

## Article

# Diversity and parasitic load of freshwater fish of the Shipstern Peninsula, Sarteneja, Corozal District, Belize, Central America from recent collections and historical archives

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**Abstract:** Belize is located within the Mesoamerican biodiversity hotspot and is an important link between critical biodiverse habitats in Central America. Despite considerable research on biodiversity in marine environments of Belize, research in freshwater environments is limited, with the most recent checklists having been published in obscure, mostly unavailable references more than 20 years ago.

Belize is currently experiencing serious degradation of some of its major freshwater resources such as the New River in the northern part of the country. These unique habitats are increasingly threatened by agro-urban development, invasive species and erratic climatic events. The economically important barrier coral reef is also experiencing impacts from the degraded freshwater watersheds that empty into the sea near the reefs.

This work addresses the paucity of documentation of the freshwater fishes of Belize, particularly for the Shipstern Peninsula area. The Shipstern Peninsula is a unique region in northern Belize, adjacent to the southern border of Mexico and the northeastern end of the Corozal Bay Wildlife Sanctuary. This paper reports on fish collected in 2015, with comparisons to historical data in surveys published in 1990, 1993, and 1997. We report on 12 genera/species collected in 2015 and provide molecular barcode data on several, which was not available in previous publications. *Floridichthys polyommus*, a new observation for the Shipstern Lagoon, and *Dajaus monticola*, a revised name and new observation for the Inland Blue Hole, were confirmed by barcodes. This update is vital to the continued management of freshwater environments in Belize, and especially for the Shipstern Peninsula and its important habitats held in trust for perpetuity for the Belizean people.

**Keywords:** biodiversity; cenote; chorros; *Dajaus monticola*; *Floridichthys polyommus*, parasitic copepod; *Poecilia*

## 1. Introduction

Although Belize is considered eco-friendly, the onslaught of ecologically negative development has reduced prime habitat. Rivers and streams are being seriously impacted by agro-urban land-use changes. Resultant changes in the Belize River watershed are having impacts on the economically important near shore and off-shore corals [1]. These corals are part of the economically and ecologically important Belize Barrier Reef Reserve System (BBRRS), a UNESCO declared World Heritage site barrier reef. Similarly, in northern Belize, the New River is experiencing serious degradation with reports of algal blooms and low levels of dissolved oxygen [2]. These conditions have created an urgent need to assess the current biodiversity and environmental health of these habitats.

The present study focuses on the northern part of Belize which has largely been ignored in previous surveys of freshwater habitats in Belize. This area encompasses the

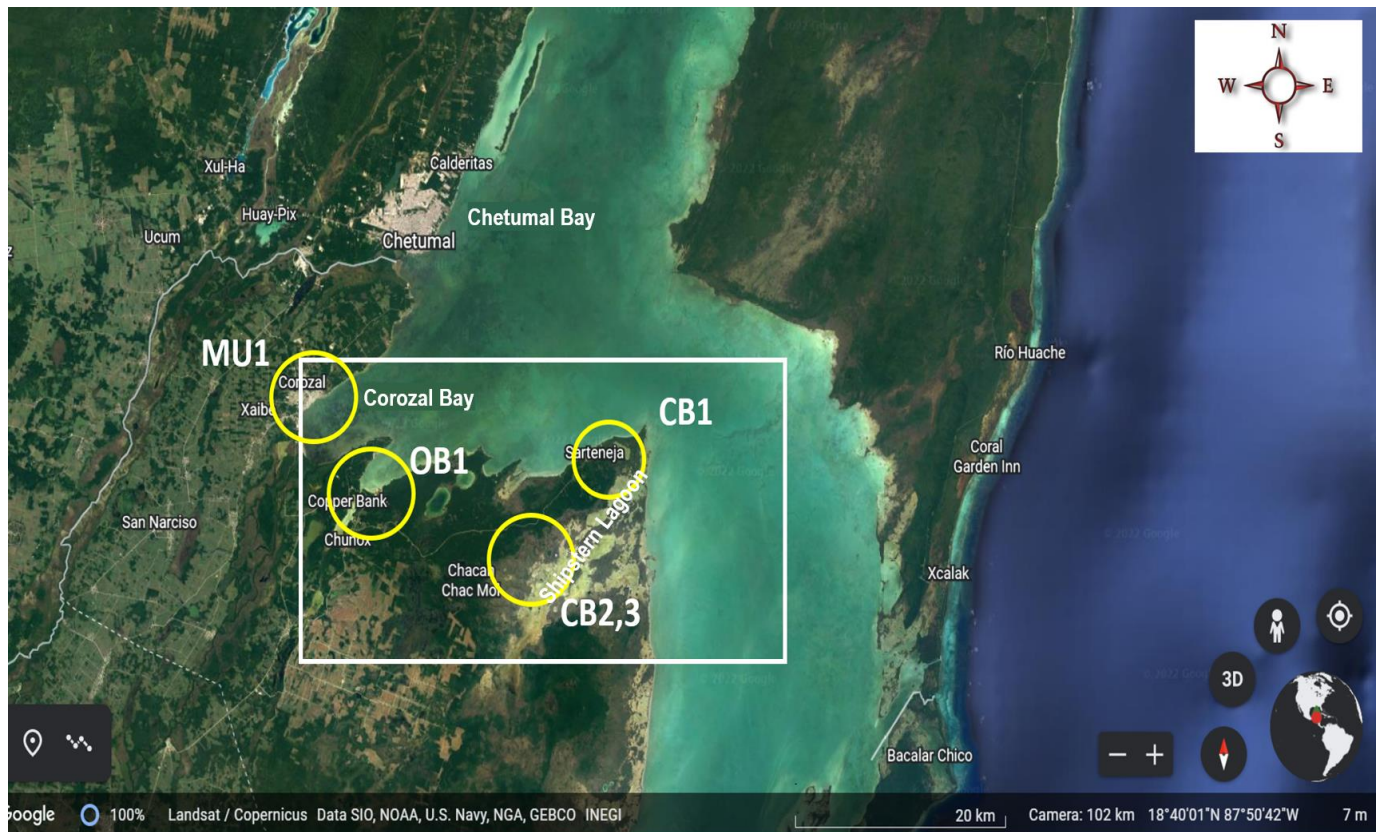
Corozal Bay Wildlife Sanctuary (CBWS) and is an important component of the North East Biological Corridor. Corozal Bay surrounds the Shipstern Peninsula and borders Sarteneja village (approximate population 3,000) and Corozal Town, (population 10,000) and is partly contained within the Shipstern Conservation Management Area (SCMA; formerly known as the Shipstern Nature Reserve). The Bay and the Shipstern Lagoon provide nurseries for fish and other organisms that spend their adult lives in the surrounding waters of Shipstern Peninsula and beyond including the barrier reef. Chetumal Bay is contiguous with Corozal Bay and adjacent to the city of Chetumal, Mexico, a metropolitan area of 160,000 people and part of a regional population of more than 225,000.

Initial work on freshwater fish of Belize by Greenfield [3], although useful, excluded fish from a significantly important region, the Shipstern Peninsula. A review by Esselman and Boles [4] noted that most limnological work in the northern wetlands of Belize had focused on plant communities, *Anopheles* mosquitoes, and archeological investigations. Fish assemblages have been studied in the Monkey River, located in southern Belize [5]. Fish communities have also been studied in adjacent areas of Mexico in the Rio Hondo [6]. These authors report an impressive 40 species captured using a harpoon method [6]. Other studies in Mexico have looked at a fish migration occurring between an artificial canal, and into the Corozal/Chetumal Bay with a variety of species being present [7]. Other recent discoveries in Corozal Bay include a newly described water mite and a copepod [8,9]. The understudied region of the Shipstern Peninsula and Corozal Bay prompted our research interest to update the current zoological lists for freshwater fish, and to report findings of potentially new parasitic copepod species for this region. Whilst added information on the freshwater fish species is necessary, such an update can also serve well in understanding the ecological dynamics of this region, and likewise to assist in improving its conservation management.

## 2. Materials and Methods

### 2.1. Sampling Locations

The Shipstern Peninsula (latitude 18.290789° N, longitude 88.239198° W, and surrounding area) is located in the Corozal District in the northernmost portion of Belize (Figure 1). Fish were collected from diverse habitats including rivers, bay, lagoons, cenotes, and chorros (distinctive urban human engineered stormwater ditches). Sampling sites identified in Figure 1 were located near the base of the peninsula (site OB1) and within the Shipstern Lagoon (sites CB2 and CB3). Samples were also taken from a small cenote in the SCMA (CB1), with approval of the SCMA management. Several specimens in this report were collected in the Blue Hole (site BHB; also known as the Inland Blue Hole), near the center of the country (17.15717° N, 88.68348° W). The Inland Blue Hole is a far inland freshwater cenote created by the collapse of the roof of an underground stream that flows via an underground cave system into the Sibun River. Collection locations of all specimens and water chemistry data (salinity, temperature, pH, and site description) are listed in Table S1.



**Figure 1.** Satellite image of the Shipstern Peninsula (Corozal District, Belize) (within the box). Sampling sites for the 2015 field work are indicated by the yellow circles. Sampling sites are labelled MU1: municipal wharf in Corozal Town; OB1: Orchid Bay residential area; CB1: Shipstern Conservation and Management Area headquarters; CB2, 3: Shipstern Conservation and Management Area region within the Shipstern Lagoon. Image captured from Google.

## 2.2. Sampling Methods, Taxonomy, and Parasitic Load Evaluation

Fish were caught with small dipnets and a minnow cast net. After capture, fish were immediately photographed and placed in isopropanol (70%) or identified and returned to the collection site (catch and release). Taxonomy was determined using the keys in Greenfield and Thomerson [3]. Comparisons were also made to Bijleveld [10]. Parasitic load on the gills and external structures was evaluated under stereomicroscope.

## 2.3. Molecular Analysis

After fish identification, a small piece of tissue was excised from the dorsal muscle of selected fish using a sterile razor blade. DNA was extracted using the Qiagen DNeasy Tissue Kit and subjected to PCR utilizing the cytochrome oxidase I (COI) "Folmer" barcode primers [11] as previously described [12]. Amplified PCR products were sequenced by GeneWiz (Plainfield, NJ, USA). Sequences were compared to previous sequences in GenBank and Barcode of Life databases by BLAST and relationships determined by phylogenetic analysis using MEGA bioinformatic software. COI barcode sequences for organisms identified by BLAST results and morphological classification were uploaded to public databases for public dissemination with accession IDs OP938589 - OP938594.

## 3. Results

### 3.1. Morphotaxonomic identifications

In the 2015 collections, out of a total of 29 specimens analyzed we found at least 12 different types of fish that were identified to at least genus level and some to species. Table

1 provides the identification of these fish and analysis conducted on these specimens. These include one specimen of *Astyanax aeneus* (colloquially known as billum) collected at site MU1 (municipal wharf of Corozal Town), a location marked by heavy traffic including water taxis and the town. Site OB1 (a canal in a residential area found near forested mangrove areas that connect to the sea) had the most cichlid species, including *C. urophthalmus* known colloquially as tuba, crana or in Maya as Xpinta and may have been mistaken for *Tilapia mossambica* when represented in postal stamps during colonial times, and 3 *Poecilia*. This was also the site of collection for *Eugerres plumieri*, known colloquially as Chiwas or stone bass.

**Table 1. Identification of Fish Collected in Belize, February, 2015**

| Specimen ID <sup>1</sup> | Taxonomic identification       | Comment   |
|--------------------------|--------------------------------|---|
| CB1F022215S1             | <i>Astyanax aeneus</i>         |   |
| CB2F022215S1             | <i>Poecilia</i> sp.            | Barcoded; found gill parasites  |
| CB2F022215S2             | <i>Poecilia</i> sp.            | Barcoded; found gill parasites  |
| CB2F022215S3             | <i>Poecilia</i> sp.            |   |
| CB2F022215S4             | <i>Gambusia</i> sp.            |   |
| CB2F022215S5             | <i>Belonesox belizanus</i>     |   |
| CB2F022215S6             | <i>Poecilia</i> sp.            |   |
| CB2F022215S7             | <i>Floridichthys polyommus</i> | Barcoded  |
| CB2F022215S8             | Needle fish                    | Found dead, no other observations   |
| CB2F022215S9             | <i>Belonesox belizanus</i>     |   |
| CB2F022215S10            | <i>Cichlasoma urophthalmus</i> |   |
| CB2F022215S11            | <i>Poecilia</i> sp.            |   |
| CB3F022215S1             | <i>Gambusia</i> sp.            |   |
| CB3F022215S2             | <i>Gambusia</i> sp.            |   |
| CB3F022215S3             | <i>Gambusia</i> sp.            |   |
| CB3F022215S4             | <i>Gambusia</i> sp.            |   |
| CB3F022215S5             | <i>Gambusia</i> sp.            |   |
| BH1F022415S1             | <i>Dajaus monticola</i>        | 1 specimen barcoded, no parasitic load found (2 other specimens were released after being photographed) |
| MU1F022715S1             | <i>Astyanax aeneus</i>         |   |
| OB1F022715S1             | <i>Cichlasoma meeki</i>        | Found gill parasites (nematode)   |
| OB1F022715S2             | <i>Astyanax aeneus</i>         |   |
| OB1F022715S3             | <i>Eugerres plumieri</i>       |   |
| OB1F022715S4             | <i>Poecilia</i> sp.            | Barcoded; found gill parasites; gut: plant matter found   |
| OB1F022715S5             | <i>Poecilia</i> sp.            | Barcoded  |
| OB1F022715S6             | <i>Poecilia</i> sp.            |   |
| OB1F022715S7             | <i>Eugerres plumieri</i>       |   |
| OB1F022715S8             | <i>Cichlasoma urophthalmus</i> | Fish released after being photographed.   |
| OB1F022715S9             | <i>Amphilophus robertsoni</i>  | Fish released after being photographed.   |
| OB1F022715S10            | <i>Cichlasoma synspilum</i>    | Fish released after being photographed.   |

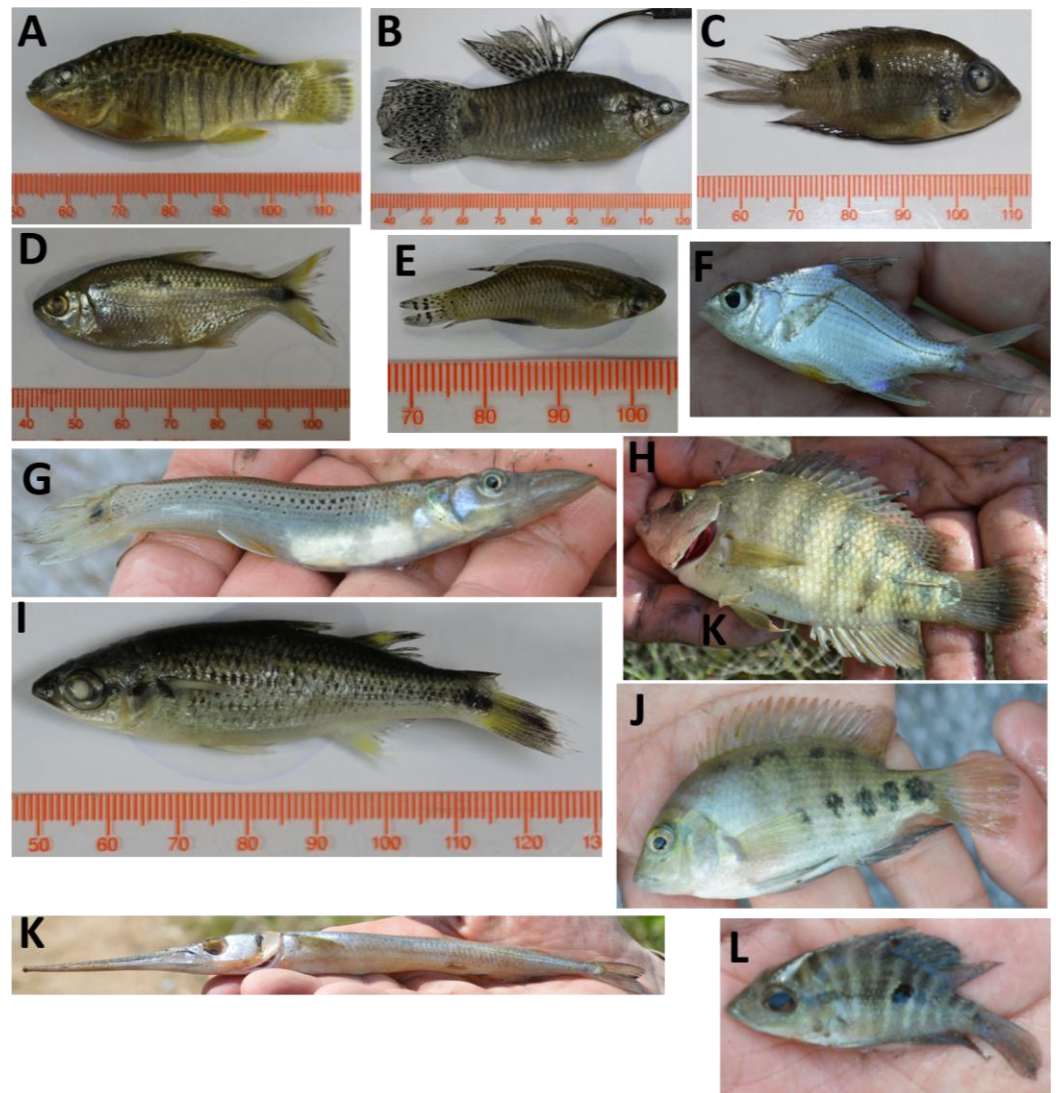
<sup>1</sup>Specimens named with the following code: AB#XMMDDYY S##, where AB# is a 3 character site identification, X identifies collection method (for this report, F for cast minnow net or dip net); MMDDYY is the date of the collection; and S## designates what number specimen for that site, method, and date. All but the last 3 specimens were checked for parasites.

Sites CB1-3 are all found within the Shipstern reserve (SCMA) and are considered the least disturbed sites. At these sites we collected the most specimens of *Gambusia* sp., commonly called mosquito fish, and *Poecilia*, known colloquially as poopsie or boglis or



mollies in the pet trade. Site CB1 is a small cenote with a salinity of 0, called Orchid Pond in the work of [10]. At CB1 we collected an *Astyanax aeneus* and observed but did not collect specimens of *Poecilia*. Site CB2 collections included a wide diversity of fish including *Gambusia* sp., *Floridichthys polyommus* and others. CB2 is a shallow canal that connects to the large Shipstern Lagoon seen in Figure 1. CB3 is a site where we collected several *Gambusia* sp. and the area where the shallow canal connects to the Shipstern Lagoon. This might be a transition zone that is especially suitable for *Gambusia* to capture prey. Photographs of representative specimens are shown in Figure 2. In the Shipstern area, the numbers of specimens identified included *Astyanax aeneus* (3), *Belenesox belizanus* (2), *Cichlasoma meeki* (1), *C. urophthalmus* (2), *Cichlasoma synspilum* (1), *Amphilophus robertsoni* (1), *Eugerres plumieri* (2), *Floridichthys polyommus* (1), *Gambusia* sp. (6), needlefish (1), *Poecilia* sp. (8). Three fish collected from the Inland Blue Hole were identified as *Dajaus monticola* (see discussion of the name below).

The types of fish collected varied from site to site. For example, five of the six *Gambusia* specimens were collected from site CB3, near the apex of the Shipstern Lagoon while none of the *Poecilia* were found at CB3. *Poecilia* were primarily found at sites CB2 and OB1, with CB2 being part of the Shipstern Lagoon canal and OB1 a mangrove canal connected to the sea. We found *D. monticola* only in the Inland Blue Hole (site BH1).



**Figure 2.** Representative fish collected during dip and cast net sampling. (A) *Floridichthys polyommus*, (B) *Poecilia* sp., (C) *Cichlasoma meeki*, (D) *Astyanax aeneus*, (E) *Gambusia*, (F) *Eugerres plumieri*, (G) *Belonesox belizanus*, (H) *Cichlasoma urophthalmus*, (I) *Dajaus monticola*, (J) *Cichlasoma synspilum*, (K) Needlefish, (L) *Amphilophus robertsoni*.

### 3.2. COI Barcodes

DNA barcodes of six specimens are reported in Table 2. The barcodes have high percentage (>99%) matches to identified barcodes in GenBank, as follows: BH1F022415S1, *Agonostomus monticola*; OB1F022715S5, *Poecilia mexicana/orri*; CB2F022215S1, *Poecilia mexicana/orri*; CB2F022215S2, *Poecilia mexicana/gillii*; CB2F022215S7, *Floridichthys polyommus*; and OB1F022715S4, *Poecilia mexicana/sphenops*. Of these six “identifications,” only the *F. polyommus* had an unambiguous agreement with our morphotaxonomic identification. Other fish specimens had either an ambiguous identification (e.g., *P. mexicana/orri*) or a different name, potentially indicating either a synonymy, incorrect, or archaic identification for the sequence.

Table 2. BLAST results of fish DNA barcodes in GenBank database

| Specimen<br>ID_taxon_GenBank accession ID                          | BLAST result<br>Query coverage, Q;<br>Percent identity, ID                            | Barcode status <sup>1</sup>   |
|--|---|-------------------------------|
| BH1F022415S1_ <i>Dajaus monticola</i> _OP938589                    | <i>Agonostomus monticola</i><br>Q: 100%; ID: 99.70%                                   | Taxonomic name needs updating |
| OB1F022715S5_ <i>Poecilia</i> sp._OP938590                         | <i>Poecilia mexicana</i><br>Q:100% ID: 99.55%;<br><i>P. orri</i> (Q:100%, ID:99.55%)  | Taxonomy unresolved           |
| CB2F022215S1_ <i>Poecilia</i> sp._OP938591                         | <i>Poecilia mexicana</i><br>Q:100 ID: 99.41%;<br><i>P. orri</i> Q:97%, ID: 100%       | Taxonomy unresolved           |
| CB2F022215S2_ <i>Poecilia</i> sp._OP938592                         | <i>Poecilia mexicana</i><br>Q: 100% ID: 99.41%;<br><i>P. gillii</i> Q:100% ID: 99.11% | Taxonomy unresolved           |
| CB2F022215S7_ <i>Floridichthys polyom-</i><br><i>mus</i> _OP938593 | <i>Floridichthys polyommus</i><br>Q: 96% ID:100%                                      | Taxonomy agreed               |
| OB1F022715S4_ <i>Poecilia</i> sp._OP938594                         | <i>Poecilia mexicana</i><br>Q: 100% ID:99.55%;<br><i>P. sphenops</i> Q: 98% ID: 100%  | Taxonomy unresolved           |

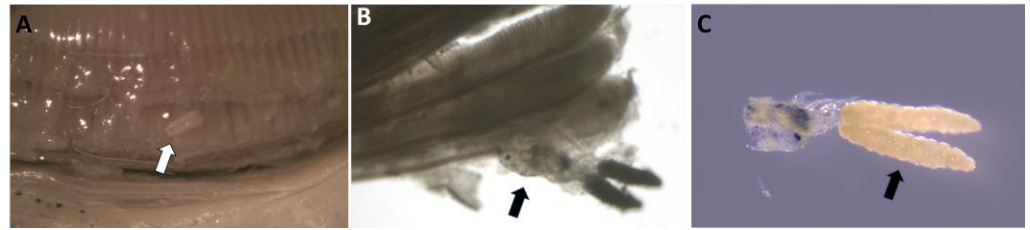
<sup>1</sup> See Discussion on the need for updated name and/or resolution of taxonomy in GenBank

3.3. External Parasites of the Freshwater Fish of the Shipstern Peninsula

Upon checking 26 of the specimens for external parasites, and parasites were found on the gill rakers of four fish, as summarized in Table 3. Parasites found on three *Poecilia* sp. were parasitic copepods. The parasite found on *C. meeki* was a nematode. Figure 3 shows the copepod parasite (identified as *Ergasilus* sp. in [13]) *in situ* in the gill rakers of a *Poecilia* specimen.

Table 3. Parasites on Fish of the Shipstern Peninsula area

| Sample ID    | Taxonomic ID        | Type of Parasite |
|--------------|---------------------|------------------|
| CB2F022215S1 | <i>Poecilia</i> sp. | copepod          |
| CB2F022215S2 | <i>Poecilia</i> sp. | copepod          |
| OB1F022715S4 | <i>Poecilia</i> sp. | copepod          |
| OB1F022715S1 | <i>C. meeki</i>     | nematode         |



**Figure 3.** Parasitic copepods on *Poecilia* sp., specimen OB1F022715S4 (a) Placement of parasitic copepod (white arrow) on *Poecilia* sp. gills seen under low power; (b) Parasitic copepod seen as attached to the gill filament (black arrow). (c) Parasitic copepod as seen removed from gill showing egg sacs (black arrow).

#### 4. Discussion

In this work 12 different types of freshwater fish were identified, mostly from collections in northern Belize. As discussed below, this paper represents the first update on the fish of the Shipstern Peninsula area in more than 30 years. Furthermore, we provide additional data on the COI barcodes of several specimens, confirming their identity and revealing or updating issues in the names of the species that are present in the area.

Prior to the present study only one other work on the biodiversity of fish of the Shipstern Peninsula area had been published in a peer-reviewed journal [14], but the journal in which the paper was published is no longer in operation. Published in 1993, Meerman [14]'s study listed 26 species; however, this reference included both marine and freshwater species in the Shipstern region. A report by Bijleveld [10] reported 15 species of freshwater fish found in the Shipstern area of Belize in 1990. Although this work was cited by others, it was not widely disseminated, and we were only able to obtain a copy by writing directly to the author, luckily still available for contact after 30 years. In response to our suggestion, Bijleveld allowed us to post his manuscript to an internet site, [https://www.ram-labwsu.org/uploads/1/3/8/6/138665805/bijleveld\\_1990\\_p\\_1-36\\_com-plete\\_with\\_cover\\_page.pdf](https://www.ram-labwsu.org/uploads/1/3/8/6/138665805/bijleveld_1990_p_1-36_com-plete_with_cover_page.pdf), so that it is now publicly available. Another publication in the 1990s was that of De Rham [15] but this work is also not available in a widely disseminated venue and is not present in search engines like the Web of Science® portal (Clarivate Analytics). In fact a search on Web of Science® with the key words "fish AND Shipstern" results in zero records. De Rham [15] reported 13 species of fish in the peninsula. Thus, the present work represents the first update on this body of literature on the fish of the Shipstern Peninsula in more than 30 years.

The 12 types of fish in the present study may be compared to Bijleveld [10] which reported 15 species of freshwater fish found in a similar area of Belize in 1990. The greater variety of fish found in Bijleveld's sampling is likely related to differences in duration and method of sampling as Bijleveld's sampling included night collections using traps baited with dead fish and collections over a longer period (8 months v. 1 week in the present study). Fish that were seen in Bijleveld [10] but not in the present study were *Synbranchus marmoratus*, *Rhamdia laticauda*, *Jordanella pulchra*, *Cyprinodon variegatus*, *Rivulus ocellatus*, and *Gobiomorus dormitator*. Bijleveld [10] also reported 5 species of cichlids, which is close to what we report in the present collections (4 species). We did not find two of his species of Cichlid including the "yellow bellied cichlid" (*Cichlasoma salvini*) nor the "bay snook" (*Petenia splendida*); however, a species that we found that Bijleveld [10] did not report was *Amphilophus robertsoni* or the false "fire-mouth cichlid". Our observations included several other species not reported by Bijleveld [10], among them *Eugerres plumieri* and *Floridichthys polyommus*. However, these two species do have precedents in northern Belize, with *Eugerres plumieri* having been reported in Shipstern Nature Reserve by Meerman and Boomsa in 1993 [14] and *F. polyommus* reported by Greenfield and Thomerson [3] in the Progresso Lagoon (latitude 18.208842 N, longitude 88.415887 W; west of Shipstern Peninsula and about 20 km due south of our site MU1). Among other species that differ in name, at least, is *Astyanax aeneus*, which was reported in Bijleveld [10] as *Astyanax fasciatus*, a



species name that is now restricted to Brazil in favor of *A. aeneus* for Central American and Mexican forms [16]. Furthermore, *A. aeneus* has been suggested to apply only to specimens on the Pacific slope and that the species of *Astyanax* found in Belize should therefore be named either *A. bacalarensis* (reported from Quintana Roo in northern Belize) or *A. belizianus*, reported from southern Belize [16]. We have reported these specimens under the name *A. aeneus*.

The fish of the continental waters of Belize were comprehensively reviewed by Greenfield and Thomerson [3] but their collections, which were prior to 1977, excluded the tip of the Shipstern Peninsula where most of our collections came from. In addition to *F. polyommus*, Greenfield and Thomerson [3] also collected the following species in the nearby Progreso Lagoon: *Petenia splendida*, *Cichlasoma urophthalmus*, *Lophiogobius cyprinoides*, *Cichlasoma meeki*, *Cichlasoma spilurum*, *Cichlasoma synspilum*, *Cichlasoma salvini*, *Eugerres plumieri*, *Cichlasoma friedrichsthalii*, *Cyprinodom artifrons*, *Belonesox belizanus*, *Astyanax aeneus*, and several *Gambusia*. Our work provides a geographic complement to the fish data of Greenfield and Thomerson [3] for Belize and an update relative to Bijleveld [10], De Rham [15], and others [14] reporting directly about the Shipstern Reserve.

Parasites that we found on four of the 26 specimens of fish that we inspected are a concern. While one nematode was found parasitizing *Cichlasoma meeki* the highest numbers of parasites were found in the gill region of *Poecilia* sp. Fish of the family Poeciliidae are an important resource for this region of Belize since they provide significant ecosystem services such as consumption of mosquito larvae. Poeciliidae are also important as ornamental fish, and copepod parasites have been found in some *Poecilia* in the pet trade which could represent a threat to the industry [17]. Given the encroaching anthropogenic impacts like farming in the area we wanted to know what was the parasitic load of these important fish. Some of the parasites on these fish were identified as parasitic copepods of the *Ergasilus* genus in another study [13]. This parasite might represent a new species. Similarly, another parasitic copepod was recently reported in the offshore waters of the Shipstern Peninsula parasitizing a polychaete marine worm [9].

*Poecilia* is thought to encompass at least 14 species in Belize according to Greenfield and Thomerson [3]. However, our DNA barcoding analysis of 4 specimens of *Poecilia* resulted in several high matches in the GenBank and BOLD databases highlighting the unresolved state of *Poecilia* taxonomy. Our specimens matched (>99% identity in the COI barcode region) up to four different possible species, underscoring the need for reliable morphotaxonomic reporting on groups such as *Poecilia*.

Another fish among our specimens that was barcoded was identified by us as *Dajaus monticola*, collected in the Inland Blue Hole. This species has been described as “the only species of mullet that ascends far inland and spends all its adult life in freshwater” as cited at <https://fishbase.mnhn.fr/summary/1085> from Kenny [18]. Consistent with this description, our collection site is found not only well inland but also well-separated from downstream populations by an underwater cave system through which the Blue Hole water flows. Whereas the GenBank database identifies this sequence as *Agonostomus monticola* (99.7% identical sequence), the BOLD taxonomy database identifies it as *Dajaus monticola*. This group’s taxonomy is a matter of discussion [19-21], with Durand et al. [19] insisting (in agreement with our assignment of *Dajaus* as the genus) that “*A. monticola* ... should be placed under a different genus name, namely *Dajaus* which is the earliest genus name available for *A. monticola* .... The genus *Agonostomus* under its present, revised definition exclusively occurs in the South-West Indian Ocean.” We agree with the published work and submitted our barcode under the name *Dajaus* (accession ID OP938589).

We also uploaded a barcode of *F. polyommus* to GenBank (accession ID OP938593). GenBank and BOLD databases have several almost identical sequences with the same identification, with one identified in Belize in the southern District of Stann Creek (BZLW5443 GenBank Accession ID JQ840504 ) but to our knowledge none of these previous submissions were collected in the Shipstern Peninsula. Based on various personal communications, we believe our specimen of *F. polyommus* might be a first report of this species in the area of the Shipstern Peninsula.



Other important fish types found by our study include *Eugerres plumieri* and many species of Cichlids, both types of fish that are economically important as a food source. *E. plumieri* is particularly interesting because we found some juvenile specimens in OB1 which is a canal connected to the sea. This area may be a nursery for this fish since the adults are known to be large schooling fish caught in the bay and heavily consumed in Belize. The multiple species of Cichlids sampled are an important finding since these have important cultural connections to the indigenous people of Belize, being called Xpinta by the Mayans and threatened by invasive *Tilapia*. It should be noted that we did not find any *Tilapia* in our collections but more monitoring is needed to ensure these are not invading these habitats.

Significant negative changes have occurred in the freshwater ecosystems of Belize since those earlier studies, a point that was already being emphasized by Esselman and Boles [4] over 20 years ago. On the other hand, some positive change has also occurred with the establishment of several reserves and biological corridors that protect more charismatic species such as jaguars which in turn protects threatened freshwater habitats found in the reserves (<https://www.worldlandtrust.org/news/2020/01/government-of-belize-sign-final-declaration-of-70000-acre-biological-corridor-in-north-east-belize/>). Given this recent establishment of the biological corridor in Belize, it is vital to have a published record of species found in the Shipstern Peninsula so that future conservation decisions can be made with historical data.

**Supplementary Materials:** The following supporting information can be downloaded at: [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: Water chemistry data, sampling labels, location and site descriptions.

**Author Contributions:** Conceptualization, A.A.V. and J.L.R.; methodology, A.A.V.; bioinformatic analysis, A.A.V. and J.L.R.; resources & taxonomy, J.T.-M. & A.A.V.; writing—original draft preparation, A.A.V.; writing—review and editing, J.L.R. & J.T.-M.; funding acquisition, A.A.V. & J.L.R. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** No live animals were involved. This project analyzed only fish caught with required permits inside the Shipstern Reserve and elsewhere in Belize and preserved as described in methods. All tests and analyses were done on dead specimens.

**Data Availability Statement:** Barcode sequences have been uploaded to GenBank, including collection locations.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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Supplement

**Table S1.** Water chemistry data and sampling site notes for fish collected in Belize, February 2015<sup>1</sup>.

| Site ID and date | Location (Latitude, Longitude) | pH   | Temperature (°C) | Salinity (ppt) | Site Description  |
|------------------|--------------------------------|------|------------------|----------------|---|
| CB1<br>2/22/2015 | 18.3164 N,<br>88.1825 W        | 7.71 | 25.5             | 0.0            | Water hole near Shipstern Reserve headquarters                          |
| CB2<br>2/22/2015 | 18.2221 N,<br>88.2224 W        | 8.30 | 27.9             | 12             | Shallow canal to Shipstern Laguna                                       |
| CB3<br>2/22/2015 | 18.2211 N,<br>88.2175 W        | 8.66 | 28.6             | 7              | Opening of the canal to Shipstern Laguna                                |
| BH1<br>2/24/2015 | 17.1572 N,<br>88.6836 W        | --   | --               | --             | Inland Blue Hole, collapsed cenote along Hummingbird Highway            |
| MU1<br>2/27/2015 | 18.3894 N,<br>88.3856 W        | --   | --               | --             | Municipal wharf Corozal Bay very disturbed urban                        |
| OB1<br>2/27/2015 | 18.3280 N<br>88.3262 W         | --   | --               | --             | manmade canal connected to sea, adjacent to Orchid Bay residential area |

<sup>1</sup>-- means the variable was not measured.