An Indo-Pacific damselfish (*Neopomacentrus cyanomos*) in the Gulf of Mexico: origin and mode of introduction

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The Indo-West Pacific (IWP) coral-reef damselfish *Neopomacentrus cyanomos* is well established across the south-west Gulf of Mexico (SwGoMx). Comparisons of mtDNA sequences of the SwGoMx population with those from conspecifics from 16 sites scattered across its native geographic 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-oceanic 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 IWP regions where many petro-platforms currently in the SwGoMx and other Atlantic offshore oilfields originated.

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31 **ABSTRACT**

32   The Indo-West Pacific (IWP) coral-reef damselfish *Neopomacentrus cyanomos* is

33 well established across the south-west Gulf of Mexico (SwGoMx). Comparisons of

34 mtDNA sequences of the SwGoMx population with those from conspecifics from 16

35 sites scattered across its native geographic range show that the SwGoMx population is

36 derived from two of four native lineages: one from the north-west Pacific Ocean, the

37 other from the northern Indian Ocean. Three hypotheses address how this species was

38 introduced to the SwGoMX: (1) aquarium release; (2) borne by cargo-ship; and (3)

39 carried by offshore petroleum platform (petro-platform). The first is unlikely because this

40 species rarely features in the aquarium trade, and "*N. cyanomos*" traded to the USA

41 from the sole IWP source we are aware of are a misidentified congener, *N. taeniurus*.

42 The second hypothesis is unlikely because shipping has not been associated with the

43 introduction of alien damselfishes, there is little international shipping between the IWP

44 and the SwGoMx, and voyages between those areas would be lengthy and along

45 environmentally unfavorable routes. Various lines of evidence support the third

46 hypothesis: (i) bio-fouled petro-platforms represent artificial reefs that can sustain large

47 and diverse populations of tropical reef-fishes, including *N. cyanomos* in the SwGoMx;

48   (ii) relocation of such platforms has been implicated in trans-oceanic introductions

49   leading to establishment of non-native populations of such fishes; and (iii) genetic

50   characteristics of the SwGoMx population indicate that it was established by a large and

51   diverse group of founders drawn from the IWP regions where many petro-platforms

52   currently in the SwGoMx and other Atlantic offshore oilfields originated.

53   **INTRODUCTION**

54   The damselfish *Neopomacentrus cyanomos* (Bleeker,1856), which is native to

55   coral reefs of the tropical Indo-West Pacific (IWP) (Allen, 1991), was first recorded in the

56   south-west Gulf of Mexico (SwGoMx) in mid-2013 (González-Gándara & Cruz-

57   Francisco, 2014). However, by then it was common on both coastal and offshore reefs

58   dispersed across at least 350 km of that area (Robertson et al., 2016 a). Since then, this

59   species has been found across most of the SwGoMx, and, most recently, on petro-

60   platforms, artificial- and natural reefs in both the north-west and north-east Gulf of

61   Mexico (Schofield, 2017). After the lionfish (*Pterois volitans*), which arguably represents

62   a single species in the west Atlantic (cf. Wilcox et al., 2017), *N. cyanomos* is only the

63   second species of IWP coral-reef fish to have established a substantial population

64   across a large area of the tropical Greater Caribbean. This paper deals with two

65   questions: (i) from where in its native range did the SwGoMx population of *N. cyanomos*

66   originate, and (ii) by what means were the fish that established that population

67   transported to the Gulf of Mexico.

68   To address these two questions we compared DNA sequences of the

69   mitochondrial DNA cytochrome c oxidase I gene (CO1 sequences) to assess

70 relationships among three groups of fish: (i) aquarium-trade fish purchased in the USA

71 that were supposedly "*N. cyanomos*" shipped from the Philippines; (ii) a large group of

72 individuals of *N. cyanomos* we collected from two reefs in the SwGoMx; and (iii)

73 samples of *N. cyanomos* collected at 16 sites scattered across most of its native range

74 in the IWP

75   We used these genetic data to assess the following predictions relating to three

76 hypothesized modes by which tropical reef-fishes have been transported long distances

77 to sites well outside their native ranges: (1) release of aquarium specimens (Semmens

78 et al., 2004; Schofield et al., 2009); (2) carriage by cargo-ship, on the hull or in ballast

79 water (González-Gándara & Cruz-Francisco, 2014); and (3) carriage by marine

80 platforms used for exploration and production of offshore oil and gas ("petro-platforms")

81 (Robertson et al., 2016 b). We predicted that if the *N. cyanomos* population in the

82 SwGoMx was established by aquarium release, then individuals from the SwGoMx

83 should match genetically to those supplied by the aquarium trade from the Philippines

84 (apparently the sole source of fish labelled as this species for the US trade to the

85 Americas during the early 2000s, the only period for which published data are available;

86 https://www.aquariumtradedata.org/, accessed July 15, 2017). Since it is likely that an

87 aquarium release of an obscure species such as *N. cyanomos*, a minor component in

88 the international fish trade, would involve a relatively small number of individuals, then

89 the *N. cyanomos* population in the SwGoMx should have low genetic diversity and show

90 evidence of having passed through a genetic bottleneck due to its expansion from a

91 small number of founders (a founder effect). Similarly, if the introduction were from

92 transport by cargo-ship, a method not conducive to transport of large numbers of

93   individual fishes in a single event, we predicted a SwGoMx population with genetic

94   characteristics essentially the same as those for a population established by aquarium

95   release, i.e. low genetic diversity, with evidence of a founder effect (Bernardi et al.,

96   2010; Seixas et. al., 2017). In contrast, we predicted that transfer by petro-platforms

97   would produce a markedly different genetic profile in the GoMx population. These

98   platforms can host substantial populations of a range of species of tropical reef-fishes

99   (Hastings, Ogren & Mabry, 1976; Ferreira, Goncalves & Coutinho, 2006; Friedlander et

100 al., 2015; and Pradella et al., 2014), and at least one small platform in the SwGoMx

101 supports a high-density population of many tens of thousands of *N. cyanomos* (Simoes

102 & Robertson, 2016). Hence a platform being transported from the IWP potentially could

103 carry a substantial number of individuals representing a cross section of the

104 population(s) of *N. cyanomos* from the IWP site(s) where it had been constructed or

105 stationed before arriving in the GoMx. A GoMx population that was genetically diverse

106 and showed no signs of having passed through a genetic bottleneck due to small

107 founder-population size would be consistent with such a mode of introduction. In

108 addition, that GoMx population should be most closely related to an IWP population(s)

109 in an area(s) that contain offshore oilfields and where petro-platforms were constructed

110 or stationed before being moved to the SwGoMx.

111 **MATERIALS AND METHODS**

112   Research at Cayo Arcas in 2016 was permitted by the Mexican Navy (SEMAR

113 DO: 112/162), which also provided logistical support for that activity. An ACUC permit

114 from the Smithsonian Tropical Research Institute (no. 2017-1107-2020) approved

115 methods used to collect specimens of *Neopomacentrus cyanomos* there.

Study species and origin of DNA sequences

116

117   The native IWP range of *N. cyanomos* extends from the Red Sea and the

118   Persian Gulf to south-east Africa, and eastward to Japan, the Philippines, the Solomon

119   Islands and New Caledonia in the West-Pacific. (Allen, 1991;

120   https://www.gbif.org/species/2398535, accessed Sept. 20, 2017). The range of *N.*

121   *cyanomos* encompasses most of the range of the entire genus, which extends only

122   further southeast, to the south-central Pacific (Allen, 1991;

123   https://www.gbif.org/species/2398526, accessed Sept. 20, 2017). No members of the

124   genus occur naturally in the Atlantic Ocean (Allen, 1991), and none are yet known from

125   the Mediterranean Sea, either naturally (Allen, 1991) or as a result of introduction (Arndt

126   & Schembri, 2015).

127   The DNA sequences of *N. cyanomos* that we analysed came from four sources:

128   (i) tissue samples of fish we collected in the Gulf of Mexico; (ii) tissue samples from fish

129   collected at various sites in the IWP that we obtained from other researchers; (iii)

130   sequences independently obtained by other researchers from different sites in the IWP

131   and compiled by the Fish Barcode of Life Initiative (FISH-BOL; http://www.fishbol.org/);

132   and (iv) tissue samples from putative aquarium-trade *N. cyanomos*.

133   The 128 sequences we analysed included 54 from documented *N. cyanomos*

134   (many identified by GR Allen), that had been collected by a variety of researchers,

135   including contributors to the FISH-BOL database, at 16 sites (1-8 fish per site) scattered

136   across most of the native IWP range. These sequences were compared to sequences

137   of 65 individuals of *N. cyanomos* we collected from the non-native population in the

138 SwGoMx on Campeche Bank, two from Madagascar reef in 2015 (Robertson et al.,

139 2016 b) and 63 from Cayo Arcas reef in 2016. After collection using the anaesthetic

140 clove-oil those 65 fish were euthanized with an overdose of that oil, and a pectoral fin

141 from each was preserved in 95% ethanol for use as a tissue sample from which we

142 obtained DNA. In addition we analysed sequences from nine aquarium-trade fish we

143 purchased in the USA that were identified by the US wholesale dealers as "*N.

144 cyanomos*" from the Philippines (eight from a single group purchased in Los Angeles,

145 California, and one from New York, New York)**. Table S1 provides information on

146 sample sites, numbers of sequences and their Genbank Accession numbers, and

147 indicates how we obtained all DNA sequences used in this study, both those from Fish

148 we collected or tissue samples sent to us by third parties, and those from other

149 contributors to the FISH-BOL database.

150 **DNA sequencing**

151   DNA extractions were performed with the NucleoSpin96 (Machery-Nagel) kit

152 according to manufacturer's specifications under automation with a Biomek NX liquid-

153 handling station (Beckman-Coulter) equipped with a filtration manifold. PCR

154 amplifications were performed on volumes of 12.5 µl compose of 6.25 µl of 10%

155 trehalose, 2 µl of ultra pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM

156 (NH4)2SO4, 20mM Tris-HCl (pH8.8), 2mM MgSO4, 0.1% Triton X-100), 0.625 µl of

157 MgCl2 (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM),

158 0.0625 µl of Taq DNA polymerase (New England Biolabs), and 2 µl of template DNA.

159 The PCR conditions consisted of 94°C for two min., 35 cycles of 94°C for 30 sec., 52°C

160 for 40 sec., and 72°C for one min., with a final extension at 72°C for 10 min. Sequences

161 from the native-range specimens (other than those available on FISH-BOL) were

162 obtained from The Biodiversity Institute of Ontario, University of Guelph, Canada, while

163 those from the SwGoMx were provided by Macrogen Inc., The Netherlands.

Phylogenetic analysis and haplotype network reconstruction

165   Nucleotide sequences were edited and manually aligned in Mega v.6.06 (Tamura

166 et al., 2013) and examined with a chromatogram. A final alignment of 594 base pairs

167 was used in the analyses. Maximum likelihood (ML) analysis was conducted with

168 RAxMLGUI v. 1.3.1 (Stamatakis, 2006; Silvestro & Michalak, 2012), performing 10,000

169 bootstrap replicates, and using the evolutionary substitution model estimated with the

170 GTR+G model, as obtained in jModelTest (Posada, 2008) according to AIC (Posada,

171 2008). The relative stability of clades was evaluated by 1,000 non-parametric bootstrap

172 replicates. *N. azysron* and *N. sororius* (Genbank # KP194962.1, and BOLD #

173 PCOM166-10, respectively) were used as out-groups. The haplotype network was

174 constructed using PopART (available at http://popart.otago.ac.nz/downloads.shtml) and

175 applying the Median-Joining method (Bandelt, Forster & Rohl, 2000).

Historical demography

177   To detect signatures of demographic changes in populations of each of the four

178 native lineages and the two lineages of *N. cyanomos* found in the SwGoMx (those

179 belonging to groups 1 and 4 in the haplotype network of Figs. 1 & 2), Tajima´s D

180 (Tajima, 1989), and Fu´s Fs (Fu & Li, 1993) neutrality tests were calculated, in DNAsp

181 v5 (Librado and Rozas, 2009). Evidence of an expanding population of a lineage

182 would be assumed if significant negative values of Tajima's D and Fu's F-statistics were

183 obtained (Mousset et al., 2004). Additionally, a pairwise mismatch distribution was

184 computed for each of those six populations in DNAsp v5 (Librado & Rozas, 2009).

185 Populations that have been historically stable are predicted to have multimodal

186 mismatch distributions, whereas those that have undergone a recent expansion are

187 predicted to be unimodal (Slatkin & Hudson, 1991). To test if the observed mismatch

188 distributions predicted a stable or an expanding population model, differences between

189 observed and expected (multimodal) distributions were evaluated using the

190 Harpending's raggedness index (Harpending et al., 1993) and the R_2 statistic (Ramos-

191 Onsins & Rozas, 2002). Diversity indices were calculated for each genetic group among

192 both the native-range and SwGoMx populations, and for combined populations, using

193 the program DNAsp v5 (Librado & Rozas, 2009).

194 **RESULTS**

195 The haplotype network for the entire set of 128 sequences is presented in Fig. 1,

196 and the locations from which individuals in the four major haplotype groups of *N.*

197 *cyanomos* identified in that figure were obtained are shown in Fig. 2. Genetic diversity

198 parameters of these different haplotype groups are displayed in Table 1.

199 The aquarium-trade specimens supposedly from the Philippines are only distantly

200 related to all *N. cyanomos* sequenced here, and were identified by us, both

201 morphologically and genetically, as a different species, *Neopomacentrus taeniurus*

202 (Bleeker, 1856), which is native to brackish non-reef environments and widely

203 distributed in the IWP (Allen, 1991). The mtDNA lineage of those aquarium-trade fish is

204 separated by 80-89 mutation steps from all four haplotype groups of *N. cyanomos*, from

205 both the native sites and the SwGoMx (Fig. 1).

206   All native-range specimens of *N. cyanomos* belonged to four closely related

207   haplotype groups and all SwGoMx fish belonged to two of those haplotype groups

208   (groups 1 & 4; Fig 1.). The native-range specimens had a total of 23 haplotypes, 12 of

209   those from groups 1 and 4 (18 of 54 individuals; Fig. 1, Table 1). The 65 SwGoMx

210   sequences included 11 haplotypes, four of which were shared with native-range

211   specimens of groups 1 and 4 (Fig. 1, Table 1). Haplotype diversity and nucleotide

212   diversity were high within each of the four genetic groups from the native-range (Table

213   1). While haplotype diversity also was high in the SwGoMx populations of groups 1 and

214   4, in each case the level was lower than in the native-range population of the same

215   group (Table 1). Nucleotide diversity in each of groups 1 and 4 of the SwGoMx

216   population was a little lower than in the native group of each case, but similar to

217   nucleotide diversity in the other two groups (2 and 3) from the native population (Table

218   1).

219   The SwGoMx population comprised roughly equal numbers of individuals drawn

220   from the two haplotype groups that are largely geographically separated in the native

221   range, with group 1 restricted to the northern Indian Ocean and group 4 found in

222   samples from Indonesia and Taiwan in the north-west Pacific Ocean (Fig. 2). The only

223   exception to this pattern of geographic separation is one individual of group 4 that was

224   collected at Gujarat, in India, at the same location as two individuals of group 1.

225   The results of the neutrality tests showed that Tajima's D and Fu's Fs values

226   were not statistically significant for any of the four groups in the native range, or for

227   either of groups 1 and 4 in the SwGoMx (Table 1). Each of those six groups showed a

228   multimodal mismatch distribution, with non-significant results for both Harpending's

229 raggedness index and the R_2 statistic (Table 1). Thus none of those six groups showed

230 evidence of having passed through a bottleneck due to a founder effect.

DISCUSSION

232 The analysis of mtDNA sequences indicate that the SwGoMx population of *N.*

233 *cyanomos* is unrelated to the aquarium-trade fish from the Philippines, which represent

234 a different species, *N. taeniurus*. Most significantly, the SwGoMx population is derived

235 from two distinct genetic lineages of *N. cyanomos* found in the native range, is

236 genetically diverse, and neither of those GoMx lineages shows signs of having passed

237 through a genetic bottleneck due to a founder effect. Lastly, the SwGoMx population is

238 derived from a combination of a lineage found in the northern Indian Ocean and another

239 from two widely separated locations in the northwest Pacific Ocean (Taiwan and

240 Indonesia). These results permit an evaluation of alternative hypotheses on the origin

241 and mode of introduction of this alien species into the Gulf of Mexico.

Aquarium-Release Hypothesis*:*

243 The aquarium-trade specimens sold as "*N. cyanomos*" proved to belong to a

244 different species. As part of a separate phylogenetic study of the genus, one of us (B

245 Victor) has obtained mtDNA CO1 sequences of specimens of all 15 nominal species of

246 *Neopomacentrus* that were collected in the field and identified by an expert in

247 damselfish taxonomy (GR Allen). The mtDNA sequences of the Philippines aquarium-

248 trade specimens are nested within a cluster of barcodes of specimens identified as *N.*

249 *taeniurus* that were obtained from multiple locations (sequences in Appendix I). In

250 addition, our morphological examinations confirmed this identification: Species of

251   *Neopomacentrus* fall into two morphological groups, one (including *N. cyanomos*) with

252   the lower edge of the suborbital bone hidden under a covering of scales, and the other

253   (including *N. taeniurus*) in which the suborbital margin is exposed and not covered by

254   scales (Allen, 1991). Specimens from the SwGoMx we examined all have a hidden

255   suborbital margin, while the aquarium-trade specimens of "*N. cyanomos*" we examined

256   all have exposed suborbitals. *N taeniurus* has a similar color pattern to, and can be

257   easily mistaken for *N. cyanomos.*

258   Aquarium-trade data presented by Rhyne et al. (2015) indicate that "*N.*

259   *cyanomos",* which is not brightly colored like many desirable aquarium species, is a very

260   small component of the aquarium trade in IWP reef-fishes to the USA (not sold in

261   sufficient numbers to be represented among the top 20 species of damselfishes), and

262   that the Philippines apparently is the sole native-range source for that trade to the USA.

263   *Neopomacentrus cyanomos* is known to occur in the Philippines, but whether or not any

264   aquarium-trade imports from there actually are that species remains uncertain.

265   Furthermore, it is not clear which mitochondrial lineage(s) of *N. cyanomos* is present in

266   the Philippines, given the proximity of that archipelago to not only of the Group 4 lineage

267   (one of the SwGoMx lineages), but also the Group 2 lineage at Western Papua (Fig. 2).

268   Even if the Group 4 lineage of *N. cyanomos* that is present in the SwGoMx is exported

269   from the Philippines in the aquarium trade to the US, the northern Indian Ocean lineage

270   (Group 1) that forms a major part of the SwGoMx population would not be expected to

271   be part of the trade.

272   While keeping marine aquarium-fish is popular and widespread in countries like

273   the US, that is not the case in Mexico (N Simoes, personal observations), where

274 attention to the international trade in marine aquarium fish is focused primarily on export

275 of native species (e.g. Lango-Reynoso et al., 2012). Any individuals of alien marine

276 fishes obtained by aquarists in Mexico likely would have been derived from US

277 wholesalers, the main global recipient in the aquarium trade. Personnel at a large public

278 aquarium at the city of Veracruz, in the SwGoMx, the only such installation in the

279 Mexican part of the Gulf of Mexico, have confirmed that *N. cyamonos* has never been

280 maintained in that facility (H. Pérez-España, pers. comm. Dec.14, 2017). Almost all

281 recorded aquarium-trade releases of exotic marine fish in or adjacent to the GoMx have

282 occurred in southern Florida, where at least 31 species (14 different families) of alien

283 reef-fishes have been recorded (Semmens et al., 2004; Schofield et al., 2009). *N.*

284 *cyanomos* would be expected to have established in southern Florida first if its GoMx

285 population was derived from aquarium release. It has not been discovered there

286 (Schofield, 2017; accessed Dec. 20, 2017), despite the fact that southeast Florida hosts

287 an abundance of divers, and is intensively monitored by the REEF organization

288 (www.reef.org) for sightings of exotic species. REEF was made aware of the presence

289 of *N. cyanomos* in the Gulf of Mexico by the publication of González-Gándara & Cruz-

290 Francisco (2014), and members of that organization have been looking for this species

291 on south Florida reefs.

292   The genetic data do not support the aquarium-release hypothesis. Both SwGoMx

293 lineages of *N. cyanomos* are genetically diverse, and the results of both the neutrality

294 tests and mismatch distributions indicate a stable population that did not pass through a

295 genetic bottleneck due to a founder effect, and hence was not established by a few fish

296 released from an aquarium.

297

Cargo-ship carriage hypothesis:

299   There are very few instances of long-distance introductions by alien

300   damselfishes. One of those evidently is due to a range expansion within the Pacific by a

301   species that associates with flotsam (*Abudefduf vaigiensis* (Quoy & Gaimard, 1825),

302   Coleman et al., 2014). Another introduction (of another *Abudefduf* species that also

303   associates with flotsam) from the Atlantic to the Mediterranean, has been attributed to

304   ship-transport by Occhipinti-Ambrogi et al. (2011), without further detail or presentation

305   of any justification (see Tsadok et al., 2015 for resolution of the identity of this species;

306   and Pajuelo et al., 2016, below).

307   Ship-transport was proposed as the means of introduction of *N. cyanomos* into

308   the SwGoMx by González-Gándara & Cruz-Francisco (2014). Movements of cargo-

309   ships in the normal course of business have been implicated in the long-distance

310   transport of exotic marine fishes well beyond their native ranges, usually in ballast water

311   (Wonham, Carlton & Ruiz, 2000), but, potentially, also in sea-chests on the outside of

312   the hulls of ships. Damselfishes are not included among the species thought to be

313   introduced by ballast water (Wonham, Carlton & Ruiz, 2000) and none were found on

314   the hulls of cargo ships examined in Brazil by Ferriera, Goncalves & Coutinho (2006).

315   Sea chests are screened containers on the outside of the hulls that house the water

316   intakes of a ship, inside which diminutive, crevice-dwelling reef-fishes such as gobies

317   and blennies have been found (Wonham, Carlton & Ruiz, 2000; Coutts & Dunham

318   2007). However, *N. cyanomos* is not a crevice-living species and typically feeds on

319   plankton in a flow of water 0.5-1 m above the substrate rather than maintaining contact

320   with it. Whether a species with these characteristics is likely to be able to survive a long

321   voyage in a sea-chest with functioning water intakes, and do so in large enough

322   numbers to establish a genetically diverse population when it arrives in a different ocean

323   is far from certain.

324   A modern cargo-ship, which typically will not have a heavily fouled hull, travelling

325   at 20-25 knots (37-46 kph) on a voyage from the western Pacific (Hong Kong) to the

326   SwGoMx via the Panama Canal would take ~19-23 days. A similar voyage would take

327   ~16-20 days from Northwest India via the Suez Canal (both calculated on http://sea-

328   distances.org, accessed Sept. 20 2017), not counting stoppages at any ports during the

329   voyage. Survivorship in ballast water is time-dependent for many marine organisms

330   (Muirhead et al., 2015) and, in addition to the lengthy duration of a transit by either

331   route, the Pacific route would pose additional environmental barriers for transport of

332   tropical reef-fish hitching a ride on the hull of a cargo-ship going to the Gulf of Mexico

333   via that route. Most such traffic goes via California (Figure S1), which has a temperate

334   climate with cold winter waters. However, average water temperatures near the major

335   port of Long Beach, California, peak at ~20^0C for several months during summer, the

336   same as average minimum winter temperatures at the latitudinal limits of the IWP range

337   of *N. cyanomos*. In addition sea temperatures off California are elevated above the

338   norm during El Niño events. Thus the sea temperature regime on the coast of California

339   would reduce, but not eliminate, the possibility of successful carriage to Mexico.

340   Second, passing through the Panama Canal (see Figure S1) exposes any hull-

341   associated organisms to the freshwater lake that constitutes most of that canal, and

342   represents an effective barrier to transfer of stenohaline reef-fishes between the oceans

343   separated by the Isthmus of Panama (although a few euryhaline, non-reef fishes that

344   live in brackish environments have made the transit; reviewed in Cohen, 2006; and

345   Robertson & Allen 2015; Robertson & Van Tassell 2015).

346   In addition, due to the fact that the Panama Canal represents a major global

347   choke point for international shipping (see Figure S1), one would expect the first

348   occurrence of marine reef-fishes transported across the Pacific in this manner to be on

349   reefs adjacent to the Pacific end of the canal, where ships often stop before making a

350   canal transit. However, there are no records of such occurrences (review in Cohen

351   2006; Robertson & Allen, 2015; Robertson & Van Tassell, 2015), at either end of the

352   canal.

353   As with the aquarium-release hypothesis, the genetic data do not support this

354   hypothesis, since both the SwGoMx lineages of *N. cyanomos* are genetically diverse,

355   and show no signs of having passed through a genetic bottleneck and hence was not

356   established by a few founders, i.e. by a few fish transported in ballast water or on the

357   hull of a cargo ship.

358   If *N. cyanomos* is capable of being carried far from its native range by cargo-ship

359   in large enough numbers to establish a genetically diverse population, that would be

360   much more likely to occur near major ports (e.g. Miami, Florida, or Panama, or

361   somewhere in the Mediterranean), than in the southwest Gulf of Mexico. However, there

362   are no indications that such has happened.

363   **Petro-platform translocation hypothesis:**

364   Offshore petro-platforms have been shown to support an abundance of reef-

365 fishes belonging to a broad variety of taxa, in the Gulf of Mexico (Hastings, Ogren &

366 Mabry, 1976; Topolski & Szedlmayer, 2004; Ajemian et al., 2015), Brazil (Ferreira,

367 Goncalves & Coutinho, 2006), California (Claisse et al., 2014; Martin & Lowe, 2010), the

368 Mediterranean (Consoli et al., 2013), west Africa (Friedlander et al., 2015), Australia

369 (Pradella et al., 2014), the Canary Islands (Pajuelo et al., 2016), and the Persian Gulf

370 (Torquato et al., 2017). In the SwGoMx, we have observed *N. cyanomos* living on two

371 such platforms, including in superabundance on a relatively lightly-fouled platform near

372 Cayo Arcas, in the southwest corner of Campeche Bank (up to ~100 individuals m^{-2};

373 Simoes & Robertson, 2016). That platform is one of several hundred platforms in the

374 area of the greatest concentration of such structures in the SwGoMx

375 (https://portal.cnih.cnh.gob.mx/iicnih/?lng=en_US, accessed Sept. 20, 2017).

376   Translocated petro-platforms have long been known to be vectors of long-

377 distance transport of sessile fouling organisms (Foster & Willan, 1979; Bax et al., 2003;

378 Hopkins & Forrest 2010; Wanless et al., 2010; Yeo et al., 2010; Mineur et al., 2014).

379 Recently, such movements have been implicated in the transoceanic transport of

380 western Atlantic reef-fishes to the Canary Islands, where a platform-servicing center has

381 been established (Falcon et al., 2015; Pajuela et al., 2016), as well as to the

382 Mediterranean (Dulcic & Dragicevic, 2013; Pajuela et al., 2016). Some reef-fish species

383 apparently carried to the Canary Islands by petro-platforms have established breeding

384 populations there (Falcon et al., 2015; Pajuelo et al., 2016). Petro-platforms may help

385 exotic reef-fishes establish off the coast of west Africa (Friedlander et al., 2014), and

386 translocated platforms may have carried eastern Atlantic reef-fishes to Brazil, where

387 some have recently become established (Pajuelo et al., 2016; Anderson et al., 2017).

388   Relocated petro-platforms originating in the Indian Ocean are thought to have

389 transported alien fishes to the Mediterranean Sea (Galil, 2008). Among the exotic reef-

390 fishes newly recorded in the Canary Islands, one species identified by Falcon et al.

391 (2015; plate 4B) as the (naturally trans-Atlantic) damselfish, *Abudefduf taurus* (Muller &

392 Troschel, 1848), actually is most likely the IWP species *A. sordidus* (Forsskal, 1775)

393 (GR Allen, pers. comm. 2016). Since *A. sordidus* is a dull-colored species that is not

394 found in the aquarium trade (Rhyne et al., 2015), this occurrence represents support for

395 the hypothesis that IWP reef-fishes are carried from their native ranges into the Atlantic

396 by petro-platforms. Another example, in the Pacific Ocean, reported by Myers &

397 Donaldson (1996) and Myers (1999, p 183), is the damselfish *Neopomacentrus*

398 *violascens* (Bleeker, 1848), which they proposed was transported from the Philippines

399 to Guam, where it is not native, by a bio-fouled military barge. It has now established a

400 population at Guam, living on bio-fouled mooring chains rather than on natural

401 substrata.

402   Are there movements to the SwGoMx of petro-platforms from the two areas of

403 the native range of *N. cyanomos* that contain the lineages of that species found in the

404 SwGoMx? The website www.infield.com/rigs (accessed Dec. 20, 2017) provides

405 information on the construction sites of drilling-platforms and drill ships currently located

406 in the offshore oilfields of different countries. A review of information on that site of the

407 origins of such platforms and ships showed that about 60% of those listed as currently

408 being used in the SwGoMx were constructed at sites within the native ranges of the two

409 lineages found in the SwGoMx. Many drilling rigs currently in use in other offshore

410 oilfields in the tropical Atlantic also were constructed at sites within the native range of

411   *N. cyanomos* (Table 2). In some cases rigs were moved from those construction sites

412   directly to the Atlantic oilfield; in other cases, particularly for older rigs, their movements

413   prior to arrival at those offshore fields are unclear. These linkages produced by

414   relocations of drilling-rigs represent only part of a global web of movements of new and

415   used drilling-rigs, drill-ships, support vessels, and parts of production platforms within

416   and between oceans that could result in establishment of exotic sessile and mobile reef

417   organisms well beyond their native ranges (Wanless et al., 2010; Yeo et al., 2010;

418   Pajuelo et al., 2016).

419   Drilling-rigs are moved long distances either by being towed by tugboats while in

420   the sea (a wet-tow), or by being transported out of the water on the deck of a heavy-lift

421   ship (a dry-tow). Although dry-tows are more commonly used for long-distance and

422   interoceanic relocations of rigs, both dry- and wet-tows have been used to move rigs

423   from the IWP to the GoMx around the southern tip of Africa (e.g. a wet-tow:

424   https://mb50.wordpress.com/tag/cape-of-good-hope/, accessed Dec. 15, 2017); a dry-

425   tow:

426   https://www.rigzone.com/news/oil_gas/a/16673/dockwise_delivers_thunder_horse_pdq

427   _to_corpus_christi/, accessed Dec. 15, 2017). Wet-towed rigs moved by this route

428   would have the potential to transport marine organisms from the IWP to the GoMx or to

429   any other Atlantic offshore oil-field.

430   The southern tip of Africa has a temperate environment that very likely inhibits

431   the transport of tropical fish from the Indian Ocean to the Atlantic Ocean. However,

432   summer temperatures at that tip are ~20^0C, similar to winter temperatures at the

433   latitudinal limits of the native geographic range of *N. cyanomos*. In addition,

434 temperatures there are distinctly warmer during El Niño years (Dufois & Rouault 2012),

435 and there are periodic leakages of warm water from the southern Indian Ocean around

436 the southern tip of Africa into the South Atlantic (Duncombe Rae, 1991).Thus

437 temperatures suitable for the rig-transport of *N. cyanomos* are intermittently available off

438 that tip.

439   Whether any of the rigs employed in the GoMx arrived there from the IWP via the

440 Suez Canal is unclear. Limitations in the capacity of that canal may oblige some rigs to

441 move between the Mediterranean and IWP via southern Africa (e.g.

442 https://www.offshoreenergytoday.com/fairstar-to-transport-ensco-105-jack-up-rig-from-

443 malta-to-singapore/, accessed Dec 15, 2017)). However, rigs have been wet-towed

444 from the Persian Gulf to northern Europe via the Suez canal (e.g.

445 https://www.rigzone.com/news/oil_gas/a/60320/aker_solutions_aker_h6e_sets_sail_for

446 _norway/, accessed Dec 15, 2017). Sea temperatures are at or above 20^0C for 6-8

447 months each year in the southern Mediterranean, which would facilitate transport of

448 tropical organisms on rigs passing through. However, the only connections between the

449 IWP and tropical Atlantic oilfields that we were able to find through web searches

450 involved relocations of rigs around southern Africa.

451   The genetic structure of the SwGoMx population of *N. cyanomos* indicates it was

452 established by two large and genetically diverse groups of founders, which did not go

453 through a genetic bottleneck during the expansion of their populations from a small

454 group of founders in that area. These results support the hypothesis that a large number

455 of fish were transported to that area, which would be most consistent with the

456 movement of one or more petro-platforms from the native range. *Neopomacentrus*

457   *cyanomos* appears to be the first known tropical reef-fish to have successfully

458   established outside its native geographic range, in another ocean, as a result of

459   transport by a petro-platform.

460   **How many introductions of *N. cyanomos* to the GoMx?**

461   The fact that the SwGoMx population of *N. cyanomos* is derived from two

462   lineages mostly confined to different oceans raises the question of whether that

463   population was derived from two well-separated sources and two separate

464   introductions. While those lineages are largely allopatric, both are present at Gujarat, in

465   northwestern India (Fig. 2). That occurrence and the absence of the Pacific lineage

466   (group 4; Fig. 2) at Sri Lanka (in an admittedly small sample of fish), which is closer to

467   the Pacific than is Gujarat, suggests that the presence of group 4 at western India may

468   not be natural. Is there evidence that offshore oil-industry activity provides connections

469   that could have carried group 4 fish from the Pacific to Gujarat?

470   There is a major, active offshore-petroleum area immediately to the south of the

471   broad Kathiawar peninsula of Gujarat that projects well into the Indian Ocean (e.g.

472   https://watchers.news/data/uploads/2012/04/Untitled1.png, accessed Sept. 15, 2017).

473   Of 41 petro-platforms used in India that are listed by www.infield.com/rigs, (accessed

474   July 15, 2017), 15 were constructed in Singapore and two in southeast China, near the

475   Taiwan sample of the Group 4 lineage of *N. cyanomos*. Further, there is a major global

476   shipbreaking site on either side of the Gujarat peninsula, one at Gaddani in Pakistan,

477   ~360 km west of Gujarat, the other at Alang, at the eastern base of that peninsula, in

478   India. A list of decommissioned vessels of various types that were sent to those two

479   sites during 2015-2016 (provided by www.shipbreakingplatform.org) shows that

480   decommissioned offshore platforms and support vessels do get taken from various

481   locations within the northwest Pacific (Indonesia, Singapore, Vietnam, south-east

482   China) to both those shipbreaking yards. In addition, other types of decommissioned

483   vessels also make similar last voyages. Decommissioned vessels transiting to a

484   shipbreaker do not have clean hulls; rather, typically they are heavily bio-fouled

485   (Davidson et al., 2008), which would facilitate long-distance transport of fishes such as

486   *N. cyanomos.*

487   Thus, there are interoceanic connections involving movements of drilling-

488   platforms, drill-ships, offshore support vessels, and decommissioned vessels of various

489   types that could be responsible for the occurrence of both lineages in the sample from

490   Gujarat. The existence of such connections supports the hypothesis that the occurrence

491   of *N. cyanomos* in the GoMx could well be due to a single introduction (the simplest

492   explanation) rather than multiple introductions from different source locations.

493   **When and where in the GoMx was *N. cyanomos* initially introduced?**

494   *Neopomacentrus cyanomos* was first recorded at Coatzacoalcos, in the

495   southwest corner of the GoMx in July 2013 by González-Gándara & de la Cruz-

496   Francisco (2014), who thought it had been introduced directly to that port from its native

497   range. However, several years later, inspection of underwater photographs taken in July

498   2013 at Cayo Arcas reef, 350 km northwest of Coatzacoalcos, showed that it was

499   present in significant numbers at both those sites at the same time in 2013 (Robertson

500   et al., 2016 a). Furthermore, this species was recorded in appreciable numbers on reefs

501 near Veracruz, 200 km northwest from Coatzacoalcos and 450 km west from Cayo

502 Arcas, in October 2014 (Robertson et al., 2016 b). These observations indicate that *N.*

503 *cyanomos* was already well established over a large area in the SwGoMx when it was

504 first discovered, and indicate that its arrival in that area occurred long before 2013. More

505 recently, *N. cyanomos* has been found to be even more widely spread across the

506 SwGoMx (Schofield, 2017, accessed Dec. 20, 2017). Between 2016 and 2017, this

507 species also was found at widely separated sites in the northern GoMx: on a natural

508 reef off Texas in the west, on a petro-platform off Louisiana, and on an artificial reef off

509 the Florida Panhandle in the east (Schofield, 2017, accessed Dec. 20, 2017).

510   The occurrence of *N. cyanomos* at two widely separated sites at the time it was

511 first discovered in the SwGoMx demonstrates that the location at which any alien

512 species is first discovered should not be equated with the site at which it was first

513 introduced. This issue is particularly applicable to *N. cyanomos*, since it is small, dully

514 colored, and readily mistaken for a native planktivorous damselfish, the brown chromis,

515 *Chromis multilineata* (Guichenot, 1853), which it resembles in size, shape, and

516 coloration. Distinguishing small juveniles of these two species in the field is more

517 difficult than distinguishing adults. The brown chromis is particularly abundant on coral

518 reefs in the SwGoMx, where it is far more numerous than *N. cyanomos* (DRR personal

519 observations), and also lives on petro-platforms in the north and south of the GoMx. In

520 that area both species often co-occur in feeding aggregations that are predominantly

521 composed of brown chromis. Small numbers of *N. cyanomos* sharing a reef (or petro-

522 platform) with many brown chromis could easily be ignored or mistaken for that species

523 by a diver who is not familiar with, and not actively searching for *N. cyanomos*.

524   Given the difficulty in detecting the timing of the arrival of *N. cyanomos* in the

525   SwGoMx, the possibility that it was initially introduced to the northern GoMx rather than

526   the southwest part cannot be ruled out at present. Drilling-platforms originated from

527   sites in the native geographic ranges of both lineages of *N. cyanomos* occur in both the

528   northern and southern GoMx (Table 2). The fact that *N. cyanomos* seems to be much

529   more common in the SwGoMx, even superabundant on petro-platforms there, and that

530   it was first detected there, does suggest that it was initially introduced there. However,

531   the greater abundance of this species in the SwGoMx may simply be due to more

532   favourable environmental conditions (warmer water and greater availability of coral

533   reefs), rather than prior occurrence there.

534   **Future directions for research**

535   Three sets of DNA barcode data from *N. cyanomos* at several sites in its native

536   range should help clarify some of the questions concerning the origins of the SwGoMx

537   population. First, as can be seen from the numbers of platforms dispatched to other

538   countries from Singapore (www.infield.com/rigs), that city-state is a major global player

539   in the business, has produced the largest number of GoMx petro-platforms that

540   originated in the IWP, and is a site for servicing of used platforms coming from various

541   areas, including the tropical Atlantic. Documenting which lineage(s) of *N. cyanomos*

542   occur in Singapore would help evaluate the potential for Singapore as a source

543   population for the Gulf of Mexico population of this damselfish. Second, a substantial

544   set of DNA-sequence data also is needed from Gujarat, to indicate the proportional

545   abundances of groups 1 and 4 lineages in the population there, and to show whether

546   group 4 haplotypes present there are shared with west Pacific sites. Third, DNA

547   barcode data from confirmed *N. cyanomos* from the Philippines would clarify the extent

548   to which *N. cyanomos* is actually involved in the aquarium trade to the Americas, and

549   whether Philippine fish belong to a DNA lineage found in the GoMx.

550   A host of other questions raised by the appearance of *N. cyanomos* in the GoMx

551   deserve attention in future research. Prime examples include: (i) Where in the GoMx

552   was *N. cyanomos* initially introduced: the northern or southern parts of that Gulf? (ii)

553   Does *N. cyanomos* represent a true invasive species that will have negative effects on

554   reef ecosystems of the Greater Caribbean, or a relatively innocuous addition to the reef-

555   fish fauna of that region (cf. Davis et al., 2011)? (iii) What factors contribute to the

556   evident success of this species in the Gulf of Mexico, on both petro-platforms and coral

557   reefs? (iv) Why is *N. cyanomos* the only known successful alien reef-fish that appears to

558   have arrived in the GoMx via relocated petro-platforms? Is that a reflection of the low

559   frequency of wet-tows of rigs via southern Africa during intermittent warm-water

560   episodes; or did other species arrive in numbers in the GoMx but fail to establish (cf.

561   Oda & Parrish, 1981), or are there other alien fish species present there that have yet to

562   be noticed (e.g. small, crevice-living, cryptic species)? (v) Has *N. cyanomos*, (or other

563   IWP reef-fishes) established any unnoticed population(s) on platforms in offshore oil

564   fields in other tropical parts of the Atlantic Ocean, such as western Africa and Brazil,

565   which might have acted as stepping stones that helped this species spread into the

566   GoMx on platforms or support vessels moved between Atlantic oil fields?

567   **CONCLUSIONS**

568   Various lines of evidence support the petro-platform translocation hypothesis:

569   petro-platforms can provide habitat for substantial populations of a broad variety of

570   tropical reef-fish; *N. cyanomos* can live in a dense population on a petro-platform; petro-

571   platforms are regularly constructed within the parts of the native range of *N. cyanomos*

572   where the SwGoMx lineages occur, and transported from there to the SwGoMx;

573   translocated platforms has been implicated as vectors of long-distance transport of

574   tropical reef-fishes, sometimes in sufficient numbers to allow them to establish breeding

575   populations in non-native areas; members of the genus *Neopomacentrus* apparently

576   can be transported long distances by bio-fouled structures in sufficient numbers to

577   establish populations outside their native ranges; and, finally, the genetic diversity of the

578   SwGoMx population of *N. cyanomos* indicates it is derived from a large and genetically

579   diverse population of founders, consistent with large numbers of fish being transported

580   by a drilling-platform. *Neopomacentrus cyanomos* has characteristics well suited to

581   being transported by a petro-platform in sufficient numbers to establish a population far

582   from its native geographic range: it is small (maximum length ~12 cm), can feed on the

583   plankton stream produced by a slow-moving platform (1-6 kts (2-11 kph); Yeo et al.,

584   2010), and can live in high-density aggregations on small amounts of relatively low-

585   profile, bio-fouling cover.

586   Heavy-lift ships, which carry offshore drilling-rigs on the deck, out of the water,

587   often are employed for long-distance relocation of rigs. The use of this existing

588   technology for *all* long-distance transport of such rigs would greatly reduce the risk of

589   any types of marine organisms being transported outside their native ranges by

590   relocated rigs. Such transport should be used not only for movements of rigs between

591   oceans such as the Indo-Pacific and Atlantic, or the Mediterranean and Atlantic, but

592   also relocations between regions with different biotas in the same ocean, such as those

593   implicated in alien-fish transfers between the eastern and western tropical Atlantic.

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Figure 1

Haplotype network

Haplotype network of *N. cyanomos* from its native range and the southwest Gulf of Mexico, and of aquarium-trade "*N. cyanomos*" (= *N. taeniurus*) from the Philippines. Number of mutational steps: each red line indicates a single step, boxed number indicates number of multiple steps. Each multicolored haplotype pie indicates the abundance of a haplotype found at multiple locations.

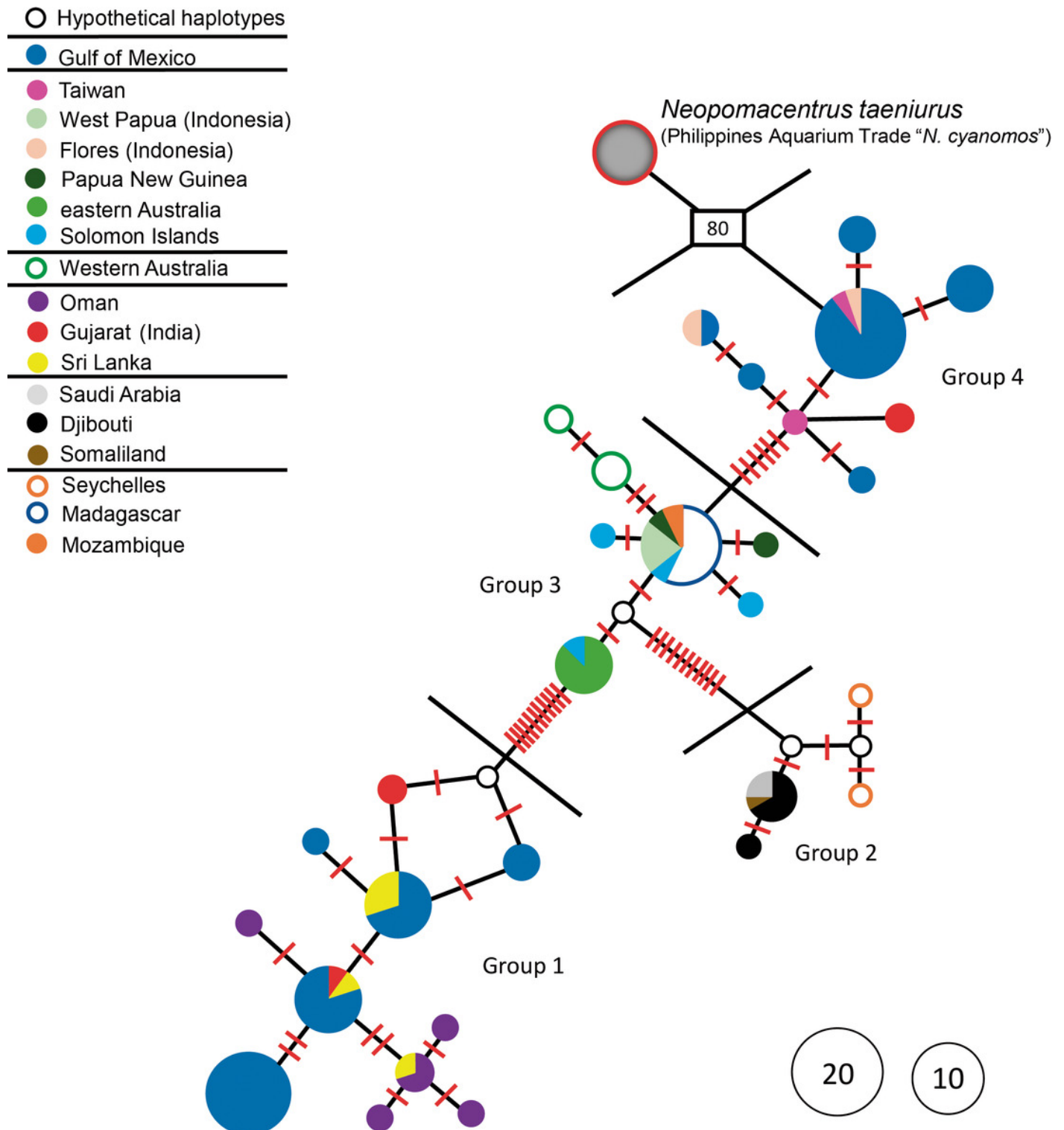
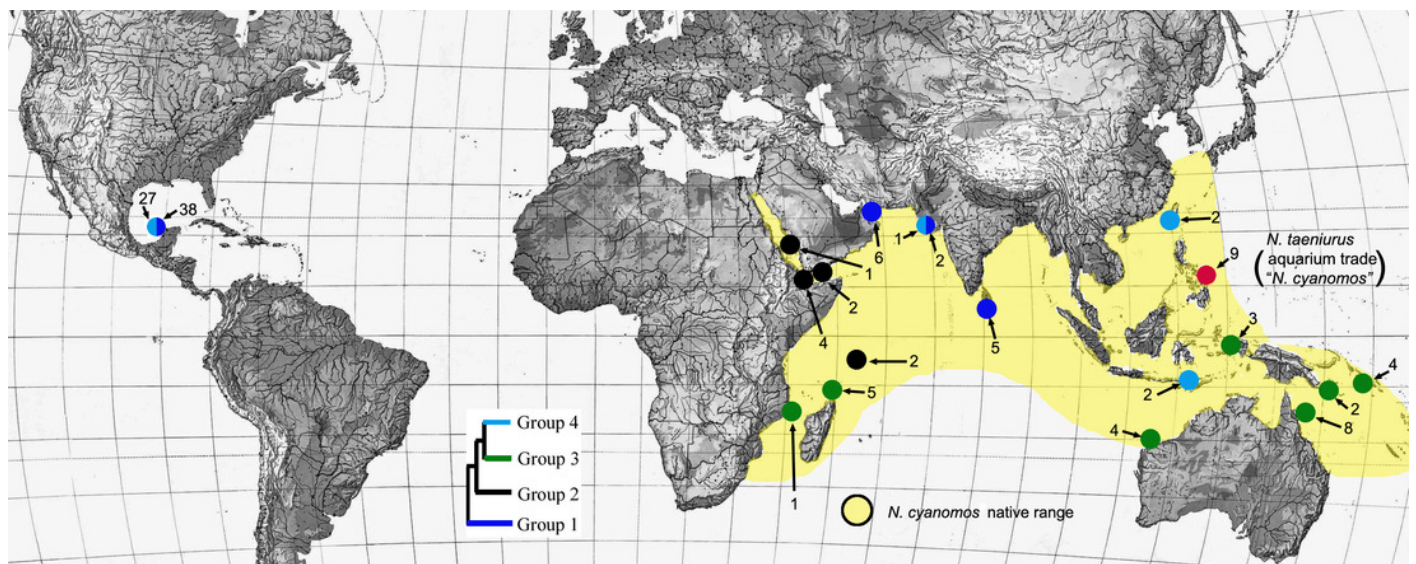



Figure 2

Locations of sites with different haplotype groups

Locations of sites within the Indo-west Pacific and the southwest Gulf of Mexico from which individuals were obtained from four different haplotype groups (see Figure 1) of *N. cyanomos,* and of aquarium-trade "*N. cyanomos*" (= *N. taeniurus*) from the Philippines. The ML (bootstrap support >85% in all cases) tree is shown as an inset. The color scheme illustrates the geographic distributions of the four major genetic lineages of *N.cyanomos.*

Genetic diversity parameters for different genetic groups of *N. cyanomos* from both the native Indo-West Pacific range and the Gulf of Mexico.

Genetic group	N	S	H	π	h	D	Fs	R_2	r
Group 1 native	13	8	8	0.0039	0.910	-0.40	-3.11	0.11	0.06
Group 2 native	9	5	4	0.0024	0.583	-0.91	-0.29	0.12	0.25
Group 3 native	29	8	7	0.0027	0.700	-0.66	-1.22	0.09	0.19
Group 4 native	5	4	4	0.0030	0.900	-0.41	-1.20	0.21	0.13
All native	54	43	23	0.0173	0.922	---	---	---	---
Group 1 GoMx	38	5	5	0.0028	0.683	-1.00	1.05	0.08	0.16
Group 4 GoMx	26	7	6	0.0020	0.603	-1.08	-1.93	0.08	0.06
All GoMx	65	27	11	0.0183	0.823	---	---	---	---
All native + GoMx	121	49	30	0.0195	0.919	---	---	---	---
Group 1 native + GoMx	51	12	11	0.0036	0.791	-0.57	-2.39	0.08	0.04
Group 4 native + GoMx	32	8	8	0.0022	0.635	-1.02	-2.70	0.08	0.05

Notes.

N= sample size; S = no. polymorphic sites; H = no. haplotypes; π = nucleotide diversity; h = haplotype diversity; D = Tajima's D; Fs = Fu's Fs statistic [all values of D and Fs were non-significant at p >0.05]; R_2 = Ramos-Onsins & Rozas' R_2 statistic, r = Harpending's raggedness index [all values of R_2 and r were non-significant at p >0.05]. Native = Indo-West Pacific native range; GoMx = Gulf of Mexico

Indo-West Pacific sources of drilling-rigs present in late 2017 on offshore oilfields of the tropical Atlantic Ocean.

Atlantic Oilfields	No. rigs	Construction site						
		Persian Gulf	Malaysia	Singapore	China	Taiwan	South Korea	Japan
Brazil	13	Yes	-	Yes	Yes	-	Yes	-
Colombia	1	Yes	-	-	-	-	-	-
Mexico	30	Yes	-	Yes	Yes	Yes	Yes	Yes
Trinidad	1	-	-	Yes	-	-	-	-
USA	17	-	-	Yes	Yes	Yes	-	-
West Africa	13	Yes	Yes	Yes	-	-	-	Yes

Notes.

1. West Africa = Angola, Cameroon, Congo, Ghana, Ivory Coast and Nigeria.
2. Yes indicates rig(s) at an Atlantic site originated from the particular construction site.
3. No. rigs = no. rigs present at an Atlantic site that were constructed at one or more Indo-West Pacific sites.
4. Venezuela is not included because no rigs of Indo-West Pacific origin are currently based there.
5. Source: http://www.infield.com/rigs/, accessed December 20, 2017. Data from this website only cover currently present rigs, and do not include rigs no longer at a site; e.g. the semi-submersible drilling-rig Deepwater Horizon, which exploded and sank off Louisiana in 2010, is not included among the list of rigs for the US section of the Gulf of Mexico.