) assigned the specimens collected in BCWR to 61 species (Appendix A Table A-S1).

3.2 Expert Morphological Identification

Morphological identifications of the specimens collected in BCWR were completed by curators of ichthyology at the University of Costa Rica and Royal Ontario Museum. Cichlid fishes were identified from samples collected in 2013, 2015, and 2019. Specimens from all taxa were identified for the 2019 collection. A total of 29 species were identified (Appendix A Table A-S2).

3.3 Barcode-based identification

I analyzed a total of 240 sequences from specimens collected in BCWR. The results of the top 3 species matches for each specimen in the BCWR collection for each database can be found in Appendix 1. The barcode approach assigned specimens to 51 different species using the Genbank database and 49 different species using the BOLD database (Appendix A Table A-S3).

Of the 240 specimens and associated sequences that were identified using the search functions in the databases, 93 were identified with high percent similarity matches (>98%) to one species. This included 19 species (*Agonostomus monticola*, *Amatitlania siquia (nigrofasciata)*, *Alfaro cultratus*, *Archocentrus centrarchus*, *Belonesox belizanus*, *Brycon costaricensis (guatemalensis)*, *Cribroheros longimanus*, *Cribroheros rostratus*, *Ctenogobius fasciatus*, *Cynodonichthys isthmensis*, *Dormitator maculatus*, *Herotilapia multispinosa*, *Hyphessobrycon tortuguerae*, *Parachromis dovii*, *Parachromis friedrichsthalii (loisellei)*, *Parachromis managuensis*, *Phallichthys amates*, *Roeboides bouchellei* and *Vieja maculicauda*). The other 147 specimens could not be identified to species level due to match similarity below 98% or high similarity matches (>98%) to multiple species. Both of these cases prevented a clear identification for the queried sequences.

3.4 Integrative Identification

My integrative approach assigned 240 specimens to 42 species (Appendix A Table A-S4). Eight specimens could only be identified to the genus level using this method: *Hypostomus* sp 1 (N10526, N10528), *Anchoviella* sp 1 (N13542, N13543, N13631, N13635), and *Eleotris* sp 1 (N13598, N13605). Identifications using the integrative approach included 5 species that were only determined using this method (*Centropomus* cf. *ensiferus*, *Centropomus pectinatus*, *Mugil* cf. *hospes*, *Parachromis dovii*, *Achirus* cf. *lineatus*) (Appendix A Table A-S4).

3.5 Comparison of Specimen identification methods

To compare the different approaches to specimen identification, the 68 specimens that had field-based identifications, expert-based identifications, and barcode-based identifications from both databases were compared to identifications provided by the integrative approach (Figure 4). I assumed that the integrative approach provides the best identification for a given specimen. Expert-based identifications produced the same identifications as the integrative approach more often than the barcode-based or field-based identifications (Figure 4). Interestingly, the barcode-based identifications and the field-based identifications both produced species level identifications that matched the integrative approach nearly 50% of the time (field-based 48.53%, BOLD database 54.41% and Genbank 51.47%) (Figure 4). Identifications by experts using morphology provided the same genus level identification as the integrative

approach nearly 100% of the time, whereas the barcode-based approach matched nearly 90% of the time (86.76 for BOLD, 91.18 for Genbank), and field-based was nearly 71% (70.59%) (Figure 4).

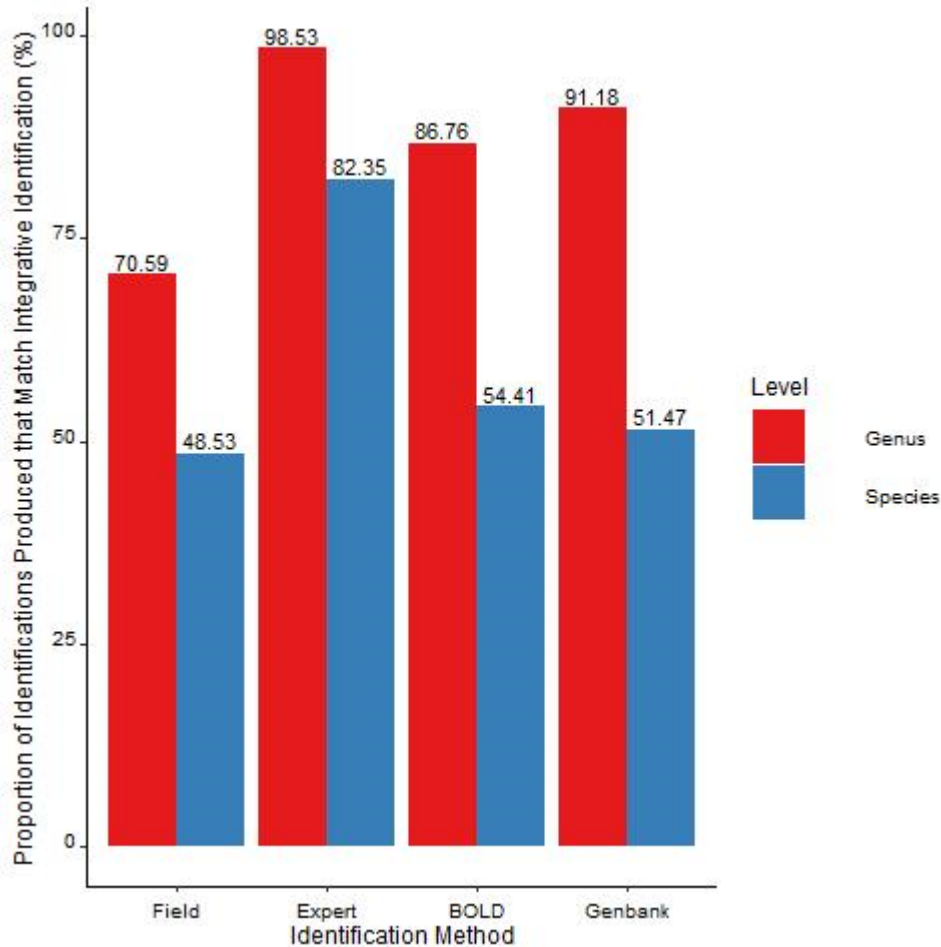


Figure 4. Comparison of identification methods for 68 specimens of the BCWR collection. Each of the four methods (field-based, expert-based and barcode-based from BOLD and Genbank) were compared to the identification provided by the integrative approach and the proportion of matching identifications is shown as a percent. This comparison was done at both the species and genus level.

I also assessed the effectiveness of identification methods by comparing the identifications for a larger pool of 240 specimens that had field-based IDs, barcode-based IDs, and integrative approach-based IDs. For this analysis, I compared field and barcode IDs to integrative-based IDs. As observed in the previous comparison, overall species identifications based on both field and barcode approaches are less accurate compared to the integrative

approach (Figure 5). The field-based approach performed similarly in this comparison as it did in the previous. Field-based IDs matched the integrative approach less than 50% (40.83%) of the time at the species level and less than 75% (72.92%) of the time at the genus level (Figure 5). As seen in the previous comparison, the field-based method and the barcode-based method had a similar success rate at the species level, where all three methods produced the same identification as the integrative approach less than 50% of the time (field-based 40.83%, BOLD 42.08%, and Genbank 43.75%) (Figure 5). The barcode-based approaches were more successful at identifying specimens to the genus level than the field-based identifications.

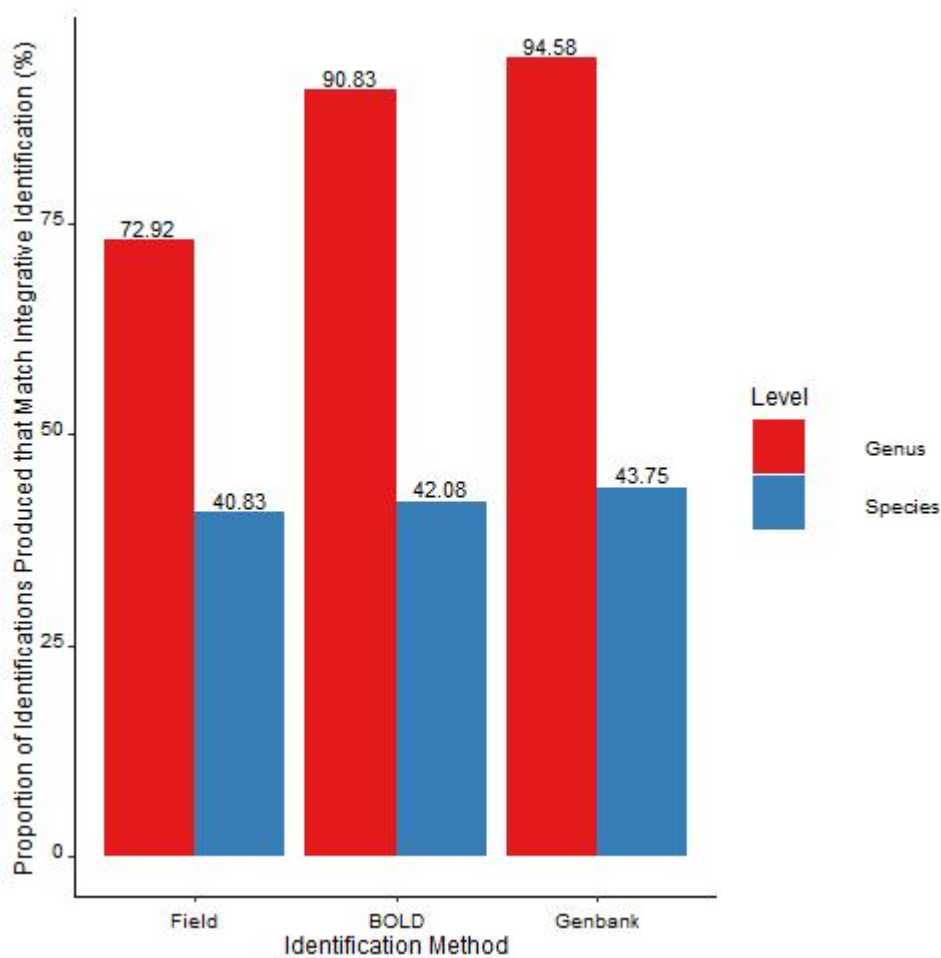


Figure 5. Comparison of identification methods for 240 specimens of the BCWR collection. Species identifications using the three methods were compared to the identification provided by the integrative approach. The proportion of matching identifications between the methods is shown as a percent. This was done at both the species and genus level.

3.6 Holistic Species List for BCWR

My holistic approach to identification that incorporated species from multiple sources of data found 51 species from 41 genera, 21 families, and 15 orders in BCWR (Table 3). The families with highest number of species in BWCR are Cichlidae, Characidae, and Poeciliidae (Figure 6). The holistic approach revealed the presence of two non-native species: the African cichlid *Oreochromis sp1* and the South American loricariid catfish *Hypostomus sp 1*. The *Hypostomus sp 1* was not known to occur in the region prior to this study. The holistic approach found 22 additions to the list of species found in BCWR by Mason (2008). The integrative approach also revealed three additions to the list of species present in BCWR compared to Angulo et al. (2013)'s list of fishes in the Tortuguero region.

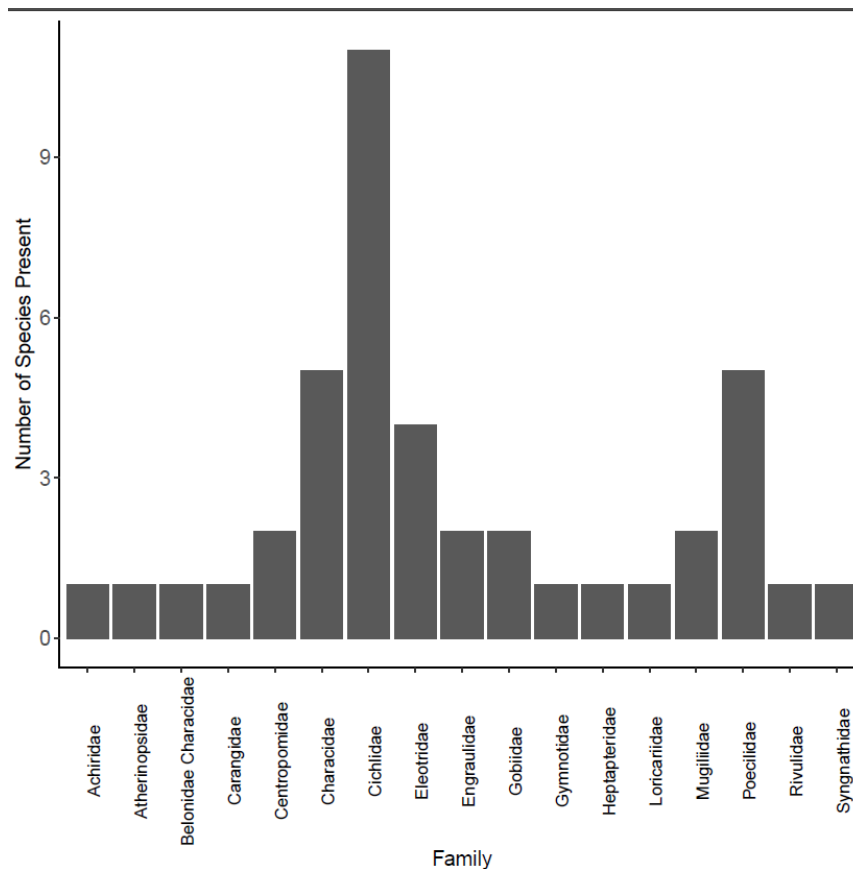


Figure 6. Number of species in each family of fishes found in BCWR.

Table 3. Species identified in the Barra del Colorado Wildlife Refuge using a holistic approach. Presence of each species at each site is indicated, with site labels matching Figure 2 and Table 1. Photo indicates that the species was included in the list based on photographic evidence.

ORDER	FAMILY	GENUS	SPECIES	A	B	C	D	E	F	G	H	I	J
CARCHARHINIFORMES	Carcharhinidae	<i>Carcharhinus</i>	<i>leucas</i>	photo									
LEPISOSTEIFORMES	Lepisosteidae	<i>Atractosteus</i>	<i>tropicus</i>	photo									
CLUPEIFORMES	Engraulidae	<i>Anchoviella</i>	<i>sp 1</i>			x							
		<i>Lycengraulis</i>	<i>grossidens</i>				x						
CHARACIFORMES	Characidae	<i>Astyanax</i>	cf. <i>nicaraguensis</i>	x	x	x	x		x	x		x	x
		<i>Astyanax</i>	<i>bransfordii</i>		x								
		<i>Brycon</i>	<i>costaricensis</i>		x							x	
		<i>Hyphessobrycon</i>	<i>tortuguerae</i>		x								
		<i>Roeboides</i>	<i>bouchelli</i>		x	x	x			x		x	
SILURIFORMES	Heptapteridae	<i>Rhamdia</i>	cf. <i>guatemalensis</i>			x	x			x		x	
	Loricariidae	<i>Hypostomus</i>	<i>sp 1</i>								x		
GYMNOTIFORMES	Gymnotidae	<i>Gymnotus</i>	cf. <i>maculosus</i>	x	x		x					x	
ATHERINIFORMES	Atherinopsidae	<i>Atherinella</i>	cf. <i>chagresi</i>		x	x	x					x	
CYPRINODONTIFORMES	Rivulidae	<i>Cynodonichthys</i>	<i>isthmensis</i>	x									
	Poeciliidae	<i>Alfaro</i>	<i>cultratus</i>	x	x	x	x					x	
		<i>Belonesox</i>	<i>belizanus</i>		x	x							x
		<i>Brachyrhaphis</i>	<i>holdridgei</i>	x			x						
		<i>Brachyrhaphis</i>	<i>parismina</i>	x									
		<i>Phallichthys</i>	<i>amates</i>	x	x	x	x						
		<i>Poecilia</i>	cf. <i>gillii</i>	x			x	x	x	x			x

		<i>Poecilia</i>	<i>sp. Tipitapa</i>						x					
BELONIFORMES	Belonidae	<i>Strongylura</i>	<i>timucu</i>		x									
		<i>Strongylura</i>	<i>notata</i>	photo										
SYNGNATHIFORMES	Syngnathidae	<i>Microphis</i>	<i>lineatus</i>		x		x					x	x	x
SYNBRANCHIFORMES	Synbranchidae	<i>Synbranchus</i>	<i>marmoratus</i>	x										
PERCIFORMES	Centropomidae	<i>Centropomus</i>	<i>cf. ensiferus</i>				x							
		<i>Centropomus</i>	<i>pectinatus</i>				x							
	Lutjanidae	<i>Lutjanus</i>	<i>griseus</i>	photo										
MUGILIFORMES	Mugilidae	<i>Agonostomus</i>	<i>monticola</i>							x				
		<i>Mugil</i>	<i>cf. hospes</i>					x						
PERCIFORMES	Cichlidae	<i>Amatitlania</i>	<i>siquia</i>	x	x		x	x						
		<i>Amphilophus</i>	<i>alfari</i>	photo										
		<i>Amphilophus</i>	<i>citrinellus</i>		x		x	x					x	x
		<i>Archocentrus</i>	<i>centrarchus</i>				x	x						x
		<i>Amphilophus</i>	<i>longimanus</i>				x			x				
		<i>Amphilophus</i>	<i>rostratus</i>			x	x	x						
		<i>Herotilapia</i>	<i>multispinosa</i>					x				x		
		<i>Oreochromis</i>	<i>sp 1</i>						x					
		<i>Parachromis</i>	<i>dovii</i>										x	
		<i>Parachromis</i>	<i>friedrichsthalii</i>	x	x		x	x						x
		<i>Parachromis</i>	<i>managuensis</i>			x	x		x					x
		<i>Vieja</i>	<i>maculicauda</i>				x							x
	Eleotridae	<i>Dormitator</i>	<i>maculatus</i>	x										

		<i>Eleotris</i>	<i>amblyopsis</i>	x	x		x							x
		<i>Eleotris</i>	<i>sp 1</i>		x		x							
		<i>Gobiomorus</i>	<i>dormitor</i>		x		x	x			x	x	x	x
	Gobiidae	<i>Awaous</i>	<i>banana</i>							x				
		<i>Ctenogobius</i>	<i>fasciatus</i>				x							
PLEURONECTIFORMES	Achiridae	<i>Achirus</i>	<i>cf. lineatus</i>									x		
		<i>Trinectes</i>	<i>paulistanus</i>				x							
TESTRAODONTIFORMES	Tetraodontidae	<i>Sphoeroides</i>	<i>testudineus</i>	photo										

3.7 Species Accumulation Curve

The species accumulation curve (SAC) for the BCWR collections shows an upward trend that has not yet met an expected horizontal asymptote (Figure 7). Extrapolation of this curve using the nls method “lomolino”, indicates an asymptote at 62 species, which is 20 more species than the observed 42. This indicates that there are still more species to find in BCWR and the current diversity assessment is incomplete. A similar result is observed for the SAC generated for the expeditions completed at site D (Figure 8).

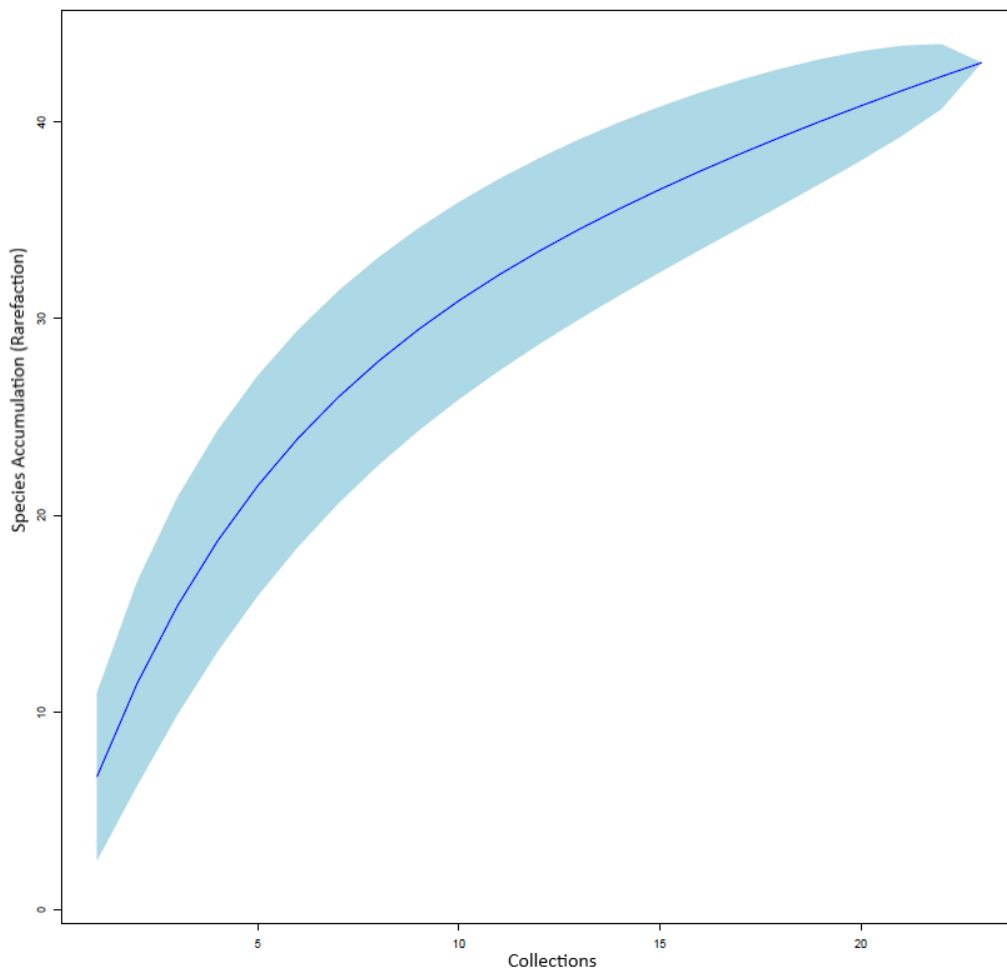


Figure 7. Species Accumulation Curve using rarefaction for the BCWR collection. Shaded area around the curve represents a 95% confidence interval.

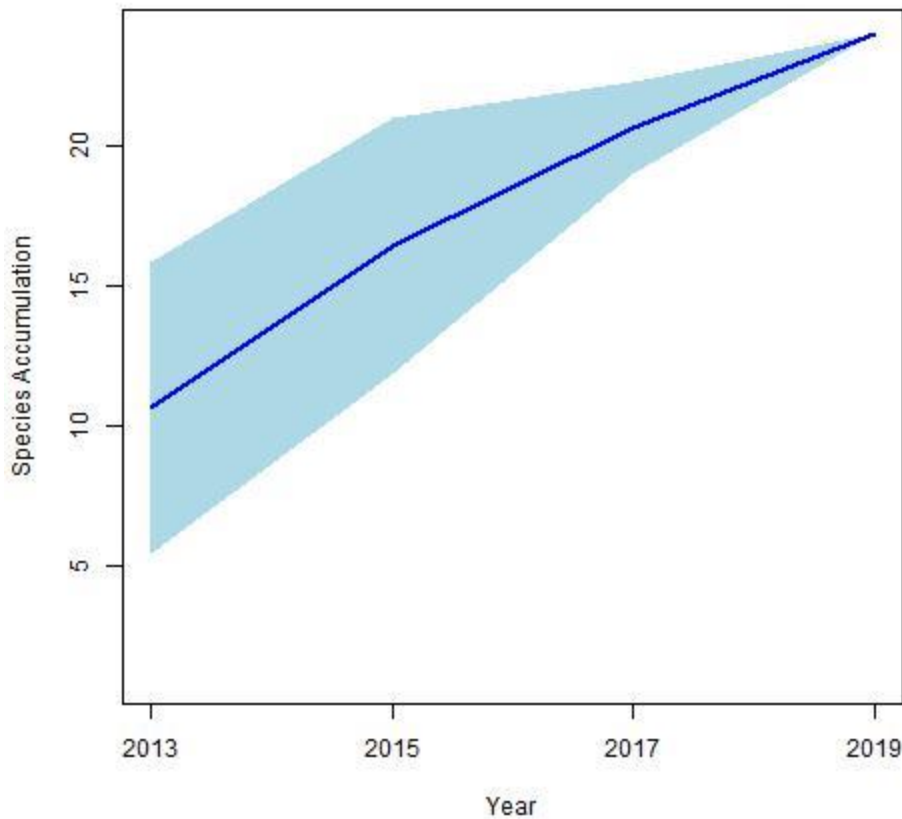


Figure 8. Species accumulation curve for Site D. Shaded area around the curve represents a 95% confidence interval

3.8 Establishment of a Curated Barcode Reference Library based on Regional Observations

My goal was to generate a barcode reference library specifically for freshwater fish species in BCWR. A list of species that could occur in the BCWR was created using observation data from previous diversity assessments and the Global Biodiversity Information Facility (GBIF) (Appendix B Table B-S2). This list represented the wider Tortuguero region since no formal studies had been done in BCWR. This regional list includes 162 species in 105 genera and 43 families. The inclusion of sequences from public databases (297 barcodes for 101 species) and from my barcodes (155 barcodes for 38 species) resulted in the reference library including 452 barcodes for 139 species (or 85%) of the regional list (Appendix C Table C-S1).

Every effort was made to obtain four sequences for each species, but for 30 of the 101 species only 1-3 sequences exist. For these species, all available sequences were used for the library. For each species represented by my barcodes all available sequences were used. Of the 162 species on the regional list, 23 had no representatives in the public databases and were therefore not included in the library.

3.9 Details of the Integrative Approach

For specimens whose expert or field identifications and database identifications did not agree and/or whose database identification was less than 98% similar to the BCWR specimen and/or was with ambiguities, I followed the protocol outlined in Figure 3. The results of these analyses can be found in Appendix B Table B-S1.

4 Discussion

My project aim was to determine the diversity of freshwater fishes in the Barra del Colorado Wildlife Refuge using an integrated approach to species identification, and a holistic approach to determine species present in the area. This project also aimed to assess the effectiveness of different species identification methods in comparison to an integrative approach for species identification. These methods included morphological analysis in the field following Bussing (1998), morphological analysis in the lab by experts, and the identification of specimens using DNA barcoding and the two databases Genbank and BOLD. I also present the first regional curated barcode library for use in future biomonitoring projects.

4.1 Fish Species Diversity in BCWR

4.1.1 Holistic Approach

Based on my holistic approach, I determined the presence of 51 species from 41 genera, 21 families, and 15 orders at BCWR (Table 3). The holistic approach adds 22 species to Mason's (2008) unpublished observations of species present in BCWR. In comparison to Angulo et al (2013), this project adds three new species records for the region: *Mugil* cf. *hospes*, *Poecilia* sp Tipitapa, and *Hypostomus* sp 1. Two species that I detected, *Hypostomus* sp 1 and *Oreochromis* sp 1, are non-native.

My holistic approach (incorporating multiple lines of evidence for the presence of a species in an area) provides a more complete understanding of the diversity in BCWR. Relying on physical specimens or molecular data excludes species from datasets. In this project, where certain species are unlikely to be captured because of the collection methods used, only including species captured by these methods excludes valuable diversity information (Lapointe et al., 2006; Kubečka et al., 2012; Oliveira et al., 2014; Diaz Pauli et al., 2015). For example, the bull shark (*Carcharhinus leucas*) is known to occur in the region (Tuma, 1976; Angulo et al., 2013), but we did not collect it because of the methods we used. However, the bull shark has been caught on hook and line and photographed in the area sampled. Thus, I include it in the list of species.

The families with the most species collected were the Characidae (5 species), the Cichlidae (11 species), and the Poeciliidae (5 species). Similar trends have been observed in other Central American countries where similar studies have been done (Valdez-Moreno et al., 2005, 2009; Matamoros et al., 2009).

4.1.2 Species Accumulation

Examination of the species accumulation curve generated for all collection efforts suggests that the full diversity of species in the region has not yet been fully sampled, and that there are additional, probably rare species to be collected in BCWR (Figure 6). This is further demonstrated by extrapolation of the SAC where the maximum number of species is 62 and current sampling found 42 species. The SAC produced by vegan treated each site visit (collection) as a separate sampling effort. The SAC produced from Site D, which was visited during each year of collection effort, shows a similar trend of accumulation (Figure 8). Overall, these two measures suggest that the present study has not fully documented the diversity of freshwater fishes in BCWR.

4.2 New Species Records in BCWR

Based on my holistic approach to determine species present in BCWR, 22 species were recorded that were not present in Mason's (2008) unpublished species list, however 18 of these species were unsurprising since they are recorded in the wider Tortuguero regions by Angulo et al. (2013). Four of the most interesting species are discussed here. The species *Mugil cf. hospes*, has not been recorded in BCWR (based on Mason (2008)) or in the wider Tortuguero region

(based on Angulo et al. (2013)). The species *Poecilia sp* Tipitapa is a putative undescribed species, first mention by Bagley et al. (2015), and has not been recorded in Costa Rica previously. I found evidence for two non-native species. One of these, *Hypostomus sp* 1, has not been recorded in Costa Rica previously. The other, *Oreochromis sp* 1 has not been recorded in BCWR (based on Mason (2008)) but has been recorded in the wider Tortuguero region (based on Angulo et al. (2013)).

4.2.1 *Mugil cf. hospes*

Mulletts (family: Mugilidae) are commercially important, widespread, and exist across coastal marine, brackish and freshwater environments in tropical and temperate seas (Fraga et al., 2007). This group is also characterized by uniform external and internal morphology, which can cause confusion and discrepancies in identifications (Fraga et al., 2007; Nirchio et al., 2018; Neves et al., 2020). The interrelationships within this family are also still debated (Xia et al., 2016). Results from this project suggest the presence of a species of *Mugil* not previously known to occur in the area: *Mugil cf. hospes*.

Initial results from the databases identified the BCWR specimens as both *Mugil hospes* and *Mugil trichodon* with less than one percent divergence (Appendix B). There were four sequences from *Mugil hospes* and one sequences from *Mugil trichodon* that produced these high similarity matches. There are other sequences from the public databases however, listed as both of these species that are more than six percent diverged from the BCWR specimens (six percent diverged for *Mugil hospes* and 18% diverged for *Mugil trichodon*) (Appendix B). There are two *Mugil* species listed by Angulo et al. (2013) listed as occurring in Tortuguero however, representatives of these sequences were more than 18% diverged from the BCWR specimens (18% for *Mugil curema* and 20% for *Mugil cephalus*). The BCWR specimens were lost due to flooding so morphological identification was not possible. Since there were more public sequences listed as *Mugil hospes* than *Mugil trichodon* that matched the BCWR specimens, but not all representatives of these species did, I labelled the BCWR specimens as *Mugil cf. hospes* where the term “cf” represents the level of uncertainty of this identification.

The distribution of the species *Mugil hospes* is still debated due to the poor diagnostic power of conventionally used morphological characters that confuse specimen identifications;

however, the species is known to occur from Columbia to Brazil in freshwater, in the coastal waters of Cuba, and in marine waters from Florida to Brazil (Ibanez et al. 2011). Ibanez et al. (2011) also state that the species occurs in Mexico. *Mugil hospes* is often confused with the *Mugil curema* due to the morphological overlap that characterizes the family (Ibanez et al., 2011; Nirchio et al., 2018). Previous studies have also found evidence suggesting the two species are genetically similar (Ibanez et al., 2011). In addition, previous studies have found multiple lineages within *Mugil hospes*, with authors noting a pacific lineage that ranges from Mexico to Ecuador, and an Atlantic lineage that ranges from Belize to Brazil (Durand et al., 2012, 2017; Nirchio et al., 2018). Nirchio and colleagues (2018) also found that specimens of *Mugil hospes* collected in the Atlantic Ocean were only 92-93% similar to those found in freshwater. Angulo and colleagues (2013) list *Mugil curema* as occurring in the wider Tortuguero region in which BCWR is located however, specimens collected in BCWR were substantially diverged (18%) from public data sequences representing *Mugil curema*. This may not be completely informative since there is evidence for distinct mitochondrial lineages within the *Mugil curema* complex (Neves et al., 2020). Given the confusion surrounding the taxonomy of this group, there is a possibility that the specimens collected in BCWR are not representative of a non-native species in the area but rather a native undocumented species unknown until molecular studies. These specimens were lost following flooding at COTERC, thus, to confirm the presence of a new species to BCWR, additional sampling is required.

4.2.2 *Poecilia sp* Tipitapa

I found evidence for the presence of a *Poecilia* species that, based on other studies, is likely an undescribed species. The single specimen was identified in the field as *Poecilia gillii*, however Genbank and BOLD identified the specimen as *Poecilia sp.* Tipitapa (99.70% similarity). The name *Poecilia sp.* Tipitapa was first proposed by Bagley et al (2015) while conducting a multilocus approach study to delimit species within the *Poecilia sphenops* complex. Bagley et al. (2015) found that the *Poecilia sp.* Tipitapa lineage was most similar to the *Poecilia mexicana* lineages, but importantly, the putative species was different from *Poecilia mexicana*, *Poecilia gillii* and *Poecila sphenops*. Specimens of *Poecilia sp.* Tipitapa were collected in the Rio Tipitapa, which flows down to Lake Nicaragua (Bagley et al., 2015). This lake drains into the San Juan River, which runs towards the Caribbean coast along the border between Nicaragua and Costa Rica, also feeding the smaller rivers and canals of BCWR, including those that we

surveyed. The connectivity between these rivers can explain the presence of *Poecilia sp* Tipitapa in BCWR. In addition, Bagley et al. (2015) explain that the initial divergence of this taxon is likely connected to the origin and isolation of the Nicaraguan depression. This is the first record of this putative species in Costa Rica.

4.2.3 *Oreochromis sp* 1

One cichlid specimen was captured in BCWR during the 2013 expedition and was identified as *Oreochromis sp* 1, primarily using genetic data. The species *Oreochromis aureus* and *Oreochromis niloticus* naturally occur in Africa and arrived in Central American (including Costa Rica) as a result of aquaculture (Angulo et al., 2013; Rognon & Guyomard, 2013; Gu et al., 2014; Bbole et al., 2018). Because this genus does not naturally occur in the area, identification of this specimen in the field using morphology resulted in the specimen being identified as *Aequidens corulespunctatus*, another member of the Cichlidae family (Bussing, 1998). Additional morphological studies of this specimen proved difficult because it was a small juvenile.

Both Genbank and BOLD identified the BCWR specimen as a member of the genus *Oreochromis*, specifically the BCWR matched sequences of both *Oreochromis niloticus* and *Oreochromis aureus*. While there are many representative sequences of both species in the public databases, inconsistencies with their identifications make using these problematic. Also, high percent similarity matches that were not part of the top three matches included public data listed as *Oreochromis niloticus* x *Oreochromis aureus*. These two species have been hybridized for aquaculture (Liu et al., 2020). There is evidence for differential introgression of mitochondrial DNA (mtDNA) from *Oreochromis aureus* to *Oreochromis niloticus* making the risk of misidentification using only mtDNA or a single marker, high (Rognon & Guyomard, 2003; Luo et al., 2021). Due to issues identified using mtDNA for identification, and issues with identification of current reference sequences, the specimen from BCWR could not be identified to the species level and is named *Oreochromis sp* 1. It seems likely that the specimen represents *Oreochromis aureus* or *Oreochromis niloticus*. In this case, DNA barcoding does aid in identification however it would be ideal to collect adult specimens to confirm the species identity and utilize their sequences for future identification using barcoding.

Although *Oreochromis* has been reported in Costa Rica (Angulo et al., 2013), this is the first record of this species from in the Barra del Colorado Wildlife Refuge. Species of *Oreochromis* have been shown to have negative effects on water systems by competing with native species (Canonico et al., 2005). *Oreochromis* species compete with native species for breeding ground and are capable of reproducing in great numbers quickly (Canonico et al., 2005). These fish also compete directly with native species for food sources and have been documented consuming larvae and eggs, putting additional pressure on native species (Bittencourt et al., 2014). Introduced *Oreochromis* species can also hybridize with native cichlid species, causing gene pool deterioration (Canonico et al., 2005). The species *Oreochromis niloticus* can also contribute to the eutrophication of water, altering ecosystems and affecting non-fish species (Figueredo & Giani, 2005). It is also concerning that the specimen collected in this project is a juvenile. This suggests that the species is breeding in BCWR. Given the likely presence of a non-native *Oreochromis* species in BCWR, a better understanding of the impacts of this species on native BCWR species should be determined.

4.2.4 *Hypostomus* sp 1

The catfish genus *Hypostomus* currently contains 140 recognized species and is one of the most species rich genera in the order Siluriformes (Soares et al., 2021). *Hypostomus* species diversity is highest in central South America, but the genus includes species with ranges that extend to Costa Rica in the north and Argentina in the south (Jardim de Queiroz et al., 2020). Several *Hypostomus* specimens were collected in BCRW, and the genus *Hypostomus* has been recorded in the wider Tortuguero region prior to this study (Bussing, 1998; Cramer et al., 2011; Angulo et al., 2013).

The specimens collected for this project were initially identified as *Hypostomus aspidolepis*, a species that is native in Costa Rica (Angulo et al., 2013). However, the BCWR specimens were approximately six percent diverged from public sequences of *Hypostomus aspidolepis*, suggesting that the BCWR sequences do not represent *Hypostomus aspidolepis* (Appendix B). Instead, the BCWR specimens were 100% similar to several sequences with different *Hypostomus* species names from South America (Appendix B Table B-S1). Despite the taxonomy in the databases, this high level of sequence similarity indicates that the BCWR and the 100% matching sequences likely represent a single species, which I name *Hypostomus* sp 1.

(Appendix B). *Hypostomus* sp 1 is approximately three percent diverged from all other *Hypostomus* sequences in Genbank and BOLD. Unfortunately, since *Hypostomus* sp 1 sequences have multiple species names in the databases, a more conclusive species identification could not be established.

Interestingly, *Hypostomus* sp 1 has now been collected in Costa Rica (this study) and on both the eastern and western side of the Andes Mountain in South America. This is an unusual distribution pattern, as the Andes serves as a geographic barrier to gene flow (Bicudo et al., 2019). Typically, closely related but distinguishable species are found on either side of the mountain ranges like the Andes (Bagley & Johnson, 2014). Additionally, *Hypostomus* is not known to be migratory. Thus, it is most likely that the distribution of this species is partly the result of human mediated introduction (Montoya-Burgos, 2003; Hoover et al., 2014; Matamoros et al., 2015; Cook-Hildreth et al., 2016). Matamoros et al. (2016) also recorded the presence of a non-native *Hypostomus* species in Nicaragua, whose introduction they attributed to the pet trade. Other species of non-native *Hypostomus* have also been recorded in Costa Rica (Solano & Arias, 2011; Fonseca-Hernández & Vargas-Alpizar, 2018). There have also been reports of an invasive *Hypostomus* species in Texas and Florida (Cook-Hildreth et al., 2016; Rob Robbins personal communication). Tests should be done to determine if the species recorded in the U.S., Nicaragua, and other parts of Costa Rica, is the same species as specimens found in BCWR.

The presence of a non-native species of *Hypostomus* in BCWR is significant. *Hypostomus* has been associated with negative ecological impacts including streambank erosion and competition with threatened species for habitat and food (Pound et al., 2011; Hoover et al., 2014). The facilitation of movement of non-native fishes via the aquarium trade is a documented issue in Costa Rica (Allen et al., 2017). The presence of juvenile specimens for this species is also concerning as it suggests this species is breeding in BCWR. This highlights the need for ecological assessments in BCWR to discern the effect on this non-native species on local ecosystems (Hoover et al., 2014; Cook-Hildreth et al., 2016; Fonseca-Hernández & Vargas-Alpizar, 2018). This case again shows the utility of DNA barcoding. The original specimens collected were juveniles and morphological identification was not possible, thus the identification of a non-native species was only possible with barcoding.

4.3 Comparison of Approaches for Species Identification

Since no previous studies had assessed the diversity of freshwater fishes in Costa Rica using a molecular approach like DNA barcoding, I wanted to determine how different approaches to specimen identification compared. As previously discussed, integrative approaches are increasing in popularity to answer a wide range of biological questions (Will et al., 2005; Gomes et al., 2015; Hernández-Mena et al., 2019; Duong et al., 2020). One use for an integrative approach is in the identification of specimens (Pires & Marinoni, 2010; Sheth & Thaker, 2017). By integrating different data sources, we can provide more robust specimen identifications (Pires & Marinoni, 2010). For the following analyses, I assumed that the identifications provided by the integrative approach are the best identifications available and used them to assess the accuracy of other identification methods.

4.3.1 DNA Barcoding

I initially hypothesized that DNA barcoding would provide better species identifications than other methods. However, for the specimens collected in BCWR, DNA barcoding did not identify all specimens. When looking at the 68 specimens that had been identified using all identification methods (field-based, expert identified in the lab, barcoded using Genbank, and BOLD), the barcode-based identifications from both databases produced the same identification as the integrative approach less than 50% of the time at the species level (Figure 4). Interestingly, the success of the barcode-based identifications was similar to the field-based identifications performed by non-experts using a key that was identified in Angulo et al. (2013) as already missing 108 species known to occur in the broader region. The databases were however able to successfully identify specimens to the genus level more than 85% of the time (Figure 4). While this provides some information about the diversity of freshwater fishes in BCWR, it does not provide the adequate detailed information authors have described as necessary for conservation of biodiversity (Dudgeon et al., 2006). Not all specimens used in this project had expert-based identifications; however, I could still compare the identifications for the 240 specimens with identifications that were field-based, barcoded using Genbank, and barcoded using BOLD (Figure 5). This comparison shows similar results, where the success rate of barcode identification using both databases was less than 50% and was similar to the success rate of field-based identifications (Figure 5).

Why is the barcoding identification approach so unsuccessful for BCWR fishes? When using the barcode databases, I observed multiple instances where the databases were inconsistent in their designation of a species match (providing multiple species as matches) or were unable to provide a close match. These difficulties had several causes, as discussed below.

In some cases, I found that specimens/barcodes in the reference libraries were likely misidentified, leading to inconsistencies in query species identifications. The genus *Gobiomorus* is a good example. Barcode-based identifications of specimens from BCWR indicated the species *Gobiomorus dormitor* and *Gobiomorus maculatus* as matches (Appendix B Table B-S1). When conducting additional analyses on this group, I found that there were two phylogenetically distinct groups of barcodes within the public data sequences, likely representing *Gobiomorus dormitor* and *Gobiomorus maculatus*. However, within each of these two groups there were sequences listed as both *Gobiomorus dormitor* and *Gobiomorus maculatus*. Since the databases could not provide an identification to determine the likely identification for my specimens, I needed to better understand the ecology and distributions of the two species. Thacker and Hardman (2005) conducted a multilocus study on Gobiodei to investigate the evolutionary relationships of these fishes. They found that within the Neotropical gobioids, there were five examples of geminate taxa occurring on either side of the isthmus of Panama (Thacker & Hardman, 2005). Within each pair, one species occurred in westward flowing Pacific drainages and the other in eastward flowing Atlantic drainages (Thacker & Hardman, 2005). Costa Rica shares a similar geologic pattern and there are distinct assemblages of fishes on either side of the Cordillera (Angulo et al., 2013). Angulo and colleagues (2013) list *Gobiomorus maculatus* as occurring on the Pacific slope, and *Gobiomorus dormitor* along the Atlantic slope following the pattern Thacker and Hardman (2005) described. Thacker and Hardman (2005) hypothesize of the split between these two species around 12.5-17.8 million years ago, suggesting ample time for divergence. In the sequences I collected from the public databases, one group represents the Atlantic and one represents the Pacific. This information strongly suggests that the two phylogenetically distinct groups of sequences in the barcode databases correspond to the two different species *Gobiomorus dormitor* and *Gobiomorus maculatus* but that samples within each group have been misidentified. Thus, although differentiating between these two species using barcode databases should be relatively straightforward, sample misidentifications mean this is not the case. Database barcode misidentifications can create a “snowball” effect, as

samples erroneously identified are themselves entered in the databases leading to more errors in later projects.

I found that some species in the barcode databases, while not necessarily misidentified, were labelled with outdated taxonomic names. An example is the Cichlidae species *Vieja maculicauda*. There are sequences in both databases listed as *Paraneetroplus maculicauda*, a genus assignment now considered invalid assignment for this species (McMahan et al., 2015). This situation is not intuitive, particularly to those who may not have a deep understanding of the taxonomy of this group. The decreased accessibility of these databases because of a requirement for deeper taxonomic understanding to sift through data such as this is problematic if the goal is conservation (Bianchi & Gonçalves, 2021). Bianchi and Gonçalves (2021) found that within the BOLD database on average 10% of misidentifications for a given taxon could be attributed to misspelling and invalid taxonomy, compromising the integrity of the database.

In many cases, there was incomplete representation of species in the barcode reference libraries. A particularly clear example of this was seen in the family Engraulidae (anchovies) (Appendix B). My initial attempts to identify these specimens using the databases default search functions did not yield any matches above 98% similarity. There are four species from this family that are listed as occurring in Tortuguero (Angulo et al., 2013) and two of these are missing from the barcode databases, including the species assigned by museum experts (*Anchoviella elongata*). Unless there is a clear match to a species, this lack of representation makes it extremely difficult to identify a specimen. In addition, without this representation it also becomes impossible to corroborate a morphological identification with other data sources such as molecular data. The databases also lack reference sequences for other species from the genera represented in Tortuguero. If there is adequate representation at the genus level, I can assign a genus level identification, however even this was difficult in this case.

The records for individual sequences in BOLD are more complete than Genbank most of the time. However, BOLD contains significant amounts of privatised data. These records are not available to the public, meaning one cannot actually check the vouchers' origin, the authors, locations, and additional details. This type of data is included in the repositories that BOLD uses to determine the identity of a queried sequence, making it more difficult for users to have

confidence in identifications. Genbank generally had fewer complete records than BOLD, but does not have privatised data meaning a user can access the records and determine which is most reliable when using the search functions to identify their specimens. When comparing the different identification methods to the integrative approach (Section 3.5) I also compared the identifications given by each database to each other. I assumed that the two databases would provide the same identification for the same specimen however this only occurred 80% of the time for species level identifications. This suggests that there are content differences between the two databases that can result in each database producing a different species identification.

Overall, I found that DNA barcoding is insufficient for identifying most specimens collected in BCWR. The low success rate of DNA barcoding for this assemblage can be attributed to the problems discussed above with current public barcode libraries. Other authors have noted similar issues with public barcode databases, highlighting the need for reviews of this data (Diaz et al., 2016; Oliveira et al., 2016; Cariani et al., 2017; Bianchi & Gonçalves, 2021; Fort et al., 2021). Authors have suggested one way to circumvent these issues is to be selective in the public data used for specimen identification (Diaz et al., 2016; Cariani et al., 2017; Oliveira et al., 2016). Building barcode reference libraries for a group or region of interest has become increasingly popular where authors create protocols for exhaustively analyzing public data to include the best public data available (ex ELASMOMED DNA Barcode Reference Library from Cariani et al (2017)) (Diaz et al., 2016; Cariani et al., 2017; Oliveira et al., 2016; Fort et al., 2021).

4.3.2 Morphology-based Identification

Morphology is the primary resource for identification of specimens and taxonomy. In hyper diverse areas like the neotropics, morphological identification can be especially difficult for non-experts and is even more so in hyper diverse groups such as freshwater fishes whose taxonomy is also sometimes not fully resolved (Schmitter-Soto, 2017). For this project, I examined and assessed two types of morphological identifications: field-based and expert-based. The field-based identifications were accomplished by students using a dichotomous key for the region by Bussing (1998). This key represents the most recent and accessible tool for non experts interested in identifying fishes in Costa Rica. The identifications given following the Bussing (1998) key were the least accurate when compared to the integrative approach (Figure 4 and Figure 5). When compared to the integrative approach, the identification done in the field using

morphology produced the same species level identification between 40% and 48% of the time and a genus level identification between 70% and 72% of the time (Figure 4 and Figure 5). This result is unsurprising given that more recent work in Costa Rica has added 108 new species that are not included in Bussing (1998) (Angulo et al., 2013). In addition, the field-based identifications were completed by students as part of an undergraduate course and were likely subject to more errors given their limited experience. This indicates that improvements to field-based identifications could derive from improvements to the Bussing (1998) key, or from additional training for the identifiers.

I also wanted to determine the success rate of expert-based morphological identifications. In this case, experts chosen were curators of fishes from natural history museums, Dr. Arturo Angulo and Dr. Hernan Lopez Fernandez. Compared to field-based and barcode-based identifications, expert-based identifications were the most successful. Expert morphology-based identifications matched the integrative identifications for specimens at the species level 82% of the time and at the genus level 98% of the time (Figure 4). The identifications provided by Dr. Lopez Fernandez were only completed for cichlids from 2013 and 2015. Dr. Angulo's work on the 2019 specimens provides the most direct comparisons since he identified all the fish specimens collected that year. These results show that expert-based identifications are more successful than barcode-based and field identifications at both the species and genus level for this region. However, experts are not always available for identification. As mentioned earlier, the taxonomic impediment is one of the biggest issues facing morphology-based identifications (Sheth & Thaker, 2017; Ricciardi et al., 2020). The number of scientists entering taxonomy and funding is decreasing, presenting a big problem as these specialists are needed for successful conservation of biodiversity (Ricciardi et al., 2020).

That expert-based identifications did not match the integrative approach at the species or genus level uniformly (Figure 4) does not spell despair for the expert-based methodology, but rather highlights how an integrative approach can guide specialists. The specimens that were not given the same identification for the integrative approach and expert based identifications included specimens of *Strongylura* and *Centropomus*. In the case of *Strongylura*, a morphological reassessment, prompted by the DNA barcode analysis, indicated that the species identity was different than initially determined based on morphology. Further analysis showed

that morphology and DNA barcodes agreed that the specimens were *Strongylura timucu*. Upon first glance, the specimens of *Centropomus* appeared to represent a single species, however molecular data suggested the presence of two species in BCWR. When this was brought to the attention of Dr. Angulo, he reassessed the specimens, revised their identifications, and confirmed the presence of two species of *Centropomus* among my specimens. Specimens belonging to the genus *Eleotris* were also not consistently identified by experts. Experts were only able to provide a genus level identification for these species due to difficulties with using conventional diagnosable characters for identification. My integrative approach however provided evidence for a species level identification (*Eleotris amblyopsis*), allowing experts to reassess the specimens to corroborate the identification.

My work demonstrates the ability of an integrative approach to work in tandem with taxonomy, and the ability of this method to highlight specimens for special attention and areas for further research. Issues arising from the taxonomic impediment are typically cited as resulting from lack of funding and interest, however when specialists do exist, they are considered the best resource for taxonomy and identification (Pires & Marinoni, 2010). While DNA barcoding may be more accessible for non-experts, currently available public barcode libraries are not adequate, and specialists are needed to identify specimens in BCWR to produce foundations for biomonitoring and conservation.

4.4 Curated Barcode Reference Library

Given the issues with current public barcode reference libraries in the identification of specimens from this project and the increasing popularity in creating region or taxon specific barcode reference libraries for identification, my BCWR Barcode Reference Library will help to bridge the gap in knowledge and bring together barcoding and taxonomy for conservation goals (Diaz et al., 2016; Oliveira et al., 2016; Cariani et al., 2017). My library contains representative sequences for 139 species from the 162 species on the regional list (Appendix B Table B-S2 and Appendix C Table C-S1). While the library contains sequences for many species, it is currently missing representatives for three species listed by the IUCN. This includes the vulnerable Mexican goby (*Ctenogobius claytonia*), the endangered Titi (*Sicydium adelum*), and the critically endangered Smalltooth Sawfish (*Pristis pectinata*). This is problematic if one of the goals of the library is biomonitoring for conservation purposes since the library as it exists currently cannot

say if the species is present. This could be solved if I can procure sequences for these species so that they can be included in the library and future projects would be able to determine if they are present in BCWR. There are also another twenty non-IUCN species without representation in the library. The library could be improved if sequences for these species could be obtained. Finally, from the 139 species with representation in the library, 101 species are represented by public data. Of these 101 species, 30 species are represented in public databases by 1-3 sequences only. I assumed that some form of representation was better than no representation at all, so despite having inclusion criteria, in these cases I chose to include sequences for these 30 species even if they did not fit all criteria. Thus, the library could be improved by including new sequences for these species that are of higher quality (based on the inclusion criteria).

4.5 Significance and Future Directions

Immediate detection of biological responses to climate change are vital to conservation and management plans (Shoo et al., 2006). Given that fish are reliable indicators of ecosystem health, we should collect sufficient baseline information on their diversity in order to assess not only range shifts, but also community health (Shoo et al., 2006; Sharma et al., 2007). In ecosystems impacted by stress, fish diversity is expected to decrease, but change cannot be noted without both frequently sampling and baseline diversity measures (Odum, 1985; Shoo et al., 2006). In addition, frequent sampling and baseline diversity measures will also allow for the introduction of invasive species and range expansions to be noted (Thomas, 2010; González-Bergonzoni et al., 2020). Aquatic communities are especially vulnerable to environmental changes, thus if protection of diversity is wanted, frequent sampling and baseline diversity measures must be taken (Araújo et al., 2018). My results demonstrate the importance of method selection and the need for proper resources to assess diversity.

For fishes, the two main methods used to identify specimens and subsequently assess the diversity of a region of interest are morphological and molecular identification. Both methods provide useful information. However, the ability to identify this diversity without issue was less than 55% when using field identification methods or current public barcode databases Genbank and BOLD. This is problematic if the goal of these methods is to determine the species present and monitor changes over time. In the case of Engraulidae (Anchovies), members of this family are important economically and biomonitoring of the populations is critical to preventing

overfishing, however the lack of representation of these species and taxonomic uncertainty makes conservation more difficult (Vicente et al., 2020). By combining different data sources to take an integrative approach, I was able to produce the best identifications for the specimens captured in BCWR. By using multiple sources of data to take a holistic approach, I was able to produce a list of species present in BCWR. I also introduce a new tool for identifying fish species in Tortuguero and BCWR. Morphological identification is extremely important for taxonomy; however, methods of non-destructive sampling may be more useful in the long run. Given that barcoding methods can be very fast and successful, once a complete and reliable library has been set up, future sampling could rely more on less destructive sampling of fishes such as fin clipping.

One possibility of future studies could be the inclusion of additional markers for taxonomic groups that were especially problematic in this project. For some groups in this project, *coI* may not be sufficient for use in assigning species identifications. Although BOLD only contains sequences for *coI*, Genbank does contain sequences from other commonly used genetic markers that could be used for identification when *coI* fails. Zhang and Hanner (2012) conducted a study assessed the potential of the mitochondrial gene *cytB* and the nuclear genes 16S and 18S for use as barcoding genes. They found that 16S and 18S suffer from base pair insertions and deletions that could cause alignment problems when comparing the user sequences to a reference library (Zhang & Hanner, 2012). Zhang and Hanner (2012) also found that while *cytB* was effective for identification, it was more difficult to create universal primers for this gene compared to *coI*. There has been success in using *cytB* for identification of non-fish groups (Kundu et al., 2020). These genes could however be helpful in cases where *coI* is not enough to assign an identity to a specimen and could be useful for problematic groups in BCWR (Parson et al., 2000; Zhang & Hanner, 2012).

I would like to test my curated barcode library against environmental DNA (eDNA) methods or bulk sampling to determine if these types of analyses could be used in future biomonitoring efforts. Since conservation plans rely on robust biodiversity estimates and a good understanding of trends in those estimates, but standard DNA barcoding practises have relied on whole taxon sampling, other sources of DNA have been explored, namely environmental DNA (eDNA) (Taberlet et al., 2012; Cristescu, 2014; Thomsen & Willerslev, 2015). The advancement

of sequencing technology has also made obtaining useful DNA from environmental samples more tangible (Cristescu, 2014). Next generation sequencing allows researchers to sequence bulk or environmental samples rather than single individuals leading to a new type of barcoding: metabarcoding (Cristescu, 2014; Cruaud et al., 2017). The time and costs associated with this method are lower than standard barcoding, which makes this method appealing for conservation and biomonitoring projects (Cristescu, 2014). The use of eDNA can also be helpful in determining the presence of rare species that are more difficult to catch (Balasingham et al., 2018; Ruppert et al., 2019; Schroeter et al., 2020). Researchers have employed this method for assessing diversity in fishes and have developed protocols and primers for sequencing (Miya et al., 2015; Gillet et al., 2018). eDNA has also been tested for long term biomonitoring projects in fishes and results from these suggest eDNA can describe communities (Hänfling et al., 2016). However, there is also evidence that current public libraries may not be sufficient for identifying species from eDNA samples, and regional libraries like mine may become more relevant as this methodology advances (Locatelli et al., 2020). Future work could explore the use of metabarcoding for neotropical fishes and collect eDNA samples to test metabarcoding efficiency for these samples.

Lastly, I would also like to continue building my barcode reference library, with additional sampling or by searching for museum tissue samples with voucher specimens, to replace species currently represented by public data with sequences from specimens captured in BCWR or the wider Tortuguero region.

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Appendices

Appendix A:

Species Lists from Four Identification Methods

Table A-S1. Species identified in BCWR using morphology in the field following Bussing (1998).

ORDER	FAMILY	SPECIES
CLUPEIFORMES	Engraulidae	<i>Anchoviella sp</i>
		<i>Engraulidae sp</i>
CHARACIFORMES	Characidae	<i>Astyanax aeneus</i>
		<i>Astyanax bransfordii</i>
		<i>Astyanax nicaraguensis</i>
		<i>Astyanax sp</i>
		<i>Bramocharax bransfordii</i>
		<i>Brycon guatemalensis</i>
		<i>Bryconamericus sp (cf. aeneus)</i>
		<i>Hyphessobrycon tortuguerae</i>
		<i>Roeboides bouchellei</i>
SILURIFORMES	Heptapteridae	<i>Rhamdia nicaraguensis</i>
	Loricariidae	<i>Hypostomus aspidolepis</i>
GYMNOTIFORMES	Gymnotidae	<i>Gymnotus cylindricus</i>
		<i>Gymnotus maculosus</i>
ATHERINIFORMES	Atherinopsidae	<i>Atherinella argentea</i>

		<i>Atherinella chagresi</i>
		<i>Atherinella milleri</i>
		<i>Atherinella sardina</i>
		<i>Atherinella sp</i>
CYPRINODONIFORMES	Rivulidae	<i>Cynodonichthys isthmensis</i>
		<i>Rivulus sp</i>
	Poeciliidae	<i>Alfaro cultratus</i>
		<i>Belonesox belizanus</i>
		<i>Phallichthys amates</i>
		<i>Poecilia cf. gillii</i>
		<i>Poecilia gillii</i>
		<i>Poecilia mexicana</i>
		<i>Poecilia sp 2</i>
		<i>Poeciliopsis elongata</i>
BELONIFORMES	Belonidae	<i>Strongylura marina</i>
		<i>Strongylura sp</i>
		<i>Strongylura timucu</i>
SYNGNATHIFORMES	Syngnathidae	<i>Microphis brachyurus</i>
		<i>Pseudophallus mindii</i>
PERCIFORMES	Centropomidae	<i>Centropomus cf. robalito</i>
		<i>Centropomus paralellus</i>
	Carangidae	<i>Trachinotus carolinus</i>
MUGILIFORMES	Mugilidae	<i>Agonostomus monticola</i>

		<i>Mugil sp</i>
PERCIFORMES	Cichlidae	<i>Aequidens coeruleopunctatus</i>
		<i>Amatitlania nigrofasciatus</i>
		<i>Amatitlania septemfasciata</i>
		<i>Amphilophus citrinellus</i>
		<i>Archocentrus centrarchus</i>
		<i>Archocentrus nigrofasciatus</i>
		<i>Archocentrus sp</i>
		<i>Astatheros rostratus</i>
		<i>Cribroheros rostratus</i>
		<i>Herotilapia multispinosa</i>
		<i>Parachromis loisellei</i>
		<i>Parachromis managuensis</i>
		<i>Parachromis sp</i>
		<i>Vieja maculicauda</i>
	Eleotridae	<i>Dormitor maculatus</i>
		<i>Eleotris amblyopsis</i>
		<i>Eleotris cf. pisonis</i>
		<i>Eleotris sp</i>
		<i>Hemieleotris latifasciatus</i>
	Gobiidae	<i>Awaous banana</i>
PLEURONECTIFORMES	Achiridae	<i>Trinectes paulistanus</i>

Table A-S2. Species identified in BCWR by experts using morphology. Collection year listed in the last 3 columns.

ORDER	FAMILY	SPECIES	2013	2015	2019
CLUPEIFORMES	Engraulidae	<i>Anchoviella elongata</i>			x
CHARACIFORMES	Characidae	<i>Astyanax bransfordii</i>			x
		<i>Astyanax nicaraguensis</i>			x
		<i>Brycon costaricensis</i>			x
		<i>Hyphessobrycon tortuguerae</i>			x
		<i>Roeboides bouchellei</i>			x
SILURIFORMES	Heptapteridae	<i>Rhamdia guatemelensis</i>			x
ATHERINIFORMES	Atherinopsidae	<i>Atherinella cf chagresi</i>			x
GYMNOTIFORMES	Gymnotidae	<i>Gymnotus cylindricus</i>			x
		<i>Gymnotus maculosus</i>			x
CYPRINODONTIFORMES	Poeciliidae	<i>Alfaro cultratus</i>			x
		<i>Beleonesox belizanus</i>			x
		<i>Phallichthys amates</i>			x
		<i>Poecilia gillii</i>			x
BELONIFORMES	Belonidae	<i>Strongylura marina</i>			x
SYNGNATHIFORMES	Syngnathidae	<i>Microphis lineatus</i>			x
PERCIFORMES	Centropomidae	<i>Centropomus ensiferus</i>			x
CARANGIFORMES	Carangidae	<i>Oligoplites saliens</i>			x
PERCIFORMES	Cichlidae	<i>Amatitlania siquia</i>			x
		<i>Amphilophus citrinellus</i>		x	x
		<i>Archocentrus centrarchus</i>	x		x

		<i>Cribroheros longimanus</i>	x	
		<i>Cribroheros rostratus</i>		x
		<i>Parachromis friedrichsthalii</i>	x	x
		<i>Parachromis managuensis</i>	x	
		<i>Vieja maculicauda</i>	x	x
PERCIFORMES	Eleotridae	<i>Dormitator maculatus</i>		x
		<i>Eleotris sp</i>		x
		<i>Gobiomorous dormitor</i>		x

Table A-S3. Species identified in BCWR using barcode databases Genbank and BOLD.

ORDER	FAMILY	SPECIES	GENBANK	BOLD
CLUPEIFORMES	Engraulidae	<i>Engraulis japonicus</i>	x	
		<i>Lycengraulis grossidens</i>	x	
CHARACIFORMES	Characidae	<i>Astyanax aeneus</i>	x	x
		<i>Astyanax bransfordii</i>	x	
		<i>Astyanax nicaraguensis</i>	x	x
		<i>Brycon guatemalensis</i>	x	x
		<i>Hyphessobrycon tortuguerae</i>	x	x
		<i>Roeboides bouchellei</i>	x	x
SILURIFORMES	Heptapteridae	<i>Rhamdia quelen</i>	x	x
	Loricariidae	<i>Hypostomus niceforoi</i>	x	
		<i>Hypostomus sp</i>		x
GYMNOTIFORMES	Gymnotidae	<i>Gymnotus cylindricus</i>	x	x
		<i>Gymnotus maculosus</i>		x
		<i>Gymnotus sp</i>		x
ATHERINIFORMES	Atherinopsidae	<i>Atherinella alvarezi</i>		x
		<i>Atherinella sp</i>	x	x
CYPRINODONTIFORMES	Rivulidae	<i>Cynodonichthys isthmensis</i>	x	x
	Poeciliidae	<i>Alfaro cultratus</i>	x	x
		<i>Belonesox belizanus</i>	x	x
		<i>Phallichthys amates</i>	x	x
		<i>Poecilia sp</i>	x	x
		<i>Poecilia gillii</i>	x	x
		<i>Poecilia mexicana</i>	x	x
		<i>Poecilia sp. Tipitapa</i>	x	
BELONIFORMES	Belonidae	<i>Strongylura timucu</i>	x	x
		<i>Strongylura marina</i>	x	x
SYNGNATHIFORMES	Syngnathidae	<i>Microphis lineatus</i>	x	x
		<i>Microphis sp</i>	x	x
PERCIFORMES	Centropomidae	<i>Centropomus mexicanus</i>	x	
		<i>Centropomus parallelus</i>	x	x
		<i>Centropomus sp</i>		x
MUGILIFORMES	Mugilidae	<i>Agonostomous monticola</i>	x	x
		<i>Mugil sp</i>	x	x
PERCIFORMES	Cichlidae	<i>Amatitlania nigrofasciata</i>	x	x
		<i>Amphilophus sp</i>	x	x
		<i>Amphilophus centrarchus</i>		x
		<i>Archocentrus centrarchus</i>	x	x
		<i>Cribroheros longimanus</i>	x	x
		<i>Cribroheros rostratus</i>	x	x
		<i>Herotilapia multispinosa</i>	x	x
		<i>Oreochromis sp</i>	x	x
		<i>Parachromis dovii</i>	x	x

		<i>Parachromis friedrichsthalii</i>		x
		<i>Parachromis loisellei</i>	x	x
		<i>Parachromis managuensis</i>	x	x
		<i>Vieja maculicauda</i>	x	x
	Eleotridae	<i>Dormitator maculatus</i>	x	x
		<i>Eleotris amblyopsis</i>	x	x
		<i>Eleotris sp</i>	x	x
		<i>Gobiomorus maculatus</i>	x	x
		<i>Gobiomorus dormitor</i>	x	x
		<i>Gobiomorus sp</i>	x	
	Gobiidae	<i>Awaous banana</i>	x	x
		<i>Ctenogobius fasciatus</i>		x
		<i>Ctenogobius sp</i>	x	
PLEURONECTIFORMES	Achiridae	<i>Achirus lineatus</i>	x	
		<i>Trinectes paulistanus</i>	x	x

Table A-S4. Species identified in BCWR using an integrative approach with presence of species at each site indicated.

ORDER	FAMILY	GENUS	SPECIES	A	B	C	D	E	F	G	H	I	J
CLUPEIFORMES	Engraulidae	<i>Anchoviella</i>	<i>sp 1</i>	x									
		<i>Lycengraulis</i>	<i>grossidens</i>				x						
CHARACIFORMES	Characidae	<i>Astyanax</i>	<i>cf. nicaraguensis</i>	x	x	x	x		x	x		x	x
		<i>Astyanax</i>	<i>bransfordii</i>		x								
		<i>Brycon</i>	<i>costaricensis</i>		x							x	
		<i>Hyphessobrycon</i>	<i>tortuguerae</i>		x								
		<i>Roeboides</i>	<i>bouchelli</i>	x	x		x			x		x	
SILURIFORMES	Heptapteridae	<i>Rhamdia</i>	<i>cf. guatemalensis</i>				x	x		x		x	
	Loricariidae	<i>Hypostomus</i>	<i>sp 1</i>									x	
GYMNOTIFORMES	Gymnotidae	<i>Gymnotus</i>	<i>cf. maculosus</i>	x	x		x					x	
ATHERINIFORMES	Atherinopsidae	<i>Atherinella</i>	<i>cf. chagresi</i>	x	x		x					x	
CYPRINODONTIFORMES	Rivulidae	<i>Cynodonichthys</i>	<i>isthmensis</i>	x									
	Poeciliidae	<i>Alfaro</i>	<i>cultratus</i>	x	x	x	x					x	
		<i>Belonesox</i>	<i>belizanus</i>	x	x								x
		<i>Phallichthys</i>	<i>amates</i>	x	x	x	x						
		<i>Poecilia</i>	<i>cf. gillii</i>	x			x	x	x	x			x
		<i>Poecilia</i>	<i>sp. Tipitapa</i>						x				
BELONIFORMES	Belonidae	<i>Strongylura</i>	<i>timucu</i>		x								
SYNGNATHIFORMES	Syngnathidae	<i>Microphis</i>	<i>lineatus</i>		x		x				x	x	x
PERCIFORMES	Centropomidae	<i>Centropomus</i>	<i>cf. ensiferus</i>				x						
		<i>Centropomus</i>	<i>pectinatus</i>				x						

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Appendix B:

Supplementary information for Results section

Clupeiformes: Engraulidae: *Anchoviella* (Anchovies)

Specimens: N13543, N13631, N13542, N13635

All anchovy specimens collected in BCWR are less than two percent diverged from one another, suggesting that they likely represent one species. The specimens were identified in the field using morphology as *Engraulidae* sp and *Anchoviella* sp. The specimens N13543, N13542 and N13635 were also identified by experts using morphology as *Anchoviella elongata*. Attempts to identify these specimens using public barcoding libraries did not provide robust identifications.

188 sequences representing 52 species from public databases were downloaded and used to analyze the four specimens in the BCWR collection. It is important to note that in this analysis many sequences and species from public data were used because standard barcode identification did not even provide robust identifications to the genus level. Four species from the Engraulidae family are known to occur in the wider Tortuguero region: *Anchoa lamprotaenia*, *Anchoa parva*, *Anchoviella elongata*, and *Lycengraulis grossidens*. In addition, *Anchoa curta*, *Anchoa lucida*, and *Anchoa macrolepidota* have been recorded in Costa Rica. The public databases only contained sequences for *Anchoa lamprotaenia*, *Lycengraulis grossidens*, and *Anchoa lucida*. Due to this, additional 49 species belonging to the Engraulidae family were used in an attempt to gain insight into the placement of the BCWR specimens within the family.

The BCWR specimens were over fourteen percent diverged from the only representatives of the *Anchoviella* genus (*Anchoviella lepidentostole* and *Anchoviella* sp) suggesting this genus may not be the best identification. These 4 specimens are also more than 14% diverged from other species included in this analysis. I also included the specimen N13449 was also included in this analysis since it is closely related to the other 4 specimens. The specimen N13449 is best identified as *Lycengraulis grossidens* with divergence at approximately 6%. While this is the

best match, the divergence between the BCWR specimen and the representatives of *Lycengraulis grossidens* is well above the standard two percent for inclusion in one species.

Due low species coverage in the public databases, molecular identification was not possible in this case. The best identification for these specimens at this time is *Anchoviella* sp 1 based on identification by experts with a note for further morphological and molecular analyses.

Characiformes: Characidae: *Astyanax* (Tetras)

Specimens: N10494, N9710, N10522, N10481, N10495, N10520, N10480, N10519, N9709, N9755, N13433, N13456, N13410, N13432, N13445, N13610, N13423, N13494, N13614, N13399, N13411, N9753, N13499, N13619, N13398, N13555, N13422, N13592, N13591, N9754, N10479

Using the K2P parameter to measure distance between sequences, the specimens collected in BCWR showed divergence values of less than two percent. This suggests that the specimens collected in BCWR likely represent one species. These specimens were identified in the field using morphology as *Astyanax aeneus*, *Bramocharax* (*Astyanax*) *bransfordii*, *Astyanax* sp (cf. *aeneus*), *Bryconamericus* sp (cf. *aeneus*), *Astyanax* cf. *aeneus*, and *Astyanax* cf. *nasutus*. Five of these specimens were also identified by experts using morphology with one specimen identified as *Astyanax bransfordii* and four as *Astyanax nicaraguensis*. The K2P distances among these five specimens is less than one percent suggesting they represent one species. Public databases were unable to provide a single species level match instead matching to multiple species at high similarity.

A total of 44 sequences from public databases were used to aid in the identification of 31 specimens in the BCWR collection. The sequences used from the public databases represented *Astyanax aeneus*, *Astyanax bransfordii*, *Astyanax orthodus*, *Astyanax cocibolca* (*nasutus*), and *Astyanax nicaraguensis*. The species *Astyanax nicaraguensis* is not known to occur in the Tortuguero region while the other species listed are (Angulo et al., 2013).

The BCWR specimens are all approximately three percent diverged from most *Astyanax aeneus* sequences in public databases, suggesting this is not the best match for these specimens. Three sequences listed as *Astyanax aeneus* in public databases are less than one percent diverged

from the BCWR specimens, suggesting these all represent one species, and these public sequences may be misidentified. In addition, the BCWR specimens are less than one percent diverged from a single reference sequences of *Astyanax bransfordii*. The BCWR specimens are also less than one percent diverged from the public sequences listed as *Astyanax nicaraguensis*. These results suggest that these public sequences and the BCWR specimens represent one species. The BCWR specimens' range in divergence (three to twenty percent) from the public database representatives of *Astyanax cocibolca* (*nasutus*) and *Astyanax orthodus*. These results suggest that public sequences of *Astyanax aeneus*, *Astyanax bransfordii*, and *Astyanax nicaraguensis* require revision to better understand the relationships between them. Based on the identifications from experts and the results of the additional analyses, the BCWR specimens have been listed as *Astyanax* cf. *nicaraguensis*.

Siluriformes: Heptapteridae: *Rhamdia* (Three-Barbeled Catfish)

Specimens: N10515, N10482, N10516, N13424, N10483, N13451

Using the K2P distance parameter to measure distance between sequences, the BCWR specimens were less than one percent diverged from one another suggesting they represent one species. The specimens were identified in the field using morphology as *Rhamdia nicaraguensis*. Some specimens from the collection were identified by experts using morphology as *Rhamdia guatemalensis* but were not sequenced successfully. Results from initial attempts to identify these specimens using public databases provided matches to a species not known to occur in Costa Rica.

An additional 183 sequences from public databases were used to aid in the identification of six specimens from the BCWR collection. The sequences taken from public databases included representatives of *Rhamdia guatemalensis*, *Rhamdia laticauda*, and *Rhamdia quelen*. The species *Rhamdia guatemalensis* and *Rhamdia laticauda* have been recorded in Tortuguero previously. The databases did not contain representative sequences for the species *Rhamdia nicaraguensis* which is also known to occur in the area and was therefore not included in the analysis.

The BCWR specimens are approximately three percent diverged from public sequences of *Rhamdia guatemalensis*. The BCWR specimens are also approximately nine percent diverged from public sequences identified as *Rhamdia laticauda*. The BCWR specimens are also approximately ten percent diverged from most public sequences listed as *Rhamdia quelen*. There are four public sequences listed as *Rhamdia quelen* that are approximately one percent diverged from the BCWR specimens, suggesting these sequences represent the same species as the BCWR specimens. The voucher specimens associated with these sequences were found in Central America. Previous studies have debated the synonymy of *Rhamdia quelen* and *Rhamdia guatemalensis* in Central America with some authors suggesting that the two species actually represent one species and other arguing *Rhamdia quelen* is the South American species and *Rhamdia guatemalensis* representing the Central American counterpart (Angulo et al., 2013). However, due to the divergence between the BCWR specimens and four *Rhamdia quelen* sequences from the remainder of *Rhamdia quelen* sequences, this does not appear to be the species these sequences represent. In addition, the divergence between the BCWR sequences and four *Rhamdia quelen* sequences from the public sequences of *Rhamdia guatemalensis* suggest these sequences also may not represent this species. Without representatives of *Rhamdia nicaraguensis*, excluding this species identification is difficult. Therefore, the BCWR sequences are listed as *Rhamdia cf. guatemalensis*.

Siluriformes: Loricariidae: *Hypostomus* (Armored Catfish)

Specimens: N10526 and N10528

Using the K2P parameter to measure distance, the BCWR specimens are less than one percent diverged suggesting that these specimens represent one species. The BCWR specimens were identified in the field using morphology as *Hypostomus aspidolepis*. The specimens were reviewed by experts but due to their juvenile state, were assigned the identification of *Hypostomus* sp 1. Initial attempts to identify the specimens using public barcoding libraries provided multiple species identifications.

A total of 207 publicly available sequences were used to aid in the identification of the two BCWR specimens. These public sequences included representation from a wide variety of accepted species and candidate species. The analysis also included two representative sequences

of *col* from Dr. Nathan Lujan. One species of *Hypostomus* is known to occur in Central American including Costa Rica: *Hypostomus aspidolepis* (Angulo et al., 2013). Measurements for the divergence between *Hypostomus aspidolepis* and the BCWR specimens is approximately six percent. This falls outside the standard two percent for inclusion. The bulk of the public sequences used were greater than three percent diverged from the BCWR sequences suggesting these are not representatives of the same species. There were nine sequences that were less than one percent diverged from the BCWR specimens. The identifications for these sequences included: *Hypostomus robinii*, *Hypostomus sp*, *Hypostomus holostictus*, *Hypostomus honda*, *Hypostomus niceforoi*, and *Hypostomus rhantos*. Most of these species had other representatives in the databases that were greater than two percent diverged from these sequences. Since the nine public sequences and the BCWR specimens are less than one percent diverged this suggests these sequences represent one species. However, it is unclear what the identification of this species is based on the publicly available data and the juvenile state of the specimens. The BCWR specimens are listed as *Hypostomus sp* 1 with notes for additional morphological and molecular analysis.

Gymnotiformes: Gymnotidae: *Gymnotus* (Knifefish)

Specimens: N9725, N13620, N9723, N13642, N13641, N10504, N10507, N13639, N9739, N9740, N10506, N10505

From the K2P distance matrix, all BCWR sequences were less than 2% diverged, indicating that these sequences most likely belong to the same species. Two species of *Gymnotus* have been identified in Costa Rica including Tortuguero: *Gymnotus cylindricus* and *Gymnotus maculosus*. Given the high percent matches between the BCWR specimens and public data for *Gymnotus cylindricus* and *Gymnotus maculosus*, I included 14 other species belonging to *Gymnotus* that are not known to occur in Costa Rica. I used 236 publicly available sequences from 16 *Gymnotus* species to help identify twelve specimens from BCWR. The twelve specimens from BCWR were identified in the field using morphology as *Gymnotus cylindricus* and *Gymnotus maculosus*.

Distance analyses between the public sequences demonstrated that sequences listed as *Gymnotus cylindricus* most closely matched the BCWR specimens (less than 1% divergence).

However, public databases also indicated a less than 1% divergence between the BCWR specimens and private sequences of *Gymnotus maculosus*. In addition, BCWR specimens identified using morphology by experts were identified as *Gymnotus maculosus*. Without full access to the *Gymnotus maculosus* sequences it is difficult to include to exclude this species as a likely identification. These specimens have been listed as *Gymnotus* cf. *maculosus* based on identifications by experts with the proviso that further morphological and molecular analyses should be undertaken.

Atheriniformes: Atherinopsidae: *Atherinella* (Neotropical Silversides)

Specimens: N9769, N13389, N13547, N13500, N13387, N10478, N9770, N10477, N13601

To begin this analysis, a distance matrix using K2P was generated to determine relationships between the BCWR specimens. From this there appear to be two groups in BCWR. The BCWR specimens were all less than 2% diverged from one another except for N13601 which was ~4% diverged from N13387, N13389, N13547 and N13500. Interestingly, N13387, N13389, N13547, and N13500 are still less than 2% diverged from N10477, N10478, N9770 and N9769. It appears that N13601 and N13387 + N13547 + N13500 + N13389 form two groups with specimens N10477 + N10478 + N9770 + N9769 covering variation between these two groups.

The BCWR specimens had been identified in the field as *Atherinella milleri*, *Atherinella argentea*, *Atherinella sardina*, *Atherinella chagresi*, and *Atherinella* sp. The specimen N13547 was also identified by experts using morphology as *Atherinella* cf. *chagresi*. The relevant literature included four species of *Atherinella* that could be present in BCWR. An additional 2 species belonging to this genus are listed in Costa Rica but not in Tortuguero. The public databases only contained representatives for three of the four species likely to occur in BCWR. Representatives for the additional two species in Costa Rica were not present in the databases. An additional seven species from closely related genera and families were added to the analysis, thus 10 species were used in this analysis. A total of 47 publicly available sequences from these 10 species were used to help identify 9 BCWR silverside specimens.

The specimens from BCWR most closely matched public reference sequences from *Atherinella alvarezi*, a species not known to occur in Costa Rica. The specimens from BCWR also closely matched a group of sequences listed as *Atherinella sp.* However, the divergence between the BCWR sequences and both of these species is ~3-4%, above the standard threshold for sequence inclusion in a species. With regards to the two groups identified, N13601 was approximately 4% diverged from *Atherinella alvarezi* and *Atherinella sp.*, while the remaining specimens were closer to 3% diverged from these two species. Given that the databases did not include representatives for all species of *Atherinella* previously listed as occurring in Costa Rica, the currently available data does not allow conclusive species identification. The sequences were identified as *Atherinella cf. chagresi* based on identifications given by experts.

Cyprinodontiformes: Poeciliidae: *Poecilia* (Mollies)

Specimens: N9760, N10523, N9766, N9758, N10524, N9744, N13392, N13442, N13441, N13473, N9745, N13393, N13395, N13394

Using the K2P parameter, the sequences from specimens found in BCWR are less than 1% diverged from one another suggesting that these specimens represent a single species. There is one exception for specimen N9760 which was between five and seven percent diverged from the other specimens collected in Costa Rica. Identifications done in the field using morphology listed these specimens as *Poecilia gillii* (or *Poecilia cf. gillii*), *Poecilia mexicana*, and *Alfaro cultratus*. Specimens that had been identified by experts failed to be successfully sequenced but were identified as *Poecilia gillii*.

A total of 198 sequences were sourced from the public databases Genbank and BOLD to help identify 14 specimens from the BCWR collection. The sequences from public databases were listed as *Poecilia gillii*, *Poecilia mexicana*, and *Poecilia* “Tipitapa”. Initial attempts to identify specimen N9760 using public data listed this specimen as most similar to public sequences identified as *Poecilia* “Tipitapa” with divergence between BCWR specimen and public data at less than one percent diverged. In addition this specimen was greater than five percent diverged from public sequences listed as *Poecilia gillii* and *Poecilia mexicana*. This suggests the best identification for this specimen is the candidate species *Poecilia* “Tipitapa”. The remaining BCWR specimens could not be identified to the species level during initial

identification attempts using public databases. Databases returned less than one percent divergences between the BCWR specimens and public data listed as both *Poecilia gillii* and *Poecilia mexicana*. In addition, public sequences listed as *Poecilia gillii* and *Poecilia mexicana* were less than one percent diverged from each other, again suggesting these species be included in the same species. This also suggests that a sizeable proportion of sequences in public databases listed as these two species are misidentified and additional analyses are required. Due to this the BCWR sequences are currently listed as *Poecilia* cf. *gillii*.

Beloniformes: Belonidae: *Strongylura* (Needlefish)

Specimens: N13391, N13599, N9768, N13644, N13645

Using the K2P parameter to assess distance between sequences, the BCWR specimens are less than one percent diverged from one another. This is below the standard two percent threshold indicating that these specimens are representatives of one species. The specimens were identified in the field using morphology as *Strongylura* sp, *Strongylura timucu*, and *Strongylura marina*. Two specimens (N13599 and N13644) were also identified by experts using morphology as *Strongylura marina*. Initial attempts to use public databases to aid in the molecular identification of these specimens failed to provide a species level match.

An additional nineteen sequences from the public databases Genbank and BOLD were used to help aid in the molecular identification of five specimens from the BCWR collection. The sequences taken from public databases represented the two species *Strongylura timucu* and *Strongylura marina*. Initial attempts to identify these specimens using public databases failed to differentiate between these two species, providing both as likely identification for the BCWR specimens. Both species are known to occur in the wider region of Tortuguero and are the only representatives of this family previously known to occur in Costa Rica. An additional sequence of the *coI* region was provided by Dr. Katherine Balasingham and was included in the analysis.

Results from the additional analyses show that the BCWR specimens are less than 1 percent diverged from most public sequences identified as *Strongylura timucu*. There are three public sequences that are approximately four percent diverged from the BCWR specimens and other public sequences identified as *Strongylura timucu*. These sequences are also approximately

17 percent diverged from most sequences from public databases identified as *Strongylura marina*. This suggests that these three public databases sequences are misidentified and do not represent *Strongylura timucu* or *Strongylura marina*. In addition, the BCWR specimens showed divergence values at greater than 15 percent diverged from most public sequences identified as *Strongylura marina*. There are two public sequences listed as *Strongylura marina* that are less than 1 percent diverged from the BCWR specimens and from most public sequences identified as *Strongylura timucu*. These sequences are also greater than 15 percent diverged from the major group of public *Strongylura marina* sequences. This suggests these sequences have been misidentified and are likely representatives of *Strongylura timucu*. The results from additional analyses suggest that the best identification for the specimens found in BCWR is *Strongylura timucu*.

Perciformes: Centropomidae: *Centropomus* (Snooks)

Specimens: N10489, N13578, N13579, N13593, N13594

To begin this analysis, a K2P distance matrix containing the BCWR specimens was generated to determine the relationships between the specimens. The specimens N13594 and N13578 form a sister group to the remaining BCWR specimens (with a divergence of ~13%). N13594 was identified by experts as *Centropomus ensiferus* while N13578 was identified in the field as *Centropomus* cf. *robalito*. However, these two sequences are 0.28% diverged from one another indicating that they are likely representatives of the same species. These two sequences are also more than 14% diverged from the other species included in the analysis, indicating that these specimens are also not representatives of the same species. The remaining BCWR specimens (N10489, N13579, and N13593) were all less than 1% diverged from each other but approximately 13% diverged from the other BCWR specimens. N13593 was identified by experts as *Centropomus ensiferus* while N10489 was identified in the field as *Centropomus parallelus* and N13579 was identified in the field as *Centropomus* cf. *robalito*. The result of the analysis of the K2P distance matrix suggests the presence of two species of *Centropomus* in BCWR.

There are five species of *Centropomus* that have been recorded in the wider Tortuguero region: *Centropomus ensiferus*, *Centropomus mexicanus*, *Centropomus parallelus*, *Centropomus*

undecimalis, and *Centropomus pectinatus*. Public databases do not contain representative sequences for *Centropomus ensiferus* thus this species could not be included in the barcode analysis. I also included 6 other species, 5 of which are known to occur in Costa Rica but not in Tortuguero.

A total of 120 publicly available sequences from 11 species were used to help identify five BCWR specimens. For the first group in BCWR, N13594 and N13578, the best match according to the database was *Centropomus pectinatus* with divergence less than 1% between the BCWR specimens and the public data. For the second group identified in BCWR (N10489, N13579, and N13593), the best match was to both *Centropomus parallelus* and *Centropomus mexicanus* at approximately 2.5% divergence between the BCWR specimens and public data. However, this divergence is outside the standard 2% threshold for inclusion suggesting these specimens are not representatives of either of these species. One of these specimens was identified by experts as *Centropomus ensiferus* but this species does not have representatives in the public databases. Based on both the data from public databases and the expert identification, the best identification for the second group of *Centropomus* identified in BCWR is *Centropomus* cf. *ensiferus*.

Mugiliformes: Mugilidae: *Mugil* (Mullet)

Specimens: N13475 and N13476

When using the K2P distance parameter to assess if sequences can be included in one species, the two BCWR specimens are less than one percent diverged from one another. This suggests that the two specimens are representatives of one species. Both specimens were identified in the field using morphology as *Mugil* sp. These specimens were ultimately lost and were not identified by experts using morphology. Despite this, tissue samples from the specimens were saved and the specimens could be identified using molecular techniques. Initial attempts to identify the specimens using public barcoding libraries proved ineffective as the libraries returned high likelihood matches to multiple species, therefore only generating a genus level match.

An additional 59 sequences from public sequence libraries were used to aid in the identification of the BCWR specimens. The sequences pulled from the public sequence libraries represented eight species: *Mugil trichodon*, *Mugil liza*, *Mugil thoburni*, *Mugil hospes*, *Mugil curema*, *Mugil cephalus*, *Joturus pichardi* and sequences belonging to an unidentified species *Mugil sp.* In Tortuguero, *Mugil curema* and *Mugil cephalus* are the documented species belonging to this genus with *Joturus puchardi* representing a closely related genera documented in the region. The remaining species represent other species from the *Mugil* genus that have representatives in the public libraries.

The results from the additional analysis show the BCWR specimens as less than one percent diverged from 6 sequences in the public databases. These included one representative of *Mugil trichodon*, four representatives of *Mugil hospes*, and one representative listed as *Mugil sp.* The BCWR specimens were greater than seventeen percent diverged from the majority of the sequences representing *Mugil trichodon* (with one exception of a sequences that was greater than two percent diverged). In addition, the BCWR specimens were greater than six percent diverged from all other representatives of *Mugil hospes* found in the public databases. A similar result occurred for other public sequences listed as *Mugil sp.* These sequences ranged from twelve to twenty percent diverged from the BCWR specimens. Finally, the BCWR specimens were greater than eighteen percent diverged from the native species *Mugil cephalus* and *Mugil curema*.

These results suggest that the public sequences that were most similar to the BCWR specimens, all represent one species, but that these public sequences have been misidentified. The BCWR specimens have been listed as *Mugil cf. hospes* with notes for additional morphology and molecular analyses.

Perciformes: Cichlidae: *Amphilophus* (Cichlid)

Specimens: N10487, N9734, N9735, N10499, N13448, N13494, N13533, N13541, N13483, N13573

The K2P distance matrix generated to determine relationships between the BCWR specimens shows all the BCWR specimens as less than 1% diverged from one another. This suggests that these specimens represent one species. Experts identified 8 of the specimens to the

species *Amphilophus citrinellus*. The first attempts to identify these specimens using public databases resulted in high similarity matches to multiple species.

From the relevant literature, there are 4 species that are known to occur in Tortuguero with an additional 5 species known to occur from other parts of Costa Rica. I was able to download representative sequences for all of these species. I also included sequences representing on additional species that was a common match in the first attempts to identify the specimens using public databases. This resulted in 37 species representing 10 species of the genus *Amphilophus*.

From the K2P distance matrix for the BCWR specimens and the public data sequences, the BCWR specimens most closely match representatives of *Amphilophus citrinellus* and *Amphilophus labiatus* at less than 1% diverged. The BCWR specimens were more than 10% diverged from the other species represented. Given the expert identification was *Amphilophus citrinellus* and *Amphilophus labiatus* is not as well represented in the databases as *Amphilophus citrinellus*, the identification given to these specimens was *Amphilophus citrinellus*.

Gobiiformes: Eleotridae: Eleotris (Spinycheek Sleepers)

Specimens: N9717, N9716, N10496, N10497, N13530, N13531, N13580, N13590, N13624, N13598, N13605, N13436

To begin, a K2P distance matrix was generated to determine relationships between the BCWR specimens. Results from this matrix show two groups within the BCWR specimens. The first group, specimens N13598 + N13605 are approximately 6% diverged from the remaining BCWR specimens. This is outside the standard 2% threshold for inclusion. The remaining BCWR specimens are less than 1% diverged from each other suggesting they represent one species. Five of the BCWR specimens were identified by experts as *Eleotris sp* including representatives from both of the groups I have identified from within the BCWR specimens.

From the relevant literature, there are 5 species known to occur in Costa Rica, and only two of these are known to occur in Tortuguero. Public databases contain representative sequences for 3 of these species. I also included representatives of *Eleotris perniger* since they

were high matches when the specimens were originally queried in the databases Genbank and BOLD.

A total of 52 publicly available sequences from 4 species were used to help identify twelve BCWR specimens. From the K2P distance matrix generated for the BCWR specimens and the public data, it seems the best match for the larger group of BCWR specimens is *Eleotris amblyopsis*. The BCWR specimens and representatives of this species are less than 1% diverged from each other, suggesting these all represent one species. The remaining two BCWR specimens that form the other group are most similar to both *Eleotris perniger* and *Eleotris pisonis* with divergences between the BCWR specimens and the public data at approximately 1%. However, the BCWR specimens did not match all public data sequences identified as *Eleotris pisonis*, suggesting that there are inconsistencies in the databases regarding the identifications of both *Eleotris pisonis* and *Eleotris perniger*. Due to these inconsistencies, the two BCWR specimens N13598 and N13605 were identified as *Eleotris* sp 1.

Gobiiformes: Eleotridae: *Gobiomorus* (Sleeper Gobies)

Specimens: N13481, N13596, N13503, N13577, N13595, N13607, N13574, N13502, N13575, N13576, N13622, N13463, N13464, N13479, N10511, N9729, N10501, N13401, N10502

Using the K2P distance parameter, the sequences belonging to specimens collected in BCWR showed values of less than 1% divergence between sequences. This supports the placement of these sequences and specimens into one species. Identifications done in the field using morphology identified these specimens as *Poecilia gillii*, *Alfaro cultratus*, *Rivulus* sp, *Hemieleotris latifasciatus*, and *Gobiomorus dormitor*. The specimen N13622 was also identified by experts as *Gobiomorus dormitor*. Initial attempts to identify these specimens using molecular techniques resulted in two likely species as identifications, *Gobiomorus maculatus* and *Gobiomorus dormitor*.

From public databases, 61 sequences were used to aid in the identification of 19 specimens in further analyses. The public data included *Gobiomorus maculatus* and *Gobiomorus dormitor*. The public data also included a third species from the same genus listed as occurring in Costa Rica: *Gobiomorus polylepis*. The public data used also included the additional species

Dormitator maculatus, *Hemieleotris latifasciata*, *Leptophilypnus fluviatilis*, and *Guavina guavina* representing closely related species known to occur in Costa Rica (Angulo et al., 2013).

The specimens from BCWR were less than 1% diverged from most of the *Gobiomorus dormitor* sequences used from public databases. However, the BCWR specimens were also between 5 and 15 percent diverged from other sequences identified as *Gobiomorus dormitor*. These other sequences were more similar to sequences listed as *Gobiomorus maculatus* than to sequences listed as *Gobiomorus dormitor*. This indicates these sequences are all likely *Gobiomorus maculatus* and there has been a misidentification in these sequences. In addition, two sequences listed as *Gobiomorus maculatus* were less than 1% diverged from the BCWR sequences and the bulk of the sequences listed as *Gobiomorus dormitor*. This indicates that these sequences are likely *Gobiomorus dormitor* and were misidentified in the public databases.

The combination of morphological identification by experts and the use of molecular data suggests that the best identification for these sequences is *Gobiomorus dormitor*.

Pleuronectiformes: Achiridae (Soles) and Paralichthidae (large-tooth flounders)

Specimens: N9730, N13496, N13497

First, I analyzed an exported K2P distance matrix to determine if the specimens N13496 and N13497 represented the same species. The two specimens are less than 2% diverged from one another, suggesting they represent the one species. When using the search functions with default parameters in Genbank and BOLD, the best match provided was less than 98% similar to the BCWR specimens. The specimen N9730 was included in this analysis due to the low percent similarity of the first identification provided by the databases (Appendix 1). All three of these specimens were not identified by experts.

From Angulo et al. (2013) and additional observational data, BCWR may have up to eight species belonging to Pleuronectiformes, and Genbank contained *col* sequences for seven of these species. Sequences for *Citharichthys uhleri* are not present in Genbank or BOLD and were therefore not included in the analysis. Given the best match to the BCWR specimens was a member of the genus *Achirus*, attempts to find public data for other species from the genus *Achirus* were made however BOLD and Genbank only contain representatives for three of nine

currently accepted species. An additional 22 species from closely related genera/families to those from the regional species list were included in the analysis. A total of 124 sequences from public databases Genbank and BOLD representing 30 species belonging to the *Achiridae* and *Paralichthidae* families to aid in identifying three specimens (N9730, N13496 and N13497) in my dataset.

To begin with the *Achiridae* specimens, based on genetic distance, specimens N13496 and N13497 are most similar to *Achirus lineatus* (~8% diverged). However, this divergence is well above the standard 2% threshold for inclusion in the same species suggesting that the BCWR species are likely not *Achirus lineatus*. While the relative similarity of the BCWR sequences to *Achirus lineatus* and the increased divergence with regard to other genera, suggests that while this is not the species represented in BCWR, it is possibly another species belonging to the same genus. There are six species of *Achirus* previously recorded in Costa Rica that do not have public barcodes, and my samples could be one of these species. The specimen N9730 is best identified as *Trinectes paulistanus*. Divergence between N9730 and public data sequences for *Trinectes paulistanus* is approximately 9%, well above the standard 2% threshold for inclusion. However, divergence for this specimen and other representatives is much larger and this suggests *Trinectes paulistanus* is the best identification for this specimen at this time.

Table B-S1. Results from search queries in Genbank and BOLD. Top three results are listed but the order is arbitrary. Items that are bolded represent the identification given to that specimen by the respective database.

ID #	Field Identification	BOLD (% Similarity)			Genbank (% Similarity)		
		1	2	3	1	2	3
9709	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100%)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)
9710	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.84)
9712	<i>Phallichthys amates</i>	<i>Phallichthys amates</i> (100)	<i>Brachyrhaphis episcopi</i> (89.7)	<i>Phallichthys tico</i> (89.03)	<i>Phallichthys amates</i> (99.85)	<i>Brachyrhaphis episcopi</i> (89.51)	<i>Phallichthys tico</i> (89.01)
9716	<i>Eleotris amblyopsis</i>	<i>Eleotris amblyopsis</i> (99.85)	<i>Eleotris pisonis</i> (99.85)	<i>Eleotris perniger</i> (94.14)	<i>Eleotris amblyopsis</i> (99.70)	<i>Eleotris perniger</i> (94.14)	<i>Eleotris pisonis</i> (93.67)
9717	<i>Eleotris amblyopsis</i>	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (100)	<i>Eleotris perniger</i> (94.29)	<i>Eleotris amblyopsis</i> (99.85)	<i>Eleotris sp.</i> (100)	<i>Eleotris perniger</i> (94.29)
9718	<i>Cynodonichthys isthmensis</i>	<i>Rivulus isthmensis</i> (99.84)	<i>Rivulus tenuis</i> (92.06)	<i>Rivulus brunneus</i> (92.02)	<i>Cynodonichthys isthmensis</i> (99.85)	<i>Rivulus sp.</i> (92.40)	<i>Cynodonichthys kuelpmanni</i> (91.96)
9723	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus maculosus</i> (99.84)	<i>Gymnotus carapo</i> (91.93)	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus carpo</i> (91.54)	<i>Gymnotus pantanal</i> (90.88)
9725	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus maculosus</i> (99.84)	<i>Gymnotus</i> (92.09)	<i>Gymnotus cylindricus</i> (99.69)	<i>Gymnotus carpo</i> (91.24)	<i>Gymnotus pantanal</i> (90.58)
9729	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)	<i>Hemieleotris latifasciata</i> (89.30)	<i>Gobimorus dormitor</i> (99.70)	<i>Gobimorus maculatus</i> (100)	<i>Hemieleotris latifasciatus</i> (89.22)
9730	<i>Trinectes paulistanus</i>	<i>Trinectes paulistanus</i> (97.61)	<i>Trinectes fluviatilis</i> (93.3)	<i>Trinectes maculatus</i> (91.60)	<i>Trinectes maculatus</i> (90.66)	<i>Trinectes paulistanus</i> (91.48)	<i>Trinectes inscriptes</i> (90.77)
9734	<i>Parachromis loisellei</i>	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus sp. labiatus</i> (100)	<i>Amphilophus sagittae</i> (100)	<i>Amphilophus amarillo</i> (99.70)	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus labiatus</i> (100)
9735	<i>Parachromis loisellei</i>	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus cf. Libiatus</i> (100)	<i>Amphilophus sagittae</i> (100)	<i>Amphilophus amarillo</i> (99.70)	<i>Amphilophus citrinellus</i> (99.70)	<i>Amphilophus labiatus</i> (100)
9736	<i>Parachromis loisellei</i>	<i>Archocentrus centrarchus</i> (100)	<i>Amphilophus citrinellus</i> (96.07)	<i>Amphilophus sagittae</i> (96.01)	<i>Archocentrus centrarchus</i> (100)	<i>Amphilophus amarillo</i> (95.71)	<i>Amphilophus citrinellus</i> (95.71)
9737	<i>Parachromis loisellei</i>	<i>Ctenogobius fasciatus</i> (99.85)	<i>Gobiosoma yucatanum</i> (99.38)	<i>Gobiomorus dormitor</i> (99.38)	<i>Ctenogobius fasciatus</i> (99.39)	<i>Ctenogobius sp.</i> (99.69)	<i>Ctenogobius boleosoma</i> (84.89)
9739	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus maculosus</i> (99.84)	<i>Gymnotus carpo</i> (91.91)	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus carpo</i> (90.83)	<i>Gymnotus pantanal</i> (90.91)
9740	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (99.84)	<i>Gymnotus maculosus</i> (99.69)	<i>Gymnotus carpo</i> (92.05)	<i>Gymnotus cylindricus</i> (99.69)	<i>Gymnotus carpo</i> (91.46)	<i>Gymnotus pantanal</i> (90.83)
9743	<i>Amatitlania nigrofasciatus</i>	<i>Archocentrus centrarchus</i> (100)	<i>Amphilophus citrinellus</i> (96.07)	<i>Amphilophus sagittae</i> (96.01)	<i>Archocentrus centrarchus</i> (99.85)	<i>Amphilophus amarillo</i> (95.46)	<i>Amphilophus citrinellus</i> (95.46)
9744	<i>Poecilia cf. gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia salvatoris</i> (99.25)	<i>Poecilia gillii</i> (100)	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia sphenops</i> (99.70)
9745	<i>Poecilia cf. gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia sphenops</i> (99.84)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia sphenops</i> (99.69)
9749	<i>Parachromis managuensis</i>	<i>Parachromis managuensis</i> (100)	<i>Parachromis friedrichsthalii</i> (99.51)	<i>Maskaheros argenteus</i> (97.09)	<i>Amphilophus amarillo</i> (93.45)	<i>Parachromis managuensis</i> (100)	

9753	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)
9754	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Astyanax aeneus</i> (99.69)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax aeneus</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.84)
9755	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (100)
9758	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia sphenops</i> (99.85)	<i>Poecilia gillii</i> (100)	<i>Poecilia mexicana</i> (100)	<i>Poecilia sphenops</i> (99.85)
9760	<i>Poecilia gillii</i>	<i>Poecilia</i> sp. (99.69)	<i>Poecilia latipunctata</i> (95.81)	<i>Poecilia sphenops</i> (95.32)	<i>Poecilia</i> sp. Tipitapa (99.70)	<i>Poecilia sphenops</i> (95.42)	<i>Poecilia gillii</i> (95.13)
9762	<i>Aequidens coeruleopunctatus</i>	<i>Oreochromis</i> sp (100)	<i>Oreochromis aureus</i> (100)	<i>Oreochromis</i> sp. TP (100)	<i>Oreochromis niloticus</i> (99.85)	<i>Oreochromis aureus</i> (99.85)	<i>Sarotherodon galilaeus</i> (99.26)
9764	<i>Awaous banana</i>	<i>Awaous banana</i> (100)	<i>Awaous tajasica</i> (97.18)	<i>Awaous transandeanus</i> (93.27%)	<i>Awaous banana</i> (99.10)	<i>Awaous ocellaris</i> (90.42)	<i>Awaous grammepomus</i> (91.06%)
9766	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia sphenops</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia sphenops</i> (99.85)
9768	<i>Strongylura timucu</i>	<i>Strongylura timucu</i> (100)	<i>Strongylura marina</i> (100)	<i>Tylosurus crocodilus</i> (86.6)	<i>Strongylura</i> sp. (100)	<i>Strongylura timucu</i> (100)	<i>Strongylura marina</i> (100)
9769	<i>Atherinella milleri</i>	<i>Atherinella alvarezi</i> (97.40)	<i>Atherinella</i> sp. 1 (97.38)	<i>Atherinella hubbsi</i> (90.05)	<i>Atherinella</i> sp. (96.96)	<i>Atherinella hubbsi</i> (89.98)	<i>Atherinella chagresi</i> (90.02)
9770	<i>Atherinella chagresi</i>	<i>Atherinella alvarezi</i> (97.16)	<i>Atherinella</i> sp. 1 (97.10)	<i>Atherinella chagresi</i> (90.48)	<i>Atherinella</i> sp. (94.83)	<i>Atherinella hubbsi</i> (88.54)	<i>Atherinella chagresi</i> (88.36)
10477	<i>Atherinella chagresi</i>	<i>Atherinella</i> sp. (96.96)	<i>Atherinella hubbsi</i> (90.45)	<i>Atherinella chagresi</i> (90.48)	<i>Atherinella alvarezi</i> (97.09)	<i>Atherinella</i> sp. 1 (97.08)	<i>Atherinella hubbsi</i> (90.23)
10478	<i>Atherinella chagresi</i>	<i>Atherinella</i> sp. (97.26)	<i>Atherinella hubbsi</i> (90.45)	<i>Atherinella chagresi</i> (90.48)	<i>Atherinella alvarezi</i> (97.41)	<i>Atherinella</i> sp. 1 (97.39)	<i>Atherinella hubbsi</i> (90.23)
10479	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)
10480	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)
10481	<i>Astyanax aeneus</i>	<i>Astyanax aeneus</i> (99.54)	<i>Bramocharax bransfordii</i> (99.38)	<i>Astyanax nicaraguensis</i> (99.38)	<i>Astyanax aeneus</i> (99.53)	<i>Bramocharax bransfordii</i> (99.53)	<i>Astyanax aeneus</i> (99.22)
10482	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.84)	<i>Rhamdia laticauda</i> (91.98)	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.98)	<i>Rhamdia laticauda</i> (96.9)
10483	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.88)	<i>Rhamdia laticauda</i> (91.91)	<i>Rhamdia quelen</i> (100)	<i>Rhamdia laticauda</i> (98.05)	<i>Rhamdia guatemalensis</i> (98.04)
10486	<i>Amphilophus citrinellus</i>	<i>Paraneetroplus maculicauda</i> (100)	<i>Paraneetroplus synspilus</i> (98.1)	<i>Vieja bifasciata</i> (99.66)	<i>Vieja maculicauda</i> (100)	<i>Vieja bifasciata</i> (99.66)	<i>Vieja melanura</i> (99.15)
10487	<i>Amphilophus citrinellus</i>	<i>Amphilophus amarillo</i> (99.85)	<i>Amphilophus citrinellus</i> (99.85)	<i>Amphilophus labiatus</i> (99.69)	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus</i> sp. (100)	<i>Amphilophus</i> cf. <i>labiatus</i> (100)
10488	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides salvadoris</i> (99.69)	<i>Roboides ilseae</i> (99.15)	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.59)
10489	<i>Centropomus parallelus</i>	<i>Centropomus parallelus</i> (97.85)	<i>Cephalopholis cruentata</i> (84.17)	<i>Aethaloperca rogaa</i> (83.48)	<i>Centropomus mexicanus</i> (99.84)	<i>Centropomus parallelus</i> (97.84)	<i>Centropomus</i> sp. (97.69)
10490	<i>Amphilophus citrinellus</i>	<i>Paraneetroplus maculicauda</i> (100)	<i>Vieja maculicauda</i> (100)	<i>Paraneetroplus synspilus</i> (98.82)	<i>Vieja maculicauda</i> (100)	<i>Vieja bifasciata</i> (99.66)	<i>Vieja melanura</i> (99.15)

10491	<i>Astatheros rostratus</i>	<i>Cribroheros rostratus</i> (99.07)	<i>Cribroheros longimanus</i> (98.61)	<i>Criberheros diquis</i> (94.13)	<i>Thorichthys rostratus</i> (99.22)	<i>Cribroheros longimanus</i> (98.9)	<i>Cribroheros diquis</i> (94.25)
10492	<i>Parachromis loisellei</i>	<i>Parachromis managuensis</i> (99.41)	<i>Parachromis dovii</i> (96.30)	<i>Amphilophus amarillo</i> (94.09)	<i>Parachromis managuensis</i> (100)	<i>Parachromis friedrichsthalii</i> (99.54)	
10494	<i>Astyanax cf. aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicarguensis</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)
10495	?	<i>Astyanax aeneus</i> (99.85)	<i>Bramocharax bransfordii</i> (99.69)	<i>Astyanax nicarguensis</i> (99.69)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.53)
10496	<i>Eleotris amblyopsis</i>	<i>Eleotris amblyopsis</i> (99.41)	<i>Eleotris sp.</i> (99.85)	<i>Eleotris perniger</i> (94.14)	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (99.54)	<i>Eleotris perniger</i> (94.14)
10497	<i>Eleotris amblyopsis</i>	<i>Eleotris amblyopsis</i> (99.58)	<i>Eleotris sp.</i> (100)	<i>Eleotris perniger</i> (94.14)	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (100)	<i>Eleotris perniger</i> (94.29)
10499	<i>Amphilophus citrinellus</i>	<i>Amphilophus amarillo</i> (100)	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus labiatus</i> (100)	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus labitus</i> (100)	<i>Amphilophus sagittae</i> (100)
10500	<i>Parachromis managuensis</i>	<i>Paraneetroplus maculicauda</i> (100)	<i>Paraneetroplus synspilus</i> (98.82)	<i>Vieja maculincauda</i> (100)	<i>Vieja maculicauda</i> (100)	<i>Vieja bifasciata</i> (99.66)	<i>Vieja melanura</i> (99.15)
10501	<i>Gobiomorus dormitor</i>	<i>Gobiomorus dormitor</i> (99.70)	<i>Gobiomorus maculatus</i> (100)	<i>Hemieleotris latifasciatus</i> (89.17)	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)	<i>Hemieleotris latifasciata</i> (89.25)
10502	<i>Gobiomorus dormitor</i>	<i>Gobiomorus dormitor</i> (99.52)	<i>Gobiomorus maculatus</i> (99.67)	<i>Hemieleotris latifasciatus</i> (88.91)	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)	<i>Hemieleotris latifasciata</i> (89.46)
10503	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.39)	<i>Alfaro huberi</i> (88.91)	<i>Phallichthys amates</i> (85.82)	<i>Alfaro cultratus</i> (98.91)	<i>Alfaro huberi</i> (88.79)	<i>Brachyrhaphis episcopi</i> (85.67)
10504	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (99.69)	<i>Gymnotus carpo</i> (90.99)	<i>Gymnotus pantanal</i> (90.61)	<i>Gymnotus cylindricus</i> (99.84)	<i>Gymnotus maculosus</i> (99.69)	<i>Gymnotus carpo</i> (92.09)
10505	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (99.85)	<i>Gymnotus carapo</i> (91.58%)	<i>Gymnotus pantanal</i> (91.06)	<i>Gymnotus cylindricus</i> (99.84)	<i>Gymnotus maculosus</i> (99.69)	<i>Gymnotus carpo</i> (92.12)
10506	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (99.84)	<i>Gymnotus carpo</i> (91.89)	<i>Gymnotus pantanal</i> (91.26)	<i>Gymnotus cylindricus</i> (99.84)	<i>Gymnotus maculosus</i> (99.68)	<i>Gymnotus carpo</i> (92.38)
10507	<i>Gymnotus cylindricus</i>	<i>Gymnotus cylindricus</i> (99.68)	<i>Gymnotus carpo</i> (91.67)	<i>Gymnotus pantanal</i> (91.04)	<i>Gymnotus maculosus</i> (99.84)	<i>Gymnotus cylindricus</i> (99.68)	<i>Gymnotus carpo</i> (92.35)
10508	<i>Pseudophallus mindii</i>	<i>Microphis brachyurus</i> (98.66)	<i>Microphis lineatus</i> (99.85)	<i>Microphis sp.</i> (99.07)	<i>Microphis brachyurus</i> (99.69)	<i>Microphis lineatus</i> (99.69)	<i>Microphis retzii</i> (83.95)
10509	<i>Pseudophallus mindii</i>	<i>Microphis brachyurus</i> (98.71)	<i>Microphis sp.</i> (99.85)	<i>Microphis lineatus</i> (99.69)	<i>Microphis lineatus</i> (99.85)	<i>Microphis brachyurus</i> (99.85)	<i>Microphis retzii</i> (84.57)
10510	<i>Herotilapia multispinosa</i>	<i>Archocentrus multispinosus</i> (100)	<i>Paraneetroplus synspilus</i> (90.83)	<i>Kronoheros umbrifer</i> (91.67)	<i>Herotilapia multispinosa</i> (100)	<i>Kronoheros umbrifer</i> (91.63)	<i>Vieja bifasciata</i> (90.99)
10511	<i>Gobiomorus dormitor</i>	<i>Gobiomorus dormitor</i> (99.56)	<i>Gobiomorus maculatus</i> (99.84)	<i>Hemieleotris latifasciatus</i> (89.07)	<i>Gobiomorus dormitor</i> (99.85)	<i>Gobiomorus maculatus</i> (99.84)	<i>Hemieleotris latifasciata</i> (89.15)
10513	<i>Astatheros sp. (cf. rostratus)</i>	<i>Cribroheros longimanus</i> (100)	<i>Criberheros rostratus</i> (98.30)	<i>Cribroheros diquis</i> (94.90)	<i>Cribroheros longimanus</i> (99.84)	<i>Thorichthys rostratus</i> (98.29)	<i>Cribroheros diquis</i> (94.88)
10514	<i>Astatheros sp. (cf. rostratus)</i>	<i>Cribroheros longimanus</i> (99.85)	<i>Criberheros rostratus</i> (98.15)	<i>Cribroheros diquis</i> (94.75)	<i>Cribroheros longimanus</i> (99.69)	<i>Thorichthys rostratus</i> (98.14)	<i>Cribroheros diquis</i> (94.73)
10515	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.84)	<i>Rhamdia laticauda</i> (91.98)	<i>Rhamdia quelen</i> (100)	<i>Rhamdia laticauda</i> (98.16)	<i>Rhamdia guatemalensis</i> (97.98)
10516	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.84)	<i>Rhamdia laticauda</i> (91.98)	<i>Rhamdia quelen</i> (100)	<i>Rhamdia laticauda</i> (98.16)	<i>Rhamdia guatemalensis</i> (97.98)
10517	<i>Agonostomus monticola</i>	<i>Agonostomus monticola</i> (100)	<i>Joturus pichardi</i> (86.57)		<i>Dajaus monticola</i> (100)	<i>Joturus pichardi</i> (87.39)	

10519	<i>Astyanax</i> sp (cf. <i>aeneus</i>)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)
10520	<i>Astyanax</i> sp (cf. <i>aeneus</i>)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax aeneus</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.69)	<i>Bramocharax bransfordii</i> (99.84)	<i>Astyanax aeneus</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.69)
10521	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides salvadoris</i> (99.69)	<i>Roeboides ilseae</i> (98.15)	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.59)
10522	<i>Bryconamericus</i> sp (cf. <i>aeneus</i>)	<i>Astyanax aeneus</i> (100)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.69)	<i>Astyanax aeneus</i> (100)	<i>Bramocharax bransfordii</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)
10523	<i>Alfaro cultratus</i>	<i>Poecilia gillii</i> (99.70)	<i>Poecilia mexicana</i> (99.70)	<i>Poecilia sphenops</i> (99.56)	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (99.7)	<i>Poecilia schphenops</i> (99.55)
10524	<i>Alfaro cultratus</i>	<i>Poecilia gillii</i> (100)	<i>Poecilia mexicana</i> (100)	<i>Poecilia orri</i> (99.40)	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia schphenops</i> (99.85)
10526	<i>Hypostomus aspidolepis</i>	<i>Hypostomus</i> sp YTR-66 (100)	<i>Hypostomus affinis</i> (100)	<i>Hypostomus niceforoi</i> (100)	<i>Hypostomus robinii</i> (99.85)	<i>Hypostomus holostictus</i> (99.85)	<i>Hypostomus</i> sp YTR (100)
10528	<i>Hypostomus aspidolepis</i>	<i>Hypostomus</i> sp YTR-66 (100)	<i>Hypostomus affinis</i> (100)	<i>Hypostomus niceforoi</i> (100)	<i>Hypostomus robinii</i> (99.70)	<i>Hypostomus holostictus</i> (99.70)	<i>Hypostomus</i> sp YTR (99.85)
13381	<i>Eleotris amblyopsis</i>	<i>Dormitator maculatus</i> (99.69)	<i>Dormitator latifrons</i> (94.46)		<i>Dormitator maculatus</i> (97.59)	<i>Dormitator latifrons</i> (92.61)	<i>Guavina micropus</i> (85.94)
13383	?	<i>Amatitlania nigrofasciata</i> (99.69)			<i>Amatitlania nigrofasciata</i> (99.09)	<i>Amatitlania myrnae</i> (97.22)	<i>Amatitlania coatepeque</i> (99.11)
13387	<i>Atherinella argentea</i>	<i>Atherinella</i> sp (97.53)	<i>Atherinella alvarezi</i> (97.41)		<i>Atherinella</i> sp 97.26	<i>Atherinella hubbsi</i> 90.45	<i>Atherinella chagresi</i> 90.48
13389	<i>Atherinella argentea</i>	<i>Atherinella</i> sp (97.53)	<i>Atherinella alvarezi</i> (97.41)		<i>Atherinella</i> sp (97.26)	<i>Atherinella hubbsi</i> (90.29)	<i>Atherinella chagresi</i> (90.33)
13390	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (98.58)	<i>Pseudoxiphophorus bimaculatus</i> (86.61)		<i>Pseudoxiphophorus bimaculatus</i> (85.95)	<i>Heterandria bimaculata</i> (85.74)	
13391	<i>Strongylura</i> sp	<i>Strongylura timucu</i> (100)	<i>Strongylura marina</i> (100)		<i>Strongylura</i> sp (98.02)	<i>Strongylura timucu</i> (98.01)	<i>Strongylura marina</i> (97.92)
13392	<i>Poecilia mexicana</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia sphenops</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia sphenops</i> (99.85)
13393	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia salvatoris</i> (99.40)	<i>Poecilia gillii</i> (99.56)	<i>Poecilia mexicana</i> (99.56)	<i>Poecilia salvatoris</i> (99.12)
13394	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia salvatoris</i> (99.40)	<i>Poecilia gillii</i> (99.70)	<i>Poecilia mexicana</i> (99.70)	<i>Poecilia salvatoris</i> (99.26)
13395	<i>Poecilia mexicana</i>	<i>Poecilia mexicana</i> (99.85)	<i>Poecilia gillii</i> (99.85)	<i>Poecilia salvatoris</i> (99.40)	<i>Poecilia gillii</i> (99.56)	<i>Poecilia mexicana</i> (99.56)	<i>Poecilia salvatoris</i> (99.12)
13396	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (98.75)	<i>Gambusia affini</i> (86.65)		<i>Belonesox belizanus</i> (98.15)	<i>Pseudoxiphophorus bimaculatus</i> (86.26)	<i>Gambusia holbrooki</i> (85.65)
13397	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (97.12)	<i>Xiphophorus variatus</i> (86.41)		<i>Belonesox belizanus</i> (95.32)	<i>Pseudoxiphophorus bimaculatus</i> (83.74)	<i>Heterandria bimaculata</i> (83.64)
13398	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.54)
13399	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Astyanax aeneus</i> (99.69)	<i>Bramocharax bransfordii</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.69)	<i>Astyanax aeneus</i> (99.54)
13401	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (99.70)	<i>Gobiomorus maculatus</i> (100)	<i>Perciformes</i> sp (94.34)

13406	<i>Herotilapia multispinosa</i>	<i>Parachromis loisellei</i> (99.36)	<i>Parachromis motaguensis</i> (98.58)	<i>Parachromis friedrichsthalii</i> (97.45)	<i>Parachromis loisellei</i> (96.46)	<i>Parachromis friedrichsthalii</i> (94.64)	<i>Amphilophus amarillo</i> (91.94)
13407	<i>Herotilapia multispinosa</i>	<i>Parachromis loisellei</i> (100)	<i>Parachromis motaguensis</i> (99.32)	<i>Parachromis friedrichsthalii</i> (98.16)	<i>Parachromis loisellei</i> (100)	<i>Parachromis friedrichsthalii</i> (98.16)	<i>Amphilophus amarillo</i> (95.45)
13408	<i>Archocentrus nigrofasciatus</i>	<i>Parachromis managuensis</i> (100)	<i>Parachromis friedrichsthalii</i> (99.54)	<i>Parachromis managuensis</i> (97.87)	<i>Parachromis dovii</i> (95.52)	<i>Amphilophus amarillo</i> (92.90)	
13409	<i>Archocentrus nigrofasciatus</i>	<i>Archocentrus centrarchus</i> (99.83)	<i>Amphilophus citrinellus</i> (95.68)	<i>Amphilophus sp rsj</i> (95.64)	<i>Archocentrus centrarchus</i> (97.99)	<i>Amphilophus amarillo</i> (93.35)	<i>Amphilophus citrinellus</i> (93.35)
13410	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.69)	<i>Bramocharax bransfordii</i> (99.69)	<i>Astyanax aeneus</i> (99.23)
13411	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13412	<i>Astatheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Criboheros alfari</i> (94.11)	<i>Cribroheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.45)	<i>Cribroheros diquis</i> (94.27)
13413	<i>Astatheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Criboheros diquis</i> (94.25)	<i>Cribroheros rostratus</i> (99.69)	<i>Cribroheros longimanus</i> (98.39)	<i>Cribroheros diquis</i> (94.14)
13414	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.37)	<i>Alfaro huberi</i> (88.68)	<i>Brachyrhaphis episcopi</i> (85.49)	<i>Alfaro cultratus</i> (96.96)	<i>Alfaro huberi</i> (86.63)	<i>Phallichthys amates</i> (83.51)
13415	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (100)	<i>Alfaro huberi</i> (88.51)	<i>Brachyrhaphis episcopi</i> (85.67)	<i>Alfaro cultratus</i> (97.72)	<i>Alfaro huberi</i> (87.23)	<i>Brachyrhaphis</i> (84.41)
13420	<i>Poeciliopsis elongata</i>	<i>Phallichthys amates</i> (99.51)	<i>Brachyrhaphis episcopi</i> (89.64)	<i>Brachyrhaphis punctifer</i> (89.30)	<i>Phallichthys amates</i> (95.68)	<i>Brachyrhaphis episcopi</i> (86.42)	<i>Phallichthys tico</i> (85.80)
13422	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)	<i>Astyanax nicaraguensis</i> (99.85)
13423	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13424	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia laticauda</i> (98.14)	<i>Rhamdia guatemalensis</i> (97.96)	<i>Rhamdia quelen</i> (99.07)	<i>Rhamdia guatemalensis</i> (96.92)	
13426	<i>Parachromis sp</i>	<i>Parachromis managuensis</i> (99.83)	<i>Parachromis friedrichsthalii</i> (99.05)	<i>Parachromis managuensis</i> (96.33)	<i>Parachromis dovii</i> (93.52)	<i>Amphilophus amarillo</i> (91.20)	
13432	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (99.84)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.85)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax aeneus</i> (99.69)
13433	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (99.85)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax aeneus</i> (99.69)
13434	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (99.13)	<i>Parachromis friedrichsthalii</i> (98.11)	<i>Parachromis loisellei</i> (98.15)	<i>Parachromis friedrichsthalii</i> (96.47)	<i>Amphilophus amarillo</i> (93.32)
13435	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (99.13)	<i>Parachromis friedrichsthalii</i> (97.95)	<i>Parachromis loisellei</i> (98.30)	<i>Parachromis friedrichsthalii</i> (96.47)	<i>Amphilophus amarillo</i> (92.47)
13436	<i>Eleotris amblyopsis</i>	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (99.54)		<i>Eleotris amblyopsis</i> (99.26)		
13437	<i>Archocentrus nigrofasciatus</i>	<i>Amatitlania nigrofasciata</i> (99.84)			<i>Amatitlania nigrofasciata</i> (98.48)	<i>Amatitlania myrnae</i> (96.63)	<i>Amatitlania coatepeque</i> (98.21)
13438	<i>Archocentrus nigrofasciatus</i>	<i>Amantitlania nigrofasciata</i> (99.84)			<i>Amantitlania nigrofasciata</i> (97.57)	<i>Amantitlania myrnae</i> (95.83)	<i>Amatitlania coatepeque</i> (97.14)
13439	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.06)	<i>Alfaro huberi</i> (89.05)	<i>Phallichthys amates</i>	<i>Alfaro cultratus</i> (96.81)	<i>Alfaro huberi</i> (87.08)	<i>Phallichthys amates</i> (83.82)
13440	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.36)	<i>Alfaro huberi</i> (88.68)	<i>Phallichthys amates</i> (85.44)	<i>Alfaro cultratus</i> (95.74)	<i>Alfaro huberi</i> (85.56)	<i>Phallichthys amates</i> (82.59)

13441	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia sphenops</i> (99.85)	<i>Poecilia gillii</i> (100)	<i>Poecilia mexicana</i> (100)	<i>Poecilia sphenops</i> (99.85)
13442	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (99.38)	<i>Poecilia gillii</i> (99.38)	<i>Poecilia sphenops</i> (99.08)	<i>Poecilia gillii</i> (96.32)	<i>Poecilia mexicana</i> (96.32)	<i>Poecilia sphenops</i> (96.02)
13444	<i>Poeciliopsis elongata</i>	<i>Phallichthys amates</i> (100)	<i>Brachyrhaphis episcopi</i> (89.65)	<i>Brachyrhaphis punctifer</i> (89.0)	<i>Phallichthys amates</i> (97.84)	<i>Brachyrhaphis episcopi</i> (87.91)	<i>Phallichthys tico</i> (87.10)
13445	<i>Bramocharax bransfordii</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.85)
13446	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (99.12)	<i>Parachromis friedrichsthalii</i> (97.95)	<i>Parachromis loisellei</i> (98.15)	<i>Parachromis friedrichsthalii</i> (96.32)	<i>Amphilophus amarillo</i> (93.30)
13447	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (98.95)	<i>Parachromis friedrichsthalii</i> (98.11)	<i>Parachromis loisellei</i> (98.15)	<i>Parachromis friedrichsthalii</i> (96.47)	<i>Amphilophus amarillo</i> (92.44)
13448	<i>Astatheros rostratus</i>	<i>Amphilophus citrinellus</i> (99.84)	<i>Amphilophus cf. labiatus</i> (99.84)	<i>Amphilophus cf. sagittae</i> (99.84)	<i>Amphilophus amarillo</i> (97.82)	<i>Amphilophus citrinellus</i> (97.82)	<i>Amphilophus labiatus</i> (98.31)
13449	?	no match			<i>Lycengraulis grossidens</i> (94.06)	<i>Anchoviella</i> sp (86.83)	
13451	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (100)	<i>Rhamdia laticauda</i> (98.16)	<i>Rhamdia guatemalensis</i> (97.98)	<i>Rhamdia quelen</i> (100)	<i>Rhamdia guatemalensis</i> (97.84)	
13452	<i>Astatheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Cribroheros diquis</i> (94.26)	<i>Cribroheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.46)	<i>Cribroheros diquis</i> (94.29)
13453	<i>Astatheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Cribroheros diquis</i> (94.26)	<i>Cribroheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.46)	<i>Cribroheros diquis</i> (94.29)
13456	<i>Bramocharax bransfordii</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aenus</i> (99.84)	<i>Bramocharax bransfordii</i> (99.54)	<i>Astyanax nicaraguensis</i> (99.38)	<i>Astyanax aeneus</i> (99.38)
13457	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (99.12)	<i>Parachromis friedrichsthalii</i> (97.92)	<i>Parachromis loisellei</i> (96.91)	<i>Parachromis friedrichsthalii</i> (95.09)	<i>Parachromis motaguensis</i> (96.26)
13463	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.05)	<i>Gobiomorus maculatus</i> (98.14)	<i>Perciformes</i> sp (92.44)
13464	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.05)	<i>Gobiomorus maculatus</i> (98.16)	<i>Perciformes</i> sp (92.44)
13465	<i>Archocentrus centrarchus</i>	<i>Vieja maculicauda</i> (100)	<i>Vieja bifasciata</i> (99.65)	<i>Vieja melanura</i> (99.30)	<i>Vieja maculicauda</i> (98.15)	<i>Vieja synspilus</i> (97.14)	<i>Vieja melanura</i> (97.29)
13466	<i>Archocentrus sp</i>	<i>Amphilophus centrarchus</i> (99.84)	<i>Amphilophus citrinellus</i> (95.80)	<i>Amphilophus sagittae</i> (95.77)	<i>Archocentrus centrarchus</i> (98.30)	<i>Amphilophus amarillo</i> (93.73)	<i>Amphilophus citrinellus</i> (93.73)
13467	<i>Archocentrus centrarchus</i>	<i>Amatitlania nigrofasciata</i> (99.69)			<i>Amatitlania nigrofasciata</i> (98.48)	<i>Amatitlania myrnae</i> (96.60)	<i>Amatitlania coatepeque</i> (99.14)
13469	<i>Poeciliopsis elongata</i>	<i>Phallichthys amates</i> (100)	<i>Brachyrhaphis episcopi</i> (89.56)	<i>Phallichthys tico</i> (89.04)	<i>Phallichthys amates</i> (100)	<i>Brachyrhaphis episcopi</i> (89.81)	<i>Phallichthys tico</i> (89.16)
13470	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.08)	<i>Alfaro huberi</i> (89.09)	<i>Phallichthys amates</i> (85.67)	<i>Alfaro cultratus</i> (99.09)	<i>Alfaro huberi</i> (89.21)	<i>Phallichthys amates</i> (85.98)
13473	<i>Poecilia gillii</i>	<i>Poecilia mexicana</i> (100)	<i>Poecilia gillii</i> (100)	<i>Poecilia sphenops</i> (99.85)	<i>Poecilia gillii</i> (100)	<i>Poecilia mexicana</i> (100)	
13475	<i>Mugil sp</i>	<i>Mugil trichodon</i> (99.84)	<i>Mugil hospes</i> (99.83)	<i>Mugil brevirostris</i> (93.53)	<i>Mugil sp</i> (98.02)	<i>Mugil trichodon</i> (98.01)	<i>Mugil hospes</i> (98.35)
13476	<i>Mugil sp</i>	<i>Mugil hospes</i> (99.66)	<i>Mugil trichodon</i> (99.22)	<i>Mugil brevirostris</i> (93.72)	<i>Mugil sp</i> (97.40)	<i>Mugil trichodon</i> (97.54)	<i>Mugil hospes</i> (98.02)
13479	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.55)	<i>Gobiomorus maculatus</i> (98.45)	<i>Perciformes</i> sp (92.97)

13481	<i>Poecilia gillii</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (95.30)	<i>Perciformes</i> (96.01)	<i>Gobiomorus maculatus</i> (96.12)
13482	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (100)	<i>Parachromis motaguensis</i> (99.32)	<i>Parachromis friedrichsthalii</i> (98.15)	<i>Parachromis loisellei</i> (99.69)	<i>Parachromis friedrichsthalii</i> (97.85)	<i>Amphilophus amarillo</i> (94.44)
13483	<i>Amphilophus citrinellus</i>	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus cf citrinellus</i> (100)	<i>Amphilophus cf libiatus</i> (100)	<i>Amphilophus amarillo</i> (99.0)	<i>Amphilophus citrinellus</i> (99.0)	<i>Amphilophus labiatus</i> (99.85)
13484	<i>Archocentrus sp</i>	<i>Herotilapia multispinosa</i> (100)	<i>Kronoheros umbrifer</i> (91.6)	<i>Vieja bifasciata</i> (90.96)	<i>Herotilapia multispinosa</i> (99.69)	<i>Vieja synspilus</i> (90.31)	<i>Rocio octofasciata</i> (89.89)
13485	<i>Archocentrus sp</i>	<i>Herotilapia multispinosa</i> (99.53)	<i>Kronoheros umbrifer</i> (91.61)	<i>Vieja bifasciata</i> (90.78)	<i>Archocentrus multispinosus</i> (98.16)	<i>Paraneetroplus synspilus</i> (88.89)	<i>Kronoheros umbrifer</i> (90.43)
13486	<i>Archocentrus centrarchus</i>	<i>Archocentrus centrarchus</i> (99.84)	<i>Amphilophus citrinellus</i> (95.85)	<i>Amphilophus sagittae</i> (95.8)	<i>Archocentrus centrarchus</i> (99.23)	<i>Amphilophus amarillo</i> (94.59)	<i>Amphilophus labiatus</i> (95.08)
13487	<i>Archocentrus centrarchus</i>	<i>Archocentrus centrarchus</i> (99.84)	<i>Amphilophus citrinellus</i> (95.86)	<i>Amphilophus sagittae</i> (95.82)	<i>Archocentrus centrarchus</i> (99.54)	<i>Amphilophus amarillo</i> (94.88)	<i>Amphilophus labiatus</i> (95.40)
13488	<i>Archocentrus nigrofasciatus</i>	<i>Amatitlania nigrofasciata</i> (97.73)			<i>Amatitlania nigrofasciata</i> (83.10)	<i>Amatitlania myrnae</i> (82.06)	<i>Amatitlania coatepeque</i> (84.13)
13489	?	<i>Thorichthys rostratus</i> (99.69)	<i>Cribroheros longimanus</i> (98.43)	<i>Cribroheros diquis</i> (94.09)	<i>Cribroheros rostratus</i> (99.38)	<i>Cribroheros longimanus</i> (97.99)	<i>Cribroheros diquis</i> (93.81)
13490	?	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Cribroheros diquis</i> (94.26)	<i>Cribroheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.45)	<i>Cribroheros diquis</i> (94.28)
13492	<i>Brycon guatemalensis</i>	<i>Brycon guatemalensis</i> (100)			<i>Brycon guatemalensis</i> (99.54)	<i>Brycon behreae</i> (98.61)	<i>Brycon chagrensis</i> (98.30)
13493	?	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus amarillo</i> (100)	<i>Amphilophus cf citrinellus</i> (100)	<i>Amphilophus amarillo</i> (100)	<i>Amphilophus citrinellus</i> (100)	<i>Amphilophus labiatus</i> (100)
13494	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.85)
13495	<i>Astyanax aeneus</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.58)	<i>Roeboides bouchellei</i> (99.69)	<i>Roeboides salvadoris</i> (99.38)	<i>Roeboides ilseae</i> (97.84)
13496	<i>Trachinotus carolinus</i>				<i>Achirus lineatus</i> (91.86)	<i>Soleonassus finis</i> (85.87)	
13497	<i>Trachinotus carolinus</i>				<i>Archirus lineatus</i> (91.65)	<i>Soleonassus finis</i> (85.71)	
13499	<i>Astyanax cf. nasutus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13500	<i>Atherinella milleri</i>	<i>Atherinella sp</i> (97.22)	<i>Atherinella alvaezi</i> (97.09)		<i>Atherinella sp</i> (96.81)	<i>Atherinella hubbsi</i> (90.31)	<i>Atherinella chagresi</i> (90.34)
13501	<i>Parachromis managuensis</i>	<i>Parachromis dovii</i> (100)	<i>Parachromis managuensis</i> (96.62)	<i>Parachromis friedrichsthalii</i> (96.47)	<i>Parachromis dovii</i> (100)	<i>Parachromis managuensis</i> (96.02)	<i>Amphilophus amarillo</i> (93.75)
13502	<i>Gobiomorus dormitor</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (92.39)	<i>Gobiomorus maculatus</i> (92.80)	
13503	<i>Alfaro cultratus</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (98.43)	<i>Gobiomorus maculatus</i> (99.84)	
13505	<i>Microphis brachyurus</i>	<i>Microphis brachyurus</i> (100)	<i>Microphis lineatus</i> (100)		<i>Microphis brachyurus</i> (98.15)	<i>Microphis lineatus</i> (99.38)	
13508	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.06)	<i>Alfaro huberi</i> (89.05)	<i>Phallichthys amates</i> (85.56)	<i>Alfaro cultratus</i> (97.26)	<i>Alfaro huberi</i> (87.54)	<i>Phallichthys amates</i> (84.28)
13510	<i>Rhamdia nicaraguensis</i>	<i>Rhamdia quelen</i> (97.55)	<i>Rhamdia guatemalensis</i> (95.98)	<i>Rhamdia laticauda</i> (95.62)	<i>Rhamdia quelen</i> (94.90)	<i>Rhamdia guatemalensis</i> (93.08)	

13523	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei</i> (99.84)	<i>Roeboides ilseae</i> (97.98)	<i>Roeboides occientalis</i> (96.43)	<i>Roeboides bouchellei</i> (99.85)	<i>Roeboides saladoris</i> (99.54)	<i>Roeboides ilseae</i> (97.99)
13525	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (98.6)	<i>Poecilia</i> sp (86.84)		<i>Belonesox belizanus</i> (98.15)	<i>Poecilia</i> sp "Tipitapa" (85.51)	
13528	<i>Pseudophallus mindii</i>	<i>Microphis brachyurus</i> (100)	<i>Microphis lineatus</i> (100)		<i>Microphis brachyurus</i> (98.01)	<i>Microphis</i> sp (99.38)	<i>Microphis lineatus</i> (99.23)
13530	<i>Eleotris cf. pisonis</i>	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (99.38)		<i>Eleotris amblyopsis</i> (97.60)	<i>Eleotris</i> sp (98.78)	
13531	<i>Eleotris cf. pisonis</i>	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (100)		<i>Eleotris amblyopsis</i> (98.45)	<i>Eleotris</i> sp (99.69)	
13532	<i>Dormitor maculatus</i>	<i>Dormitor maculatus</i> (99.69)			<i>Dormitor maculatus</i> (98.30)	<i>Dormitor latifrons</i> (93.32)	
13533	?a	<i>Amphilophus citrinellus</i> (99.84)	<i>Amphilophus cf citrinellus</i> (99.84)	<i>Amphilophus cf libiatus</i> (99.84)	<i>Amphilophus amarillo</i> (96.87)	<i>Amphilophus citrinellus</i> (96.87)	<i>Amphilophus libiatus</i> (98)
13535	?c	<i>Archocentrus centrarchus</i> (99.69)	<i>Amphilophus citrinellus</i> (96.06)	<i>Amphilophus sagittae</i> (96.01)	<i>Archocentrus centrarchus</i> (99.54)	<i>Amphilophus amarillo</i> (95.26)	<i>Amphilophus citrinellus</i> (95.26)
13538	?d	<i>Vieja maculicauda</i> (98.71)	<i>Vieja bifasciata</i> (98.34)	<i>Vieja melanura</i> (97.87)	<i>Vieja maculicauda</i> (93.14)	<i>Paraneotroplus synspilus</i> (91.65)	<i>Vieja melanura</i> (92.13)
13539	?e	<i>Archocentrus centrarchus</i> (99.34)	<i>Mayaheros urophthalmus</i> (96.67)	<i>Amphilophus trimaculatus</i> (96.55)	<i>Archocentrus centrarchus</i> (94.55)	<i>Amphilophus amarillo</i> (90.27)	<i>Amphilophus citrinellus</i> (90.27)
13540	?f	<i>Archocentrus centrarchus</i> (98.33)	<i>Amphilophus citrinellus</i> (95.74)	<i>Amphilophus sagittae</i> (95.46)	<i>Archocentrus centrarchus</i> (94.91)	<i>Amphilophus amarillo</i> (90.74)	<i>Amphilophus citrinellus</i> (90.74)
13541	?g	<i>Amphilophus cf. citrinellus</i> (100)	<i>Amphilophus</i> sp. (100)	<i>Amphilophus cf. labiatus</i> (100)	<i>Amphilophus amarillo</i> (96.03)	<i>Amphilophus citrinellus</i> (96.03)	<i>Amphilophus labiatus</i> (97.09)
13542	<i>Engraulidae</i> sp	no match			<i>Engraulis japonicus</i> (89.05)		
13543	<i>Engraulidae</i> sp	no match			<i>Engraulis japonicus</i> (89.63)		
13547	<i>Atherinella sardina</i>	<i>Atherinella alvarezi</i> (97.41)	<i>Atherinella</i> sp (97.38)		<i>Atherinella</i> sp (97.11)	<i>Atherinella hubbsi</i> (90.14)	<i>Atherinella chagresi</i> (90.28)
13548	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.58)	<i>Roeboides bouchellei</i> (100)	<i>Roeboides salvadoris</i> (99.69)	<i>Roeboides ilseae</i> (98.15)
13549	<i>Alfaro cultratus</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.59)	<i>Roeboides bouchellei</i> (100)	<i>Roeboides salvadoris</i> (99.69)	<i>Roeboides ilseae</i> (98.15)
13550	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (98.72)	<i>Alfaro huberi</i> (88.52)	<i>Xiphophorus nezahualcoyotl</i> (85.29)	<i>Alfaro cultratus</i> (95.44)	<i>Alfaro huberi</i> (85.56)	<i>Phallichthys amates</i> (82.74)
13552	<i>Cribroheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Cribroheros diquis</i> (94.26)	<i>Criboheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.46)	<i>Cribroheros diquis</i> (94.29)
13553	<i>Cribroheros rostratus</i>	<i>Thorichthys rostratus</i> (99.84)	<i>Cribroheros longimanus</i> (98.59)	<i>Cribroheros diquis</i> (94.26)	<i>Cribroheros rostratus</i> (99.85)	<i>Cribroheros longimanus</i> (98.46)	<i>Cribroheros diquis</i> (94.29)
13555	<i>Astyanax nicaraguensis</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13564	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.68)	<i>Parachromis motaguensis</i> (99.12)	<i>Parachromis friedrichsthalii</i> (97.75)	<i>Parachromis loisellei</i> (96.15)	<i>Parachromis friedrichsthalii</i> (94.33)	<i>Amphilophus amarillo</i> (91.35)
13565	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.47)	<i>Parachromis motaguensis</i> (98.94)	<i>Parachromis friedrichsthalii</i> (97.52)	<i>Parachromis loisellei</i> (96.14)	<i>Parachromis friedrichsthalii</i> (94.33)	<i>Amphilophus amarillo</i> (91.19)
13567	<i>Archocentrus centrarchus</i>	<i>Archocentrus centrarchus</i> (99.65)	<i>Amphilophus citrinellus</i> (95.58)	<i>Amphilophus cf citrinellus</i> (95.56)	<i>Archocentrus centrarchus</i> (94.76)	<i>Amphilophus amarillo</i> (90.50)	<i>Amphilophus citrinellus</i> (90.50)

13570	<i>Vieja maculicauda</i>	<i>Vieja maculicauda</i> (99.12)	<i>Vieja bifasciata</i> (98.76)	<i>Vieja melanura</i> (98.13)	<i>Vieja maculicauda</i> (95.06)	<i>Paraneetroplus synspleius</i> (94.16)	
13573	<i>Amphilophus citrinellus</i>	<i>Amphilophus citrinellus</i> (99.81)	<i>Amphilophus cf citrinellus</i> (99.81)	<i>Amphilophus cf labiatus</i> (99.69)	<i>Amphilophus amarillo</i> (98.01)	<i>Amphilophus citrinellus</i> (98.01)	<i>Amphilophus labiatus</i> (98.77)
13574	<i>Rivulus sp</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.59)	<i>Perciformes sp</i> (98.62)	<i>Gobiomorus maculatus</i> (98.61)
13575	<i>Rivulus sp</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.59)	<i>Perciformes sp</i> (98.62)	<i>Gobiomorus maculatus</i> (98.61)
13576	<i>Rivulus sp</i>	<i>Gobiomorus dormitor</i> (100)	<i>Gobiomorus maculatus</i> (99.84)		<i>Gobiomorus dormitor</i> (96.88)	<i>Perciformes sp</i> (98.17)	<i>Gobiomorus maculatus</i> (98.15)
13577	<i>Rivulus sp</i>	<i>Gobiomorus dormitor</i> (99.85)	<i>Gobiomorus maculatus</i> (99.84)		<i>Gobiomorus dormitor</i> (97.87)	<i>Perciformes sp</i> (99.08)	<i>Gobiomorus maculatus</i> (99.22)
13578	<i>Centropomus cf. robalito</i>	<i>Centropomus sp</i> (99.69)	<i>Centropomus viridis</i> (95.32)	<i>Centropomus medius</i> (95.32)	<i>Centropomus parallelus</i> (86.31)	<i>Centropomus undecimalis</i> (85.19)	
13579	<i>Centropomus cf. robalito</i>	<i>Centropomus parallelus</i> (99.69)	<i>Centropomus cruentata</i> (99.69)	<i>Centropomus mexicanus</i> (99.51)	<i>Centropomus parallelus</i> (94.94)	<i>Cephalopholis boenak</i> (81.34)	
13580	<i>Eleotris sp</i>	<i>Eleotris amblyopsis</i> (99.53)	<i>Eleotris pisonis</i> (99.06)		<i>Eleotris amblyopsis</i> (96.07)	<i>Eleotris sp</i> (97.41)	
13584	<i>Amphilophus citrinellus</i>	<i>Microphis lineatus</i> (99.03)	<i>Microphis brachyurus</i> (99.03)		<i>Microphis brachyurus</i> (93.61)	<i>Microphis lineatus</i> (94.88)	<i>Oostethus brachyurus</i> (95.15)
13585	<i>Pseudophallus mindii</i>	<i>Microphis brachyurus</i> (98.84)	<i>Microphis lineatus</i> (98.56)		<i>Microphis brachyurus</i> (94.03)	<i>Microphis sp</i> (95.94)	<i>Microphis lineatus</i> (95.79)
13586	<i>Archocentrus centrarchus</i>	<i>Archocentrus centrarchus</i> (99.83)	<i>Amphilophus citrinellus</i> (95.66)	<i>Amphilophus cf citrinellus</i> (95.64)	<i>Archocentrus centrarchus</i> (97.99)	<i>Amphilophus amarillo</i> (93.47)	<i>Amphilophus citrinellus</i> (93.47)
13587	<i>Archocentrus centrarchus</i>	<i>Parachromis loisellei</i> (99.84)	<i>Parachromis motaguensis</i> (99.14)	<i>Parachromis friedrichsthalii</i> (97.97)	<i>Parachromis loisellei</i> (98.15)	<i>Parachromis friedrichsthalii</i> (96.32)	<i>Amphilophus amarillo</i> (93.05)
13588	<i>Parachromis loisellei</i>	<i>Parachromis loisellei</i> (99.69)	<i>Parachromis motaguensis</i> (99.14)	<i>Parachromis friedrichsthalii</i> (97.82)	<i>Parachromis loisellei</i> (97.85)	<i>Parachromis friedrichsthalii</i> (96.30)	<i>Amphilophus amarillo</i> (92.77)
13590	<i>Eleotris sp 1</i>	<i>Eleotris amblyopsis</i> (100)	<i>Eleotris pisonis</i> (100)		<i>Eleotris amblyopsis</i> (98.72)	<i>Eleotris sp</i> (99.69)	
13591	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13592	<i>Astyanax aenus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13593	<i>Centropomus cf. robalito</i>	<i>Centropomus parallelus</i> (99.85)	<i>Cephalopholis cruentata</i> (99.85)	<i>Centropomus mexicanus</i> (99.68)	<i>Centropomus parallelus</i> (97.39)	<i>Cephalopholis hemistiktos</i> (82.94)	
13594	<i>Centropomus cf. robalito</i>	<i>Centropomus sp</i> (99.85)	<i>Centropomus viridis</i> (95.67)	<i>Centropomus medius</i> (95.67)	<i>Centropomus parallelus</i> (87.69)	<i>Centropomus undecimalis</i> (86.73)	
13595	<i>Rivulus sp</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (98.58)	<i>Perciformes sp</i> (99.69)	<i>Gobiomorus maculatus</i> (99.69)
13596	<i>Rivulus sp</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (98.58)	<i>Perciformes sp</i> (99.69)	<i>Gobiomorus maculatus</i> (99.69)
13598	<i>Eleotris sp 2</i>	<i>Eleotris pisonis</i> (100)	<i>Eleotris perniger</i> (100)		<i>Eleotris amblyopsis</i> (98.58)	<i>Eleotris perniger</i> (99.85)	
13599	<i>Strongylura marina</i>	<i>Strongylura timucu</i> (100)	<i>Strongylura marina</i> (100)		<i>Strongylura sp</i> (99.69)	<i>Strongylura timucu</i> (99.69)	<i>Strongylura marina</i> (99.68)
13601	<i>Atherinella sp</i>	<i>no match</i>			<i>Atherinella sp</i> (93.31)	<i>Atherinella hubbsi</i> (87.89)	<i>Atherinella chagresi</i> (87.74)

13602	<i>Pseudophallus mindii</i>	<i>Microphis lineatus</i> (99.06)	<i>Microphis brachyurus</i> (99.03)		<i>Microphis brachyurus</i> (97.16)	<i>Microphis lineatus</i> (98.61)	<i>Microphis</i> sp (98.45)
13603	<i>Brycon guatemalensis</i>	<i>Brycon guatemalensis</i> (100)			<i>Brycon guatemalensis</i> (99.85)	<i>Brycon behreae</i> (98.92)	<i>Brycon</i> sp (98.91)
13604	<i>Hyphessobrycon tortuguerae</i>	<i>Hyphessobrycon tortuguerae</i> (100)	<i>Hyphessobrycon compressus</i> (91.55)	<i>Hyphessobrycon</i> sp (86.98)	<i>Hyphessobrycon tortuguerae</i> (100)	<i>Hyphessobrycon compressus</i> (89.88)	<i>Hyphessobrycon savagei</i> (86.29)
13605	<i>Eleotris</i> sp	<i>Eleotris pisonis</i> (99.84)	<i>Eleotris perniger</i> (99.70)		<i>Eleotris amblyopsis</i> (97.87)	<i>Eleotris perniger</i> (99.25)	<i>Eleotris pisonis</i> (99.38)
13606	<i>Brycon guatemalensis</i>	<i>Brycon guatemalensis</i> (100)			<i>Brycon guatemalensis</i> (99.85)	<i>Brycon behreae</i> (98.92)	<i>Brycon chagrensis</i> (98.61)
13609	<i>Astyanax aeneus</i>	<i>Brycon guatemalensis</i> (100)			<i>Brycon guatemalensis</i> (100)	<i>Brycon behreae</i> (99.07)	<i>Brycon chgrensis</i> (98.77)
13610	<i>Astyanax aeneus</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13611	<i>Hyphessobrycon tortuguerae</i>	<i>Hyphessobrycon tortuguerae</i> (99.84)	<i>Hyphessobrycon compressus</i> (91.86)	<i>Hyphessobrycon</i> sp (87.13)	<i>Hyphessobrycon tortuguerae</i> (99.85)	<i>Hyphessobrycon compressus</i> (89.72)	<i>Hyphessobrycon columnianus</i> (86.04)
13612	<i>Amantitania septemfasciata</i>	<i>Amantitania nigrofasciata</i> (99.85)			<i>Amantitania nigrofasciata</i> (99.54)	<i>Amantitania myrnae</i> (97.75)	<i>Amphilophus amarillo</i> (92.90)
13613	<i>Pseudophallus mindii</i>	<i>Microphis brachyurus</i> (99.67)	<i>Microphis lineatus</i> (99.53)		<i>Microphis brachyurus</i> (97.15)	<i>Microphis</i> sp (98.15)	<i>Microphis lineatus</i> (97.99)
13614	<i>Astyanax aeneus</i>	<i>Astyanax nicaraguensis</i> (99.85)	<i>Bramocharax bransfordii</i> (99.84)	<i>Astyanax aeneus</i> (99.69)	<i>Astyanax nicaraguensis</i> (99.85)	<i>Bramocharax bransfordii</i> (99.85)	<i>Astyanax aeneus</i> (99.69)
13615	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.38)	<i>Alfaro huberi</i> (88.75)	<i>Brachyrhaphis episcopi</i> (85.63)	<i>Alfaro cultratus</i> (99.09)	<i>Alfaro huberi</i> (88.60)	<i>Phallichthys amates</i> (85.52)
13616	<i>Alfaro cultratus</i>	<i>Alfaro cultratus</i> (99.38)	<i>Alfaro huberi</i> (88.77)	<i>Brachyrhaphis episcopi</i> (85.65)	<i>Alfaro cultratus</i> (99.24)	<i>Alfaro huberi</i> (88.75)	<i>Phallichthys amates</i> (85.67)
13619	<i>Astyanax bransfordii</i>	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax nicaraguensis</i> (100)	<i>Astyanax aeneus</i> (99.84)	<i>Astyanax nicaraguensis</i> (100)	<i>Bramocharax bransfordii</i> (100)	<i>Astyanax aeneus</i> (99.85)
13620	<i>Gymnotus maculosus</i>	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus maculosus</i> (99.84)	<i>Gymnotus carpo</i> (92.11)	<i>Gymnotus cylindricus</i> (100)	<i>Gymnotus carapo</i> (90.97)	<i>Gymnotus pantanal</i> (90.91)
13621	<i>Amantitania septemfasciata</i>	<i>Amantitania nigrofasciata</i> (99.85)			<i>Amantitania nigrofasciata</i> (98.94)	<i>Amantitania myrae</i> (97.07)	<i>Amantitania coatepeque</i> (99.11)
13622	<i>Hemieleotris latifasciatus</i>	<i>Gobiomorus maculatus</i> (100)	<i>Gobiomorus dormitor</i> (100)		<i>Gobiomorus dormitor</i> (97.87)	<i>Perciformes</i> (98.83)	<i>Gobiomorus maculatus</i> (98.92)
13623	<i>Poecilia</i> sp 2	<i>Phallichthys amates</i> (99.84)	<i>Brachyrhaphis episcopi</i> (89.62)	<i>Brachyrhaphis punctifer</i> (88.82)	<i>Phallichthys amates</i> (99.54)	<i>Brachyrhaphis episcopi</i> (89.51)	<i>Phallichthys tico</i> (88.70)
13624	<i>Eleotris</i> sp	<i>Eleotris amblyopsis</i> (99.69)	<i>Eleotris pisonis</i> (99.69)		<i>Eleotris amblyopsis</i> (97.61)	<i>Eleotris</i> sp (98.78)	
13630	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (98.44)	<i>Gambusia affinis</i> (86.68)		<i>Belonesox belizanus</i> (98.15)	<i>Poecilia</i> sp "Tipitapa" (85.51)	
13631	<i>Anchoviella</i> sp	no match			<i>Engraulis japonicus</i> (89.96)	<i>Engraulis encrasicolus</i> (88.92)	
13633	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei</i> (100)	<i>Roeboides ilseae</i> (98.14)	<i>Roeboides occidentalis</i> (96.59)	<i>Roeboides bouchellei</i> (100)	<i>Roeboides salvadoris</i> (99.69)	<i>Roeboides ilseae</i> (98.15)
13634	<i>Belonesox belizanus</i>	<i>Belonesox belizanus</i> (97.95)	<i>Gambusia affinis</i> (86.69)		<i>Belonesox belizanus</i> (97.20)	<i>Gambusia holbrooki</i> (84.84)	
13635	<i>Anchoviella</i> sp	no match			<i>Engraulis japonicus</i> (89.96)	<i>Engraulis encrasicolus</i> (88.92)	

13637	<i>Roeboides bouchellei</i>	<i>Roeboides bouchellei (100)</i>	<i>Roeboides ilseae (98.14)</i>	<i>Roeboides occidentalis (96.59)</i>	<i>Roeboides bouchellei (100)</i>	<i>Roeboides salvadoris (99.69)</i>	<i>Roeboides ilseae (98.15)</i>
13639	<i>Gymnotus maculosus</i>	<i>Gymnotus cylindricus (99.53)</i>	<i>Gymnotus maculosus (99.38)</i>	<i>Gymnotus carapo (91.89)</i>	<i>Gymnotus cylindricus (99.54)</i>	<i>Gymnotus carapo (90.94)</i>	<i>Gymnotus pantanal (91.06)</i>
13641	<i>Gymnotus maculosus</i>	<i>Gymnotus cylindricus (99.84)</i>	<i>Gymnotus maculosus (99.69)</i>	<i>Gymnotus carapo (91.89)</i>	<i>Gymnotus cylindricus (99.85)</i>	<i>Gymnotus carapo (90.95)</i>	<i>Gymnotus pantanal (91.06)</i>
13642	<i>Gymnotus maculosus</i>	<i>Gymnotus cylindricus (99.84)</i>	<i>Gymnotus maculosus (99.69)</i>	<i>Gymnotus carapo (91.80)</i>	<i>Gymnotus cylindricus (99.69)</i>	<i>Gymnotus carapo (90.80)</i>	<i>Gymnotus pantanal (90.91)</i>
13644	<i>Strongylura marina</i>	<i>Strongylura timucu (100)</i>	<i>Strongylura marina (100)</i>		<i>Strongylura sp (97.71)</i>	<i>Strongylura timucu (97.70)</i>	<i>Strongylura marina (97.76)</i>
13645	<i>Strongylura marina</i>	<i>Strongylura timucu (100)</i>	<i>Strongylura marina (100)</i>		<i>Strongylura sp (97.72)</i>	<i>Strongylura timucu (97.71)</i>	<i>Strongylura marina (97.77)</i>

Table B-S2. Regional List of Species Based on Literature

Class	Order	Family	Genus	Species	Source
Actinopterygii	Albuliformes	Albulidae	<i>Albula</i>	<i>vulpes</i>	(Angulo et al., 2013)
Actinopterygii	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>rostrata</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Anguilliformes	Ophichthidae	<i>Myrophis</i>	<i>platyrhynchus</i>	(GBIF)
Actinopterygii	Anguilliformes	Ophichthidae	<i>Myrophis</i>	<i>punctatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Atheriniformes	Atherinopsidae	<i>Atherinella</i>	<i>blackburni</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Atheriniformes	Atherinopsidae	<i>Atherinella</i>	<i>chagresi</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Atheriniformes	Atherinopsidae	<i>Atherinella</i>	<i>hubbsi</i>	(Angulo et al., 2013)
Actinopterygii	Atheriniformes	Atherinopsidae	<i>Atherinella</i>	<i>milleri</i>	(Angulo et al., 2013), (Winemiller & Leslie, 1992), (GBIF), (Mason, 2008)
Actinopterygii	Batrachoidiformes	Batachoididae	<i>Porichthys</i>	<i>pauciradiatus</i>	(GBIF)
Actinopterygii	Batrachoidiformes	Batachoididae	<i>Porichthys</i>	<i>plectrodon</i>	(GBIF)
Actinopterygii	Batrachoidiformes	Batrachoididae	<i>Batrachoides</i>	<i>gilberti</i>	(GBIF)
Actinopterygii	Batrachoidiformes	Batrachoididae	<i>Batrachoides</i>	<i>surinamensis</i>	(GBIF)
Actinopterygii	Beloniformes	Belonidae	<i>Strongylura</i>	<i>marina</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Beloniformes	Belonidae	<i>Strongylura</i>	<i>timucu</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)

Actinopterygii	Beloniformes	Hemiramphidae	<i>Hemiramphus</i>	<i>brasiliensis</i>	(Angulo et al., 2013)
Actinopterygii	Beloniformes	Hyporhamphus	<i>Hyporhamphus</i>	<i>roberti</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>aeneus</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>cocibolca</i>	(Angulo et al., 2013)
Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>fasciatus</i>	(Winemiller & Leslie, 1992), (GBIF)
Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>nasutus</i>	(GBIF)
Actinopterygii	Characiformes	Characidae	<i>Bramocharax</i>	<i>bransfordii</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Characiformes	Characidae	<i>Brycon</i>	<i>costaricensis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Characiformes	Characidae	<i>Bryconamericus</i>	<i>scleroparius</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Characiformes	Characidae	<i>Carlana</i>	<i>eigenmanni</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Characiformes	Characidae	<i>Hyphessobrycon</i>	<i>tortuguerae</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Characiformes	Characidae	<i>Roeboides</i>	<i>bouchellei</i>	(Angulo et al., 2013), (Mason, 2008)
Actinopterygii	Clupeiformes	Clupeidae	<i>Dorosoma</i>	<i>chavesi</i>	(Angulo et al., 2013)
Actinopterygii	Clupeiformes	Clupeidae	<i>Harengula</i>	<i>clupeola</i>	(GBIF)
Actinopterygii	Clupeiformes	Clupeidae	<i>Harengula</i>	<i>jaguana</i>	(GBIF)
Actinopterygii	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>aurita</i>	(GBIF)
Actinopterygii	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>brasiliensis</i>	(Angulo et al., 2013)

Actinopterygii	Clupeiformes	Engraulidae	<i>Anchoa</i>	<i>lamprotaenia</i>	(Winemiller & Leslie, 1992), (GBIF)
Actinopterygii	Clupeiformes	Engraulidae	<i>Anchoa</i>	<i>parva</i>	(Mason, 2008)
Actinopterygii	Clupeiformes	Engraulidae	<i>Anchoviella</i>	<i>elongata</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Clupeiformes	Engraulidae	<i>Cetengraulis</i>	<i>edentulus</i>	(GBIF)
Actinopterygii	Clupeiformes	Engraulidae	<i>Lycengraulis</i>	<i>grossidens</i>	(Angulo et al., 2013)
Actinopterygii	Clupeiformes	Pristigasteridae	<i>Chirocentron</i>	<i>bleekermanus</i>	(GBIF)
Actinopterygii	Clupeiformes	Pristigasteridae	<i>Odontognathus</i>	<i>compressus</i>	(GBIF)
Actinopterygii	Clupeiformes	Pristigasteridae	<i>Pellona</i>	<i>harroweri</i>	(GBIF)
Actinopterygii	Cyprinodontiformes	Cynodonichthys	<i>Cynodonichthys</i>	<i>isthmensis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Alfaro</i>	<i>cultratus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Belonesox</i>	<i>belizanus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Brachyrhaphis</i>	<i>holdridgei</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Brachyrhaphis</i>	<i>parismina</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Phallichthys</i>	<i>amates</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Poecilia</i>	<i>gillii</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Poecilia</i>	<i>mexicana</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Poecilia</i>	<i>sphenops</i>	(GBIF)

Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Priapichthys</i>	<i>annectens</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Xenophallus</i>	<i>umbratilis</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Elopiformes	Megalopidae	<i>Megalops</i>	<i>atlanticus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Gobiesociformes	Gobiesocidae	<i>Gobiesox</i>	<i>nudus</i>	(Angulo et al., 2013)
Actinopterygii	Gymnotiformes	Gymnotidae	<i>Gymnotus</i>	<i>cylindricus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Gymnotiformes	Gymnotidae	<i>Gymnotus</i>	<i>maculosus</i>	(Angulo et al., 2013), (Mason, 2008)
Actinopterygii	Lepisosteiformes	Lepisosteidae	<i>Atractosteus</i>	<i>tropicus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Mugiliformes	Mugilide	<i>Agonostomus</i>	<i>monticola</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Mugiliformes	Mugilide	<i>Joturus</i>	<i>pichardi</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Mugiliformes	Mugilide	<i>Mugil</i>	<i>cephalus</i>	(Angulo et al., 2013)
Actinopterygii	Mugiliformes	Mugilide	<i>Mugil</i>	<i>curema</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Carangidae	<i>Caranx</i>	<i>hippos</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Carangidae	<i>Caranx</i>	<i>latus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Carangidae	<i>Hemicaranx</i>	<i>amblyrhynchus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Carangidae	<i>Oligoplites</i>	<i>palometa</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Carangidae	<i>Oligoplites</i>	<i>saurus</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Carangidae	<i>Selene</i>	<i>setapinnis</i>	(GBIF)

Actinopterygii	Perciformes	Carangidae	<i>Selene</i>	<i>vomer</i>	(GBIF)
Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>carolinus</i>	(GBIF)
Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>falcatus</i>	(GBIF)
Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>goodei</i>	(GBIF)
Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>ensiferus</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>mexicanus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>parallelus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>pectinatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>undecimalis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Eleotridae	<i>Dormitator</i>	<i>maculatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Eleotridae	<i>Eleotris</i>	<i>amblyopsis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Eleotridae	<i>Eleotris</i>	<i>pisonis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Eleotridae	<i>Gobiomorus</i>	<i>dormitor</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Eleotridae	<i>Guavina</i>	<i>guavina</i>	(GBIF)
Actinopterygii	Perciformes	Eleotridae	<i>Leptophilypnus</i>	<i>fluviatilis</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Gerreidae	<i>Diapterus</i>	<i>auratus</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Gerreidae	<i>Diapterus</i>	<i>rhombeus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)

Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>argenteus</i>	(GBIF)
Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>harengulus</i>	(GBIF)
Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>melanopterus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gerreidae	<i>Eugerres</i>	<i>plumieri</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gerreidae	<i>Gerres</i>	<i>cinereus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Gobiidae	<i>Awaous</i>	<i>banana</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Gobiidae	<i>Awaous</i>	<i>tajasica</i>	(GBIF)
Actinopterygii	Perciformes	Gobiidae	<i>Bathygobius</i>	<i>soporator</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>boleosoma</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>claytonia</i> ¹	(GBIF)
Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>fasciatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>pseudofasciatus</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>smaragdus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Gobiidae	<i>Evorthodus</i>	<i>lyricus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)

¹ Listed by IUCN as vulnerable

Actinopterygii	Perciformes	Gobiidae	<i>Gobiooides</i>	<i>broussonnetii</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Gobiidae	<i>Gobiosoma</i>	<i>spes</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Gobiidae	<i>Sicydium</i>	<i>adelum</i> ²	(Angulo et al., 2013)
Actinopterygii	Perciformes	Haemulidae	<i>Conodon</i>	<i>nobilis</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Haemulidae	<i>Haemulopsis</i>	<i>corvinaeformis</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Haemulidae	<i>Pomadour</i>	<i>croco</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Kyphosidae	<i>Kyphosus</i>	<i>sectatrix</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>analis</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>griseus</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>jocu</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Microdesmidae	<i>Microdesmus</i>	<i>carri</i>	(GBIF)
Actinopterygii	Perciformes	Polynemidae	<i>Polydactylus</i>	<i>virginicus</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Perciformes	Sciaenidae	<i>Bairdiella</i>	<i>ronchus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Sciaenidae	<i>Larimus</i>	<i>breviceps</i>	(Angulo et al., 2013), (GBIF)

² Listed by IUCN as Endangered

Actinopterygii	Perciformes	Sciaenidae	<i>Menticirrhus</i>	<i>americanus</i>	(GBIF)
Actinopterygii	Perciformes	Sciaenidae	<i>Menticirrhus</i>	<i>littoralis</i>	(GBIF)
Actinopterygii	Perciformes	Sciaenidae	<i>Micropogonias</i>	<i>furnieri</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Serranidae	<i>Epinephelus</i>	<i>guttatus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Uranoscopidae	<i>Astroscopus</i>	<i>y-graecum</i>	(GBIF)
Actinopterygii	Perciformes	Cichlidae	<i>Amatitlania</i>	<i>septemfasciata</i>	(Winemiller & Leslie, 1992), (GBIF)
Actinopterygii	Perciformes	Cichlidae	<i>Amatitlania</i>	<i>siquia</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>alfari</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>citrinellus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>longimanus</i>	(Angulo et al., 2013), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>rostratus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Archocentrus</i>	<i>centrarchus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Archocentrus</i>	<i>multispinosa</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Hypsophrys</i>	<i>nematopus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Cichlidae	<i>Hypsophrys</i>	<i>nicaraguensis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Perciformes	Cichlidae	<i>Oreochromis</i>	<i>niloticus</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Cichlidae	<i>Parachromis</i>	<i>dovii</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)

Actinopterygii	Perciformes	Cichlidae	<i>Parachromis</i>	<i>friedrichsthalii</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Parachromis</i>	<i>managuensis</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Perciformes	Cichlidae	<i>Petenia</i>	<i>splendida</i>	(GBIF)
Actinopterygii	Perciformes	Cichlidae	<i>Rocio</i>	<i>octofasciata</i>	(GBIF)
Actinopterygii	Perciformes	Cichlidae	<i>Tomocichla</i>	<i>tuba</i>	(Angulo et al., 2013)
Actinopterygii	Perciformes	Cichlidae	<i>Vieja</i>	<i>maculicauda</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Perciformes	Echeneidae	<i>Echeneis</i>	<i>naucrates</i>	(GBIF)
Actinopterygii	Pleuronectiformes	Achiridae	<i>Achirus</i>	<i>declivis</i>	(Angulo et al., 2013), (GBIF)
Actinopterygii	Pleuronectiformes	Achiridae	<i>Achirus</i>	<i>lineatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Pleuronectiformes	Achiridae	<i>Trichromis</i>	<i>salvini</i>	(GBIF)
Actinopterygii	Pleuronectiformes	Achiridae	<i>Trinectes</i>	<i>paulistanus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Pleuronectiformes	Cynoglossidae	<i>Symphurus</i>	<i>plagusia</i>	(GBIF)
Actinopterygii	Pleuronectiformes	Paralichthyidae	<i>Citharichthys</i>	<i>arenaceus</i>	(Angulo et al., 2013)
Actinopterygii	Pleuronectiformes	Paralichthyidae	<i>Citharichthys</i>	<i>spilopterus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Pleuronectiformes	Paralichthyidae	<i>Citharichthys</i>	<i>uhleri</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Siluriformes	Ariidae	<i>Bagre</i>	<i>bagre</i>	(GBIF)
Actinopterygii	Siluriformes	Ariidae	<i>Bagre</i>	<i>marinus</i>	(GBIF)

Actinopterygii	Siluriformes	Ariidae	<i>Sciades</i>	<i>assimilis</i>	(Angulo et al., 2013)
Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>guatemalensis</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992), (Mason, 2008)
Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>laticauda</i>	(Angulo et al., 2013), (Mason, 2008)
Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>nicaraguensis</i>	(Angulo et al., 2013)
Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>quelen</i>	(GBIF)
Actinopterygii	Synbranchiformes	Synbranchidae	<i>Synbranchus</i>	<i>marmoratus</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Syngnathiformes	Syngnathidae	<i>Microphis</i>	<i>brachyurus</i>	(GBIF)
Actinopterygii	Syngnathiformes	Syngnathidae	<i>Microphis</i>	<i>lineatus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Actinopterygii	Syngnathiformes	Syngnathidae	<i>Pseudophallus</i>	<i>mindii</i>	(Angulo et al., 2013), (GBIF), (Mason, 2008)
Actinopterygii	Syngnathiformes	Syngnathidae	<i>Syngnathus</i>	<i>scovelli</i>	(Angulo et al., 2013)
Actinopterygii	Tetraodontiformes	Tetraodontidae	<i>Sphoeroides</i>	<i>testudineus</i>	(Angulo et al., 2013), (GBIF), (Winemiller & Leslie, 1992)
Elasmobranchii	Carcharhiniformes	Carcharhinidae	<i>Carcharhinus</i>	<i>leucas</i>	(Angulo et al., 2013), (Mason, 2008)
Elasmobranchii	Rhinopristiformes	Pristidae	<i>Pristis</i>	<i>pectinata</i> ³	(Angulo et al., 2013)
Elasmobranchii	Rhinopristiformes	Pristidae	<i>Pristis</i>	<i>pristis</i>	(Angulo et al., 2013)

³ Listed by IUCN as Critically Endangered

Appendix C:

Genbank and BOLD sequences used for Curated Reference Library

Table C-S1. Publicly available *coI* sequences used for the curated reference library.

Identification Number	Class	Order	Family	Genus	Species	Museum ID / Sample ID	Institution	Upload Date	Location	Date Collected	Sequence length	Source	Publication
GU22552 6.1	Actinopterygii	Albuliformes	Albulidae	<i>Albula</i>	<i>vulpes</i>	ECOCH5912	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Belize	2006-12-12	652	GENBANK/BOLD	Martha Valdez-Moreno, Lourdes Vásquez-Yeomans, Manuel Elías-Gutiérrez, Natalia V. Ivanova and Paul D. N. Hebert Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management Marine and Freshwater Research 2010-06-10;61(6):655-671
GU67269 7.1				<i>Albula</i>	<i>vulpes</i>	ECOCH6227	El Colegio de la Frontera Sur, Unidad Chetumal	05-Jan-21	Mexico	2009-02-13	601	GENBANK/BOLD	International Barcode of Life (iBOL)
KF92957 7.1				<i>Albula</i>	<i>vulpes</i>	KUT 231	University of Kansas, Biodiversity Research Center	11-Feb-14	Belize	1991-07-20	652	GENBANK/BOLD	University of Kansas Biodiversity Institute barcoding initiative
GU22436 7.1				<i>Albula</i>	<i>vulpes</i>	ECO-CH LP 3613	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Mexico	2004-10-15	652	GENBANK/BOLD	Martha Valdez-Moreno, Lourdes Vásquez-Yeomans, Manuel Elías-Gutiérrez, Natalia V. Ivanova and Paul D. N. Hebert Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management Marine and

													Freshwater Research 2010-06-10;61(6):655-671
EU52391 8.1	Actinopterygii	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>rostrata</i>	ROM-80156	Royal Ontario Museum	05-Mar-12	Quebec, Canada	2001-08-22	652	GENBANK/ BOLD	Sujeevan Ratnasingham and Paul D. N. Hebert A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System PLoS ONE 2013-07-13;8(7):e66213 J. April, R. L. Mayden, R. H. Hanner and L. Bernatchez Genetic calibration of species diversity among North America's freshwater fishes Proceedings of the National Academy of Sciences 2011-06-13;108(26):10602-10607 Nicolas Hubert, Robert Hanner, Erling Holm, Nicholas E. Mandrak, Eric Taylor, Mary Burridge, Douglas Watkinson, Pierre Dumont, Allen Curry, Paul Bentzen, Junbin Zhang, Julien April and Louis Bernatchez Identifying Canadian Freshwater Fishes through DNA Barcodes PLoS ONE 2008-06-18;3(6):e2490
MT45503 6.1				<i>Anguilla</i>	<i>rostrata</i>	SERCFISH0168	Smithsonian Institution, National Museum of Natural History	06-Aug-20	Anne Arundel County, Maryland, USA	29-May-02	602	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
KX45933 3.1				<i>Anguilla</i>	<i>rostrata</i>	SERCFISH0165	Smithsonian Institution, National Museum of Natural History	19-Aug-19	Anne Arundel County, Maryland, USA	2012-05-17	655	GENBANK/ BOLD	Robert Aguilar, Matthew B. Ogburn, Amy C. Driskell, Lee A. Weigt, Mary C. Groves, Anson H. Hines Gutsy genetics: identification of digested piscine prey items in the stomach contents of sympatric native and introduced warmwater catfishes via DNA barcoding Environmental Biology of Fishes 2016-09-19;4(100):325-336
MG9366 70.1				<i>Anguilla</i>	<i>rostrata</i>	STRI-05692	Smithsonian Tropical	13-Mar-18	Chagres, Colon, Panama	1998-03-11	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.

							Research Institute						
JQ84017 7.1	Actinopterygii	Anguilliformes	Ophichthidae	<i>Myrophis</i>	<i>platyrhynchus</i>	USNM:FISH:BZL W4386	Smithsonian Institution	24- Jul- 12	Stann Creek District, Belize	2004- 03-04	628	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
JQ84017 8.1				<i>Myrophis</i>	<i>platyrhynchus</i>	USNM:FISH:BZL W4244	Smithsonian Institution	24- Jul- 12	Stann Creek District, Belize	2004- 02-29	632	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
JQ84173 1.1				<i>Myrophis</i>	<i>platyrhynchus</i>	USNM:FISH:BLZ 8392	Smithsonian Institution	24- Jul- 12	Stann Creek District, Belize	2008- 05-27	655	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
JQ84094 4.1	Actinopterygii	Anguilliformes	Ophichthidae	<i>Myrophis</i>	<i>punctatus</i>	USNM:FISH:433 477	Smithsonian Institution, National Museum of Natural History	24- Jul- 12	Stann Creek District, Belize	2006- 04-01	650	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
JQ84260 4.1				<i>Myrophis</i>	<i>punctatus</i>	SMSA7155	Smithsonian Institution, National Museum of Natural History	24- Jul- 12	St. Lucie County, Florida, USA	N/A	644	GENBANK	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
MG4960 91.1	Actinopterygii	Atheriniformes	Atherinopsidae	<i>Atherinella</i>	<i>hubbsi</i>	STRI-02094	Smithsonian Tropical Research Institute	26- Nov- 18	San Juan, Guanacaste, Costa Rica	1990- 01-20	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.

MG4960 92.1				<i>Atherinella</i>	<i>hubbsi</i>	STRI-02094	Smithsonian Tropical Research Institute	26- Nov- 18	San Juan, Guanacaste, Costa Rica	1990- 01-20	648	GENBANK/ BOLD	Birmingham,E., Reina,R.G. and Sanjur,O.
JQ84097 6.1	Actinopterygii	Batrachoidiformes	Batrachoididae	<i>Porichthys</i>	<i>pauciradialis</i>	USNM:Fish:416307	Smithsonian Institution, National Museum of Natural History	24- Jul- 12	Belize	2006- 04-07	651	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
KF93030 5.1	Actinopterygii	Batrachoidiformes	Batrachoididae	<i>Porichthys</i>	<i>plectrodon</i>	KU 30140	University of Kansas, Biodiversity Research Center	11- Feb- 14	Texas, United States	2002- 06-17	652	GENBANK/ BOLD	University of Kansas Biodiversity Institute barcoding initiative
HQ93701 9.1	Actinopterygii	Beleoniiformes	Belontiidae	<i>Strongylura</i>	<i>marina</i>	162812	Florida Museum of Natural History, Ichthyology Department	25- Jul- 16	Seminole, Georgia, United States	N/A	652	GENBANK/ BOLD	Julien April, Robert H. Hanner, Richard L. Mayden and Louis Bernatchez Metabolic Rate and Climatic Fluctuations Shape Continental Wide Pattern of Genetic Divergence and Biodiversity in Fishes PLoS ONE 2013-06-21;8(7):e70296 J. April, R. L. Mayden, R. H. Hanner and L. Bernatchez Genetic calibration of species diversity among North America's freshwater fishes Proceedings of the National Academy of Sciences 2011-06-13;108(26):10602-10607
MT45505 0.1				<i>Strongylura</i>	<i>marina</i>	USNM:FISH:423890	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06- Aug- 20	Somerset County, Maryland, USA	13- Jun-12	655	GENBANK	Aguilar,R., Ogburn,M.B., Weigt,L.A., Driskell,A.C., Macdonald,K.S. and Hines,A.H. Chesapeake Bay Barcode Initiative (CBBi): Fishes of the greater Chesapeake Bay
JQ84308 1.1				<i>Strongylura</i>	<i>marina</i>	USNM:FISH:TOB9369	Smithsonian Institution	24- Jul- 12	Tobago, Trinidad and Tobago	2009- 03-20	613	GENBANK/ BOLD	Weigt,L.A., Baldwin,C.C., Driskell,A., Smith,D.G., Ormos,A. and Reyier,E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic

													coverage PLoS ONE 7 (7), E41059 (2012)
HQ57329 6.1				<i>Strongylura</i>	<i>marina</i>	ECOCH6548	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Anahuac, Nuevo Leon, Mexico	2010-02-28	624	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ84159 9.1	Actinopterygii	Beloniformes	Hemirampidae	<i>Hemirampus</i>	<i>brasiliensis</i>	USNM:FISH:BLZ 8007	Smithsonian Institution	24-Jul-12	Stann Creek District, Belize	2004-05-14	633	GENBANK/BOLD	Weigt, L.A., Baldwin, C.C., Driskell, A., Smith, D.G., Ormos, A. and Reyier, E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
JQ84216 4.1				<i>Hemirampus</i>	<i>brasiliensis</i>	USNM:FISH:CUR 8119	Smithsonian Institution	24-Jul-12	Curacao	2004-03-11	655	GENBANK/BOLD	Weigt, L.A., Baldwin, C.C., Driskell, A., Smith, D.G., Ormos, A. and Reyier, E.A. Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage PLoS ONE 7 (7), E41059 (2012)
MT45504 4.1	Elasmobranchii	Carcharhiniformes	Carcharhinidae	<i>Carcharhinus</i>	<i>leucas</i>	USNM:FISH:451 240 / SERCFISH1350	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	North Carolina, USA	N/A	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
EU39861 6.1				<i>Carcharhinus</i>	<i>leucas</i>	BW-A2536	CSIRO, Australian National Fish Collection	26-Jul-16	Cilicap, Java, Indonesia	2002-08-20	649	GENBANK/BOLD	Ward, R.D., Holmes, B.H., White, W.T. and Last, P.R. (2008) DNA Barcoding Australian Chondrichthyans: Results and Potential Uses in Conservation. Mar. Freshw. Res. 59, 57-71
JF493060 .1				<i>Carcharhinus</i>	<i>leucas</i>	ADC08 Smith 9.9 #7	South African Institute for Aquatic Biodiversity	25-Jul-16	South Africa	N/A	648	GENBANK/BOLD	Steinke, D., Zemlak, T.S., Connell, A.D., Heemstra, P.C. and Hebert, P.D.N.
MG4960 82.1	Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>aeneus</i>	STRI-00070	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Costa Rica	1990-01-16	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjurjo, O.

									Puntarenas, Costa Rica				
GU80698 4.1				<i>Astyanax</i>	<i>aeneus</i>	ECOCH6205	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Tulum, Quintana Roo, Mexico	2008-08-07	639	GENBANK/BOLD	International Barcode of Life (iBOL)
EU75162 5.1				<i>Astyanax</i>	<i>aeneus</i>	ECOCH5526	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-08-22	652	GENBANK/BOLD	Valdez-Moreno, M.; Ivanova, N. V.; Elías-Gutiérrez, M.; Contreras-Balderas, S.; Hebert, P. D. N. Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of Fish Biology 2009-01-29;74(2):377-402
MG9366 95.1				<i>Astyanax</i>	<i>aeneus</i>	STRI-00134	Smithsonian Tropical Research Institute	13-Mar-18	Santa Maria, Herrera, Panama	1991-01-12	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
KY26699 1.1	Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>fasciatus</i>	48890	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	05-Jan-17	Buritizeiro, Minas Gerais, Brazil	2010-07-16	633	GENBANK/BOLD	Rossini, B.C., Oliveira, C.A., Melo, F.A., Bertaco, V.A., Astarloa, J.M., Rosso, J.J., Foresti, F. and Oliveira, C. (2016). Highlighting <i>Astyanax</i> Species Diversity through DNA Barcoding. PLoS ONE 11 (12), E0167203
JN98872 9.1				<i>Astyanax</i>	<i>fasciatus</i>	35894	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	04-Apr-13	Upper Parana Basin, Goias, Brazil	2008-10-15	636	GENBANK/BOLD	Pereira, L.H., Hanner, R., Foresti, F. and Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Henet. 12. 20
KY26723 9.1				<i>Astyanax</i>	<i>fasciatus</i>	59647	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	05-Jan-17	Porto da Folha/SE, Sergipe, Brazil	2010-11-22	683	GENBANK/BOLD	Rossini BC, Oliveira CA, Melo FA, Bertaco VA, Astarloa JM, Rosso JJ, Foresti F, Oliveira C. Highlighting <i>Astyanax</i> Species Diversity through DNA Barcoding. PLoS One. 2016 Dec 19;11(12):e0167203.
MG4961 09.1	Actinopterygii	Characiformes	Characidae	<i>Bramocharax</i>	<i>bransfordii</i>	STRI-00909	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-14	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.

MG4961 15.1	Actinopterygii	Characiformes	Characidae	<i>Brycon</i>	<i>costaricensis</i>	STRI-00237	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-14	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
EU75172 6.1				<i>Brycon</i>	<i>costaricensis</i>	ECOCH5797	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Alta Verapaz, Guatemala	2006-03-06	652	GENBANK/BOLD	Valdez-Moreno, M.; Ivanova, N. V.; Elías-Gutiérrez, M.; Contreras-Balderas, S.; Hebert, P. D. N. Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of Fish Biology 2009-01-29;74(2):377-402
MG4961 14.1				<i>Brycon</i>	<i>costaricensis</i>	STRI-05876	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantica Norte, San Juan, Nicaragua	1997-03-10	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
EU75172 7.1				<i>Brycon</i>	<i>costaricensis</i>	ECOCH5797	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Alta Verapaz, Guatemala	2006-03-06	652	GENBANK/BOLD	Valdez-Moreno, M.; Ivanova, N. V.; Elías-Gutiérrez, M.; Contreras-Balderas, S.; Hebert, P. D. N. Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of Fish Biology 2009-01-29;74(2):377-402
MG9367 93.1	Actinopterygii	Characiformes	Characidae	<i>Bryconame ricus</i>	<i>scleropari us</i>	STRI-00534	Smithsonian Tropical Research Institute	13-Mar-18	Bocas, Bocas Del Toro, Panama	1995-07-12	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 11.1				<i>Bryconame ricus</i>	<i>scleropari us</i>	STRI-00581	Smithsonian Tropical Research Institute	26-Nov-18	Bocas, Costa Rica	1990-01-20	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 10.1				<i>Bryconame ricus</i>	<i>scleropari us</i>	STRI-05902	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantica Sur, San Juan, Nicaragua	1997-03-01	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 16.1	Actinopterygii	Characiformes	Characidae	<i>Carlana</i>	<i>eigenmani</i>	STRI-00911	Smithsonian Tropical Research Institute	26-Nov-18	Rio San Juan, Costa Rica	1994-01-15	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.

MG9368 27.1				<i>Carlana</i>	<i>eigenmani</i>	STRI-00914	Smithsonian Tropical Research Institute	13- Mar- 18	Chagres, San Blas, Panama	1996- 02-15	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 17.1				<i>Carlana</i>	<i>eigenmani</i>	STRI-05913	Smithsonian Tropical Research Institute	26- Nov- 18	San Juan, Chontales, Nicaragua	2001- 03-13	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG9368 21.1				<i>Carlana</i>	<i>eigenmani</i>	STRI-00913	Smithsonian Tropical Research Institute	13- Mar- 18	Chagres, San Blas, Panama	1996- 02-13	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG9372 32.1	Actinopterygii	Characiformes	Characidae	<i>Roeboidea</i>	<i>bouchellei</i>	STRI-00464	Smithsonian Tropical Research Institute	13- Mar- 18	Chiriqui, Panama	1990- 03-13	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4962 27.1				<i>Roeboidea</i>	<i>bouchellei</i>	STRI-00426	Smithsonian Tropical Research Institute	26- Nov- 18	Estero Real- Tempisque, Puntarenas, Costa Rica	1990- 01-16	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 36.1	Actinopterygii	Clupeiformes	Clupeidae	<i>Dorosoma</i>	<i>chavesi</i>	cSTRI-08622	Smithsonian Tropical Research Institute	26- Nov- 18	Region Autonoma Atlantica Sur, San Juan, Nicaragua	1997- 03-13	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 37.1				<i>Dorosoma</i>	<i>chavesi</i>	cSTRI-08622	Smithsonian Tropical Research Institute	26- Nov- 18	Region Autonoma Atlantica Sur, San Juan, Nicaragua	1997- 03-13	638	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
JQ36537 0.1	Actinopterygii	Clupeiformes	Clupeidae	<i>Harengula</i>	<i>clupeola</i>	46930	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13- Sep- 12	Sao Paulo, Brazil	2010- 09-14	652	GENBANK/ BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020

GU22450 0.1				<i>Harengula</i>	<i>clupeola</i>	ECOCH5491	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-08-05	652	GENBANK/ BOLD	Martha Valdez-Moreno, Lourdes Vásquez-Yeomans, Manuel Elías-Gutiérrez, Natalia V. Ivanova and Paul D. N. Hebert Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management Marine and Freshwater Research 2010-06-10;61(6):655-671
GU22560 9.1				<i>Harengula</i>	<i>clupeola</i>	ECOCH6030	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2010-03-07	647	GENBANK/ BOLD	Martha Valdez-Moreno, Lourdes Vásquez-Yeomans, Manuel Elías-Gutiérrez, Natalia V. Ivanova and Paul D. N. Hebert Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management Marine and Freshwater Research 2010-06-10;61(6):655-671
KF92995 9.1	Actinopterygii	Clupeiformes	Clupeidae	<i>Harengula</i>	<i>jaguana</i>	KU 35363	University of Kansas, Biodiversity Research Center	11-Feb-14	Monroe, Florida, United States	2004-10-07	652	GENBANK/ BOLD	Bentley, A.C. and Wiley, E.O. University of Kansas Biodiversity Institute barcoding initiative
GU22532 5.1				<i>Harengula</i>	<i>jaguana</i>	ECOCH5511	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-08-24	652	GENBANK/ BOLD	Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N. V., & Hebert, P. D. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. Marine and Freshwater Research, 61(6), 655-671.
HQ57568 8.1				<i>Harengula</i>	<i>jaguana</i>	ECOCH6423	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Carmen, Campeche , Mexico	2010-04-20	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
KM53851 1.1	Actinopterygii	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>aurita</i>	SaAu301	Agricultural Research Organizatio	15-Sep-15	Israel	2009-09-16	663	GENBANK/ BOLD	Shirak, A., Dor, L., Seroussi, E., Ron, M., Hulata, G. and Golani, D.

							n, Volcani Center						DNA barcoding of Mediterranean fish species
BCOLL413-08				<i>Sardinella</i>	<i>aurita</i>	391423	Smithsonian Institution	20-Feb-08	North Carolina, United States	2006-09-09	652	BOLD	Bruce B. Collette
NPRS090-15				<i>Sardinella</i>	<i>aurita</i>	UNMDP 1311	Universidad Nacional de Mar del Plata	N/A	Mar Chiquita Lagoon, Buenos Aires, Argentina	N/A	671	BOLD	Stern, N., Douek, J., Goren, M., Rinkevich, B With no gap to mind: A shallow genealogy within the world's most widespread small pelagic fish. Ecography 2017-03-13;41:491-504
AM911174.1	Actinopterygii	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>brasiliensis</i>	N/A	Dpt of Marine Food Sciences & Techniques	26-Jul-16	N/A	N/A	594	GENBANK	Jérôme, M., Martinsohn, J. T., Ortega, D., Carreau, P., Verrez-Bagnis, V., & Mouchel, O. (2008). Toward fish and seafood traceability: anchovy species determination in fish products by molecular markers and support through a public domain database. Journal of agricultural and food chemistry, 56(10), 3460-3469.
JQ841067.1	Actinopterygii	Clupeiformes	Engraulidae	<i>Anchoa</i>	<i>lamprotaenia</i>	USNM:FISH:BLZ 7157	Smithsonian Institution	24-Jul-12	Stann Creek District, Belize	2007-01-16	624	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ842366.1				<i>Anchoa</i>	<i>lamprotaenia</i>	SMSA7191	Laboratories of Analytical Biology, Smithsonian Inst.	24-Jul-12	St. Lucie County, Florida, USA	N/A	601	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MXV803-15				<i>Anchoa</i>	<i>lamprotaenia</i>	ECO-CH P 7658	El Colegio de la Frontera Sur, Unidad Chetumal	2015-12-04	Sabancuy, Campeche, Mexico	2015-03-24	652	BOLD	Martha Valdez-Moreno

KU28876 5.1	Actinopterygii	Clupeiformes	Engraulidae	<i>Lycengraulis</i>	<i>grossidens</i>	MG ZV-P 128-1	Instituto de Biología Molecular y Celular de Rosario (IB)	18-Apr-16	N/A	N/A	642	GENBANK	Díaz, J., Villanova, G. V., Brancolini, F., Del Pazo, F., Posner, V. M., Grimberg, A., & Arranz, S. E. (2016). First DNA Barcode Reference Library for the Identification of South American Freshwater Fish from the Lower Paraná River. PLoS one, 11(7), e0157419.
EU07444 8.1				<i>Lycengraulis</i>	<i>grossidens</i>	INIDEP-T 0281	Universidad Nacional de Mar del Plata	22-Dec-11	Buenos Aires, Argentina	2006-02-08	652	GENBANK/ BOLD	Mabragaña, E., Díaz de Astarloa, J. M., Hanner, R., Zhang, J., & González Castro, M. (2011). DNA barcoding identifies Argentine fishes from marine and brackish waters. PLoS one, 6(12), e28655.
JEQUI261 -13				<i>Lycengraulis</i>	<i>grossidens</i>	JEQUI_C177	Pontificia Universidad Católica de Minas Gerais	2013-06-29	Jequitinhonha, Bahia, Brazil	2013-03-30	573	BOLD	Pugedo, M.L., de Andrade Neto, F.R., Pessali, T.C. et al. Integrative taxonomy supports new candidate fish species in a poorly studied neotropical region: the Jequitinhonha River Basin. Genetica 144, 341–349 (2016).
JX124858 .1	Actinopterygii	Clupeiformes	Pristigasteridae	<i>Pellona</i>	<i>harroweri</i>	48609	Universidad Estadual Paulista, Lab de Biología e Genética de Peixes	01-Jun-13	Rio de Janeiro, Brazil	2010-10-21	652	GENBANK/ BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
JX124859 .1				<i>Pellona</i>	<i>harroweri</i>	48611	Universidad Estadual Paulista, Lab de Biología e Genética de Peixes	01-Jun-13	Rio de Janeiro, Brazil	2010-10-21	652	GENBANK/ BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
MG4960 62.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Alfaro</i>	<i>cultratus</i>	STRI-02272	Smithsonian Tropical Research Institute	26-Nov-18	Bocas, Costa Rica	1990-01-20	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjurjo, O.

MG4960 63.1				<i>Alfaro</i>	<i>cultratus</i>	STRI-06359	Smithsonian Tropical Research Institute	26- Nov- 18	Region Autonoma Atlantica Norte, San Juan, Nicaragua	1997- 03-10	638	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG9366 50.1				<i>Alfaro</i>	<i>cultratus</i>	STRI-02247	Smithsonian Tropical Research Institute	13- Mar- 18	Bocas, Bocas del Toro, Panama	1993- 08-19	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
LC15312 0.1				<i>Alfaro</i>	<i>cultratus</i>	SDNCU:SDNCU- A2780	Mieko Suzuki- Matsubara Nagoya City University	20- May- 16	Higashiya ma Zoo, Aichi, Japan	N/A	658	GENBANK	Higashiyama Zoo DNA Barcode Project
EU75166 3.1	Actinopter ygii	Cyprinodontif ormes	Poeciliidae	<i>Belonesox</i>	<i>belizanus</i>	ECOCH5538	El Colegio de la Frontera Sur, Unidad Chetumal	26- Jul- 16	Bacalar, Quintana Roo, Mexico	2005- 08-31	652	GENBANK/ BOLD	Valdez-Moreno, M.; Ivanova, N. V.; Elías-Gutiérrez, M.; Contreras- Balderas, S.; Hebert, P. D. N. Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of Fish Biology 2009-01- 29;74(2):377-402
JQ84042 8.1				<i>Belonesox</i>	<i>belizanus</i>	USNM:Fish:423 163	Smithsonian Institution, National Museum of Natural History	24- Jul- 12	Stann Creek District, Belize	2005- 05-08	655	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MG4960 97.1				<i>Belonesox</i>	<i>belizanus</i>	STRI-02261	Smithsonian Tropical Research Institute	26- Nov- 18	NON-LCA, Honduras	1995- 04-30	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4960 99.1	Actinopter ygii	Cyprinodontif ormes	Poeciliidae	<i>Brachyrha phis</i>	<i>holdridgei</i>	STRI-06376	Smithsonian Tropical Research Institute	26- Nov- 18	ATL -- San Juan NI, Rio San Juan, Nicaragua	2001- 03-14	655	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 00.1				<i>Brachyrha phis</i>	<i>holdridgei</i>	STRI-02409	Smithsonian Tropical Research Institute	26- Nov- 18	ATL -- San Juan CR, Heredia, Costa Rica	1994- 01-20	648	GENBANK/ BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.

MG4961 01.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Brachyrhaphis</i>	<i>parismina</i>	STRI-02410	Smithsonian Tropical Research Institute	26-Nov-18	ATL -- San Juan CR, Heredia, Costa Rica	1994-01-20	655	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 02.1				<i>Brachyrhaphis</i>	<i>parismina</i>	STRI-02410	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-19	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4961 87.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Phallichthys</i>	<i>amates</i>	STRI-02442	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantico Sur, San Juan, Nicaragua	1997-02-28	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG9370 94.1				<i>Phallichthys</i>	<i>amates</i>	STRI-02431	Smithsonian Tropical Research Institute	13-Mar-18	WAP, Bocas Del Toro, Panama	1999-07-15	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
HQ56457 9.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Poecilia</i>	<i>mexicana</i>	CINV-NEC7380	Centro de Investigacion y de Estudios Avanzados del IPN, Unidad Merida	21-May-19	Campeche, Campeche, Mexico	N/A	652	GENBANK/BOLD	International Barcode of Life (iBOL)
HQ57318 2.1				<i>Poecilia</i>	<i>mexicana</i>	ECOCH6386	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Gral. Teran, Nuevo Leon, Mexico	2009-10-10	658	GENBANK/BOLD	International Barcode of Life (iBOL)
MG4961 94.1				<i>Poecilia</i>	<i>mexicana</i>	STRI-06472	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantico Sur, San Juan, Nicaragua	1997-02-28	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
KP70041 8.1				<i>Poecilia</i>	<i>mexicana</i>	1205	Tulane/Xavier Center for Bioenvironmental Research, Tulane University	25-Jun-15	Rio Canas, Costa Rica	N/A	683	GENBANK	Bagley, J. C., Alda, F., Breitman, M. F., Bermingham, E., van den Berghe, E. P., & Johnson, J. B. (2015). Assessing species boundaries using multilocus species delimitation in a morphologically conserved group of neotropical freshwater fishes,

													the <i>Poecilia sphenops</i> species complex (Poeciliidae). PLoS one, 10(4), e0121139.
HQ57582 7.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Poecilia</i>	<i>sphenops</i>	ECOCH6483	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Progreso, Yucatan, Mexico	2010-04-22	652	GENBANK/BOLD	International Barcode of Life (iBOL)
KP70043 6.1				<i>Poecilia</i>	<i>sphenops</i>	8314	Tulane/Xavier Center for Bioenvironmental Research, Tulane University	25-Jun-15	Nueva Ocotepeque, Lempa, Honduras	N/A	683	GENBANK	Bagley, J. C., Alda, F., Breitman, M. F., Bermingham, E., van den Berghe, E. P., & Johnson, J. B. (2015). Assessing species boundaries using multilocus species delimitation in a morphologically conserved group of neotropical freshwater fishes, the <i>Poecilia sphenops</i> species complex (Poeciliidae). PLoS one, 10(4), e0121139.
JX968661 .1				<i>Poecilia</i>	<i>sphenops</i>	stri7780	Smithsonian Tropical Research Institute	20-Mar-13	Guatemala	N/A	651	GENBANK	Alda, F., Reina, R. G., Doadrio, I., & Bermingham, E. (2013). Phylogeny and biogeography of the <i>Poecilia sphenops</i> species complex (Actinopterygii, Poeciliidae) in Central America. Molecular phylogenetics and evolution, 66(3), 1011–1026.
MG9371 47.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Priapichthys</i>	<i>annectens</i>	STRI-02620	Smithsonian Tropical Research Institute	13-Mar-18	WAP, Bocas Del Toro, Panama	2000-03-23	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG9371 48.1				<i>Priapichthys</i>	<i>annectens</i>	STRI-06507	Smithsonian Tropical Research Institute	13-Mar-18	WAP, Bocas Del Toro, Panama	2002-04-06	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4962 51.1	Actinopterygii	Cyprinodontiformes	Poeciliidae	<i>Xenophallus</i>	<i>umbratilis</i>	STRI-02398	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Heredia, Costa Rica	1994-01-21	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG4962 52.1				<i>Xenophallus</i>	<i>umbratilis</i>	STRI-02398	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-20	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.

MK18570 0.1	Actinopterygii	Elopiformes	Megalopidae	<i>Megalops</i>	<i>atlanticus</i>	CPM-IEOCD:14	Instituto Espanol de Oceanografia, Cadiz	27-Nov-19	FAO 27, Andalucia, Atlantic Ocean	2018-08-27	652	GENBANK/BOLD	Banon,R., Farias,C., Gonzalez-Ortegon,E., Arronte,J.C., Varela,J.L., Arias,A., Barros-Garcia,D. and De Carlos,A. New record and revised list of <i>Megalops atlanticus</i> (Elopiformes:Megalopidae) from Atlantic European waters. <i>Cybum</i> 43 (2), 203-207 (2019)
JQ84127 8.1				<i>Megalops</i>	<i>atlanticus</i>	USNM:FISH:BLZ 7876	Smithsonian Institution	24-Jul-12	Belize District, Belize	2007-10-04	649	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. <i>PloS one</i> , 7(7), e41059.
HM3798 24.1				<i>Megalops</i>	<i>atlanticus</i>	ECOCH6320	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Minatitlan , Veracruz, Mexico	2009-07-17	652	GENBANK/BOLD	International Barcode of Life (iBOL)
KY65642 1.1	Actinopterygii	Gobiesociformes	Gobiesocidae	<i>Gobiesox</i>	<i>nudus</i>	N/A	Department of Wildlife and Fisheries Sciences, Texas A&M University	14-May-17	N/A	N/A	684	GENBANK	Conway, K. W., Kim, D., Rüber, L., Espinosa Pérez, H. S., & Hastings, P. A. (2017). Molecular systematics of the New World clingfish genus <i>Gobiesox</i> (Teleostei: Gobiesocidae) and the origin of a freshwater clade. <i>Molecular phylogenetics and evolution</i> , 112, 138–147.
MN1951 27.1	Actinopterygii	Gymnotiformes	Gymnotidae	<i>Gymnotus</i>	<i>cylindricus</i>	STRI4852	Smithsonian Institution	21-Sep-19	N/A	N/A	568	GENBANK	de Santana, C. D., Crampton, W., Dillman, C. B., Frederico, R. G., Sabaj, M. H., Covain, R., Ready, J., Zuanon, J., de Oliveira, R. R., Mendes-Júnior, R. N., Bastos, D. A., Teixeira, T. F., Mol, J., Ohara, W., Castro, N., Peixoto, L. A., Nagamachi, C., Sousa, L., Montag, L., Ribeiro, F., ... Wosiacki, W. B. (2019). Unexpected species diversity in electric eels with a description of the strongest living bioelectricity generator. <i>Nature communications</i> , 10(1), 4000.

KJ135116.1				<i>Gymnotus</i>	<i>cylindricus</i>	AM302	Biology, McGill University	13-Aug-14	Panama	N/A	648	GENBANK	Picq, S., Alda, F., Krahe, R., & Bermingham, E. (2014). Miocene and Pliocene colonization of the Central American Isthmus by the weakly electric fish <i>B. rachyhypopomus occidentalis</i> (Hypopomidae, Gymnotiformes). <i>Journal of Biogeography</i> , 41(8), 1520-1532.
MG496151.1				<i>Gymnotus</i>	<i>cylindricus</i>	STRI07773	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantico Sur, San Juan, Nicaragua	1997-03-02	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
JN853332.1	Actinopterygii	Lepisosteiformes	Lepisosteidae	<i>Atractosteus</i>	<i>tropicus</i>	YFTC:21070	Ecology and Evolutionary Biology, University of Michigan	03-May-12	N/A	N/A	604	GENBANK	Wright, J. J., David, S. R., & Near, T. J. (2012). Gene trees, species trees, and morphology converge on a similar phylogeny of living gars (Actinopterygii: Holostei: Lepisosteidae), an ancient clade of ray-finned fishes. <i>Molecular phylogenetics and evolution</i> , 63(3), 848–856.
MK950847.1	Actinopterygii	Mugiliformes	Mugilide	<i>Agonostomus</i>	<i>monticola</i>	TCWC 20062.01	Wildlife and Fisheries Sciences, Texas A&M University	22-Dec-19	Brazos River, Texas, USA	04-Nov-16	688	GENBANK	Kubicek KM, Pinion AK, Conway KW (2019) New records of the Mountain Mullet, <i>Dajaus monticola</i> (Bancroft, 1834), and an overview of historical records in Texas. <i>Check List</i> 15(3): 471-478.
MG496128.1				<i>Agonostomus</i>	<i>monticola</i>	STRI-06288	Smithsonian Tropical Research Institute	26-Nov-18	Region Autonoma Atlantica Norte, San Juan, Nicaragua	1997-03-09	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG937019.1	Actinopterygii	Mugiliformes	Mugilide	<i>Joturus</i>	<i>pichardi</i>	STRI-02165	Smithsonian Tropical Research Institute	13-Mar-18	Chagres, San Blas, Panama	1991-02-21	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.
MG937020.1				<i>Joturus</i>	<i>pichardi</i>	STRI-02166	Smithsonian Tropical Research Institute	13-Mar-18	Chagres, San Blas, Panama	1991-02-15	648	GENBANK/BOLD	Bermingham, E., Reina, R.G. and Sanjur, O.

MN3389 59.1	Actinopterygii	Mugiliformes	Mugilide	<i>Mugil</i>	<i>cephalus</i>	N/A	Division de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana	31-Aug-20	N/A	N/A	635	GENBANK	Colín, A., Hernández-Pérez, Z., Guevara-Chumacero, L. M., Castañeda-Rico, S., Serrato-Díaz, A., & Ibáñez, A. L. (2020). Are striped mullet (<i>Mugil cephalus</i>) philopatric?. Marine Biology, 167(1), 1-15.
MT45510 5.1				<i>Mugil</i>	<i>cephalus</i>	USNM:FISH:425040	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	St. Mary's County, Maryland, USA	12-Jul-12	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
HQ57326 2.1				<i>Mugil</i>	<i>cephalus</i>	ECOCH6530	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Juarez, Nuevo Leon, Mexico	2010-02-27	652	GENBANK/BOLD	International Barcode of Life (iBOL)
MT45501 5.1	Actinopterygii	Mugiliformes	Mugilide	<i>Mugil</i>	<i>curema</i>	USNM:FISH:429752	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Virginia Beach County, Virginia, USA	21-Aug-13	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
HM3798 21.1				<i>Mugil</i>	<i>curema</i>	ECOCH6319	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Minatitlán, Veracruz, Mexico	2009-07-17	650	GENBANK/BOLD	International Barcode of Life (iBOL)
MG4961 69.1				<i>Mugil</i>	<i>curema</i>	STRI-05429	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Rivas, Nicaragua	1997-03-16	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
MT45521 6.1	Actinopterygii	Perciformes	Carangidae	<i>Caranx</i>	<i>hippos</i>	USNM:FISH:429757	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Virginia Beach County, Virginia, USA	21-Aug-13	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)

HM3797 98.1				<i>Caranx</i>	<i>hippos</i>	ECOCH6309	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Coatzacoalcos, Veracruz, Mexico	2009-07-15	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JX124748 .1				<i>Caranx</i>	<i>hippos</i>	35145	Universidad e Estadual Paulista, Lab de Biología e Genética de Peixes	01-Jun-13	Sao Paulo, Brazil	2008-12-11	652	GENBANK/BOLD	Ribeiro,A.D.E.O., Oliveira,C. and Ribeiro,A.D.
JQ36525 3.1	Actinopterygii	Perciformes	Carangidae	<i>Caranx</i>	<i>latus</i>	40537	Universidad e Estadual Paulista, Lab de Biología e Genética de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-03-26	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JQ84043 8.1				<i>Caranx</i>	<i>latus</i>	USNM:Fish:423123	Smithsonian Institution, National Museum of Natural History	24-Jul-12	Stann Creek District, Belize	2005-05-02	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ36538 3.1	Actinopterygii	Perciformes	Carangidae	<i>Hemicaranx</i>	<i>amblyrhynchus</i>	40595	Universidad e Estadual Paulista, Lab de Biología e Genética de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-06-21	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
GU22564 9.1	Actinopterygii	Perciformes	Carangidae	<i>Oligoplites</i>	<i>saurus</i>	ECOCH	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Mexico	2007-06-03	652	GENBANK/BOLD	Martha Valdez-Moreno, Lourdes Vásquez-Yeomans, Manuel Elías-Gutiérrez, Natalia V. Ivanova and Paul D. N. Hebert Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management Marine and Freshwater Research 2010-06-10;61(6):655-671
JQ84261 3.1				<i>Oligoplites</i>	<i>saurus</i>	SMSA7262	Laboratories of Analytical	N/A	St. Lucie County,	N/A	655	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos,

							Biology, Smithsonian Inst.		Florida, USA				A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS one, 7(7), e41059.
MT45541 5.1	Actinopterygii	Perciformes	Carangidae	<i>Selene</i>	<i>setapinnis</i>	USNM:FISH:423 942	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06- Aug- 20	Poquoson County, Virginia, USA	13- Sep- 12	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
GU22503 6.1				<i>Selene</i>	<i>setapinnis</i>	ECO-CH LP 3828	El Colegio de la Frontera Sur, Unidad Chetumal	25- Jul- 16	Quintana Roo, Mexico	2005- 09-28	652	GENBANK/ BOLD	Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N. V., & Hebert, P. D. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. Marine and Freshwater Research, 61(6), 655-671.
KF93042 6.1				<i>Selene</i>	<i>setapinnis</i>	KU 30094	University of Kansas, Biodiversity Research Center	11- Feb- 14	Texas, United States	2002- 06-24	648	GENBANK/ BOLD	University of Kansas Biodiversity Institute barcoding initiative
KC01591 5.1				<i>Selene</i>	<i>setapinnis</i>	ARC 25447	Dalhousie University	10- Mar- 13	Nova Scotia, Canada	N/A	616	GENBANK/ BOLD	McCusker, M. R., Denti, D., Van Guelpen, L., Kenchington, E., & Bentzen, P. (2013). Barcoding Atlantic Canada's commonly encountered marine fishes. Molecular ecology resources, 13(2), 177-188.
MT45505 9.1	Actinopterygii	Perciformes	Carangidae	<i>Selene</i>	<i>vomer</i>	USNM:FISH:431 003	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06- Aug- 20	Northampton County, Virginia, USA	16- Aug- 13	640	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
MG8379 85.1				<i>Selene</i>	<i>vomer</i>	ECOCH7192	El Colegio de la Frontera	07- May- 18	Distrito Federal, Mexico	2010- 12-28	652	GENBANK/ BOLD	Sarmiento-Camacho, S., & Valdez-Moreno, M. (2018). DNA barcode identification of commercial fish

							Sur, Unidad Chetumal						sold in Mexican markets. Genome, 61(6), 457–466.
KF93042 7.1				<i>Selene</i>	<i>vomer</i>	KU 29658	University of Kansas, Biodiversity Research Center	11-Feb-14	United States	2001-11-16	641	GENBANK/ BOLD	University of Kansas Biodiversity Institute barcoding initiative
JQ36556 2.1				<i>Selene</i>	<i>vomer</i>	40578	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-05-08	652	GENBANK/ BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
MT45501 6.1	Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>carolinus</i>	USNM:FISH:429764	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Virginia Beach County, Virginia, USA	20-Aug-13	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
KF46124 4.1				<i>Trachinotus</i>	<i>carolinus</i>	394197	Smithsonian Institution, National Museum of Natural History	27-Jul-14	Alabama, United States	2008-07-20	655	GENBANK/ BOLD	Deeds, J. R., Handy, S. M., Fry Jr, F., Granade, H., Williams, J. T., Powers, M., ... & Weigt, L. A. (2014). Protocol for building a reference standard sequence library for DNA-based seafood identification. Journal of AOAC International, 97(6), 1626-1633.
JX124911 .1				<i>Trachinotus</i>	<i>carolinus</i>	48521	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	01-Jun-13	Rio de Janeiro, Brazil	2010-10-19	652	GENBANK/ BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
MT45593 3.1	Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>falcatus</i>	USNM:FISH:429773	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Newport News County, Virginia, USA	30-Jul-13	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)

HM379788.1				<i>Trachinotus</i>	<i>falcatus</i>	ECOCH6354	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Othon P. Blanco, Quintana Roo, Mexico	2009-11-19	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ365600.1				<i>Trachinotus</i>	<i>falcatus</i>	40555	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-04-14	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
HQ575796.1	Actinopterygii	Perciformes	Carangidae	<i>Trachinotus</i>	<i>goodei</i>	ECOCH6473	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Carmen, Campeche, Mexico	2010-04-22	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ365604.1				<i>Trachinotus</i>	<i>goodei</i>	51302	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2011-02-16	651	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JQ841417.1				<i>Trachinotus</i>	<i>goodei</i>	USNM:FISH:BLZ 7873	Smithsonian Institution	24-Jul-12	Belize District, Belize	2007-10-04	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ365271.1	Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>parallelus</i>	40520	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-01-27	629	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JQ365276.1	Actinopterygii	Perciformes	Centropomidae	<i>Centropomus</i>	<i>undecimalis</i>	40602	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-08-29	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.

JQ84110 2.1				<i>Centropomus</i>	<i>undecimalis</i>	USNM:FISH:BLZ 7878	Smithsonian Institution	24-Jul-12	Belize District, Belize	2007-10-04	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
HM3797 95.1				<i>Centropomus</i>	<i>undecimalis</i>	ECOCH6305	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Coatzacoalcos, Veracruz, Mexico	2009-07-15	652	GENBANK/BOLD	International Barcode of Life (iBOL)
MT45524 8.1	Actinopterygii	Perciformes	Eleotridae	<i>Dormitator</i>	<i>maculatus</i>	USNM:FISH:447322	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Carteret County, North Carolina, USA	16-Sep-17	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
MT45509 3.1	Actinopterygii	Perciformes	Eleotridae	<i>Eleotris</i>	<i>amblyopsis</i>	USNM:FISH:447320	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Carteret County, North Carolina, USA	16-Sep-17	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
MG9369 06.1	Actinopterygii	Perciformes	Eleotridae	<i>Eleotris</i>	<i>pissonis</i>	STRI-04340	Smithsonian Tropical Research Institute	13-Mar-18	Chagres, Colon, Panama	1992-11-25	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjur, O.
GU67272 1.1				<i>Eleotris</i>	<i>pissonis</i>	ECOCH6304	El Colegio de la Frontera Sur, Unidad Chetumal	05-Jan-21	Othon P. Blanco, Quintana Roo, Mexico	2009-05-03	629	GENBANK/BOLD	International Barcode of Life (iBOL)
GU67272 2.1	Actinopterygii	Perciformes	Eleotridae	<i>Gobiomorus</i>	<i>dormitor</i>	ECOCH6226	El Colegio de la Frontera Sur, Unidad Chetumal	05-Jan-21	Bacalar, Quintana Roo, Mexico	2008-07-25	636	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ84051 6.1	Actinopterygii	Perciformes	Eleotridae	<i>Guavina</i>	<i>guavina</i>	USNM:Fish:423146	Smithsonian Institution, National Museum of	24-Jul-12	Stann Creek District, Belize	2005-05-07	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess

							Natural History						Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
AY72212 8.1	Actinopterygii	Perciformes	Eleotridae	<i>Leptophilypnus</i>	<i>fluviatilis</i>	N/A	Ichthyology, Natural History Museum of Los Angeles County	15-Dec-15	N/A	N/A	1255	GENBANK	Thacker, C. E., & Hardman, M. A. (2005). Molecular phylogeny of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidae). Molecular phylogenetics and evolution, 37(3), 858–871.
AY72213 0.1				<i>Leptophilypnus</i>	<i>fluviatilis</i>	N/A	Ichthyology, Natural History Museum of Los Angeles County	15-Dec-15	N/A	N/A	1255	GENBANK	Thacker, C. E., & Hardman, M. A. (2005). Molecular phylogeny of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidae). Molecular phylogenetics and evolution, 37(3), 858–871.
AY72212 9.1				<i>Leptophilypnus</i>	<i>fluviatilis</i>	N/A	Ichthyology, Natural History Museum of Los Angeles County	15-Dec-15	N/A	N/A	1255	GENBANK	Thacker, C. E., & Hardman, M. A. (2005). Molecular phylogeny of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidae). Molecular phylogenetics and evolution, 37(3), 858–871.
HM3797 99.1	Actinopterygii	Perciformes	Gerreidae	<i>Diapterus</i>	<i>auratus</i>	ECOCH6310	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Coatzacoatlcos, Veracruz, Mexico	2009-07-15	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ84186 5.1				<i>Diapterus</i>	<i>auratus</i>	USNM:FISH:FCC 8051	Smithsonian Institution	24-Jul-12	Brevard County, Florida, United States	2008-04-08	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.

BAHIA19 7-14				<i>Diapterus</i>	<i>auratus</i>	1580	Universidade Estadual do Sudoeste da Bahia	2014-05-23	Bahia, Brazil	2011-01-20	655	BOLD	José Henrique Souza Galdino Brandão, Jamille de Araújo Bitencourt, Flávia Borges Santos, Luciana Almeida Watanabe, Horácio Schneider, Iracilda Sampaio, Paulo Roberto Antunes de Mello Affonso DNA barcoding of coastal ichthyofauna from Bahia, northeastern Brazil, South Atlantic: High efficiency for systematics and identification of cryptic diversity Biochemical Systematics and Ecology 2016-03-02;65(1): 214–224
HQ57567 7.1	Actinopterygii	Perciformes	Gerreidae	<i>Diapterus</i>	<i>rhombeus</i>	ECOCH6419	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Carmen, Campeche, Mexico	2010-04-20	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
JX124777 .1				<i>Diapterus</i>	<i>rhombeus</i>	41573	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes	01-Jun-13	Sao Paulo, Brazil	2008-12-11	652	GENBANK/ BOLD	Ribeiro, A. D. O., Caires, R. A., Mariguela, T. C., Pereira, L. H. G., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular Ecology Resources, 12(6), 1012-1020.
GU67269 2.1	Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>argenteus</i>	ECOCH6235	El Colegio de la Frontera Sur, Unidad Chetumal	05-Jan-21	Isla Mujeres, Quintana Roo, Mexico	2009-05-24	655	GENBANK/ BOLD	International Barcode of Life (iBOL)
HM3797 62.1				<i>Eucinostomus</i>	<i>argenteus</i>	ECOCH6258	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Isla Mujeres, Quintana Roo, Mexico	2009-05-22	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
JQ84189 3.1	Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>harengulus</i>	USNM:FISH:FCC 8057	Smithsonian Institution	24-Jul-12	Brevard County, Florida, United States	2008-04-08	615	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS one, 7(7), e41059.

MT45520 5.1	Actinopterygii	Perciformes	Gerreidae	<i>Eucinostomus</i>	<i>melanopterus</i>	USNM:FISH:431076	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Virginia Beach County, Virginia, USA	21-Aug-13	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
JN31379 3.1				<i>Eucinostomus</i>	<i>melanopterus</i>	ECO-CH P 7173	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Isla Mujeres, Quintana Roo, Mexico	2011-02-03	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JQ84189 6.1				<i>Eucinostomus</i>	<i>melanopterus</i>	USNM:FISH:FCC8074	Smithsonian Institution	24-Jul-12	Brevard County, Florida, United States	2008-06-17	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
GU67269 5.1	Actinopterygii	Perciformes	Gerreidae	<i>Eugerres</i>	<i>plumieri</i>	ECOCH6172	El Colegio de la Frontera Sur, Unidad Chetumal	05-Jan-21	Othon P. Blanco, Quintana Roo, Mexico	2008-08-21	652	GENBANK/BOLD	International Barcode of Life (iBOL)
HM3798 19.1				<i>Eugerres</i>	<i>plumieri</i>	ECOCH6318	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Minatitlán, Veracruz, Mexico	2009-07-17	649	GENBANK/BOLD	International Barcode of Life (iBOL)
GU22526 0.1				<i>Eugerres</i>	<i>plumieri</i>	ECOCH5509	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-08-22	652	GENBANK/BOLD	Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N. V., & Hebert, P. D. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. Marine and Freshwater Research, 61(6), 655-671.
GU25560 4.1	Actinopterygii	Perciformes	Gerreidae	<i>Gerres</i>	<i>cinereus</i>	GENT:ODC1056	Biología Vegetal (Botánica), Universidad	04-Sep-15	Banyuls, Languedoc-Roussillon, France	N/A	874	GENBANK	Tronholm, A., Steen, F., Tyberghein, L., Leliaert, F., Verbruggen, H., Antonia Ribera Siguan, M., & De Clerck, O. (2010). Species delimitation,

							de La Laguna						taxonomy, and biogeography of Dictyota in Europe (Dictyotales, Phaeophyceae) 1. Journal of phycology, 46(6), 1301-1321.
BAHA220-08				<i>Gerres</i>	<i>cinereus</i>	USNM:FISH:BAH8220	Smithsonian Institution	2008-07-23	Berry Islands, Bahamas	2008-06-18	651	BOLD	Weigt, Lee A.; Baldwin, Carole C.; Driskell, Amy; Smith, David G.; Ormos, Andrea; Reyier, Eric A. Using DNA Barcoding to Assess Caribbean Reef Fish Biodiversity: Expanding Taxonomic and Geographic Coverage PLoS ONE 2012-07-16;7(7):e41059
BZLWA403-06				<i>Gerres</i>	<i>cinereus</i>	USNM:FISH:BZLW4405	Smithsonian Institution	2010-01-22	Stann Creek District, Belize	2004-03-05	655	GENBANK/BOLD	N/A
MT455114.1	Actinopterygii	Perciformes	Gobiidae	<i>Awaous</i>	<i>banana</i>	USNM:FISH:447325	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Carteret County, North Carolina, USA	18-Jan-17	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBi)
KF929911.1				<i>Awaous</i>	<i>banana</i>	USNM 343870	University of Kansas, Biodiversity Research Center	11-Feb-14	Belize	1991-07-10	652	GENBANK/BOLD	University of Kansas Biodiversity Institute barcoding initiative
JQ839767.1				<i>Awaous</i>	<i>banana</i>	USNM:FISH:BAH8220	Smithsonian Institution	24-Jul-12	Berry Islands, Bahamas	2008-06-18	651	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MUCU119-14	Actinopterygii	Perciformes	Gobiidae	<i>Awaous</i>	<i>tajasica</i>	MCNIP-1478	Pontificia Universidade Catolica de Minas Gerais, Museu de Ciencias Naturais	2014-04-16	Nanuque, Minas Gerais, Brazil	N/A	396	BOLD	N/A

MUCU13 5-14				<i>Awaous</i>	<i>tajasica</i>	MCNIP-1478	Pontificia Universidad e Catolica de Minas Gerais, Museu de Ciencias Naturais	2014 -05- 18	Nanuque, Minas Gerais, Brazil	N/A	591	BOLD	N/A
MUCU14 8-14				<i>Awaous</i>	<i>tajasica</i>	MCNIP-1478	Pontificia Universidad e Catolica de Minas Gerais, Museu de Ciencias Naturais	2014 -05- 23	Nanuque, Minas Gerais, Brazil		681	BOLD	N/A
HM7483 66.1	Actinopterygii	Perciformes	Gobiidae	<i>Bathygobius</i>	<i>soporator</i>	USNM:FISH:397 597	Laboratories of Analytical Biology, Smithsonian Institution	23- Sep- 13	USA	30- Oct-09	655	GENBANK	Tornabene, L., Baldwin, C., Weigt, L. A., & Pezold, F. (2010). Exploring the diversity of western Atlantic <i>Bathygobius</i> (Teleostei: Gobiidae) with cytochrome c oxidase-I, with descriptions of two new species. <i>aqua</i> , 16(4), 141-170.
JQ36524 6.1				<i>Bathygobius</i>	<i>soporator</i>	40571	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13- Sep- 12	Sao Paulo, Brazil	2010- 05-07	652	GENBANK/ BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. <i>Molecular ecology resources</i> , 12(6), 1012–1020.
ANGBF58 59-12				<i>Bathygobius</i>	<i>soporator</i>	AMNH:Fish:251 647a	Mined from GenBank, NCBI	N/A	Puerto Rico	2009- 05-25	655	BOLD	N/A
JQ84242 5.1	Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>boleosoma</i>	SMSA7179	Laboratories of Analytical Biology, Smithsonian Institution	24- Jul- 12	St. Lucie County, Florida, USA	N/A	591	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. <i>PloS one</i> , 7(7), e41059.
MT45574 7.1				<i>Ctenogobius</i>	<i>boleosoma</i>	USNM:FISH:447 313	Fisheries Conservation Lab, Smithsonian	06- Aug- 20	Carteret County, North	17- Sep- 17	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)

							Environmental Research Center		Carolina, USA				
MLIII197-08				<i>Ctenogobius</i>	<i>boleosoma</i>	ECO-CH LP 3845	El Colegio de la Frontera Sur, Unidad Chetumal	2008-10-06	Othon P. Blanco, Quintana Roo, Mexico	2008-01-12	652	BOLD	N/A
MT88840 4.1	Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>fasciatus</i>	ECO-CH-Z	El Colegio de la Frontera Sur, Unidad Chetumal	26-Sep-20	Bacalar, Quintana Roo, Mexico	2017-05-26	650	GENBANK/BOLD	Montes-Ortiz L, Elias-Gutierrez M. Faunistic survey of the zooplankton community in an oligotrophic sinkhole, Cenote Azul (Quintana Roo, Mexico), using different sampling methods, and documented with DNA barcodes: Zooplankton baseline in a karstic system. J Limnol 77, 428-440 (2018)
MG4502 93.1				<i>Ctenogobius</i>	<i>fasciatus</i>	ECO-CH-LP 23021	El Colegio de la Frontera Sur, Unidad Chetumal	27-Mar-18	Bacalar, Quintana Roo, Mexico	2015-04-02	647	GENBANK/BOLD	Elías-Gutiérrez, M., Valdez-Moreno, M., Topan, J., Young, M. R., & Cohuo-Colli, J. A. (2018). Improved protocols to accelerate the assembly of DNA barcode reference libraries for freshwater zooplankton. Ecology and evolution, 8(5), 3002–3018.
JQ84243 3.1	Actinopterygii	Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>smaragdus</i>	SMSA7176	Laboratories of Analytical Biology, Smithsonian Institution	24-Jul-12	St. Lucie County, Florida, USA	N/A	578	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ84243 5.1				<i>Ctenogobius</i>	<i>smaragdus</i>	SMSA7177	Laboratories of Analytical Biology, Smithsonian Institution	24-Jul-12	St. Lucie County, Florida, USA	N/A	593	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ84243 4.1				<i>Ctenogobius</i>	<i>smaragdus</i>	SMSA7178	Laboratories of Analytical Biology,	24-Jul-12	St. Lucie County, Florida, USA	N/A	603	GENBANK	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess

							Smithsonian Institution						Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS one, 7(7), e41059.
MT45530 6.1	Actinopterygii	Perciformes	Gobiidae	<i>Evorthodus</i>	<i>lyricus</i>	USNM:FISH:447 356	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Carteret County, North Carolina, USA	16-Sep-17	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBi)
JQ84246 9.1				<i>Evorthodus</i>	<i>lyricus</i>	SMSA7548	Laboratories of Analytical Biology, Smithsonian Institution	24-Jul-12	St. Lucie County, Florida, USA	N/A	622	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS one, 7(7), e41059.
JN02672 7.1	Actinopterygii	Perciformes	Gobiidae	<i>Gobioides</i>	<i>broussonnetii</i>	GOBR-Petshop-1	Laval University	25-Jul-16	Pet Shop, Quebec City, Canada	N/A	658	GENBANK/BOLD	April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America's freshwater fishes. Proceedings of the National Academy of Sciences of the United States of America, 108(26), 10602–10607. Julien April, Robert H. Hanner, Richard L. Mayden and Louis Bernatchez Metabolic Rate and Climatic Fluctuations Shape Continental Wide Pattern of Genetic Divergence and Biodiversity in Fishes PLoS ONE 2013-06-21;8(7):e70296
JN02672 6.1				<i>Gobioides</i>	<i>broussonnetii</i>	GOBR-Petshop-2	Laval University	25-Jul-16	Pet Shop, Quebec City, Canada	N/A	658	GENBANK/BOLD	April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America's freshwater fishes. Proceedings of the National Academy of Sciences of the United States of America, 108(26), 10602–10607. Julien April, Robert H. Hanner, Richard L. Mayden and Louis Bernatchez Metabolic Rate and Climatic

													Fluctuations Shape Continental Wide Pattern of Genetic Divergence and Biodiversity in Fishes PLoS ONE 2013-06-21;8(7):e70296
JN02672 5.1				<i>Gobioides</i>	<i>broussonnetii</i>	GOBR-Petshop-3	Laval University	25-Jul-16	Pet Shop, Quebec City, Canada	N/A	650	GENBANK/BOLD	April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America's freshwater fishes. Proceedings of the National Academy of Sciences of the United States of America, 108(26), 10602–10607. Julien April, Robert H. Hanner, Richard L. Mayden and Louis Bernatchez Metabolic Rate and Climatic Fluctuations Shape Continental Wide Pattern of Genetic Divergence and Biodiversity in Fishes PLoS ONE 2013-06-21;8(7):e70296
KT27851 8.1	Actinopterygii	Perciformes	Gobiidae	<i>Gobiosoma</i>	<i>spes</i>	N/A	College of Science & Engineering, Texas A&M University	28-Oct-15	N/A	N/A	1019	GENBANK/BOLD	Van Tassell, J. L., Joyeux, J. C., Macieira, R. M., & Tornabene, L. (2015). Status of Gobiosoma (Teleostei: Gobiidae) from Brazil: description of a new species, redescription of G. hemigymnum, molecular phylogeny of the genus, and key to Atlantic species. Zootaxa, 4007(4), 451–480.
KT27855 4.1				<i>Gobiosoma</i>	<i>spes</i>	N/A	College of Science & Engineering, Texas A&M University	28-Oct-15	N/A	N/A	1019	GENBANK/BOLD	Van Tassell, J. L., Joyeux, J. C., Macieira, R. M., & Tornabene, L. (2015). Status of Gobiosoma (Teleostei: Gobiidae) from Brazil: description of a new species, redescription of G. hemigymnum, molecular phylogeny of the genus, and key to Atlantic species. Zootaxa, 4007(4), 451–480.
HQ57570 8.1	Actinopterygii	Perciformes	Haemulidae	<i>Conodon</i>	<i>nobilis</i>	ECOCH6428	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Carmen, Campeche, Mexico	2010-04-20	652	GENBANK/BOLD	International Barcode of Life (iBOL)

JX124759.1				<i>Conodon</i>	<i>nobilis</i>	48659	Universidad e Estadual Paulista, Lab de Biologia e Genetica de Peixes	01-Jun-13	Rio de Janeiro, Brazil	2010-10-21	652	GENBANK/BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
BAHIA072-14	Actinopterygii	Perciformes	Haemulidae	<i>Haemulopsis</i>	<i>corvinaeformis</i>	3791	Universidad e Estadual do Sudoeste da Bahia	2014-06-10	Bahia, Brazil	2013-01-09	655	BOLD	José Henrique Souza Galdino Brandão, Jamille de Araújo Bitencourt, Flávia Borges Santos, Luciana Almeida Watanabe, Horácio Schneider, Iracilda Sampaio, Paulo Roberto Antunes de Mello Affonso DNA barcoding of coastal ichthyofauna from Bahia, northeastern Brazil, South Atlantic: High efficiency for systematics and identification of cryptic diversity Biochemical Systematics and Ecology 2016-03-02;65(1): 214–224
JQ365497.1				<i>Haemulopsis</i>	<i>corvinaeformis</i>	40607	Universidad e Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-08-29	652	GENBANK/BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
JQ741309.1	Actinopterygii	Perciformes	Haemulidae	<i>Pomadasys</i>	<i>crocro</i>	N/A	Centro de Investigaciones Biologicas del Noroeste	27-Dec-12	N/A	N/A	517	GENBANK	Tavera, J. J., Acero P, A., Balart, E. F., & Bernardi, G. (2012). Molecular phylogeny of grunts (Teleostei, Haemulidae), with an emphasis on the ecology, evolution, and speciation history of new world species. BMC evolutionary biology, 12, 57.
JQ365390.1	Actinopterygii	Perciformes	Kyphosidae	<i>Kyphosus</i>	<i>sectatrix</i>	40576	Universidad e Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-05-08	652	GENBANK/BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of

													Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
JQ36539 1.1				<i>Kyphosus</i>	<i>sectatrix</i>	40575	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-05-08	652	GENBANK/BOLD	Amanda de Oliveira Ribeiro, Rodrigo Antunes Caires, Tatiane Casagrande Mariguela, Luiz Henrique Garcia Pereira, Robert Hanner and Claudio Oliveira DNA barcodes identify marine fishes of Sao Paulo State, Brazil Molecular Ecology Resources 2012-09-08;12(6):1012-1020
JQ84291 2.1				<i>Kyphosus</i>	<i>sectatrix</i>	USNM:FISH:TO B9235	Smithsonian Institution	24-Jul-12	Tobago, Trinidad and Tobago	2009-03-18	655	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS one, 7(7), e41059.
JQ83980 1.1				<i>Kyphosus</i>	<i>sectatrix</i>	USNM:FISH:BA H8219	Smithsonian Institution	24-Jul-12	Berry Islands, Bahamas	2008-06-18	618	GENBANK/BOLD	Weigt, Lee A.; Baldwin, Carole C.; Driskell, Amy; Smith, David G.; Ormos, Andrea; Reyier, Eric A. Using DNA Barcoding to Assess Caribbean Reef Fish Biodiversity: Expanding Taxonomic and Geographic Coverage PLoS ONE 2012-07-16;7(7):e41059
FJ998454 .1	Actinopterygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i> analis</i>	SIO-09-174	Scripps Institution of Oceanography	24-Jul-16	Belize	2006-01-01	652	GENBANK/BOLD	Victor, B. C., Hanner, R., Shivji, M., Hyde, J. and Caldwell, C. Identification of the larval and juvenile stages of the cubera snapper, <i>Lutjanus cyanopterus</i> , using mtDNA barcoding. Zootaxa 2215, 24-36 (2009)
GU22534 9.1				<i>Lutjanus</i>	<i> analis</i>	ECOCH5704	El Colegio de la Frontera Sur, Unidad Chetumal	25-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-11-30	650	GENBANK/BOLD	Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N. V., & Hebert, P. D. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. Marine and Freshwater Research, 61(6), 655-671.

JQ84125 2.1				<i>Lutjanus</i>	<i>analis</i>	USNM:FISH:BLZ 7865	Smithsonian Institution	24- Jul- 12	Belize District, Belize	2007- 10-04	655	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MT45516 4.1	Actinopter ygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>griseus</i>	USNM:FISH:447 402	Fisheries Conservatio n Lab, Smithsonian Environmen tal Research Center	06- Aug- 20	Ann Arundel County, Maryland, USA	18- Oct-18	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
JN31199 9.1				<i>Lutjanus</i>	<i>griseus</i>	ECO-CH P 7147	El Colegio de la Frontera Sur, Unidad Chetumal	21- May- 19	Isla Mujeres, Quintana Roo, Mexico	2011- 02-02	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
GU22491 4.1				<i>Lutjanus</i>	<i>griseus</i>	ECO-CH LP 3694	El Colegio de la Frontera Sur, Unidad Chetumal	25- Jul- 16	Othon P. Blanco, Quintana Roo, Mexico	2005- 10-01	652	GENBANK/ BOLD	Valdez-Moreno, M., Vásquez- Yeomans, L., Elías-Gutiérrez, M., Ivanova, N. V., & Hebert, P. D. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. Marine and Freshwater Research, 61(6), 655- 671.
FJ998474 .1	Actinopter ygii	Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>jocu</i>	n7529al230	Research Collection of Benjamin Victor	24- Jul- 16	Colon, Panama	2007- 05-29	652	GENBANK/ BOLD	Victor, B. C., Hanner, R., Shivji, M., Hyde, J., & Caldwell, C. (2009). Identification of the larval and juvenile stages of the Cubera Snapper, <i>Lutjanus cyanopterus</i> , using DNA barcoding. Zootaxa, 2215(2), 24.
JQ84014 1.1				<i>Lutjanus</i>	<i>jocu</i>	USNM:FISH:BZL W4402	Smithsonian Institution	24- Jul- 12	Stann Creek District, Belize	2004- 03-04	655	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059

BAHIA26 1-14				<i>Lutjanus</i>	<i>jocu</i>	1529	Universidade Estadual do Sudoeste da Bahia	2014-06-11	Bahia, Brazil	2011-01-20	683	BOLD	José Henrique Souza Galdino Brandão, Jamille de Araújo Bitencourt, Flávia Borges Santos, Luciana Almeida Watanabe, Horácio Schneider, Iracilda Sampaio, Paulo Roberto Antunes de Mello Affonso DNA barcoding of coastal ichthyofauna from Bahia, northeastern Brazil, South Atlantic: High efficiency for systematics and identification of cryptic diversity Biochemical Systematics and Ecology 2016-03-02;65(1): 214–224
JQ84061 2.1	Actinopterygii	Perciformes	Microdesmidae	<i>Microdesmus</i>	<i>carri</i>	USNM:Fish:420307	Smithsonian Institution, National Museum of Natural History	24-Jul-12	Stann Creek District, Belize	2005-05-05	655	GENBANK/ BOLD	Weight, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ84128 1.1				<i>Microdesmus</i>	<i>carri</i>	USNM:FISH:BLZ 7177	Smithsonian Institution	24-Jul-12	Belize	2007-01-16	624	GENBANK/ BOLD	Weight, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ84061 1.1				<i>Microdesmus</i>	<i>carri</i>	USNM:Fish:420279	Smithsonian Institution, National Museum of Natural History	24-Jul-12	Stann Creek District, Belize	2005-05-08	655	GENBANK/ BOLD	Weight, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
JQ84172 1.1				<i>Microdesmus</i>	<i>carri</i>	USNM:FISH:BLZ 8322	Smithsonian Institution	24-Jul-12	Stann Creek District, Belize	2008-05-24	655	GENBANK/ BOLD	Weight, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.

JQ36549 5.1	Actinopterygii	Perciformes	Polynemidae	<i>Polydactylus</i>	<i>virginicus</i>	40523	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes	13-Sep-12	Sao Paulo, Brazil	2010-02-16	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JQ84021 9.1				<i>Polydactylus</i>	<i>virginicus</i>	USNM:FISH:BZW4427	Smithsonian Institution	24-Jul-12	Stann Creek District, Belize	2004-03-05	630	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MXIII100-08				<i>Polydactylus</i>	<i>virginicus</i>	ECOCH6102	El Colegio de la Frontera Sur, Unidad Chetumal	2008-10-04	Othon P. Blanco, Quintana Roo, Mexico	2008-04-06	652	BOLD	N/A
TOBA245-09				<i>Polydactylus</i>	<i>virginicus</i>	USNM:FISH:TOB9245	Smithsonian Institution	2009-05-05	Tobago, Trinidad and Tobago	2009-03-18	592	BOLD	N/A
HM3798 64.1	Actinopterygii	Perciformes	Sciaenidae	<i>Bairdiella</i>	<i>ronchus</i>	ECOCH6360	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Othon P. Blanco, Quintana Roo, Mexico	2009-11-27	652	GENBANK/BOLD	International Barcode of Life (iBOL)
JX124740 .1				<i>Bairdiella</i>	<i>ronchus</i>	40524	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes	01-Jun-13	Sao Paulo, Brazil	2010-02-16	652	GENBANK/BOLD	N/A
JX124796 .1	Actinopterygii	Perciformes	Sciaenidae	<i>Larimus</i>	<i>breviceps</i>	48649	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes	01-Jun-13	Rio de Janeiro, Brazil	2010-10-21	652	GENBANK/BOLD	N/A
MT45506 4.1	Actinopterygii	Perciformes	Sciaenidae	<i>Menticirrhus</i>	<i>americanus</i>	USNM:FISH:425023	Fisheries Conservation Lab, Smithsonian	06-Aug-20	Somerset County, Maryland, USA	28-Aug-12	623	GENBANK	Chesapeake Bay Barcode Initiative (CBBi)

							Environmental Research Center						
HQ57573 2.1				<i>Menticirrhus</i>	<i>americanus</i>	ECOCH6433	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Carmen, Campeche, Mexico	2010-04-21	652	GENBANK/BOLD	International Barcode of Life (iBOL)
KF93012 1.1				<i>Menticirrhus</i>	<i>americanus</i>	MCZ 156777	University of Kansas, Biodiversity Research Center	11-Feb-14	Belize	1999-07-05	652	GENBANK/BOLD	University of Kansas Biodiversity Institute barcoding initiative
JN31380 5.1	Actinopterygii	Perciformes	Sciaenidae	<i>Menticirrhus</i>	<i>littoralis</i>	ECO-CH P 7186	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Isla Mujeres, Quintana Roo, Mexico	2011-02-01	652	GENBANK/BOLD	International Barcode of Life (iBOL)
MT45560 7.1				<i>Menticirrhus</i>	<i>littoralis</i>	USNM:FISH:431085	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Virginia Beach County, Virginia, USA	20-Aug-13	653	GENBANK	Chesapeake Bay Barcode Initiative (CBBI)
GU70242 9.1	Actinopterygii	Perciformes	Sciaenidae	<i>Micropogonias</i>	<i>furnieri</i>	35037	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2008-12-11	652	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
EU07448 2.1				<i>Micropogonias</i>	<i>furnieri</i>	INIDEP-T 0288	Universidad Nacional de Mar del Plata	22-Dec-11	Buenos Aires, Argentina	2006-03-30	652	GENBANK/BOLD	Mabragaña, E., Díaz de Astarloa, J. M., Hanner, R., Zhang, J., & González Castro, M. (2011). DNA barcoding identifies Argentine fishes from marine and brackish waters. PLoS one, 6(12), e28655.
KF92985 0.1	Actinopterygii	Perciformes	Serranidae	<i>Epinephelus</i>	<i>guttatus</i>	KUT 72	University of Kansas, Biodiversity Research Center	11-Feb-14	Belize	1991-07-11	652	GENBANK/BOLD	University of Kansas Biodiversity Institute barcoding initiative

JQ84004 7.1				<i>Epinephelus</i>	<i>guttatus</i>	USNM:FISH:BZL W4106	Smithsonian Institution	24- Jul- 12	Stann Creek District, Belize	2004- 02-24	655	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MXV545- 14				<i>Epinephelus</i>	<i>guttatus</i>	ECO-CH	El Colegio de la Frontera Sur, Unidad Campeche	2014 -06- 09	Campeche , Mexico	2014- 06-09	300	BOLD	N/A
MG4960 74.1	Actinopterygii	Perciformes	Cichlidae	<i>Amatitlani</i> <i>a</i>	<i>septemfasciata</i>	STRI-03006	Smithsonian Tropical Research Institute	26- Nov- 18	Estero Real- Tempisque, Limon, Costa Rica	1990- 01-20	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG4960 75.1				<i>Amatitlani</i> <i>a</i>	<i>septemfasciata</i>	STRI-03005	Smithsonian Tropical Research Institute	26- Nov- 18	San Juan, Guanacaste, Costa Rica	1990- 01-19	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
GU81726 0.1				<i>Amatitlani</i> <i>a</i>	<i>septemfasciata</i>	UMMZ 243191	Museum of Natural Science, Louisiana State University	25- Jul- 16	N/A	N/A	590	GENBANK	Chakrabarty, P., & Albert, J. S. (2011). Eighteen. Not So Fast A New Take on the Great American Biotic Interchange. In Historical biogeography of Neotropical freshwater fishes (pp. 293-306). University of California Press.
MG4960 67.1	Actinopterygii	Perciformes	Cichlidae	<i>Amatitlani</i> <i>a</i>	<i>siquia</i>	STRI-02982	Smithsonian Tropical Research Institute	26- Nov- 18	Bocas, Costa Rica	1990- 01-20	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG4961 18.1	Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>alfari</i>	STRI-04221	Smithsonian Tropical Research Institute	26- Nov- 18	Region Autonoma Atlantico Sur, San Juan, Nicaragua	1997- 02-28	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG4960 76.1	Actinopterygii	Perciformes	Cichlidae	<i>Amphilophus</i>	<i>citrinellus</i>	STRI-02953	Smithsonian Tropical Research Institute	26- Nov- 18	Region Autonoma Atlantico Sur, San Juan, Nicaragua	1997- 02-28	648	GENBANK/ BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.

MG4961 53.1	Actinopterygii	Perciformes	Cichlidae	<i>Archocentrus</i>	<i>multispinosus</i>	STRI-06092	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Chinandega, Costa Rica	1997-02-25	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
MG4961 70.1	Actinopterygii	Perciformes	Cichlidae	<i>Hypsophrys</i>	<i>nematopus</i>	STRI-04209	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-14	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
MG4961 64.1	Actinopterygii	Perciformes	Cichlidae	<i>Hypsophrys</i>	<i>nicaraguensis</i>	STRI-06095	Smithsonian Tropical Research Institute	26-Nov-18	Region Autónoma Atlántico Sur, San Juan, Nicaragua	1997-03-13	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
MG4961 65.1				<i>Hypsophrys</i>	<i>nicaraguensis</i>	STRI-04260	Smithsonian Tropical Research Institute	26-Nov-18	San Juan, Guanacaste, Costa Rica	1990-01-20	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
DQ11920 2.1				<i>Hypsophrys</i>	<i>nicaraguensis</i>	UMMZ 243188	Museum of Zoology, University of Michigan	24-May-06	N/A	N/A	590	GENBANK/BOLD	Chakrabarty P. (2006). Systematics and historical biogeography of Greater Antillean Cichlidae. Molecular phylogenetics and evolution, 39(3), 619–627.
MG4961 73.1	Actinopterygii	Perciformes	Cichlidae	<i>Parachromis</i>	<i>dovii</i>	STRI-03038	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Guanacaste, Costa Rica	1990-01-14	648	GENBANK/BOLD	Birmingham, E., Reina, R.G. and Sanjurjo, O.
EU75190 1.1	Actinopterygii	Perciformes	Cichlidae	<i>Petenia</i>	<i>splendida</i>	ECOCH5826	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Peten, Guatemala	2006-03-09	652	GENBANK/BOLD	Valdez-Moreno, M., Ivanova, N. V., Elías-Gutiérrez, M., Contreras-Balderas, S., & Hebert, P. D. (2009). Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of fish biology, 74(2), 377–402.
GU80703 8.1				<i>Petenia</i>	<i>splendida</i>	ECOCH6159	El Colegio de la Frontera	21-May-19	Quintana Roo, Mexico	2008-06-26	637	GENBANK/BOLD	International Barcode of Life (iBOL)

							Sur, Unidad Chetumal						
EU75175 2.1	Actinopterygii	Perciformes	Cichlidae	<i>Rocio</i>	<i>octofasciata</i>	ECOCH5476	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Bacalar, Quintana Roo, Mexico	2005-06-16	652	GENBANK/BOLD	Valdez-Moreno, M., Ivanova, N. V., Elías-Gutiérrez, M., Contreras-Balderas, S., & Hebert, P. D. (2009). Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. <i>Journal of fish biology</i> , 74(2), 377–402.
JN02839 8.1				<i>Rocio</i>	<i>octofasciata</i>	CIOC-Petshop-1	Laval University	25-Jul-16	Pet Shop, Quebec City, Canada	N/A	658	GENBANK/BOLD	April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America's freshwater fishes. <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 108(26), 10602–10607.
MT45601 7.1	Actinopterygii	Perciformes	Echeneidae	<i>Echeneis</i>	<i>naucrates</i>	USNM:FISH:429 839	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	Northampton County, Virginia, USA	14-Sep-12	655	GENBANK	Chesapeake Bay Barcode Initiative (CBBi)
MK65747 0.1				<i>Echeneis</i>	<i>naucrates</i>	USNM:FISH:438 462	Smithsonian Institution, National Museum of Natural History	19-Jun-19	Tuamotu-Gambier, French Polynesia	2010-10-05	655	GENBANK/BOLD	Trottin, E. D., Williams, J., Pitassy, D., Driskell, A., Hubert, N., Viviani, J., ... & Planes, S. (2019). A DNA barcode reference library of the French Polynesian shore fishes.
HQ95653 2.1				<i>Echeneis</i>	<i>naucrates</i>	CSIRO H 6517-04	CSIRO, Australian National Fish Collection	21-May-19	Queensland, Australia	2003-11-25	648	GENBANK/BOLD	International Barcode of Life (iBOL)
JN31382 8.1				<i>Echeneis</i>	<i>naucrates</i>	ECO-CH P 7226	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Isla Mujeres, Quintana Roo, Mexico	2011-02-04	652	GENBANK/BOLD	International Barcode of Life (iBOL)
MH8989 50.1	Actinopterygii	Pleuronectiformes	Achiridae	<i>Achirus</i>	<i>declivis</i>	MUFAL1686	Instituto de Ciencias Biológicas e da Saúde,	31-Oct-19	Alagoas State, Brazil	N/A	424	GENBANK	Matias,G.D.A., Mott,T., Fabre,N.N., Neves,J.M.M. and Torres,R.A.

							Universidad e Federal de Alagoas						
MH8989 49.1				<i>Achirus</i>	<i>declivis</i>	MUFAL1812	Instituto de Ciencias Biologicas e da Saude, Universidad e Federal de Alagoas	31-Oct-19	Alagoas State, Brazil	N/A	424	GENBANK	Matias,G.D.A., Mott,T., Fabre,N.N., Neves,J.M.M. and Torres,R.A.
MH8989 48.1				<i>Achirus</i>	<i>declivis</i>	MUFAL1811	Instituto de Ciencias Biologicas e da Saude, Universidad e Federal de Alagoas	31-Oct-19	Alagoas State, Brazil	N/A	424	GENBANK	Matias,G.D.A., Mott,T., Fabre,N.N., Neves,J.M.M. and Torres,R.A.
MH8989 47.1				<i>Achirus</i>	<i>declivis</i>	MUFAL1810	Instituto de Ciencias Biologicas e da Saude, Universidad e Federal de Alagoas	31-Oct-19	Alagoas State, Brazil	N/A	424	GENBANK	Matias,G.D.A., Mott,T., Fabre,N.N., Neves,J.M.M. and Torres,R.A.
KT31006 7.1	Actinopterygii	Pleuronectiformes	Achiridae	<i>Trinectes</i>	<i>paulistanus</i>	GEA.ICT 01710	Zoology, Universidad e do Estado do Rio de Janeiro	24-Jan-16	Brazil	13-May-09	650	GENBANK	Cantalice,K.M., Amaral,C.R.L., Pereira,F., Amorim,A., Silva,D.A. and Carvalho,E.F.
KT31006 6.1				<i>Trinectes</i>	<i>paulistanus</i>	GEA.ICT 01711	Zoology, Universidad e do Estado do Rio de Janeiro	24-Jan-16	Brazil	13-May-09	659	GENBANK/BOLD	Cantalice,K.M., Amaral,C.R.L., Pereira,F., Amorim,A., Silva,D.A. and Carvalho,E.F.
KT31006 5.1				<i>Trinectes</i>	<i>paulistanus</i>	GEA.ICT 01712	Zoology, Universidad e do Estado do Rio de Janeiro	24-Jan-16	Brazil	13-May-09	659	GENBANK/BOLD	Cantalice,K.M., Amaral,C.R.L., Pereira,F., Amorim,A., Silva,D.A. and Carvalho,E.F.
GU80704 8.1	Actinopterygii	Pleuronectiformes	Achiridae	<i>Trichromis</i>	<i>salvini</i>	ECOCH6158	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Felipe Carrillo Puerto, Qunitana Roo, Mexico	2008-06-27	627	GENBANK/BOLD	International Barcode of Life (iBOL)

EU75175 7.1				<i>Trichromis</i>	<i>salvini</i>	ECOCH5794	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Alta Verapaz, Guatemala	2006-03-05	652	GENBANK/ BOLD	Valdez-Moreno, M., Ivanova, N. V., Elías-Gutiérrez, M., Contreras-Balderas, S., & Hebert, P. D. (2009). Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. <i>Journal of fish biology</i> , 74(2), 377–402
JN02496 6.1				<i>Trichromis</i>	<i>salvini</i>	CISA-Petshop-2	Laval University	25-Jul-16	Pet Shop, Quebec City, Canada	N/A	658	GENBANK/ BOLD	April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America's freshwater fishes. <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 108(26), 10602–10607
MT45560 5.1	Actinopterygii	Pleuronectiformes	Cynoglossidae	<i>Symphurus</i>	<i>plagusia</i>	USNM:FISH:425008	Fisheries Conservation Lab, Smithsonian Environmental Research Center	06-Aug-20	USA: Virginia	13-Aug-12	555	GENBANK/ BOLD	N/A
JQ84272 6.1				<i>Symphurus</i>	<i>plagusia</i>	SMSA7552	National Museum of Natural History, Smithsonian Inst	24-Jul-12	USA: Florida	N/A	639	GENBANK/ BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. <i>PloS one</i> , 7(7), e41059.
JX516095 .1	Actinopterygii	Pleuronectiformes	Paralichthyidae	<i>Citharichthys</i>	<i>arenaceus</i>	n762bc130	Research Collection of Benjamin Victor	15-Mar-13	Colon, Panama	2007-06-02	652	GENBANK/ BOLD	Victor, B. and Wellington, G.M. <i>Citharichthys darwini</i> n. sp., a new endemic flatfish from the Galapagos Archipelago (Teleostei: Pleuronectiformes: Paralichthyidae). <i>J Ocean Sci Found</i> 6, 19-32 (2013)
HQ98785 2.1				<i>Citharichthys</i>	<i>arenaceus</i>	n7529af124	Research Collection of Benjamin Victor	20-Nov-12	Colon, Panama	2007-05-29	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
HQ57571 3.1	Actinopterygii	Pleuronectiformes	Paralichthyidae	<i>Citharichthys</i>	<i>spilopteruss</i>	ECOCH6429	El Colegio de la Frontera	21-May-19	Carmen, Campeche, Mexico	2010-04-20	647	GENBANK/ BOLD	International Barcode of Life (iBOL)

							Sur, Unidad Chetumal						
GU70234 7.1				<i>Citharichthys</i>	<i>spilopteruss</i>	41593	Universidad e Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2008-12-11	652	GENBANK/ BOLD	Ribeiro, A. O., Caires, R. A., Mariguella, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JX516096 .1				<i>Citharichthys</i>	<i>spilopteruss</i>	n7529acs551	Research Collection of Benjamin Victor	15-Mar-13	Colon, Panama	2007-05-29	652	GENBANK/ BOLD	Victor, B. and Wellington, G.M. <i>Citharichthys darwini</i> n. sp., a new endemic flatfish from the Galapagos Archipelago (Teleostei: Pleuronectiformes: Paralichthyidae). J Ocean Sci Found 6, 19-32 (2013)
HQ57329 7.1	Actinopterygii	Siluriformes	Ariidae	<i>Bagre</i>	<i>marinus</i>	ECOCH6549	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Lampazos de Naranjo, Nuevo Leon, Mexico	2010-02-28	652	GENBANK/ BOLD	International Barcode of Life (iBOL)
KF46113 5.1				<i>Bagre</i>	<i>marinus</i>	394170	Smithsonian Institution, National Museum of Natural History	27-Jul-14	Alabama, United States	2008-07-19	655	GENBANK/ BOLD	N/A
MG8378 82.1				<i>Bagre</i>	<i>marinus</i>	ECOCH7192	El Colegio de la Frontera Sur, Unidad Chetumal	07-May-18	Distrito Federal, Mexico	2010-12-28	652	GENBANK/ BOLD	Sarmiento-Camacho, S., & Valdez-Moreno, M. (2018). DNA barcode identification of commercial fish sold in Mexican markets. Genome, 61(6), 457–466.
EU75183 9.1	Actinopterygii	Siluriformes	Ariidae	<i>Sciades</i>	<i>assimilis</i>	ECOCH5512	El Colegio de la Frontera Sur, Unidad Chetumal	26-Jul-16	Othon P. Blanco, Quintana Roo, Mexico	2005-08-27	652	GENBANK/ BOLD	Valdez-Moreno, M., Ivanova, N. V., Elías-Gutiérrez, M., Contreras-Balderas, S., & Hebert, P. D. (2009). Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of fish biology, 74(2), 377–402.
GU22565 4.1				<i>Sciades</i>	<i>assimilis</i>	ECOCH6033	El Colegio de la Frontera	25-Jul-16	Othon P. Blanco, Quintana	2007-07-21	652	GENBANK/ BOLD	Valdez-Moreno, M., Ivanova, N. V., Elías-Gutiérrez, M., Contreras-Balderas, S., & Hebert, P. D. (2009). Probing diversity in

							Sur, Unidad Chetumal		Roo, Mexico				freshwater fishes from Mexico and Guatemala with DNA barcodes. Journal of fish biology, 74(2), 377–402.
MG4962 13.1	Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>laticauda</i>	STRI-00750	Smithsonian Tropical Research Institute	26-Nov-18	Bocas, Costa Rica	1990-01-20	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG9371 81.1				<i>Rhamdia</i>	<i>laticauda</i>	STRI-00745	Smithsonian Tropical Research Institute	13-Mar-18	Chiriqui, Panama	1989-08-20	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MXV775-15				<i>Rhamdia</i>	<i>laticauda</i>	ECO-CH P 7690	El Colegio de la Frontera Sur, Unidad Chetumal	2015-12-04	District Cayo, Cotton Tree, Belize	2015-02-14	652	BOLD	N/A
MG4962 39.1	Actinopterygii	Synbranchiformes	Synbranchidae	<i>Synbranchus</i>	<i>marmoratus</i>	STRI-02818	Smithsonian Tropical Research Institute	26-Nov-18	Bocas, Costa Rica	1990-01-20	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
HM4049 79.1				<i>Synbranchus</i>	<i>marmoratus</i>	DCC41826	Universidade Estadual Paulista	25-Jul-16	Fortuna de Minas, Minas Gerais, Brazil	2005-02-09	652	GENBANK/BOLD	de Carvalho, D. C., Oliveira, D. A., Pompeu, P. S., Leal, C. G., Oliveira, C., & Hanner, R. (2011). Deep barcode divergence in Brazilian freshwater fishes: the case of the São Francisco River basin. Mitochondrial DNA, 22 Suppl 1, 80–86.
JX111854 .1				<i>Synbranchus</i>	<i>marmoratus</i>	UNMDP-T 0376	Universidad Nacional de Mar del Plata	26-Oct-12	Buenos Aires, Argentina	2010-09-01	652	GENBANK/BOLD	Rosso, J. J., Mabrugaña, E., Castro, M. G., & de Astarloa, J. M. (2012). DNA barcoding Neotropical fishes: recent advances from the Pampa Plain, Argentina. Molecular ecology resources, 12(6), 999–1011.
MG9370 55.1	Actinopterygii	Syngnathiformes	Syngnathidae	<i>Microphis</i>	<i>brachyurus</i>	STRI-02738	Smithsonian Tropical Research Institute	13-Mar-18	WAP, Bocas Del Toro, Panama	1998-02-21	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
KU69262 0.1				<i>Microphis</i>	<i>brachyurus</i>	MZB-BIF00334	Indonesian Institute of Sciences, Research	20-Apr-16	Jawat Barat, Indonesia	2012-11-22	652	GENBANK/BOLD	Dahrudin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R., & Hubert, N. (2017). Revisiting the ichthyodiversity of Java and Bali

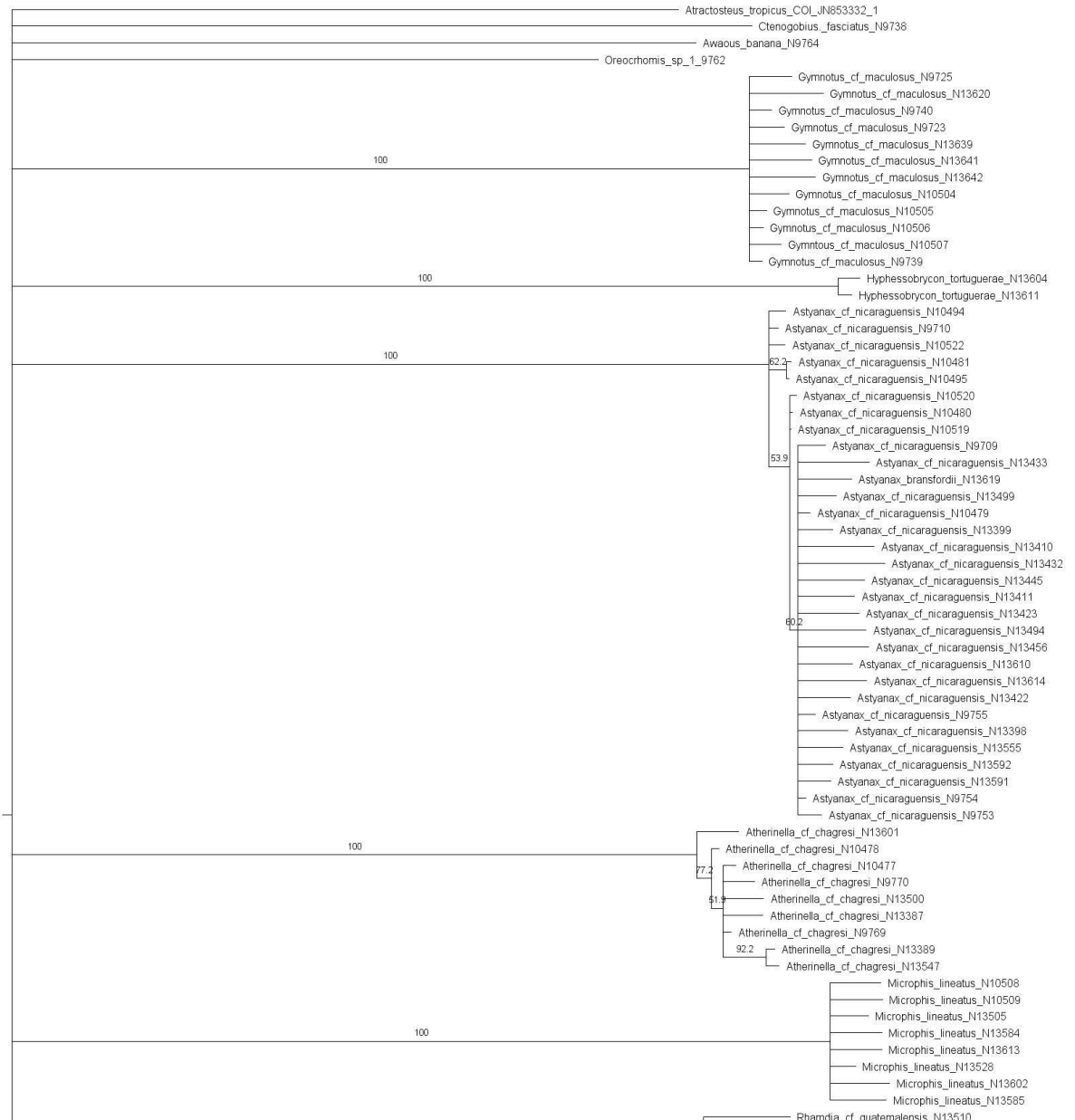
							Centre for Biology						through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Molecular ecology resources, 17(2), 288–299.
JQ84128 3.1				<i>Microphis</i>	<i>brachyurus</i>	USNM:FISH:BLZ 7175	Smithsonian Institution	24-Jul-12	Belize	2007-01-16	624	GENBANK/BOLD	Weigt, L. A., Baldwin, C. C., Driskell, A., Smith, D. G., Ormos, A., & Reyier, E. A. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PloS one, 7(7), e41059.
MG9371 68.1	Actinopterygii	Syngnathiformes	Syngnathidae	<i>Pseudophyllus</i>	<i>mindii</i>	STRI-02753	Smithsonian Tropical Research Institute	13-Mar-18	Chagres, San Blas, Panama	1990-05-13	648	GENBANK/BOLD	Bermingham, E., Reina, R. G. and Sanjurjo, O.
HQ57323 9.1	Actinopterygii	Syngnathiformes	Syngnathidae	<i>Syngnathus</i>	<i>scovelli</i>	ECOCH6520	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	San Fernando, Tamaulipas, Mexico	2010-04-25	652	GENBANK/BOLD	International Barcode of Life (iBOL)
SMSA326 -09				<i>Syngnathus</i>	<i>scovelli</i>	USNM:FISH:SM S7326	Smithsonian Institution	2007-08-04	St. Lucie County, Florida, USA	N/A	616	BOLD	N/A
HM3797 69.1	Actinopterygii	Tetraodontiformes	Tetraodontidae	<i>Sphoeroides</i>	<i>testudineus</i>	ECOCH6350	El Colegio de la Frontera Sur, Unidad Chetumal	21-May-19	Othon P. Blanco, Quintana Roo, Mexico	2009-06-12	623	GENBANK/BOLD	International Barcode of Life (iBOL)
KF93045 5.1				<i>Sphoeroides</i>	<i>testudineus</i>	KU 34277	University of Kansas, Biodiversity Research Center	11-Feb-14	Belize	1999-07-04	652	GENBANK/BOLD	University of Kansas Biodiversity Institute barcoding initiative
GU70232 7.1				<i>Sphoeroides</i>	<i>testudineus</i>	41590	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes	13-Sep-12	Sao Paulo, Brazil	2008-12-11	630	GENBANK/BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.

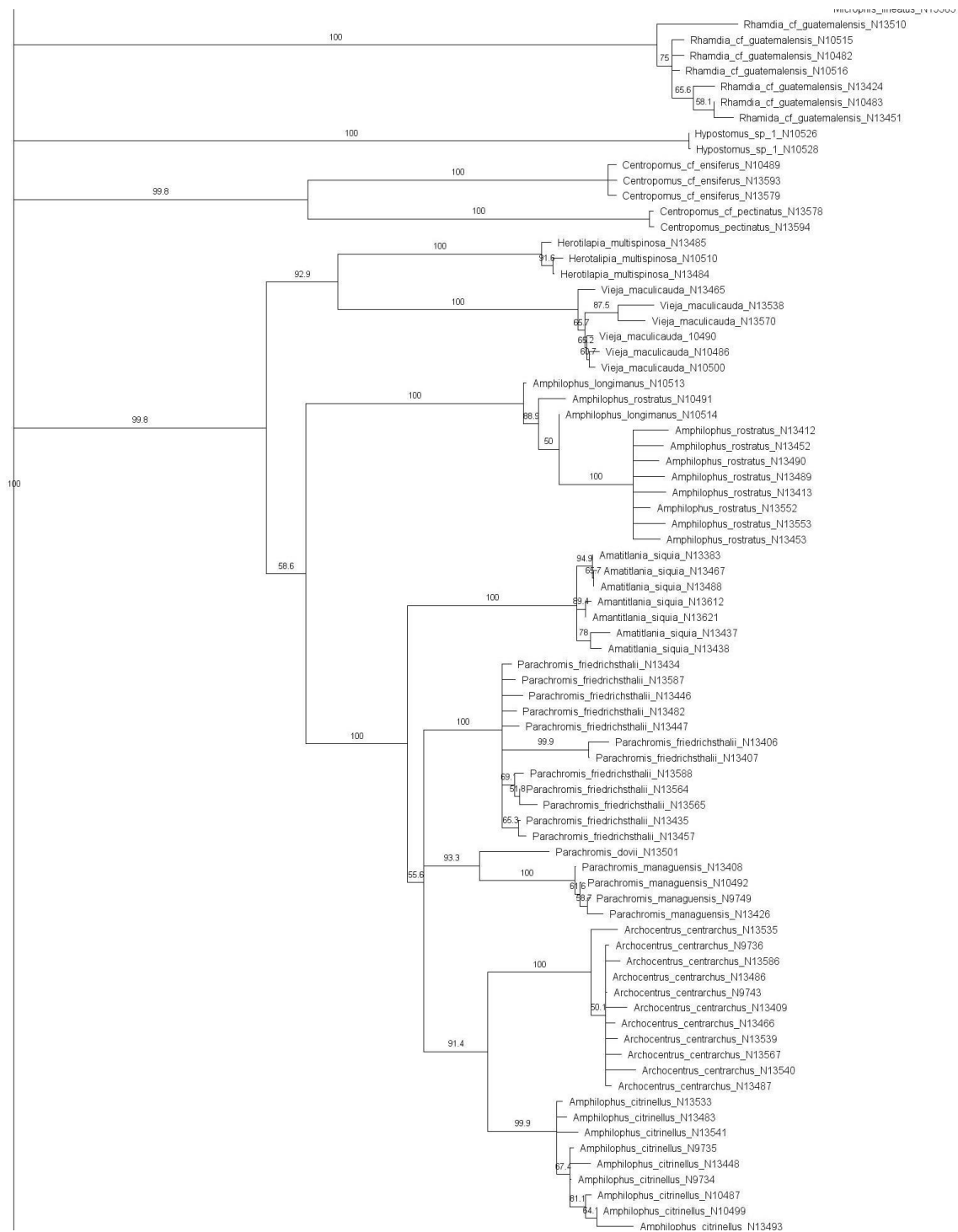
BCOLL26 2-06	Actinopterygii	Perciformes	Uranoscopidae	<i>Astroscoptus</i>	<i>y-graecum</i>	USNM-388248- AL200110-077	Smithsonian Institution	2006-09-27	United States	N/A	651	GENBANK/ BOLD	N/A
FJ439390 .1	Actinopterygii	Characiformes	Characidae	<i>Astyanax</i>	<i>nasutus</i>	A175	Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales	24-Jul-16	N/A	N/A	655	GENBANK	Ornelas-García, C. P., Domínguez-Domínguez, O., & Doadrio, I. (2008). Evolutionary history of the fish genus <i>Astyanax</i> Baird & Girard (1854)(Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC evolutionary biology, 8(1), 1-17.
FJ439388 .1				<i>Astyanax</i>	<i>nasutus</i>	A1480	Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales	24-Jul-16	N/A	N/A	655	GENBANK/ BOLD	Ornelas-García, C. P., Domínguez-Domínguez, O., & Doadrio, I. (2008). Evolutionary history of the fish genus <i>Astyanax</i> Baird & Girard (1854)(Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC evolutionary biology, 8(1), 1-17.
FJ439387 .1				<i>Astyanax</i>	<i>nasutus</i>	A1499	Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales	24-Jul-16	N/A	N/A	655	GENBANK/ BOLD	Ornelas-García, C. P., Domínguez-Domínguez, O., & Doadrio, I. (2008). Evolutionary history of the fish genus <i>Astyanax</i> Baird & Girard (1854)(Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC evolutionary biology, 8(1), 1-17.
GU70239 6.1	Actinopterygii	Siluriformes	Ariidae	<i>Bagre</i>	<i>bagre</i>	35128	Universidade Estadual Paulista, Lab de Biología e Genética de Peixes	13-Sep-12	Sao Paulo, Brazil	2008-12-12	652	GENBANK/ BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.
JQ36523 4.1				<i>Bagre</i>	<i>bagre</i>	51383	Universidade Estadual Paulista, Lab de Biología e Genética de Peixes	13-Sep-12	Sao Paulo, Brazil	2011-02-16	652	GENBANK/ BOLD	Ribeiro, A. O., Caires, R. A., Mariguela, T. C., Pereira, L. H., Hanner, R., & Oliveira, C. (2012). DNA barcodes identify marine fishes of São Paulo State, Brazil. Molecular ecology resources, 12(6), 1012–1020.

MH8989 52.1	Actinopterygii	Clupeiformes	Pristigasteridae	<i>Chirocentron</i>	<i>bleekeri</i>	MUFAL1807	Instituto de Ciencias Biológicas e da Saúde, Universidade Federal de Alagoas	31-Oct-19	Alagoas State, Brazil	N/A	480	GENBANK	Matias,G.D.A., Mott,T., Fabre,N.N., Neves,J.M.M. and Torres,R.A.
MG4962 18.1	Actinopterygii	Siluriformes	Heptapteridae	<i>Rhamdia</i>	<i>quelen</i>	STRI-00761	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Puntarenas, Costa Rica	1990-01-16	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG4962 19.1				<i>Rhamdia</i>	<i>quelen</i>	stri-13478	Smithsonian Tropical Research Institute	26-Nov-18	Estero Real-Tempisque, Esteli, Nicaragua	1997-03-12	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
MG9371 83.1				<i>Rhamdia</i>	<i>quelen</i>	STRI-00808	Smithsonian Tropical Research Institute	13-Mar-18	Santa Maria, Herrera, Panama	1995-01-13	648	GENBANK/BOLD	Bermingham,E., Reina,R.G. and Sanjur,O.
GU70154 1.1				<i>Rhamdia</i>	<i>quelen</i>	35805	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes	26-Jun-13	Upper Parana Basin, Goiás, Brazil	2008-10-14	652	GENBANK/BOLD	Pereira, L. H., Hanner, R., Foresti, F., & Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna?. BMC genetics, 14, 20.
IRREK497-08	Elasmobranchii	Rhinopristiformes	Pristidae	<i>Pristis</i>	<i>pectinata</i>	GN2736	Florida State University	2009-02-04	Andros Islands, Bahamas	N/A	652	BOLD	N/A
GU67358 4.1	Elasmobranchii	Rhinopristiformes	Pristidae	<i>Pristis</i>	<i>pristis</i>	BW-A6287	Biodiversity Institute of Ontario	05-Jan-21	Great Barrier Reef, Queensland, Australia	1993-07-15	652	GENBANK/BOLD	International Barcode of Life (iBOL)

Appendix D:

Neighbor-Joining Tree of BCWR Barcode Sequences





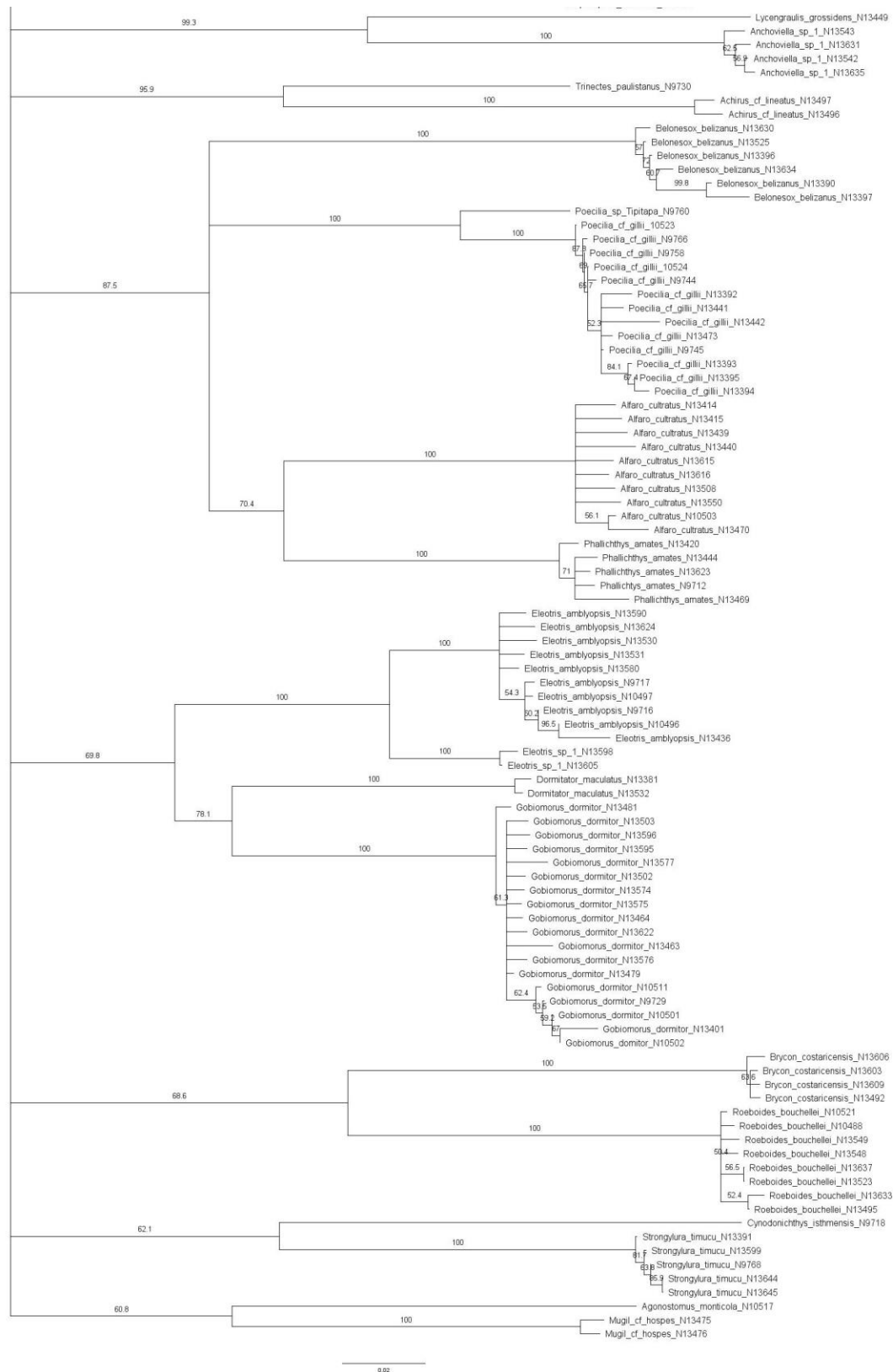


Figure D-S1. Neighborhood-joining tree for BCWR barcode sequences. Consensus values less than 50% are not shown on the tree.