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The imperiled fish fauna in the Nicaragua Canal zone

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Abstract: *Large-scale infrastructure projects commonly have large effects on the environment. The planned construction of the Nicaragua Canal will irreversibly alter the aquatic environment of Nicaragua in many ways. Two distinct drainage basins (San Juan and Punta Gorda) will be connected and numerous ecosystems will be altered. Considering the project's far-reaching environmental effects, too few studies on biodiversity have been performed to date. This limits provision of robust environmental impact assessments. We explored the geographic distribution of taxonomic and genetic diversity of freshwater fish species (*Poecilia *spp.,* Amatitlania siquia, Hypsophrys nematopus, Brycon guatemalensis, and Roeboides bouchellei*) across the Nicaragua Canal zone. We collected population samples in affected areas (San Juan, Punta Gorda, and Escondido drainage basins), investigated species composition of 2 drainage basins and performed genetic analyses (genetic diversity, analysis of molecular variance) based on mitochondrial* cytb*. Freshwater fish faunas differed substantially between drainage basins (Jaccard similarity = 0.33). Most populations from distinct drainage basins were genetically differentiated. Removing the geographic barrier between these basins will promote biotic homogenization and the loss of unique genetic diversity. We found species in areas where they were not known to exist, including an undescribed, highly distinct clade of live bearing fish (*Poecilia*). Our results indicate that the Nicaragua Canal likely will have strong impacts on Nicaragua's freshwater biodiversity. However, knowledge about the extent of these impacts is lacking, which highlights the need for more thorough investigations before the environment is altered irreversibly.*

Keywords: Central America, freshwater biodiversity, genetic diversity, Poeciliidae, population differentiation, population genetics

La Fauna Ictiológica en Riesgo en la Zona del Canal de Nicaragua

Resumen: *Los proyectos de infraestructura a gran escala tienen comúnmente grandes efectos sobre el ambiente. La construcción planeada del Canal de Nicaragua alterará de manera irreversible el ambiente acuático de Nicaragua de muchas formas. Dos distintas cuencas de drenado (San Juan y Punta Gorda) estarán conectadas y numerosos ecosistemas serán alterados. Si se consideran los efectos ambientales trascendentales del proyecto, a la fecha se han realizado pocos estudios sobre la biodiversidad. Esto limita el suministro de valoraciones generales sobre el impacto ambiental. Exploramos la distribución geográfica de la diversidad genética y taxonómica de las especies de peces de agua dulce (*Poecilia *spp.,* Amatitlania siquia, Hypsophrys nematopus, Brycon guatemalensis, *y* Roeboides bouchellei*) a lo largo de la zona del Canal de Nicaragua. Colectamos muestras de poblaciones en las áreas afectadas (las cuencas de drenado de San Juan, Punta Gorda y Escondido), investigamos la composición de especies de dos cuencas de drenado y realizamos análisis genéticos (diversidad genética, análisis de la variación molecular) basados en el* cytb *mitocondrial. Las faunas ictiológicas de agua dulce difirieron sustancialmente entre las cuencas de drenado (similitud de Jaccard = 0.33). La mayoría de las poblaciones de las distintas cuencas de drenado estuvieron diferenciadas genéticamente. Remover la barrera geográfica entre estas cuencas promoverá la homogenización biótica y la pérdida de la diversidad genética única. Encontramos especies en áreas en las que no se sabía que existían, incluyendo un clado no descrito y altamente distinto de peces vivíparos (*Poecilia*). Nuestros resultados indican que el Canal de Nicaragua tendrá impactos fuertes sobre la biodiversidad de agua dulce de Nicaragua. Sin*

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embargo, el conocimiento sobre la extensión de estos impactos es escaso, lo que resalta la necesidad de más investigaciones rigurosas antes de que el ambiente sea alterado de manera irreversible.

Palabras Clave: América Central, biodiversidad de agua dulce, diferenciación poblacional, diversidad genética, genética poblacional, Poeciliidae

Introduction

Human-induced changes to the environment are now one of the major driving forces behind evolutionary change (Palumbi 2001; Davies & Davies 2010), and they commonly have drastic effects on the integrity of natural populations and threaten the maintenance of biodiversity (Carroll et al. 2014). This applies specifically when environmental changes occur too quickly for species to adapt to, as currently seen on a large scale in what has been called Earth's sixth mass extinction (Barnosky et al. 2011). Particularly, biodiversity in freshwater environments is far more threatened than biodiversity in terrestrial environments (Ricciardi & Rasmussen 1999; Sala et al. 2000). This is alarming because some freshwater ecosystems have exceptionally high species richness. Although freshwater habitats cover only 0.8% of Earth's surface (Gleick 1996), they harbor approximately 6% of all species described (Hawksworth 1995). Major threats are overexploitation, water pollution, waterflow modification, destruction or degradation of habitat, and establishment and spread of nonnative species (Dudgeon et al. 2006). The planned construction of the Nicaragua Canal may entail all these major threats and adversely affect the aquatic fauna of Nicaragua (Meyer and Huete-Pérez 2014; Brett et al. 2015; Huete-Pérez et al. 2015).

In July 2013, the Hong Kong Nicaragua Canal Development Group (HKND) of China was granted the concession to build an interoceanic shipping canal, called the Nicaragua Canal, across the Central American isthmus through Nicaragua connecting the Caribbean Sea and the Pacific Ocean (ERM 2014). The concession grants HKND rights to use any water body, including Lake Nicaragua, the largest freshwater lake in Central America (and 19th largest worldwide) with 8400 km^2 of surface area (Schwoerbel 2013). The lake's water volume is only 80 km^3 because the average depth is 15 m and the maximum depth is 50 m (Barluenga and Meyer 2010). The fish fauna of the lake is well characterized (Koenig et al. 1976; Barluenga and Meyer 2010); over 40 species have been described (Astorqui 1976; Bussing 1976). However, the level of knowledge on the fish fauna in the remote eastern regions of Nicaragua is relatively low. The prospective construction and operation of the canal are of major environmental concern due to the threat the canal poses to the fauna of Lake Nicaragua, adjacent rivers, and several natural reserves (Huete-Pérez et al. 2013; Meyer & Huete-Pérez 2014). Moreover, Lake Nicaragua's freshwater fishes are of socioeconomic importance because fishing constitutes the livelihood of many Nicaraguans (Davies 1976; Huete-Pérez et al. 2013).

The canal will start in the Caribbean Sea at Río Punta Gorda, follow Río Tule, cross Lake Nicaragua, head westward via Río Las Lajas and enter the Pacific Ocean close to the small village of Brito (Fig. 1). Hence, the Punta Gorda and San Juan Basins will be connected (ERM 2014), promoting alterations in species' distribution and ecosystem structure. Multiple routes were proposed; 2 of the rejected routes included the Escondido Basin (Fig. 1). The final decision on the route via Río Punta Gorda was released in the environmental and social impact assessment (ESIA) report in late 2015 and was based mainly on technical and economic grounds (ERM 2015). However, the data presented in the ESIA report, according to a panel of Nicaraguan and international scientists, do not provide sufficient information for a proper evaluation of the effects of the Nicaragua Canal on Nicaraguan biodiversity (Huete-Pérez et al. 2016). Thus, there have been appeals to conduct independent studies on a broad taxonomic scale for different ecosystems in affected areas (Huete-Pérez et al. 2015).

Construction of the canal will break a geographic barrier between two distinct drainage basins, promoting homogenization of freshwater biotas across the affected regions. Biotic homogenization increases the genetic, taxonomic, and functional similarity of previously distinct biotas (Olden 2006) through introduction of nonnative species, extirpation of native species, and changes to land cover (Rahel 2002). In the case of fishes (Rahel 2000; Vitule et al. 2012), biotic homogenization is associated primarily with human actions (e.g., canal building, ballast water discharge, and introduction of nonnative species for recreational use) and commonly leads to a decrease in biodiversity (McKinney & Lockwood 1999). Successful establishment of invasive species is explained mainly by human disturbance of the environment (Leprieur et al. 2008). For example, in the Mediterranean Sea species invasions occur via the Suez Canal and have had adverse ecological and social consequences (Galil et al. 2015).

The aquatic landscape of Nicaragua is characterized by a number of rivers and lakes. It has been suggested that its fish fauna is similar across distinct drainage basins (Bussing 1976), which indicates low levels of beta diversity. This contention has not been tested adequately. Thus, we surveyed 3 representative teleost families to determine their geographic distribution and population

*Figure 1. Proposed route (*solid line*) and alternative routes (dashed lines) of the Nicaragua Canal. The 3 drainage basins involved are San Juan (red), Punta Gorda (blue), and Escondido (yellow). Fish-sampling locations are marked with open diamonds.*

structure (Supporting Information) across Escondido, Punta Gorda, and San Juan Basins (Fig. 1). These families represent a broad taxonomic range of freshwater fishes in Nicaragua. The Punta Gorda and San Juan Basins will be part of the Nicaragua Canal zone.

The studied species belong to the most species-rich and widespread teleost families in Central America. In Nicaragua, 31 Cichlidae, 7 Characidae, and 8 Poeciliidae species have been described (Froese 2014). Relative to other countries in Central America, the number of poeciliid and characid species appears relatively small (Supporting Information). For example, only 1 poeciliid species (*P. mexicana*) has been described for Nicaragua, whereas 6 in Guatemala, 3 in Costa Rica, and 6 in Panama have been described. These numbers indicate low species diversity across Nicaragua (Bussing 1976). In contrast, recent biogeographic investigations restricted to the Pacific slope found several species in the *Poecilia sphenops* and *Poecilia mexicana* species complexes (Alda et al. 2013; Bagley et al. 2015). Bagley et al. (2015) detected a third genetic cluster, referred to as *Poecilia* sp. Tipitapa, that falls outside the *P. shenops* and *P. mexicana* species complexes. This suggests knowledge is lacking on the geographic distribution of aquatic biodiversity in Nicaragua and on how species' distributions will be affected by the canal. Therefore, we sought to shed light on species' distribution and patterns of population structure across three distinct drainage basins. We aimed to inform understanding of the effects of artificially connecting distinct basins on the freshwater fauna of Nicaragua and to contribute to the evaluation of the canal's route through Punta Gorda and Lake Nicaragua from a conservation standpoint.

Methods

Study Area and Sample Collection

We sampled 2 poeciliids (*Poecilia* spp. cluster 2, *Poecilia* spp. cluster 4), 2 cichlids (*Amatitlania siquia*, *Hypsophrys nematopus*), and 2 characids (*Brycon guatemalensis*, *Roeboides bouchellei*). We chose these species based on their geographic distribution across Nicaragua and because their ecological characteristics (maximum size, trophic level, and reproductive strategy) differ (Supporting Information). Information about species' distribution was obtained from the literature and was based on our findings in the field.

We aimed to collect 25 specimens per species at each location. However, this was not achieved for all populations. Samples for interdrainage comparisons were from single-sample sites for the Punta Gorda and Escondido Basins and 6 sites from the San Juan Basin (Supporting Information). In August 2014, we collected fishes with seine nets (6 m long, 1-cm mesh size), gill nets (mesh sizes from 1 to 4 cm), and cast nets. We labeled each specimen with a unique identification number and stored muscle tissue or fin clips in ethanol for later DNA extraction. We identified species in the field visually and subsequently by *cytochrome b* (*cytb*) sequencing.

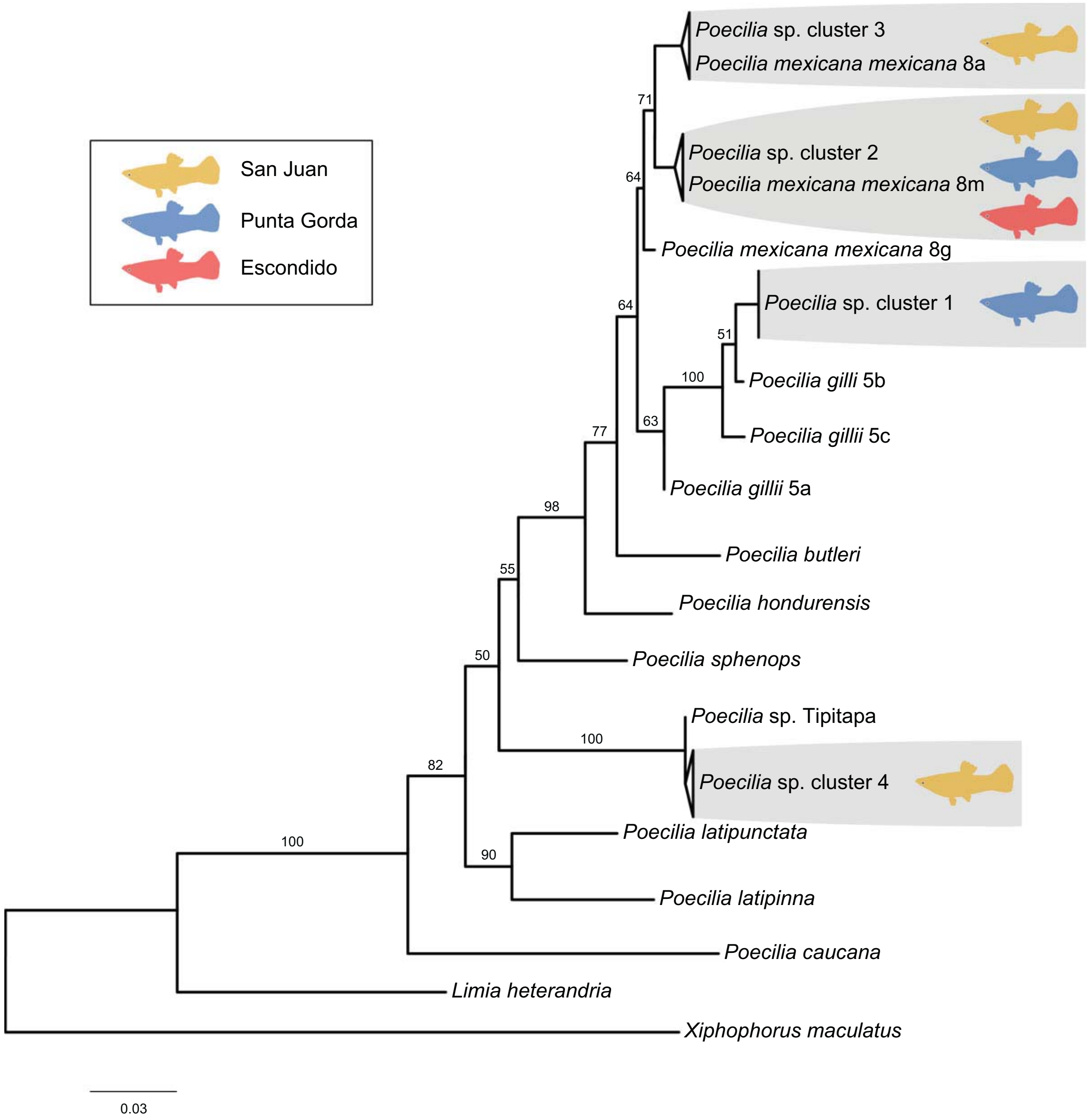

Figure 2. Phylogeny of Poecilia *from 3 drainage basins (San Juan, Punta Gorda, and Escondido) in Nicaragua based on* cytb *sequence data (1086 bp) (grey shading, samples collected for this study). Species identity was assigned based on Bagley et al. (2015).*

DNA Analyses

We used polymerase chain reaction to amplify mitochondrial *cytb* (DreamTaq DNA Polymerase, Life Technologies, Carlsbad, California, USA). The size of the amplified PCR products was approximately 1200 basepairs (bp), primers are listed in Supporting Information. Primer annealing temperatures were 54 °C (*Poecilia* spp.) or 58 °C (*A. siquia*, *H. nematopus*, *B. guatemalensis*, *R. bouchellei*). We sequenced purified templates on an Applied Biosystems3130xl Genetic Analyzer (Life Technologies). The quality of sequencing reads was checked manually and reads were trimmed and assembled with SeqMan Pro (DNASTAR, Madison, Wisconsin, USA). The trimmed DNA sequences used for all analyses had total lengths of 1086 bp (*Poecilia.* spp.), 1044 bp (*A. siquia*), 1065 bp (*H. nematopus*), 973 bp (*B. guatemalensis*), and 991 bp (*R. bouchellei*). The *cytb* sequences for phylogenetic analyses were obtained from GenBank (Supporting Information). For *Poecilia*, we assigned samples to known species based on published sequences (Fig. 2, Supporting Information) according to a recent phylogeny (Bagley et al. 2015). We aligned *cytb* sequences and built maximum likelihood phylogenetic trees with SeaView version 4 (Gouy et al. 2010). We used the GTR model of nucleotide substitution to reconstruct phylogenetic trees. We created median joining haplotype networks with PopART version 1.7 (PopArt, Dunedin, New Zealand). Hierarchical clustering of genetic diversity was investigated using analysis of molecular variance (AMOVA) in Arlequin version 3.5.2.1 (Excoffier et al. 2005). Measures of population differentiation (F_{ST}), haplotype diversity, and nucleotide diversity were calculated with DnaSP version 5 (Librado & Rozas 2009) and Arlequin version 3.5.2.1.

Results

Geographic distribution Based on our field data and the ESIA (ERM 2015), at least 27 species occurred in both affected drainage basins, 24 species were exclusive to Punta Gorda, and 31 species were exclusive to San Juan (Jaccard similarity coefficient of 0.33 [Supporting Information]). Two nonnative species recently introduced to Lake Nicaragua, the Nile Tilapia (*Oreochromis niloticus)* and the devil fish (*Hypostomus panamensis*), were widespread across the San Juan Basin. The characids *R. bouchellei* and *B. guatemalensis* and the cichlid *A. siquia* occurred in all 3 drainage basins. The cichlid *H. nematopus* occurred in multiple locations in the San Juan Basin.

We identified 4 monophyletic cluster within *Poecilia*, these corresponded to *P. gillii* (*P.* sp. cluster 1), *P. mexicana* clade 8m (*P.* sp. cluster 2), *P. mexicana* clade 8a (*P.* sp. cluster 3), and *P.* sp. Tipitapa (*P.* sp. cluster 4) (sensu Bagley et al. 2015) (Fig. 2, Supporting Information). The *P.* sp. cluster 1 was restricted to the Punta Gorda Basin, and *P.* sp. cluster 3 and cluster 4 were restricted to the San Juan Basin. In contrast, *P.* sp. cluster 2 occurred in all 3 basins. In the small creek Río Caño Chiquito, a tributary to Río Punta Gorda, 2 genetic clusters, *P. gillii* (cluster 1) and *P. mexicana* clade 8m (cluster 2), co-occurred. In Río Las Lajas, 3 different clusters co-occurred, *P.* sp. cluster 2, *P.* sp. cluster 3, and *P.* sp. cluster 4. The still undescribed *P.* sp. Tipitapa (cluster 4) from Río Tipitapa at the northern shore of Lake Nicaragua (Bagley et al. 2015) was widespread across the San Juan Basin and had high levels of haplotype diversity (Fig. 3f & Supporting Information).

Genetic Diversity

In most species, a large proportion of genetic diversity was explained by drainage basin of origin (Fig. 3 & Table 1). For the cichlid *A. siquia* and the poeciliid *P.* sp. cluster 2, a significant proportion of genetic diversity, 71% and 89% respectively, was attributable to drainage basin of origin (Table 1). The *A. siquia* population from Punta Gorda was highly differentiated from those in Escondido and San Juan, whereas populations from Escondido and San Juan were less divergent. (Fig. 3a, Supporting Information). Moreover, haplotype diversity varied strongly across drainage basins, and both haplotype and nucleotide diversity were much lower in Punta Gorda (Supporting Information). In contrast, genetic diversity was less variable in the other species (Supporting Information). Differences in drainage basin explained a high (63%) but only marginally significant ($P = 0.065$) proportion of genetic diversity for the characid *B. guatemalensis*. Populations from Escondido and Punta Gorda possessed a single shared haplotype, whereas haplotype diversity was higher in populations from San Juan (Fig. 3b & Supporting Information). For *R. bouchellei*, the crystal tetra, 96% of diversity was attributable to variation within populations, whereas 1% and 3% of diversity was attributable, respectively, to variation among populations within the same basin and among populations from distinct basins (Table 1). This pattern of low differentiation among populations from the same drainage basins also applied to the poeciliid *P.* sp. cluster 4 (*P.* sp. Tipitapa), indicating low levels of genetic differentiation within the San Juan Basin. However, the cichlid *H. nematopus*, found at 3 locations within the San Juan Basin had higher levels of differentiation among populations (Table 1).

Table 1. Results of the hierarchical analyses of genetic diversity in 6 fish species from the Nicaragua Canal zone.[a]

	Percent diversity attributable to variation			
Species[a]	*within populations*	*among populations within basin*	*within basins*	*among basins*
A. siquia	–	–	29	71[b]
P. mexicana clade 8m	–	–	11	89[b]
B. guatemalensis	37	0	–	63[c]
R. bouchellei	96	1	–	3
H. nematopus	79	21[b]	–	–
P. sp Tipitapa	97	3	–	–

[a] *For the first 4 species, populations from all 3 drainage basins (San Juan, Punta Gorda, and Escondio) are compared. The last 4 species are represented by multiple populations within the same basin (San Juan).*

[b] $p < 0.001$.

[c] $p < 0.1$.

Populations within the same basin showed almost no genetic differentiation, whereas comparisons across basins exhibited higher differentiation. The characid *B. guatemalensis* (no differentiation between Punta Gorda and Escondido) and the cichlid *H. nematopus* (high differentiation between Río Las Lajas and other San Juan populations) were exceptions to this pattern.

Discussion

Nicaragua is part of the Mesoamerican biodiversity hotspot and has particularly high levels of vertebrate endemism (Myers et al. 2000). Moreover, the Neotropics

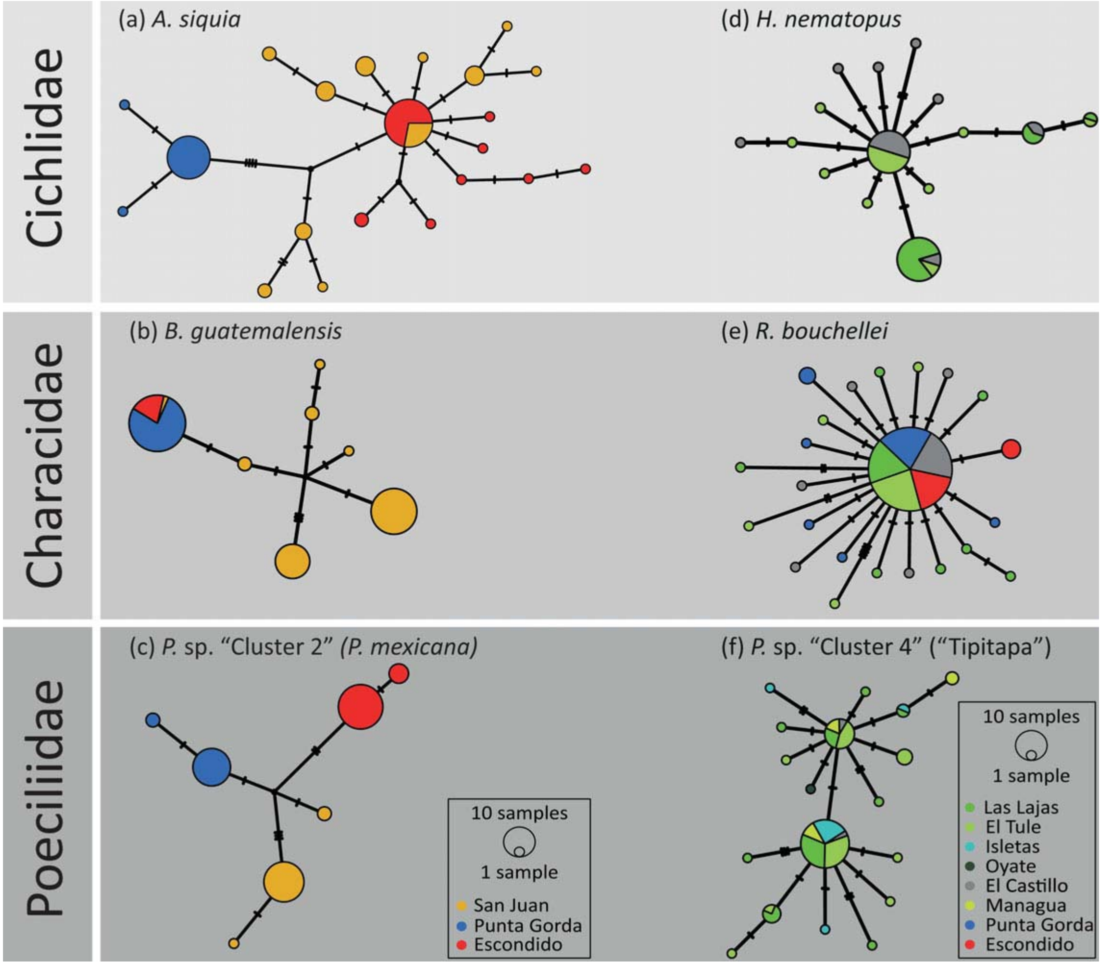

Figure 3. Median joining haplotype networks for freshwater fish populations from (a–c, e) 3 distinct drainage basins and from (d–f) multiple locations within the San Juan drainage Basin. Circle sizes represent sample sizes for each haplotype.

harbor a large proportion of freshwater fishes' global taxonomic and functional diversity (Toussaint et al. 2016). Functional diversity plays a major role in maintaining ecosystem functioning and services relevant to humans (Diaz & Cabido 2001). The Nicaragua Canal will have large effects on the environment and poses a threat to Nicaragua's biodiversity (Meyer & Huete-Pérez 2014). In particular, connecting the Punta Gorda and San Juan drainage Basins will substantially affect the freshwater fauna. Based on the Freshwater Ecoregions of the World (FEOW) map, the San Juan and Punta Gorda Basins are within the San Juan ecoregion (Ecoregion 205) (Abell et al. 2008). Ecoregions are defined as areas harboring distinct assemblages of species communities and are viewed as conservation units. Hence, it is assumed that the 2 drainage basins have relatively similar freshwater faunas. Yet, data from the ESIA reveals a Jaccard similarity coefficient of 0.33 for freshwater fishes (ERM 2015), suggesting that both basins have only one-third of species in common (Supporting Information). After the canal is built, species assemblages are likely to become more similar due to biotic homogenization (Olden 2006). Yet the scope of these effects remains unknown because there is still a lack of information on the distribution of taxonomic and genetic diversity of freshwater fishes in affected areas.

Habitat Alteration and Destruction

Water pollution, destruction or degradation of habitat, and water-flow modification are among the major threats to freshwater biodiversity (Dudgeon et al. 2006). The canal will likely lead to increased water pollution (e.g., through construction works, oil spills, and ship traffic) and cause significant alteration of habitat along its route. Multiple rivers in the canal zone, representing unique and distinct ecosystems, will be turned into more lake-like environments. This homogenization of habitats and connection of distinct drainage basins can increase the likelihood of biotic homogenization caused by widespread, generalist species replacing locally adapted species in affected areas (Rahel 2000; Vitule et al. 2012). Further, destruction of habitats along the route will affect populations of numerous species, possibly posing a risk to biodiversity in these areas.

For example, 3 different genetic clusters of *Poecilia* co-occur in Río Las Lajas. According to a recent phylogeny on fishes of the genus *Poecilia* in Central America (Bagley et al. 2015), these genetic clusters refer to *P. mexicana* clade 8a, *P. mexicana* clade 8m, and *P.* sp. Tipitapa (Fig. 2, Supporting Information). This small river on the eastern shore of Lake Nicaragua will be part of the canal and therefore disappear in its current form. Río Las Lajas harbors elevated nucleotide and haplotype diversity for most of the species we investigated (Supporting Information). Dredging of the river during construction works will irreversibly destroy this habitat, putting the persistence of these populations at risk. In the Punta Gorda Basin, the *Poecilia* sp. cluster 1 from Río Caño Chiquito shows only 1 *cytb* haplotype. This clade may be highly threatened by rapid environmental changes because reduced genetic diversity negatively affects a population's ability to adapt to environmental changes (Sgro et al. 2011) and decreases survival chances under stress conditions (Frankham 2005).

Invasion by Nonnative Species

The canal will facilitate colonization of novel habitats by nonnative species, as seen in similar scenarios (Kolar & Lodge 2000; Holeck et al. 2004). This may happen either through species' range expansion after the two drainage basins are connected or through introduction of new species via ballast-water discharge. Establishment of nonnative species has detrimental effects on ecosystems (e.g., eutrophication, extirpation of native species, and biotic homogenization [Figueredo & Giani 2005; Menezes et al. 2012; Vitule et al. 2012]). Altered environments, such as those expected for the Punta Gorda region after construction of the canal, face a higher risk of invasive species becoming established (Moyle & Light 1996; Johnson et al. 2008). Moreover, the expected homogenization of the environment is predicted to further increase the speed of species invasions (Garcia-Ramos & Rodriguez 2002). Changes in abiotic factors, especially water-flow regime, may increase the risk of successful establishment of invasive species (Baltz & Moyle 1993). Nonnative species can replace native species after reduction of water-flow velocity and variability caused by the construction of a dam (Marchetti & Moyle 2001). Establishment success of introduced species is also determined by propagule pressure (Williamson 1996; Lockwood et al. 2009), which reduces effects of demographic stochasticity. Propagule pressure is determined by propagule size (number of individuals) and propagule number (number of release events) (Simberloff 2009). Because the canal will form a permanent connection between distinct drainage basins, it can be assumed that propagule pressure will be high and that this will facilitate colonization and establishment.

Our results show that even within the well-studied genus *Poecilia*, too little is known about the geographic distribution of species in Nicaragua, specifically in the Punta Gorda region. For example, out of the 2 genetic clusters we found in Punta Gorda, only 1 was previously described for Nicaragua. The other cluster grouped with a clade of *P. gillii* from Costa Rica (clade 5b in Bagley et al. 2015). This cluster was not found in the San Juan and Escondido Basins. A recently discovered undescribed species (*P.* sp. "Tipitapa" Bagley et al. 2015) is known only from the San Juan Basin. Our results show that *P.* sp. Tipitapa is widespread across the San Juan Basin and occurs at least in Lake Nicaragua (El Tule and Isletas), Lake Managua, and three adjacent rivers (Río San Juan, Río Las Lajas, and Río Oyate), but it was not detected in the Punta Gorda Basin. An artificial connection of the two basins will enhance the potential for range expansions and contact between previously allopatric species. This, in turn, may cause extirpations related to competitive exclusion between ecologically similar species (Hardin 1960). Because two-thirds of freshwater fish species in the San Juan and Punta Gorda Basins are restricted to one or the other of the 2 basins, removing the geographic barrier could cause strong shifts in ecological dynamics for the better part of Nicaragua's freshwater fauna.

Several nonnative species have been introduced to Lake Nicaragua, most notably tilapia (*Oreochromis niloticus*, *O. mossambicus*, and *O. aureus*) and devil fish (*Hypostomus panamensis*). Tilapia have been listed as one of the most invasive groups of species in the world (Lowe et al. 2000), and their occurrence in Lake Nicaragua is correlated with a decrease in abundance of native cichlid fishes (McKaye et al. 1995). Similarly, based on accounts of local fishers, the occurrence of the devil fish is accompanied by a decrease in abundance of native species, especially of the commercially important guapote (*Parachromis managuensis*). However, the ecological impacts of this species have not been investigated formally. All the aforementioned species appear to be highly invasive in Nicaragua and have spread across the entire San Juan Basin within a few years (McKaye et al. 1995, personal observation) but have not been reported in the Punta Gorda Basin to date. Hence, the construction of the canal will facilitate colonization by removing biogeographic barriers (Rahel 2002), most likely posing a threat to the local fauna.

Hybridization between Genetically Distinct Populations

Contact between genetically distinct populations could lead to (intra- and interspecific) hybridization and cause genetic homogenization of previously differentiated gene pools (Olden et al. 2004). This would manifest as a decrease in allelic composition, an increase in similarity of allelic frequencies, and possibly compromised local adaptation (Storfer 1999). We detected high levels of differentiation among drainage basins for taxonomically and ecologically diverse species. Assuming our results are generally applicable across a broad taxonomic range, populations from distinct basins may represent independent evolutionary significant units (Fraser & Bernatchez 2001). However, populations within the same drainage basin showed no significant genetic differentiation (Figs. 3d–f), even across large geographic distances of up to 180 km (e.g., from Lake Managua to El Tule or from Río Las Lajas to El Castillo). These results suggest that connecting the two basins will most likely be accompanied by genetic homogenization for multiple species in the Nicaragua Canal zone. At least 27 fish species occur in both basins (Supporting Information), which illustrates the large scale on which unique genetic diversity may be lost across Nicaragua's freshwater fauna.

Political Issues Concerning the Nicaragua Canal

Unfortunately, environmental concerns are commonly not addressed properly in the course of political decision making. There are many cases of artificial waterways connecting distinct drainage basins in which associated risks for biodiversity were not taken into account (Zhan et al. 2015). For instance, the Brazilian government plans to transfer water between isolated river basins to combat the effects of a severe drought without accounting for environmental consequences (Vitule et al. 2015). Nicaraguan freshwater biodiversity will face serious threats once construction of the canal begins. The concession to build and operate the canal was given to the infrastructure development firm HKND by the Nicaraguan government without competitive bidding. Moreover, the environmental consultancy ERM was commissioned by HKND to conduct the environmental and social impact assessment (ESIA) only after the concession was already granted. In general, the ESIA is not comprehensive. It lacks, for example, data on stratigraphy of the lake and a seismic risk assessment and does not address water quality. These problems have been pointed out by a panel of Nicaraguan and international scientists who participated in a workshop in Managua organized by the Academy of Sciences of Nicaragua in 2015 (Huete-Pérez et al. 2016). The ESIA also does not properly account for effects of the canal on distinct localized freshwater faunas in the 2 affected drainage basins or the detrimental effects of biotic homogenization for these regions. The threats this project poses to multiple ecosystems and their freshwater biodiversity are profound and their effects will be irreversible. Thus, a sustainable management concept should be implemented. The associated effects of the canal on biodiversity remain unclear. Hence, the precautionary principle should be applied; the burden of proof that an action is not harmful to the environment is on those taking action. This concept is endorsed in the UN World Charter for Nature (United Nations 1982) and calls are being made for its application to become common practice internationally, especially in the context of comparable mega projects. However, its efficacy to counteract the current loss of biodiversity and its proper incorporation into political decision making remain controversial (Myers 1993; Cooney 2004; Kanongdate et al. 2012).

We found that populations from distinct drainage basins were genetically differentiated and that species assemblages differed substantially among basins. These results highlight the risk of genetic homogenization and biotic invasion associated with the Nicaragua Canal. Breaking down the geographic barrier between the Punta Gorda and San Juan Basins, as the canal would, is worrisome from a conservation standpoint and may have drastic consequences for Nicaragua's freshwater fauna, that is, loss of unique genetic diversity on a large scale. The canal will surely imperil the status quo ante. However, due to the lack of knowledge of the biogeography of species and phylogeographic distribution of genetic variation within the Nicaragua Canal zone, it is currently impossible to adequately predict consequences of the canal on Nicaragua's biodiversity. Therefore, it is of great urgency to determine which endemic species or genetically unique populations may be threatened by its construction. Hence, we call for more thorough and independent investigations of biodiversity in the ecosystems affected by the Nicaragua Canal. Otherwise, many more, putatively still unknown, species might be threatened by extinction.

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Supporting Information

Numbers of described Cichlidae, Characidae, and Poeciliidae species; ecology and reproductive features of our study species; sample sizes and sampling locations; *cytochrome b* primers; GenBank accession numbers of

Poecilia spp.; a list of species occurring in Punta Gorda and San Juan Basins; F_{ST} values among populations; nucleotide and haplotype diversity (Appendix S1); pictures of our study species; and a full phylogeny of the *Poecilia sphenops* species complex (Appendix S2) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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