An Indo-Pacific damselfish in the Gulf of Mexico: origin and mode of introduction.

D Ross Robertson[1], Omar Dominguez-Dominguez[2,3], Benjamin Victor[4], Nuno Simoes[5-7]

1. Smithsonian Tropical Research Institute, Balboa, Republic of Panamá

2. Laboratorio de Biología Acuática, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mujica S/N, Morelia Michoacán, 58030, México.

3. Laboratorio Nacional de Análisis y Síntesis Ecológica para la Conservación de Recursos Genéticos de México, Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, Apartado Postal 27-3 (Xangari), 58089, Morelia, Michoacán, México.

4. Guy Harvey Research Institute, Nova Southeastern University, Ft Lauderdale, Florida 33314, USA

5. UMDI-Sisal, Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal 97355, Yucatán, México

6. Laboratorio Nacional de Resiliencia Costera, Unidad Académica de Yucatán, Universidad Nacional Autónoma de México, Sisal, 97355, Yucatán, México

7. International Chair for Coastal and Marine Studies in Mexico, Harte Research Institute for Gulf of Mexico Studies, Texas A&M University-Corpus Christi, 6300 Ocean Drive, Unit 5869 Corpus Christi, Texas 78412

Corresponding Author: D Ross Robertson, drr@stri.org

ABSTRACT

The Indo-west Pacific (IWP) coral-reef damselfish *Neopomacentrus cyanomos* is well established across the southwest Gulf of Mexico (SwGoMx). Comparisons of mtDNA sequences of the SwGoMx population with those from conspecifics from 16 sites scattered across its native range show that the SwGoMx population is derived from two of four native lineages: one from the north-west Pacific Ocean, the other from the northern Indian Ocean. Three hypotheses address how this species was introduced to the SwGoMX: (1) aquarium release; (2) borne by cargo-ship; and (3) carried by offshore petroleum platform (petro-platform). The first is unlikely because this species rarely features in the aquarium trade, and "*N. cyanomos*" traded to the USA from the sole IWP source we are aware of are a misidentified congener, *N. taeniurus*. The second hypothesis is unlikely because shipping has not been associated with the introduction of alien damselfishes, there is little international shipping between the IWP and the SwGoMx, and voyages between those areas would be lengthy and along environmentally unfavorable routes. Various lines of evidence support the third hypothesis: (i) bio-fouled petro-platforms represent artificial reefs that can sustain

large and diverse populations of tropical reef-fishes, including *N. cyanomos* in the SwGoMx; (ii) relocation of such platforms has been implicated in trans-ocean introductions leading to establishment of non-native populations of such fishes; and (iii) genetic characteristics of the SwGoMx population indicate that it was established by a large and diverse group of founders drawn from the regions where many SwGoMx petro-platforms originated.

INTRODUCTION

The damselfish *Neopomacentrus cyanomos* (Bleeker,1856), which is native to coral reefs of the tropical Indian Ocean and West-Pacific Ocean (Allen, 1991), was first recorded in the southwest Gulf of Mexico (SwGoMx) in mid-2013 (González-Gándara & Cruz-Francisco, 2014). However, by then it was common on both coastal and offshore reefs dispersed across at least 350 km of that area (Robertson et al., 2016 a). Since then, this species has been found across most of the SwGoMx, and, most recently, in both the northwest and northeast Gulf of Mexico (USGS, 2017, accessed October 10, 2017). After the lionfish (*Pterois volitans*), this is only the second species of Indo-west Pacific coral-reef fish to have established a substantial population across a large area of the tropical Greater Caribbean.

Kommentar [Reviewer1]: There are two species of lionfish that invaded the Gulf of Mexico, *Pterois volitans* and *Pterois miles* (Côté et al. 2013). Thus, *N. cyanomos* would be the third coral-reef fish of Indo-Pacific to invade the Gulf of Mexico.
- Côté IM, Green SJ, Hixon MA. 2013. Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. *Biological Conservation* **164**, 50-61.

This paper deals with two questions: (i) from where in its native range did the SWGoMx population of *N. cyanomos* originate, and (ii) by what means were the fish that established that population transported to the Gulf of Mexico.

Kommentar [Reviewer2]: This paragraph should be the last of introduction section.

To address these two questions we compared DNA sequences of the mitochondrial DNA cytochrome c oxidase I gene (CO1 sequences) to assess relationships among three groups of fish: (i) aquarium-trade fish purchased in the USA that were supposedly ~~said to~~ be “*N. cyanomos”* shipped ~~there~~ from the Philippines; (ii) a large group of individuals of *N. cyanomos* collected from two reefs in the SwGoMx~~,~~; and (iii) samples of *N. cyanomos* collected at 16 sites scattered across most of its native range in~~,~~ Indo-west Pacific ~~range~~.

We used ~~these~~ genetic data to assess ~~the following predictions relating to~~ three hypotheses ~~hypothetized modes~~ by which marine fishes have been transported by long distances to ~~well~~ outside their native ranges (see González-Gándara & Cruz-Francisco, 2014; Robertson et al., 2016 b): (1) release of aquarium specimens; (2) carriage by cargo-ship, on the hull or in ballast water; and (3) carriage by marine platforms used for exploration and production of offshore oil and gas ("petro-platforms"). We predicted that if the *N. cyanomos* population in the SWGoMx was established by aquarium release, then individuals from that area should match genetically to those supplied by the aquarium trade from the Philippines (apparently the sole source of fish labelled as this species for the US trade to the Americas during the early 2000s, the only period for which published data are available, see https://www.aquariumtradedata.org/, accessed July 15, 2017). Since it is likely that an aquarium release of an obscure species such as *N. cyanomos*, a minor component in the international fish trade (see discussion), would involve a relatively small number of individuals, then the *N. cyanomos* population in the SWGoMx should have low genetic diversity and show evidence of having passed through a genetic bottleneck due to its expansion from a small number of founders. Similarly, if the introduction were from transport by cargo-ship, a method not conducive to transport of large numbers of individual fishes in a single event (see discussion), we predicted a SWGoMx population with genetic characteristics essentially the same as those for a population established by aquarium release, i.e. low genetic diversity, with evidence of having passed through a genetic bottleneck/founder effect as the population expanded from a few founders (referece). In contrast, we predicted that transfer by petro-platforms

Kommentar [Reviewer3]: You should include some examples of biological introductions caused by aquarium trade, as the lion fish.

Kommentar [Reviewer4]: Suggested references to reinforce your null expectation.
- Seixas VC, Zanol J, Magalhães WF, Paiva PC. 2017. Genetic diversity of *Timarete punctata* (Annelida: Cirratulidae): Detection of pseudo-cryptic species and a potential biological invader. *Estuarine, Coastal and Shelf Science* **197**, 214-220.
- Chandler EA, McDowell JR, Graves JE. 2008. Genetically monomorphic invasive populations of the rapa whelk, *Rapana venosa*. *Molecular Ecology* **17**, 4079-4091.

would produce a markedly different genetic profile in the GoMx population. These platforms can host substantial populations of a range of species of tropical reef-fishes (reference) (see discussion), and at least one small platform in the SWGoMx supports a population of many thousands of *N. cyanomos* (Simoes & Robertson, 2016). Hence a platform being transported from the IWP potentially could carry a substantial number of individuals representing a cross section of the population(s) of *N. cyanomos* from the IWP site(s) where it had been constructed or stationed before arriving in the GoMx. A GoMx population that was genetically diverse and showed no signs of having passed through a genetic bottleneck due to small founder population size would be consistent with such a mode of introduction. In addition, that GoMx population should be most closely related to an IWP population(s) in an area(s) that contain offshore oilfields and/or where petro-platforms were constructed or stationed before being moved to the SwGoMx.

~~METHODS~~Material & Methods

Kommentar [Reviewer5]: Change to stay in accordance with the author intructions.

The native range of *N. cyanomos* extends from the Red Sea and the Persian Gulf to south-east Africa, and eastward to Japan, the Philippines, the Solomon Islands and New Caledonia in the West-Pacific. (Allen, 1991; and see https://www.gbif.org/species/2398535, accessed September 20 2017). The range of *N. cyanomos* encompasses most of the range of the entire genus, which extends only further southeast, to the south central Pacific (Allen, 1991; and see https://www.gbif.org/species/2398526, accessed September 20 2017). No members of the genus occur naturally in the Atlantic Ocean (Allen, 1991), and none are known from the Mediterranean Sea, either naturally (Allen, 1991) or as a result of introduction (Arndt & Schembri, 2015).

The specimens used for DNA sequencing in our analysis comprised nine aquarium-trade fish purchased in the USA that were identified by the US wholesale dealers as "*N. cyanomos*" ~~originating~~ from the Philippines~~;~~ (eight from a single group ~~purchased from a dealer by BV~~ in Los Angeles, California, and one ~~from a dealer~~ in New York, New York). In addition, 54 documented *N. cyanomos* (many identified by GR Allen), that had been collected by a variety of researchers, including some contributors to the Fish Barcode of Life project (Fish-BOL), at 16 sites (1-8 fish per site) distributed ~~scattered across~~ over most of the native range in the Indo-west Pacific ~~range~~ were also sequenced (Table S1). These sequences were compared to ~~sequences of~~ 65 sequences of ~~individuals of~~ *N. cyanomos* individuals ~~collected~~ from the non-native population ~~on Campeche Bank,~~ in the SwGoMx (Campeche Bank)~~using clove-oil anaesthetic~~, two from Madagascar reef in 2015 (see Robertson et al., 2016 b) and 63 ~~of them~~ from Cayo Arcas reef collected in 2016. After collection those 65 fish were euthanized with an overdose of clove-oil anaesthetic and preserved in 95% ethanol.

Kommentar [Reviewer6]: This paragraph should be transfer to the Introduction Section.

Kommentar [Reviewer7]: What is BV? Benjamin Victor? You do not need specify who bought the fishes.

Kommentar [Reviewer8]: This phase is confuse. Considering rewritten. In the first time I didn't understanding if you sequenced this or downloaded from Fish-BOL database.

DNA sequences

Total genomic DNA was isolated with the Qiagen BioSprint Dneasy Tissue and Blood Kit (Qiagen, Valencia, CA, USA), following the manufacturer’s protocol. A fragment of 594 bp of cytochrome oxidase subunit I (COI~~: 594 pb~~) was amplified using the primers Fish-F1 and Fish-R1 ~~following ~~(Ward et al.,~~ (~~2005). Polymerase chain reactions (PCRs) were performed in a volume of 12.5 µl containing 4.25 µl ultrapure water, 0.5 µl of each 0.2 µM primer, 6.25 µl Dream Taq Green PCR Master Mix 2x (Thermo Scientific), and 1 µl (ca. 10-100 ng) of DNA template. The PCR products were purified using ExoSAP-IT (USB Corp.) and sequenced in~~submitted to~~ Macrogen Inc. and in~~ for sequencing. The native populations of *N. cyanomos* and the aquarium-trade specimens were sequenced at~~ The Biodiversity Institute of Ontario, University of Guelph, Canada. A 652-bp segment was amplified from the 5′ -region of the mitochondrial cytochrome c oxidase (COI) gene using a variety of primers (Ivanova et al., 2007). DNA extractions were performed with the NucleoSpin96 (Machery-Nagel) kit according to manufacturer’s specifications under automation with a Biomek NX liquid-handling station (Beckman-Coulter) equipped with a filtration manifold. PCR amplifications were performed on 12.5 µl volumes including 6.25 µl of 10% trehalose, 2 µl of ~~ultra ~~ultra-pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM $(NH_4)_2SO_4$, 20mM Tris-HCl (pH8.8), 2mM $MgSO_4$, 0.1% Triton X-100), 0.625 µl of $MgCl_2$ (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM), 0.0625 µl of Taq DNA polymerase (New England Biolabs), and 2 µl of template DNA. The PCR conditions consisted of 94°C for two min., 35 cycles of 94°C for 30 s~~ec~~., 52°C for

Kommentar [Reviewer9]: I don’t understanding. You used two regions of COI gene? Why you have a fragment of 594 and other of 652?

Kommentar [Reviewer10]: Again? Structure your methodology in parts, first put all DNA extractions protocols used, after inform the genes and primers used to amplify the regions follow by concentrations of PCR and thermocycler conditions. In last were the PCR products were sequenced.

40 s~~ec~~., and 72°C for one min., with a final extension at 72°C for 10 min. Specimen information and barcode sequence data for these specimens were compiled using the Barcode of Life Data Systems (Ratnasingham & Hebert, 2007; Ward, Hanner & Herbert, 2009).

Phylogenetic analysis and haplotype network reconstruction

Nucleotide sequences were edited and manually aligned in Mega v.6.06 (Tamura et al., 2013) and examined with a chromatogram. Maximum likelihood (ML) analysis was conducted with RAxMLGUI v. 1.3.1 (Stamatakis, 2006; Silvestro & Michalak, 2012), performing 10,000 bootstrap repetitions and using the evolutionary substitution model estimated with the ~~General Time-Reversible model (Tavaré, 1986) + gamma (~~GTR+G~~),~~ model, as obtained in jModelTest (Posada, 2008) according to . *N. azysron* and *N. sororius* (Genbank # KP194962.1, and BOLD # PCOM166-10, respectively) were used as out-groups. The haplotype network was constructed using PopART (Leigh & Bryant, 2015) ~~(available at htt://popart.otago.ac.nz)~~ and applying the Median-Joining method (Bandelt, Forster & Rohl, ~~2000~~1999).

Historical demography

To detect signatures of demographic changes in populations ~~of the two lineages of~~ *N. cyanomos* ~~found in the SWGoMx (those belonging to groups 1 and 4 in the haplotype network of Figures 1 & 2)~~, Tajima´s D (Tajima, 1989) and Fu´s Fs (Fu y Li, 1993) neutrality tests were calculated, with significance levels set at $\alpha=0.05$ and 10,000 random permutations as implemented in ARLEQUIN 3.5.1.2 (Excoffier and Lincher, 2010). Evidence of an expanding population of either lineage would be assumed if significant negative values of Tajima's D and Fu's F-

Kommentar [Reviewer11]: Please provide the parameters used.

Kommentar [Reviewer12]: Which criteria you used to choice the best substitution model, AIC, BIC, …?

Kommentar [Reviewer13]: When starting a phase with the species name not abbreviate.

Kommentar [Reviewer14]: Check the website to the entire reference.

Kommentar [Reviewer15]: Why not include a Bayesian Skyline Plot to investigate historical demographic changes? It is less speculative than neutrality tests. You can use the package BEAST/BEAuTi to do this.

Kommentar [Reviewer16]: Do not enter in Results section.

statistics were obtained (Mousset et al., 2004). Additionally, a pairwise mismatch distribution was computed. Populations that have been historically stable are predicted to have multimodal mismatch distributions, whereas those that have undergone a recent expansion are predicted to be unimodal (Slatkin & Hudson, 1991). To test if the observed mismatch distributions predicted a stable or an expanding population model, differences between observed and expected distributions were evaluated using the Harpending's raggedness index (raggedness) (Harppending et al., 1993) and the R2 statistic (Ramos-Onsins & Rozas, 2002).

Kommentar [Reviewer17]: Also with ARLEQUIN?

RESULTS

We obtained 128 ~~mtDNA~~ COI sequences of *N. cyanomos*, ~~from~~ 54 ~~specimens of *N. cyanomos*~~ from 16 sites over its ~~scattered across this species~~' native range, nine ~~and~~ from ~~9 fish provided by~~ two aquarium-trade shops~~dealers~~ in the USA~~,~~ (identified as "*N. cyanomos*" ~~shipped~~ from ~~the~~ Philippines) and 65 from~~.~~ ~~For comparison, we sequenced 65 specimens of *N. cyanomos* collected on~~ two reefs in the SwGoMx. The haplotype network for the entire set of specimens is presented in Figure 1, and the locations from which individuals in the different haplotype groups were obtained are shown in Figure 2.

Kommentar [Reviewer18]: First you can provide the total number of sequences obtained and after specify the number of sequences.

Include a table with the genetic diversity parameters and add the number of sequences by each group of specimens in this table. This will make the visualization of your dataset clearer.

The aquarium-trade specimens, supposedly, from the Philippines are ~~only~~ distantly related to all *N. cyanomos* sequenced here~~in the study~~ and were identified morphologically ~~(by BV)~~ and genetically as ~~a different species,~~ *Neopomacentrus taeniurus* (Bleeker, 1856), which is native to brackish non-reef environments and

Kommentar [Reviewer19]: You can't completely trust in the information of the aquarium-trade shops.

Kommentar [Reviewer20]: Just mentioned the researcher who identified the samples if he/she is not one of the authors.

widespread distributed in the Indo-West Pacific Ocean (Allen, 1991). Their mtDNA lineage is separated by 80-89 mutations from all four haplotype groups of ~~native-range~~ *N. cyanomos*, from native and non-native sites~~as well as from the SwGoMx fish~~ (~~Figure~~ Fig. 1).

Kommentar [Reviewer21]: I think it would be interesting include a separated figure with the ML tree. But this is a minor detail.

All native-range specimens of *N. cyanomos* belong to four closely related haplotype groups that included a total of 23 haplotypes, 12 of those from groups 1 and 4 (18 of 54 individuals; ~~Figures~~ Fig. 1 & 2). The 65 SwGoMx specimens included 11 haplotypes, four of which were shared with native-range specimens, all members of groups 1 and 4 (see Figure 1). Genetic diversity was high among the native-range specimens, with haplotype diversity 0.913 and nucleotide diversity 0.0468. Within native-range Groups 1 & 4, haplotype diversity was high (0.942) and nucleotide diversity lower (0.0168), and a similar pattern occurred within the SwGoMx specimens (haplotype diversity 0.823; nucleotide diversity 0.0183).

Kommentar [Reviewer22]: What means the numbers indicate by arrows in the Fig. 2?

Kommentar [Reviewer23]: Provide the program used to calculate these parameters.

Kommentar [Reviewer24]: It be interesting provide genetic diversity parameters as the number of haplotypes, haplotype diversity and nucleotide diversity in a table. In addition you can add in this table the values of neutrality test and mismatch distribution.

The SwGoMx population comprises roughly equal numbers of individuals drawn from the two haplotype groups, 1 ~~&~~ and 4, which are largely geographically separated in the native range, with group 1 restricted to the northern Indian Ocean and group 4 found in samples from Indonesia and Taiwan in the north-west Pacific Ocean (~~Figure~~ Fig. 2). The only exception to this pattern of geographic separation is two individuals of group 4 that were collected at Gujarat, in India, at the same location as one individual of group 1.

The results of the neutrality tests for the SwGoMx haplotype groups showed that the Tajima's D and Fu's Fs values were not statistically significant for either group 1 (Tajima's D = -0,082, $p>0.05$ and Fu's Fs 1.05, $p>0.05$) or group 4 (Tajima's D = -1.08, $p>0.05$ and Fu's Fs -1.93, $p>0.05$). Each of those groups showed a multimodal mismatch distribution, with non-significant results in both Harpending's raggedness index and the R2 statistic ($p > 0.05$ in both cases).

DISCUSSION

The analysis of mtDNA sequences indicate that the SwGoMx population of *N. cyanomos*, is unrelated to the aquarium-trade fish from the Philippines, which represent a different species, *N. taeniurus*. Most significantly, the SwGoMx population is derived from two distinct genetic lineages found in the native range, is genetically diverse, and shows no signs of having passed through a genetic bottleneck. Lastly, the SwGoMx population is derived from a combination of a lineage found in the northern Indian Ocean and another from two widely separated locations in the northwest Pacific Ocean (Taiwan and Indonesia). These results permit an evaluation of alternative hypotheses on the origin and mode of introduction of this non-native species into the Gulf of Mexico.

Kommentar [Reviewer25]: You do not test historical demography changes considering only SwGoMx sequences. So you can't say that the SWGoMx no passed through a genetic bottleneck.

Aquarium-Release Hypothesis*:*

The aquarium-trade specimens sold as "*N. cyanomos"* proved to belong to a different species, *N. taeniurus*. As part of a separate phylogeographic study of the genus, ~~BV~~ has obtained mtDNA COI sequences of specimens of all 15 nominal species of *Neopomacentrus* that were collected in the field and identified by experts in damselfish taxonomy. The mtDNA sequences of the Philippines aquarium-trade specimens are nested within a cluster of barcodes of *N. taeniurus*

Kommentar [Reviewer26]: Use unpublished data or in prep. BV is also an author of this article.

(~~BV~~ unpublished data). In addition, a morphological examination confirms this identification; species of *Neopomacentrus* fall into two morphological groups, one (including *N. cyanomos*) with the lower edge of the suborbital bone hidden under a covering of scales, and the other (including *N. taeniurus*) in which the suborbital margin is exposed and not covered by scales. Specimens from the SwGoMx ~~(examined by DRR)~~ have a hidden suborbital margin, while the aquarium-trade specimens of "*N. cyanomos*" ~~(examined by BV)~~ have exposed suborbitals. *N taeniurus* has a similar ~~color~~colour pattern to, and can be easily mistaken for *N. cyanomos.*

Aquarium-trade data presented by Rhyne et al. (2015) indicate that "*N. cyanomos",* which is not brightly ~~colored~~coloured like many desirable aquarium species, is a very small component of the aquarium trade in damselfishes to the USA (not sold in sufficient numbers to be represented among the top 20 species of damselfishes), and that the Philippines apparently is the sole native-range source for that trade to the USA. *Neopomacentrus cyanomos* is known to occur in the Philippines, but whether or not any aquarium-trade imports from there actually are that species remains uncertain. Furthermore, it is not clear which mitochondrial lineage(s) of *N. cyanomos* is present in the Philippines, given the proximity of that Archipelago not only of the Group 4 lineage (one of the SWGoMx lineages), but also the Group 2 lineage at western Irian Jaya (~~Figure~~ Fig. 1), which is not. Even if the Group 4 lineage that is present in the SwGoMx were exported in the aquarium trade to the US, the northern Indian Ocean lineage (Group 1) that forms a major part of the SwGoMx population would not be expected to be part of the trade.

While keeping marine aquarium-fish is popular in the US, that is not the case in Mexico (~~NS~~ Pers. Obs.), where *N. cyanomos* was first recorded here. Any individuals of this~~at~~ species potentially obtained by aquarists in Mexico likely would have been derived from US wholesalers, the main global recipient in the aquarium trade. Almost all recorded aquarium-trade releases of exotic marine fish in the vicinity of the GoMx have occurred in southeast Florida, where many such species are known (Semmens et al., 2004), and *N. cyanomos* would be expected to have established there first if they were derived from aquarium release. However, southeast Florida hosts an abundance of divers, and is intensively monitored by the REEF organization (www.reef.org) for sightings of exotic species. REEF was made aware of the presence of *N. cyanomos* in the Gulf of Mexico by the publication of González-Gándara & Cruz-Francisco (2014). To date, there remain no records of *N. cyanomos* in south Florida (USGS, 2017, accessed October 9 2017).

The genetic data do not support the aquarium-release hypothesis. Both SwGoMx lineages of *N. cyanomos* are genetically diverse, and the results of both the neutrality tests and mismatch distributions suggest a stable population that did not pass through a genetic bottleneck, and hence was not established by a few founders, i.e. by a few fish released from an aquarium.

Kommentar [Reviewer27]:

Kommentar [Reviewer28]: I suggest change the term genetic bottleneck to founder effect. Note, for an invasive species is expected to find a population that was found recently and not a population that suffer a genetic bottleneck (that is frequently associate to a population decrease). In the neutrality tests and mismatch distribution the same signal can be interpreted as a genetic bottleneck or founder effect. In this case, would be expected find a founder effected (low genetic diversity). So in your case the term founder effect would be more appropriate. So, with a signal of founder effect was not found, how your results can be interpreted? Multiple invasions?

Cargo-ship carriage hypothesis:

Movements of cargo-shipping in the normal course of business have been implicated in the long distance transport of exotic marine fishes ~~well~~ beyond their native ranges, usually in ballast water (Wonham et al., 2000), but, potentially, also in sea-chests on the hulls of ships. Such ship transportation~~shipping~~ was proposed as the means of introduction of *N. cyanomos* into the SwGoMx ~~by ~~(González-Gándara & Cruz-Francisco,~~ (~~ 2014). Damselfishes are not included among the species thought to be introduced by ballast water (Wonham, Carlton & Ruiz, 2000) and none were found on the hulls of cargo ships examined in Brazil ~~by ~~(Ferriera, Goncalves & Coutinho~~ (~~, 2006). There are very few cases~~instances~~ of long-distance introductions reported to~~by~~ alien damselfishes. One of those evidently is due to natural range expansion in a species that associates with flotsam (Coleman et al., 2014). Another introduction (of a species that also associates with flotsam) from the Atlantic to the Mediterranean, has been attributed to ship transport “~~shipping~~” by Occhipinti-Ambrogi et al. (2011), without the presentation of any justification (see Tsadok et al., 2015 for resolution of the identity of this species; and see also Pajuelo et al., 2016, below).

Kommentar [Reviewer29]: Rafting dispersion? Is rafting considered as a natural range expansion? If is a natural range expansion such case can't be considered as a biological invasion (or introduction).
This phase contradicts the next.

Kommentar [Reviewer30]: Which species? Please give the species name or any information about which taxon you are referring to.

A modern cargo-ship, which typically will not have a heavily fouled hull, travelling at 20-25 knots on a voyage from the western Pacific (Hong Kong) to the SwGoMx via the Panama Canal would take ~19-23 days, and ~16-20 days from Northwest India via the Suez Canal (Calculated on http://sea-distances.org, accessed ~~September ~~Sep. 20, 2017), not counting stoppages at any ports during the voyage. Survivorship in ballast water is time-dependent for many marine organisms (~~see ~~Muirhead et al., 2015) and, in addition to the lengthy duration of a transit by either route, the Pacific route would pose additional environmental barriers for any tropical reef fishes that hitched a ride on the hull of a cargo-ship

Kommentar [Reviewer31]: What is this?

going to the Gulf of Mexico via that route. Most such traffic goes via California (see Figure S1), which has a colder temperate environment unsuitable for survival of coral-reef fishes. Second, passing through the Panama Canal (see Figure S1) exposes any hull-associated organisms to the freshwater lake that constitutes most of that canal, and represents an effective barrier to transfer of tropical reef-fishes between the oceans separated by the Isthmus of Panama (although a few euryhaline, non-reef fishes that live in brackish environments have made the transit; reviewed in Cohen, 2006; and see Robertson & Allen 2015; Robertson & Van Tassell 2015).

In addition, due to the fact that the Panama Canal represents a major global chokepoint for international shipping (see Figure S1), one would expect the first occurrence of marine reef-fishes transported across the Pacific in this manner to be on reefs adjacent to the Pacific end of the canal, where ships often stop before making a canal transit. However, there are no records of such occurrences (review in Cohen 2006; and see Robertson & Allen, 2015, Robertson & Van Tassell, 2015), at either end of the canal.

As in the aquarium-release hypothesis, the genetic data do not support this hypothesis, since both the SwGoMx lineages of *N. cyanomos* are genetically diverse, and the results of both the neutrality tests and mismatch distributions suggest a stable population that shows no signs of having passed through a genetic bottleneck and hence was not established by a few founders, i.e. by a few fish transported on the hull of a cargo ship.

~~Petro~~Oil-platform translocation hypothesis:

Offshore petro-platforms have been shown to support an abundance of reef fishes belonging to a broad variety of taxa, in the Gulf of Mexico (Hastings, Ogren & Mabry, 1976; Topolski & Szedlmayer, 2004; Ajemian et al., 2015), Brazil (Ferreira, Goncalves & Coutinho, 2006), California (Claisse et al., 2014, Martin & Lowe, 2010), the Mediterranean (Consoli et al., 2013), west Africa (Friedlander et al., 2015), Australia (Pradella et al., 2014), the Canary Islands (Pajuelo et al., 2016), and the Persian Gulf (Torquato et al., 2017). In the SwGoMx, we have observed *N. cyanomos* living on such platforms, including in superabundance on a relatively lightly-fouled platform near Cayo Arcas, in the southwest corner of Campeche Bank (up to 100 individuals m^{-2}; see video of Simoes & Robertson, 2016). That platform is one of several hundred platforms in the area of the greatest concentration of such structures in the SwGoMx (https://portal.cnih.cnh.gob.mx/iicnih/?lng=en_US, accessed September 20, 2017).

Translocated ~~petro~~oil-platforms have long been known to be vectors of long-distance transport of sessile fouling organisms (e.g. ~~see~~ Foster & Willan, 1979; Bax et al., 2003; Hopkins & Forrest 2010; Wanless et al., 2010; Yeo et al., 2010; Mineur et al., 2014). Recently, however, such translocation has been implicated in transoceanic transport of western Atlantic reef fishes to the Canary Islands, where a platform-servicing center has been established (Falcon et al., 2015; Pajuela et al., 2016), as well as to the Mediterranean (Dulcic & Dragicevic, 2013; Pajuela et al., 2016). Petro-platforms have also transported non-native fishes from Australia and the Red Sea through the Suez Canal to the Mediterranean Sea (Galil, 2008).

Some reef-fish species apparently carried to the Canary Islands in this way have established breeding populations there (Falcon et al., 2015; Pajuelo et al., 2016). Petro-platforms may help exotic reef-fishes establish in west Africa (Friedlander et al., 2014), and Pajuelo et al. (2016) suggested that translocated platforms may have carried eastern Atlantic reef-fishes to Brazil, where some have recently become established.

Among the exotic reef fishes newly recorded in the Canary Islands, one species identified by Falcon et al. (2015; see plate 4B) as the (naturally trans-Atlantic) damselfish, *Abudefduf taurus*, actually is most likely the Indo-west Pacific species *A. sordidus* (GR Allen pers. comm. 2016). Since *A. sordidus* is a dull-colored species that is not found in the aquarium trade (see Rhyne et al., 2015), this occurrence represents support for the hypothesis that Indo-west Pacific reef fishes are carried from their native ranges into the Atlantic by petro-platforms. Another example, in the Pacific ocean, reported by Myers & Donaldson (1996) and Myers (1999, p 183), is the damselfish *Neopomacentrus violascens* (Bleeker, 1848), which they proposed was transported to Guam, where it is not native, from the Philippines by a bio-fouled military barge. It has now established a population at Guam, living on bio-fouled mooring chains rather than on natural substrata.

Are there movements to the SwGoMx of petro-platforms from the two areas of the native range of *N. cyanomos* that contain the lineages of that species found in the SwGoMx? The website www.infield.com/rigs (accessed July 15, 2017) provides information on the construction sites of petro-platforms and drill ships found in different countries. A review of information on that site of the origins of such platforms and drill-ships showed that about 60% of those listed as being used in the area currently occupied by *N. cyanomos* in the SwGoMx were constructed at sites within the native ranges of the two lineages found in the SwGoMx (see Figure S2). These linkages represent only part of a global web of movements of relocated new and used petro-platforms and drill-ships within and between oceans that could result in establishment of exotic sessile and mobile reef organisms well beyond their native ranges (e.g. ~~see~~ Wanless et al., 2010; Yeo et al., 2010; Pajuelo et al., 2016).

The genetic structure of the SwGoMx population of *N. cyanomos* indicates it was established by two large and genetically diverse groups of founders, which did not go through a genetic bottleneck during the expansion of their populations in that area. These results support the hypothesis that a large number of fish were transported, which would be most consistent with the movement of one or more petro-platforms from the native range.

Formatiert: Hervorheben

How many introductions to the GoMx?

The fact that the SwGoMx population of *N. cyanomos* is derived from two lineages mostly confined to different oceans raises ~~the~~ questions of whether ~~that~~ population was derived from two well-separated sources and two separate introductions. While those lineages are largely allopatric, both are present at Gujarat, in northwestern India (~~see Figure~~ Fig. 2). This~~at~~ occurrence and the absence of ~~the~~ Pacific lineage (group 4 of figure 2) at Sri Lanka (in an admittedly small sample of fish), which is closer to the Pacific than Gujarat, suggests that the presence of group 4 at western India may not be natural. Is there evidence that indicates that oil-industry activity provides connections that could have carried group 4 fish from the Pacific to Gujarat?

There is a major active offshore-petroleum area immediately to the south of the broad Kathiawar ~~península~~peninsula of Gujarat that projects well into the Indian Ocean (e.g. ~~see~~ https://watchers.news/data/uploads/2012/04/Untitled1.png). Of 41 petro-platforms used in India that are listed by www.infield.com/rigs, (accessed July 15, 2017), 15 were constructed in Singapore and two in southeast China, near the Taiwan sample of the Group 4 lineage of *N. cyanomos*. Further, there is a major global shipbreaking site on either side of the Gujarat peninsula, one at Gaddani in Pakistan, ~360 km from Gujarat, the other at Alang, at the eastern base of that peninsula in India. A list of decommissioned vessels of various types that were sent to those two sites during 2015-2016 (provided by www.shipbreakingplatform.org) shows that decommissioned offshore platforms and support vessels do get taken from various locations within the north-west Pacific (Indonesia, Singapore, Vietnam, SE China) to both those shipbreaking yards. In addition, other types of decommissioned vessels also make similar last voyages. Decommissioned vessels transiting to a shipbreaker do not have clean hulls; rather, typically they are heavily bio-fouled (Davidson et al. 2008), which would facilitate long-distance transport of fishes such as *N. cyanomos.*

Thus there are interoceanic connections involving movements of petro-platforms, drill-ships, offshore support vessels, and decommissioned vessels of various types that could be responsible for the occurrence of both lineages in the sample from Gujarat. These findings support the hypothesis that the occurrence of *N. cyanomos* in the GoMx is due to a single introduction (the simplest explanation) rather than multiple introductions from different source locations.

Where in the GoMx was *N. cyanomos* initially introduced?

Neopomacentrus cyanomos was first recorded at Coatzacoalcos, in the southwest corner of the GoMx in July 2013 by González-Gándara & de la Cruz-Francisco (2014), who thought it had been introduced directly to that port from its native range. However, several years later, inspection of underwater photographs taken at Cayo Arcas reef, 350 km northwest of Coatzacoalcos, showed that it was present in significant numbers at both those sites at the same time in 2013 (see Robertson et al., 2016 a). Furthermore, this species was recorded in appreciable numbers on reefs near Veracruz, 200 km northwest from Coatzacoalcos and 450 km west from Cayo Arcas, in October 2014 (see Robertson et al., 2016 b). These observations indicate that *N. cyanomos* was already well established over a large area in the SwGoMx when it was first discovered. Since then, *N. cyanomos* has been found to be even more widely spread across the SwGoMx, and is evidently spreading eastwards on reefs on the Campeche Bank. In the latter half of 2017, it was found at widely separated sites in the northern GoMx, off Texas in the west and off the Florida Panhandle in the east (USGS 2017, accessed October 8 2017).

The occurrence of *N. cyanomos* at two widely separated sites at the time it was first discovered in the GoMx clearly demonstrates that the location at which a non-native species is first discovered cannot be equated with the location where it was first introduced. This issue is particularly applicable to *N. cyanomos*, since it is small, dully colored, and often mistaken for a native planktivorous damselfish, the Brown Chromis, *Chromis multilineata* (Guichenot, 1853), which it resembles in size, shape, and coloration. Distinguishing small juveniles of these two species in the field is more difficult than distinguishing adults. The ~~Brown~~ brown ~~Chromis~~ chromis is particularly abundant on reefs in the SwGoMx, where it is far more numerous than *N. cyanomos*. (~~DRR~~ Pers. Obs.) There, both species often co-occur in feeding aggregations that are predominantly composed of ~~Brown~~ brown ~~Chromis~~chromis. Small numbers of *N. cyanomos* sharing a reef with many Brown Chromis could easily be mistaken for Brown Chromis by a diver who is not familiar with both species and not actively searching for *N. cyanomos*.

Kommentar [Reviewer32]: Lower case

Given the difficulty in detecting the first introduction of *N. cyanomos* in the SwGoMx, the possibility that *N. cyanomos* was initially introduced to the northern GoMx rather than the southwest part cannot be ruled out. Inspection of data on www.infield.com/rigs (accessed September 2017) about the origins of petro-platforms used in the northern GoMx shows that about 1/3 of them were constructed at Singapore and China, but none in the Indian Ocean. Thus connections exist between the native range of *N. cyanomos* and both parts of the GoMx, although they are stronger with the SWGoMx, where there is direct evidence of Indian Ocean petro-platform origins. The fact that *N. cyanomos* seems to be much more abundant in the SwGoMx, even superabundant on petro-platforms there, suggests that it was initially introduced there.

As can be seen from the numbers of platforms dispatched to other countries from Singapore (www.infield.com/rigs), that city state is a major global player in the business, has produced the largest number of GoMx petro-platforms that originated in the Indo-West Pacific, and is a site for servicing of old platforms coming from various areas, including the Atlantic. Documenting which lineage(s) of *N. cyanomos* occur in Singapore would help evaluation of the potential for Singapore as a source population for the Gulf of Mexico population of this damselfish. A substantial set of DNA sequence data is also needed from Gujarat, to indicate the proportional abundances of group 1 and 4 lineages in the population there, and to show whether group 4 haplotypes present there are shared with west Pacific sites. In addition, barcode data on confirmed *N. cyanomos* from the Philippines would clarify the extent to which *N. cyanomos* is actually involved in the aquarium trade to the Americas.

CONCLUSIONS

While there are no solid data consistent with either the aquarium-release or cargo-ship transport hypotheses, various lines of evidence support the petro-platform translocation hypothesis: petro-platforms can provide habitat for substantial populations of a broad variety of tropical reef fishes; *N. cyanomos* can live in a dense population on a lightly fouled petro-platform; petro-platforms are regularly constructed within the parts of the native range of *N. cyanomos* where the SwGoMx lineages occur, and transported from there to the SwGoMx; translocation of such platforms has been implicated as vectors of long-distance transport of tropical reef-fishes, sometimes in sufficient numbers to allow them to establish breeding populations in non-native areas; *Neopomacentrus* species apparently can be transported long distances by bio-fouled structures in sufficient numbers to establish populations outside their native ranges; and, finally, the genetic structure of the SwGoMx population of *N. cyanomos* indicates it is derived from a large and genetically diverse population of founders, consistent with large numbers of fish being transported by a petro-platform. *Neopomacentrus cyanomos* has characteristics well suited to petro-platform transport: it is small (maximum length ~10cm), can feed on the plankton stream produced by a slow-moving platform (1-6 kts; see Yeo *et al* 2010), and can live in high-density aggregations on small amounts of relatively low-profile bio-fouling cover.

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