UNIVERSIDADE FEDERAL DE ALAGOAS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM DIVERSIDADE BIOLÓGICA E CONSERVAÇÃO NOS TRÓPICOS

JESSIKA MARIA DE MOURA NEVES

DIVERSIDADE E CONSERVAÇÃO DE TAINHAS (ACTINOPTERYGII: MUGILIDAE) NA PROVÍNCIA ATLÂNTICO SUDOESTE TROPICAL: UMA ABORDAGEM INTEGRATIVA

MACEIÓ - ALAGOAS
2020

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, como requisito para obtenção do título de Doutora em CIÊNCIAS BIOLÓGICAS, área de concentração em Conservação da Biodiversidade Tropical.

Orientadora: Profa. Dra. Tamí Mott
Coorientadores: Profa. Dra. Nidia Noemi Fabré
Prof. Dr. Ricardo José Pereira

MACEIÓ - ALAGOAS
2020

Catalogação na fonte
Universidade Federal de Alagoas
Biblioteca Central
Divisão de Tratamento Técnico
Bibliotecária: Taciana Sousa dos Santos – CRB-4 – 2062

N518d Neves, Jessika Maria de Moura.
Diversidade e conservação de tainhas (Actinopterygii: Mugilidae) na província Atlântico Sudoeste Tropical: uma abordagem integrativa / Jessika Maria de Moura Neves. – 2020.
246 f. : il., figs. e tabs. color.

Orientadora: Tamí Mott
Coorientadores: Nidia Noemi Fabré, Ricardo José Pereira.
Tese (Doutorado em Ciências Biológicas) – Universidade Federal de Alagoas. Instituto de Ciências Biológicas e da Saúde. Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos. Maceió, 2021.

Inclui bibliografias.
Anexos: f. 144-246.

1. Tainha (Peixe). 2. Taxonomia integrativa. 3. Morfologia externa. 4. DNA barcoding. 5. Marcadores DArT-seq. 6. Conservação. I. Título.

CDU: 57: 639.2

Folha de aprovação

Jessika Maria de Moura Neves

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Tese aprovada em 17 de dezembro de 2020.

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MACEIÓ - AL
Dezembro/ 2020

Dedico esta tese aos meus pais, que sempre acreditaram em mim, mais do que eu mesma.

AGRADECIMENTOS

Não foi sozinha que eu cheguei até aqui e, por isso, gostaria expressar minha gratidão a todos que fizeram parte deste processo:

Primeiramente à minha instituição, a Universidade Federal de Alagoas, que me acolheu em 2008 quando eu era uma adolescente cheia de sonhos e dúvidas. De lá pra cá, a Ufal tem sido a minha casa e tudo que sou academicamente eu devo às oportunidades que me foram dadas por ela. Eu me agarrei a cada uma delas e fiz o melhor que pude, na esperança de fazer algo bom pela sociedade, e continuarei dando o meu melhor como servidora. Sempre defenderei a universidade pública, gratuita e de qualidade!

Ao programa de pós-graduação em Diversidade Biológica e Conservação nos Trópicos (DIBICT) que me deu a chance de fazer mestrado e doutorado sem sair de perto da minha família. Estendo meus agradecimentos a quem está nos bastidores do DIBICT e faz tudo acontecer: a super secretária Julliene Gonçalves, o técnico Alex Wanderley e Leide que era responsável pela limpeza quando comecei esta empreitada;

Aos órgãos que fomentaram a minha pesquisa e a minha formação: Capes, CNPq e Fapeal;

Aos meus três orientadores: Dra. Tamí Mott, Dra. Nidia Fabré e Dr. Ricardo Pereira, por todos os ensinamentos, não apenas os acadêmicos. Agradeço especialmente ao Ricardo por ter me acolhido durante período de doutorado sanduíche em sua instituição Ludwig-Maximilians-Universität München (LMU), me recebendo em seu grupo de pesquisa, me ajudando a socializar e a superar as minhas dificuldades;

A todos que me acolheram na LMU, com gestos de gentileza que jamais vou conseguir retribuir: Dr. Oliver Hawlitschek, Dra. Marina Querejeta, Msc. Carmen Argudo, Kathleen Webster, Johanna Schmalhofer, Amelie Höcherl, Irina Formoso, Zachary Nolen, Peter Middle, Callum Thomas, Hanna Algora, Cillian Nolan, Dr. Claire Peart, Dr. Sergio Tusso, Dr. Joshua Peñalba, Dra. Ana Catalán, Dr. Sebastian Höhna, Dr. Saurabh Pophaly, Dr. Dirk Metzler e Ingrid Kroiss;

Às amizades brasileiras que me ajudaram a manter um pouco da sanidade na Alemanha: Bárbara Ricci e Ana Luiza Aguiar, e à minha família alemã que me acolheu em meio a tantos contratempos: Aleksandra Woszek, Frederic Gorges, o pequeno Luis e Ekaterina. Agradeço também à minha tia Rubinha que me ajudou muito nessa aventura que foi viver na Alemanha e aos meus primos Marconi Oliveira e Gueberson Moura que me ajudaram na busca ao passaporte perdido (rsrs);

A todos que contribuíram para melhorar a qualidade deste trabalho: Dr. Marcelo Sturaro, Dr. Sergio Maia, Dr. Adrian Garda, Dr. Uedson Jacobina, Dr. Kim Barão, Dr. Henrique Batalha, Dra. Júlia Verba e Dra. Tereza Tomé, a quem também sou muito grata por não ter me deixado morrer de frio na Alemanha;

A todos da equipe PELD APACC, que foram de extrema importância para o bom andamento desse trabalho, principalmente Dr. Richard Ladle, Dra. Ana Malhado, Dr. Davi Teles, Dra. Bárbara Pinheiro, Dr. Ricardo Miranda e Dr. João Campos-Silva;

Ao professor Dr. Renato Gaban-Lima, por quem tenho grande amizade, e que tive o prazer de ter como supervisor do estágio docência;

A todos os meus amigos que ajudaram no processamento das amostras: Victor, Gilmar, Joyce, Ivan, Rawelly, Tainá, Bruno, Anny, Myrna, Mônica, Adel, Reginaldo, Matheus Raimundo, Camila Navarro, Lavínia, e Marcos Dubeux;

Aos meus companheiros do laboratório de biologia integrativa: Andre, Luana, Grazi e principalmente João, que me ajudaram em diversas etapas, seja colocando a mão na massa, dando conselhos ou apoio moral;

Aos pescadores que me ajudaram nas coletas: Cicero, Zé do Bosque e sua esposa Amara. Agradeço também ao Alan (esposo da Tamí) que saiu diversas vezes na caçada às valiosas tainhas;

A Alfredo Perez, que me acompanhou nos campos e com toda paciência me ensinou a identificar as tainhas e analisar os otólitos;

Aos demais companheiros de curso e/ou copa que me proporcionaram ótimos momentos ao longo desses anos: Ciro, Gustavo, Aldo, Karol, Dani, Rafa, Victor, Beth, Gilmar, Ivan, Norah, Diogo, Samantha, Jordana, Jayne, Lucas, Maxwell, Felipe e Arthur;

A todos os professores que tive ao longo da minha vida pois, com certeza, cada um contribuiu para o que sou hoje;

Aos meus compadres e amigos Héllder Medino e Gesika Matias e meus afilhados maravilhosos Miguel e Giovanna por todo apoio e carinho;

Ao meu xuxuzinho, Yumi Asakura, e Gustavo Paulino que vêm me aturando desde 2008 e são os melhores amigos que alguém poderia ter;

Ao Gaio, uma amizade inesperada que se fortaleceu ao longo desses 4 anos, por quem eu tenho muito carinho;

À minha família incrível que sempre me apoiou e me deu forças para chegar até aqui: meu pai Augusto, minha mãe Áurea, meus irmãos Augusto e Kaius, minhas cunhadas Mayara e Daiane e meus sobrinhos Gabriel, Luana e Laura (ainda na barriga da mamãe Dai). Amo demais todos vocês!

O meu agradecimento a minha mãe tem que ser estendido porque, com toda certeza, eu não teria chegado aqui sem ela. Foram momentos muito difíceis ao longo desses quatro anos, mas sempre nos apoiamos uma na outra para superar todos eles. O seu suporte, emocional, financeiro e gastronômico (rsrs) foram fundamentais para que eu pudesse alcançar este objetivo.

Aos meus anjos de quatro patas: Jade e Mike, que não têm noção do quanto são importantes para mim;

A Deus e todas as forças do universo por me permitirem chegar até aqui e não me deixarem desistir;

Muito obrigada!

“Não é sobre chegar no topo do mundo e saber que venceu
É sobre escalar e sentir que o caminho te fortaleceu
É sobre ser abrigo e também ter morada em outros corações
E assim ter amigos contigo em todas as situações”
Ana Vilela

“Aquilo que é impenetrável para nós existe de fato. Por trás dos segredos da natureza há algo sutil, intangível e inexplicável. A veneração a essa força que está além de tudo o que podemos compreender é a minha religião.”

Albert Einstein

RESUMO

O mundo está enfrentando uma crise da biodiversidade generalizada que afeta particularmente os ambientes aquáticos devido à sobre-explotação pesqueira, aliada à poluição e à perda de habitat. Uma estratégia para reduzir a perda de espécies é o estabelecimento de áreas protegidas (AP). No entanto, para que as APs sejam bem-sucedidas é necessário conhecer a biodiversidade que está sendo protegida para designar ações de manejo eficazes. A Área de Proteção Ambiental Costa dos Corais (APACC) é a maior AP costeira do Brasil, localizada na província Atlântico Sudoeste Tropical, e tem como um dos objetivos ordenar a atividade pesqueira, de modo a reduzir seu impacto. Dentro da APACC um grupo de peixes muito visado pela pesca artesanal são as tainhas. Entretanto, é difícil mensurar se esta atividade pesqueira tem impactado as populações naturais de tainha, uma vez que várias espécies ocorrem em simpatria nesta região e é desafiador distingui-las utilizando caracteres de morfologia externa devido à grande similaridade entre elas. Nesta tese foi utilizada uma abordagem integrativa, onde dados da plataforma GenBank e sequências de DNA inéditas foram utilizados para construir uma filogenia temporalmente calibrada para representantes do gênero *Mugil*. Além disso, dados de morfologia externa, DNA *barcoding* e sequenciamento de nova geração pelo método *DArT-seq* foram utilizados para delimitar as espécies de tainha que ocorrem na APACC e para fornecer dados para subsidiar ações de manejo. Com esses dados foi possível concluir que, embora as espécies possuam morfologia muito conservada, elas se diversificaram há aproximadamente 30 milhões de anos. Também foi possível corroborar a existência de espécies crípticas e parafiletismo em *M. cephalus*, *M. curema* e *M. rubrioculus*. Os dados de morfologia externa, DNA *barcoding* e *DArT-seq* confirmam a existência de cinco linhagens de tainhas na APACC, correspondendo às espécies: *M. brevirostris*, *M. curema*, *M. curvidens*, *M. liza* e *M. rubrioculus*. O registro histórico da espécie *M. incilis* na área revelou se tratar de identificação incorreta de indivíduos de *M. curema*. Com a análise de milhares de marcadores genômicos foi possível concluir que não existe fluxo gênico interespecífico, bem como não existe barreira ao fluxo gênico entre indivíduos coletados em estuários distantes 38km. Estimativas do tamanho efetivo populacional (N_e) de cada espécie revelaram alterações espécie-específicas ao longo do tempo. Desse modo, é sugerido que cada espécie deve ter um plano de manejo diferente. No entanto, como a identificação das espécies no momento do desembarque pesqueiro é dificultada pela ausência de caracteres diagnósticos, uma sugestão é definir, dentro da APACC, áreas onde a pesca é permitida, intercaladas com áreas de proteção integral, garantindo a manutenção da variabilidade genética dos estoques a longo prazo. Este estudo demonstra como a taxonomia integrativa, incluindo ferramentas moleculares e morfológicas, é essencial para estimar a biodiversidade em áreas protegidas, fornecendo dados importantes para o estabelecimento de ações de manejo e conservação.

Palavras-chave: taxonomia integrativa, estase morfológica, DNA *barcoding*, *DArT-seq*, datação molecular, conservação.

ABSTRACT

The world is facing a widespread biodiversity crisis that particularly affects aquatic environments due to overexploitation of fisheries coupled with pollution and habitat loss. A strategy to reduce species loss is to establish protected areas (PA). However, in order for PAs be successful, it is necessary to know the biodiversity that is being protected to designate effective management actions. The Costa dos Corais Environmental Protection Area (APACC) is the largest coastal PA in Brazil, located in the Atlantic Southwestern Tropical province, and one of its objectives is to order fishing activity to reduce its impact. Within APACC, a group of fish mostly targeted by artisanal fisheries is the mullet group. Nevertheless, it is difficult to measure whether those fisheries activities had impacted the natural mullet populations, since several species occur in sympatry in this region and it is challenging to distinguish them using characters of external morphology due to the great similarity among them. In this thesis an integrative approach was used, where data from the GenBank platform and new DNA sequences were used to build a temporally calibrated phylogeny for mullets of the *Mugil* genus. Furthermore, data of external morphology, DNA barcoding and new generation sequencing by the *DArT-seq* methodology were used to delimit mullet species occurring in APACC, providing data to support management actions. With these data it was possible to conclude that, although the species have very conserved morphology, they diversified approximately 30 million years ago. It was also possible to corroborate the existence of cryptic species and paraphyly in *M. cephalus*, *M. curema* and *M. rubrioculus*. Data from external morphology, DNA barcoding and *DArT-seq* confirm the existence of five lineages of mullets in APACC, corresponding to the species: *M. brevirostris*, *M. curema*, *M. curvidens*, *M. liza* and *M. rubrioculus*. The historical record of the species *M. incilis* in the area revealed to be incorrect identification of individuals of *M. curema*. With the analysis of thousands of genomic markers, it was possible to conclude that there is no interspecific gene flow, as well as there is no barrier to gene flow between individuals collected in 38km apart estuaries. Estimation of the effective population size (N_e) of each species revealed that it has not remained constant over time and was not uniform for all species. Thus, it is suggested that each species should have a different management plan. However, as the identification of species at the time of fisheries landings is hampered by the absence of diagnostic characters, a suggestion is to define, within the APACC, areas where fishery is allowed interspersed with areas of integral protection, ensuring the maintenance of long-term genetic variability of the stocks. This study demonstrates how integrative taxonomy, including molecular and morphological tools, is essential for estimating biodiversity in protected areas, providing important data for the establishment of management and conservation actions.

Keywords: integrative taxonomy, morphological stasis, DNA barcoding, *DArT-seq*, molecular dating, conservation.

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1 APRESENTAÇÃO

Tainhas são peixes costeiros da família Mugilidae, ordem Mugiliformes, que ocorrem no mundo todo (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016). São estuarino-dependentes, ou seja, passam grande parte do seu ciclo de vida na água doce dos rios e estuários e migram para o oceano no período de reprodução (NORDLIE, 2016), tendo grande importância nos fluxos de matéria orgânica entre esses habitats (LEBRETON *et al.*, 2011). Uma característica marcante dos mugilídeos é a estase morfológica dentro da família, que faz com que seja um desafio identificar as espécies com base em caracteres de morfologia externa (DURAND; SHEN; *et al.*, 2012).

Os peixes dessa família são alvo da pesca comercial em todos os lugares em que ocorrem (CROSETTI, 2016; MENEZES, 1983), estando entre os cinco tipos de peixes mais comercializados no Brasil (MPA, 2011). A pesca coloca essas espécies sob forte ameaça devido à redução de seus estoques, levando à perda de variabilidade genética, o que deixa as espécies mais suscetíveis a extinções locais (HAUSER *et al.*, 2002). Isso também pode ocasionar a redução do tamanho dos indivíduos de uma população e início da maturação sexual em indivíduos jovens (KUPARINEN; MERILÄ, 2007). Todos esses fatores fazem com que seja urgente o estabelecimento de medidas que assegurem a manutenção dos estoques de tainhas a longo prazo.

Uma das ferramentas mais importantes para evitar ou reduzir a perda de biodiversidade é a implementação de áreas protegidas (SINGH, 2002). O Brasil possui mais de 300 áreas protegidas no ambiente marinho e costeiro (SCHIAVETTI *et al.*, 2013), sendo a maior área protegida costeira denominada ‘Área de Proteção Ambiental Costa dos Corais’ (APACC), com mais de 400 mil ha de área e 120 km de praias e mangues (SOUZA, C. D. DE; BATISTA; FABRÉ, 2012). Um dos principais objetivos da APACC é garantir o uso sustentável dos recursos naturais, seja esse uso direto (como a pesca, por exemplo) ou indireto (como turismo) (ICMBIO, 2013).

Diversas espécies de tainha ocorrem em simpatria na APACC (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015; TORRES *et al.*, 2008). Essa co-ocorrência pode ser explicada por separações de nicho trófico (LELOC’H *et al.*, 2015) e por diferenças no período reprodutivo (RANGELY, 2011), que reduziriam os níveis de

competição interespecífica. No entanto, como é difícil delimitar essas espécies simpátricas usando caracteres morfológicos, a identificação das espécies no desembarque pesqueiro e na comercialização se torna um desafio. Por esse motivo, todos os mugilídeos comercializados na APACC são classificados em uma única categoria denominada ‘tainha’ para fins de estatística pesqueira (IBAMA, 2007), dificultando o estabelecimento de ações de manejo e conservação das diferentes espécies.

Além das dificuldades de identificar corretamente as espécies de tainha, análises moleculares utilizando marcadores mitocondriais e nucleares têm apontado a existência de linhagens crípticas dentro desse grupo (DURAND; SHEN; *et al.*, 2012; DURAND; BORSA, 2015; FRAGA *et al.*, 2007; HERAS; ROLDÁN; CASTRO, 2009; XIA, R.; DURAND; FU, 2016). No entanto, outros estudos refutaram essa hipótese integrando dados morfológicos e marcadores mitocondriais, mostrando que as espécies estudadas são compostas de uma única linhagem (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015; SICCHA-RAMIREZ *et al.*, 2014).

De fato, para a delimitação de espécies crípticas, é indicado o uso de várias fontes de informação independentes para avaliar possíveis contradições entre diferentes conjuntos de dados, como aspectos ecológicos, reprodutivos, morfológicos e genéticos (FIŠER; ROBINSON; MALARD, 2018). Essa abordagem é denominada taxonomia integrativa e vem sendo aplicada com sucesso em vários estudos para delimitar espécies e revelar diversidade críptica (ANJOS *et al.*, 2020; ARRIBAS *et al.*, 2013; HAELEWATERS; DE KESEL; PFISTER, 2018). Entretanto, é preciso ter cuidado ao se escolher os conjuntos de dados que serão analisados para não gerar interpretações enviesadas. No que diz respeito aos aspectos genéticos, por exemplo, a taxa de substituição nucleotídica varia em cada genoma (mitocondrial e nuclear) e até mesmo em diferentes genes, o que pode levar a respostas incongruentes entre estudos com diferentes marcadores (TOEWS; BRELSFORD, 2012). Por isso, a escolha do marcador molecular a ser utilizado em cada estudo depende do objetivo e do nível taxonômico almejado (PARKER *et al.*, 1998).

Uma abordagem que pode se mostrar promissora para o estudo da diversidade das tainhas é o ‘Sequenciamento de Fragmento de DNA Associado a Enzimas de Restrição’ (*RAD-seq*) (BAIRD *et al.*, 2008), uma técnica inovadora de ‘Sequenciamento de Nova Geração’ (NGS) que tem sido muito utilizada em estudos

de ecologia, evolução e conservação (ANDREWS *et al.*, 2016). Várias técnicas surgiram após o *RAD-seq*, como o *DArT-seq*, uma técnica muito semelhante, mas com algumas diferenças metodológicas (SANSALONI *et al.*, 2011). Essa abordagem tem se mostrado adequada para avaliação de diversidade inter e intraespecífica (AL-BREIKI *et al.*, 2018; COUCH *et al.*, 2016; MAKOMBU *et al.*, 2019; MARTIN *et al.*, 2019), sendo também útil para estimar índices demográficos pretéritos e atuais (PAZMIÑO *et al.*, 2017; SOUZA, F. H. S. DE *et al.*, 2019), dados importantes para o manejo de espécies ameaçadas e exploradas comercialmente.

Na presente pesquisa foi primeiramente realizada uma análise filogenética temporalmente calibrada das espécies do gênero *Mugil*, utilizando sequências disponíveis no GenBank e sequências inéditas, para entender melhor a história evolutiva do grupo. Em seguida dados morfológicos, dados de DNA mitocondrial e de NGS pelo método *DArT-seq* foram usados para desvendar a diversidade das tainhas presentes na APACC, delimitando possíveis linhagens crípticas e estimando parâmetros de demografia histórica. Com esses dados é possível subsidiar ações de manejo eficazes para garantir a continuação dos estoques de tainha a longo prazo na APACC.

2 REVISÃO DE LITERATURA

2.1 Crise da biodiversidade

A expansão das populações humanas tem levado ao aumento da exploração de recursos naturais, bem como a mudanças no uso da terra (BAKKER; SVENNING, 2018; SINGH, 2002). Essas atividades resultaram em séria ameaça à biodiversidade global, impulsionada pela perda de habitat, introdução de espécies invasoras, poluição, mudanças climáticas e sobre-explotação em níveis superiores ao reabastecimento natural (MORA; ZAPATA, 2013; WWF, 2016). Por esses motivos, a taxa de extinção é cerca de 3 ordens de grandeza maior do que a estimada por registros fósseis (MORA; ZAPATA, 2013; SINGH, 2002) e inúmeras espécies estão desaparecendo antes mesmo de serem conhecidas (SINGH, 2002). Nos ambientes aquáticos, essa crise levou à redução da abundância média de populações em cerca de 36% em habitats marinhos e 81% em habitats de água doce entre 1970 e 2012 (WWF, 2016). A principal ameaça para as espécies de água doce é a perda de habitat, enquanto para espécies marinhas tem sido a sobre-explotação por meio da pesca extrativa (WWF, 2016).

2.1.1 Pesca extrativa

O setor pesqueiro é uma fonte de renda e meio de subsistência para milhões de pessoas ao redor do mundo (FAO, 2010), sendo também fonte de proteínas, micronutrientes e ácidos graxos essenciais (FAO, 2005). Existem duas formas para obtenção dessa fonte de proteína: por meio da pesca extrativa ou por cultivo, uma técnica denominada aquicultura.

A pesca extrativa pode ser definida como a retirada de organismos aquáticos da natureza, podendo ser em pequena escala, também denominada pesca artesanal, ou em grande escala, denominada pesca industrial (IBAMA, 2007). A aquicultura se refere ao processo de criação de organismos aquáticos em cativeiro, podendo ser realizada no mar (maricultura) ou em águas continentais (aquicultura continental) (IBAMA, 2007).

Analisando a evolução da produção do pescado no Brasil no período entre 1960 e 2010 (Fig. 1.1) pode-se notar um grande aumento entre os anos de 1965 e 1985, seguido por um grande declínio até 1990, voltando a aumentar devido à produção por aquicultura, indicando exaustão dos estoques pesqueiros marinhos e continentais no Brasil (VIANA, 2013).

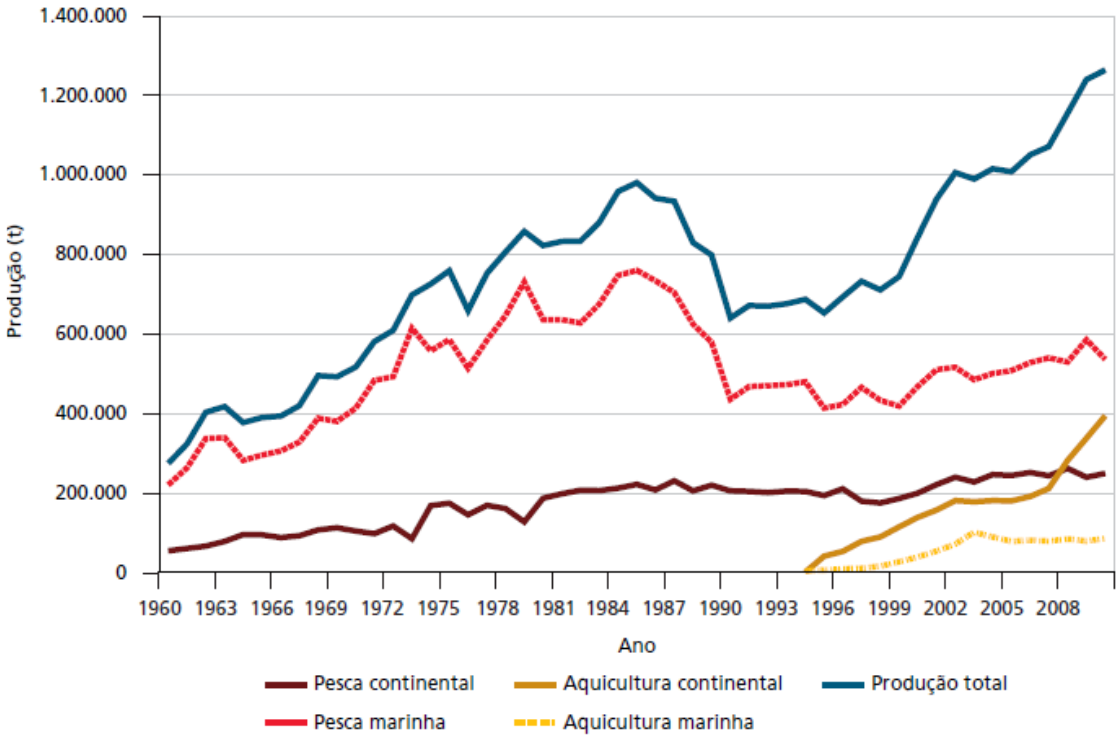

Figura 1.1 – Produção da pesca extrativa e da aquicultura em água marinhas e continentais do Brasil, no período entre 1960 e 2010 (fonte: VIANA, 2013).

Mais da metade do pescado capturado no mundo é derivado da pesca artesanal (FAO, 2010), caracterizada pelo uso de tecnologia simples e baixo investimento de capital (BATISTA *et al.*, 2014), ocorrendo em pequena escala e com uso de métodos tradicionais (HAWKINS; ROBERTS, 2004). Cerca de 90% das pessoas registradas como pescadores ou piscicultores pela Organização de Alimento e Agricultura das Nações Unidas são classificados dentro da modalidade artesanal (FAO, 2004). Essa atividade é, portanto, fonte de subsistência local, segurança alimentar e alívio da pobreza para muitas comunidades (OLIVEIRA JÚNIOR *et al.*, 2016). Apesar da pesca artesanal ser considerada menos impactante ao meio ambiente quando comparada à pesca industrial (FAO, 2005), já foi demonstrado que

esta prática pode também afetar os níveis de biomassa e composição das comunidades (GOETZE *et al.*, 2011; HAWKINS; ROBERTS, 2004).

Essa modalidade de pesca é mais comum na região costeira das regiões tropicais, onde há grande diversidade de espécies, porém baixa abundância (BATISTA *et al.*, 2014). No Brasil, estima-se que 40% da produção pesqueira seja oriunda desse tipo de pesca, sendo predominante nas regiões norte (81%) e nordeste (65%) (IBAMA, 2007). No estado de Alagoas, por exemplo, 79% do pescado produzido é oriundo da pesca artesanal, sendo o 15º estado com maior produção nessa modalidade, onde aproximadamente 21 mil pescadores são registrados em 36 colônias oficiais (ALAGOAS, 2008) e 57 comunidades pesqueiras (IBAMA, 2007). Entretanto, além dos impactos causados às comunidades ícticas pelos pescadores locais, há também evidências de que a área é explorada por frotas pesqueiras oriundas de diversos estados do Brasil (RANGELY, 2010), aumentando a pressão nos estoques locais.

2.1.2 Impactos da Pesca

De acordo com dados recentes, 31,4% dos estoques de peixes estão em níveis biologicamente insustentáveis ou sobre-explotados (FAO, 2016). A sobre-explotação dos estoques tende a reduzir a proporção de indivíduos maiores e em estágio de maturação mais avançados nas populações, promovendo a maturação sexual de indivíduos jovens e ocasionando a reprodução de indivíduos menores e com baixa fecundidade (KUPARINEN; MERILÄ, 2007), alterando assim características importantes da população. Além disso, a sobre-explotação e a redução dos estoques dos grandes peixes carnívoros leva ao chamado *fishing down,* ou seja, o direcionamento da pesca para níveis inferiores da cadeia trófica, como peixes planctívoros e aqueles que se alimentam de pequenos invertebrados, o que causa grandes mudanças em toda a estrutura trófica das comunidades (LIANG; PAULY, 2020; PAULY *et al.*, 1998). Desse modo, é necessário o manejo da pesca para garantir a manutenção dos estoques locais de peixe a curto prazo (CARVALHO; HAUSER, 1994), evitando o *fishing down* e a redução do tamanho dos indivíduos na população.

Outra grave consequência da pesca excessiva é a redução dos níveis de variabilidade genética, e consequente perda de alelos responsáveis pela adaptação

das espécies a novos ambientes (HAUSER *et al.*, 2002), o que pode ocasionar o colapso da população. Assim, a longo prazo, é necessário o manejo não apenas das populações locais de peixe, mas também da diversidade genética entre e dentro das populações (CARVALHO; HAUSER, 1994).

Populações maiores tendem a apresentar maior diversidade genética do que populações pequenas e períodos prolongados com baixo tamanho populacional levam à redução da diversidade genética (OVENDEN *et al.*, 2013). No entanto, essa questão vai além do tamanho populacional censitário (N_c), uma vez que é o tamanho efetivo populacional (N_e)[1] o que determina a capacidade de uma população se manter ao longo do tempo evolutivo (DUSSEX; ROBERTSON, 2018; HAUSER *et al.*, 2002; WANG, J.; SANTIAGO; CABALLERO, 2016) e, geralmente, o N_e é muito reduzido em relação ao N_c (HAUSER *et al.*, 2002). A diversidade genética neutra (aquela que não é afetada pela seleção natural contida em uma população) deve ser proporcional ao produto N_e x μ, onde μ é a taxa de mutação por geração. Dessa forma, seria possível acessar valores de N_e entre as espécies a partir da diversidade genética (GALTIER, NICOLAS; ROUSSELLE, 2020). Espécies com N_c maior e estável ao longo das gerações tendem a apresentar maiores níveis de diversidade genética e, portanto, maiores valores de N_e (LEFFLER *et al.*, 2012). Entretanto, N_c não é o único fator que influencia o N_e, este também pode ser afetado pela desproporção entre indivíduos machos e fêmeas de uma população, aumento dos níveis de endogamia, variação no número de descendentes entre os indivíduos da população, estruturação genética (CHARLESWORTH, 2009) e também pelo sistema de acasalamento (NUNNEY, 1993), fatores que não necessariamente levam a redução de N_c.

Para o pargo da Nova Zelândia, *Pagrus auratus* (Sparidae), por exemplo, há evidências de perda de diversidade genética estimada pelos níveis de heterozigosidade e número médio de alelos ao longo dos 50 anos desde o início da sua exploração (HAUSER *et al.*, 2002). Essa perda de diversidade genética foi surpreendente visto que o tamanho mínimo estimado da população censitária seria de mais de 3 milhões de peixes, evidenciando que o N_e é várias ordens de grandeza menor que o N_c, o que provavelmente foi causado pela pesca excessiva (HAUSER *et al.*, 2002). Um outro estudo comparou a diversidade genética de espécies de peixes marinhos com registro de sobrepesca e espécies filogeneticamente próximas que não possuem registro de sobrepesca (PINSKY; PALUMBI, 2014). Os dados apontaram

1. Tamanho efetivo populacional (N_e) é uma estimativa dos indivíduos que contribuem geneticamente para as gerações futuras, fornecendo informações sobre a história demográfica que ajudam a avaliar as probabilidades de extinção ou manutenção das populações (DUSSEX; ROBERTSON, 2018; WANG, J.; SANTIAGO; CABALLERO, 2016).

que a pesca excessiva pode ser a responsável pela redução da variabilidade genética em 32 espécies, em uma proporção pequena, mas significativa. A redução encontrada de 2% nos níveis médios de heterozigosidade (p=0,03) indica que as populações sofreram redução populacional recente, o que, em longo prazo, pode impactar o potencial evolutivo das espécies (PINSKY; PALUMBI, 2014).

Órgãos gestores de pesca, historicamente, não tem dado a devida atenção ao manejo da diversidade genética dos estoques pesqueiros, o que leva a incertezas sobre como a sobre-explotação afeta as espécies no nível molecular (PINSKY; PALUMBI, 2014). Esse fato soma-se a outras dificuldades do manejo da pesca artesanal: primeiro, a grande rotatividade de pescadores e dos apetrechos utilizados tornam difíceis o levantamento e a análise dos dados; segundo, o impacto das pescarias artesanais frequentemente se sobrepõem ao de outras atividades costeiras como o turismo, pesca industrial e recreativa, navegação e mineração; e por fim, o desenvolvimento de políticas para o manejo precisa levar em conta fatores locais socioeconômicos, culturais e históricos para obter êxito (BATISTA *et al.*, 2014; OLIVEIRA JÚNIOR *et al.*, 2016).

2.1.3 O papel das áreas protegidas

Uma das principais estratégias mundiais de manejo e conservação para evitar a perda de biodiversidade é o estabelecimento de Áreas Protegidas (AP) (SINGH, 2002). Embora o número destas áreas venha aumentando nas últimas décadas (WATSON *et al.*, 2014), essa expansão não atinge os diferentes biomas em proporções iguais (VIEIRA, R. R. S.; PRESSEY; LOYOLA, 2019). Além disso, estudos globais apontam para um déficit significativo de eficácia, onde muitas áreas protegidas não alcançam objetivos básicos, havendo evidências de que populações de espécies ameaçadas estão em declínio em algumas AP (WATSON *et al.*, 2014). Este fato se mostra contraditório, pois uma AP bem-sucedida deveria garantir a proteção da biodiversidade em escalas ecológicas e evolutivas (PINHEIRO *et al.*, 2019).

Em relação aos ecossistemas marinhos, a implementação de APs vem aumentando em todo o mundo, com dois objetivos principais: proteger a biodiversidade e regular a pesca (STORI; SHINODA; TURRA, 2019). Todavia, esse aumento tem sido muito mais lento do que o relativo a ambientes terrestres (WATSON

et al., 2014). No Brasil existem 336 áreas costeiras e marinhas protegidas, sendo aproximadamente 16 milhões de hectares de área protegida (SCHIAVETTI *et al.*, 2013). A maior AP costeira do Brasil é denominada 'Área de Proteção Ambiental Costa dos Corais' (APACC), criada em 1997, com mais de 400 mil ha e cerca de 120 km de praias e manguezais (BRASIL, 1997). Está localizada na província Atlântico Sudoeste Tropical (SPALDING *et al.*, 2007), abrangendo oito municípios do estado de Alagoas e quatro municípios do estado de Pernambuco (BRASIL, 1997). Ocupa 1,3% da costa nacional, incluindo em parte um importante sistema de recife de coral do continente americano (GARZÓN-FERREIRA *et al.*, 2002). A APACC é organizada em parcelas denominadas 'Zonas', designadas com base nas demandas locais, onde cada uma exige ações de gerenciamento diferentes (ICMBIO, 2013).

Uma das grandes demandas, não apenas da APACC, mas em todas as regiões costeiras dos trópicos, são as pescarias artesanais (BATISTA *et al.*, 2014), uma atividade muito desenvolvida nesta AP. Embora não existam dados de estatística pesqueira para a APACC, em Alagoas a espécie mais capturada no ano de 2007 foi a tainha (*Mugil* spp.) (1.295,5 toneladas) e em Pernambuco a manjuba *Opisthonema oglinum* (Clupeidae) (734,5 toneladas), ambas oriundas exclusivamente da pesca artesanal (IBAMA, 2007). Esses dados reforçam a importância do manejo da pesca artesanal nessa AP. No entanto, muitos aspectos do manejo da pesca dependem da identificação precisa das espécies exploradas e não exploradas, uma vez que os indivíduos alvo da pesca, os indivíduos capturados não-intencionalmente ou os afetados de outra forma, precisam ser identificados corretamente para manter registros precisos e dessa forma contribuir no manejo da pesca (OVENDEN *et al.*, 2013) e garantir a efetividade das APs.

2.2 Caracterizando a biodiversidade

A falta de informações taxonômicas acuradas sobre as espécies economicamente exploradas inviabiliza o desenvolvimento de planos de conservação e manejo a longo prazo, pois para isso é imprescindível saber quais espécies estão envolvidas e se existem subpopulações dentro dessas espécies e como identificá-las (FAO, 2013)

A função básica e também o desafio da taxonomia alfa é caracterizar as unidades que compõe a biodiversidade de uma determinada região — isto é, determinar os limites entre espécies ou populações subespecíficas (SEIFERT; RITZ; CSOSZ, 2014). Isso pode ser feito utilizando diferentes fontes de dados, como morfologia externa, anatomia interna e genética, entre outras, cada uma apresentando vantagens e desvantagens (FAO, 2013).

2.2.1 Dados morfológicos

A abordagem morfológica visa conhecer as espécies, entender suas características e diferenciá-las através de caracteres morfológicos selecionados (DUNN, 2003). Ela se faz essencial, uma vez que a base da classificação dos seres vivos é a semelhança fenotípica e as relações filogenéticas entre as espécies (SARASWATI; SRINIVASAN, 2016). Dessa forma, os limites entre variação fenotípica intraespecífica (de acordo com estágio ontogenético, sexo, e condições de habitat) e a variação interespecífica são estabelecidos através da análise dos caracteres morfológicos (SARMIENTO; STINER; MOWBRAY, 2002).

A análise de caracteres morfológicos ao longo dos diferentes níveis taxonômicos também ajuda a entender como se deu a evolução desses caracteres, mostrando onde ocorreram as mudanças de estado. De modo geral, quanto mais diferenças morfológicas acumuladas entre dois táxons relacionados, mais alto é o nível taxonômico que compartilham (SARMIENTO; STINER; MOWBRAY, 2002). Existem vários métodos para quantificar o acúmulo de diferenças morfológicas entre os táxons e a escolha do método depende do objetivo da análise. É importante também ter em mente que as técnicas estatísticas possuem pressupostos e é necessário saber se os caracteres escolhidos atendem a esses pressupostos (SARASWATI; SRINIVASAN, 2016).

Existem muitas vantagens na utilização de dados morfológicos para a caracterização da biodiversidade e estabelecimento de relações filogenéticas. Entre elas está a possibilidade de analisar um grande número de indivíduos a um baixo custo, incluindo espécimes preservados em museus — o que é mais difícil para análises moleculares, por exemplo, que possuem um alto custo e requerem amostras

frescas (HILLIS; WIENS, 2000). Além disso, o estudo da morfologia é a única maneira de incluir a informação de grande parte dos fósseis nas filogenias, sendo imprescindível para a polarização dos caracteres e para o estabelecimento das relações entre os grupos viventes (HILLIS; WIENS, 2000). Ademais, muitas situações práticas (como condições degradantes de armazenamento) excluem o acesso a outro tipo de informações como genéticas ou bioquímicas (ČANDEK; KUNTNER, 2015).

A caracterização da biodiversidade com base em caracteres morfológicos funciona bem quando as diferenças fenotípicas entre os organismos são óbvias, mas é passível de erros à medida em que exista sobreposição de dados em qualquer um dos caracteres em consideração, o que é comum de ocorrer em espécies crípticas (SEIFERT; RITZ; CSOSZ, 2014).

2.2.2 Espécies crípticas

De acordo com o conceito geral de linhagem, as espécies não são entidades isoladas, mas fragmentos temporais de uma linhagem evolutiva que pode ou não ser morfológica e ecologicamente divergente (DE QUEIROZ, 1998). Isso ocorre porque as taxas de divergência genética e morfológica não são correlacionadas, pois mutações genéticas ocorrem principalmente de maneira neutra e estocástica, acumulando-se em uma linhagem a uma taxa fixa, ao passo que as modificações morfológicas sofrem influência direta do ambiente e não são previsíveis ao longo do tempo (ČANDEK; KUNTNER, 2015; KALIONTZOPOULOU; PINHO; MARTÍNEZ-FREIRÍA, 2018). Três mecanismos podem estar por trás da ausência de modificações morfológicas entre as linhagens: 1) divergência recente: as linhagens divergiram há pouco tempo e as modificações morfológicas ainda não são evidentes; 2) conservadorismo de nicho: a evolução de nicho e, portanto, a diferenciação morfológica entre as linhagens é contida pela seleção natural em um fenômeno conhecido como estase morfológica; 3) convergência morfológica: a similaridade morfológica pode ter surgido de maneira independente em resposta a pressões seletivas semelhantes (FIŠER; ROBINSON; MALARD, 2018). Nesses casos, os caracteres morfológicos para diagnóstico das espécies não são evidentes e a identificação destas pode ser difícil, apesar da divergência genética (OVENDEN *et al.*, 2013).

Quando duas ou mais linhagens são classificadas como a mesma espécie devido à ausência de caracteres morfológicos distintos, chamamos de linhagens crípticas ou espécies crípticas (DETWILER; BOS; MINCHELLA, 2010). Desse modo, um complexo de espécies pode incluir várias linhagens crípticas, podendo ser totalmente indistinguíveis morfologicamente ou apresentar pequenas variações morfológicas (DELIĆ *et al.*, 2017; KORSHUNOVA *et al.*, 2017). Existem ainda as chamadas espécies pseudo-crípticas, que são espécies muito parecidas morfologicamente que podem ser distinguidas umas das outras após uma análise cuidadosa dos caracteres morfológicos, no entanto, essas espécies apresentam altos níveis de identificação incorreta (MANN; EVANS, 2008).

Por esse motivo, a utilização de dados genéticos tem se mostrado uma abordagem mais promissora para avaliar divergências inter e intraespecíficas em linhagens próximas do que uma abordagem baseada apenas em dados morfológicos (COATES; BYRNE; MORITZ, 2018), levando a um aumento exponencial na descrição de espécies crípticas e pseudo-crípticas marcadas por uma notável estase morfológica. Por exemplo, quatro linhagens crípticas foram encontradas com base em análises genéticas, revelando táxons potencialmente não descritos no gênero *Eucinostomus* (Gerreidae), um grupo de peixes composto por 11 espécies com relevância comercial para a pesca local, identificadas por diferenças morfológicas sutis e sobrepostas (JACOBINA *et al.*, 2020).

Nesse contexto, a genética é uma ferramenta versátil e muito útil para o manejo pesqueiro de espécies crípticas e pseudo-crípticas, uma vez delimitadas as linhagens com dados moleculares, caracteres morfológicos diagnósticos confiáveis podem ser identificados (OVENDEN *et al.*, 2013).

2.2.3 Taxonomia integrativa

Taxonomia integrativa é uma abordagem indicada para explorar a diversidade em complexos de espécies crípticas (PADIAL *et al.*, 2010), onde diferentes conjuntos de dados são utilizados para avaliar a biodiversidade (FIŠER; ROBINSON; MALARD, 2018). Por exemplo, o uso de dados morfológicos e genéticos corroborou a presença de 15 candidatas a novas espécies de peixes na bacia no Rio Jequitinhonha (estado de Minas Gerais, Brasil) sendo duas delas espécies crípticas (PUGEDO *et al.*, 2016).

Outro exemplo de estudo utilizando a taxonomia integrativa foi feito com o bagre *Hypostomus chrysostiktos* (Loricariidae), que após a análise de diferentes fontes de dados (caracteres merísticos, morfometria geométrica, citogenética e análises moleculares) foi realocada ao gênero *Pterygoplichthys* (Loricariidae) (ANJOS *et al.*, 2020). Esses exemplos mostram como a taxonomia integrativa pode contribuir para a identificação de espécies e sinalizar candidatas a novas espécies a partir de linhagens crípticas (PUGEDO *et al.*, 2016). Isso é de vital importância, visto que espécies são unidades fundamentais dos planos de conservação e por isso precisam ser bem delimitadas (COATES; BYRNE; MORITZ, 2018).

Além disso, no que diz respeito a planos de manejo e conservação, recomenda-se também que estudos taxonômicos integrando dados genéticos sejam utilizados, pois a heterogeneidade intra-específica é muito importante para a persistência a longo prazo das espécies (uma vez que essa heterogeneidade torna as espécies mais resilientes a mudanças ambientais) (SVANCARA *et al.*, 2005). Por esse motivo, investigar a presença de diversidade críptica dentro de espécies ameaçadas ou comercialmente exploradas é imprescindível (BICKFORD *et al.*, 2006), bem como analisar seus parâmetros populacionais, como taxas de migração e abundância (OLIVIER *et al.*, 2010).

2.2.4 Ferramentas moleculares

Nas últimas três décadas, a utilização de ferramentas moleculares permitiu testar se a diversidade de espécies obtida pela análise de caracteres morfológicos reflete linhagens evolutivas independentes (AVISE, 2009; MORITZ *et al.*, 2018; MORITZ; POTTER, 2013). Para esses estudos moleculares, qualquer parte do genoma pode fornecer informações importantes (PLEINES; JAKOB; BLATTNER, 2009; TAUTZ *et al.*, 2003). Entretanto, é recomendado o uso combinado de diferentes marcadores, numa tentativa de entender a história evolutiva das espécies de maneira mais completa, pois diferentes regiões do genoma podem apresentar padrões evolutivos distintos, de acordo com eventos de seleção natural, hibridização e assimetrias sexuais (TOEWS; BRELSFORD, 2012).

No ramo da pesca, estudos genéticos têm sido utilizados para resolver a falta de padronização entre nomes científicos, nomes populares e nomes de mercado e,

além disso, a análise da estrutura genética do estoque tem revelado a escala de manejo mais apropriada para cada espécie (OVENDEN *et al.*, 2013). Quatro tipos de marcadores moleculares têm sido historicamente muito utilizados para responder a esses diferentes objetivos: aloenzimas, DNA mitocondrial (DNAmt), microssatélites e, mais recentemente, polimorfismos de nucleotídeo único (SNPs) (HAUSER; SEEB, 2008). No entanto, para escolher o marcador molecular mais apropriado para cada abordagem, três questões devem ser levadas em conta: o número de *loci* independentes, níveis de variabilidade detectados em cada *locus*, e como as pressões seletivas podem estar moldando a variabilidade genética (HAUSER; SEEB, 2008).

2.2.4.1 Aloenzimas

Aloenzimas são enzimas codificadas por diferentes alelos no mesmo *locus* que possuem a mesma função, mas têm diferentes padrões de migração de eletroforese (MONTEIRO; MARCET; DORN, 2010). A variação nas aloenzimas é detectável separando-as em gel por eletroforese (HAUSER; SEEB, 2008). Esse método foi desenvolvido no final da década de 1970 e, por não exigir equipamentos ou reagentes caros, foi amplamente utilizado para caracterizar a variação genética entre populações e para identificar espécies (KRAUSE; BRAND, 2016). Nas décadas de 80 e 90 foi bastante utilizado em espécies de peixe para fins de conservação (KUCUKTAS; LIU, 2007), mas foi gradualmente sendo substituído por marcadores mais variáveis (MONTEIRO; MARCET; DORN, 2010).

Para analisar as aloenzimas, proteínas são extraídas e isoladas de cada indivíduo, aplicadas em um gel e submetidas à eletroforese, assim as proteínas são separadas com base na carga e no tamanho. O gel é então corado para uma enzima particular e bandas discretas são produzidas para diferentes aloenzimas (MONTEIRO; MARCET; DORN, 2010). Uma das desvantagens de usar aloenzimas é que as bandas (alelos) que têm a mesma carga elétrica e migram para o mesmo ponto no gel podem não ser homólogas (ou seja, convergência evolutiva). Além disso, a interpretação dos géis se torna difícil quando as bandas são fracas ou próximas umas das outras (STEPIEN; KOCHER, 1997).

2.2.4.2 DNA mitocondrial (DNAmt)

Marcadores de DNAmt são utilizados para responder questões em diferentes escalas temporais, desde eventos recentes (por exemplo para avaliar estruturação genética populacional), até eventos antigos (como relações filogenéticas em diferentes níveis taxonômicos hierárquicos) (BALLARD; WHITLOCK, 2004). O DNAmt também é bastante utilizado na identificação e delimitação de espécies (e.g. HEBERT *et al.*, 2003). Um exemplo de gene mitocondrial muito utilizado em pesquisas sobre delimitação de espécies nas últimas décadas é o Citocromo C Oxidase subunidade I (COI). Esse gene possui grande amplitude de sinais filogenéticos, pois a sua evolução é rápida o suficiente para permitir a discriminação de espécies relacionadas, bem como grupos filogeográficos dentro de uma mesma espécie (HEBERT *et al.*, 2003). Por esses motivos, foi proposta a utilização desse marcador como um código de barras genético (DNA *barcode*) em um sistema global de bioidentificação (HEBERT *et al.*, 2003).

A meta inicial do projeto DNA *barcode* foi a criação de uma biblioteca de referência de sequências *barcode* para todas as espécies conhecidas. '*The Barcode of Life Data System*' (BOLD – http://www.barcodinglife.org) é um depósito para os registros de sequências *barcode*, fornecendo um veículo que facilita a colaboração entre a comunidade científica (RATNASINGHAM; HEBERT, 2007), já tendo sido depositadas sequências de mais de 316 mil espécies. No entanto, esse marcador possui a desvantagem de caracterizar uma história uniparental (herança matrilineal), além do que a sequência pode conter heteroplasmia[2] (KMIEC; WOLOSZYNSKA; JANSKA, 2006) e as linhagens são rapidamente separáveis devido à alta taxa de variabilidade (ZHANG, D.; HEWITT, 1996). A alta variabilidade, por sua vez, traz o problema da saturação[3] (ARBOGAST *et al.*, 2002; XIA, X., 2009) o que pode gerar resultados equivocados para divergências mais antigas.

2.2.4.3 Microssatélites

Microssatélites, ou repetições de sequência simples (SSRs), são repetições em tandem que possuem de um a seis pares de bases que estão presentes em genomas

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2. Heteroplasmia é designada como um estado em que mais de um genótipo mitocondrial ocorre em um organismo (KMIEC; WOLOSZYNSKA; JANSKA, 2006).

3. Saturação ocorre quando o gene é tão variável que várias substituições nucleotídicas ocorrem no mesmo sítio, reduzindo a informação filogenética do marcador (ARBOGAST et al., 2002; XIA, X., 2009).

eucariotos e procariotos, tanto em regiões codificadoras como não codificadoras, e geralmente são caracterizadas por um alto grau de polimorfismo (ZANE; BARGELLONI; PATARNELLO, 2002). Uma vez que as sequências flanqueadoras dos microssatélites são conservadas, esses marcadores podem ser obtidos por meio de PCR usando um par de *primers* que flanqueiam o microssatélite (YOUNG; VIVIER, 2010). Essa técnica é particularmente útil devido à abundância dos microssatélites no genoma, alto grau de variabilidade e reprodutibilidade dos marcadores (YOUNG; VIVIER, 2010). Esses marcadores retratam processos contemporâneos e podem descrever melhor algumas características ecológicas e traços de história de vida como transporte e dispersão de indivíduos (MAI *et al.*, 2014).

O método possui algumas desvantagens como a necessidade do conhecimento prévio do genoma da espécie para o desenvolvimento de marcadores espécie-específico (ZANE; BARGELLONI; PATARNELLO, 2002), o que torna o método trabalhoso e caro. Além disso, como os microssatélites podem ter estruturas e/ou história evolutivas complexas, é possível que alelos de tamanho idêntico sejam produtos de linhagens evolutivas diferentes. Isso pode resultar em inferências falsas, onde alelos de mesmo tamanho são considerados homólogos, mas na verdade constituem homoplasias (CHAMBERS; MACAVOY, 2000). Embora esses marcadores sejam muito utilizados em estudos de genética populacional (MONTEIRO; MARCET; DORN, 2010), não são os mais indicados para reconstruções filogenéticas devido aos modelos mutacionais empregados (CHAMBERS; MACAVOY, 2000).

2.2.4.4 Polimorfismos de nucleotídeos únicos (SNPs)

As tecnologias de sequenciamento de DNA de nova geração (NGS) têm um impacto cada vez maior no manejo da pesca, pois fornecem grandes volumes de dados (aumentando o poder das análises), além de velocidade de processamento e economia sem precedentes (OVENDEN *et al.*, 2013). Diversos métodos de NGS se baseiam nos polimorfismos de nucleotídeos únicos (SNPs), que são milhares de variações únicas de nucleotídeos espalhadas pelo genoma que podem ser utilizados para detectar diferenças dentro e entre populações (NORRGARD; SCHULTZ, 2008).

‘Sequenciamento de Fragmento de DNA Associado à Enzima de Restrição’ (*RAD-seq*) é um método de sequenciamento de nova geração que permite o rápido

sequenciamento de milhares de SNPs em centenas de indivíduos com custo reduzido (BAIRD *et al.*, 2008). Este método é capaz de gerar uma representação do genoma por meio do sequenciamento de SNPs próximos a sítios reconhecidos por enzimas de restrição ao longo de todo o genoma (BAIRD *et al.*, 2008). Dentro da técnica de *RAD-seq* existem vários métodos para desenvolvimento da biblioteca genômica mas, no método tradicional, o DNA genômico é digerido por uma enzima de restrição, depois são adicionados adaptadores contendo sequências *barcode* (usadas para identificar as amostras que são amplificadas e sequenciadas juntas em uma única biblioteca, em um método denominado *multiplex*) e em seguida ocorre o corte mecânico para reduzir os fragmentos no comprimento apropriado para o sequenciamento, que varia de acordo com a metodologia utilizada (ANDREWS *et al.*, 2016). Dentre todas as vantagens do método por *RAD-Seq*, duas se destacam: 1) não é necessário conhecimento prévio do genoma estudado e nem um genoma de referência, uma vez que as enzimas de restrição digerem o DNA em posições conhecidas; 2) excelente relação entre custo e benefício para o sequenciamento de vários marcadores distribuídos por todo o genoma em muitos indivíduos (ANDERSON *et al.*, 2017; DA FONSECA *et al.*, 2016).

Vários métodos de sequenciamento de nova geração foram desenvolvidos utilizando o princípio descrito para o *RAD-seq*. Um destes métodos foi o *DArT-seq* desenvolvido pela empresa '*Diversity Arrays Technology*' (SANSALONI *et al.*, 2011). Essa abordagem é muito semelhante à anterior, mas difere pela utilização de duas enzimas de restrição, que cortam o DNA em diferentes pontos e geram o fragmento que será sequenciado (KILLIAN *et al.*, 2012). Vários pares de enzimas de restrição são testados, otimizando o método para cada espécie, visando as enzimas com maiores índices de reprodutibilidade dos fragmentos gerados. Nesse método, o fragmento final consiste em cerca de 75 pb, contendo um ou mais SNPs (COUCH *et al.*, 2016) (Fig. 1.2).

DArT-seq tem sido utilizado em estudos de genética da conservação, caracterizando estrutura populacional (JUNGE *et al.*, 2019; MARTIN *et al.*, 2019), verificando a mistura genética e hibridização (GEORGES *et al.*, 2018; MAKOMBU *et al.*, 2019; PAZMIÑO *et al.*, 2019) e avaliando os índices de demografia histórica e N_e (PAZMIÑO *et al.*, 2017; SOUZA, F. H. S. DE *et al.*, 2019). Dessa forma, os dados gerados pelo método do *DArT-seq* podem fornecer informações valiosas para

conservação e manejo, tendo sido aplicado a espécies de grande importância econômica, historicamente exploradas. Alguns exemplos são a lagosta *Panulirus homarus* (Palinuridae), explorada no Oriente Médio (AL-BREIKI *et al.*, 2018); os tubarões *Carcharhinus brachyurus* e *C. obscurus* (Carcharhinidae) (JUNGE *et al.*, 2019), e camarões do gênero *Macrobrachium* (Palaemonidae) (MAKOMBU *et al.*, 2019), explorados globalmente; e peixes do gênero *Osteoglossum* (Osteoglossidae) comercializados na região amazônica (SOUZA, F. H. S. DE *et al.*, 2019).

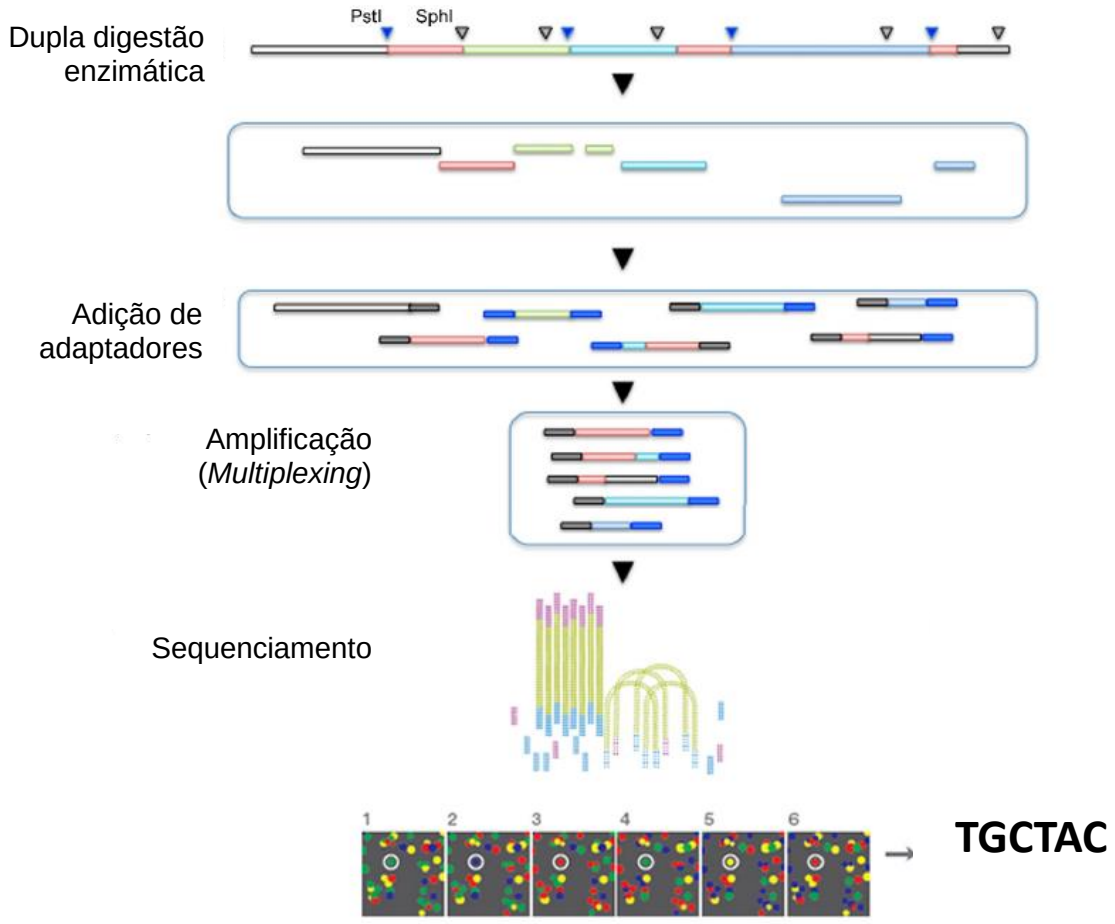

Figura 1.2 – Ilustração do método de preparação da biblioteca por *DArT-seq*, desde o momento da digestão enzimática, ligação de adaptadores, amplificação e sequenciamento (adaptado de GRUBER; GEORGES, 2018).

Apesar da eficácia evidenciada pelos estudos listados, existem críticas em torno das técnicas baseadas na metodologia *RAD-seq* devido à possível baixa cobertura em sequências com genomas grandes e perda de polimorfismos importantes durante as etapas de bioinformática (LOWRY *et al.*, 2017). Além disso,

existem ainda críticas relacionadas às dificuldades no estabelecimento de homologias devido à baixa quantidade de sítios conservados (CARIOU; DURET; CHARLAT, 2013). Entretanto, outros autores advogam em favor da técnica, alegando que é eficaz mesmo considerando suas limitações pois, é uma abordagem poderosa e eficiente que fornece uma grande quantidade de marcadores (CATCHEN *et al.*, 2017), sendo suficiente para estudar diferentes questões em populações naturais. Além disso, é possível adaptar a técnica para diferentes conjuntos de dados e sistemas específicos (ANDREWS *et al.*, 2016). O uso de um genoma de referência garante maior confiabilidade aos resultados, bem como a escolha correta dos softwares de análises e suas configurações (SHAFER *et al.*, 2017).

2.3 Tainhas: espécies marcadas por estase morfológica

Os peixes conhecidos como tainhas (Mugiliformes: Mugilidae) são alvo da pesca comercial ao longo de toda a sua distribuição (CROSETTI, 2016; PACHECO-ALMANZAR *et al.*, 2017; WHITFIELD; PANFILI; DURAND, 2012). Dados globais de 2013 apontam que foram capturadas aproximadamente 130 mil toneladas de tainhas naquele ano (CROSETTI, 2016). Além da ameaça da pesca, essas espécies geralmente são mais impactadas devido ao seu comportamento migratório (JONSSON; WAPLES; FRIEDLAND, 1999), estando portanto suscetíveis às ameaças das águas continentais e dos oceanos, sendo com frequência capturadas em grandes números quando agrupadas. A proposição de planos de manejo para esse grupo se torna um desafio, porque diversas espécies ocorrem em simpatria (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015) e é difícil diferenciá-las utilizando caracteres de morfologia externa, pois são marcadas por estase morfológica (DURAND; SHEN; *et al.*, 2012).

2.3.1 Diversidade e biologia das tainhas

São chamadas de tainhas todas as espécies alocadas na ordem Mugiliformes, representada por uma única família, Mugilidae. A família possui 4 subfamílias e 25 gêneros (XIA, R.; DURAND; FU, 2016) e conta atualmente com 79 espécies válidas (ESCHMEYER; FONG, 2020). São peixes costeiros que ocorrem em água tropicais,

subtropicais e temperadas (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016). No entanto, muitos autores discordam sobre o número e a distribuição geográfica de várias espécies desse grupo devido à morfologia externa conservada, que dificulta a delimitação acurada das espécies (DURAND; BORSA, 2015).

São peixes eurihalinos e euritérmicos (SECKENDORFF; AZEVEDO, 2007) e algumas espécies habitam água doce e salobra durante a maior parte do seu ciclo de vida, migrando para esse ambiente no início do desenvolvimento, mas retornam ao mar para reproduzir, sendo por isso referidas como espécies catádromas (NORDLIE, 2016). Além dessa migração sazonal para reprodução, mugilídeos também podem migrar das áreas costeiras (onde se alimentam) para o mar aberto (LELOC'H *et al.*, 2015), desempenhando um papel importante nos fluxos de matéria orgânica entre esses habitats (LEBRETON *et al.*, 2011).

O período reprodutivo das tainhas varia entre as espécies (TORRES *et al.*, 2008). Em Alagoas, por exemplo, a reprodução de *Mugil curema* Valenciennes, 1836 ocorre no primeiro trimestre do ano, de *M. curvidens* Valenciennes, 1836 no segundo trimestre (RANGELY, 2011; TORRES *et al.*, 2008) e *M. liza* Valenciennes, 1836 no terceiro trimestre (durante a estação chuvosa) (SOUSA; FABRÉ; BATISTA, 2015). Além disso, as espécies podem exibir diferentes preferência de habitats. Por exemplo, foi verificado que no rio Tramandaí, no estado do Rio Grande do Sul, indivíduos de *M. curema* estão associados a águas de alta salinidade, enquanto indivíduos de *M. liza* estão associados a águas menos salinas (MAI *et al.*, 2018).

A dieta do grupo também varia conforme a espécie, de acordo com o habitat que estão colonizando, mas os adultos são bentófagos e frequentemente se alimentam de produtores primários (por exemplo, microfitobentos, material vegetal fresco ou detrítico) ou meiofauna (LEBRETON *et al.*, 2011). Apesar de haver sobreposição na dieta dos mugilídeos, a competição entre espécies simpátricas pode ser reduzida devido a diferenças no tamanho das partículas preferidas por cada espécie (LELOC'H *et al.*, 2015). Essa preferência trófica aliada a diferenças no comportamento reprodutivo (ALBIERI; ARAÚJO; UEHARA, 2010; IBÁÑEZ-AGUIRRE, 1993; SHEN; CHANG; DURAND, 2015), e diferentes usos de hábitat (MAI *et al.*, 2018; SHEN; CHANG; DURAND, 2015), podem ser os fatores que viabilizam a co-ocorrência dessas espécies morfologicamente similares e filogeneticamente próximas.

2.3.2 Espécies crípticas e relações filogenéticas no gênero *Mugil*

As espécies de Mugilidae são caracterizadas por uma morfologia externa notavelmente uniforme, assim como, a anatomia interna pouco variável. Essa uniformidade resulta em confusão contínua de identificação e classificação (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016). Diferentes autores analisaram diversas estruturas em mugilídeos buscando caracteres diagnósticos. Essas estruturas incluem: presença de pálpebra adiposa, ceco pilórico, dentição, formato do estômago, formato da cabeça, boca e lábios, mandíbula, ossos pré-orbirais, narinas, órgãos faringobranquiais, escamas, raios e espinhos das nadadeiras, número de voltas intestinais, otólitos, morfologia dos canais da linha lateral cefálica, pigmentação e padrões de melanóforos (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016; IBAÑEZ; COWX; O'HIGGINS, 2007; IBÁÑEZ; GALLARDO-CABELLO, 2005). No entanto, a família possui grande plasticidade fenotípica, não havendo um limite claro entre diferenças intra e interespecíficas (HARRISON *et al.*, 2007). Esse fato levou à descrição de 304 espécies dentro da família (ESCHMEYER; FONG, 2020), mas a maioria acabou sendo posteriormente sinonimizada devido à falta de evidências para sustentar sua validade (DURAND; CHEN; *et al.*, 2012).

Das espécies sinonimizadas, mais da metade foram originalmente descritas no gênero *Mugil* (FROESE; PAULY, 2020), que é o mais especioso da família, atualmente com 16 espécies validas (FROESE; PAULY, 2020). O gênero possui uma distribuição circunglobal em águas tropicais e temperadas (THOMSON, 1997) e, recentemente, uma chave dicotômica foi desenvolvida para a identificação de oito espécies de tainha do gênero *Mugil* que ocorrem no Caribe e na costa leste da América do Sul, com base em caracteres merísticos e morfométricos que, em princípio, deveriam ser capazes de distinguir todas as espécies simpátricas (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015). Entretanto, as diferenças de caracteres são muitas vezes reduzidas entre algumas espécies, polimórficas dentro de outras e, em muitos casos, aplicáveis a apenas um estágio ontogenético específico (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016). Além da morfologia extremamente conservada, vários estudos filogenéticos apontam que algumas espécies de *Mugil* são parafiléticas, constituindo complexos de espécies formados por diversas linhagens

crípticas (DURAND; CHEN; *et al.*, 2012; DURAND; SHEN; *et al.*, 2012; XIA, R.; DURAND; FU, 2016), onde o número de linhagens crípticas no gênero pode chegar a 17 (DURAND; BORSA, 2015).

A espécie de tainha com maior número de estudos é *Mugil cephalus* Linnaeus, 1758, espécie tipo do gênero (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016) com localidade tipo no Mediterrâneo (ESCHMEYER; FONG, 2020). É o mugilídeo com a distribuição geográfica mais ampla, ocorrendo nos oceanos, estuários e rios do mundo todo entre as latitudes 42°N e 42°S (WHITFIELD; PANFILI; DURAND, 2012), mas essa distribuição é disjunta (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016), e a espécie não ocorre na porção leste da América do Sul (MENEZES; OLIVEIRA; NIRCHIO, 2010). Vários estudos apontam que *M. cephalus* é um complexo de espécies composto por várias linhagens crípticas, incluindo *M. liza* (localidade tipo Caribe, (ESCHMEYER; FONG, 2020)) (DURAND *et al.*, 2017; DURAND; SHEN; *et al.*, 2012; DURAND; BORSA, 2015; HERAS; ROLDÁN; CASTRO, 2009; WHITFIELD; PANFILI; DURAND, 2012).

Mugil curema apresenta localidade tipo na Bahia, Brasil (ESCHMEYER; FONG, 2020). Esta espécie foi recuperada como um complexo de espécies por vários estudos, incluindo outras espécies taxonomicamente reconhecidas como *M. incilis* Hancock, 1830 (localidade tipo Guiana (ESCHMEYER; FONG, 2020)), *M. margaritae* Menezes, Nirchio, Oliveira & Siccharamirez, 2015 (localidade tipo ilha de Margarida (ESCHMEYER; FONG, 2020)) e *M. thoburni* Jordan & Starks, 1896 (localidade tipo Galápagos (ESCHMEYER; FONG, 2020)) (DURAND; SHEN; *et al.*, 2012; FRAGA *et al.*, 2007; HERAS; ROLDÁN; CASTRO, 2009; NIRCHIO *et al.*, 2017; SICCHA-RAMIREZ *et al.*, 2014). *Mugil margaritae* na verdade era considerada uma linhagem de *M. curema* e, somente em 2015, foi descrita como espécie nova com base em caracteres morfológicos e genéticos (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015). Interessante notar que neste complexo de espécies são encontradas também diferenças no padrão citogenético (NIRCHIO *et al.*, 2017): apesar da maioria dos membros da família Mugilidae possuir a mesma estrutura cariotípica básica com 2n = 48 (como é o caso de *M. cephalus* e *M. liza*, por exemplo), várias linhagens do complexo *M. curema* divergiram desse padrão (GALETTI JR.; AGUILAR; MOLINA, 2000). *Mugil curema stricto sensu* possui 2n = 28 (NIRCHIO *et al.*, 2005), enquanto a linhagem do pacífico tem seu cariótipo caracterizado por 2n = 46 (NIRCHIO *et al.*,

2017). Já *M. incilis* tem o cariótipo caracterizado por 2n = 28 (GALETTI JR.; AGUILAR; MOLINA, 2000), enquanto *M. margaritae* é caracterizada por 2n = 24. Diferenças no cariótipo contribuem para o isolamento reprodutivo pós-zigótico e são características utilizadas para descrever espécies crípticas (DINCĂ *et al.*, 2011).

Foi detectada também uma linhagem críptica em *M. rubrioculus* Harrison, Nirchio, Oliveira, Ron & Gaviria, 2007 (localidade tipo Venezuela (ESCHMEYER; FONG, 2020)) no Pacífico (DURAND; BORSA, 2015), embora alguns autores não considerem a existência dessa espécie nessa região (BARLETTA; DANTAS, 2016; MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015). Além desses casos, recentemente foi descrito um complexo de espécies envolvendo *M. hospes* Jordan & Culver, 1895 (localidade tipo México (ESCHMEYER; FONG, 2020)) e *M. brevirostris* (Ribeiro, 1915) (localidade tipo Brasil (ESCHMEYER; FONG, 2020)), com a descrição de uma linhagem críptica composta por indivíduos identificados como *M. hospes* de Belize e Venezuela, sendo grupo irmão de *M. brevirostris*, tornando *M. hospes* parafilético (NIRCHIO *et al.*, 2018).

2.3.3 Tainhas da província Atlântico Sudoeste Tropical

Na província Atlântico Sudoeste Tropical ocorre um único gênero de tainha, *Mugil* (BARLETTA; DANTAS, 2016), mas o número de espécies continua ser debatido (Fig. 1.3). *Mugil cephalus* e *M. platanus* (Günther, 1880) já fcoram registrados, mas posteriormente foi descoberto que ambos os casos se tratavam de identificações incorretas de *M. liza* (MENEZES; OLIVEIRA; NIRCHIO, 2010). Indivíduos de *M. hospes* também foram registrados nesta província, mas depois descobriu-se que se tratavam de espécimes de *M. brevirostris* (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015). Outro exemplo é *M. incilis*, cuja presença na região permanece em debate: uma revisão de espécies de tainhas da América do Sul indicou que essa espécie não atinge a província (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015), enquanto alguns autores continuam registrando essa espécie na área (BARLETTA; DANTAS, 2016; FREIRE; ARAÚJO, 2016).

Desse modo, as espécies com ocorrência confirmada na província Atlântico Sudoeste Tropical são cinco: *M. brevirostris*; *M. curema*; *M. curvidens*; *M. rubrioculus*; e *M. liza. Mugil brevirostris* é endêmica do Brasil, ocorrendo do Amapá até o Rio

Grande do Sul; *M. curema* ocorre no Atlântico Oeste, desde o Canadá até Argentina, no Atlântico leste, do Senegal ao Congo, e no Pacífico leste, da Califórnia ao Chile; *M. curvidens* ocorre no Atlântico sul, do Pará até o Rio de Janeiro; *M. liza* ocorre no Atlântico Oeste, do Golfo do México até a Argentina; e *M. rubrioculus* ocorre do sul do Caribe até a costa nordeste do Brasil (DURAND; WHITFIELD, 2016; MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015; PACHECO-ALMANZAR *et al.*, 2016). Essas cinco espécies já foram registradas simpatricamente na APACC (DA SILVA, V. E. L. *et al.*, 2017, 2018; DA SILVA, V. E. L.; FABRÉ, 2019), onde existe também o registro histórico de *M. incilis* (TORRES *et al.*, 2008) (Fig. 1.3).

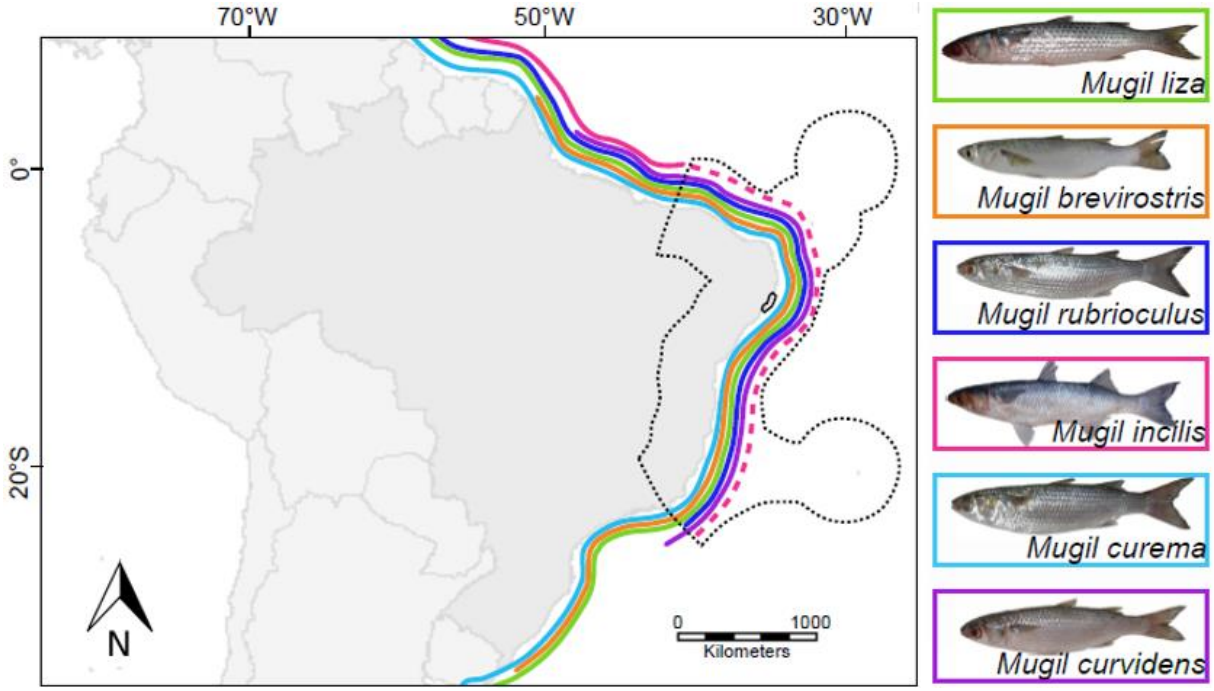

Figura 1.3 – Mapa de distribuição das tainhas na província Atlântico Sudoeste Tropical (representada pela área contornada pela linha preta pontilhada), destacando a Área de Proteção Ambiental Costa dos Corais (marcada pelo contorno em preto). Cada linha colorida contínua representa a distribuição de uma espécie de acordo com revisão taxonômica recente (MENEZES; DE OLIVEIRA; SICCHA-RAMIREZ, 2015). A linha rosa tracejada representa a incerteza sobre a ocorrência de *Mugil incilis* na região. (Foto de *M. incilis* por A. Carvalho).

2.3.4 Pesca da tainha

A similaridade morfológica entre as tainhas, o grande número de linhagens crípticas e a distribuição simpátrica de várias espécies, dificultam a legislação e o controle da pesca desse grupo. Devido a essa dificuldade taxonômica, todas as

espécies de tainha são atualmente agrupadas como *Mugil* spp. para fins de estatística pesqueira no Brasil (MPA, 2011).

As tainhas são comercialmente explorados em todas as regiões que ocorrem, constituindo parte importante da alimentação humana (CROSETTI, 2016; MENEZES, 1983). Relatos da importância da tainha na alimentação existem desde o século XVI (SECKENDORFF; AZEVEDO, 2007). No Brasil, elas são capturadas tanto em rios e estuários como no oceano, com uma grande diversidade de apetrechos e métodos de pesca, especialmente por pescadores artesanais, mas também são alvo importante da pesca industrial no sul e sudeste do país (SECKENDORFF; AZEVEDO, 2007). Os principais apetrechos de pesca artesanal de tainha no nordeste são: rede de cerco, rede de espera e tarrafa, com malhas de diferentes tamanhos para captura de espécies diferentes de tainha (TORRES *et al.*, 2007). Pescadores artesanais relatam que já houve redução da abundância das tainhas, entretanto eles atribuem essa redução à pesca industrial (SOUZA, M. R.; BARRELLA, 2001).

A pesca industrial é caracterizada pela coleta mecânica, resultando em altas taxas de captura (GRANZOTTO *et al.*, 2004; JACQUET; PAULY, 2008). Essa modalidade é predominante em regiões temperadas, onde há alta abundância e baixa diversidade de espécies (BATISTA *et al.*, 2014). No Brasil, apenas 25% do pescado é oriundo desta prática (IBAMA, 2007), sendo essa concentrada nas regiões sul e sudeste. A captura é realizada, principalmente, por grandes frotas especializadas em determinadas espécies (GIULIETTI; ASSUMPÇÃO, 1995). Quando se trata de tainha, o alvo principal é *M. liza*, que passou de uma produção de 1.000 tonelada na década de 1980, para 4.100 toneladas, a partir do ano 2000 (MPA/MMA, 2015), e já está sofrendo significativa redução de estoque (SANT'ANA *et al.*, 2017; VIEIRA, J. *et al.*, 2019).

Devido à alta importância comercial da espécie, um estudo de genética de populações utilizando seis genes mitocondriais buscou avaliar se existem subdivisões populacionais em *M. liza* ao longo do sul do Caribe e América do sul e concluiu que essa espécie é composta por uma única população (SICCHA-RAMIREZ *et al.*, 2014). No entanto, um outro estudo utilizando microssatélites concluiu que existem duas populações geneticamente estruturadas, uma composta pelos indivíduos coletados no Rio de Janeiro e outra composta pelos indivíduos coletados em São Paulo, Santa Catarina, Rio Grande do Sul e Buenos Aires (MAI *et al.*, 2014). Dados desse estudo

foram utilizados para embasar o plano de gestão para a pesca sustentável de *M. liza* no sudeste e sul do Brasil, o qual inclui medidas importantes como proibição da pesca com rede em cada um dos estoques em diferentes épocas do ano, redefinição do tamanho mínimo de captura, estabelecimento de áreas de exclusão da pesca com rede e limitação do esforço de pesca (MPA/MMA, 2015). Esse resultado evidencia mais uma vez como a escolha do marcador é importante, fazendo toda a diferença para subsidiar o manejo e conservação.

2.3.5 Importância das tainhas na APACC

Embora não exista pesca industrial na APACC, a pesca das tainhas tem grande importância socioeconômica, visto que representa cerca de 21% da pesca artesanal no estado de Alagoas (que abrange 8 dos 12 municípios dessa AP) (IBAMA, 2007). Nessa modalidade de pesca, várias espécies de tainha são capturadas e caracteres como tipo de escama, cor do olho, formato da cabeça, da cauda e do corpo, são utilizados pelos pescadores para distinguir as espécies (MARQUES, 1991).

Apesar de se tratar de uma área protegida, não existe o controle da captura de tainhas na região e, sendo um recurso vulnerável, pode haver prejuízos caso seja explorada indevidamente (SECKENDORFF; AZEVEDO, 2007). No entanto, para o desenvolvimento de planos de manejo a nível específico, são necessários dados que promovam a correta caracterização das espécies, além de informações sobre as características populacionais (RODRIGUES-FILHO *et al.*, 2009).

Para a delimitação acurada de espécies com potenciais linhagens crípticas e cujos caracteres morfológicos tem baixo poder diagnóstico (DURAND; SHEN; *et al.*, 2012) é fundamental a utilização de uma abordagem de taxonomia integrativa (ARRIBAS *et al.*, 2013). A análise de diferentes fontes de dados, incluindo técnicas de sequenciamento de nova geração (que têm se mostrado ideais para delimitar linhagens evolutivas quando os caracteres morfológicos não são suficientes (PANTE *et al.*, 2015)), podem ser a chave para a delimitação das espécies de tainha na APACC. Além disso, esses dados podem fornecer informações populacionais importantes para subsidiar um plano de manejo eficaz que permita a conservação das espécies a longo prazo, garantindo assim, além da perpetuação das espécies, a atividade e renda de milhares de pescadores artesanais.

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3 OBJETIVOS

3.1 Objetivos gerais

O objetivo geral desta tese foi caracterizar linhagens evolutivas de tainhas na APACC utilizando dados genéticos disponíveis nas plataformas GenBank e Bold system, dados de morfologia externa, DNA *barcoding* e de *DArT-seq*, a fim de embasar a conservação dos estoques pesqueiros.

Esta tese foi dividida em três capítulos, cada um correspondente a um dos seguintes objetivos gerais:

1. Datação molecular: estimar o tempo de divergência entre as linhagens do gênero *Mugil*;

2. Diversidade: determinar quantas linhagens evolutivas de tainhas ocorrem simpatricamente em um sistema estuarino tropical utilizando DNA *barcoding* e dados de morfologia externa;

3. Demografia: avaliar o tamanho efetivo populacional (N_e) e as alterações demográficas históricas utilizando milhares de fragmentos genômicos obtidos por *DArT-seq*.

3.2 Objetivos específicos

Capítulo 1: Divergência genética profunda e parafilia em espécies crípticas de peixes *Mugil* (Actinopterygii: Mugilidae)

1) Identificar o número de linhagens evolutivamente independentes no gênero *Mugil*;

2) Estimar o tempo de diversificação dos principais clados.

Capítulo 2: Taxonomia integrativa revela extremo conservadorismo morfológico em espécies simpátricas de *Mugil* no Atlântico Sudoeste Tropical

1) Determinar a precisão da delimitação das espécies com base na morfologia externa;

2) Estimar quantas linhagens evolutivas ocorrem simpatricamente na APACC;

3) Correlacionar a similaridade morfológica com o tempo desde a divergência entre as espécies.

Capítulo 3: Métodos genômicos revelam níveis ocultos de diversidade em espécies de peixes de alta importância ecológica e econômica

1) Determinar o número de espécies de *Mugil* na APACC;

2) Quantificar os padrões de conectividade genética entre as espécies de tainha simpátricas no mesmo estuário e dentro de uma espécie em dois estuários;

3) Estimar o tamanho efetivo populacional relativo entre as espécies de tainha e inferir se estes são determinados por histórias demográficas espécie-específicas.

4 CAPÍTULO 1

Deep genetic divergence and paraphyly in cryptic species of *Mugil* fishes (Actinopterygii: Mugilidae)[4]

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Abstract

Morphological conservatism among closely related species often results in incongruent taxonomic classification between studies, leading to disagreements about the inferred evolutionary history of the species. This is the case in *Mugil* fishes, where extreme morphologic conservatism contrasts with wide distributions and high genetic divergence. To understand how these morphologically similar species evolved, it is necessary to 1) test whether taxonomically recognised species are independently evolving lineages, and 2) evaluate the timing and geographic context of the diversification of these lineages. Based on three mitochondrial genes and fossil data, we estimated a comprehensive time-calibrated phylogeny for 13 out of the 16 valid species of *Mugil* and tested the monophyly of each species. Our results indicate that the diversification of *Mugil* began nearly 30 MYA, during a period of large temperature fluctuations. *Mugil cephalus*, *M. curema* and *M. rubrioculus* all form relatively old groups (between 5 to 10 MYA) and form paraphyletic entities. Our study reinforces the general finding that morphologically and ecologically similar species may have long and independent evolutionary histories, which must be considered when assessing the evolution and conservation of such ecologically and economically important species.

Keywords: conservation, fossil calibration, grey mullets, molecular dating, monophyly, species complex.

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4. Artigo publicado na revista Systematics and Biodiversity (qualis A2, percentil 77% pelo Scopus em novembro de 2020): Neves, J. M. M., Almeida, J. P. F. A., Sturaro, M. J., Fabré, N. N., Pereira, R. J., & Mott, T. (2020). Deep genetic divergence and paraphyly in cryptic species of *Mugil* fishes (Actinopterygii: Mugilidae). *Systematics and Biodiversity*, 18(2), 116–128. doi: 10.1080/14772000.2020.1729892

4.1 INTRODUCTION

Species diversity results from the interplay between the temporal splitting of evolutionary lineages and their interaction with the environment (Brooker, 2006; Cavender-Bares, Kozak, Fine, & Kembel, 2009; Losos, 2008). In this context, species should not be considered as isolated entities, but rather as temporal fragments of an evolutionary lineage that may (or may not) be morphologically and ecologically divergent (De Queiroz, 1998). Indeed, genetic divergence of independent lineages is not always reflected by phenotypic differentiation because constraints of adaptation to similar environments may lead to morphological conservatism (Lavoué et al., 2011). Thus, robust assessment of species diversity first requires genetic analyses, ideally focused on functionally neutral loci that are not subject to strong selective pressures (Kaliontzopoulou, Pinho, & Martínez-Freiría, 2018). These genetic relationships can later be compared with morphological patterns of diversity, which are more likely to be affected by natural selection (Lande, 1976).

Over the last three decades, the application of genetic tools throughout the tree of life has allowed testing whether species diversity reflected by morphological criteria is an indicator of independent evolutionary lineages (Avise, 2009; Moritz & Potter, 2013; Moritz et al., 2018). A continuously growing number of studies reveals that levels of species diversification are significantly higher in molecular markers, relative to morphological characters (Coates, Byrne, & Moritz, 2018). This has been accompanied by an exponential increase in the description of cryptic species (Jacobina et al., 2013; Lima, Freitas, Araujo, & Solé-Cava, 2005; Victor, 2014), i.e. genetically distinct but morphologically similar species (Poulin & Pérez-Ponce de León, 2017).

Although molecular studies are biased towards temperate areas of the globe, particularly in the northern hemisphere, studies in tropical areas have also shown that species diversity is largely underappreciated, and that even previously known biodiversity hotspots harbour a large number of cryptic species (e.g. Carnaval, Hickerson, Haddad, Rodrigues, & Moritz, 2009; Gaither & Rocha, 2013; Moritz et al., 2018; Vieites et al., 2009). Marine biomes seem to be particularly rich in cryptic species, as high levels of genetic divergence are observed between presumably morphologically identical species of shrimps, rays and bony fishes (Bilgin, Utkan,

Kalkan, Karhan, & Bekbolet, 2015; Borsa, Arlyza, Hoareau, & Shen, 2018; Bucciarelli, Golani, & Bernardi, 2002; Craig et al., 2009; Healey et al., 2018). Therefore, there is a need to evaluate the genetic patterns of diversity in tropical regions, where biodiversity is often understudied and where human exploitation is often less regulated (Hortal, de Bello, Diniz-filho, Lewinsohn, & Ladle, 2015; Jackson et al., 2001).

A good candidate for genetic analyses of cryptic diversity is the genus *Mugil* (Linnaeus, 1758), a geographically widespread species complex of marine fishes known commonly as grey mullets that are characterized by a remarkable level of morphological similarity (Durand, Shen, et al., 2012). To date, more than 200 species names have been proposed within *Mugil*, but currently only 16 are taxonomically recognised as valid (Froese & Pauly, 2019). This enormous uncertainty may stem from the fact that external morphological characters are few between the different species and are clearly not sufficient to distinguish the diversity of grey mullets species (Durand & Borsa, 2015). The lack of easily distinguishable diagnostic features has led to considerable taxonomic confusion and to the underestimation of species level diversity within *Mugil*.

Species of *Mugil* are considered of ecological significance because they promote organic matter fluxes between estuarine and oceanic environments (Lebreton, Richard, Parlier, Guillou, & Blanchard, 2011). They are also economically important, being commercially exploited throughout their distribution (Menezes, 1983). To improve the management and conservation of *Mugil*, a detailed understanding of the historical processes that shape the current morphological and genetic diversity within the entire genus is required.

Several phylogenetic studies of *Mugil* have highlighted that some taxonomically recognised species are paraphyletic and harbour independently evolving cryptic lineages (Durand & Borsa, 2015; Durand, Chen, Shen, Fu, & Borsa, 2012; Durand, Shen, et al., 2012; Xia, Durand, & Fu, 2016). But it remains unclear about how many lineages should be recognized, when these lineages diversified, and whether their diversification is coincident with major biogeographic events. We conducted a systematic review of publicly available DNA sequences of the *Mugil* genus in addition of new samples and performed a time-calibrated molecular phylogenetic analysis to: 1) identify the number of independently evolving lineages, in addition to the

taxonomically recognised species, and 2) estimate the time of diversification of major clades. Our results also help to elucidate the complex relationships between these species and shed light on evolutionary scenarios that could have led to the diversification of grey mullets.

4.2 MATERIALS AND METHODS

4.2.1 Data sampling

Using the Web of Science search engine, we identified all genetic studies of the genus *Mugil* with sequences deposited in GenBank (Benson et al., 2014).

We chose the individuals with the highest number of genes sequenced and more precise sample collection details, and the genes most represented across taxonomically recognized species. These selection criteria resulted in a dataset of three mitochondrial genes: 16S ribosomal RNA (16S), Cytochrome c oxidase subunit I (COI) and Cytochrome b (CYTB) (accession numbers in Table S4.1). Sequences for 12 of the 16 species were recorded in this database: *Mugil bananensis* (Pellegrin, 1927); *M. capurrii* (Perugia, 1892); *M. cephalus* Linnaeus, 1758, *M. curema* Valenciennes, 1836; *M. curvidens* Valenciennes, 1836; *M. hospes* Jordan & Culver, 1895; *M. incilis* Hancock, 1830; *M. liza* Valenciennes, 1836; *M. margaritae* Menezes, Nirchio, Oliveira & Siccha-Ramirez, 2015; *M. rubrioculus* Harrison, Nirchio, Oliveira, Ron & Gaviria, 2007; *M. thoburni* Jordan & Starks, 1896 and *M. trichodon* Poey, 1875.

This sampling encompassed a total of 52 individuals, including multiple individuals from the three species where potentially cryptic lineages have been suggested in the literature (*M. cephalus*, *M. curema* and *M. rubrioculus*; Durand & Borsa, 2015), and at least one individual from all the remaining lineages with available genetic data (*M. bananensis*, *M. capurrii*, *M. curvidens*, *M. hospes*, *M. incilis*, *M. liza*, *M. margaritae*, *M. thoburni* and *M. trichodon*). We also included one individual of the closely related taxa *Chaenomugil proboscideus* (Günther, 1861), *Dajaus monticola* (Bancroft, 1834), *Joturus pichardi* Poey, 1860, *Agonostomus catalai* Pellegrin, 1932, *Cestreus goldiei* (MacLeay, 1883) and *Neomyxus leuciscus* (Günther, 1872), to be

used as outgroups (Durand, Chen, et al., 2012; Durand, Shen, et al., 2012; Xia et al., 2016) in phylogenetic analyses.

Because some of the published sequences lack precise collection data or voucher specimens, we confirmed the identification of these specimens by sampling 17 new specimens belonging to eight species: *Mugil brevirostris*, *M. cephalus*, *M. curema, M. curvidens*, *M. hospes*, *M. incilis*, *M. liza*, and *M. rubrioculus* (Table S4.1). All the individuals were morphologically identified using a published identification key (Menezes, De Oliveira, & Siccha-Ramirez, 2015) or by personal communication with specialists. When possible, vouchers were deposited at zoological collections. Total genomic DNA was extracted from muscle tissue using the phenol-chloroform method (Sambrook, Fritsch, & Maniatis, 1989). A fragment of the barcode COI gene was amplified following the protocol of Neves et al. (2016). We performed Sanger sequencing only with the forward primer FISH-BCL (Baldwin, Mounts, Smith, & Weigt, 2009). This data was aligned with the 51 COI sequences available from the 52 individuals previously selected to calculate genetic distance and to build a Bayesian and a Maximum Likelihood phylogenetic trees.

Furthermore, in order to test the congruence between the mitochondrial topology and a nuclear topology, we retrieved 12 nuclear markers from GenBank: TMO4C4, SH3PX3, ENC1, GLYT, MYH6, PTR, RAG1, RNF213, SIDKEY, PLAG2, RHOD and SREB2. This sampling included 14 specimens belonging to 10 out of the 12 species included in the mitochondrial data set (Table S4.2). Importantly, all these individuals or sampling localities were also present in our new mitochondrial topology so that the two topologies could be better compared.

4.2.2 Specimen identification

The COI gene is the most common reference in barcoding projects of actinopterygian (ray-finned) fishes (e.g., Brandão et al., 2016; de Carvalho Lima, Egito, Feijó, de Arruda Mauro, & Ferraz, 2017; Pereira, Hanner, Foresti, & Oliveira, 2013; Ribeiro et al., 2012), thus we used this marker to calculate intra and interspecific genetic distances and to build the phylogenetic trees. Following the DNA barcode protocol, genetic divergence was inferred using the Kimura's two-parameter (K2P)

evolutionary model implemented in the software MEGA 7 (Kumar, Stecher, & Tamura, 2016), with a standard threshold of 2% between species (Hubert et al., 2008; Pereira et al., 2013; Rosso, Mabragaña, González Castro, & Díaz de Astarloa, 2012). For this analysis and phylogenetic trees, we used the 51 COI sequences from GenBank plus the 17 individuals sequenced here (Table S4.1).

We also performed Maximum Likelihood and Bayesian phylogenetic analyses to check the positions of sequences from GenBank related to our reference sequences, using only *Chaenomugil proboscideus* and *Neomyxus leuciscus* as outgroups. Maximum Likelihood trees were estimated using RAxML-HPC2 v8.2.12 (Stamatakis, 2014) at the CIPRES Science Gateway (Miller et al., 2010). We used a GTR+G model of evolution and a data partition by gene. Branches supports were evaluated through 1,000 rapid bootstrapping replicates.

The Bayesian analysis was implemented in MrBayes v3.2.6 (Ronquist et al., 2012). We estimated the best-fitting evolutionary models with PartitionFinder v2.1.1 (Lanfear et al., 2012) where we set a partition by codon position. The best models were TIMEF+I, F81+I and TRN+G for the first, second and third codon positions of COI gene, respectively. Two independent runs with four Markov chains starting from a random tree were performed, each composed by 10 million generations sampled every 1,000. The first 25% of trees were discarded as burn-in and nodes with posterior probability values higher than 0.95 were considered well-supported. Trees were visualized using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

4.2.3 Mitochondrial topology and divergence time estimation

Mitochondrial DNA sequences were aligned using the Q-INS-I algorithm for 16S and the G-INS-I algorithm for COI and CYTB, implemented in MAFFT v7.310 (Katoh & Standley, 2013). All positions of the 16S gene with internal gaps were removed due to ambiguities in the alignment. Subsequently, evolutionary models were selected for each gene through the Bayesian Information Criterion (BIC) implemented in PartitionFinder v2.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012). The best-fitting evolutionary models for each gene were 16S: SYM+I+G; COI: TRN+I+G+X; CYTB: HKY+I+G+X. Genes belonging to the same individual were then concatenated.

Topology and divergence times were co-estimated with a Bayesian approach and the support values for the branches were calculated through Bayesian posterior probabilities (PP), as implemented in BEAST v2.4.8 (Bouckaert et al., 2014) using the CIPRES Science Gateway v3.3 (Miller, Pfeiffer, & Schwartz, 2010). For dating estimates we used the Fossilized Birth-Death (FBD) model, so that all species are recognised as part of the same macro-evolutionary process and the estimation of diversification time is more robust (Heath, Huelsenbeck, & Stadler, 2014). We used a log-normal relaxed clock (Drummond, Ho, Phillips, & Rambaut, 2006), and nucleotide substitution rates of 1% for 16S, 1.2% for COI, and 2% for CYTB (Aboim, Menezes, Schlitt, & Rogers, 2005; Tringali, Bert, Seyoum, Bermingham, & Bartolacci, 1999).

The oldest known fossil representing *Mugil* is *M. princeps* from the Paleogene epoch, ~30–40 million years (MYA) (Patterson, 1993 as cited in McMahan et al., 2013). We used this fossil as a calibration point, including it as the most recent common ancestor (MRCA) of all *Mugil* species, to limit the age of the node clustering all members of *Mugil*. This calibration point was set to a log-normal prior and to a 95% probability range of 30–40 MYA (mean (M) = 3.55, standard deviation (S) = 0.071). The prior for the origin of the family was set to 64 MYA based on a prior study (Santini, May, Carnevale, & Moore, 2015).Three independent runs of the Bayesian analysis were performed, each composed of 200 million generations sampled every 20,000 generations. Stationarity and effective sample sizes (ESS > 200) were checked using Tracer v1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Logs and trees were combined using LogCombiner v2.4.5, after removing the initial 10% of the generation as burn-in. A maximum clade credibility tree was generated using TreeAnnotator v2.4.5 (Bouckaert et al., 2014). The inferred tree was visualised in the program FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and nodes were considered as significantly supported when posterior probabilities (PP) were above 0.95. Nodes representing the same lineage were collapsed.

4.2.4 Monophyly test and species delimitation

In order to test the monophyly of the taxonomically recognised species of *Mugil* we used the Shimodaira-Hasegawa test (SH) (SHIMODAIRA; HASEGAWA, 1999),

based on the three concatenated mitochondrial genes. This test evaluates the power of distinct topologies to explain the observed data by comparing the Maximum Likelihood (ML) tree where species are free to be paraphyletic, to alternative trees where species are constrained to be monophyletic (GOLDMAN; ANDERSON; RODRIGO, 2000). The ML analysis was performed using RAxML HPC2 v8.2.12 (STAMATAKIS, 2014) through the CIPRES gateway (MILLER; PFEIFFER; SCHWARTZ, 2010), using a general time-reversible (GTR+G) evolutionary model and a data partition by gene. Nodes supports were evaluated using 1,000 rapid bootstrapping replicates (BS). The best ML tree (Fig. S4.1) was then compared with three alternative trees where the following species were forced to be monophyletic: *Mugil cephalus*, *M. curema* and *M. rubrioculus*.

We also estimated species boundaries solely based on genetic divergence, irrespective of taxonomically recognised species. We used the Poisson Tree Processes model applied in a Bayesian analysis referred as bPTP (ZHANG, J. *et al.*, 2013). The bPTP method estimates species divergence based on the substitution rate per site between branching events, which is reflected in branch lengths (ARRIGONI *et al.*, 2016; ZHANG, J. *et al.*, 2013). The analysis was performed on the online server (https://species.h-its.org/), with 100,000 MCMC and 10% burn-in. The outgroups were removed as this analysis concerns only to *Mugil* lineages.

4.2.5 Congruence between mitochondrial and nuclear topologies

In order to test the monophyly of the taxonomically recognised species of *Mugil* we used the Shimodaira-Hasegawa test (SH) (Shimodaira & Hasegawa, 1999), based on the three concatenated mitochondrial genes. This test evaluates the power of distinct topologies to explain the observed data by comparing the Maximum Likelihood (ML) tree where species are free to be paraphyletic, to alternative trees where species are constrained to be monophyletic (Goldman, Anderson, & Rodrigo, 2000). The ML analysis was performed using RAxML HPC2 v8.2.12 (Stamatakis, 2014) through the CIPRES gateway (Miller et al., 2010), using a general time-reversible (GTR+G) evolutionary model and a data partition by gene. Nodes supports were evaluated using 1,000 rapid bootstrapping replicates (BS). The best ML tree (Fig. S4.1) was then

compared with three alternative trees where the following species were forced to be monophyletic: *Mugil cephalus*, *M. curema* and *M. rubrioculus*.

We also estimated species boundaries solely based on genetic divergence, irrespective of taxonomically recognised species. We used the Poisson Tree Processes model applied in a Bayesian analysis referred as bPTP (Zhang, Kapli, Pavlidis, & Stamatakis, 2013). The bPTP method estimates species divergence based on the substitution rate per site between branching events, which is reflected in branch lengths (Arrigoni et al., 2016; Zhang et al., 2013). The analysis was performed on the online server (https://species.h-its.org/), with 100,000 MCMC and 10% burn-in. The outgroups were removed as this analysis concerns only to *Mugil* lineages.

4.3 RESULTS

4.3.1 Specimen identification

By comparing 569 base pairs (bp) of the barcode COI gene between the published specimens with the reference specimens collected and morphologically analysed here, we were able to assess and correct several of the taxonomic identification for sequences of *Mugil* available on GenBank.

Average interspecific genetic distance was 16.9%. The lowest divergence was observed between *M. cephalus* and *M. liza* (2.8%) and the highest between *M. liza* and *M. hospes* (21.6%) (Table S4.3). For the 11 species for which we had more than one individual, mean intraspecific divergence was generally very low, bellow 1%. The three exceptions were *M. cephalus* (3.5%), *M*. *curema* (3.8%) and *M. rubrioculus* (2.6%), largely due to the paraphyly discussed below.

The low divergence within species and high divergence between species resulted in well-supported clades allowing for accurate species identification based solely on COI (Fig. S4.2).

Our results show two misidentifications of sequences derived from the GenBank. The *M. hospes* sequence from the GenBank collected in Brazil (Individual 42659 – LBP 10004) showed a genetic divergence of 7.9% relative to *M. hospes* from GenBank collected in Belize (Individual 306) and with our reference *M. hospes*

collected in Gulf of Mexico. Yet, this sequence is only 0.9% divergent from our reference *M. brevirostris* collected in Brazil (Table S4.4). This strongly supports the hypothesis that the putative *M. hospes* from Brazil (Individual 42659) is in fact *M. brevirostris.* Other misidentification was related to individuals of *M. curema*: the putative *M. curema* (Individual 30070 – LBP 6065) is genetically more similar to *M. margaritae* sequences (mean divergence=0.09%), rather than to conspecific sequences (mean divergence= 4.4%) (Table S4.4). All specimens of *M. cephalus* and *M. rubrioculus* were correctly identified, as they cluster with our reference individuals (Fig. S4.2). The two misidentified specimens (one of *M. hospes* and one of *M. curema*) were referred by their corrected species names in our analyses.

4.3.2 Topology and divergence time

The final matrix of mitochondrial sequences had a total of 1,939 bp: 557 of 16S (144 variable sites and 118 informative sites); 598 of COI (229 variable sites and 212 informative sites); and 784 of CYTB (351 variable sites and 313 informative sites).

The diversification of the *Mugil* species sampled here was estimated to have begun around ~30 MYA (23.7–40.0). The Bayesian analysis recovered *Mugil trichodon* was sister-group of all other congeneric species with strong statistical support (PP=0.95). All remaining species were recovered in two main clades: one including *M. bananensis*, *M. capurrii, M. cephalus* and *M. liza* (PP=0.93), with an estimated diversification time of ~26 MYA (17.7–34.8), and a second clade that includes *M. curema*, *M. margaritae*, *M. thoburni*, *M. incilis*, *M. curvidens*, *M. hospes, M. brevirostris* and *M. rubrioculus* (PP=0.99) with an estimated diversification time of ~23 MYA (15.6–31.5) (Fig. 4.1).

Within the first clade, *M. liza* was nested within *M. cephalus*, making *M. cephalus* a paraphyletic species (PP=1). The divergence of this clade was estimated to have initiated ~4.5 MYA (2.8–6.6). *Mugil cephalus* + *M. liza* are sister taxa to *M. bananensis* + *M. capurrii* (PP=1), the latter diverging about ~16 MYA (8.4–23.4).

In the second clade, two groups were recovered (PP=1 for both clades). In the first one, *M. curema* was paraphyletic, as both *M. thoburni* (PP=0.99) and *M. margaritae* (PP=0.99) were nested within it. These three lineages started diversifying

about ~6 MYA (3.6–8.5). These species are sister species to *M. incilis* (PP=1), and together, are sister taxa to *M. curvidens* (PP=1). In the second clade, *M. hospes* and *M. brevirostris* were recovered together and nested within a paraphyletic *M. rubrioculus* (PP=1), with diversification starting approximately ~10 MYA (5.7–14.0).

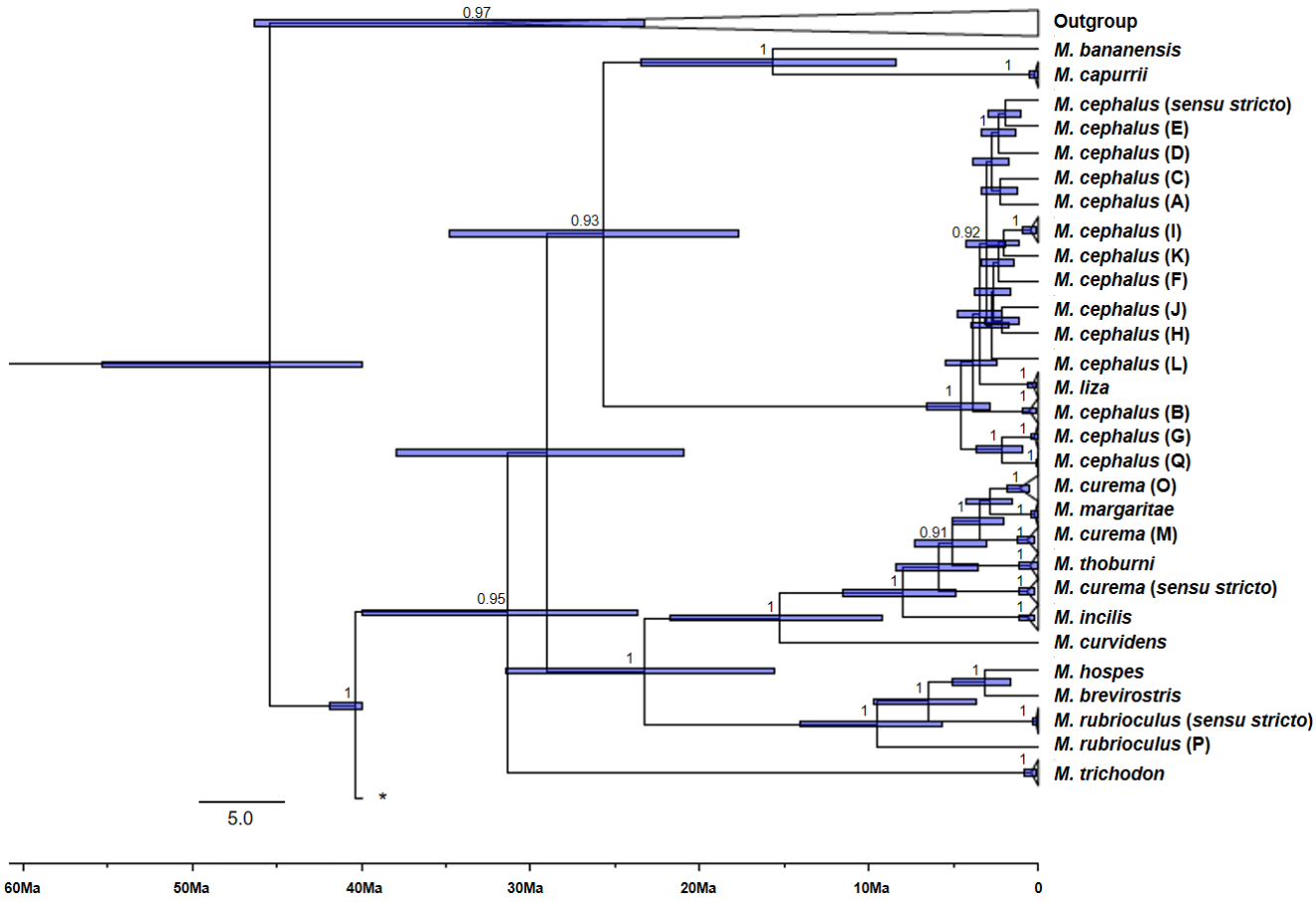

Figure 4.1 - Bayesian tree using fossilized birth-death model showing the relationships between 13 *Mugil* species based on 1939 bp of 16S, COI and CYTB mitochondrial genes. Letters in parentheses represent lineages described by Durand & Borsa, 2015. Posterior probability values are shown in near the nodes (values under 0.9 were omitted). Node bars represent 95% Highest Posterior Density (HPD) intervals of node ages. Geologic scale is plotted on the bottom in millions of years. Scale bar indicates 5 substitutions/site. * Calibration point with fossil data.

4.3.3 Monophyly test and species delimitation

The Maximum Likelihood tree based on the three mitochondrial genes corroborated the Bayesian analysis and recovered the same paraphyletic groups (Fig.

S4.1). *Mugil cephalus* is paraphyletic in relation to *M. liza* (BS=100), and its monophyly was rejected by the SH test (best tree -lnL= 15853.04, alternative tree -lnL= 15869.04, difference -lnL= 15.99, P-value<0.05). Likewise, *Mugil curema* is also paraphyletic with respect to *M. thoburni* and *M. margaritae*, although not so well-supported (BS=60). The monophyly of *M. curema* was also rejected by the SH test (alternative tree -lnL= 15878.07, difference -lnL= 25.03, P-value<0.02). *Mugil rubrioculus* was paraphyletic in relation to *M. hospes* and *M. brevirostris* in the most likely tree (BS=100), but this tree was not significantly better than the alternative tree where *M. rubrioculus* was forced to be monophyletic (alternative tree -lnL= 15860.03, difference -lnL= 6.99, P-value>0.05).

Based on the three studied mitochondrial genes, and disregarding current taxonomic nomenclature, the bPTP analysis estimated a total of 30 independently evolving lineages (Table S4.5). Four of these had low support (PP≤0.94). From the remaining 26 well-supported lineages (PP≥0.95), 13 are cryptic lineages of *M. cephalus*, two lineages of *M. rubrioculus*, two of *M. curema*, and the remaining are the taxonomically recognised *M. bananensis*, *M. brevirostris, M. capurrii*, *M. curvidens*, *M. hospes, M. incilis*, *M. margaritae*, *M. liza* and *M. trichodon*.

4.3.4 Congruence between mitochondrial and nuclear topologies

Bayesian and ML topologies for nuclear and mitochondrial reduced datasets were very similar (Fig. S4.3). The only differences between them were the positioning of *Mugil bananensis*, *M. capurrii* and *M. incilis*. *Mugil bananensis* and *M. capurrii* were recovered in a sister group relationship with high support using either the mitochondrial (PP=1, BS=98) or the nuclear (PP=1, BS=99) datasets, however while in the mitochondrial topology this clade represents the sister group to a clade including *M. cephalus* and *M. liza* (PP=1, BS=81), in the nuclear this clade represents the sister group to a larger clade, comprising *M. curema*, *M. incilis* and *M. thoburni* (PP=1, BS=86). In the mitochondrial topology, *M. incilis* was recovered as sister to a clade including *M. curema* and *M. thoburni* (PP=1, BS=100), while it was sister to *M. curema* (PP=1, BS=95) in the nuclear topology. The remaining clades recovered were the same between the mitochondrial and nuclear topologies (Fig. S4.3). In both topologies

M. trichodon is sister to all other species of *Mugil* (PP=1, BS=100), *M. cephalus* was paraphyletic with *M. liza* nested within it (PP=1, BS=100) and *M. hospes* and *M. rubrioculus* were recovered together (PP=1, BS=100).

4.4 DISCUSSION

4.4.1 Taxonomically recognised species of *Mugil* encompass multiple highly divergent evolutionary lineages

Despite high morphological similarity, average interspecific divergence between the species of *Mugil* included in our investigation was 16.9%, based on the COI gene. Similar divergence levels have previously been reported among congeneric species of other genera (Wang, Guo, Liu, Fan, & Liu, 2012), suggesting that this may be a general pattern in marine fishes. Nevertheless, within a few *Mugil* species intraspecific divergences was higher than the 2% threshold typically observed for the COI gene (Hubert et al., 2008; Pereira et al., 2013; Rosso et al., 2012): 3.5% within *M. cephalus*, 3.8% within *M*. *curema*, 2.6% within *M. rubrioculus.* This result suggests that currently taxonomically recognised species actually encompass several independently evolving lineages. In agreement with this conclusion, our estimated phylogenetic tree for the diversification of the genus recovered the same three species as paraphyletic. Our results are consistent with previous phylogenetic studies (Durand & Borsa, 2015; Durand, Chen, et al., 2012; Durand, Shen, et al., 2012; Siccha-Ramirez, Menezes, Nirchio, Foresti, & Oliveira, 2014; Xia et al., 2016) that also suggested that the diversity of the genus *Mugil* has been significantly underestimated. This congruence was expected, since we used the same genes and included the same individuals from those studies in this larger dataset.

In our study, we explicitly tested the hypothesis of cryptic evolutionary lineages by delineating evolutionarily independent units irrespective of current species level classification (bPTP analysis), and tested the monophyly of the currently named species (SH test). The bPTP analysis strongly supported the hypotheses that *M. cephalus* is a species complex. According to Durand and Borsa (2015), diversity in *M. cephalus* was allocated into 15 lineages, including one taxonomically recognised as

M. liza. In the present study, from the 15 lineages, 14 were recovered as independent lineages with high support (PP≥0.95); *M. cephalus* lineage from Gulf of Mexico ("*Mugil* sp. B" – individuals 344, 347, 373) had slightly lower support (PP=0.93) (Table S4.5). *Mugil curema* has also been described as a species complex containing multiple independently evolving lineages such as *M. incilis*, *M. thoburni, M. margaritae*, "*Mugil* sp. M", "*Mugil* sp. O" and *M. curema sensu stricto* (Durand & Borsa, 2015; Durand, Chen, et al., 2012; Durand, Shen, et al., 2012; Nirchio et al., 2017; Siccha-Ramirez et al., 2014; Xia et al., 2016), all of these were also included in our study. Our analysis recovered *M. incilis* as well as *M. margaritae* as well-supported lineages (PP=0.99), but *M. thoburni* had slightly low support (PP=0.94). The individuals of *M. curema* from East Pacific, which should be part of the "*Mugil* sp. O" lineage, were split into two lineages, both with high support. The individuals of *M. curema* from East Atlantic should comprise the lineage "*Mugil* sp. M", but this lineage had low support (PP=0.75). Individuals from *M. curema sensu stricto* also had slightly low support (PP=0.94). Regarding *M. rubrioculus*, our bPTP analysis recovered two lineages with high support: "*Mugil* sp. P" from Pacific waters, and *M. rubrioculus sensu stricto*.

A recent review based on traditional morphological traits and mitochondrial DNA proposed that specimens from Brazil previously classified as *M. hospes* actually belong to *M. brevirostris* (Menezes et al., 2015). Our phylogeny and genetic distance results as well as species delimitation tests corroborate this hypothesis. Our results revealed that the sequence of *M. hospes* from Brazil from GenBank was more similar to our reference sequences of *M. brevirostris* from the type locality (0.9%) than to other *M. hospes* (7.9%). Furthermore, the phylogenetic analysis showed that they constitute a clade (those with only moderate statistical support PP=0.88), supporting the hypothesis that *M. hospes* from Brazil is in fact *M. brevirostris*. Our results also support an identification error among *M. curema* individuals. *Mugil margaritae* is endemic of an island in Venezuela and was described only in 2015 (Menezes et al., 2015), before the description, members of this species were considered to represent *M. curema* (Nirchio et al., 2017). The putative *M. curema* (Individual 30070) was collected from Venezuela prior to the description of *M. margaritae* and for this reason could not be correctly assigned.

To explicitly test the current classification as a phylogenetic hypothesis, we used the SH test and compared our estimated ML tree with alternative trees where species were forced to be monophyletic. On this basis, we statistically rejected the monophyly of *M. cephalus* and *M. curema* (P-values<0.05). The monophyly of *M. rubrioculus* could not be rejected relative to the most likely paraphyletic tree (P-value>0.05), despite the slightly high intraspecific genetic distances (2.6%).

The use of mitochondrial markers alone in species delimitation has been criticised by several authors, because mitochondrial trees may be discordant from nuclear trees due to hybridization, demographic differences between males and females, or simply due to selection on mitochondria (reviewed in Galtier, Nabholz, Glémin, & Hurst, 2009). Xia et al. (2016) concatenated 12 nuclear and three mitochondrial markers to reconstruct a phylogeny of 10 species of *Mugil*. Despite their much smaller taxonomic sampling, both at the species and lineage levels, their proposed relationships did not significantly differ from that obtained solely with mitochondrial markers here. In that concatenated analysis, *M. cephalus* remains paraphyletic in relation to *M. liza* (the remaining paraphyletic taxa were not sampled). By repeating this analysis only with the 12 nuclear genes we also obtained a topology mostly congruent with our mitochondrial tree (Fig. S4.3, see online supplemental material S1). The differences between the trees were: the position of *M. capurrii* and *M. bananensis*, which can be attributed to incomplete lineage sorting due to the slower evolving nature of the nuclear markers; and the position of *M. incilis* related to *M. curema* and *M. thoburni* which can be attributed either to incomplete lineage sorting or to gene flow between them. Yet, a broader sampling and further analyses would certainly help to elucidate these differences. Nevertheless, this similarity between the mitochondrial and nuclear topologies suggests that evolutionary processes specific to the mitochondria do not strongly affect the estimated phylogenetic relationships of *Mugil*, at least at this relatively deep time scale, and that mitochondrial DNA can be used as a proxy for phylogenetic inference of our target species. However, we would like to stress that assessing potential gene flow between closely related species of *Mugil* will require mitochondrial and multiple nuclear loci for the same individuals. The minor discordance that we found would need to be tested in this way in the future.

Together, our results reinforce the idea that several species of *Mugil* are comprised by multiple, independently evolving lineages. The recognition of these lineages is essential from a conservation standpoint, since species that are currently recognized as holding large genetic diversity likely reflect multiple evolutionary lineages with little genetic diversity within each. Based on this point, we also suggest a thorough review of the morphology of these lineages, in an attempt to better delineate them in the future.

4.4.2 Timing of diversification of *Mugil* species

Our time-calibrated phylogeny offers the first insights into the timing of the diversification of the genus *Mugil*. Our results suggest that the extant species of *Mugil* sampled here started diversifying ~30 MYA (23.7–40.0). This diversification coincides with the Eocene-Oligocene transition period (33.5–34 MYA), characterized by fluctuations in sea level and global climate change. During this time, atmospheric air and sea surface temperatures at high latitudes are hypothesised to have cooled by approximately 5 °C, while the thermocline and deep waters of the Southern Ocean and Indian Ocean may have cooled 2–3 °C (Williams et al., 2013). As sea surface temperature is the main factor limiting dispersal of mugilids (Durand & Whitfield, 2016), it is likely that these changes resulted in bottlenecks and local extinctions, which may have contributed to the deep phylogenetic divergence between lineages of *Mugil* found in this study. This cooling period likely drove the diversification of benthic invertebrates, probably as a result of increased nutrient availability due to erosive processes, changes in ocean circulation, tectonic events and upwelling (Williams et al., 2013). A greater diversity of food sources followed by the warm climate of the early and middle Miocene (12–23 MYA) (Lyle et al., 2008), may have further contributed to lineage diversification, as grey mullets are benthic microphages (LeLoc'h, Durand, Diop, & Panfili, 2015) and probably benefited from the larger variety of food available.

4.4.3 *Mugil cephalus* species complex

Mugil cephalus was recovered as paraphyletic with respect to *M. liza* (PP=1). As *M. liza* has previously been recognized as taxonomically distinct based on meristic

features and morphometric measurements (Menezes, Oliveira, & Nirchio, 2010; Pacheco-Almanzar, Simons, Espinosa-Pérez, Chiappa-Carrara, & Ibáñez, 2016), we suggest that *M. cephalus* should be considered as a species complex with several lineages, including *M. liza.* This is in agreement with Durand and Borsa (2015), who showed evidence that *M. cephalus* is comprised of at least fifteen lineages.

Long oceanic distances should represent barriers to the dispersal of cosmopolitan coastal species such as grey mullets (Livi, Sola, & Crosetti, 2011), but *M. cephalus* seems to represent an exception to this rule (Durand, Shen, et al., 2012). This species occupies several habitats and is considered eurytopic (Whitfield, Panfili, & Durand, 2012). Partly as a consequence of its wide distribution, 31 different species names have been used for this species (Whitfield et al., 2012). This strongly suggests that a taxonomic review of this group is urgently needed in order to revalidate some of the proposed names, according to the described evolutionary lineages.

If *M. cephalus* was a single cosmopolitan generalist species, it would be expected to display low genetic structure (Horne & Van Herwerden, 2013). Our analyses suggest the opposite and indicate the presence of at least 14 lineages. Additionally, different levels of tolerance (regarding water temperature, turbidity and salinity) among different populations of *M. cephalus* reinforce the view that this species is actually a set of ecologically specialised lineages that have colonized different regions and have been subject to different selective pressures (Whitfield et al., 2012).

Our study shows that the diversification of *M. cephalus* began at ~5 MYA (2.8–6.6 MYA). The most derived lineage of this species (0.0–0.14 MYA) is found in the Eastern Pacific region (Galapagos). Our results contrast with previous findings based on a small fragment of CYTB that suggested that the most derived *M. cephalus* population comes from Western Australia (Livi et al., 2011), and that the likely centre of origin of the taxon was Eastern Australia (Western Pacific), during the most recent glacial cycle, approximately 120 KYA. However, we estimate that the lineage from Eastern Australia originated about ~2 MYA. This contrasting divergence times is probably due to the broader data sampling used here.

Our results suggest that the diversification of the Moroccan lineage of *M. cephalus* (type locality) from other lineages occurred ca 1.0–3.0 MYA, after the

opening of the Mediterranean Sea to the Atlantic waters through the Gibraltar Strait (5.3 MYA; Moreno, Faria, & Rocha, 2014).

4.4.4 *Mugil curema* species complex

Our study shows that the diversification of the *Mugil curema* complex began nearly ~6 MYA. The ancestral lineage is from the Pacific region (0.5–1.9 MYA) and the most derived lineage is its sister lineage, from the Western Atlantic (0.05–0.5 MYA). *Mugil thoburni* is also an endemic lineage of the Eastern Pacific, occurring at the Galápagos Islands (Barletta & Dantas, 2016). Due to geographic proximity, we would expect this species to be phylogenetically closer to the *M. curema* lineage from the Pacific, but our results show that they are not sister species. From this, we infer that *M. curema* dispersed to the Pacific two times, likely before the total closure of Central America Seaway, 3–12 MYA (Byrne, Chapleau, & Aris-Brosou, 2018). *Mugil margaritae* was nested within the Pacific lineage with low support (PP=0.78) and the estimation of its phylogenetic position awaits additional samplings.

Differences in chromosome number are one of the tools used to discriminate cryptic diversity within morphologically similar lineages (Dincă, Lukhtanov, Talavera, & Vila, 2011; Milhomem et al., 2008). And indeed, during their divergence, lineages of *M. curema* acquired many cytogenetic differences (Durand, Shen, et al., 2012; Nirchio et al., 2017). Most members of the Mugilidae family maintained the same basic karyotypic structure of all Perciformes, with the diploid number 2n=48 and a fixed number of chromosome arms, i.e. fundamental number (NF)=48. Nevertheless, several lineages of the *M. curema* have diverged from this pattern. For example, *M. incilis* has a karyotype characterised as 2n=28 (Galetti Jr., Aguilar, & Molina, 2000), while *M. margaritae* is characterised by 2n=24 and *M. curema sensu stricto* (from the Western Atlantic coast) is characterised as 2n=28 (Nirchio, Cipriano, Cestari, & Fenocchio, 2005). The Pacific lineage of *M. curema* ("*Mugil* sp. O") was also investigated through cytogenetic approaches (Nirchio et al., 2017) and its karyotype was characterised as 2n=46, although it remained NF=48. These karyotypic differences can be related to ecological preferences (Pramual & Kuvangkadilok, 2012) and may result in reproductive isolation (Galetti Jr. et al., 2000). Further studies are

required to verify if there are morphological differences within these lineages. Our analyses, together with such observed patterns of cytogenetic divergence, show that *M. curema* is a species complex comprised of multiple, deeply divergent lineages.

4.4.5 *Mugil rubrioculus* species complex

Our study recovered *Mugil rubrioculus* as a paraphyletic taxon, as the Western Atlantic lineage is sister taxon of the clade *M. hospes* + *M. brevirostris* (PP=1) and they are both sister taxa of the *M. rubrioculus* lineage from Eastern Pacific. Several authors doubt the existence of *M. rubrioculus* in the Pacific Ocean (Barletta & Dantas, 2016; Menezes et al., 2015), and Durand and Borsa (2015) suggested that the Pacific clade represents a distinct lineage and temporarily named it as "*Mugil* sp. P". We found 5.9% of genetic divergence between these lineages (Table S4.4) supported by bPTP results, strongly suggesting that they represent cryptic lineages.

According to our results, the divergence between the Pacific and the Atlantic lineages occurred about ~10MYA (5.7–14.0). This date fits the estimated period of Central America Seaway closure between 3–12 MYA (Byrne et al., 2018), and the separation of the lineages may have been caused by this geological event.

The genetic distance between *M. brevirostris* and *M. hospes*, based on COI gene, was 7.9%, corroborating a previous study that found a 7.7% divergence on COI gene between these species (Nirchio, Paim, Milana, Rossi, & Oliveira, 2018). The divergence of *M. hospes* and *M. brevirostris* lineages occurred about ~3 MYA (1.56–5.0), and a possible cause for the split of these species is the well-known geological barrier between the Caribbean and Atlantic fauna (Rocha, 2003), the opening of the Amazon River around 5–6 MYA (Joyeux, Floeter, Ferreira, & Gasparini, 2001).

4.5 FINAL CONSIDERATIONS

Despite the considerable morphological conservatism among species of *Mugil*, we show that genetic differences are very high between species, diverging from ~30 MYA to ~5 MYA. Such old divergence times are also observed within species (up to ~10 MYA). Several independently evolving lineages were recovered within *M.*

cephalus, including *M. liza*. In the same way, *M. rubrioculus* is composed of two different lineages that make the species paraphyletic with respect to *M. hospes and M. brevirostris. Mugil curema* is also a species complex recovered as paraphyletic with respect to *M. margaritae* and *M. thoburni*.

As several species of *Mugil* are evolving under similar ecological pressures, conservatism of the morphological traits commonly used in fish taxonomy hides a remarkable level of genetic diversity generated due to dispersal and vicariance events. In conclusion, these results indicate that the evolutionary history of multiple cryptic lineages within taxonomically recognised species must be taken into account for management and conservation of *Mugil* fishes.

ACKNOWLEDGEMENTS

JMMN thanks to scholarship provided by Coordination of Improvement of Higher Level Personnel - CAPES (#88887.137815/2017-00 and #88881.189448/2018-01) TM thanks to CNPq fellowship (#309904/2015–3) and FAPEAL fellowship (#60030 000406/2017). JPFAA thanks to FAPEAL (#23038.023347/2016-74). We would also like to thank to the editors: Elliot Shubert and Kevin Conway, and the two anonymous reviewers for the constructive comments on the previous version of the manuscript, as well R. Ladle and C. Thomas for revising the grammar, and Dr. Ana L. Ibáñez and Dr. Alfredo P. Lozano for the tissue samples kindly provided.

FUNDING

This work is part of the Long Term Ecological Research – Brazil site PELD-CCAL (Projeto Ecológico de Longa Duração – Costa dos Corais, Alagoas) funded by the Brazilian National Council for Scientific and Technological Development – CNPq (#441657/2016-8), the Research Support Foundation of the State of Alagoas – FAPEAL (#60030 1564/2016), and BAYLAT.

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5 CAPÍTULO 2

Integrative taxonomy reveals extreme morphological conservatism in sympatric *Mugil* species from the Tropical Southwestern Atlantic[5]

Jessika M. M. Neves, Alfredo Perez, Nidia N. Fabré, Ricardo J. Pereira, Tamí Mott

Abstract

The biodiversity crisis has had particularly harsh impacts on marine environments. However, there is still considerable uncertainty about how many species have been seriously impacted and the effectiveness of protection measures (e.g. marine protected areas or MPAs) due to high levels of cryptic species in many taxa. Here, we employ an integrative taxonomy approach to mullet species in the genus *Mugil*. In addition to its high economic value, this genus is notable for having diversified ~29 million years ago without marked morphological and ecological divergence. We obtained 129 specimens of *Mugil* from the Coral Coast MPA, the largest of its kind in the tropical Southwestern Atlantic marine province. Although morphometric and meristic traits revealed six taxonomically recognized species, only five mitochondrial lineages were observed. All individuals morphologically identified as *M. incilis* belonged to the mitochondrial lineage of *M. curema*, which is consistent with misidentification of morphologically similar species and an overestimation of species diversity. Remarkably, *Mugil* species in our sample that diverged up to ~23 million years ago are also the most morphologically similar (*M. curema* and *M. rubrioculus*), suggesting extreme morphological conservatism, possibly driven by similarities in habitat use and life history traits. This study demonstrates the potential utility of integrative taxonomy (including DNA barcoding) for contributing to the conservation and sustainable use of natural resources.

Keywords: conservation, Tropical Southwestern Atlantic province, DNA barcoding, morphological stasis, cryptic species.

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5. Artigo publicado na revista Journal of Zoological Systematics and Evolutionary Research (qualis A2, percentil 85% pelo Scopus em novembro de 2020): Neves, J. M. M., Perez, A., Fabré, N. N., Pereira, R. J., & Mott, T. (2020). Integrative taxonomy reveals extreme morphologic conservatism in species of *Mugil* fishes in a protected area. *Journal of Zoological Systematics and Evolutionary Research*. doi: 10.1111/JZS.12421

5.1 INTRODUCTION

Increases in human populations and consumption patterns have led to overexploitation of natural resources and habitat loss (Bakker & Svenning, 2018; Jørgensen, Folke, & Carroll, 2019; Singh, 2002), culminating in pressing threats to global biodiversity (Mora & Zapata, 2013). The biodiversity crisis has disproportionally impacted aquatic environments, where populations have declined to 36% of pre-exploitation levels in marine habitats and 81% in freshwater habitats (WWF, 2016).Various strategies have been developed to mitigate the loss of marine biodiversity, the most important of which is the establishment of Marine Protected Areas (MPAs), which aim to protect distinct habitat types and limit the exploitation of key species by fisheries (Stori, Shinoda, & Turra, 2019). However, due to widespread cryptic diversity in many marine taxa (Cox, Moore, & Ladle, 2016), it is often unclear how many species are at risk and how many are actually protected by these strategies. This is particularly the case in tropical regions, where marine diversity is higher but less studied (Oliveira Júnior et al., 2016).

The Brazilian coast has high levels of marine biodiversity due to its physiographic complexity (Brandini, 2014). Nevertheless, this region remains understudied due to a combination of its large size, high diversity of habitats, and low sampling effort (Couto, Da Silveira, & Rocha, 2003). This lack of knowledge is particularly problematic given that 80% of marine resources are overexploited in Brazil (Miloslavich et al., 2011), making sustainable management actions in marine ecosystems a pressing goal. Brazilian coastal biodiversity is distributed along three marine provinces (Spalding et al., 2007). The Tropical Southwestern Atlantic contains the enormous Coral Coast MPA (designated for sustainable use of natural resources), extending for 120 km (ICMBio, 2013) and occupying 1.3% of the national coast (Garzón-Ferreira et al., 2002). Like all sustainable use MPAs, the Coral Coast environmental managers need to carefully balance conservation needs with those of resource uses, a task that is complicated by the high levels of cryptic diversity among exploited fish populations – especially grey mullet (*Mugil* sp.).

Grey mullet are targeted by commercial fisheries throughout their global distribution (Pacheco-Almanzar, Ramírez-Saad, Velázquez-Aragón, Serrato, &

Ibáñez, 2017; Whitfield, Panfili, & Durand, 2012) and within the states that embrace this Southwestern Atlantic MPA they account of ~14% of the artisanal fisheries catch (IBAMA, 2007). *Mugil* spp. live in freshwater and brackish habitats during most of their life cycle, migrating to the sea to reproduce (Nordlie, 2016), and thus play an important role in organic matter fluxes between those habitats (Lebreton, Richard, Parlier, Guillou, & Blanchard, 2011). Due to this migratory behaviour, these species often face a higher extinction risk than other groups (Jonsson, Waples, & Friedland, 1999).

Grey mullet exploitation is particularly difficult to manage due to their very high levels of morphological similarity (Durand, Shen, et al., 2012) and their sympatric distribution (e.g. Chang & Iizuka, 2012; Mai, dos Santos, Lemos, & Vieira, 2018). Current Brazilian fisheries regulations consequently lump all grey mullet species together (as *Mugil* spp.) (MPA, 2011). Recently, a dichotomous key was developed for the identification of mullet species from the Atlantic South Caribbean and South America. This key is based on meristic and morphometric traits that, in principle, can distinguish all sympatric species (Menezes, De Oliveira, & Siccha-Ramirez, 2015). However, many of the traits in the key hardly differ between species, some are polymorphic within species, and in many cases are applicable to a single ontogenetic stage (González-Castro & Ghasemzadeh, 2016). A more robust approach to resolve the challenging taxonomy and systematics of mullets is to integrate different sources of evidence (Padial, Miralles, De la Riva, & Vences, 2010), such as genetic and morphometric traits.

Notwithstanding the existence of traditional taxonomic keys, classical external morphological characters are clearly not sufficient to delimit grey mullet species (Durand & Borsa, 2015). Consequently, many authors disagree on the number and geographic distribution of each species, including those in the Tropical Southwestern Atlantic marine province. For example, *Mugil cephalus* Linnaeus, 1758 and *M. platanus* (Günther, 1880) were previously described in the region but were later found to be *M. liza* Valenciennes, 1836 (Menezes, Oliveira, & Nirchio, 2010). *Mugil hospes* Jordan & Culver, 1895 was also recorded in the province but was later identified as *M. brevirostris* (Ribeiro, 1915) (Menezes et al., 2015). The presence of *M. incilis* Hancock, 1830 in this region remains uncertain: a review of mullet species of South America indicated that this species does not reach the Tropical Southwestern Atlantic (Menezes

et al., 2015), while some authors continue to record the species in the area (e.g. Barletta & Dantas, 2016; Freire & Araújo, 2016).

To guarantee the sustainable management of this ecological and economically important group it is important to determine how many species occur in this area and how accurate the species delimitation is based on external morphology. To this end we integrate genetic and morphological data through an integrative taxonomy approach. Specifically, we use a fast-evolving mitochondrial marker to detect cryptic lineages and compare these results to morphological traits that putatively distinguish the species recognised in this region. Our aim was to answer the following questions: 1) how accurate is the species delimitation based on external morphology?; 2) how many evolutionary lineages are sympatric in the Coral Coast MPA?; 3) is morphological similarity correlated with time since divergence?

5.2 MATERIALS AND METHODS

5.2.1 Sampling

We acquired 129 *Mugil* specimens from local fishermen at two rivers: Santo Antonio river and Manguaba river, both situated within the Coral Coast MPA in the Tropical Southwestern Atlantic marine province (Fig. 5.1). For each individual, a sample of muscle tissue was preserved in 96% ethanol and stored at -20°C. All specimens were fixed in 10% formalin and preserved in 70% ethanol and archived at the zoological collection of the Natural History Museum from Federal University of Alagoas (Table S5.1 and Table S5.2 for voucher numbers).

The research was carried out in accordance with the principles of the Basel Declaration and following the recommendations of Brazilian Animal Protection Law. Sampling procedures were approved by the System of Biodiversity Authorization and Information (SISBIO committee) under the license # 56293-1.

Figure 5.1 - Map of the studied area. Tropical Southwestern Atlantic province is highlighted in red. The sampling sites, Santo Antonio River and Manguaba river, are pointed out with red dots.

5.2.2 Morphological delimitation

All specimens were identified following the identification key of Menezes et al. (2015). Accordingly, we selected eleven morphological traits (seven continuous and four categorical) to perform statistical analyses (Table 5.1, Table S5.2).

5.2.2.1 Multivariate morphological analyses without a priori species identification

In order to estimate the morphological variance in our samples, and to estimate the traits that better distinguish individuals from each species, we performed a Multiple Factor Analysis (MFA). This multivariate analysis includes both categorical and continuous variables and classifies individuals according to statistically uncorrelated

multidimensional axis without *a priori* classification into morphological species, while also estimating how much each trait contributes to these axes. This analysis was performed using the FactoMineR package (Lê, Josse, & Husson, 2008) in R, with all 11 traits together, grouping the different meristic traits from each fin, as number of rays and spines from anal fin (AFS+AFR); and unbranched and branched rays at pectoral and second dorsal fin (PFUR+PFBR; SDFUR+SDFBR), and plotting a 95% confidence level ellipse. We did not perform any transformation of continuous variables, as meristic characters are usually independent of the size of the organism and tend to be fixed in the early stages of growth.

Table 5.1 - Morphological traits used to identify *Mugil* species from the Tropical Southwestern Atlantic marine province.

Code	**Trait**	**State**
AFS	Number of anal fin spines	Continuous
AFR	Number of anal fin rays	Continuous
PFUR	Number of pectoral fin unbranched rays	Continuous
PFBR	Number of pectoral fin branched rays	Continuous
OSR	Number of oblique scale rows from dorsal limit of pectoral fin base to the base of caudal fin	Continuous
SDFUR	Number of second dorsal fin unbranched rays	Continuous
SDFBR	Number of second dorsal fin branched rays	Continuous
SSDAF	Presence of scales at the second dorsal and anal fins	Categorical:
		Restricted to anterior basal portion
		Densely scaled
DFDF	Distance from the origin of the first dorsal fin to the caudal fin base	Categorical:
		Yes - origin of the first dorsal fin to the caudal fin base longer than the origin of the first dorsal fin to snout tip
		No - origin of the first dorsal fin to the caudal fin base shorter than the origin of the first dorsal fin to snout tip
DTPF	Distance from the tip of pectoral fin to the origin of the first dorsal fin	Categorical:
		Yes - tip of pectoral fin reaching the origin of the first dorsal fin
		No - tip of pectoral fin not reaching the origin of the first dorsal fin
PDSF	Presence of dark spot on some fin	Categorical:
		Absent
		Present only at dorsal fin
		Present only at pectoral fin
		Present at both dorsal and pectoral fins

5.2.2.2 Multivariate morphological analyses with a priori species identification

We tested if the species identified using the identification key were significantly distinct considering the same 11 morphological traits and using a one-way Analysis of Similarity (ANOSIM). We grouped individuals *a priori* based on morphological identification and percentage dissimilarity was computed between each pair of morphological species. The ANOSIM was performed in the Paleontological Statistics Software (PAST) v.3.0 using the Bray-Curtis measure of similarity, with 9,999 permutations, and a Bonferroni correction of the p-values for multiple pairwise comparisons (Hammer, Harper, & Ryan, 2001) and considering $\alpha = 0.05$.

5.2.3 Mitochondrial evolutionary lineages

Total genomic DNA was extracted with the phenol-chloroform method (Sambrook, Fritsch, & Maniatis, 1989). A fragment of the mitochondrial gene *cytochrome c oxidase subunit 1* (*COI*) was amplified using the primers FISH-BCL (5-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5-TAAACTTCAGGGTGACCAAAAAATCA) (Baldwin, Mounts, Smith, & Weigt, 2009) following the protocol of Neves et al. (2016). We then performed Sanger sequencing only for the forward strand. This fast evolving gene is often used to diagnose species (Hebert et al., 2003) and has been shown to clearly distinguish cryptic species of *Mugil* (Delrieu-Trottin et al., 2020; Durand, Hubert, Shen, & Borsa, 2017). All 129 sequences were edited with BioEdit v.3.3.19 (Hall, 1999) and then translated into amino acids to verify the lack of stop codons. To confirm the absence of contaminations in the sequenced fragments, we used the blastn tool from GenBank (Benson et al., 2014). Sequences were aligned with MAFFT v.7.427 (Katoh & Standley, 2013). The final alignment is provided as supporting information (Additional Data S1).

To estimate the number of evolutionary lineages, we performed a Bayesian phylogenetic analysis. The best evolutionary model was estimated by PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012), allowing a codon partition. As reference, we included sequences from GenBank representing all *Mugil* species available in this data bank to better understand the phylogenetic relationships of the lineages sampled in

the MPA, choosing the specimen sampled closest to the type locality of each species (accession numbers: JQ060523 (*M. bananensis* (Pellegrin, 1927)), JQ060525 (*M. capurrii* (Perugia, 1892)), JQ060562 (*M. cephalus*), JX185205 (*M. curema* Valenciennes, 1836), KJ702390 (*M. curvidens* Valenciennes, 1836), JQ060607 (*M. hospes*), JX185182 (*M. incilis*), JX185158 (*M. liza*), JX185210 (*M. rubrioculus* Harrison, Nirchio, Oliveira, Ron & Gaviria, 2007), JX559534 (*M. thoburni* Jordan & Starks, 1896), JX185179 (*M. trichodon* Poey, 1875)). As outgroups, we also included one sequence from *Neomyxus leuciscus* (Günther, 1872) (JQ060618) and one from *Chaenomugil proboscideus* (Günther, 1861) (JQ060409). The analysis was performed in MrBayes v.3.2.6 (Ronquist et al., 2012) with 10 million generations, sampled every 1,000 trees, with a 25% burn-in. The output was analysed in Tracer v.1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) and the tree was visualised in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). Posterior probabilities (PP) were used as node support values; PP values equal to or above 0.95 were considered as high support.

To determine a non-arbitrary threshold for genetic differentiation between species, we compared our sequences with all available sequences at the Barcode of Life Data systems (BOLD, www.boldsystems.org), broadening the geographic scope of our dataset. The approach used to determine this threshold was the Barcode Index Number (BIN), an algorithm that combines single linkage and Markov clustering to assign each sequence to a cluster of sequences available in the BOLD database without a priori classification into species. To access this tool, we created a new project at BOLD named “Mullets From Alagoas”.

In order to evaluate the genetic diversity within and between evolutionary lineages, the individuals were re-classified into evolutionary lineages according to the phylogenetic tree and the BINs. We calculated pairwise genetic distances between individuals within and between lineages using the MEGA software, with the best fitting evolutionary model estimated by PartitionFinder. Through a function called Local Minima from the Spider package in R (Brown et al., 2012), we estimated the threshold between intraspecific and interspecific genetic distances, using an alignment composed of our samples and publicly available sequences for the BINs assigned to our sequences.

To compute the pairwise percentage of morphological dissimilarity between the evolutionary lineages identified above, the ANOSIM test was repeated with the individuals grouped *a priori* according to the mitochondrial lineage. The results of the two ANOSIM tests were compared through p-values and the dissimilarity index (R-values), which varies from 0 (most similar) to 1 (most different).

5.2.4 Correlating morphological similarity with genetic divergence

To test if the morphological similarity between two pairs of species is correlated to the time since divergence, we used a linear regression model. First, for estimating morphological distances, we calculated the Euclidian distance using the scores of the first five MFA dimensions for each pair of individuals as a measure of morphological divergence, using the dendextend package (Galili, 2015) in R, and built a dendrogram to visualise the relation between individuals. Second, for estimating genetic distances, we calculated genetic divergence between each pair of individuals (with the same evolutionary model estimated before, using the ape package in R (Paradis, Claude, & Strimmer, 2004). Then, we built separate matrices of genetic divergence for each pair of species.

Using those two matrices, we calculated standard deviation and the average of genetic and morphological divergence for each pair of species. We tested the correlation between those average values using a linear regression model and plotted the average points and standard deviation bars in a dispersion graph. Data normality was assessed using residuals of the regression analysis through Shapiro-Wilk test and homocedasticity was tested by plotting fitted values against the residuals of the regression analysis. Both analyses were performed in R.

5.3 RESULTS

5.3.1 Morphological delimitation

The classification of *Mugil* species following the identification key of Menezes et al. (2015) resulted in the classification of individuals into 6 morphological species:

16 *M. brevirostris*, 34 *M. curema*, 31 *M. curvidens*, 6 *M. incilis*, 14 *M. liza* and 28 *M. rubrioculus*.

5.3.1.2 Multivariate morphological analyses without a priori species identification

The first five dimensions of MFA explained 85.3% of the data variability. Dimensions 1 explained 27.9% of data variance, and the traits that contributed most to this axis were: PDSF, OSR, SSDAF, and AFS+AFR, in that order, reflecting a separation between the individuals taxonomically identified as *M. brevirostris*, *M. curvidens* and *M. liza*, and an overlap between *M. curema*, *M. incilis* and *M. rubrioculus*. Dimension 2 explained 21.5% of the morphological variance, and the traits that contributed the most to this axis were: DTPF, PFUR+PFBR, SDFUR+SDFBR, and SSDAF, in that order, reflecting a separation between *M. brevirostris* and *M. liza*, and an overlap between *M. curema*, *M. curvidens*, *M. incilis* and *M. rubrioculus* (Fig. 5.2). The relationships between dimensions 1 to 5 can be seen in figure S5.1.

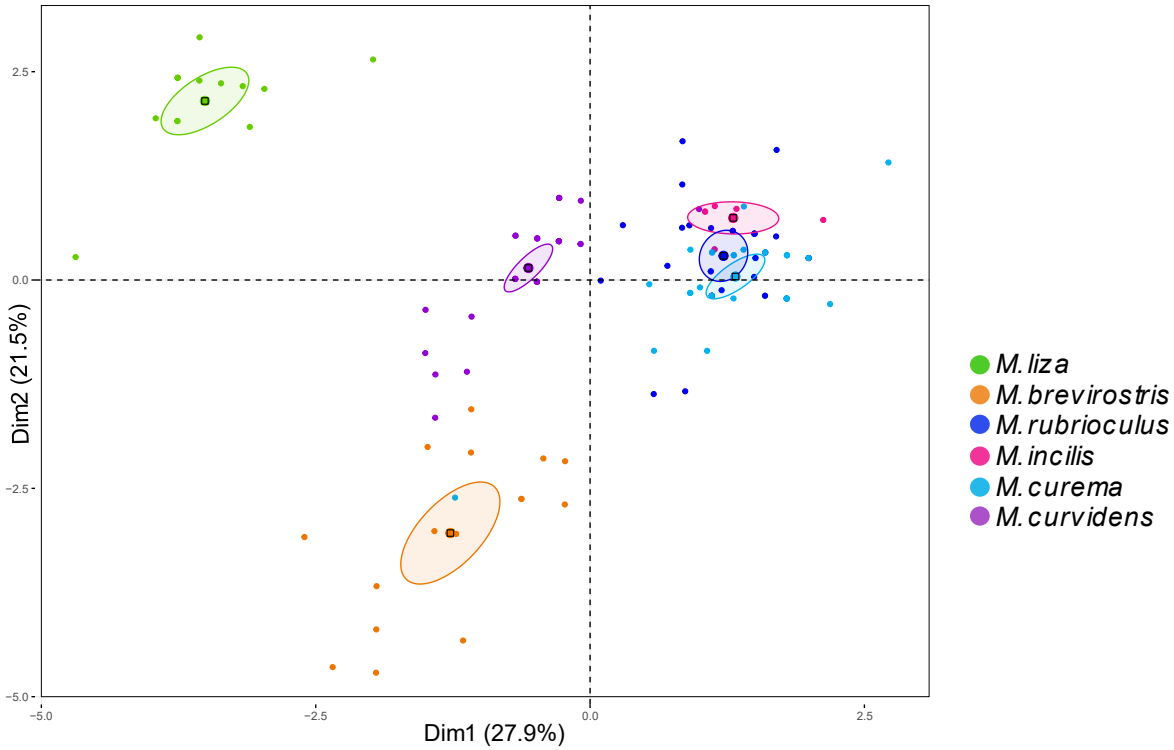

Figure 5.2 - Plot of dimensions 1 and 2 of the Multiple factor analysis (MFA). Ellipses represent 95% confidence level. It is possible to observe an overlap between individuals morphologically identified as *Mugil curema*, *M. incilis* and *M. rubrioculus*.

5.3.1.3 Multivariate morphological analyses with a priori species identification

There was significant morphological dissimilarity between the six morphological species (R= 0.63, p=0.0001). Significant dissimilarities were detected in most of the pairs of species (p≤0.01), with the exception of *M. curema* and *M. incilis* (p=0.39), and *M. curema* and *M. rubrioculus* (p=0.14).

5.3.2 Mitochondrial evolutionary lineages

We obtained a COI fragment of variable size, between 679-691bp, as we sequenced only the forward strand. Our trimmed alignment was composed of 569bp, 162 of which are variable. We found a different evolutionary model for each codon position (position 1: TIMEF +G; position 2: F81 + I; position 3: TRN +G). The phylogenetic tree (Fig. 5.3) recovered four highly supported lineages: *M. brevirostris* (PP=1), *M. curema* + *M. incilis* (PP=0.99), *M. curvidens* (PP=1), and *M. rubrioculus* (PP=1); while *M. liza* had a low support value (PP=0.84). The phylogenetic tree pointed to some disagreements with the assignment based on the morphological traits, as some individuals morphologically identified as *M. curvidens*, *M. incilis* and *M. rubrioculus*, nested within the clade of *M. curema*, and one individual morphologically identified as *M. curema* nested within the *M. curvidens* clade (Fig.5.3, Table 5.2). Most reference sequences of *Mugil* species from GenBank nested with specimens morphologically identified as the same species in this study. The exceptions were all the individuals morphologically identified as *M. incilis*, which nested with the reference sequence of *M. curema*, instead of with the reference sequence of *M. inicilis*.

The BIN clustering corroborated our phylogenetic analysis, showing the presence of five genetic groups among our sequences (BINs: AAB3698, AAA7840, ACC0101, ACE4593, AAZ7695) (Table 5.2, Table S5.2). These results showed that 2.94% (1 out of 34) of individuals were misidentified as *M. curema*; 3.22% (1 out of 31) were misidentified as *M. curvidens*; 100% (all 6 individuals) were misidentified as *M. incilis*; and 35.71% (10 out of 28) were misidentified as *M. rubrioculus*. The morphological identifications of *M. brevirostris* and *M. liza* were 100% accurate.

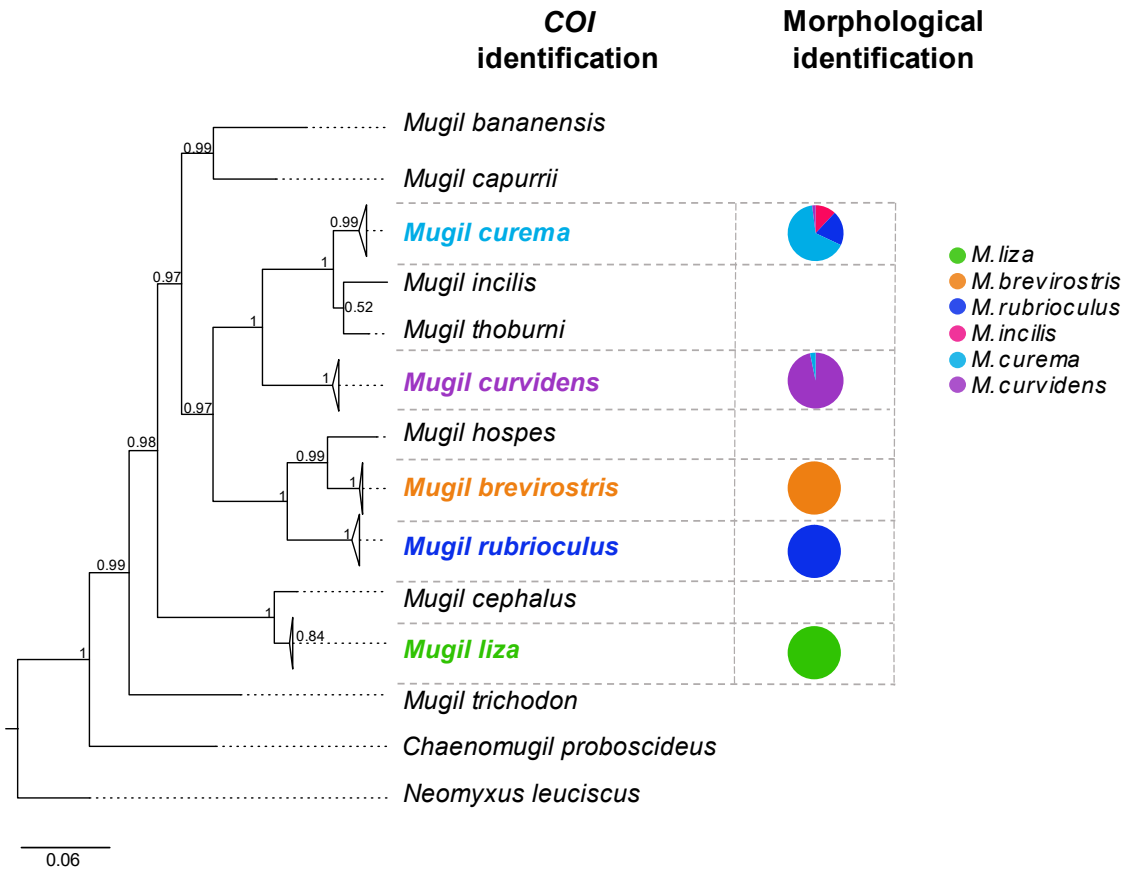

Figure 5.3 - Phylogeny based on the Bayesian method. Lineages in black only contain the reference sequences from GenBank. Colored lineages contain the reference sequences plus our sequences in collapsed branches. Pie charts represent the amount of misassignment among our samples. Numbers at the nodes are the posterior probabilities (PP). Scale bar indicates 0.06 substitutions/site.

Table 5.2 - Identification of *Mugil* individuals from the Tropical Southwestern Atlantic marine province according to morphological and COI identification.

Species	Morphological identification	COI identification (BIN and Bayesian)
M. brevirostris	16 (SA)	16 (SA)
M. curema	31 (SA) + 3 (MB)	47 (SA) + 2 (MB)
M. curvidens	16 (SA) + 15 (MB)	15 (SA) + 16 (MB)
M. incilis	6 (SA)	0
M. liza	14 (SA)	14 (SA)
M. rubrioculus	28 (SA)	18 (SA)

SA – Santo Antonio river; MB – Manguaba river.

TN93 was the best fitting evolutionary model when we assumed that all sites at the COI gene evolved according to the same pattern. Intraspecific genetic distances calculated using this model were low, with *M. brevirostris* and *M. liza* being the least variable species (0.05%), and *M. curema* the most variable one (0.22%). The interspecific distances (Table 5.3) were high, ranging between 11% (between *M.*

brevirostris and *M. rubrioculus*) and 21.4% (between *M. brevirostris* and *M. liza*, and between *M. liza* and *M. rubrioculus*). All pairwise genetic distances between specimens can be seen in table S5.3. The Local Minima analysis confirmed our previous results, establishing a threshold between intra and interspecific distance of 6.25%.

When we analysed all individuals grouped according to the five mitochondrial lineages (*M. brevirostris*, *M. curema* + *M. incilis*, *M. curvidens*, *M. liza*, and *M. rubrioculus*) with ANOSIM, the estimated R-value was 0.72 (p=0.0001), indicating significant morphological dissimilarity between all lineages. When comparing each pair of mitochondrial lineages, all comparisons had p=0.001, indicating significant morphological dissimilarity between all pairs of species.

Table 5.3 - Interspecific genetic distance between *Mugil* species from the Tropical Southwestern Atlantic marine province.

	M. brevirostris	*M. curema*	*M. curvidens*	*M. liza*
M. curema	0.204			
M. curvidens	0.199	0.135		
M. liza	0.214	0.182	0.213	
M. rubrioculus	0.112	0.195	0.190	0.214

5.3.3 Correlating morphological similarity with genetic divergence

The dendrogram (Fig. S5.2) based on Euclidian distance matrix (Table S5.4) clustered together the individuals morphologically classified as *M. liza*, *M. brevirostris* and *M. curvidens*, showing admixture between the individuals identified as *M. curema* and *M. rubrioculus*. When we correct the individual identifications based on COI, those clusters are more evident. The correlation between genetic and morphological distance was not significant (R^2= -0.09, p=0.64), as noticeable in the dispersal plot (Fig. 5.4).

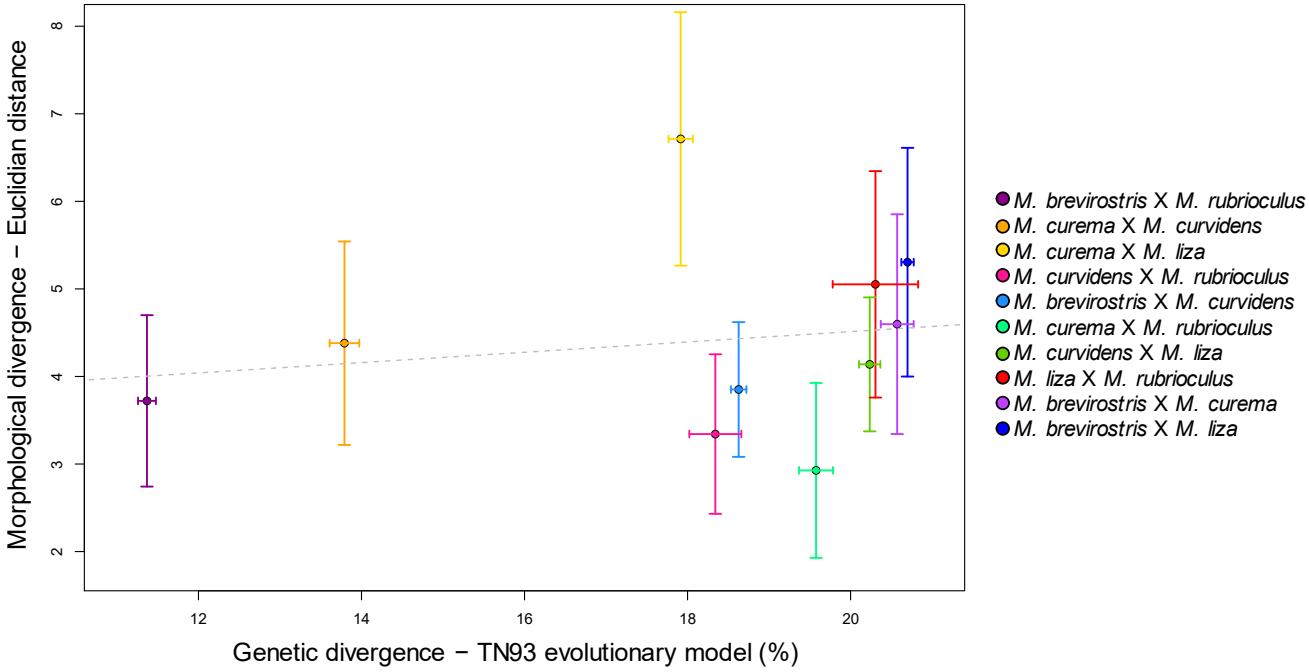

Figure 5.4 - Dispersal plot of genetic and morphological divergence per pair of species. Genetic distance was calculated using TN93 evolutionary model and is represented in percentage. Morphological distance was calculated through Euclidian distance using the scores of multiple factor analysis. The grey line represents the linear regression model.

5.4 DISCUSSION

Marine Protected Areas (MPAs) are a powerful tool to avert the overexploitation of natural resources and reduce habitat degradation (Agardy, di Sciara, & Christie, 2011). However, in order to establish sustainable conservation and management actions in MPAs, it is vital that managers know the identity and number of species a reserve contains. This is particularly challenging for morphologically similar species, such as grey mullet, that occur sympatrically throughout the Tropical Southwestern Atlantic province. As taxonomists usually rely on modest variance in morphological traits, specimen misidentification can lead to erroneous distribution limits and population estimates with potentially serious consequences for the management and conservation of fish stocks.

5.4.1 How accurate is the species delimitation based on external morphology?

Recent studies have pointed to the presence of six taxonomically recognised *Mugil* species in the Coral Coast MPA: *Mugil brevirostris*, *M. curema*, *M. curvidens*, *M. incilis*, *M. liza*, *M. rubrioculus* (da Silva, Teixeira, Batista, & Fabré, 2017, 2018; Torres et al., 2008), although the distribution of *M. incilis* has been contested (Menezes et al., 2015). Our study used 11 morphological traits to test whether the individuals identified as belonging to these six species are morphologically different. We performed a MFA, with no *a priori* hypothesis, where we had two expectations: 1) individuals morphologically identified as the same species should cluster together; and 2) morphological traits that appear earlier in the dichotomous key should contribute more towards each axis.

Dimensions 1 and 2 (Fig. 5.2) showed that the individuals taxonomically identified as *M. brevirostris*, *M. curvidens* and *M. liza* clustered together, although there were some points spread beyond the 95% confidence level ellipse. In contrast, our results indicate a big overlap among the individuals morphologically identified as *M. curema*, *M. incilis* and *M. rubrioculus*. The traits that most contribute to these two axes were: PDSF, OSR, SSDAF, AFS+AFR (dimension 1); DTPF, PFUR+PFBR, SDFUR+SDFBR, and SSDAF (dimension 2). Thus, our results strongly suggest that the taxonomically recognised species are difficult to distinguish based on external morphology alone.

Concerning the second expectation, the first trait in the key is the number of spines and rays in the anal fin (AFS+AFR), which separates species in two groups: species which have III, 8 (three spines and eight rays) at anal fin (*M. curvidens* and *M. liza*), and species which adults have III, 9 (three spines and nine rays) in anal fin (*M. brevirostris*, *M. curema*, *M. incilis* and *M. rubrioculus*). This trait (AFS+AFR) is the fourth to contribute to the first dimension and is a good diagnostic trait being useful to distinguish between *M. curvidens* (III, 8) and *M. curema* (III, 9). However, we found some notable discrepancies. For example, individual TA203 was morphologically identified as *M. curema* due to the presence III, 9 at the anal fin, but according to *COI* it is *M. curvidens*. Individual TA226 was morphologically identified as *M. curvidens* due to the presence of III, 8 at the anal fin, but was genetically identified as *M. curema*. We also found one individual (TA212), morphologically classified as *M. rubrioculus* due to the presence of other diagnostic traits, although having III, 8 at the anal fin. This

identification was genetically corroborated. Slight differences in the morphology of *Mugil* species such as these have already been described and interpreted as indicative of population structure (Pacheco-Almanzar, Simons, Espinosa-Pérez, Chiappa-Carrara, & Ibáñez, 2016).

The second trait in the identification key is SSDAF. SSDAF restricted to the anterior basal portion is a diagnostic trait of *M. liza*. This trait has a high contribution to dimensions 1 and 2, yet it is hard to evaluate in the field, limiting its use in taxonomy. The third and fourth step at the key rely on OSR and SDFUR+SDFBR, delimitating species that were filtered by the previous steps. In agreement, we find that OSR has a relatively large contribution to the dimension 1 and SDFUR+SDFBR to dimension 2. These are not good diagnostic traits as the counting of these meristic characters may overlap between species. The fifth step describes DTPF, to discriminate between *M. brevirostris* (in which the tip of pectoral fin should reach the origin of the first dorsal fin) and the remaining species (in which the tip of pectoral fin should not reach the origin of the first dorsal fin). Our results confirm that this is a good diagnostic trait, as *M. brevirostris* is the only species to possess it and the trait loads heavily on dimension 2.

The last step in the key is PDSF, which separates *M. curema* from *M. rubrioculus*, the two most similar species. This is the trait that most contributes to the dimension 1, which separates *M. brevirostris*, *M. curvidens* and *M. liza* but overlaps *M. curema* and *M. rubrioculus*. This overlap is probably due to the confusion regarding this trait. According to the taxonomic key, the description of *M. rubrioculus* reads “conspicuous black spot present on anterodorsal portion of second (soft) dorsal fin contrasting with lighter color of remainder of fin; basal portion of pectoral fin dusky or with small usually inconspicuous dark spot covering bases of two unbranched rays and of dorsalmost six or seven branched rays”. When this pattern was found, we stated the character PDSF as present only on the dorsal fin. In contrast, *M. curema* should display “anterodorsal portion of second dorsal fin uniformly dark or slightly darker than remainder of fin, but not in form of distinct dark spot; conspicuous dark spot extending over most of basal portion of pectoral fin covering bases of dorsalmost nine or ten branched rays”. When this pattern was found we recorded the character PDSF as present only on the pectoral fin. However, we found that some *M. rubrioculus* individuals do not have the black spot on the second dorsal fin, while it can be present

on some *M. curema* specimens. Likewise, the black spot on the basal portion of the pectoral fin was found on some *M. rubrioculus* specimens but was not always evident on *M. curema* individuals (Fig.5.5).

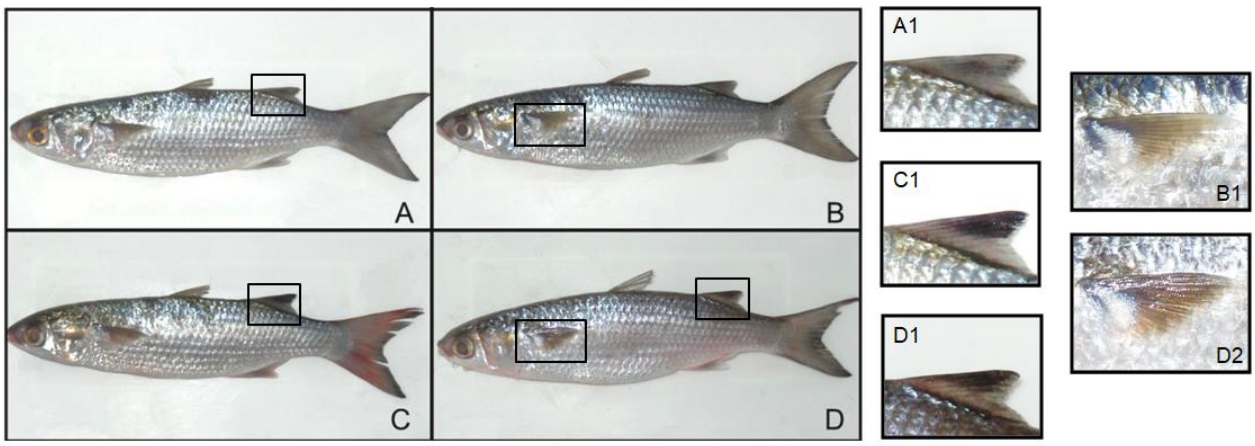

Figure 5.5 - *Mugil* specimens highlighting the subjectivity of the presence of a dark spot on some fins (PDSF) as a diagnostic trait. - A. Specimen TA029 belonging to *M. rubrioculus* mitochondrial lineage with a uniform lighter coloration on the second dorsal fin (A1). -B. Specimen TA031 belonging to *M. curema* mitochondrial lineage with a uniform colouration on the second dorsal and with a black spot on the basal portion of the pectoral fin (B1). - C. Specimen TA036 belonging to *M. rubrioculus* mitochondrial lineage with a black spot in the second dorsal fin (C1). – D. Specimen TA063 belonging to *M. curema* mitochondrial lineage with a black spot on the second dorsal fin (D1) and a black spot on the basal portion of the pectoral fin not so obvious (D2).

ANOSIM is a multidimensional analysis with *a priori* hypotheses, where we grouped the individuals according to taxonomic identification key and expected to find significant dissimilarities between groups. This test detected an overall dissimilarity (R=0.63, p=0.0001), but did not detect significant morphological dissimilarities between *M. curema* and *M. incilis* (p=0.39), or between *M. curema* and *M. rubrioculus* (p=0.14) - the three species that overlapped in the MFA analysis.

Although external morphology is the principal means to delimitate *Mugil* species, the characters often used may overlap, making it hard to establish a boundary between intra and interspecific differences, and thus resulting in taxonomic conflict (Harrison, Nirchio, Oliveira, Ron, & Gaviria, 2007). In the present study, the highest rates of misidentification were related to *M. curema* individuals, erroneously assigned either as *M. curvidens*, *M. incilis* or as *M. rubrioculus*. In fact, *M. curema* and *M. rubrioculus* are the most difficult species to morphologically distinguish. The main trait used to discriminate them is the colour of the fresh specimens (Harrison et al., 2007),

leading to frequent mistakes in the identification of preserved specimens. *Mugil incilis* is also known for being easily misidentified as *M. curema* (González-Castro & Ghasemzadeh, 2016), undoubtedly contributing to uncertainties about the distribution of *M. incilis*. Moreover, this species is a lineage nested within the *M. curema* species complex (Durand, Shen, et al., 2012; Nirchio et al., 2017).

5.4.2 How many evolutionary lineages are sympatric in this MPA?

Molecular variation in the *COI* gene has proven to be a powerful tool to resolve complicated phylogenetic relationships, estimate divergence times, and identify mislabelled specimens in *Mugil* (Delrieu-Trottin et al., 2020; Durand, Chen, Shen, Fu, & Borsa, 2012; Durand et al., 2017; Neves et al., 2020). Thus, to identify evolutionary lineages, we performed a Bayesian phylogenetic analysis with posterior probabilities (PP) based on the *COI* gene.

Our samples nested with the reference sequences of *M. brevirostris*, *M. curema*, *M. curvidens* and *M. rubrioculus* with a PP>0.95 (Fig. 5.3). The clade including the reference *M. liza* had a slightly lower support (PP=0.84), which can be justified by the complex relationship between *M. liza* and its sibling taxa, *M. cephalus*, described in previous studies (Durand & Borsa, 2015; Neves et al., 2020). The species delimitation threshold provided by the BOLD platform corroborated the results of our phylogenetic analysis. By comparing our morphological and the genetic results, we observed an important discrepancy: according to the mitochondrial lineages, our dataset is composed of 16 *M. brevirostris*, 50 *M. curema*, 31 *M. curvidens*, 14 *M. liza* and 18 *M. rubrioculus*; yet , according to the dichotomous key, these individuals are identified as 16 *M. brevirostris*, 34 *M. curema*, 31 *M. curvidens*, 6 *M. incilis*, 14 *M. liza* and 28 *M. rubrioculus*. These numbers reveal a high level of misidentification between the three species that could not be easily discriminated based on morphological traits: *M. curema*, *M. incilis* and *M. rubrioculus*.

Our molecular results are consistent with the absence of a *M. incilis* mitochondrial lineage among the sampled individuals. All individuals that were morphologically identified as *M. incilis* shared the same mitochondrial lineage as *M. curema* individuals in the phylogenetic tree and in the BOLD species delimitation

method. Multidimensional analyses where the individuals were grouped according to the mitochondrial lineage also corroborated these results. We found a stronger morphological dissimilarity between each pair of species when consider the molecular identification (R=0.72), relative to when we consider the morphological identification (R=0.63). This result could indicate the lack of *M. incilis* in the studied region. Alternatively, such discrepancy could be explained by hybridisation with full mitochondrial introgression from *M. curema* into *M. incilis*. We consider this latter hypothesis highly unlikely, as these species have extremely divergent karyotypes (28 chromosomes for *M. curema* and 48 for *M. incilis* (Hett et al., 2011; Nirchio, Cipriano, Cestari, & Fenocchio, 2005), which likely constitutes a major chromosomal incompatibility between these highly divergent species.

5.4.3 Is morphological similarity correlated with time since divergence?

As external morphology is constrained by natural selection, it is unlikely to evolve neutrally. The evaluation of only this source of information could lead to an underestimation of biodiversity (Lefébure, Douady, Gouy, & Gibert, 2006). In an attempt to avoid this bias, there is a move to apply integrative taxonomy approach, where the combination of independent data sets contributes to a more systematic assessment of biodiversity (Dayrat, 2005; Pante, Schoelinck, & Puillandre, 2015).

We measured morphological and genetic divergences between species of *Mugil* to test the hypothesis that more phylogenetically distant pairs of species are also more morphologically distinct. However, we did not observe a significant correlation between molecular and morphological divergence (p=0.64), refuting our hypothesis (Fig. 5.4). Notably, two species with high genetic divergence (*M. curema* and *M. rubrioculus*; 19.5% divergent in *COI*), which diverged ~23 MYA (Neves et al., 2020), are morphologically very similar according to the MFA and to the ANOSIM results. In contrast, more recently diverged species, such as *M. brevirostris* and *M. rubrioculus*, can be easily distinguished morphologically.

Such a lack of correlation between morphological differences and time since divergence can be explained by convergent evolution or by phylogenetic niche conservatism (Fišer, Robinson, & Malard, 2018). Convergent evolution acts when

phylogenetically distant species evolve similar morphology due to similar environmental pressures, while in phylogenetic niche conservatism the evolution of morphology in related species is constrained by stabilising selection (Fišer et al., 2018). These processes may act synergistically, causing and maintaining the high levels of morphological similarity in *Mugil* species. In any case, due to these evolutionary constraints the morphological traits evaluated in the present study do not have enough taxonomic power to delimit species of *Mugil*.

5.4.4 Implications for conservation

A biodiversity inventory is the first step for establishing effective conservation measures (Arribas, Andújar, Sánchez-Fernández, Abellán, & Millán, 2013). In light of this, we used an integrative taxonomy approach based on 11 morphological traits and one mitochondrial gene to describe the diversity of *Mugil* in a large MPA in the Tropical Southwestern Atlantic marine province. Our results indicate the presence of five lineages, as two morphological species (*M. curema* and *M. incilis*) constitute a single evolutionary lineage, where all individuals morphologically identified as *M. incilis* belong to the mitochondrial lineage of *M. curema*. The most parsimonious explanation is the absence of *M. incilis* in the MPA and historical misidentification, which corroborates the study from Menezes et al. (2015).

The application of integrative taxonomy also revealed a high rate of misidentification between *M. curema* and *M. rubrioculus*. This finding has important implications for research, conservation and fisheries management actions concerning these species. We strongly recommend that future studies and monitoring initiatives in this ecologically and culturally important MPA (Miranda et al., 2020) should adopt an integrative taxonomic approach that incorporates molecular tools to correctly assign mullet specimens to species (Schlick-Steiner et al., 2010).

ACKNOWLEDGMENTS

This work is part of the Long Term Ecological Research – Brazil site PELD-CCAL (Projeto Ecológico de Longa Duração – Costa dos Corais, Alagoas) funded by the

Brazilian National Council for Scientific and Technological Development – CNPq (#441657/2016-8), the Brazilian Coordination for the Improvement of Higher Education Personnel - PELD/CAPES (#23038.000452/2017-16) and the Research Support Foundation of the State of Alagoas – FAPEAL (#60030.1564/2016). This work was also funded by Bayerisches Hochschulzentrum für Lateinamerika - BAYLAT (#914-20.1.1). JMMN thanks to scholarship provided by Coordination of Improvement of Higher Level Personnel - CAPES (#88887.137815/2017-00 and #88881.189448/2018-01) TM thanks to CNPq fellowship (#309904/2015–3 and # 312291/2018-3) and FAPEAL fellowship (#60030.000406/2017). We thank Adrian Garda, Sergio Lima and Uedson Jacobina for the contributions to the improvement of this work. We also would like to thank to the editor Stephan Koblmüller and the two anonymous reviewers for the constructive comments on the previous version of the manuscript, as well C. Peart and R. Ladle for the thoughtful proofreading. We are very grateful to the fishermen that provided the specimens, and also to the whole team from LABI, LAEPP and LACOM.

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6 CAPÍTULO 3

Genomic methods reveal hidden levels of diversity in fish species of high ecologic and economic importance[6]

Jessika M. M. Neves, Zachary J. Nolen, Nidia N. Fabré, Tamí Mott, Ricardo J. Pereira

Abstract

Human overexploitation of natural resources has placed conservation and management as one of the most pressing challenges in modern societies, particularly regarding highly vulnerable marine ecosystems. Although a large effort has been made to establish sustainable conservation measures worldwide, many species are morphologically identical, making it challenging to evaluate their number, the level of genetic connectivity between species, and the relative levels of genetic diversity within each species. We answer these questions using morphologically similar species of the genus *Mugil* that are sympatric in the largest coastal Marine Protected Area in the Tropical Southwestern Atlantic marine province. Population structure analyses show the existence of five highly differentiated species (F_{ST} > 0.9), consistent with old divergence despite strong morphologic conservatism. Sympatric individuals are assigned to single clusters and show strong concordance among hundreds of independent gene trees, consistent with full reproductive isolation. Differences of genetic diversity within species suggest that effective population sizes differ up to two-fold, probably reflecting differences in the magnitude of population expansions during the independent evolutionary history of these species. Together, our results show that these morphologically similar species have been evolving independently over long periods of time and strongly differ in their demographic history. Recognizing such hidden patterns of diversity can direct science-based regulations for management and conservation of species of economic and ecological important that cannot be studied using traditional approaches.

Keywords: Conservation, Evolution, Marine Protected Area, Demography, Grey Mullets.

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6. Artigo submetido à revista Heredity (qualis A3, percentil 74% pelo Scopus em novembro de 2020).

6.1 INTRODUCTION

Human overexploitation of natural resources has placed conservation and management as one of the most pressing challenges in modern societies (Mora and Zapata 2013). The heavy targeting by fisheries is one of the causes of more than 36% of decline in marine populations and 81% of freshwater populations during the last four decades (WWF 2016). This has not only caused a direct decrease in fish stocks, but also an imbalance on ecosystem functioning, as fish species play an important role in the fluxes of energy and matter between trophic levels and the environment (Holmlund and Hammer 1999). Thus, understanding the distribution of fish species of high ecologic and economic importance, their population sizes, and the genetic connectivity between and within species, is fundamental for establishing strategies to protect biological diversity and the evolutionary processes that sustain it (Crandall et al. 2000; Moritz 2002; Cook and Sgrò 2019).

Addressing these questions is particularly challenging in morphologically conserved species because they are difficult to distinguish based on external morphology alone. This prevents a straightforward assessment of biodiversity and threatens fisheries sustainability (Garcia-Vazquez et al. 2012; Lyon et al. 2018) as difficulties in species identification further limit the implementation of protective measures by legislators and fishery managers. Studies integrating morphologic and genetic information from fast evolving mitochondrial genes are revealing an exponentially growing number of morphologically cryptic species (Sáez and Lozano 2005) and showing that their levels of genetic diversity often vary strongly across the species' distribution (Carnaval et al. 2009). Such discoveries of hidden genetic diversity have been reported in previously known biodiversity hotspots in the tropics, such as in the Amazon (Benzaquem et al. 2015) and in marine ecosystems (Asgharian et al. 2011; Brandão et al. 2016). Although many cryptic species have allopatric distributions, many others are partially or fully sympatric (McBride et al. 2009; Moritz et al. 2018). This suggests that genetic and/or ecological differences maintain species boundaries, and thus that species that are morphologically similar may play a meaningful role in the ecosystem (Struck et al. 2018). What is less clear is whether those species are evolving in complete genetic isolation in sympatry, conditioning their

evolutionary trajectory (Seehausen et al. 1997; Taylor et al. 2006), and whether they strongly differ in demographic history, determining their adaptive potential (Pinsky and Palumbi 2014).

Despite major advances provided by mitochondrial studies in identifying morphologically conserved species and mapping their distribution (Ward et al. 2009), mitochondrial markers cannot be used to assess demographic processes relevant for evolution and conservation (Galtier et al. 2009). Among other constraints, mitochondrial diversity can be biased by processes disproportionally affecting females (Prugnolle and de Meeus 2002), by natural selection (Ballard and Whitlock 2004), or by mitochondrial introgression between closely related species (Toews and Brelsford 2012). It is thus necessary to use hundreds or thousands of independent nuclear markers to reconstruct accurately the evolutionary history of those species and to better inform sustainable conservation measures (Grewe et al. 2015; Allendorf 2017; Grundler et al. 2019). Recent advances in sequencing technology and statistical methods now offer unprecedented opportunities for the field of conservation biology, providing new insights into the relative abundance of species (Hansson and Westerberg 2008), genetic connectivity (Pedersen et al. 2018), and even in their adaptive genetic variation (Librado et al. 2017). Understanding these processes remains an important task in marine ecosystems where overfishing is found to be correlated with lower genetic diversity, which in turn can affect species' adaptation capacity (Pinsky and Palumbi 2014).

To infer the evolutionary processes underlying the genetic diversity of cryptic marine fish of high ecological and commercial importance, we focus on a group of sympatric species of the genus *Mugil*, commonly known as mullets. These species are heavily targeted by traditional and industrial fisheries (Whitfield et al. 2012; Pacheco-Almanzar et al. 2017), with total harvest reaching about 140k tons in 2013 worldwide (Crosetti 2016). *Mugil* species live in fresh and brackish waters during most of their life cycle, migrating to the sea to reproduce (Nordlie 2016). Thus, they play a fundamental role in transferring energy between estuaries and coastal systems (Lebreton et al. 2011), helping in the maintenance of biological productivity and, consequently, the yield of other fisheries.

Several morphologically similar species of *Mugil* occur sympatrically in tropical and subtropical waters, making it necessary to use genetic information to better understand the number of species and their distribution (Durand et al. 2012b; Durand and Borsa 2015; Xia et al. 2016). Such challenges in assessing species richness and diversity also occur within important Marine Protected Areas (MPAs) that were designed specifically to protect species of high ecologic and economic importance, such as the Coral Coast MPA, the largest coastal Tropical Southwestern marine province (Souza et al. 2012). In this MPA, sympatric species are extremely challenging to identify based on current taxonomic keys that use external morphology alone (Menezes et al. 2015) (Fig. 6.1), resulting in up to 14% of individuals receiving conflicting morphological and mitochondrial classifications (Neves et al. 2020b). Most of the conflicts (94%) occur in individuals that carry the mitochondrial COI barcoding gene haplotype associated with *M. curema* but are morphologically assigned to *M. rubrioculus*, *M. incilis* or to *M. curvidens* (Neves et al. 2020b). Although *M. rubrioculus* and *M. curema* are remarkably similar in external morphology (Fig. 6.1), they have diverged 29 million years ago (mya) (Neves et al. 2020a), reflecting a strong morphological conservatism in the evolution of these species. Such morphological similarity leads to strong disagreements among biologists regarding the number and distribution of these species (Durand et al. 2012a; Menezes et al. 2015; Pacheco-Almanzar et al. 2016), and reinforces the difficulties in establishing species-specific regulations for fisheries. Currently, *Mugil* species are targeted by traditional fisheries throughout the MPA without species-specific quotas. Nevertheless, data from the most commercially valuable species (*M. curema* and *M. liza*) south of this MPA have shown significant decreases on census sizes in the last 25 years (Mendonça and Bonfante 2011; Sant'Ana et al. 2017; Vieira et al. 2019), suggesting that human impact is leading to a decrease in the abundance of these species. It is yet unclear how different species of *Mugil* differ in the levels of intraspecific genetic variability.

Here, we use double-digest restriction of genomic DNA associated with high throughput sequencing to genotype thousands of markers across nominal species of *Mugil* that are sympatric in the Coral Coast MPA to: (i) determine the number of *Mugil* species in the area, (ii) quantify patterns of genetic connectivity within species across two estuaries and between sympatric species in the same estuary, and (iii) estimate

relative effective population size across species and infer if these are determined by species-specific demographic histories. Our results provide new insights into the evolution of these species and provide guidelines for sustainable management.

6.2 MATERIALS AND METHODS

6.2.1 Sampling

6.2.1.1 Specimen collection

We used 94 muscle tissue samples of *Mugil* species that occur sympatrically in the Coral Coast MPA (Fig. 6.1): 14 *Mugil liza,* 16 *Mugil brevirostris*, 17 *Mugil rubrioculus*, 17 *Mugil curema* and 30 *Mugil curvidens*, all assigned based on a diagnostic COI barcoding gene (Table S6.1). From a previous study showing conflict between mitochondrial and morphological classifications (Neves et al. 2020b), we chose a subset of individuals where morphological and mitochondrial concordance was largest, to avoid biases due to misclassification. Nevertheless, we also included two individuals that were mitochondrially classified as *M. curema* but that have the morphological traits of *M. incilis*.

While most specimens were collected in a partial reserve of the MPA where traditional fisheries exploit *Mugil* without restrictions (Santo Antonio estuary), 15 of the 30 individuals of *Mugil curvidens* were collected in an estuary 38km away where fisheries have some restrictions to protect manatee populations (Manguaba estuary; Fig. S6.1). We included this population to test whether populations of *M. curvidens* from different estuaries function as a single panmictic population, or whether some barrier to gene flow exists between locations.

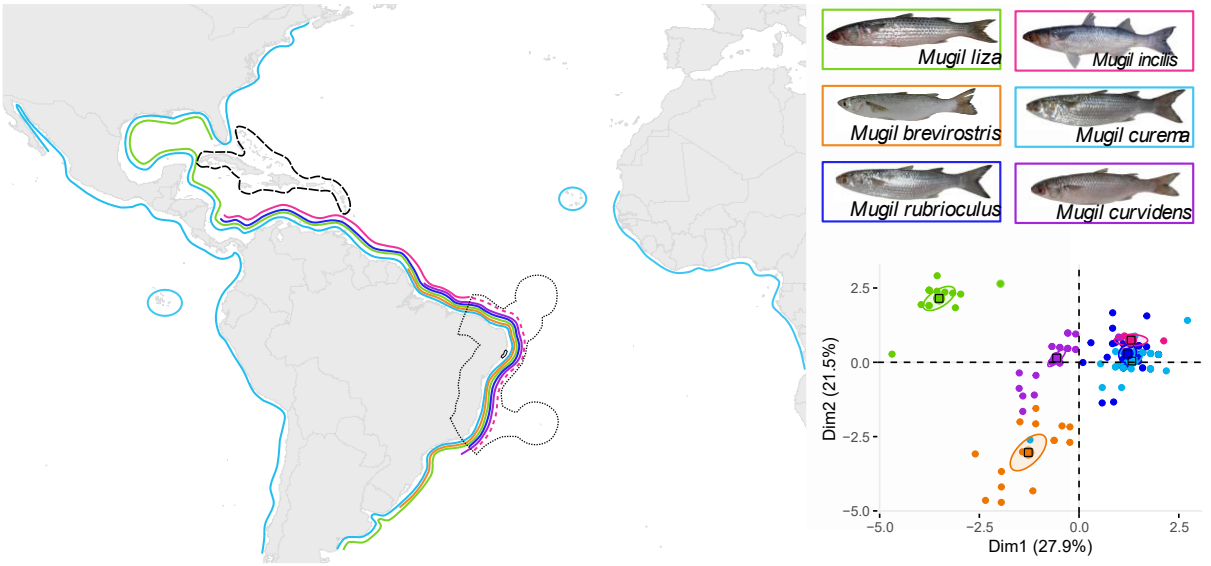

Figure 6.1 - Distribution of the six species of *Mugil* fishes that are potentially sympatric at the Coral Coast MPA (highlighted in solid black line) (Menezes et al. 2015; Barletta and Dantas 2016; Durand and Whitfield 2016; Pacheco-Almanzar et al. 2016). Dashed pink line indicates the area where the occurrence of *M. incilis* has been debated (Menezes et al. 2015). Dotted black line represents the Tropical Southwestern Atlantic province limit. Dashed black line indicates that individuals of all species except *M. brevirostris* may occur. *M. incilis* photo by A. Carvalho. The plot on the right corner represents dimensions 1 and 2 of a multiple factor analysis based on morphological traits (Neves et al. 2020b), ellipses represent 95% confidence level.

6.2.1.2 Genomic sequencing

Tissue samples were sent to DArT™ (Diversity Array Technology), who performed DNA extraction, tested two combinations of enzymes (PstI/HpaII and PstI/SphI), performed high-throughput sequencing, assembled the loci, and called genotypes for the 94 individuals. Based on a subset of 8 samples from 4 species, we chose to genotype all samples for the enzymes PstI/SphI, since this combination showed the highest reproducibility (Table S6.2).

Each individual was characterized by an array of SNPs, where 0 is homozygous for the major allele, 1 is heterozygous, 2 is homozygous for the minor allele, and - represents missing data. Each SNPs is characterized by: reproducibility (proportion of technical replicate assay pairs for which the marker score is consistent), call rate (the proportion of individuals scored for that locus), and polymorphism information content (PIC: index for evaluating the informative extent of a SNP marker, varying between zero for no allelic variation and 1.0 for maximum allele variation).

Because the focal species have diverged between ~29 to ~6 mya (Neves et al. 2020a) and some have diverged in chromosome number and structure (Galetti Jr. et al. 2000; Nirchio et al. 2005, 2017; Rossi et al. 2005), it is possible that the restriction enzymes will not cut the same genomic regions across species. We tested for biases on the distribution of missing data by plotting the missing data per individual and the call rate per species, using the package dartR (Gruber et al. 2018) in R software (R Core Team 2020).

6.2.1.3 Data filtering

We used the package dartR (Gruber et al. 2018) to filter the data and to produce the input files for all downstream analyses. We retained the SNPs with the following criteria: 1) SNPs with reproducibility above 97%, to reduce genotyping error; 2) one SNP per locus favoring SNPs with higher PIC values, to assure statistical independence among SNPs; 3) SNPs that are in Hardy-Weinberg equilibrium, to satisfy a central assumption of population genetic methods; and 4) loci with trimmed sequence tags that are distinct enough to avoid paralogous loci.

Because missing data was not equally distributed among species (Fig. S6.2), for performing comparative analyses across species we built datasets with three stringency levels for the 6 *Mugil* species (hereafter “6sp”): “0MD”, the most stringent filtering without missing data using a call rate of 100%; “20MD”, a conservative filtering allowing for a maximum of 20% missing data using a call rate of 80%; and “40MD”, a less stringent filtering allowing for a maximum of 40% missing data using a call rate of 60%. Because *M. liza* contained most of the missing data (Fig. S6.2), we repeated this process excluding *M. liza* (“5sp”), without missing data. This resulted in 4 datasets for studies of genetic variability between species: 6sp_0MD, 6sp_20MD, 6sp_40MD, 5sp_0MD.

To analyze the genetic variability within species, we have produced datasets following the criteria described above for no missing data. This resulted in 5 species specific datasets: liza_0MD, brevirostris_0MD, rubrioculus_0MD, curema_0MD, and curvidens_0MD. The individuals of *M. incilis* were found to belong to *M. curema* and therefore were included in that species dataset (see Results; Fig. 6.2).

For analyses that also require invariable sites (“inva”), we produced a dataset including 5 species supported by our genetic data, where all variable and invariable sites are present (skipping criterium 2 above), and randomly assigning the heterozygous sites; 0MD_inva.

All filtered datasets and respective analyses are summarized in Table S6.3.

6.2.2 Analyses

6.2.2.1 Population structure

In order to assess how many evolutionary lineages are present in our sampling, we performed two analyses of population structure using the 4 comparative datasets, without *a priori* information on grouping of the individuals. First, we performed a Principal Coordinates Analysis (PCoA) to visualize how the genetic variance is distributed among samples based on presence or absence of alleles, using the package dartR (Gruber et al. 2018) in R software. Second, we estimated the number of genetic clusters and tested if there is ongoing hybridization between them, based on Hardy-Weinberg equilibrium. We used STRUCTURE v.2.3.4 (Pritchard et al. 2000) to assign individuals to one or more K ancestral populations, varying K between 1 and 10, with 5 replicates for each K. We considered 10k iterations as burn-in, 10k MCMC steps, independent allelic frequencies, and no prior on the assignment of individuals. We chose the most likely K based on log-likelihood values (Pritchard et al. 2000). The graphic output was built using Clumpak (Kopelman et al. 2015). We repeated this second test for the dataset with *Mugil curvidens* (curvidens_0MD) to test whether there is population structure between the two estuaries.

For both analyses, we expect that individuals will be assigned either to 6 clusters, as suggested by the taxonomic key based on external morphology, or in 5 clusters, as suggested by the mitochondrial clades (Table S6.1; Neves et al. 2020b). If species hybridize in sympatry, we expect that individuals sampled in Santo Antonio estuary will be assigned to more than one cluster.

6.2.2.2 Phylogenetic relationships

We evaluated phylogenetic relationships among species based on our genomic data, both using phylogenomic and population genomic methods, that differ in their assumptions. First, we built a Maximum Likelihood (ML) phylogenetic tree describing the relationships among all the 94 individuals, without an *a priori* classification of these into species. We used dartR to produce a fasta alignment for each of the six species comparative datasets (6sp_0MD, 6sp_20MD, and 6sp_40MD) containing a concatenation of all loci, with a random allele in heterozygous sites. We used RAxML v.8 (Stamatakis 2014) through CIPRES gateway (Miller et al. 2010) to perform 1 000 bootstrap replicates (bs), with the GTR+GAMMA model. We visualized the ML tree using FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/), rooting the tree with *M. liza* (Neves et al. 2020a). We expect individuals of the same species to form monophyletic clades, either reflecting their mitochondrial or morphological classification.

Second, we built a coalescent species tree describing the relationships between the species included in our sampling, using SNAPP (Bryant et al. 2012). We used the most stringent dataset without missing data (6sp_0MD) to generate a nexus alignment where individuals were grouped *a priori* according to the 5 genetic clusters inferred by our population structure analyses (Fig. 6.2). This analysis was performed in Beauti and Beast software v.2.5.2 (Bouckaert et al. 2019), using the following parameters: 1 million MCMC generations, recorded every 1 000 generations, and two replicated runs to assess convergence. All the other parameters were set as default. LogCombiner v.2.5.2 was used to combine the two generated trees, and TreeAnnotator v.2.5.2 was used to generate a maximum clade credibility tree and access posterior probabilities values (PP) of every node. The species tree was visualized in DensiTree v.2.0 (Bouckaert 2010) and maximum clade credibility tree was visualized in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

6.2.2.3 Genetic variability

We estimated genetic variability between and within species. To ensure an unbiased comparison among species, we used the comparative dataset without

missing data (6sp_0MD). First, we estimated genetic differentiation between all pairwise comparisons of species, using the fixation index F_{ST} (Wright 1943), as implemented in StAMPP package (Pembleton et al. 2013). We grouped the individuals into species (based on the population structure analyses above) and considered the two sampling locations of *M. curvidens* as distinct populations. Second, we estimated several indices of genetic variability within each species, as a proxy for relative differences in effective population size (Ne). Using the dartR package and the dataset 0MD_inva, which allowed more than one SNP per loci, we estimated expected heterozygosity (He) and observed heterozygosity (Ho). Using the DNAsp software v.6.12.03 (Rozas et al. 2017) and the dataset including invariable sites (0MD_inva), we estimated nucleotide diversity (θ and π), number of singletons, and departures from demographic stability with Tajima's D. Because $\pi = 4\ Ne\ \mu$, where μ is the mutation rate per nucleotide site per generation, ratios of diversity indexes calculated from the same loci directly reflect relative Ne.

6.2.2.4 Demographic history

We inferred the demographic history that better explains the observed patterns of genetic variability within each of the five species specific datasets.

We used the diffusion approximation methods implemented in δaδi (Gutenkunst et al. 2009) to simulate patterns of intraspecific genetic variability under 4 models of increasing demographic complexity: 1) a *neutral* model, assuming a constant population size; 2) a *two-epoch* model, describing an instantaneous change in population size (Ne1) at a certain time (T1); 3) a *bottlegrowth* model, describing an instantaneous size change similar to the previous one, but followed by a period of continuous population size change to the present size (Ne2); and 4) a *three-epoch* model, describing two instantaneous size changes at times T1 and T2. To apply these models to our observed data, we converted our datasets into a VCF file using the radiator package (Gosselin 2017), and further into the δaδi's format (.snp) using the python script available at (https://github.com/CoBiG2/RAD_Tools/blob/master/vcf2DaDi.py). To explore the parameter space properly while assuring convergence, we used the following

parameters: initial values of 1 for Ne and of 0.5 for T, lower boundaries for Ne and T set at 0.001, and upper boundaries for Ne set at 100. We used several rounds of parameter optimizations, using the highest likelihood parameters of the previous round as the initial parameters of the next through the δaδi pipeline developed by Portik et al. (2017). We replicated this approach three times per species and model combination to ensure convergence on model selection and parameter estimation. To select the most likely demographic model for each species while accounting for the different number of parameters of the 4 models, we used the Akaike information criterion (AIC) (Link and Barker 2006). Standard deviation for each parameter was calculated through the Fisher information matrix uncertainty estimation implemented in δaδi (Coffman et al. 2016).

6.3 RESULTS

6.3.1 Data filtering

Our raw data (Fig. S6.2) was composed of 55 507 loci of ~69bp with 71 585 SNPs. We observed a large amount of missing data (53%) that is not homogeneously distributed across species. By plotting the call rate of all loci by species (Fig. S6.3) we consistently observed bi-modal distributions, showing that loci are either always or never called across individuals of the same species and thus that genotyping is highly consistent for individuals of the same species. The most divergent species, *Mugil liza*, shows the largest amount of missing data (85%), followed by *M. curema* (72%). This phylogenetic signal on the distribution of missing data is consistent with the chosen enzymes not cutting highly divergent genomes, rather than a methodological error.

Considering all individuals together (i.e. in the 6sp datasets), we found 7 762 SNPs with 40%MD, 3,508 SNPs with 20%MD and 987 SNPs with 0%MD (Fig. S6.4). By removing individuals of *Mugil liza* (i.e. in the 5sp_0MD), the number of SNPs increases more than two-fold (2,332 SNPs).

When considering each individual assigned to each of the five mitochondrial clades separately, the number of SNPs is still considerably large, despite allowing for

no missing data: 555 SNPs for *M. liza,* 756 for *M. brevirostris*, 1 389 for *M. rubrioculus,* 918 for *M. curema*, and 3 240 for *M. curvidens*.

When considering all sites with 0% MD, including invariable sites and multiple SNPs per locus, our alignment has 82 148 bp and 1 293 SNPs.

6.3.2 Analyses

6.3.2.1 Population structure

The first four dimensions of the PCoA using the comparative dataset without missing data (6sp_0MD) explained 95.3% of the data variability (Fig. 6.2A). In general, individuals from the same species clustered together. Individuals of *M. curvidens* sampled at the two localities also clustered together. The 2 individuals that are morphologically *M. incilis* but have the mitochondrial lineage of *M. curema* cluster with all the individuals of *M. curema*. PC1 explains 41% of the variance, reflecting a strong separation of *M. liza* and all other species. PC2 explains 27.5% the variance, with *M. curvidens* and *M. brevirostris* in each extreme. PC3 explains 18% the variance, with *M. curema* and *M. curvidens* in each extreme. PC4 explains 8.8% the variance, further separating *M. brevirostris* and *M. rubrioculus*. Allowing for missing data did not change these results (Fig. S6.5).

In agreement, our STRUCTURE analyses show that the sampled individuals are assigned to five well-differentiated clusters, with the highest likelihood values at K=5 (Fig. 6.2B; Fig. S6.6). The clusters correspond perfectly with the five mitochondrial lineages, with the 2 individuals morphologically classified as *M. incilis* being assigned to the *M. curema* cluster. The two populations of *M. curvidens* are assigned to the same cluster. Our results show no sign of ongoing hybridization, as every individual is entirely assigned to a single cluster. These results remained constant when performing the same analysis without *M. liza* (2 332 SNPs, Fig. S6.7A) or when only considering *M. curvidens* (3 240 SNPs, Fig. S6.7B).

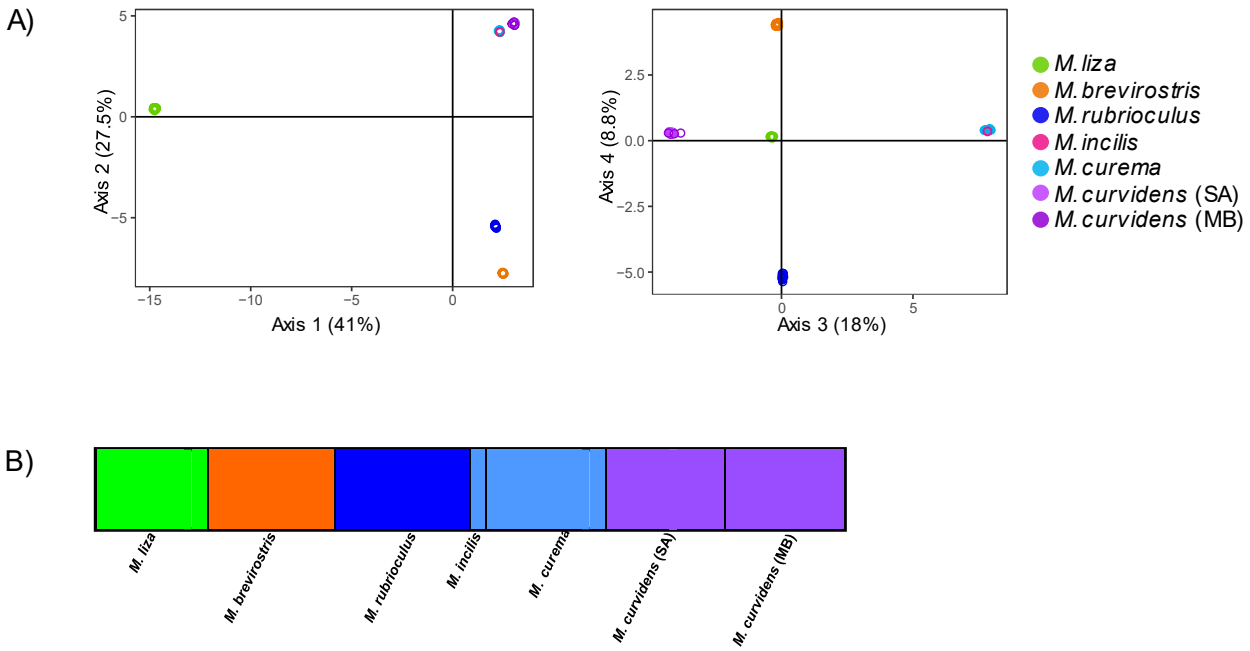

Figure 6.2 - Population structure analyses performed with 94 *Mugil* individuals and the dataset with 0% missing data (987 SNPs). A) PCoA analysis; B) Structure analysis. In both analyses, individuals morphologically identified as *Mugil incilis* belong to the same evolutionary lineages as *M. curema* individuals, and there is no sign of population genetic structure between *M. curvidens* sampled at the two estuaries (SA – Santo Antonio; MB – Manguaba).

6.3.2.2 Phylogenetic relationships

Our ML tree (Fig. 6.3A) recovered five well supported clades (bs= 100), with a consistent topology to what was previously described using a fragment of the mitochondrial COI gene (Neves et al. 2020b). The *M. curvidens* individuals sampled in the Manguaba and Santo Antonio estuaries form a single clade, suggesting no population-level divergence. Also in agreement with mtDNA, the two individuals morphologically identified as *M. incilis* and the four individuals morphologically identified as *M. rubrioculus* nested within the clade of *M. curema*, being sister of *M. curvidens*. *Mugil brevirostris* is sister to *M. rubrioculus*, having the shortest branch lengths between species. The analysis adding missing data showed the same topology (Fig. S6.8).

Our estimated species tree (Fig. S6.9) showed the same topology and relative branch lengths of the ML tree, with the two most recent splits between species being

well supported (pp > 0.99) and the basal split between *M. brevirostris* + *M. rubrioculus* and *M. curema* + *M. curvidens* being less supported (pp = 0.49). The large majority of SNPs follow the same topology as the species tree. There is no gene tree discordance at the two most recent splits, and there is a small fraction of genes that follow two alternative topologies at basal splits (Fig. 6.3B).

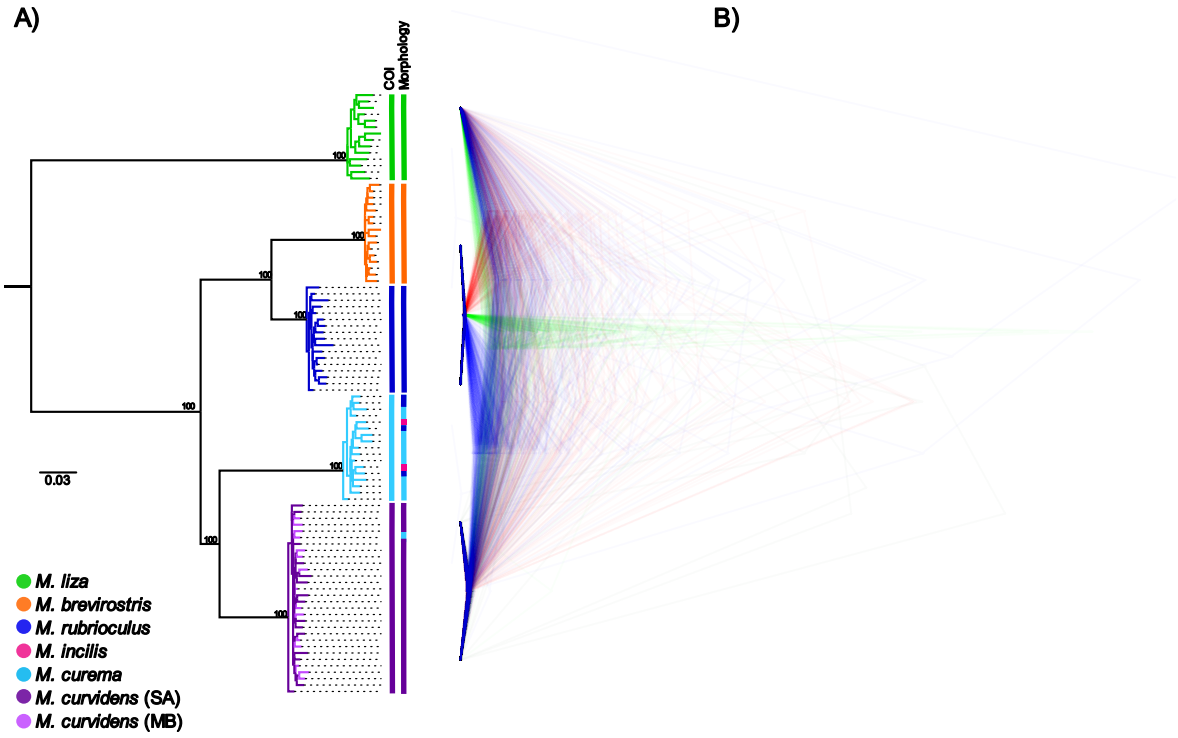

Figure 6.3 - Phylogenetic history of *Mugil* species. A) Maximum Likelihood phylogeny; numbers on nodes represent bootstrap support. B) Coalescent species tree; cloudogram representing the individual trees of 987 independent SNPs with colors related to alternative topologies; the rare cases of deep coalescence were cut off. The bars in the middle refer to the individuals identification based on COI gene and morphological traits (Neves et al. 2020b).

6.3.2.3 Genetic variability

Our measures of genetic variability between populations showed extremely high F_{ST} values between the 5 species identified by our population structure analyses (Table S6.4), showing that most SNPs are fixed between species. The minimum differentiation was observed between *M. brevirostris* and *M. rubrioculus* (F_{ST} = 0.855), and the maximum was between *M. liza* and *M. brevirostris* (F_{ST} = 0.951). Again, the

individuals morphologically assigned to *M. incilis* showed very low differentiation relative to *M. curema* (F_{ST} = 0.025), despite the low sample size of *M. incilis*. The two sampling localities of *M. curvidens* are also genetically similar (F_{ST} = 0.005).

Our estimated levels of genetic variability within species showed consistent patterns across summary statistics (Table 6.1). *Mugil brevirostris* consistently showed the lowest values across all diversity indexes, while *M. liza* showed the highest diversity, except for observed heterozygosity. All species show a negative Tajima's D, reflecting an excess of singletons across all populations. According to Tajima's D estimations, only *M. curvidens* show a significant deviation from the neutral expectations of a population with a constant Ne ($p < 0.05$).

Table 6.1 - Summary statistics of *Mugil* lineages from Tropical Southwestern Atlantic marine province.

Species	Diversity indexes					Demographic indexes	
	Ho	He	S	θ	π	Singletons	Tajima's D
M. liza	0.0196	0.0255	121	0.00046	0.00041	53	-0.51493
M. brevirostris	0.01348	0.01374	63	0.00023	0.0002	30	-0.55128
M. rubrioculus	0.0248	0.01859	109	0.00039	0.00029	62	-1.08718
M. curema	0.01405	0.01836	99	0.00036	0.0003	49	-0.70757
M. curvidens	0.01801	0.0144	137	0.00042	0.00022	79	-1.81368*

He – expected heterozygosity;
Ho – observed heterozygosity;
θ and π - nucleotide diversity;
S - number of polymorphic sites;
Tajima's D value with a * is statistically significant ($p<0.05$).

6.3.2.4 Demographic history

The demographic modelling rejected the *neutral* model of constant population size for all species, in favor of two similar models showing a recent range expansion: *two-epoch* and *bottlegrowth* (Fig. 6.4). The comparisons of observed and simulated Site Frequency Spectra (SFS) under each demographic scenario (Fig. S6.10) showed that we observe an excess of singletons and a deficit of low frequency SNPs across all species, when assuming a constant effective population size. The fit of the SFS is resolved by including an instantaneous change in effective population size (Ne1) at time T1 for all species, with an additional continuous change in population size (Ne2) for *M. brevirostris*, *M. curema*, and *M. curvidens*. The AIC weights (Fig. 6.4, Table

S6.5) support these two simpler models showing a change in effective population size and show no support for a more complex model of two changes in Ne (*three-epoch* model). The parameter estimations for the model with the best fit always show an increase of current Ne across species relative to ancestral Ne (Table S6.6); between 2-fold for *M. liza* and 10-fold for *M. brevirostris* and *M. curvidens*.

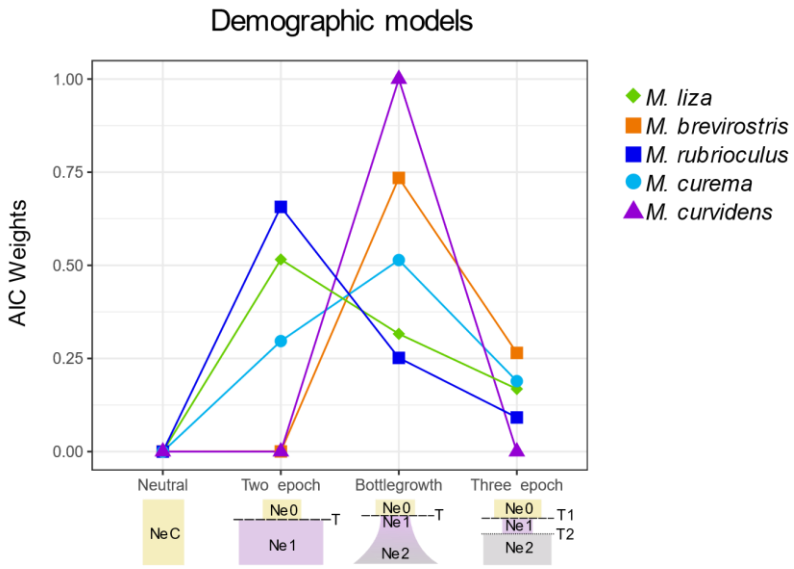

Figure 6.4 - Demographic history of the 5 *Mugil* species. Weighted support for alternative demographic models according to Akaike's information criterion (AIC).

6.4 DISCUSSION

Numerous efforts have been made to protect and manage species of high economic and ecologic importance. Yet, designing sustainable and science-based measures for managing wild populations requires a fundamental knowledge on the number of species, their genetic connectivity and their levels of genetic variability. This is particularly challenging in morphologically similar species such as the *Mugil* fishes, which are heavily targeted by fisheries and play an essential role in the ocean carbon cycling (Lebreton et al. 2011). Here, we use a genomic approach to resolve these evolutionary questions and inform measures for managing these species in the largest coastal Marine Protected Area (MPA) in the Tropical Southwestern Atlantic province.

6.4.1 Five species of *Mugil* occur in sympatry and show high vagility

The number of species of *Mugil* that are sympatric in the Coral Coast MPA in Brazil has been debated (Fig. 6.1) (Menezes et al. 2015; Barletta and Dantas 2016). A previous study integrating mitochondrial and morphologic data reported a conflict between the classification of morphologically similar species, such as *M. curema*, *M rubrioculus* and *M. incilis*, leading to a different assessment of the number of species within this MPA (Table S6.1; Neves et al. 2020b). Assessing the number of independent evolutionary lineages in this highly cryptic species complex required extensive sampling of the nuclear genome, as presented in this study.

Our population structure analyses conclusively show that all sampled specimens belong to five well defined genetic clusters (Fig. S6.6), irrespective of the amount of missing data allowed (from 2 332 to 987 SNPs; Fig. S6.7A), and of the assumptions of the clustering method (Fig. 6.2). These clusters align perfectly with the barcoding gene COI (Fig. 6.3A; Table S6.1), showing that this gene can reliably distinguish between the five species that cannot always be differentiated using external morphological data (Fig. 6.1). Importantly, the two individuals morphologically assigned to *M. incilis* are entirely assigned to *M. curema*, ruling out the hypothesis of mitochondrial introgression through hybridization, and reducing the number of species in the study area to five: *M. liza*, *M. brevirostris*, *M. rubrioculus*, *M. curema* and *M. curvidens*.

Our results also show that there is no population structure in *M. curvidens* sampled in the two estuaries 38 km apart. This result is robust to a 2-fold increase of the number of SNPs by considering this species alone (Fig. S6.7B). This shows that the heterogenous habitat composed by coral reefs and recurrent plume of sediments from the rivers (Passos et al. 2016) does not restrict gene flow and that these two areas with different conservation regimes harbor a single panmictic population of *M. curvidens*. Although this result is perhaps not surprising because adults of *Mugil* species migrate long distances along the shoreline (Livi et al. 2011), and because dispersal also occurs passively through pelagic eggs and larvae (Livi et al. 2011; Whitfield et al. 2012), this hypothesis had not been tested at the genomic level in *Mugil*. Such level of genetic connectivity across heterogeneous habitats contrasts with genomic studies in other marine species (Riginos and Nachman 2001; Hauser and

Carvalho 2008; Selkoe et al. 2016), and has relevant implications for conservation, as discussed below.

6.4.2 *Mugil* species show complete reproductive isolation despite full sympatry

According to a mitochondrial study (Neves et al. 2020a), these focal species of *Mugil* have diverged between ~29 mya, when *M. liza* split from the remaining species, and ~6 mya, when *M. brevirostris* and *M. rubrioculus* diverged. Despite such a long time since initial divergence, it has not been tested if these lineages are reproductively isolated from each other in areas of sympatry.

Our assignment tests show that every individual is assigned to a single cluster, irrespective of the stringency of the filtering (Fig. 6.2B, Fig. S6.7A). Importantly, every individual that showed a mismatch between the morphological and mitochondrial assignments (Table S6.1; Fig. 6.3A) is also assigned to a single cluster in strict agreement with the mitochondrial barcoding gene COI (probability of assignment > 0.99). This shows that sympatric species of *Mugil* are completely reproductively isolated, despite morphological and spatial overlap.

Our maximum likelihood phylogenetic analysis using concatenated loci (Fig. 6.3A) shows a topology and branch lengths that are in large agreement with those previously estimated from mitochondrial loci (Neves et al. 2020a), irrespective of the missing data allowed (Fig. S6.8). This tree shows that *M. brevirostris* and *M. rubrioculus* diverged most recently, followed by the split between *M. curema* (which includes the "*M. incilis*" individuals discussed above) and *M. curvidens* (which includes both sampling localities). Interestingly, most SNPs are fixed among all species pairwise comparisons, reflected in the extremely high values of fixation indices among the 5 species or clades (Table S6.4), and consistent with old divergence without gene flow.

By using a coalescent approach that co-estimates topologies for independent SNPs and the species tree they are embedded in (Razkin et al. 2016), we can infer the number of topologies that disagree with the species tree. Our results show that the vast majority of the gene trees agree with the species tree (Fig. 6.3B), particularly at the two most recent species splits described above. A small fraction of two alternative topologies reflects disagreements in the deeper nodes of the tree. Given that these

nodes are estimated to date between ~29 and ~23 mya (Neves et al. 2020a), that there is no evidence for shared variants between more closely related taxa (Table S6.4), and that there is no hybridization between species (Fig. 6.2B), we interpret this disagreement as reflective of incomplete lineage sorting.

Together, our results show that these five species of *Mugil* have experienced a long period of independent evolutionary history, with reduced levels of incomplete lineage sorting and no hybridization. Although these species lack any geographic barriers, use the same macrohabitat and are morphologically similar, several studies of *Mugil* species have shed some light on possible reproductive barriers that might contribute to the strong reproductive isolation reported here. Previous studies have shown that they differ in spawning time and habitat preferences (Albieri et al. 2010; Mai et al. 2018), possibly constituting a behavioral isolation barrier. Diet studies have shown that sympatric species of *Mugil*, although all limno-benthophagous, can present differential particle size selection to avoid competition (LeLoc'h et al. 2015), possibly constituting an ecological isolating barrier. Furthermore, cytogenetic studies have shown that some of these species differ in the number and arrangement of chromosomes (Rossi et al. 2005; Harrison et al. 2007; Nirchio et al. 2017), possibly constituting a genetic isolating barrier. Although the relative contribution of these barriers has not been tested, our results show that they result in complete reproductive isolation between sympatric *Mugil* species.

6.4.3 Species differ in their relative abundance and demographic expansion

Information regarding population size change is fundamental for understanding the evolutionary history of a species and to delineate conservation strategies (Ramakrishnan et al. 2005; Dussex and Robertson 2018). Yet, inferring the evolutionary processes underlying the patterns of genetic variation within species requires hundreds or thousands of genetic markers sampled randomly across the genome.

Using genomic data, we show that *M. brevirostris* has the lowest genetic diversity according to every diversity index, reflecting the smallest effective population size of the five species (Table 6.1). *Mugil liza* has the highest diversity in He, θ and π,

corresponding to twice the Ne of *M. brevirostris*. Considering these indices, *M. rubrioculus*, *M. curema* and *M. curvidens* have intermediate intraspecific diversity, corresponding to ~1.3 to 1.7 times the Ne of *M. brevirostris*. However, caution is needed when interpreting Ne, since these estimates of (long-term) effective population size are not expected to scale linearly with (contemporary) census sizes (Leffler et al. 2012).

Our demographic modelling (Fig. 6.4) show that the *neutral* demographic scenario was rejected for every species in favor of scenarios with an increase of Ne over time (a sudden change of Ne for *M. liza* and *M. rubrioculus*, and an incremental change of Ne for *M. brevirostris*, *M. curema*, and *M. curvidens*). The simulated patterns of variability are very similar under these two models of range expansion (Fig. S6.10, Table S6.5) and thus caution is needed in trying to distinguish between them. Genomic studies in other exploited fish species have found similar demographic scenarios of demographic expansion (e.g. in the Atlantic herring (Barrio et al. 2016), in the North American Lake Whitefish (Rougeux et al. 2017)).

This genomic signature of demographic expansion is in strong contrast with reports of reduction in census population size among *Mugil* species (Mendonça and Bonfante 2011; Sant'Ana et al. 2017; Vieira et al. 2019). It is possible, though, that populations exhibit higher Ne values than census population sizes, as a result of larger ancestral populations few generations ago (Braude and Templeton 2009). Together, our results suggest that current patterns of genetic diversity in wild populations are highly determined by historical changes in Ne associated with the Quaternary ice age, and thus future studies of genetic diversity in protected areas must consider the effect of these important historical events. Higher Ne values can also be associated with maturation time lengthened (Nunney 1993), which can happen when juveniles maturate earlier due to overexploitation (Kuparinen and Merilä 2007), or with differences in geographical range among species (Leffler et al. 2012). Despite a generalized demographic expansion, our results also show that its magnitude was remarkably different across species, between 2.7-fold for *M. liza* to 10-fold increase for *M. curvidens* and *M. brevirostris* (Table S6.6). This suggest that, although these species are morphologically similar and coexist in the same habitats, their evolutionary

histories can be quite different, affecting their levels of standing genetic variation, and hence their adaptive potential (Wang et al. 2016).

6.4.4 Implications for conservation

Although governmental institutions and legislators are willing to implement new science-based regulations, a fundamental knowledge on the number of species, their genetic connectivity, and their relative abundance are lacking. Considering that Mugilidae fishes are particularly challenging to identify morphologically, yet are the main fish target in tropical artisanal fisheries (Batista et al. 2014), our results provide important guidelines for conservation.

We show that *M. incilis* is absent from the Coral Coast MPA, in agreement with a previous study based on morphological and mitochondrial data (Menezes et al. 2015). This implies that conservation efforts inside this MPA should focus only on five *Mugil* species and that *M. incilis* has a more restricted distribution than previously thought in northern waters.

Currently, fisheries in northeastern Brazil report capture of all *Mugil* species under the same category (9 219.5 tons in 2007; (IBAMA 2007)). Irrespective of their unknown current census population sizes, our results suggest that these species have different effective population sizes (Table 6.1), and that the magnitude of demographic expansion highly differs across species. Although our results suggest that each species of *Mugil* should have a specific protection status, this is extremely challenging to implement in such a morphologically conserved group of species. Yet, our finding that species classification using thousands of genomic markers is in strict agreement with species classification based on single a fragment of the COI gene (Neves et al. 2020b) represents an important validation for future monitorization studies using hundreds or thousands of samples. This mitochondrial barcoding gene provides a cost-effective tool to monitor fisheries bycatch, and thus assess species abundance over time and establish sustainable protective measure for such ecologically and economically important species.

ACKNOWLEDGMENTS

This work is part of the Long Term Ecological Research – Brazil site PELD-CCAL (Projeto Ecológico de Longa Duração – Costa dos Corais, Alagoas) funded by the Brazilian National Council for Scientific and Technological Development – CNPq (#441657/2016-8), the Brazilian Coordination for the Improvement of Higher Education Personnel - PELD/CAPES (#23038.000452/2017-16) and the Research Support Foundation of the State of Alagoas – FAPEAL (#60030.1564/2016). This network was also funded by Bayerisches Hochschulzentrum für Lateinamerika - BAYLAT (#914-20.1.1). JMMN thanks to scholarship provided by Coordination of Improvement of Higher Level Personnel - CAPES (#88887.137815/2017-00 and #88881.189448/2018-01), TM thanks to CNPq fellowship (#309904/2015–3 and # 312291/2018-3) and FAPEAL fellowship (#60030.000406/2017). We thank C. Peart and S. Tusso for the discussion on bioinformatics, and J. Verba and M. Querejeta for comments in an early version of this manuscript. We are very grateful to the fishermen that provided the specimens and also to the whole team from LABI, LAEPP and LACOM who helped processing these.

DATA ARCHIVING

All individuals sampled for this study were deposited in the ichthyology collection of the Alagoas university; Voucher number, sampling locations, morphological data and COI Genbank accession numbers are listed in Table S6.1. All the raw genomic data, filtered datasets, and infiles for all the analyses were deposited in GitHub https://github.com/JMNeves/mugil_dart

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7. CONCLUSÕES GERAIS

Apesar das tainhas possuírem a morfologia externa bastante conservada, as diferenças genéticas são muito altas entre as espécies de *Mugil*. Analisando dados de 13 das 16 espécies do gênero, foi possível concluir que estas espécies divergiram entre 30 e 5 milhões de anos atrás. Três espécies foram recuperadas parafiléticas e possuem linhagens crípticas: *M. cephalus* (15 linhagens, incluindo *M. liza*), *M. curema* (5 linhagens, incluindo *M. thoburni* e *M. margaritae*) e *M. rubrioculus* (2 linhagens, sendo parafilético com *M. hospes* e *M. brevirostris*).

Na Área de Proteção Ambiental Costa do Corais (APACC) foi possível identificar seis espécies de *Mugil* com base nos caracteres morfológicos descritos em chave de identificação. No entanto, análises multivariadas não conseguiram estabelecer divergências morfológicas significativas entre todas elas. O gene mitocondrial COI foi então analisado para validar as identificações morfológicas, seguindo o protocolo de DNA *barcoding*. Utilizando esta abordagem integrativa através do contraste entre os dois conjuntos de dados, foram encontrados erros de identificação devido a sobreposição de caracteres morfológicos entre diferentes espécies. Além disso, embora tenham sido identificados seis grupos morfológicos, os dados genéticos confirmam a existência de apenas cinco linhagens mitocondriais de *Mugil* na APACC: *M. brevirostris*, *M. curema*, *M. curvidens*, *M. liza* e *M. rubrioculus*. Os indivíduos previamente identificados morfologicamente como *M. incilis* pertencem à mesma linhagem mitocondrial de *M. curema*, indicando que *M. incilis* não atinge essa província, e que existe historicamente a identificação incorreta dessa espécie na área.

Embora a divergência entre as espécies de tainhas tenha ocorrido há muito tempo, sua morfologia é bastante conservada, o que pode ser explicado por convergência evolutiva e/ou conservadorismo de nicho filogenético, que podem estar agindo sinergicamente. Desse modo, os traços utilizados atualmente para delimitação das espécies sofrem modificações pequenas, com baixo poder taxonômico. Outros traços morfológicos devem ser testados na tentativa de melhor delimitar as espécies desse grupo de alta importância econômica e ecológica. Por exemplo, morfometria geométrica do corpo e das escamas provaram ser ferramentas úteis para discriminar

tainhas em níveis específico e populacional (GONZÁLEZ-CASTRO; GHASEMZADEH, 2016).

Dados genômicos obtidos pela técnica *DArT-seq* corroboram os dados mitocondriais, indicando a existência de apenas cinco linhagens evolutivas do gênero *Mugil* na APACC, não havendo sinal de fluxo gênico interespecífico, nem tampouco barreira ao fluxo gênico entre indivíduos de *M. curvidens* coletados em diferentes rios separados por 38 km. Relações filogenéticas baseadas nesses dados corroboram a topologia estimada apenas com o gene mitocondrial COI.

A estimativa do tamanho efetivo populacional (N_e) sugere que as espécies não têm N_e semelhantes, com *M. liza* tendo o maior N_e e *M brevirostris* o menor. Esse resultado pode ser surpreendente, visto que *M. liza* é a espécie mais cobiçada pela pesca, no entanto, não deve ser interpretado como favorável à pesca não regulamentada, pois esses valores refletem mudanças demográficas ao longo de milhares de anos, muito antes do impacto humano nessa região.

O fato de não haver barreiras ao fluxo gênico entre indivíduos de *M. curvidens* amostrados em diferentes rios, aponta que o estabelecimento de áreas de reserva integral intercaladas por áreas onde a pesca é permitida dentro da APACC poderia manter o fluxo gênico ao longo do ecossistema, mantendo populações viáveis numa escala temporal ecológica e evolutiva ao mesmo tempo que garantiria a subsistência dos pescadores especializados na pesca da tainha. Desse modo, levando em consideração as dificuldades práticas de identificar espécies de *Mugil* nos desembarques e estabelecer cotas pesqueiras, essa pode ser uma maneira eficaz de preservar essas espécies a longo prazo. Com essas informações, é possível direcionar um plano de manejo cientificamente embasado para garantir a manutenção de espécies simpátricas de *Mugil* de maneira sustentável.

ANEXOS

Table S4.2 - Sequences of Mugilidae used in the mitochondrial analyses. Lineage column is according to Durand and Borsa (2015).

Reference[a]	Species	Sampling location	Individual	Voucher	16S	COI	CYTB	Lineage
III	*Mugil bananensis*	Saloum Estuary, Senegal	286	MNHN 2009-0733	JQ060769	JQ060523	JQ060267	
III	*Mugil capurii*	St Louis, Senegal Estuary, Senegal	282	–	HM143894	JQ060525	JQ060269	
III	*Mugil capurii*	Dakhla, Western Sahara	283	–	HM143895	JQ060526	JQ060270	
I, III	*Mugil cephalus*	Lima, Peru	349	–	JQ060774	HQ149714	JQ060295	G
I, III	*Mugil cephalus*	Charleston, South Carolina, USA	347	–	JQ060787	HQ149710	JQ060294	B
I, III	*Mugil cephalus*	Indian River, Florida, USA	344		JQ060786	HQ149711	JQ060293	B
I, III	*Mugil cephalus*	Hawaii	350	–	JQ060820	JQ060549	JQ060296	D
I, III	*Mugil cephalus*	Kaoping Estuary, Taiwan	328	NMMBP11549	JQ060801	JQ060539	JQ060283	I
I, III	*Mugil cephalus*	Kone, New Caledonia	386	–	JQ060783	JQ060570	JQ060319	L
I, III	*Mugil cephalus*	Laguna Madre, Mexico	373	–	JQ060788	JQ060562	JQ060310	B
I, III	*Mugil cephalus*	Kaoping Estuary, Taiwan	329	NMMBP11545	JQ060789	JQ060540	JQ060284	C
I, III	*Mugil cephalus*	Swansea, Sydney, Australia	367	–	JQ060813	JQ060558	JQ060305	H
I, III	*Mugil cephalus*	Pateu Dam, New Zeland	362	–	JQ060806	JQ060555	JQ060302	F
I, III	*Mugil cephalus*	La Paz, Mexico	371	–	JQ060775	HQ149715	JQ060307	G
I, III	*Mugil cephalus*	Great Fish Estuary, South Africa	342	–	JQ060810	JQ060545	JQ060289	E
I, III	*Mugil cephalus*	Coast of Iquique, Chilie	375	UDOV-290	JQ060790	JQ060563	JQ060311	A
I, III	*Mugil cephalus*	Merja Zerga, Morocco	314	–	JQ060815	JQ060529	JQ060273	*M. cephalus*
I, III	*Mugil cephalus*	Boran Dabon, Forecariah Estuary, Guinea	337	_	JQ060798	JQ060543	JQ060287	J

I, III	*Mugil cephalus*	Peel Harvey Estuary, Australia	368	–	JQ060785	JQ060559	JQ060306	K
I, III	*Mugil cephalus*	Pearl River, China	358	–	JQ060803	JQ060553	JQ060300	I
II	*Mugil cephalus*	Ecuador: Bahia Divine, Isla Santa Cruz, Galapagos Isld	6446	–	JX559527	JX559532	JX559523	Q
II	*Mugil cephalus*	Ecuador: Bahia Divine, Isla Santa Cruz, Galapagos Isld	6450	–	JX559528	JX559533	JX559524	Q
I, III	*Mugil curema*	Foundiougne, Saloum Estuary, Senegal	392	-	JQ060845	JQ060577	JQ060326	M
I, III	*Mugil curema*	Saloum Estuary, Senegal	390	MNHN 2009-0732	JQ060843	JQ060575	JQ060324	M
I, III	*Mugil curema*	La Paz, Mexico	406	–	JQ060830	JQ060588	JQ060337	O
I, III	*Mugil curema*	Itapissuma, Brazil	408	–	JQ060828	JQ060590	JQ060339	*M. curema*
I, III	*Mugil curema*	Sand flats at mouth of Cerique Estuary, El Salvador	426	KU:KUI:40290	JQ060850	JQ060600	JQ060349	O
I, III	*Mugil curema*	Chorreras Bay, Panama, Pacific coast	294	UDOV-187(d)	JQ060833	JQ060574	JQ060323	O
I, III	*Mugil curema*	Chorreras Bay, Panama, Pacific coast	293	UDOV-186(d)	JQ060832	JQ060573	JQ060322	O
I, III	*Mugil curema*	La Restinga, Margarita Island, Venezuela	414	UDOV-201 (b)	JQ060824	JQ060593	JQ060342	*M. curema*
V	*Mugil curema* (misidentification of *M. margaritae*)	Venezuela	30070	LBP6065	JX000539	JX185196	JX185275	
V	*Mugil curema*	Brazil	46939	LBP10004	JX000548	JX185205	JX185284	
IV	*Mugil curvidens*		1	114039	KJ702381	KJ702390		
III	*Mugil hospes*	West side of Turneffe Caye, Belize	306	–	JQ060857	JQ060607	JQ060356	

V	*Mugil hospes* (misidentification of *M. brevirostris*)	Brazil	42659	LBP6061	JX000559	JX185216	JX185295	
V	*Mugil incilis*	Brazil	42657	LBP9061	JX000525	JX185182	JX185266	
V	*Mugil incilis*	Venezuela	785		JX000528.1	JX185185		
III	*Mugil incilis*	Kourou, French Guyana	299	–	JQ060859	JQ060609	JQ060358	*M. incilis*
I, III	*Mugil liza*	La Restiga, Margarita Island, Venezuela	298	UDOV-89	JQ060861	HQ149713	JQ060360	*M. liza*
I, III	*Mugil liza*	Arroyo Zanja, Uruguay	378	NRM-56496	JQ060862	JQ060610	JQ060361	*M. liza*
I, III	*Mugil liza*	Laguna del Diaro, Uruguay	379	NRM-55970	JQ060863	JQ060611	JQ060362	*M. liza*
V	*Mugil liza*	Brazil	33447	LBP6849	JX000501	JX185158	JX185243	
V	*Mugil liza*	Argentina	27726	LBP6279	JX000503.1	JX185160.1	JX185245	
I, III	*Mugil margaritae*	Boca del Rio, Margarita Island, Venezuela	401	UDOV-73 (c)	JQ060840	JQ060584	JQ060333	N
I, III	*Mugil margaritae*	La Restinga, Margarita Island, Venezuela	400	UDOV-70 (c)	JQ060839	JQ060583	JQ060332	N
V	*Mugil rubrioculus*	Venezuela	30086	LBP6063	JX000553	JX185210	JX185289	
V	*Mugil rubrioculus*	Brazil	42655	LBP9060	JX000555.1	JX185212.1	JX185291	
III	*Mugil rubrioculus*	Chorreras Bay, Pacific coast of Panama	305b	UDOV-190	JQ060865	JQ060613	JQ060364	P
V	*Mugil trichodon*	Venezuela	30770	LBP6066	JX000522	JX185179	JX185264	
I, III	*Mugil trichodon*	Boca del Rio, Margarita Island, Venezuela	291	UDOV-72	JQ060866	JQ060614	JQ060365	
VI	*Mugil thoburni*	Ecuador: Santa Cruz Isld, Galápagos	6447	–	KF375061	–	KF375141	

II	*Mugil thoburni*	Ecuador: Santa Cruz Isld, Galápagos	6435	–	JX559529	JX559534	JX559525	*M. thoburni*
III	*Agonostomus catalai*	Comoros: Koundre River,Anjouan	23	MNHN 2006-614	JQ060643	JQ060394	JQ060138	
III	*Cestraeus goldiei*	Cagayan Province, Philippines	12	–	JQ060655	JQ060406	JQ060149	
III	*Chaenomugil proboscideus*	La paz, Mexico	002c	–	JQ060658.1	JQ060409.1	JQ060152.1	
III	*Dajaus monticola*	La Trilla River, Aragua State, Venezuela	031b	STRI 21827	JQ060650	JQ060399	JQ060143	
I, III	*Joturus pichardi*	Changuinola River, Boca del Toro, Panama	22	STRI 21850	JQ060694	JQ060446	JQ060189	
I, III	*Neomyxus leuciscus*	Arutua Atoll, Tuamotu	003b	MNHN 2009-1681	JQ060870	JQ060618	JQ060369	
This study	*Mugil cephalus*	Pacific Coast. Cuyutlán Lagoon, Colima, Mexico	I2	–	–	MN505186	–	*M.l cephalus*
This study	*Mugil cephalus*	Pacific Coast. Cuyutlán Lagoon, Colima, Mexico	I3	–	–	MN505187	–	*M. cephalus*
This study	*Mugil curema*	Barra de Sto. Antônio, Alagoas, Brazil	TA038	MUFAL2179	–	MK837984	–	*M. curema*
This study	*Mugil curema*	Barra de Sto. Antônio, Alagoas, Brazil	TA094	MUFAL2235	–	MK838025	–	*M. curema*
This study	*Mugil curema*	Barra de Sto. Antônio, Alagoas, Brazil	TA180	MUFAL2122	–	MK838061	–	
This study	*Mugil liza*	Barra de Sto. Antônio, Alagoas, Brazil	TA159	MUFAL2300	–	MK838052	–	
This study	*Mugil liza*	Barra de Sto. Antônio, Alagoas, Brazil	TA228	MUFAL2359	–	MK838096	–	
This study	*Mugil incilis*	Zulia, Venezuela	MI01	–	–	MN505188	–	
This study	*Mugil incilis*	Zulia, Venezuela	MI02	–	–	MN505189	–	

This study	*Mugil rubrioculus*	Barra de Sto. Antônio, Alagoas, Brazil	TA153	MUFAL2294	–	MK838047	–
This study	*Mugil rubrioculus*	Barra de Sto. Antônio, Alagoas, Brazil	TA210	MUFAL2341	–	MK838078	–
This study	*Mugil hospes*	Gulf of Mexico, Alvarado Lagoon, Veracruz	A214	–	–	MN505190	–
This study	*Mugil hospes*	Gulf of Mexico, Alvarado Lagoon, Veracruz	A218	–	–	MN505191	–
This study	*Mugil brevirostris*	Barra de Sto. Antônio, Alagoas, Brazil	TA109	MUFAL2250	–	MK838033	–
This study	*Mugil brevirostris*	Barra de Sto. Antônio, Alagoas, Brazil	TA110	MUFAL2251	–	MK838034	–
This study	*Mugil curvidens*	Porto de Pedras, Alagoas, Brazil	TA187	MUFAL2318	–	MK838064	–
This study	*Mugil curvidens*	Barra de Sto. Antônio, Alagoas, Brazil	TA230	MUFAL2361	–	MK838098	–

[a]Source references for mtDNA sequences were as follows: I – Durand & Borsa, 2015; II – Durand, Chen, et al., 2012; III – Durand, Shen, et al., 2012; IV – Menezes et al., 2015; V – Siccha-Ramirez et al., 2014; VI – Xia et al., 2016.

Table S4.2 - Nuclear dataset sampling and respective GenBank accession numbers.

Species	Specimen	Voucher	Lineage	RAG1	ENC1	Myh6	Ptr	Glyt	SH3PX3	Sidkey
Mugil cephalus	RX2	FDZM20070901	C	KF375586	KF375213	KF375365	KF375508	KF375290	KF375806	KF375880
Mugil cephalus	6446	-	Q	KF375589	KF375216	KF375367	KF375511	KF375293	KF375809	KF375883
Mugil curema	294	UDOV-187	O	KF375588	KF375215	-	KF375510	KF375292	KF375808	KF375882
Mugil curema	392	-	M	KF375587	KF375214	KF375366	KF375509	KF375291	KF375807	KF375881
Mugil liza	298b	-	-	KF375592	KF375219	KF375370	KF375514	KF375296	KF375812	KF375886
Mugil liza	378	-	-	KF375593	KF375220	KF375371	KF375515	KF375297	KF375813	KF375887
Mugil incilis	299b	-	-	KF375591	KF375218	KF375369	KF375513	KF375295	KF375811	KF375885
Mugil rubrioculus	305b	UDOV-177	P	KF375594	KF375221	KF375372	KF375516	KF375298	KF375814	KF375888
Mugil hospes	306	KUKUIT5833	-	KF375590	KF375217	KF375368	KF375512	KF375294	KF375810	KF375884
Mugil trichodon	291b	UDOV-81	-	KF375595	KF375222	KF375373	KF375517	KF375299	KF375815	KF375889
Mugil thoburni	6435	-	-	KF375596	KF375223	KF375374	KF375518	KF375300	KF375816	KF375890
Mugil thoburni	6447	-	-	KF375597	KF375224	KF375375	KF375519	KF375301	KF375817	KF375891
Mugil capurii	283b	-	-	KF375585	KF375212	KF375364	KF375507	KF375289	KF375805	KF375879
Mugil bananensis	286b	-	-	KF375584	KF375211	KF375363	KF375506	KF375288	KF375804	KF375878
Chaenomugil proboscideus	002f	-	-	KF375560	KF375188	-	KF375483	KF375265	KF375780	KF375857
Neomyxus leuciscus	003b	MNHN 2009-1681	-	KF375601	KF375228	KF375379	KF375523	KF375305	KF375820	KF375895

Table S4.2 continued - Nuclear dataset sampling and respective GenBank accession numbers.

Species	Specimen	Voucher	Lineage	Rhodopsin	TMO-4C4	Plag2	Sreb2	RNF213
Mugil cephalus	RX2	FDZM20070901	C	KF375660	KF376024	KF375435	KF375953	KF375732
Mugil cephalus	6446	-	Q	KF375663	KF376027	KF375438	KF375956	KF375735
Mugil curema	294	UDOV-187	O	KF375662	KF376026	KF375437	KF375955	KF375734
Mugil curema	392	-	M	KF375661	KF376025	KF375436	KF375954	KF375733
Mugil liza	298b	-	-	KF375666	KF376030	KF375441	KF375959	KF375738
Mugil liza	378	-	-	KF375667	KF376031	KF375442	KF375960	KF375739
Mugil incilis	299b	-	-	KF375665	KF376029	KF375440	KF375958	KF375737
Mugil rubrioculus	305b	UDOV-177	P	KF375668	KF376032	KF375443	KF375961	KF375740
Mugil hospes	306	KUKUIT5833	-	KF375664	KF376028	KF375439	KF375957	KF375736
Mugil trichodon	291b	UDOV-81	-	KF375669	KF376033	KF375444	KF375962	KF375741
Mugil thoburni	6435	-	-	KF375670	KF376034	KF375445	KF375963	KF375742
Mugil thoburni	6447	-	-	KF375671	KF376035	KF375446	KF375964	KF375743
Mugil capurii	283b	-	-	KF375659	KF376023	KF375434	KF375952	KF375731
Mugil bananensis	286b	-	-	KF375658	KF376022	KF375433	KF375951	KF375730
Chaenomugil proboscideus	002f	-	-	KF375638	KF376001	-	KF375932	KF375710
Neomyxus leuciscus	003b	MNHN 2009-1681	-	KF375675	KF376038	KF375447	KF375966	KF375745

Table S4.3 - Interspecific genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *M. curema*													
2. *M. margaritae*	0.044												
3. *M. thoburni*	0.055	0.055											
4. *M. incilis*	0.074	0.081	0.058										
5. *M. curvidens*	0.136	0.129	0.134	0.143									
6. *M. rubrioculus*	0.197	0.182	0.180	0.200	0.180								
7. *M. brevirostris*	0.204	0.200	0.195	0.204	0.181	0.115							
8. *M. hospes*	0.205	0.199	0.189	0.187	0.173	0.129	0.074						
9. *M. liza*	0.184	0.180	0.192	0.195	0.201	0.197	0.204	0.216					
10. *M. cephalus*	0.185	0.177	0.190	0.195	0.200	0.195	0.202	0.210	0.028				
11. *M. capurrii*	0.160	0.157	0.169	0.178	0.156	0.160	0.170	0.157	0.180	0.180			
12. *M. bananensis*	0.196	0.187	0.182	0.183	0.195	0.157	0.175	0.191	0.162	0.164	0.131		
13. *M. trichodon*	0.207	0.199	0.197	0.202	0.195	0.190	0.206	0.192	0.193	0.194	0.158	0.202	
outgroup	0.219	0.212	0.210	0.215	0.200	0.219	0.197	0.216	0.209	0.213	0.184	0.197	0.205

Table S4.4 - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. M_curema_TA094																		
2. M_curema_414	0.000																	
3. M_curema_TA180	0.004	0.004																
4. M_curema_408	0.004	0.004	0.000															
5. M_curema_TA038	0.005	0.005	0.002	0.002														
6. M_curema_46939	0.005	0.005	0.002	0.002	0.004													
7. M_curema_30070	0.048	0.048	0.044	0.044	0.046	0.046												
8. M_margaritae_400	0.048	0.048	0.044	0.044	0.046	0.046	0.000											
9. M_margaritae_401	0.050	0.050	0.046	0.046	0.048	0.048	0.002	0.002										
10. M_curema_406	0.064	0.064	0.060	0.060	0.062	0.062	0.040	0.040	0.038									
11. M_curema_426	0.062	0.062	0.058	0.058	0.056	0.060	0.039	0.039	0.037	0.016								
12. M_curema_293	0.062	0.062	0.058	0.058	0.056	0.060	0.039	0.039	0.037	0.016	0.000							
13. M_curema_294	0.062	0.062	0.058	0.058	0.056	0.060	0.039	0.039	0.037	0.016	0.000	0.000						
14. M_curema_390	0.058	0.058	0.058	0.058	0.060	0.056	0.050	0.050	0.052	0.050	0.040	0.040	0.040					
15. M_curema_392	0.054	0.054	0.054	0.054	0.056	0.052	0.046	0.046	0.044	0.042	0.033	0.033	0.033	0.007				
16. M_thoburni_6435	0.052	0.052	0.048	0.048	0.046	0.050	0.056	0.056	0.054	0.064	0.058	0.058	0.058	0.066	0.058			
17. M_incilis_MI01	0.062	0.062	0.066	0.066	0.068	0.068	0.082	0.082	0.080	0.090	0.088	0.088	0.088	0.078	0.070	0.058		
18. M_incilis_MI02	0.062	0.062	0.066	0.066	0.068	0.068	0.082	0.082	0.080	0.090	0.088	0.088	0.088	0.078	0.070	0.058	0.000	

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19. M_incilis_42657	0.062	0.062	0.066	0.066	0.068	0.068	0.082	0.082	0.080	0.090	0.088	0.088	0.088	0.078	0.070	0.058	0.000	0.000
20. M_incilis_785	0.060	0.060	0.064	0.064	0.066	0.066	0.080	0.080	0.078	0.088	0.086	0.086	0.086	0.076	0.068	0.056	0.002	0.002
21. M_incilis_299	0.060	0.060	0.064	0.064	0.066	0.066	0.080	0.080	0.082	0.092	0.090	0.090	0.090	0.076	0.072	0.060	0.002	0.002
22. M_curvidens_TA230	0.137	0.137	0.133	0.133	0.135	0.135	0.129	0.129	0.126	0.137	0.131	0.131	0.131	0.144	0.135	0.133	0.144	0.144
23. M_curvidens_1	0.140	0.140	0.135	0.135	0.137	0.137	0.131	0.131	0.129	0.140	0.133	0.133	0.133	0.142	0.133	0.135	0.142	0.142
24. M_curvidens_TA187	0.140	0.140	0.135	0.135	0.137	0.137	0.131	0.131	0.129	0.140	0.133	0.133	0.133	0.142	0.133	0.135	0.142	0.142
25. M_rubrioculus_TA210	0.199	0.199	0.194	0.194	0.194	0.192	0.184	0.184	0.182	0.199	0.199	0.199	0.199	0.204	0.199	0.181	0.199	0.199
26. M_rubrioculus_30086	0.197	0.197	0.192	0.192	0.192	0.189	0.182	0.182	0.179	0.196	0.197	0.197	0.197	0.202	0.197	0.179	0.197	0.197
27. M_rubrioculus_TA153	0.197	0.197	0.192	0.192	0.192	0.189	0.187	0.187	0.184	0.201	0.197	0.197	0.197	0.202	0.197	0.179	0.197	0.197
28. M_rubrioculus_42655	0.197	0.197	0.192	0.192	0.192	0.189	0.182	0.182	0.179	0.201	0.202	0.202	0.202	0.202	0.197	0.179	0.197	0.197
29. M_rubrioculus_305b	0.192	0.192	0.187	0.187	0.187	0.184	0.181	0.181	0.179	0.201	0.212	0.212	0.212	0.206	0.201	0.184	0.212	0.212
30. M_brevirostris_TA109	0.203	0.203	0.198	0.198	0.195	0.195	0.200	0.200	0.198	0.213	0.210	0.210	0.210	0.203	0.200	0.195	0.200	0.200
31. M_brevirostris_TA110	0.206	0.206	0.200	0.200	0.198	0.198	0.203	0.203	0.200	0.215	0.213	0.213	0.213	0.205	0.203	0.197	0.203	0.203
32. M_hospes_42659	0.203	0.203	0.198	0.198	0.195	0.195	0.200	0.200	0.197	0.212	0.210	0.210	0.210	0.207	0.205	0.192	0.210	0.210
33. M_hospes_A214	0.205	0.205	0.200	0.200	0.200	0.197	0.199	0.199	0.197	0.207	0.212	0.212	0.212	0.206	0.199	0.189	0.187	0.187
34. M_hospes_A218	0.205	0.205	0.200	0.200	0.200	0.197	0.199	0.199	0.197	0.207	0.212	0.212	0.212	0.206	0.199	0.189	0.187	0.187
35. M_hospes_306	0.205	0.205	0.200	0.200	0.200	0.197	0.199	0.199	0.197	0.207	0.212	0.212	0.212	0.206	0.199	0.189	0.187	0.187
36. M_liza_TA159	0.180	0.180	0.182	0.182	0.185	0.180	0.182	0.182	0.182	0.192	0.197	0.197	0.197	0.184	0.182	0.194	0.197	0.197

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
37. M_liza_33447	0.177	0.177	0.180	0.180	0.182	0.177	0.180	0.180	0.180	0.189	0.194	0.194	0.194	0.181	0.180	0.192	0.194	0.194
38. M_liza_27726	0.177	0.177	0.180	0.180	0.182	0.177	0.180	0.180	0.180	0.189	0.194	0.194	0.194	0.181	0.180	0.192	0.194	0.194
39. M_liza_379	0.177	0.177	0.180	0.180	0.182	0.177	0.180	0.180	0.180	0.189	0.194	0.194	0.194	0.181	0.180	0.192	0.194	0.194
40. M_liza_TA228	0.180	0.180	0.182	0.182	0.185	0.180	0.182	0.182	0.182	0.192	0.197	0.197	0.197	0.184	0.182	0.194	0.197	0.197
41. M_cephalus_367	0.177	0.177	0.180	0.180	0.182	0.177	0.175	0.175	0.175	0.184	0.189	0.189	0.189	0.181	0.180	0.192	0.194	0.194
42. M_cephalus_362	0.175	0.175	0.177	0.177	0.180	0.175	0.172	0.172	0.172	0.182	0.187	0.187	0.187	0.179	0.177	0.189	0.192	0.192
43. M_cephalus_328	0.182	0.182	0.184	0.184	0.187	0.182	0.179	0.179	0.179	0.189	0.194	0.194	0.194	0.186	0.184	0.191	0.194	0.194
44. M_liza_298	0.177	0.177	0.180	0.180	0.182	0.177	0.180	0.180	0.180	0.189	0.194	0.194	0.194	0.181	0.180	0.192	0.194	0.194
45. M_liza_378	0.175	0.175	0.177	0.177	0.180	0.175	0.177	0.177	0.177	0.189	0.192	0.192	0.192	0.179	0.177	0.189	0.192	0.192
46. M_cephalus_358	0.177	0.177	0.180	0.180	0.182	0.177	0.174	0.174	0.174	0.184	0.189	0.189	0.189	0.181	0.179	0.191	0.189	0.189
47. M_cephalus_350	0.187	0.187	0.190	0.190	0.192	0.187	0.189	0.189	0.189	0.199	0.205	0.205	0.205	0.196	0.194	0.207	0.210	0.210
48. M_cephalus_337	0.170	0.170	0.173	0.173	0.175	0.170	0.167	0.167	0.167	0.179	0.182	0.182	0.182	0.174	0.172	0.184	0.192	0.192
49. M_cephalus_314	0.185	0.185	0.182	0.182	0.185	0.180	0.177	0.177	0.177	0.187	0.192	0.192	0.192	0.189	0.187	0.194	0.197	0.197
50. M_cephalus_329	0.180	0.180	0.177	0.177	0.180	0.175	0.172	0.172	0.172	0.192	0.197	0.197	0.197	0.194	0.192	0.194	0.202	0.202
51. M_cephalus_342	0.180	0.180	0.183	0.183	0.180	0.180	0.177	0.177	0.177	0.187	0.187	0.187	0.187	0.187	0.185	0.187	0.187	0.187
52. M_cephalus_375	0.187	0.187	0.189	0.189	0.192	0.187	0.172	0.172	0.172	0.184	0.187	0.187	0.187	0.184	0.177	0.191	0.194	0.194
53. M_cephalus_368	0.187	0.187	0.184	0.184	0.187	0.182	0.179	0.179	0.179	0.189	0.194	0.194	0.194	0.191	0.189	0.191	0.194	0.194

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
54. M_cephalus_373	0.184	0.184	0.182	0.182	0.180	0.180	0.177	0.177	0.177	0.181	0.182	0.182	0.182	0.179	0.182	0.184	0.192	0.192
55. M_cephalus_347	0.187	0.187	0.184	0.184	0.182	0.182	0.179	0.179	0.179	0.184	0.184	0.184	0.184	0.182	0.184	0.186	0.189	0.189
56. M_cephalus_344	0.192	0.192	0.189	0.189	0.187	0.187	0.184	0.184	0.184	0.189	0.189	0.189	0.189	0.187	0.189	0.191	0.194	0.194
57. M_cephalus_I2	0.179	0.179	0.182	0.182	0.179	0.179	0.177	0.177	0.177	0.191	0.194	0.194	0.194	0.191	0.189	0.189	0.196	0.196
58. M_cephalus_349	0.177	0.177	0.179	0.179	0.177	0.177	0.174	0.174	0.174	0.189	0.191	0.191	0.191	0.188	0.186	0.186	0.194	0.194
59. M_cephalus_I3	0.177	0.177	0.179	0.179	0.177	0.177	0.174	0.174	0.174	0.189	0.191	0.191	0.191	0.188	0.186	0.186	0.194	0.194
60. M_cephalus_371	0.179	0.179	0.182	0.182	0.179	0.179	0.177	0.177	0.177	0.191	0.194	0.194	0.194	0.191	0.189	0.184	0.191	0.191
61. M_cephalus_386	0.177	0.177	0.180	0.180	0.177	0.177	0.165	0.165	0.165	0.169	0.170	0.170	0.170	0.167	0.165	0.181	0.189	0.189
62. M_cephalus_6446	0.185	0.185	0.187	0.187	0.185	0.185	0.187	0.187	0.187	0.192	0.194	0.194	0.194	0.191	0.189	0.194	0.199	0.199
63. M_cephalus_6450	0.185	0.185	0.187	0.187	0.185	0.185	0.187	0.187	0.187	0.192	0.194	0.194	0.194	0.191	0.189	0.194	0.199	0.199
64. M_capurrii_282	0.157	0.157	0.157	0.157	0.157	0.155	0.157	0.157	0.157	0.171	0.167	0.167	0.167	0.159	0.153	0.169	0.178	0.178
65. M_capurrii_283	0.157	0.157	0.157	0.157	0.157	0.155	0.157	0.157	0.157	0.171	0.167	0.167	0.167	0.159	0.153	0.169	0.178	0.178
66. M_bananensis_286	0.197	0.197	0.200	0.200	0.202	0.197	0.187	0.187	0.187	0.192	0.197	0.197	0.197	0.194	0.187	0.182	0.184	0.184
67. M_trichodon_30770	0.204	0.204	0.201	0.201	0.204	0.199	0.201	0.201	0.201	0.216	0.219	0.219	0.219	0.210	0.203	0.198	0.203	0.203
68. M_trichodon_291	0.201	0.201	0.199	0.199	0.201	0.196	0.198	0.198	0.198	0.213	0.216	0.216	0.216	0.208	0.201	0.195	0.201	0.201
69. C_proboscideus_002c	0.212	0.212	0.215	0.215	0.212	0.212	0.207	0.207	0.207	0.210	0.207	0.207	0.207	0.197	0.199	0.212	0.217	0.217
70. N_leuciscus_003b	0.237	0.237	0.232	0.232	0.234	0.229	0.218	0.218	0.216	0.226	0.234	0.234	0.234	0.216	0.211	0.208	0.213	0.213

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
19. M_incilis_42657																		
20. M_incilis_785	0.002																	
21. M_incilis_299	0.002	0.004																
22. M_curvidens_TA230	0.144	0.142	0.146															
23. M_curvidens_1	0.142	0.139	0.144	0.002														
24. M_curvidens_TA187	0.142	0.139	0.144	0.002	0.000													
25. M_rubrioculus_TA210	0.199	0.197	0.202	0.180	0.180	0.180												
26. M_rubrioculus_30086	0.197	0.194	0.199	0.178	0.178	0.178	0.002											
27. M_rubrioculus_TA153	0.197	0.194	0.199	0.178	0.178	0.178	0.002	0.004										
28. M_rubrioculus_42655	0.197	0.194	0.199	0.183	0.183	0.183	0.005	0.004	0.007									
29. M_rubrioculus_305b	0.212	0.209	0.214	0.183	0.183	0.183	0.060	0.058	0.062	0.058								
30. M_brevirostris_TA109	0.200	0.198	0.203	0.179	0.179	0.179	0.113	0.111	0.111	0.115	0.140							
31. M_brevirostris_TA110	0.203	0.200	0.205	0.181	0.181	0.181	0.113	0.111	0.111	0.115	0.140	0.002						
32. M_hospes_42659	0.210	0.207	0.213	0.183	0.183	0.183	0.103	0.100	0.100	0.105	0.129	0.011	0.009					
33. M_hospes_A214	0.187	0.184	0.189	0.173	0.173	0.173	0.126	0.124	0.124	0.128	0.144	0.070	0.072	0.080				
34. M_hospes_A218	0.187	0.184	0.189	0.173	0.173	0.173	0.126	0.124	0.124	0.128	0.144	0.070	0.072	0.080	0.000			
35. M_hospes_306	0.187	0.184	0.189	0.173	0.173	0.173	0.126	0.124	0.124	0.128	0.144	0.070	0.072	0.080	0.000	0.000		
36. M_liza_TA159	0.197	0.199	0.197	0.201	0.204	0.204	0.201	0.199	0.201	0.199	0.196	0.206	0.206	0.208	0.218	0.218	0.218	

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
37. M_liza_33447	0.194	0.197	0.194	0.199	0.201	0.201	0.199	0.196	0.199	0.196	0.193	0.203	0.203	0.206	0.215	0.215	0.215	0.002
38. M_liza_27726	0.194	0.197	0.194	0.199	0.201	0.201	0.199	0.196	0.199	0.196	0.193	0.203	0.203	0.206	0.215	0.215	0.215	0.002
39. M_liza_379	0.194	0.197	0.194	0.199	0.201	0.201	0.199	0.196	0.199	0.196	0.193	0.203	0.203	0.206	0.215	0.215	0.215	0.002
40. M_liza_TA228	0.197	0.199	0.197	0.201	0.204	0.204	0.201	0.199	0.201	0.199	0.196	0.206	0.206	0.208	0.218	0.218	0.218	0.004
41. M_cephalus_367	0.194	0.197	0.194	0.199	0.201	0.201	0.209	0.206	0.209	0.206	0.198	0.198	0.198	0.200	0.210	0.210	0.210	0.013
42. M_cephalus_362	0.192	0.194	0.192	0.206	0.209	0.209	0.206	0.204	0.206	0.204	0.196	0.201	0.201	0.203	0.213	0.213	0.213	0.020
43. M_cephalus_328	0.194	0.197	0.194	0.201	0.204	0.204	0.203	0.201	0.203	0.201	0.193	0.198	0.198	0.200	0.207	0.207	0.207	0.020
44. M_liza_298	0.194	0.197	0.194	0.199	0.201	0.201	0.199	0.196	0.199	0.196	0.193	0.203	0.203	0.206	0.215	0.215	0.215	0.002
45. M_liza_378	0.192	0.194	0.192	0.196	0.199	0.199	0.196	0.194	0.196	0.194	0.191	0.201	0.201	0.203	0.213	0.213	0.213	0.004
46. M_cephalus_358	0.189	0.192	0.189	0.201	0.204	0.204	0.203	0.201	0.203	0.201	0.193	0.203	0.203	0.205	0.213	0.213	0.213	0.016
47. M_cephalus_350	0.210	0.212	0.210	0.212	0.214	0.214	0.196	0.194	0.196	0.189	0.186	0.201	0.201	0.203	0.213	0.213	0.213	0.024
48. M_cephalus_337	0.192	0.189	0.192	0.191	0.194	0.194	0.191	0.189	0.191	0.189	0.181	0.196	0.196	0.198	0.208	0.208	0.208	0.024
49. M_cephalus_314	0.197	0.199	0.197	0.191	0.194	0.194	0.196	0.194	0.196	0.194	0.186	0.191	0.191	0.193	0.198	0.198	0.198	0.027
50. M_cephalus_329	0.202	0.205	0.202	0.201	0.204	0.204	0.196	0.194	0.196	0.194	0.186	0.191	0.191	0.193	0.208	0.208	0.208	0.025
51. M_cephalus_342	0.187	0.189	0.187	0.197	0.199	0.199	0.198	0.196	0.198	0.196	0.193	0.195	0.195	0.198	0.205	0.205	0.205	0.022
52. M_cephalus_375	0.194	0.197	0.194	0.204	0.206	0.206	0.198	0.196	0.198	0.196	0.188	0.198	0.198	0.200	0.206	0.206	0.206	0.027
53. M_cephalus_368	0.194	0.196	0.194	0.193	0.196	0.196	0.188	0.186	0.188	0.186	0.183	0.198	0.198	0.200	0.200	0.200	0.200	0.035

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
54. M_cephalus_373	0.192	0.194	0.192	0.201	0.204	0.204	0.189	0.186	0.189	0.181	0.194	0.206	0.206	0.208	0.226	0.226	0.226	0.033
55. M_cephalus_347	0.189	0.192	0.189	0.199	0.201	0.201	0.191	0.189	0.191	0.184	0.196	0.209	0.209	0.211	0.223	0.223	0.223	0.035
56. M_cephalus_344	0.194	0.197	0.194	0.194	0.196	0.196	0.191	0.189	0.191	0.184	0.196	0.214	0.214	0.216	0.228	0.228	0.228	0.035
57. M_cephalus_I2	0.196	0.194	0.196	0.188	0.191	0.191	0.200	0.198	0.200	0.198	0.183	0.203	0.203	0.205	0.202	0.202	0.202	0.035
58. M_cephalus_349	0.194	0.191	0.194	0.191	0.193	0.193	0.203	0.200	0.203	0.200	0.185	0.205	0.205	0.207	0.205	0.205	0.205	0.037
59. M_cephalus_I3	0.194	0.191	0.194	0.191	0.193	0.193	0.203	0.200	0.203	0.200	0.185	0.205	0.205	0.207	0.205	0.205	0.205	0.037
60. M_cephalus_371	0.191	0.189	0.191	0.191	0.193	0.193	0.200	0.198	0.200	0.198	0.183	0.203	0.203	0.205	0.202	0.202	0.202	0.038
61. M_cephalus_386	0.189	0.192	0.189	0.195	0.198	0.198	0.195	0.193	0.195	0.193	0.180	0.202	0.202	0.205	0.217	0.217	0.217	0.044
62. M_cephalus_6446	0.199	0.202	0.199	0.206	0.209	0.209	0.204	0.201	0.204	0.201	0.186	0.208	0.208	0.211	0.210	0.210	0.210	0.031
63. M_cephalus_6450	0.199	0.202	0.199	0.206	0.209	0.209	0.204	0.201	0.204	0.201	0.186	0.208	0.208	0.211	0.210	0.210	0.210	0.031
64. M_capurrii_282	0.178	0.176	0.178	0.158	0.155	0.155	0.158	0.160	0.156	0.163	0.165	0.171	0.171	0.169	0.157	0.157	0.157	0.183
65. M_capurrii_283	0.178	0.176	0.178	0.158	0.155	0.155	0.158	0.160	0.156	0.163	0.165	0.171	0.171	0.169	0.157	0.157	0.157	0.183
66. M_bananensis_286	0.184	0.181	0.184	0.197	0.194	0.194	0.156	0.154	0.154	0.154	0.170	0.179	0.179	0.167	0.191	0.191	0.191	0.164
67. M_trichodon_30770	0.203	0.201	0.203	0.198	0.196	0.196	0.189	0.192	0.187	0.194	0.182	0.202	0.202	0.211	0.191	0.191	0.191	0.196
68. M_trichodon_291	0.201	0.198	0.201	0.196	0.193	0.193	0.192	0.194	0.189	0.197	0.184	0.204	0.204	0.214	0.194	0.194	0.194	0.194
69. C_proboscideus_002c	0.217	0.215	0.217	0.194	0.197	0.197	0.219	0.222	0.222	0.219	0.240	0.207	0.209	0.209	0.234	0.234	0.234	0.201
70. N_leuciscus_003b	0.213	0.211	0.216	0.206	0.203	0.203	0.212	0.215	0.210	0.217	0.215	0.184	0.182	0.189	0.198	0.198	0.198	0.222

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
37. M_liza_33447																	
38. M_liza_27726	0.000																
39. M_liza_379	0.000	0.000															
40. M_liza_TA228	0.002	0.002	0.002														
41. M_cephalus_367	0.011	0.011	0.011	0.013													
42. M_cephalus_362	0.018	0.018	0.018	0.020	0.011												
43. M_cephalus_328	0.018	0.018	0.018	0.020	0.011	0.011											
44. M_liza_298	0.000	0.000	0.000	0.002	0.011	0.018	0.018										
45. M_liza_378	0.002	0.002	0.002	0.004	0.013	0.020	0.020	0.002									
46. M_cephalus_358	0.014	0.014	0.014	0.016	0.011	0.011	0.004	0.014	0.016								
47. M_cephalus_350	0.022	0.022	0.022	0.024	0.018	0.018	0.022	0.022	0.024	0.018							
48. M_cephalus_337	0.022	0.022	0.022	0.024	0.018	0.022	0.022	0.022	0.020	0.018	0.022						
49. M_cephalus_314	0.025	0.025	0.025	0.027	0.018	0.022	0.022	0.025	0.027	0.018	0.029	0.029					
50. M_cephalus_329	0.024	0.024	0.024	0.025	0.020	0.020	0.027	0.024	0.025	0.023	0.027	0.027	0.027				
51. M_cephalus_342	0.020	0.020	0.020	0.022	0.020	0.020	0.020	0.020	0.022	0.016	0.027	0.027	0.023	0.029			
52. M_cephalus_375	0.025	0.025	0.025	0.027	0.022	0.022	0.025	0.025	0.023	0.022	0.029	0.025	0.029	0.027	0.027		
53. M_cephalus_368	0.033	0.033	0.033	0.035	0.031	0.031	0.023	0.033	0.035	0.025	0.040	0.037	0.037	0.035	0.038	0.042	

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
54. M_cephalus_373	0.031	0.031	0.031	0.033	0.038	0.038	0.037	0.031	0.033	0.033	0.042	0.046	0.042	0.040	0.040	0.046	0.042
55. M_cephalus_347	0.033	0.033	0.033	0.035	0.040	0.040	0.035	0.033	0.035	0.031	0.044	0.048	0.040	0.042	0.038	0.048	0.040
56. M_cephalus_344	0.033	0.033	0.033	0.035	0.040	0.044	0.038	0.033	0.035	0.035	0.044	0.052	0.044	0.042	0.042	0.052	0.044
57. M_cephalus_I2	0.033	0.033	0.033	0.035	0.033	0.037	0.036	0.033	0.035	0.033	0.040	0.035	0.044	0.040	0.038	0.048	0.040
58. M_cephalus_349	0.035	0.035	0.035	0.037	0.035	0.035	0.038	0.035	0.037	0.035	0.042	0.037	0.046	0.038	0.040	0.050	0.042
59. M_cephalus_I3	0.035	0.035	0.035	0.037	0.035	0.035	0.038	0.035	0.037	0.035	0.042	0.037	0.046	0.038	0.040	0.050	0.042
60. M_cephalus_371	0.037	0.037	0.037	0.038	0.037	0.037	0.040	0.037	0.038	0.036	0.044	0.038	0.048	0.040	0.042	0.052	0.044
61. M_cephalus_386	0.042	0.042	0.042	0.044	0.036	0.040	0.040	0.042	0.044	0.038	0.048	0.044	0.046	0.052	0.044	0.046	0.050
62. M_cephalus_6446	0.029	0.029	0.029	0.031	0.029	0.037	0.036	0.029	0.031	0.033	0.037	0.040	0.038	0.042	0.038	0.040	0.052
63. M_cephalus_6450	0.029	0.029	0.029	0.031	0.029	0.037	0.036	0.029	0.031	0.033	0.037	0.040	0.038	0.042	0.038	0.040	0.052
64. M_capurrii_282	0.180	0.180	0.180	0.183	0.185	0.183	0.185	0.180	0.178	0.180	0.188	0.178	0.168	0.183	0.178	0.175	0.170
65. M_capurrii_283	0.180	0.180	0.180	0.183	0.185	0.183	0.185	0.180	0.178	0.180	0.188	0.178	0.168	0.183	0.178	0.175	0.170
66. M_bananensis_286	0.162	0.162	0.162	0.159	0.157	0.164	0.162	0.162	0.159	0.162	0.164	0.159	0.152	0.162	0.155	0.162	0.164
67. M_trichodon_30770	0.194	0.194	0.194	0.196	0.196	0.194	0.191	0.194	0.191	0.191	0.201	0.186	0.177	0.194	0.196	0.194	0.184
68. M_trichodon_291	0.191	0.191	0.191	0.194	0.194	0.191	0.189	0.191	0.189	0.189	0.199	0.184	0.174	0.191	0.194	0.191	0.181
69. C_proboscideus_002c	0.199	0.199	0.199	0.201	0.206	0.206	0.201	0.199	0.199	0.201	0.201	0.201	0.204	0.209	0.196	0.203	0.201
70. N_leuciscus_003b	0.219	0.219	0.219	0.219	0.225	0.230	0.222	0.219	0.217	0.224	0.222	0.219	0.212	0.225	0.227	0.217	0.207

Table S4.4 continued - Pairwise genetic distances between *Mugil* lineages estimated using 569 base pairs of the mitochondrial gene COI and Kimura's two parameter evolutionary model.

	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
54. M_cephalus_373																
55. M_cephalus_347	0.002															
56. M_cephalus_344	0.005	0.004														
57. M_cephalus_I2	0.050	0.048	0.048													
58. M_cephalus_349	0.052	0.050	0.050	0.002												
59. M_cephalus_I3	0.052	0.050	0.050	0.002	0.000											
60. M_cephalus_371	0.054	0.052	0.052	0.004	0.002	0.002										
61. M_cephalus_386	0.060	0.062	0.066	0.054	0.056	0.056	0.058									
62. M_cephalus_6446	0.048	0.050	0.050	0.022	0.023	0.023	0.025	0.050								
63. M_cephalus_6450	0.048	0.050	0.050	0.022	0.023	0.023	0.025	0.050	0.000							
64. M_capurrii_282	0.185	0.182	0.187	0.170	0.172	0.172	0.175	0.187	0.185	0.185						
65. M_capurrii_283	0.185	0.182	0.187	0.170	0.172	0.172	0.175	0.187	0.185	0.185	0.000					
66. M_bananensis_286	0.169	0.166	0.162	0.169	0.171	0.171	0.169	0.159	0.171	0.171	0.131	0.131				
67. M_trichodon_30770	0.191	0.189	0.194	0.196	0.198	0.198	0.201	0.208	0.212	0.212	0.159	0.159	0.201			
68. M_trichodon_291	0.189	0.186	0.191	0.193	0.196	0.196	0.198	0.211	0.209	0.209	0.157	0.157	0.203	0.002		
69. C_proboscideus_002c	0.196	0.194	0.199	0.206	0.208	0.208	0.206	0.215	0.221	0.221	0.175	0.175	0.220	0.212	0.209	
70. N_leuciscus_003b	0.215	0.212	0.212	0.219	0.221	0.221	0.219	0.240	0.224	0.224	0.193	0.193	0.174	0.201	0.199	0.204

Table S4.5 - Bayesian Poisson Tree Processes (bPTP) results. PP - Posterior probabilities. Lineages according to Durand and Borsa (2015). * lineages with support bellow 0.95.

Individuals	**Support**	**Lineage**
Mugil_trichodon_30770, Mugil_trichodon_291	PP = 0.95	*M. trichodon*
Mugil_bananensis_286	PP = 1	*M. bananensis*
Mugil_capurrii_282, Mugil_capurrii_283	PP = 0.98	*M. capurrii*
Mugil_cephalus_349, Mugil_cephalus_371	PP = 0.99	G
Mugil_cephalus_329	PP = 1	C
Mugil_cephalus_344, Mugil_cephalus_347, Mugil_cephalus_373	PP = 0.93*	B
Mugil_cephalus_375	PP = 1	A
Mugil_cephalus_386	PP = 1	L
Mugil_cephalus_328, Mugil_cephalus_358	PP = 0.97	I
Mugil_cephalus_368	PP = 1	K
Mugil_cephalus_362	PP = 1	F
Mugil_cephalus_314	PP = 1	*M. cephalus*
Mugil_cephalus_342	PP = 1	E
Mugil_cephalus_350	PP = 1	D
Mugil_cephalus_337	PP = 1	J
Mugil_cephalus_367	PP = 1	H
Mugil_cephalus_6446, Mugil_cephalus_6450	PP = 0.99	Q
Mugil_liza_298, Mugil_liza_378, Mugil_liza_379, Mugil_liza_27726, Mugil_liza_33447	PP = 0.99	*M. liza*
Mugil_curema_406	PP = 0.96	O
Mugil_curema_408, Mugil_curema_414, Mugil_curema_46939	PP = 0.94*	*M. curema**
Mugil_curema_390, Mugil_curema_392	PP = 0.75*	M*
Mugil_margaritae_400, Mugil_margaritae_30070, Mugil_margaritae_401	PP = 0.99	*M. margaritae*
Mugil_curema_293, Mugil_curema_294, Mugil_curema_426	PP = 0.95	O
Mugil_thoburni_6435, Mugil_thoburni_6447	PP = 0.94*	*M. thoburni**
Mugil_incilis_299, Mugil_incilis_42657, Mugil_incilis_785	PP = 0.99	*M. incilis*
Mugil_curvidens_1	PP = 1	*M. curvidens*
Mugil_rubrioculus_30086, Mugil_rubrioculus_42655	PP = 0.99	*M. rubrioculus*
Mugil_rubrioculus_305b	PP = 1	P
Mugil_hospes_306	PP = 1	*M. hospes*
Mugil_brevirostris_42659	PP = 1	*M. brevirostris*

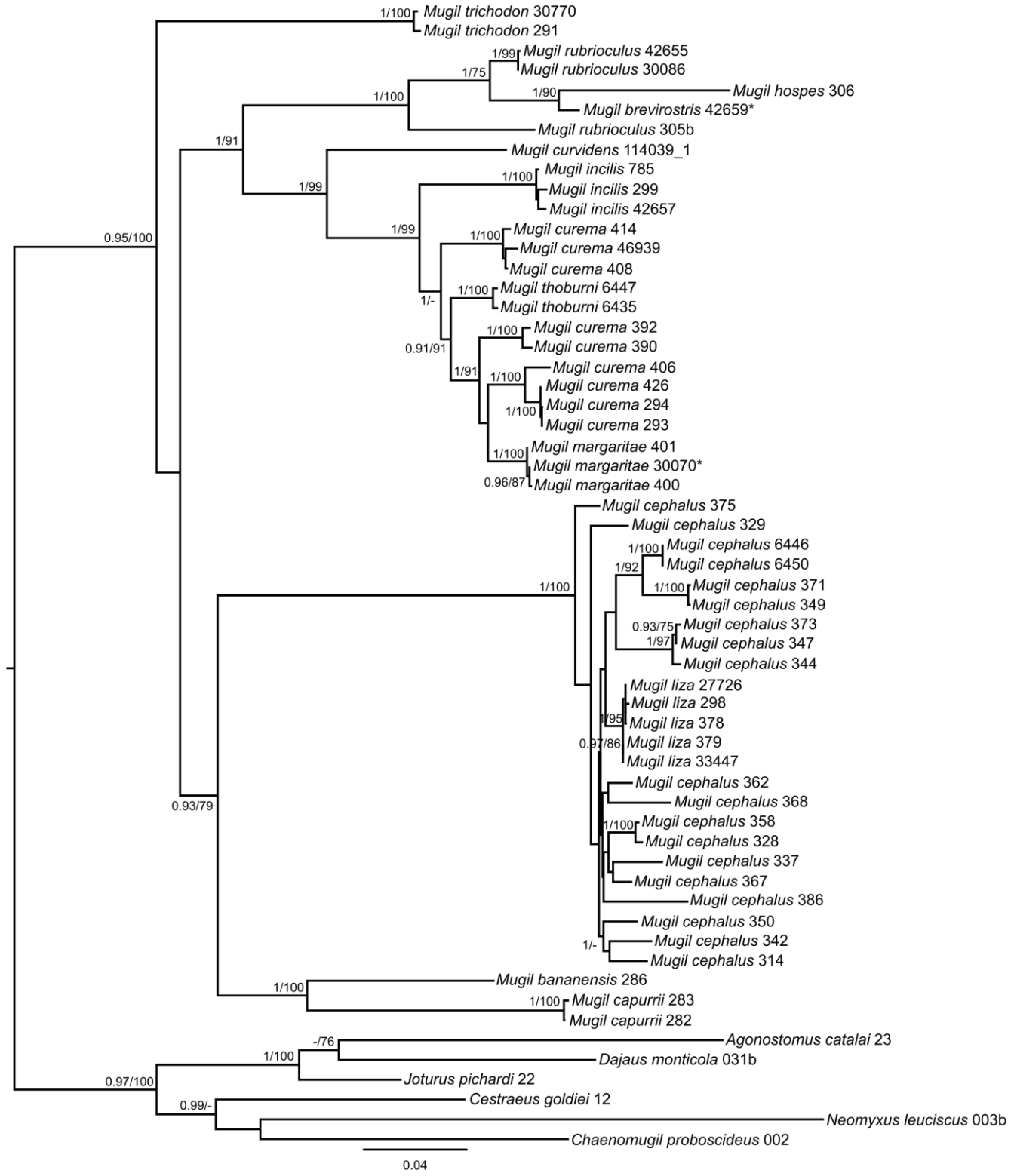

Figure S4.1 - Maximum likelihood tree generated from a concatenated matrix including 1,939 base pairs of the 16S, COI and CYTB mitochondrial genes. Values next to nodes indicate posterior probabilities obtained through a molecular dated phylogeny performed in BEAST and bootstrap values from the ML analysis. Asterisks indicate specimens with corrected identifications.

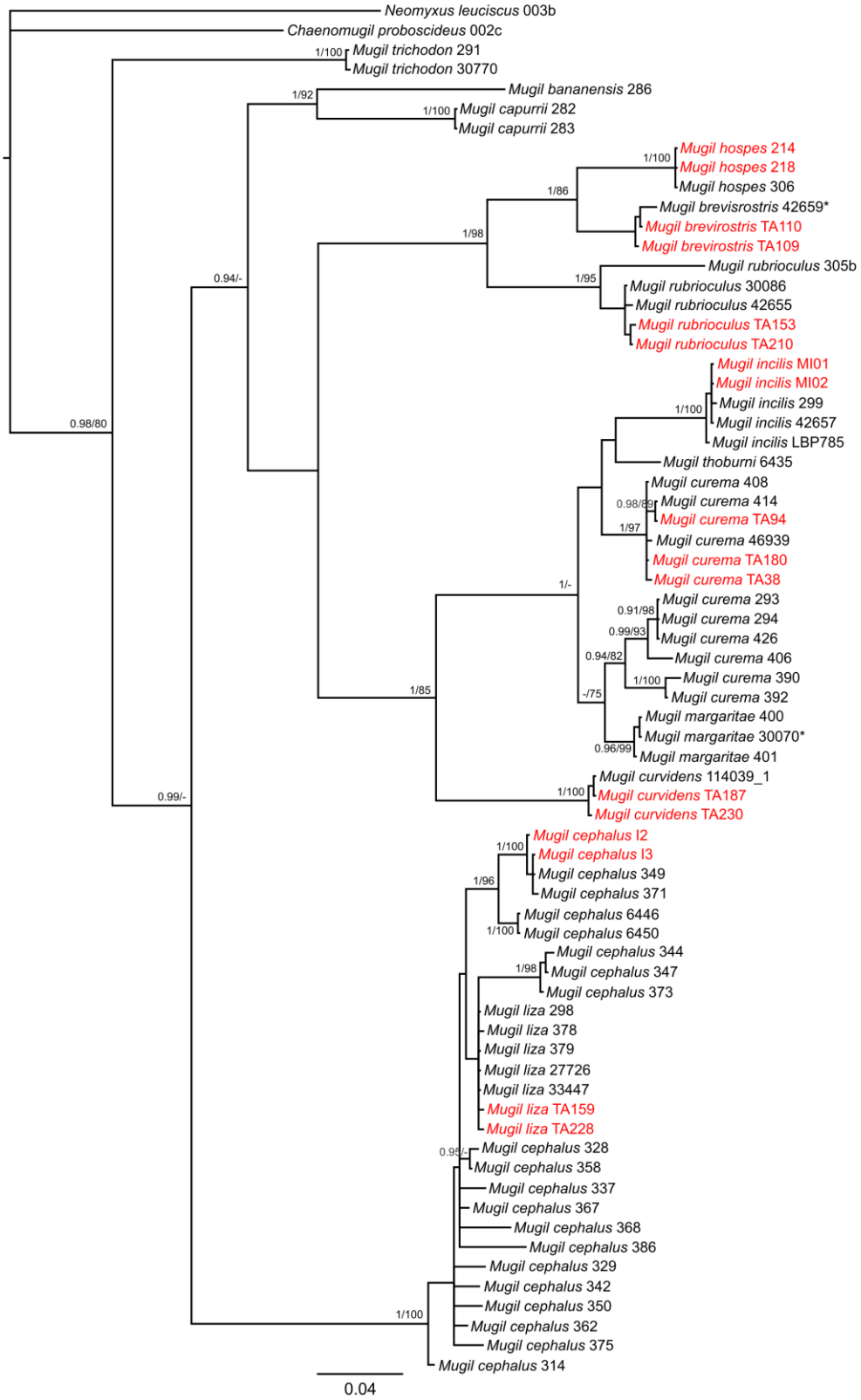

Figure S4.2 - Bayesian topology of the genus *Mugil*, reconstructed using 569 base pairs of the mitochondrial gene COI. Values next to the nodes indicate posterior probabilities and bootstrap values from Bayesian and ML analyses. Specimens collected by us or by partners are in red. Asterisks indicate specimens with corrected identifications.

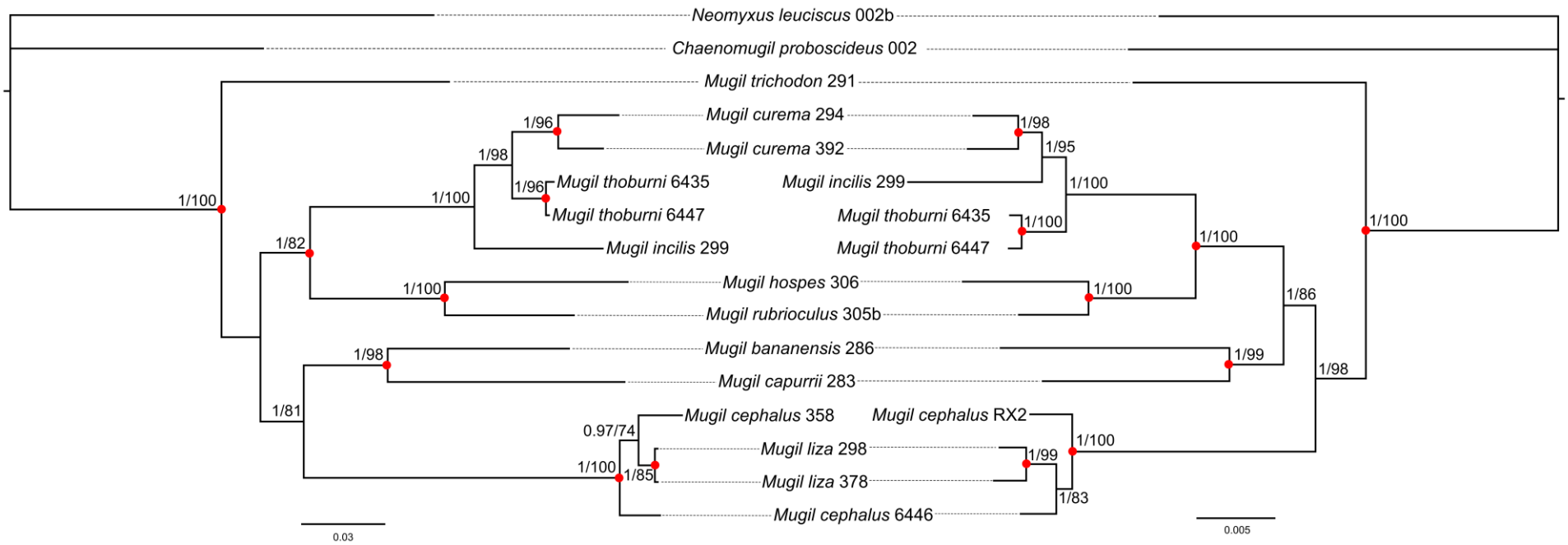

Figure S4.3 - Bayesian topologies of *Mugil*, reconstructed from 1,929 base pairs of the mitochondrial genes 16S rRNA, COI and CYTB (left) and 9,843 base pairs from 12 nuclear markers (right). Values next to the nodes indicate posterior probabilities and bootstrap values from Bayesian and Maximum Likelihood analyses. Red dots indicate congruent nodes between the topologies.

Table S5.1 - Specimen information from *Mugil* species from the Tropical Southwestern Atlantic marine province.

ID	Voucher	GenBank access number	BIN	COI identification (BIN and Bayesian)	Mophological identification	Locality
TA025	MUFAL2166	MK837973	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA026	MUFAL2167	MK837974	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA027	MUFAL2168	MK837975	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA029	MUFAL2170	MK837976	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA030	MUFAL2171	MK837977	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA031	MUFAL2172	MK837978	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA032	MUFAL2173	MK837979	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA033	MUFAL2174	MK837980	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA035	MUFAL2176	MK837981	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA036	MUFAL2177	MK837982	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA037	MUFAL2178	MK837983	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA038	MUFAL2179	MK837984	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA039	MUFAL2180	MK837985	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA041	MUFAL2182	MK837986	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA043	MUFAL2184	MK837987	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA044	MUFAL2185	MK837988	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA045	MUFAL2186	MK837989	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA046	MUFAL2187	MK837990	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA047	MUFAL2188	MK837991	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA048	MUFAL2189	MK837992	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA050	MUFAL2191	MK837993	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA051	MUFAL2192	MK837994	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA053	MUFAL2194	MK837995	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA056	MUFAL2197	MK837996	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA059	MUFAL2200	MK837997	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA060	MUFAL2201	MK837998	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA061	MUFAL2202	MK837999	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA063	MUFAL2204	MK838000	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA067	MUFAL2208	MK838001	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA069	MUFAL2210	MK838002	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA070	MUFAL2211	MK838003	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA071	MUFAL2212	MK838004	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA072	MUFAL2213	MK838005	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA073	MUFAL2214	MK838006	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA074	MUFAL2215	MK838007	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA075	MUFAL2216	MK838008	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA076	MUFAL2217	MK838009	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA077	MUFAL2218	MK838010	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA078	MUFAL2219	MK838011	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA079	MUFAL2220	MK838012	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA080	MUFAL2221	MK838013	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA082	MUFAL2223	MK838014	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA083	MUFAL2224	MK838015	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA084	MUFAL2225	MK838016	AAB3698	*M. curema*	*M. rubrioculus*	Santo Antonio
TA086	MUFAL2227	MK838017	AAB3698	*M. curema*	*M. curema*	Santo Antonio

TA087	MUFAL2228	MK838018	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA088	MUFAL2229	MK838019	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA089	MUFAL2230	MK838020	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA090	MUFAL2231	MK838021	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA091	MUFAL2232	MK838022	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA092	MUFAL2233	MK838023	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA093	MUFAL2234	MK838024	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA094	MUFAL2235	MK838025	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA095	MUFAL2236	MK838026	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA096	MUFAL2237	MK838027	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA097	MUFAL2238	MK838028	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA098	MUFAL2239	MK838029	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA099	MUFAL2240	MK838030	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA107	MUFAL2248	MK838031	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA108	MUFAL2249	MK838032	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA109	MUFAL2250	MK838033	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA110	MUFAL2251	MK838034	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA111	MUFAL2252	MK838035	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA112	MUFAL2253	MK838036	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA113	MUFAL2254	MK838037	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA114	MUFAL2255	MK838038	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA115	MUFAL2256	MK838039	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA116	MUFAL2257	MK838040	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA117	MUFAL2258	MK838041	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA118	MUFAL2259	MK838042	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA120	MUFAL2261	MK838043	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA121	MUFAL2262	MK838044	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA122	MUFAL2263	MK838045	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA123	MUFAL2264	MK838046	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA153	MUFAL2294	MK838047	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA154	MUFAL2295	MK388724	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA155	MUFAL2296	MK838048	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA156	MUFAL2297	MK838049	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA157	MUFAL2298	MK838050	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA158	MUFAL2299	MK838051	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA159	MUFAL2300	MK838052	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA160	MUFAL2301	MK838053	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA161	MUFAL2302	MK838054	AAB3698	*M. curema*	*M. curema*	Manguaba
TA162	MUFAL2303	MK838055	AAB3698	*M. curema*	*M. curema*	Manguaba
TA163	MUFAL2304	MK838056	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA164	MUFAL2305	MK838057	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA165	MUFAL2306	MK838058	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA176	MUFAL2118	MK838059	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA177	MUFAL2119	MK838060	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA180	MUFAL2122	MK838061	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA181	MUFAL2123	MK838062	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA182	MUFAL2124	MK838063	AAB3698	*M. curema*	*M. incilis*	Santo Antonio
TA187	MUFAL2318	MK838064	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA189	MUFAL2320	MK838065	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA190	MUFAL2321	MK838066	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba

TA192	MUFAL2323	MK838067	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA193	MUFAL2324	MK838068	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA194	MUFAL2325	MK838069	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA195	MUFAL2326	MK838070	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA197	MUFAL2328	MK838071	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA198	MUFAL2329	MK838072	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA199	MUFAL2330	MK838073	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA201	MUFAL2332	MK838074	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA203	MUFAL2334	MK838075	ACC0101	*M. curvidens*	*M. curema*	Manguaba
TA204	MUFAL2335	MK838076	ACC0101	*M. curvidens*	*M. curvidens*	Manguaba
TA209	MUFAL2340	MK838077	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA210	MUFAL2341	MK838078	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA211	MUFAL2342	MK838079	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA212	MUFAL2343	MK838080	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA213	MUFAL2344	MK838081	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA214	MUFAL2345	MK838082	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA215	MUFAL2346	MK838083	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA216	MUFAL2347	MK838084	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA217	MUFAL2348	MK838085	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA218	MUFAL2349	MK838086	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA219	MUFAL2350	MK838087	AAZ7695	*M. brevirostris*	*M. brevirostris*	Santo Antonio
TA220	MUFAL2351	MK838088	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA221	MUFAL2352	MK838089	AAB3698	*M. curema*	*M. curema*	Santo Antonio
TA222	MUFAL2353	MK838090	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA223	MUFAL2354	MK838091	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA224	MUFAL2355	MK838092	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA225	MUFAL2356	MK838093	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA226	MUFAL2357	MK838094	AAB3698	*M. curema*	*M. curvidens*	Santo Antonio
TA227	MUFAL2358	MK838095	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA228	MUFAL2359	MK838096	ACE4593	*M. liza*	*M. liza*	Santo Antonio
TA229	MUFAL2360	MK838097	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA230	MUFAL2361	MK838098	ACC0101	*M. curvidens*	*M. curvidens*	Santo Antonio
TA234	MUFAL2365	MK838099	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio
TA235	MUFAL2366	MK838100	AAA7840	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio

Table S5.2 - Specimen information and morphological data from *Mugil* species from the Tropical Southwestern Atlantic marine province.

ID	COI identification (BIN and Bayesian)	Mophological identification	Locality	AFS	AFR	PFUR	PFBR	SDFUR	SDFBR	OSR	DFDF	DTPF	SSDAF	PDSF	Standard length (mm)
TA025	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	35	no	no	densely	dorsal	198
TA026	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	38	no	no	densely	both	204
TA027	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	dorsal	212
TA029	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	35	no	no	densely	absent	256
TA030	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	dorsal	248
TA031	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	9	39	no	no	densely	both	251
TA032	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	15	1	8	35	yes	no	densely	dorsal	265
TA033	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	36	no	no	densely	pectoral	254
TA035	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	38	yes	no	densely	both	262
TA036	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	38	no	no	densely	both	241
TA037	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	14	1	8	39	yes	no	densely	pectoral	264
TA038	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	both	235
TA039	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	both	229
TA041	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	34	no	no	densely	both	276
TA043	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	14	1	8	42	yes	no	densely	both	233
TA044	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	40	no	no	densely	both	245
TA045	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	14	1	8	39	yes	no	densely	pectoral	240
TA046	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	38	no	no	densely	both	229
TA047	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	both	265
TA048	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	pectoral	272
TA050	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	pectoral	249
TA051	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	13	1	8	38	yes	no	densely	both	241
TA053	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	pectoral	253

TA056	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	pectoral	247
TA059	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	14	1	8	38	yes	no	densely	both	275
TA060	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	36	no	no	densely	pectoral	235
TA061	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	38	no	no	densely	pectoral	244
TA063	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	38	no	no	densely	both	259
TA067	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	33	no	no	densely	pectoral	135
TA069	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	11	1	8	32	no	no	densely	pectoral	129
TA070	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	7	32	no	no	densely	pectoral	155
TA071	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	33	no	no	densely	pectoral	171
TA072	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	34	no	no	densely	pectoral	165
TA073	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	7	37	no	no	densely	pectoral	214
TA074	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	38	no	no	densely	both	185
TA075	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	38	no	no	densely	pectoral	192
TA076	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	both	194
TA077	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	pectoral	205
TA078	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	both	201
TA079	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	38	no	no	densely	both	195
TA080	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	pectoral	201
TA082	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	35	no	no	densely	dorsal	265
TA083	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	37	yes	no	densely	dorsal	232
TA084	*M. curema*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	35	no	no	densely	both	225
TA086	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	7	36	no	no	densely	pectoral	205
TA087	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	38	no	no	densely	pectoral	205
TA088	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	35	no	no	densely	pectoral	185
TA089	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	both	265
TA090	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	dorsal	272
TA091	*M. liza*	*M. liza*	Santo Antonio	3	8	2	13	1	8	31	yes	no	restricted	absent	413
TA092	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	31	yes	no	restricted	absent	428

TA093	*M. liza*	*M. liza*	Santo Antonio	3	8	2	15	1	8	38	yes	no	restricted	absent	427
TA094	*M. curema*	*M. curema*	Santo Antonio	3	9	2	13	1	8	37	no	no	densely	pectoral	220
TA095	*M. liza*	*M. liza*	Santo Antonio	3	8	2	12	1	7	30	yes	no	restricted	absent	349
TA096	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	33	no	no	densely	pectoral	244
TA097	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	34	no	no	densely	pectoral	230
TA098	*M. curema*	*M. curema*	Santo Antonio	3	9	2	12	1	8	36	yes	no	densely	both	235
TA099	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	11	1	7	33	no	no	densely	pectoral	212
TA107	*M. liza*	*M. liza*	Santo Antonio	3	8	2	13	1	8	31	yes	no	restricted	absent	340
TA108	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	32	yes	no	restricted	absent	350
TA109	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	8	38	no	yes	densely	absent	149
TA110	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	8	35	yes	yes	densely	absent	142
TA111	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	11	1	8	33	no	yes	densely	absent	126
TA112	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	10	1	7	34	no	yes	densely	absent	116
TA113	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	11	1	8	34	no	yes	densely	absent	120
TA114	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	11	1	7	37	no	yes	densely	absent	119
TA115	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	11	1	7	33	no	yes	densely	absent	116
TA116	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	10	1	7	32	no	yes	densely	absent	114
TA117	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	7	32	no	yes	densely	absent	89
TA118	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	7	32	yes	yes	densely	absent	89
TA120	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	30	yes	no	restricted	absent	347
TA121	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	34	yes	no	restricted	absent	388
TA122	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	13	1	8	32	no	no	densely	pectoral	250
TA123	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	30	no	no	restricted	absent	380
TA153	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	16	1	8	35	no	no	densely	dorsal	252
TA154	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	8	37	yes	yes	densely	absent	174
TA155	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	8	36	no	yes	densely	absent	167
TA156	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	12	1	8	36	no	yes	densely	absent	155
TA157	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	13	1	8	37	no	yes	densely	absent	145

TA158	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	13	1	8	36	no	yes	densely	absent	136
TA159	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	30	yes	no	restricted	absent	387
TA160	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	33	yes	no	restricted	absent	405
TA161	*M. curema*	*M. curema*	Manguaba	3	9	2	14	1	7	36	no	no	densely	both	162
TA162	*M. curema*	*M. curema*	Manguaba	3	9	2	14	1	8	36	no	no	densely	pectoral	181
TA163	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	14	1	8	33	no	no	densely	pectoral	184
TA164	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	34	no	no	densely	pectoral	186
TA165	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	14	1	8	34	no	no	densely	pectoral	182
TA176	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	35	no	no	densely	both	252
TA177	*M. curema*	*M. curema*	Santo Antonio	3	9	2	15	1	8	34	no	no	densely	both	255
TA180	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	both	241
TA181	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	both	235
TA182	*M. curema*	*M. incilis*	Santo Antonio	3	9	2	14	1	8	37	yes	no	densely	both	280
TA187	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	14	1	8	33	no	no	densely	pectoral	172
TA189	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	35	no	no	densely	pectoral	165
TA190	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	34	no	no	densely	pectoral	163
TA192	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	14	1	8	33	no	no	densely	pectoral	177
TA193	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	12	1	8	33	no	no	densely	pectoral	165
TA194	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	12	1	8	33	no	no	densely	pectoral	166
TA195	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	33	no	no	densely	pectoral	177
TA197	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	34	no	no	densely	pectoral	175
TA198	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	32	no	no	densely	pectoral	182
TA199	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	13	1	8	35	no	no	densely	pectoral	180
TA201	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	12	1	7	31	no	no	densely	both	159
TA203	*M. curvidens*	*M. curema*	Manguaba	3	9	2	11	1	7	34	no	no	densely	absent	174
TA204	*M. curvidens*	*M. curvidens*	Manguaba	3	8	2	12	1	8	33	no	no	densely	absent	171
TA209	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	36	yes	no	densely	dorsal	224
TA210	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	dorsal	238

TA211	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	dorsal	229
TA212	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	8	2	13	1	8	35	no	no	densely	dorsal	227
TA213	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	37	no	no	densely	dorsal	212
TA214	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	36	no	no	densely	dorsal	235
TA215	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	34	no	no	densely	dorsal	215
TA216	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	7	36	no	no	densely	both	235
TA217	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	35	no	no	densely	dorsal	220
TA218	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	35	no	no	densely	dorsal	240
TA219	*M. brevirostris*	*M. brevirostris*	Santo Antonio	3	9	2	13	1	8	36	yes	yes	densely	absent	220
TA220	*M. liza*	*M. liza*	Santo Antonio	3	8	2	13	1	8	30	yes	no	restricted	absent	370
TA221	*M. curema*	*M. curema*	Santo Antonio	3	9	2	14	1	8	38	no	no	densely	both	230
TA222	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	34	no	no	densely	pectoral	227
TA223	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	13	1	8	34	no	no	densely	pectoral	200
TA224	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	13	1	8	33	no	no	densely	pectoral	200
TA225	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	12	1	8	34	no	no	densely	pectoral	180
TA226	*M. curema*	*M. curvidens*	Santo Antonio	3	8	2	14	1	8	37	no	no	densely	both	230
TA227	*M. liza*	*M. liza*	Santo Antonio	3	8	2	14	1	8	30	yes	no	restricted	absent	401
TA228	*M. liza*	*M. liza*	Santo Antonio	3	8	2	15	1	8	30	yes	no	restricted	absent	435
TA229	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	13	1	8	33	no	no	densely	pectoral	190
TA230	*M. curvidens*	*M. curvidens*	Santo Antonio	3	8	2	11	1	8	34	no	no	densely	absent	127
TA234	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	13	1	8	33	no	no	densely	dorsal	233
TA235	*M. rubrioculus*	*M. rubrioculus*	Santo Antonio	3	9	2	14	1	8	33	no	no	densely	dorsal	239

Table S5.3 - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA025																		
TA026	0.002																	
TA027	0.004	0.005																
TA029	0.199	0.202	0.194															
TA030	0.196	0.199	0.191	0.002														
TA031	0.002	0.000	0.005	0.202	0.199													
TA032	0.196	0.199	0.191	0.002	0.000	0.199												
TA033	0.002	0.000	0.005	0.202	0.199	0.000	0.199											
TA035	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004										
TA036	0.196	0.199	0.191	0.002	0.000	0.199	0.000	0.199	0.194									
TA037	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194								
TA038	0.004	0.005	0.004	0.197	0.194	0.005	0.194	0.005	0.002	0.194	0.002							
TA039	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004						
TA041	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002					
TA043	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000				
TA044	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002			
TA045	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002		
TA046	0.002	0.004	0.005	0.199	0.196	0.004	0.196	0.004	0.004	0.196	0.004	0.005	0.002	0.004	0.004	0.002	0.004	

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA047	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA048	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA050	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA051	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA053	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA056	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA059	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002
TA060	0.002	0.004	0.005	0.196	0.193	0.004	0.193	0.004	0.004	0.193	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA061	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA063	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002
TA067	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA069	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA070	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA071	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA072	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA073	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA074	0.002	0.004	0.005	0.199	0.196	0.004	0.196	0.004	0.004	0.196	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.000
TA075	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA076	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002
TA077	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA078	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA079	0.002	0.004	0.002	0.198	0.195	0.004	0.195	0.004	0.000	0.195	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA080	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA082	0.196	0.199	0.191	0.002	0.000	0.199	0.000	0.199	0.194	0.000	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196
TA083	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA084	0.004	0.005	0.004	0.194	0.191	0.005	0.191	0.005	0.002	0.191	0.002	0.004	0.004	0.002	0.002	0.004	0.002	0.005
TA086	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA087	0.002	0.004	0.005	0.197	0.194	0.004	0.194	0.004	0.004	0.194	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA088	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA089	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA090	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA091	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA092	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA093	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA094	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA095	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA096	0.141	0.143	0.141	0.185	0.182	0.143	0.182	0.143	0.139	0.182	0.139	0.141	0.141	0.139	0.139	0.141	0.139	0.141
TA097	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA098	0.004	0.005	0.004	0.194	0.192	0.005	0.192	0.005	0.002	0.192	0.002	0.004	0.004	0.002	0.002	0.004	0.002	0.005
TA099	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA107	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA108	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA109	0.204	0.207	0.204	0.115	0.113	0.207	0.113	0.207	0.202	0.113	0.202	0.199	0.204	0.202	0.202	0.204	0.202	0.204
TA110	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA111	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA112	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA113	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA114	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA115	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA116	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA117	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA118	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA120	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA121	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA122	0.139	0.141	0.139	0.185	0.182	0.141	0.182	0.141	0.137	0.182	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA123	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA153	0.196	0.199	0.191	0.002	0.000	0.199	0.000	0.199	0.194	0.000	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196
TA154	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA155	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA156	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA157	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA158	0.209	0.212	0.209	0.117	0.115	0.212	0.115	0.212	0.207	0.115	0.207	0.204	0.209	0.207	0.207	0.209	0.207	0.209
TA159	0.180	0.180	0.182	0.202	0.202	0.180	0.202	0.180	0.182	0.202	0.182	0.185	0.180	0.182	0.182	0.180	0.182	0.180
TA160	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA161	0.004	0.005	0.004	0.194	0.191	0.005	0.191	0.005	0.002	0.191	0.002	0.004	0.004	0.002	0.002	0.004	0.002	0.005
TA162	0.002	0.004	0.005	0.199	0.196	0.004	0.196	0.004	0.004	0.196	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.000
TA163	0.137	0.139	0.137	0.183	0.180	0.139	0.180	0.139	0.135	0.180	0.135	0.137	0.137	0.135	0.135	0.137	0.135	0.137
TA164	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA165	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA176	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA177	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002
TA180	0.002	0.004	0.002	0.197	0.194	0.004	0.194	0.004	0.000	0.194	0.000	0.002	0.002	0.000	0.000	0.002	0.000	0.004
TA181	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA182	0.002	0.004	0.005	0.199	0.196	0.004	0.196	0.004	0.004	0.196	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.000
TA187	0.140	0.142	0.140	0.184	0.181	0.142	0.181	0.142	0.138	0.181	0.138	0.140	0.140	0.138	0.138	0.140	0.138	0.140
TA189	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA190	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA192	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA193	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA194	0.137	0.139	0.137	0.183	0.180	0.139	0.180	0.139	0.135	0.180	0.135	0.137	0.137	0.135	0.135	0.137	0.135	0.137
TA195	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA197	0.141	0.143	0.141	0.185	0.182	0.143	0.182	0.143	0.139	0.182	0.139	0.141	0.141	0.139	0.139	0.141	0.139	0.141
TA198	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA199	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA201	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA203	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA204	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA209	0.199	0.202	0.194	0.000	0.002	0.202	0.002	0.202	0.197	0.002	0.197	0.197	0.199	0.197	0.197	0.199	0.197	0.199
TA210	0.199	0.202	0.194	0.000	0.002	0.202	0.002	0.202	0.197	0.002	0.197	0.197	0.199	0.197	0.197	0.199	0.197	0.199
TA211	0.195	0.198	0.190	0.002	0.000	0.198	0.000	0.198	0.193	0.000	0.193	0.193	0.195	0.193	0.193	0.195	0.193	0.195
TA212	0.199	0.202	0.194	0.000	0.002	0.202	0.002	0.202	0.197	0.002	0.197	0.197	0.199	0.197	0.197	0.199	0.197	0.199
TA213	0.196	0.199	0.191	0.002	0.004	0.199	0.004	0.199	0.194	0.004	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA214	0.196	0.199	0.191	0.002	0.004	0.199	0.004	0.199	0.194	0.004	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196
TA215	0.196	0.199	0.191	0.002	0.004	0.199	0.004	0.199	0.194	0.004	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196
TA216	0.195	0.198	0.190	0.002	0.000	0.198	0.000	0.198	0.193	0.000	0.193	0.193	0.195	0.193	0.193	0.195	0.193	0.195
TA217	0.195	0.198	0.190	0.002	0.000	0.198	0.000	0.198	0.193	0.000	0.193	0.193	0.195	0.193	0.193	0.195	0.193	0.195
TA218	0.195	0.198	0.190	0.002	0.000	0.198	0.000	0.198	0.193	0.000	0.193	0.193	0.195	0.193	0.193	0.195	0.193	0.195
TA219	0.207	0.209	0.207	0.115	0.113	0.209	0.113	0.209	0.204	0.113	0.204	0.202	0.207	0.204	0.204	0.207	0.204	0.207
TA220	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA221	0.000	0.002	0.004	0.199	0.196	0.002	0.196	0.002	0.002	0.196	0.002	0.004	0.000	0.002	0.002	0.000	0.002	0.002
TA222	0.137	0.139	0.137	0.183	0.180	0.139	0.180	0.139	0.135	0.180	0.135	0.137	0.137	0.135	0.135	0.137	0.135	0.137
TA223	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA224	0.135	0.138	0.135	0.193	0.190	0.138	0.190	0.138	0.133	0.190	0.133	0.135	0.135	0.133	0.133	0.135	0.133	0.135
TA225	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA226	0.002	0.000	0.005	0.202	0.199	0.000	0.199	0.000	0.004	0.199	0.004	0.005	0.002	0.004	0.004	0.002	0.004	0.004
TA227	0.178	0.178	0.180	0.200	0.200	0.178	0.200	0.178	0.180	0.200	0.180	0.182	0.178	0.180	0.180	0.178	0.180	0.178
TA228	0.180	0.180	0.182	0.202	0.202	0.180	0.202	0.180	0.182	0.202	0.182	0.185	0.180	0.182	0.182	0.180	0.182	0.180
TA229	0.139	0.141	0.139	0.183	0.180	0.141	0.180	0.141	0.137	0.180	0.137	0.139	0.139	0.137	0.137	0.139	0.137	0.139
TA230	0.137	0.139	0.137	0.183	0.180	0.139	0.180	0.139	0.135	0.180	0.135	0.137	0.137	0.135	0.135	0.137	0.135	0.137
TA234	0.199	0.202	0.194	0.000	0.002	0.202	0.002	0.202	0.197	0.002	0.197	0.197	0.199	0.197	0.197	0.199	0.197	0.199
TA235	0.196	0.199	0.191	0.002	0.000	0.199	0.000	0.199	0.194	0.000	0.194	0.194	0.196	0.194	0.194	0.196	0.194	0.196

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA047																		
TA048	0.000																	
TA050	0.004	0.004																
TA051	0.000	0.000	0.004															
TA053	0.000	0.000	0.004	0.000														
TA056	0.000	0.000	0.004	0.000	0.000													
TA059	0.002	0.002	0.002	0.002	0.002	0.002												
TA060	0.004	0.004	0.004	0.004	0.004	0.004	0.002											
TA061	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004										
TA063	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002									
TA067	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139								
TA069	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000							
TA070	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000						
TA071	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000					
TA072	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000				
TA073	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137			
TA074	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.139	0.139	0.139	0.139	0.139	0.004		
TA075	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002	0.000	0.139	0.139	0.139	0.139	0.139	0.002	0.002	

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA076	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002	0.000	0.139	0.139	0.139	0.139	0.139	0.002	0.002	0.000
TA077	0.004	0.004	0.000	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.141	0.141	0.141	0.141	0.141	0.004	0.004	0.002
TA078	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA079	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.138	0.138	0.138	0.138	0.138	0.000	0.004	0.002
TA080	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA082	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196
TA083	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA084	0.002	0.002	0.005	0.002	0.002	0.002	0.004	0.005	0.002	0.004	0.134	0.134	0.134	0.134	0.134	0.002	0.005	0.004
TA086	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA087	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.137	0.137	0.137	0.137	0.137	0.004	0.004	0.002
TA088	0.004	0.004	0.000	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.141	0.141	0.141	0.141	0.141	0.004	0.004	0.002
TA089	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA090	0.004	0.004	0.000	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.141	0.141	0.141	0.141	0.141	0.004	0.004	0.002
TA091	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA092	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA093	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA094	0.004	0.004	0.000	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.141	0.141	0.141	0.141	0.141	0.004	0.004	0.002
TA095	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA096	0.139	0.139	0.143	0.139	0.139	0.139	0.141	0.138	0.139	0.141	0.002	0.002	0.002	0.002	0.002	0.139	0.141	0.141
TA097	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA098	0.002	0.002	0.005	0.002	0.002	0.002	0.004	0.005	0.002	0.004	0.135	0.135	0.135	0.135	0.135	0.002	0.005	0.004
TA099	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA107	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA108	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA109	0.202	0.202	0.207	0.202	0.202	0.202	0.204	0.201	0.202	0.204	0.184	0.184	0.184	0.184	0.184	0.202	0.204	0.204
TA110	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA111	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA112	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA113	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA114	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA115	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA116	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA117	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA118	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA120	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA121	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA122	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.004	0.004	0.004	0.004	0.004	0.137	0.139	0.139
TA123	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA153	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196
TA154	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA155	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA156	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA157	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA158	0.207	0.207	0.212	0.207	0.207	0.207	0.209	0.206	0.207	0.209	0.184	0.184	0.184	0.184	0.184	0.207	0.209	0.209
TA159	0.182	0.182	0.180	0.182	0.182	0.182	0.180	0.177	0.182	0.180	0.204	0.204	0.204	0.204	0.204	0.182	0.180	0.180
TA160	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA161	0.002	0.002	0.005	0.002	0.002	0.002	0.004	0.005	0.002	0.004	0.134	0.134	0.134	0.134	0.134	0.002	0.005	0.004
TA162	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.139	0.139	0.139	0.139	0.139	0.004	0.000	0.002
TA163	0.135	0.135	0.139	0.135	0.135	0.135	0.137	0.134	0.135	0.137	0.002	0.002	0.002	0.002	0.002	0.135	0.137	0.137
TA164	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA165	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA176	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA177	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002	0.000	0.139	0.139	0.139	0.139	0.139	0.002	0.002	0.000
TA180	0.000	0.000	0.004	0.000	0.000	0.000	0.002	0.004	0.000	0.002	0.137	0.137	0.137	0.137	0.137	0.000	0.004	0.002
TA181	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002	0.000	0.139	0.139	0.139	0.139	0.139	0.002	0.002	0.000

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA182	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.139	0.139	0.139	0.139	0.139	0.004	0.000	0.002
TA187	0.138	0.138	0.142	0.138	0.138	0.138	0.140	0.137	0.138	0.140	0.000	0.000	0.000	0.000	0.000	0.138	0.140	0.140
TA189	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA190	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA192	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA193	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA194	0.135	0.135	0.139	0.135	0.135	0.135	0.137	0.134	0.135	0.137	0.002	0.002	0.002	0.002	0.002	0.135	0.137	0.137
TA195	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA197	0.139	0.139	0.143	0.139	0.139	0.139	0.141	0.138	0.139	0.141	0.002	0.002	0.002	0.002	0.002	0.139	0.141	0.141
TA198	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA199	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA201	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA203	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA204	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA209	0.197	0.197	0.202	0.197	0.197	0.197	0.199	0.196	0.197	0.199	0.183	0.183	0.183	0.183	0.183	0.197	0.199	0.199
TA210	0.197	0.197	0.202	0.197	0.197	0.197	0.199	0.196	0.197	0.199	0.183	0.183	0.183	0.183	0.183	0.197	0.199	0.199
TA211	0.193	0.193	0.198	0.193	0.193	0.193	0.195	0.195	0.193	0.195	0.190	0.190	0.190	0.190	0.190	0.193	0.195	0.195
TA212	0.197	0.197	0.202	0.197	0.197	0.197	0.199	0.196	0.197	0.199	0.183	0.183	0.183	0.183	0.183	0.197	0.199	0.199
TA213	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA214	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196
TA215	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196
TA216	0.193	0.193	0.198	0.193	0.193	0.193	0.195	0.195	0.193	0.195	0.190	0.190	0.190	0.190	0.190	0.193	0.195	0.195
TA217	0.193	0.193	0.198	0.193	0.193	0.193	0.195	0.195	0.193	0.195	0.190	0.190	0.190	0.190	0.190	0.193	0.195	0.195
TA218	0.193	0.193	0.198	0.193	0.193	0.193	0.195	0.195	0.193	0.195	0.189	0.189	0.189	0.189	0.189	0.193	0.195	0.195
TA219	0.204	0.204	0.209	0.204	0.204	0.204	0.207	0.204	0.204	0.207	0.186	0.186	0.186	0.186	0.186	0.204	0.207	0.207
TA220	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA221	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.002	0.000	0.139	0.139	0.139	0.139	0.139	0.002	0.002	0.000
TA222	0.135	0.135	0.139	0.135	0.135	0.135	0.137	0.134	0.135	0.137	0.002	0.002	0.002	0.002	0.002	0.135	0.137	0.137
TA223	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA224	0.133	0.133	0.138	0.133	0.133	0.133	0.135	0.135	0.133	0.135	0.000	0.000	0.000	0.000	0.000	0.133	0.135	0.135
TA225	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA226	0.004	0.004	0.000	0.004	0.004	0.004	0.002	0.004	0.004	0.002	0.141	0.141	0.141	0.141	0.141	0.004	0.004	0.002
TA227	0.180	0.180	0.178	0.180	0.180	0.180	0.178	0.175	0.180	0.178	0.202	0.202	0.202	0.202	0.202	0.180	0.178	0.178
TA228	0.182	0.182	0.180	0.182	0.182	0.182	0.180	0.177	0.182	0.180	0.204	0.204	0.204	0.204	0.204	0.182	0.180	0.180
TA229	0.137	0.137	0.141	0.137	0.137	0.137	0.139	0.136	0.137	0.139	0.000	0.000	0.000	0.000	0.000	0.137	0.139	0.139
TA230	0.135	0.135	0.139	0.135	0.135	0.135	0.137	0.134	0.135	0.137	0.002	0.002	0.002	0.002	0.002	0.135	0.137	0.137
TA234	0.197	0.197	0.202	0.197	0.197	0.197	0.199	0.196	0.197	0.199	0.183	0.183	0.183	0.183	0.183	0.197	0.199	0.199
TA235	0.194	0.194	0.199	0.194	0.194	0.194	0.196	0.193	0.194	0.196	0.180	0.180	0.180	0.180	0.180	0.194	0.196	0.196

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA076																		
TA077	0.002																	
TA078	0.002	0.004																
TA079	0.002	0.004	0.000															
TA080	0.002	0.004	0.000	0.000														
TA082	0.196	0.199	0.194	0.195	0.194													
TA083	0.002	0.004	0.000	0.000	0.000	0.194												
TA084	0.004	0.005	0.002	0.002	0.002	0.191	0.002											
TA086	0.002	0.004	0.000	0.000	0.000	0.194	0.000	0.002										
TA087	0.002	0.004	0.004	0.004	0.004	0.194	0.004	0.005	0.004									
TA088	0.002	0.000	0.004	0.004	0.004	0.199	0.004	0.005	0.004	0.004								
TA089	0.002	0.004	0.000	0.000	0.000	0.194	0.000	0.002	0.000	0.004	0.004							
TA090	0.002	0.000	0.004	0.004	0.004	0.199	0.004	0.005	0.004	0.004	0.000	0.004						
TA091	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178					
TA092	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000				
TA093	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000			
TA094	0.002	0.000	0.004	0.004	0.004	0.199	0.004	0.005	0.004	0.004	0.000	0.004	0.000	0.178	0.178	0.178		
TA095	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA096	0.141	0.143	0.139	0.140	0.139	0.182	0.139	0.136	0.139	0.139	0.143	0.139	0.143	0.204	0.204	0.204	0.143	0.204
TA097	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA098	0.004	0.005	0.002	0.002	0.002	0.192	0.002	0.004	0.002	0.005	0.005	0.002	0.005	0.182	0.182	0.182	0.005	0.182
TA099	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA107	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA108	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA109	0.204	0.207	0.202	0.201	0.202	0.113	0.202	0.199	0.202	0.202	0.207	0.202	0.207	0.207	0.207	0.207	0.207	0.207
TA110	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA111	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA112	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA113	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA114	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA115	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA116	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA117	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA118	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA120	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA121	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA122	0.139	0.141	0.137	0.138	0.137	0.182	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA123	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA153	0.196	0.199	0.194	0.195	0.194	0.000	0.194	0.191	0.194	0.194	0.199	0.194	0.199	0.200	0.200	0.200	0.199	0.200
TA154	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA155	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA156	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA157	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA158	0.209	0.212	0.207	0.206	0.207	0.115	0.207	0.204	0.207	0.207	0.212	0.207	0.212	0.209	0.209	0.209	0.212	0.209
TA159	0.180	0.180	0.182	0.183	0.182	0.202	0.182	0.185	0.182	0.178	0.180	0.182	0.180	0.002	0.002	0.002	0.180	0.002
TA160	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA161	0.004	0.005	0.002	0.002	0.002	0.191	0.002	0.004	0.002	0.005	0.005	0.002	0.005	0.180	0.180	0.180	0.005	0.180
TA162	0.002	0.004	0.004	0.004	0.004	0.196	0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.178	0.178	0.178	0.004	0.178
TA163	0.137	0.139	0.135	0.136	0.135	0.180	0.135	0.132	0.135	0.135	0.139	0.135	0.139	0.199	0.199	0.199	0.139	0.199
TA164	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA165	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA176	0.002	0.004	0.000	0.000	0.000	0.194	0.000	0.002	0.000	0.004	0.004	0.000	0.004	0.180	0.180	0.180	0.004	0.180
TA177	0.000	0.002	0.002	0.002	0.002	0.196	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.178	0.178	0.178	0.002	0.178
TA180	0.002	0.004	0.000	0.000	0.000	0.194	0.000	0.002	0.000	0.004	0.004	0.000	0.004	0.180	0.180	0.180	0.004	0.180
TA181	0.000	0.002	0.002	0.002	0.002	0.196	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.178	0.178	0.178	0.002	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA182	0.002	0.004	0.004	0.004	0.004	0.196	0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.178	0.178	0.178	0.004	0.178
TA187	0.140	0.142	0.138	0.138	0.138	0.181	0.138	0.135	0.138	0.138	0.142	0.138	0.142	0.203	0.203	0.203	0.142	0.203
TA189	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA190	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA192	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA193	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA194	0.137	0.139	0.135	0.136	0.135	0.180	0.135	0.132	0.135	0.135	0.139	0.135	0.139	0.199	0.199	0.199	0.139	0.199
TA195	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA197	0.141	0.143	0.139	0.140	0.139	0.182	0.139	0.136	0.139	0.139	0.143	0.139	0.143	0.204	0.204	0.204	0.143	0.204
TA198	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA199	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA201	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA203	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA204	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA209	0.199	0.202	0.197	0.198	0.197	0.002	0.197	0.194	0.197	0.197	0.202	0.197	0.202	0.200	0.200	0.200	0.202	0.200
TA210	0.199	0.202	0.197	0.198	0.197	0.002	0.197	0.194	0.197	0.197	0.202	0.197	0.202	0.200	0.200	0.200	0.202	0.200
TA211	0.195	0.198	0.193	0.193	0.193	0.000	0.193	0.190	0.193	0.193	0.198	0.193	0.198	0.214	0.214	0.214	0.198	0.214
TA212	0.199	0.202	0.197	0.198	0.197	0.002	0.197	0.194	0.197	0.197	0.202	0.197	0.202	0.200	0.200	0.200	0.202	0.200
TA213	0.196	0.199	0.194	0.195	0.194	0.004	0.194	0.191	0.194	0.194	0.199	0.194	0.199	0.197	0.197	0.197	0.199	0.197

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA214	0.196	0.199	0.194	0.195	0.194	0.004	0.194	0.191	0.194	0.194	0.199	0.194	0.199	0.197	0.197	0.197	0.199	0.197
TA215	0.196	0.199	0.194	0.195	0.194	0.004	0.194	0.191	0.194	0.194	0.199	0.194	0.199	0.197	0.197	0.197	0.199	0.197
TA216	0.195	0.198	0.193	0.193	0.193	0.000	0.193	0.190	0.193	0.193	0.198	0.193	0.198	0.214	0.214	0.214	0.198	0.214
TA217	0.195	0.198	0.193	0.193	0.193	0.000	0.193	0.190	0.193	0.193	0.198	0.193	0.198	0.214	0.214	0.214	0.198	0.214
TA218	0.195	0.198	0.193	0.193	0.193	0.000	0.193	0.190	0.193	0.193	0.198	0.193	0.198	0.214	0.214	0.214	0.198	0.214
TA219	0.207	0.209	0.204	0.203	0.204	0.113	0.204	0.202	0.204	0.204	0.209	0.204	0.209	0.207	0.207	0.207	0.209	0.207
TA220	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA221	0.000	0.002	0.002	0.002	0.002	0.196	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.178	0.178	0.178	0.002	0.178
TA222	0.137	0.139	0.135	0.136	0.135	0.180	0.135	0.132	0.135	0.135	0.139	0.135	0.139	0.199	0.199	0.199	0.139	0.199
TA223	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA224	0.135	0.138	0.133	0.133	0.133	0.190	0.133	0.130	0.133	0.133	0.138	0.133	0.138	0.211	0.211	0.211	0.138	0.211
TA225	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA226	0.002	0.000	0.004	0.004	0.004	0.199	0.004	0.005	0.004	0.004	0.000	0.004	0.000	0.178	0.178	0.178	0.000	0.178
TA227	0.178	0.178	0.180	0.181	0.180	0.200	0.180	0.182	0.180	0.175	0.178	0.180	0.178	0.000	0.000	0.000	0.178	0.000
TA228	0.180	0.180	0.182	0.183	0.182	0.202	0.182	0.185	0.182	0.178	0.180	0.182	0.180	0.002	0.002	0.002	0.180	0.002
TA229	0.139	0.141	0.137	0.138	0.137	0.180	0.137	0.134	0.137	0.137	0.141	0.137	0.141	0.202	0.202	0.202	0.141	0.202
TA230	0.137	0.139	0.135	0.136	0.135	0.180	0.135	0.132	0.135	0.135	0.139	0.135	0.139	0.199	0.199	0.199	0.139	0.199
TA234	0.199	0.202	0.197	0.198	0.197	0.002	0.197	0.194	0.197	0.197	0.202	0.197	0.202	0.200	0.200	0.200	0.202	0.200
TA235	0.196	0.199	0.194	0.195	0.194	0.000	0.194	0.191	0.194	0.194	0.199	0.194	0.199	0.200	0.200	0.200	0.199	0.200

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA096																		
TA097	0.002																	
TA098	0.137	0.135																
TA099	0.002	0.000	0.135															
TA107	0.204	0.202	0.182	0.202														
TA108	0.204	0.202	0.182	0.202	0.000													
TA109	0.186	0.184	0.199	0.184	0.207	0.207												
TA110	0.188	0.186	0.202	0.186	0.207	0.207	0.002											
TA111	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000										
TA112	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000									
TA113	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000								
TA114	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000							
TA115	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000						
TA116	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000					
TA117	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
TA118	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
TA120	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207		
TA121	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.000	

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA122	0.005	0.004	0.135	0.004	0.202	0.202	0.186	0.188	0.188	0.188	0.188	0.188	0.188	0.188	0.188	0.188	0.202	0.202
TA123	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.000	0.000
TA153	0.182	0.180	0.192	0.180	0.200	0.200	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.200	0.200
TA154	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.207
TA155	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.207
TA156	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.207
TA157	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.207
TA158	0.186	0.184	0.204	0.184	0.209	0.209	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.209	0.209
TA159	0.206	0.204	0.184	0.204	0.002	0.002	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.002	0.002
TA160	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.000	0.000
TA161	0.136	0.134	0.004	0.134	0.180	0.180	0.199	0.202	0.202	0.202	0.202	0.202	0.202	0.202	0.202	0.202	0.180	0.180
TA162	0.141	0.139	0.005	0.139	0.178	0.178	0.204	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.178	0.178
TA163	0.004	0.002	0.133	0.002	0.199	0.199	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.199	0.199
TA164	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA165	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA176	0.139	0.137	0.002	0.137	0.180	0.180	0.202	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.180	0.180
TA177	0.141	0.139	0.004	0.139	0.178	0.178	0.204	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.178	0.178
TA180	0.139	0.137	0.002	0.137	0.180	0.180	0.202	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.180	0.180
TA181	0.141	0.139	0.004	0.139	0.178	0.178	0.204	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.178	0.178

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA182	0.141	0.139	0.005	0.139	0.178	0.178	0.204	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.178	0.178
TA187	0.002	0.000	0.136	0.000	0.203	0.203	0.183	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.203	0.203
TA189	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA190	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA192	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA193	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA194	0.004	0.002	0.133	0.002	0.199	0.199	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.199	0.199
TA195	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA197	0.004	0.002	0.137	0.002	0.204	0.204	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.204	0.204
TA198	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA199	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA201	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA203	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA204	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA209	0.185	0.183	0.194	0.183	0.200	0.200	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.200	0.200
TA210	0.185	0.183	0.194	0.183	0.200	0.200	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.200	0.200
TA211	0.192	0.190	0.193	0.190	0.214	0.214	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.214	0.214
TA212	0.185	0.183	0.194	0.183	0.200	0.200	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.200	0.200
TA213	0.182	0.180	0.192	0.180	0.197	0.197	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.197	0.197

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA214	0.182	0.180	0.192	0.180	0.197	0.197	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.197	0.197
TA215	0.182	0.180	0.192	0.180	0.197	0.197	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.197	0.197
TA216	0.192	0.190	0.193	0.190	0.214	0.214	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.214	0.214
TA217	0.192	0.190	0.193	0.190	0.214	0.214	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.214	0.214
TA218	0.192	0.189	0.193	0.189	0.214	0.214	0.112	0.112	0.112	0.112	0.112	0.112	0.112	0.112	0.112	0.112	0.214	0.214
TA219	0.188	0.186	0.202	0.186	0.207	0.207	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.207	0.207
TA220	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.000	0.000
TA221	0.141	0.139	0.004	0.139	0.178	0.178	0.204	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.178	0.178
TA222	0.004	0.002	0.133	0.002	0.199	0.199	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.199	0.199
TA223	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA224	0.002	0.000	0.133	0.000	0.211	0.211	0.195	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.211	0.211
TA225	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA226	0.143	0.141	0.005	0.141	0.178	0.178	0.207	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.178	0.178
TA227	0.204	0.202	0.182	0.202	0.000	0.000	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.207	0.000	0.000
TA228	0.206	0.204	0.184	0.204	0.002	0.002	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.002	0.002
TA229	0.002	0.000	0.135	0.000	0.202	0.202	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.202	0.202
TA230	0.004	0.002	0.133	0.002	0.199	0.199	0.184	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.199	0.199
TA234	0.185	0.183	0.194	0.183	0.200	0.200	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.200	0.200
TA235	0.182	0.180	0.192	0.180	0.200	0.200	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.200	0.200

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA122																		
TA123	0.202																	
TA153	0.182	0.200																
TA154	0.188	0.207	0.113															
TA155	0.188	0.207	0.113	0.000														
TA156	0.188	0.207	0.113	0.000	0.000													
TA157	0.188	0.207	0.113	0.000	0.000	0.000												
TA158	0.186	0.209	0.115	0.002	0.002	0.002	0.002											
TA159	0.204	0.002	0.202	0.209	0.209	0.209	0.209	0.211										
TA160	0.202	0.000	0.200	0.207	0.207	0.207	0.207	0.209	0.002									
TA161	0.134	0.180	0.191	0.202	0.202	0.202	0.202	0.204	0.182	0.180								
TA162	0.139	0.178	0.196	0.207	0.207	0.207	0.207	0.209	0.180	0.178	0.005							
TA163	0.002	0.199	0.180	0.186	0.186	0.186	0.186	0.184	0.202	0.199	0.132	0.137						
TA164	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002					
TA165	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000				
TA176	0.137	0.180	0.194	0.204	0.204	0.204	0.204	0.207	0.182	0.180	0.002	0.004	0.135	0.137	0.137			
TA177	0.139	0.178	0.196	0.207	0.207	0.207	0.207	0.209	0.180	0.178	0.004	0.002	0.137	0.139	0.139	0.002		
TA180	0.137	0.180	0.194	0.204	0.204	0.204	0.204	0.207	0.182	0.180	0.002	0.004	0.135	0.137	0.137	0.000	0.002	
TA181	0.139	0.178	0.196	0.207	0.207	0.207	0.207	0.209	0.180	0.178	0.004	0.002	0.137	0.139	0.139	0.002	0.000	0.002

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA182	0.139	0.178	0.196	0.207	0.207	0.207	0.207	0.209	0.180	0.178	0.005	0.000	0.137	0.139	0.139	0.004	0.002	0.004
TA187	0.004	0.203	0.181	0.185	0.185	0.185	0.185	0.183	0.205	0.203	0.135	0.140	0.002	0.000	0.000	0.138	0.140	0.138
TA189	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA190	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA192	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA193	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA194	0.002	0.199	0.180	0.186	0.186	0.186	0.186	0.184	0.202	0.199	0.132	0.137	0.000	0.002	0.002	0.135	0.137	0.135
TA195	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA197	0.005	0.204	0.182	0.186	0.186	0.186	0.186	0.184	0.206	0.204	0.136	0.141	0.004	0.002	0.002	0.139	0.141	0.139
TA198	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA199	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA201	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA203	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA204	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA209	0.185	0.200	0.002	0.115	0.115	0.115	0.115	0.117	0.202	0.200	0.194	0.199	0.183	0.183	0.183	0.197	0.199	0.197
TA210	0.185	0.200	0.002	0.115	0.115	0.115	0.115	0.117	0.202	0.200	0.194	0.199	0.183	0.183	0.183	0.197	0.199	0.197
TA211	0.192	0.214	0.000	0.113	0.113	0.113	0.113	0.115	0.217	0.214	0.190	0.195	0.190	0.190	0.190	0.193	0.195	0.193
TA212	0.185	0.200	0.002	0.115	0.115	0.115	0.115	0.117	0.202	0.200	0.194	0.199	0.183	0.183	0.183	0.197	0.199	0.197
TA213	0.182	0.197	0.004	0.113	0.113	0.113	0.113	0.115	0.199	0.197	0.191	0.196	0.180	0.180	0.180	0.194	0.196	0.194

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA214	0.182	0.197	0.004	0.113	0.113	0.113	0.113	0.115	0.199	0.197	0.191	0.196	0.180	0.180	0.180	0.194	0.196	0.194
TA215	0.182	0.197	0.004	0.113	0.113	0.113	0.113	0.115	0.199	0.197	0.191	0.196	0.180	0.180	0.180	0.194	0.196	0.194
TA216	0.192	0.214	0.000	0.113	0.113	0.113	0.113	0.115	0.217	0.214	0.190	0.195	0.190	0.190	0.190	0.193	0.195	0.193
TA217	0.192	0.214	0.000	0.113	0.113	0.113	0.113	0.115	0.217	0.214	0.190	0.195	0.190	0.190	0.190	0.193	0.195	0.193
TA218	0.192	0.214	0.000	0.112	0.112	0.112	0.112	0.114	0.217	0.214	0.190	0.195	0.189	0.189	0.189	0.193	0.195	0.193
TA219	0.188	0.207	0.113	0.000	0.000	0.000	0.000	0.002	0.209	0.207	0.202	0.207	0.186	0.186	0.186	0.204	0.207	0.204
TA220	0.202	0.000	0.200	0.207	0.207	0.207	0.207	0.209	0.002	0.000	0.180	0.178	0.199	0.202	0.202	0.180	0.178	0.180
TA221	0.139	0.178	0.196	0.207	0.207	0.207	0.207	0.209	0.180	0.178	0.004	0.002	0.137	0.139	0.139	0.002	0.000	0.002
TA222	0.002	0.199	0.180	0.186	0.186	0.186	0.186	0.184	0.202	0.199	0.132	0.137	0.000	0.002	0.002	0.135	0.137	0.135
TA223	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA224	0.004	0.211	0.190	0.198	0.198	0.198	0.198	0.196	0.213	0.211	0.130	0.135	0.002	0.000	0.000	0.133	0.135	0.133
TA225	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA226	0.141	0.178	0.199	0.209	0.209	0.209	0.209	0.212	0.180	0.178	0.005	0.004	0.139	0.141	0.141	0.004	0.002	0.004
TA227	0.202	0.000	0.200	0.207	0.207	0.207	0.207	0.209	0.002	0.000	0.180	0.178	0.199	0.202	0.202	0.180	0.178	0.180
TA228	0.204	0.002	0.202	0.209	0.209	0.209	0.209	0.211	0.004	0.002	0.182	0.180	0.202	0.204	0.204	0.182	0.180	0.182
TA229	0.004	0.202	0.180	0.186	0.186	0.186	0.186	0.184	0.204	0.202	0.134	0.139	0.002	0.000	0.000	0.137	0.139	0.137
TA230	0.002	0.199	0.180	0.186	0.186	0.186	0.186	0.184	0.202	0.199	0.132	0.137	0.000	0.002	0.002	0.135	0.137	0.135
TA234	0.185	0.200	0.002	0.115	0.115	0.115	0.115	0.117	0.202	0.200	0.194	0.199	0.183	0.183	0.183	0.197	0.199	0.197
TA235	0.182	0.200	0.000	0.113	0.113	0.113	0.113	0.115	0.202	0.200	0.191	0.196	0.180	0.180	0.180	0.194	0.196	0.194

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA181	TA182	TA187	TA189	TA190	TA192	TA193	TA194	TA195	TA197	TA198	TA199	TA201	TA203	TA204	TA209	TA210	TA211	TA212
TA182	0.002																		
TA187	0.140	0.140																	
TA189	0.139	0.139	0.000																
TA190	0.139	0.139	0.000	0.000															
TA192	0.139	0.139	0.000	0.000	0.000														
TA193	0.139	0.139	0.000	0.000	0.000	0.000													
TA194	0.137	0.137	0.002	0.002	0.002	0.002	0.002												
TA195	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002											
TA197	0.141	0.141	0.002	0.002	0.002	0.002	0.002	0.004	0.002										
TA198	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002									
TA199	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000								
TA201	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000							
TA203	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000						
TA204	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000					
TA209	0.199	0.199	0.184	0.183	0.183	0.183	0.183	0.183	0.183	0.185	0.183	0.183	0.183	0.183	0.183				
TA210	0.199	0.199	0.184	0.183	0.183	0.183	0.183	0.183	0.183	0.185	0.183	0.183	0.183	0.183	0.183	0.000			
TA211	0.195	0.195	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.002	0.002		
TA212	0.199	0.199	0.184	0.183	0.183	0.183	0.183	0.183	0.183	0.185	0.183	0.183	0.183	0.183	0.183	0.000	0.000	0.002	
TA213	0.196	0.196	0.181	0.180	0.180	0.180	0.180	0.180	0.180	0.182	0.180	0.180	0.180	0.180	0.180	0.002	0.002	0.004	0.002

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA181	TA182	TA187	TA189	TA190	TA192	TA193	TA194	TA195	TA197	TA198	TA199	TA201	TA203	TA204	TA209	TA210	TA211	TA212
TA214	0.196	0.196	0.181	0.180	0.180	0.180	0.180	0.180	0.180	0.182	0.180	0.180	0.180	0.180	0.180	0.002	0.002	0.004	0.002
TA215	0.196	0.196	0.181	0.180	0.180	0.180	0.180	0.180	0.180	0.182	0.180	0.180	0.180	0.180	0.180	0.002	0.002	0.004	0.002
TA216	0.195	0.195	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.002	0.002	0.000	0.002
TA217	0.195	0.195	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.002	0.002	0.000	0.002
TA218	0.195	0.195	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.189	0.002	0.002	0.000	0.002
TA219	0.207	0.207	0.185	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.186	0.115	0.115	0.113	0.115
TA220	0.178	0.178	0.203	0.202	0.202	0.202	0.202	0.199	0.202	0.204	0.202	0.202	0.202	0.202	0.202	0.200	0.200	0.214	0.200
TA221	0.000	0.002	0.140	0.139	0.139	0.139	0.139	0.137	0.139	0.141	0.139	0.139	0.139	0.139	0.139	0.199	0.199	0.195	0.199
TA222	0.137	0.137	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.183	0.183	0.190	0.183
TA223	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.183	0.183	0.190	0.183
TA224	0.135	0.135	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.193	0.193	0.190	0.193
TA225	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.183	0.183	0.190	0.183
TA226	0.002	0.004	0.142	0.141	0.141	0.141	0.141	0.139	0.141	0.143	0.141	0.141	0.141	0.141	0.141	0.202	0.202	0.198	0.202
TA227	0.178	0.178	0.203	0.202	0.202	0.202	0.202	0.199	0.202	0.204	0.202	0.202	0.202	0.202	0.202	0.200	0.200	0.214	0.200
TA228	0.180	0.180	0.205	0.204	0.204	0.204	0.204	0.202	0.204	0.206	0.204	0.204	0.204	0.204	0.204	0.202	0.202	0.217	0.202
TA229	0.139	0.139	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.183	0.183	0.190	0.183
TA230	0.137	0.137	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.183	0.183	0.190	0.183
TA234	0.199	0.199	0.184	0.183	0.183	0.183	0.183	0.183	0.183	0.185	0.183	0.183	0.183	0.183	0.183	0.000	0.000	0.002	0.000
TA235	0.196	0.196	0.181	0.180	0.180	0.180	0.180	0.180	0.180	0.182	0.180	0.180	0.180	0.180	0.180	0.002	0.002	0.000	0.002

Table S5.3 continued - Pairwise genetic distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated using TN93 evolutionary model.

	TA213	TA214	TA215	TA216	TA217	TA218	TA219	TA220	TA221	TA222	TA223	TA224	TA225	TA226	TA227	TA228	TA229	TA230	TA234
TA214	0.000																		
TA215	0.000	0.000																	
TA216	0.004	0.004	0.004																
TA217	0.004	0.004	0.004	0.000															
TA218	0.004	0.004	0.004	0.000	0.000														
TA219	0.113	0.113	0.113	0.113	0.113	0.112													
TA220	0.197	0.197	0.197	0.214	0.214	0.214	0.207												
TA221	0.196	0.196	0.196	0.195	0.195	0.195	0.207	0.178											
TA222	0.180	0.180	0.180	0.190	0.190	0.189	0.186	0.199	0.137										
TA223	0.180	0.180	0.180	0.190	0.190	0.189	0.186	0.202	0.139	0.002									
TA224	0.190	0.190	0.190	0.190	0.190	0.189	0.198	0.211	0.135	0.002	0.000								
TA225	0.180	0.180	0.180	0.190	0.190	0.189	0.186	0.202	0.139	0.002	0.000	0.000							
TA226	0.199	0.199	0.199	0.198	0.198	0.198	0.209	0.178	0.002	0.139	0.141	0.138	0.141						
TA227	0.197	0.197	0.197	0.214	0.214	0.214	0.207	0.000	0.178	0.199	0.202	0.211	0.202	0.178					
TA228	0.199	0.199	0.199	0.217	0.217	0.217	0.209	0.002	0.180	0.202	0.204	0.213	0.204	0.180	0.002				
TA229	0.180	0.180	0.180	0.190	0.190	0.189	0.186	0.202	0.139	0.002	0.000	0.000	0.000	0.141	0.202	0.204			
TA230	0.180	0.180	0.180	0.190	0.190	0.189	0.186	0.199	0.137	0.000	0.002	0.002	0.002	0.139	0.199	0.202	0.002		
TA234	0.002	0.002	0.002	0.002	0.002	0.002	0.115	0.200	0.199	0.183	0.183	0.193	0.183	0.202	0.200	0.202	0.183	0.183	
TA235	0.004	0.004	0.004	0.000	0.000	0.000	0.113	0.200	0.196	0.180	0.180	0.190	0.180	0.199	0.200	0.202	0.180	0.180	0.002

Table S5.4 - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA025																		
TA026	3.494																	
TA027	0.672	3.480																
TA029	2.364	2.335	2.563															
TA030	0.513	3.245	0.573	2.344														
TA031	4.660	2.988	4.354	3.678	4.302													
TA032	2.176	3.998	1.760	2.842	2.183	4.410												
TA033	2.742	2.229	2.978	1.798	2.673	3.512	3.914											
TA035	4.011	1.445	3.914	2.381	3.798	2.960	3.787	3.079										
TA036	3.494	0.000	3.480	2.335	3.245	2.988	3.998	2.229	1.445									
TA037	3.236	1.905	3.140	1.640	3.033	2.131	3.229	1.960	1.821	1.905								
TA038	3.362	0.257	3.376	2.174	3.145	3.125	3.898	2.093	1.465	0.257	1.869							
TA039	3.339	0.573	3.245	2.298	3.101	2.852	3.615	2.333	1.317	0.573	1.746	0.569						
TA041	3.063	1.027	3.171	1.828	2.960	3.613	3.691	1.848	1.764	1.027	1.969	0.770	1.049					
TA043	4.672	2.088	4.424	3.376	4.357	2.463	4.200	3.893	1.274	2.088	2.384	2.237	1.923	2.782				
TA044	3.796	0.513	3.732	2.701	3.494	2.768	4.237	2.556	1.538	0.513	2.072	0.770	0.856	1.540	1.860			
TA045	3.236	1.905	3.140	1.640	3.033	2.131	3.229	1.960	1.821	1.905	0.000	1.869	1.746	1.969	2.384	2.072		
TA046	3.494	0.000	3.480	2.335	3.245	2.988	3.998	2.229	1.445	0.000	1.905	0.257	0.573	1.027	2.088	0.513	1.905	

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA047	3.212	0.682	3.145	2.151	3.007	3.006	3.514	2.220	1.365	0.682	1.727	0.573	0.257	0.843	2.102	1.065	1.727	0.682
TA048	2.812	2.093	3.010	1.855	2.696	3.314	3.948	0.257	2.984	2.093	1.860	1.982	2.220	1.832	3.740	2.384	1.860	2.093
TA050	2.660	1.992	2.742	1.822	2.513	2.945	3.576	0.672	2.795	1.992	1.529	1.891	1.982	1.786	3.465	2.269	1.529	1.992
TA051	4.011	1.445	3.914	2.381	3.798	2.960	3.787	3.079	0.000	1.445	1.821	1.465	1.317	1.764	1.274	1.538	1.821	1.445
TA053	2.574	2.119	2.696	1.747	2.476	3.157	3.529	0.569	2.886	2.119	1.631	1.992	2.093	1.785	3.621	2.436	1.631	2.119
TA056	2.660	1.992	2.742	1.822	2.513	2.945	3.576	0.672	2.795	1.992	1.529	1.891	1.982	1.786	3.465	2.269	1.529	1.992
TA059	4.083	1.757	3.898	2.638	3.858	2.804	3.599	3.358	0.569	1.757	1.899	1.791	1.465	2.088	1.027	1.800	1.899	1.757
TA060	2.742	2.229	2.978	1.798	2.673	3.512	3.914	0.000	3.079	2.229	1.960	2.093	2.333	1.848	3.893	2.556	1.960	2.229
TA061	2.766	1.891	2.812	1.928	2.574	2.741	3.640	0.843	2.726	1.891	1.465	1.821	1.899	1.823	3.322	2.119	1.465	1.891
TA063	3.494	0.000	3.480	2.335	3.245	2.988	3.998	2.229	1.445	0.000	1.905	0.257	0.573	1.027	2.088	0.513	1.905	0.000
TA067	3.912	3.979	4.311	3.028	4.097	5.248	5.077	2.107	4.585	3.979	3.568	3.785	4.048	3.253	5.603	4.388	3.568	3.979
TA069	4.311	4.434	4.771	3.452	4.522	5.834	5.581	2.611	5.051	4.434	4.131	4.238	4.553	3.693	6.114	4.844	4.131	4.434
TA070	4.759	4.917	5.280	4.319	5.053	7.201	6.099	3.891	5.562	4.917	5.327	4.686	5.027	4.012	6.737	5.385	5.327	4.917
TA071	3.912	3.979	4.311	3.028	4.097	5.248	5.077	2.107	4.585	3.979	3.568	3.785	4.048	3.253	5.603	4.388	3.568	3.979
TA072	3.843	3.785	4.225	2.906	3.997	5.027	5.012	1.892	4.418	3.785	3.380	3.598	3.866	3.098	5.413	4.181	3.380	3.785
TA073	3.318	2.741	3.732	2.918	3.394	5.414	4.760	2.493	3.691	2.741	3.755	2.547	2.945	2.048	4.711	3.157	3.755	2.741
TA074	3.481	0.569	3.362	2.463	3.212	2.713	3.731	2.468	1.317	0.569	1.801	0.672	0.257	1.274	1.764	0.682	1.801	0.569
TA075	2.766	1.891	2.812	1.928	2.574	2.741	3.640	0.843	2.726	1.891	1.465	1.821	1.899	1.823	3.322	2.119	1.465	1.891

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA076	3.212	0.682	3.145	2.151	3.007	3.006	3.514	2.220	1.365	0.682	1.727	0.573	0.257	0.843	2.102	1.065	1.727	0.682
TA077	2.812	2.093	3.010	1.855	2.696	3.314	3.948	0.257	2.984	2.093	1.860	1.982	2.220	1.832	3.740	2.384	1.860	2.093
TA078	3.362	0.257	3.376	2.174	3.145	3.125	3.898	2.093	1.465	0.257	1.869	0.000	0.569	0.770	2.237	0.770	1.869	0.257
TA079	3.481	0.569	3.362	2.463	3.212	2.713	3.731	2.468	1.317	0.569	1.801	0.672	0.257	1.274	1.764	0.682	1.801	0.569
TA080	2.812	2.093	3.010	1.855	2.696	3.314	3.948	0.257	2.984	2.093	1.860	1.982	2.220	1.832	3.740	2.384	1.860	2.093
TA082	0.000	3.494	0.672	2.364	0.513	4.660	2.176	2.742	4.011	3.494	3.236	3.362	3.339	3.063	4.672	3.796	3.236	3.494
TA083	1.529	3.288	1.317	1.975	1.445	4.065	1.147	3.139	3.245	3.288	2.660	3.188	3.035	3.002	3.800	3.536	2.660	3.288
TA084	3.145	0.770	3.220	1.917	3.001	3.439	3.744	1.899	1.631	0.770	1.902	0.513	0.843	0.257	2.588	1.284	1.902	0.770
TA086	3.021	2.648	3.394	2.741	3.126	5.262	4.349	2.421	3.527	2.648	3.563	2.432	2.741	1.847	4.550	3.099	3.563	2.648
TA087	2.902	1.982	3.064	1.944	2.742	3.124	3.999	0.513	2.908	1.982	1.791	1.899	2.132	1.853	3.599	2.229	1.791	1.982
TA088	2.513	2.269	2.673	1.708	2.466	3.374	3.501	0.573	2.997	2.269	1.764	2.119	2.229	1.821	3.788	2.618	1.764	2.269
TA089	3.212	0.682	3.145	2.151	3.007	3.006	3.514	2.220	1.365	0.682	1.727	0.573	0.257	0.843	2.102	1.065	1.727	0.682
TA090	0.672	3.480	0.000	2.563	0.573	4.354	1.760	2.978	3.914	3.480	3.140	3.376	3.245	3.171	4.424	3.732	3.140	3.480
TA091	5.808	6.041	5.988	4.370	6.064	6.855	5.197	5.491	5.324	6.041	5.052	5.848	5.859	5.303	6.318	6.440	5.052	6.041
TA092	5.812	6.079	5.932	4.455	6.057	6.749	5.008	5.605	5.304	6.079	5.027	5.893	5.848	5.369	6.229	6.466	5.027	6.079
TA093	5.370	5.064	5.310	3.791	5.458	5.194	4.166	5.046	4.024	5.064	3.829	4.939	4.775	4.631	4.675	5.342	3.829	5.064
TA094	2.812	2.093	3.010	1.855	2.696	3.314	3.948	0.257	2.984	2.093	1.860	1.982	2.220	1.832	3.740	2.384	1.860	2.093
TA095	6.607	6.855	6.960	5.518	6.945	8.680	6.555	6.497	6.411	6.855	6.636	6.628	6.779	5.962	7.592	7.315	6.636	6.855

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA096	3.912	3.979	4.311	3.028	4.097	5.248	5.077	2.107	4.585	3.979	3.568	3.785	4.048	3.253	5.603	4.388	3.568	3.979
TA097	3.843	3.785	4.225	2.906	3.997	5.027	5.012	1.892	4.418	3.785	3.380	3.598	3.866	3.098	5.413	4.181	3.380	3.785
TA098	3.823	1.461	3.866	1.960	3.689	3.498	3.882	2.726	0.843	1.461	1.916	1.367	1.493	1.365	2.099	1.750	1.916	1.461
TA099	5.013	5.027	5.556	4.499	5.280	7.353	6.445	4.011	5.720	5.027	5.512	4.808	5.198	4.180	6.890	5.473	5.512	5.027
TA107	5.808	6.041	5.988	4.370	6.064	6.855	5.197	5.491	5.324	6.041	5.052	5.848	5.859	5.303	6.318	6.440	5.052	6.041
TA108	5.702	5.893	5.808	4.291	5.930	6.524	4.868	5.462	5.090	5.893	4.821	5.712	5.661	5.207	5.999	6.270	4.821	5.893
TA109	4.516	4.141	4.851	3.172	4.395	5.214	5.377	3.788	4.254	4.141	3.964	4.113	4.486	4.125	4.950	4.244	3.964	4.141
TA110	4.901	4.678	5.203	3.249	4.872	5.653	5.231	4.423	4.317	4.678	4.177	4.610	4.896	4.488	5.115	4.853	4.177	4.678
TA111	4.762	4.885	5.243	3.540	4.800	6.295	5.809	4.124	5.045	4.885	4.704	4.787	5.203	4.566	5.974	5.115	4.704	4.885
TA112	6.041	6.026	6.602	5.236	6.153	8.271	7.306	5.686	6.338	6.026	6.557	5.904	6.387	5.593	7.362	6.295	6.557	6.026
TA113	4.764	4.787	5.227	3.517	4.774	6.157	5.801	4.088	4.950	4.787	4.623	4.700	5.118	4.519	5.845	4.995	4.623	4.787
TA114	5.463	5.214	5.971	4.577	5.505	7.421	6.664	5.094	5.512	5.214	5.810	5.117	5.593	4.896	6.462	5.438	5.810	5.214
TA115	5.593	5.701	6.153	4.806	5.726	7.966	6.805	5.353	5.973	5.701	6.186	5.565	6.026	5.214	7.028	5.995	6.186	5.701
TA116	6.153	6.295	6.733	5.399	6.305	8.558	7.415	5.859	6.593	6.295	6.775	6.157	6.630	5.791	7.660	6.592	6.775	6.295
TA117	5.170	5.416	5.726	4.409	5.327	7.692	6.317	5.064	5.642	5.416	5.848	5.267	5.701	4.872	6.727	5.738	5.848	5.416
TA118	5.496	5.569	5.966	4.387	5.645	7.654	6.153	5.454	5.416	5.569	5.786	5.423	5.787	5.039	6.489	5.884	5.786	5.569
TA120	5.930	6.270	6.064	4.628	6.193	6.977	5.156	5.755	5.521	6.270	5.238	6.079	6.041	5.537	6.461	6.666	5.238	6.270
TA121	5.513	5.537	5.588	3.991	5.702	6.079	4.620	5.203	4.675	5.537	4.425	5.369	5.303	4.910	5.547	5.893	4.425	5.537

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA122	3.745	3.992	4.097	2.962	3.955	5.150	4.758	2.166	4.521	3.992	3.453	3.789	3.979	3.226	5.524	4.416	3.453	3.992
TA123	4.954	5.521	5.179	3.803	5.264	6.490	4.635	4.676	5.083	5.521	4.583	5.304	5.324	4.675	6.143	5.966	4.583	5.521
TA153	1.708	4.047	1.113	3.261	1.677	4.495	1.317	3.754	4.271	4.047	3.538	3.957	3.682	3.780	4.614	4.268	3.538	4.047
TA154	4.982	4.555	5.243	3.317	4.901	5.412	5.285	4.442	4.185	4.555	4.088	4.514	4.793	4.479	4.885	4.678	4.088	4.555
TA155	4.395	4.244	4.779	3.056	4.331	5.438	5.298	3.730	4.352	4.244	4.021	4.185	4.566	4.101	5.150	4.404	4.021	4.244
TA156	4.395	4.244	4.779	3.056	4.331	5.438	5.298	3.730	4.352	4.244	4.021	4.185	4.566	4.101	5.150	4.404	4.021	4.244
TA157	4.085	3.851	4.395	2.692	3.969	4.872	4.833	3.490	3.885	3.851	3.533	3.812	4.141	3.797	4.604	3.979	3.533	3.851
TA158	4.019	3.907	4.356	2.625	3.934	4.994	4.789	3.459	3.940	3.907	3.566	3.851	4.185	3.784	4.713	4.066	3.566	3.907
TA159	5.930	6.270	6.064	4.628	6.193	6.977	5.156	5.755	5.521	6.270	5.238	6.079	6.041	5.537	6.461	6.666	5.238	6.270
TA160	5.602	5.712	5.693	4.135	5.812	6.300	4.739	5.328	4.880	5.712	4.620	5.537	5.478	5.054	5.772	6.079	4.620	5.712
TA161	4.120	2.674	4.279	3.640	4.105	5.561	4.792	3.824	3.184	2.674	4.144	2.538	2.713	2.258	4.021	2.994	4.144	2.674
TA162	2.574	2.119	2.696	1.747	2.476	3.157	3.529	0.569	2.886	2.119	1.631	1.992	2.093	1.785	3.621	2.436	1.631	2.119
TA163	3.488	3.678	3.745	2.729	3.660	4.631	4.324	1.932	4.167	3.678	3.001	3.485	3.593	2.961	5.095	4.087	3.001	3.678
TA164	3.596	3.593	3.912	2.706	3.745	4.686	4.617	1.722	4.174	3.593	3.049	3.405	3.598	2.903	5.130	3.992	3.049	3.593
TA165	3.427	3.485	3.662	2.617	3.566	4.393	4.262	1.731	3.998	3.485	2.797	3.299	3.405	2.812	4.898	3.879	2.797	3.485
TA176	3.101	0.856	3.063	2.026	2.933	3.174	3.430	2.132	1.457	0.856	1.746	0.682	0.513	0.672	2.295	1.290	1.746	0.856
TA177	3.058	1.364	2.933	2.175	2.913	3.174	3.106	2.412	1.603	1.364	1.807	1.234	0.856	1.139	2.325	1.713	1.807	1.364
TA180	3.339	0.573	3.245	2.298	3.101	2.852	3.615	2.333	1.317	0.573	1.746	0.569	0.000	1.049	1.923	0.856	1.746	0.573
TA181	3.339	0.573	3.245	2.298	3.101	2.852	3.615	2.333	1.317	0.573	1.746	0.569	0.000	1.049	1.923	0.856	1.746	0.573

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA182	3.964	1.760	3.798	2.486	3.767	2.940	3.480	3.262	0.573	1.760	1.848	1.757	1.445	1.960	1.284	1.875	1.848	1.760
TA187	3.488	3.678	3.745	2.729	3.660	4.631	4.324	1.932	4.167	3.678	3.001	3.485	3.593	2.961	5.095	4.087	3.001	3.678
TA189	3.547	3.405	3.843	2.607	3.662	4.458	4.567	1.516	4.014	3.405	2.860	3.226	3.420	2.764	4.942	3.789	2.860	3.405
TA190	3.596	3.593	3.912	2.706	3.745	4.686	4.617	1.722	4.174	3.593	3.049	3.405	3.598	2.903	5.130	3.992	3.049	3.593
TA192	3.488	3.678	3.745	2.729	3.660	4.631	4.324	1.932	4.167	3.678	3.001	3.485	3.593	2.961	5.095	4.087	3.001	3.678
TA193	3.912	3.979	4.311	3.028	4.097	5.248	5.077	2.107	4.585	3.979	3.568	3.785	4.048	3.253	5.603	4.388	3.568	3.979
TA194	3.912	3.979	4.311	3.028	4.097	5.248	5.077	2.107	4.585	3.979	3.568	3.785	4.048	3.253	5.603	4.388	3.568	3.979
TA195	3.662	3.789	3.997	2.825	3.842	4.917	4.681	1.940	4.344	3.789	3.247	3.593	3.785	3.058	5.325	4.201	3.247	3.789
TA197	3.596	3.593	3.912	2.706	3.745	4.686	4.617	1.722	4.174	3.593	3.049	3.405	3.598	2.903	5.130	3.992	3.049	3.593
TA198	3.745	3.992	4.097	2.962	3.955	5.150	4.758	2.166	4.521	3.992	3.453	3.789	3.979	3.226	5.524	4.416	3.453	3.992
TA199	3.547	3.405	3.843	2.607	3.662	4.458	4.567	1.516	4.014	3.405	2.860	3.226	3.420	2.764	4.942	3.789	2.860	3.405
TA201	5.084	4.542	5.496	4.463	5.313	7.223	6.059	4.424	4.995	4.542	5.383	4.321	4.622	3.685	6.145	4.994	5.383	4.542
TA203	4.426	4.381	4.965	3.744	4.612	6.994	5.663	4.116	4.798	4.381	5.086	4.193	4.618	3.678	5.958	4.777	5.086	4.381
TA204	3.630	3.839	4.075	2.201	3.846	5.318	4.494	2.438	4.038	3.839	3.302	3.627	3.911	3.034	5.200	4.277	3.302	3.839
TA209	1.791	3.615	1.445	2.379	1.760	4.202	0.573	3.501	3.480	3.615	2.902	3.514	3.288	3.315	3.964	3.860	2.902	3.615
TA210	0.672	3.480	0.000	2.563	0.573	4.354	1.760	2.978	3.914	3.480	3.140	3.376	3.245	3.171	4.424	3.732	3.140	3.480
TA211	0.672	3.480	0.000	2.563	0.573	4.354	1.760	2.978	3.914	3.480	3.140	3.376	3.245	3.171	4.424	3.732	3.140	3.480
TA212	1.328	3.816	1.689	2.382	1.722	4.921	2.618	2.490	4.297	3.816	3.277	3.632	3.634	3.141	5.122	4.208	3.277	3.816
TA213	0.843	3.376	0.257	2.579	0.569	4.181	1.800	2.968	3.823	3.376	3.058	3.289	3.145	3.142	4.276	3.599	3.058	3.376

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA025	TA026	TA027	TA029	TA030	TA031	TA032	TA033	TA035	TA036	TA037	TA038	TA039	TA041	TA043	TA044	TA045	TA046
TA214	0.672	3.480	0.000	2.563	0.573	4.354	1.760	2.978	3.914	3.480	3.140	3.376	3.245	3.171	4.424	3.732	3.140	3.480
TA215	0.573	3.732	0.513	2.609	0.856	4.723	1.791	3.064	4.138	3.732	3.358	3.599	3.494	3.289	4.749	4.034	3.358	3.732
TA216	4.287	2.852	4.519	3.734	4.286	5.814	5.132	3.848	3.434	2.852	4.344	2.713	2.988	2.416	4.327	3.174	4.344	2.852
TA217	0.569	3.599	0.257	2.573	0.682	4.535	1.757	3.010	4.020	3.599	3.241	3.480	3.362	3.220	4.582	3.877	3.241	3.599
TA218	0.569	3.599	0.257	2.573	0.682	4.535	1.757	3.010	4.020	3.599	3.241	3.480	3.362	3.220	4.582	3.877	3.241	3.599
TA219	4.676	4.380	4.901	2.989	4.605	5.157	4.809	4.279	3.907	4.380	3.773	4.329	4.555	4.267	4.617	4.524	3.773	4.380
TA220	5.932	6.238	6.124	4.553	6.204	7.084	5.346	5.650	5.547	6.238	5.267	6.041	6.057	5.478	6.551	6.645	5.267	6.238
TA221	3.481	0.569	3.362	2.463	3.212	2.713	3.731	2.468	1.317	0.569	1.801	0.672	0.257	1.274	1.764	0.682	1.801	0.569
TA222	3.843	3.785	4.225	2.906	3.997	5.027	5.012	1.892	4.418	3.785	3.380	3.598	3.866	3.098	5.413	4.181	3.380	3.785
TA223	3.596	3.593	3.912	2.706	3.745	4.686	4.617	1.722	4.174	3.593	3.049	3.405	3.598	2.903	5.130	3.992	3.049	3.593
TA224	3.662	3.789	3.997	2.825	3.842	4.917	4.681	1.940	4.344	3.789	3.247	3.593	3.785	3.058	5.325	4.201	3.247	3.789
TA225	3.843	3.785	4.225	2.906	3.997	5.027	5.012	1.892	4.418	3.785	3.380	3.598	3.866	3.098	5.413	4.181	3.380	3.785
TA226	3.552	1.546	3.555	2.252	3.467	3.216	3.859	1.957	1.952	1.546	1.739	1.385	1.328	1.136	2.797	1.932	1.739	1.546
TA227	5.930	6.270	6.064	4.628	6.193	6.977	5.156	5.755	5.521	6.270	5.238	6.079	6.041	5.537	6.461	6.666	5.238	6.270
TA228	5.983	6.353	6.057	4.771	6.233	6.916	5.024	5.913	5.555	6.353	5.270	6.169	6.079	5.652	6.421	6.735	5.270	6.353
TA229	3.662	3.789	3.997	2.825	3.842	4.917	4.681	1.940	4.344	3.789	3.247	3.593	3.785	3.058	5.325	4.201	3.247	3.789
TA230	3.889	3.911	4.367	2.431	4.075	5.474	4.899	2.521	4.189	3.911	3.518	3.713	4.064	3.171	5.347	4.326	3.518	3.911
TA234	0.513	3.796	1.049	2.492	1.027	5.045	2.287	2.902	4.276	3.796	3.503	3.639	3.635	3.245	5.019	4.140	3.503	3.796
TA235	0.682	3.877	0.770	2.669	1.065	4.917	1.860	3.137	4.269	3.877	3.490	3.732	3.639	3.376	4.924	4.200	3.490	3.877

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA047																		
TA048	2.132																	
TA050	1.899	0.569																
TA051	1.365	2.984	2.795															
TA053	1.982	0.573	0.257	2.886														
TA056	1.899	0.569	0.000	2.795	0.257													
TA059	1.529	3.262	2.984	0.569	3.079	2.984												
TA060	2.220	0.257	0.672	3.079	0.569	0.672	3.358											
TA061	1.848	0.672	0.257	2.726	0.513	0.257	2.908	0.843										
TA063	0.682	2.093	1.992	1.445	2.119	1.992	1.757	2.229	1.891									
TA067	3.866	2.331	2.611	4.585	2.401	2.611	4.881	2.107	2.829	3.979								
TA069	4.370	2.829	3.178	5.051	2.976	3.178	5.388	2.611	3.388	4.434	0.672							
TA070	4.808	4.103	4.413	5.562	4.209	4.413	5.915	3.891	4.622	4.917	2.713	2.421						
TA071	3.866	2.331	2.611	4.585	2.401	2.611	4.881	2.107	2.829	3.979	0.000	0.672	2.713					
TA072	3.693	2.107	2.401	4.418	2.201	2.401	4.717	1.892	2.611	3.785	0.257	0.843	2.852	0.257				
TA073	2.778	2.593	2.869	3.691	2.768	2.869	4.068	2.493	2.988	2.741	3.027	3.159	2.560	3.027	2.958			
TA074	0.513	2.333	2.093	1.317	2.229	2.093	1.445	2.468	1.982	0.569	4.238	4.743	5.248	4.238	4.048	3.124		
TA075	1.848	0.672	0.257	2.726	0.513	0.257	2.908	0.843	0.000	1.891	2.829	3.388	4.622	2.829	2.611	2.988	1.982	

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA076	0.000	2.132	1.899	1.365	1.982	1.899	1.529	2.220	1.848	0.682	3.866	4.370	4.808	3.866	3.693	2.778	0.513	1.848
TA077	2.132	0.000	0.569	2.984	0.573	0.569	3.262	0.257	0.672	2.093	2.331	2.829	4.103	2.331	2.107	2.593	2.333	0.672
TA078	0.573	1.982	1.891	1.465	1.992	1.891	1.791	2.093	1.821	0.257	3.785	4.238	4.686	3.785	3.598	2.547	0.672	1.821
TA079	0.513	2.333	2.093	1.317	2.229	2.093	1.445	2.468	1.982	0.569	4.238	4.743	5.248	4.238	4.048	3.124	0.000	1.982
TA080	2.132	0.000	0.569	2.984	0.573	0.569	3.262	0.257	0.672	2.093	2.331	2.829	4.103	2.331	2.107	2.593	2.333	0.672
TA082	3.212	2.812	2.660	4.011	2.574	2.660	4.083	2.742	2.766	3.494	3.912	4.311	4.759	3.912	3.843	3.318	3.481	2.766
TA083	2.938	3.159	2.890	3.245	2.857	2.890	3.212	3.139	2.945	3.288	4.490	4.949	5.522	4.490	4.400	3.978	3.149	2.945
TA084	0.672	1.848	1.785	1.631	1.821	1.785	1.960	1.899	1.786	0.770	3.420	3.866	4.233	3.420	3.253	2.197	1.049	1.786
TA086	2.547	2.538	2.713	3.527	2.593	2.713	3.844	2.421	2.852	2.648	2.981	3.197	2.611	2.981	2.923	0.573	2.945	2.852
TA087	2.072	0.257	0.573	2.908	0.682	0.573	3.183	0.513	0.569	1.982	2.560	3.053	4.321	2.560	2.331	2.713	2.220	0.569
TA088	2.093	0.682	0.513	2.997	0.257	0.513	3.192	0.573	0.770	2.269	2.201	2.782	4.011	2.201	2.014	2.688	2.384	0.770
TA089	0.000	2.132	1.899	1.365	1.982	1.899	1.529	2.220	1.848	0.682	3.866	4.370	4.808	3.866	3.693	2.778	0.513	1.848
TA090	3.145	3.010	2.742	3.914	2.696	2.742	3.898	2.978	2.812	3.480	4.311	4.771	5.280	4.311	4.225	3.732	3.362	2.812
TA091	5.666	5.650	5.602	5.324	5.435	5.602	5.405	5.491	5.774	6.041	4.896	5.058	5.533	4.896	4.982	5.976	6.057	5.774
TA092	5.661	5.755	5.650	5.304	5.491	5.650	5.324	5.605	5.816	6.079	5.122	5.343	5.841	5.122	5.198	6.172	6.041	5.816
TA093	4.650	5.117	4.890	4.024	4.809	4.890	3.916	5.046	4.983	5.064	5.269	5.669	6.468	5.269	5.250	6.030	4.910	4.983
TA094	2.132	0.000	0.569	2.984	0.573	0.569	3.262	0.257	0.672	2.093	2.331	2.829	4.103	2.331	2.107	2.593	2.333	0.672
TA095	6.555	6.690	6.790	6.411	6.595	6.790	6.623	6.497	6.988	6.855	5.630	5.535	4.729	5.630	5.771	5.774	7.005	6.988

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA096	3.866	2.331	2.611	4.585	2.401	2.611	4.881	2.107	2.829	3.979	0.000	0.672	2.713	0.000	0.257	3.027	4.238	2.829
TA097	3.693	2.107	2.401	4.418	2.201	2.401	4.717	1.892	2.611	3.785	0.257	0.843	2.852	0.257	0.000	2.958	4.048	2.611
TA098	1.426	2.679	2.619	0.843	2.655	2.619	1.344	2.726	2.607	1.461	4.014	4.418	4.835	4.014	3.865	3.093	1.599	2.607
TA099	4.994	4.209	4.575	5.720	4.387	4.575	6.111	4.011	4.770	5.027	2.869	2.493	0.573	2.869	2.988	2.611	5.406	4.770
TA107	5.666	5.650	5.602	5.324	5.435	5.602	5.405	5.491	5.774	6.041	4.896	5.058	5.533	4.896	4.982	5.976	6.057	5.774
TA108	5.478	5.605	5.491	5.090	5.340	5.491	5.105	5.462	5.650	5.893	5.058	5.300	5.845	5.058	5.122	6.079	5.848	5.650
TA109	4.468	3.750	4.074	4.254	4.101	4.074	4.695	3.788	4.062	4.141	4.742	4.803	5.716	4.742	4.612	4.398	4.519	4.062
TA110	4.838	4.436	4.660	4.317	4.641	4.660	4.700	4.423	4.693	4.678	5.059	5.125	5.936	5.059	4.978	4.982	4.966	4.693
TA111	5.118	4.177	4.575	5.045	4.520	4.575	5.509	4.124	4.643	4.885	4.418	4.293	4.992	4.418	4.362	4.320	5.300	4.643
TA112	6.278	5.768	6.207	6.338	6.126	6.207	6.844	5.686	6.297	6.026	5.687	5.383	4.816	5.687	5.688	4.520	6.505	6.297
TA113	5.044	4.124	4.520	4.950	4.480	4.520	5.417	4.088	4.575	4.787	4.488	4.387	5.124	4.488	4.418	4.337	5.203	4.575
TA114	5.509	5.141	5.554	5.512	5.505	5.554	6.018	5.094	5.615	5.214	5.453	5.260	4.894	5.453	5.412	4.062	5.687	5.615
TA115	5.904	5.446	5.859	5.973	5.768	5.859	6.462	5.353	5.961	5.701	5.362	5.089	4.488	5.362	5.369	4.177	6.157	5.961
TA116	6.505	5.961	6.396	6.593	6.297	6.396	7.089	5.859	6.504	6.295	5.701	5.362	4.684	5.701	5.725	4.643	6.763	6.504
TA117	5.565	5.169	5.550	5.642	5.446	5.550	6.109	5.064	5.663	5.416	5.080	4.845	4.212	5.080	5.095	3.887	5.844	5.663
TA118	5.653	5.553	5.852	5.416	5.753	5.852	5.844	5.454	5.960	5.569	5.531	5.378	4.909	5.531	5.545	4.562	5.930	5.960
TA120	5.848	5.913	5.816	5.521	5.650	5.816	5.547	5.755	5.989	6.270	5.198	5.398	5.848	5.198	5.286	6.274	6.238	5.989
TA121	5.133	5.328	5.197	4.675	5.062	5.197	4.678	5.203	5.340	5.537	4.967	5.250	5.888	4.967	5.006	5.923	5.478	5.340

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA122	3.785	2.399	2.560	4.521	2.331	2.560	4.760	2.166	2.795	3.992	0.573	1.139	2.869	0.573	0.682	3.178	4.181	2.795
TA123	5.105	4.869	4.821	5.083	4.620	4.821	5.175	4.676	5.027	5.521	3.885	4.068	4.549	3.885	4.001	5.149	5.547	5.027
TA153	3.593	3.780	3.393	4.271	3.354	3.393	4.108	3.754	3.451	4.047	4.988	5.503	5.997	4.988	4.915	4.577	3.787	3.451
TA154	4.761	4.425	4.636	4.185	4.646	4.636	4.566	4.442	4.641	4.555	5.254	5.354	6.218	5.254	5.151	5.086	4.838	4.641
TA155	4.519	3.726	4.067	4.352	4.062	4.067	4.797	3.730	4.088	4.244	4.494	4.516	5.382	4.494	4.387	4.244	4.626	4.088
TA156	4.519	3.726	4.067	4.352	4.062	4.067	4.797	3.730	4.088	4.244	4.494	4.516	5.382	4.494	4.387	4.244	4.626	4.088
TA157	4.113	3.459	3.730	3.885	3.750	3.730	4.296	3.490	3.726	3.851	4.516	4.636	5.578	4.516	4.388	4.225	4.185	3.726
TA158	4.141	3.447	3.726	3.940	3.730	3.726	4.352	3.459	3.741	3.907	4.388	4.490	5.410	4.388	4.271	4.147	4.244	3.741
TA159	5.848	5.913	5.816	5.521	5.650	5.816	5.547	5.755	5.989	6.270	5.198	5.398	5.848	5.198	5.286	6.274	6.238	5.989
TA160	5.303	5.462	5.340	4.880	5.197	5.340	4.890	5.328	5.491	5.712	5.006	5.269	5.861	5.006	5.058	5.996	5.661	5.491
TA161	2.593	3.849	3.881	3.184	3.848	3.881	3.434	3.824	3.930	2.674	4.715	4.962	4.048	4.715	4.637	2.132	2.852	3.930
TA162	1.982	0.573	0.257	2.886	0.000	0.257	3.079	0.569	0.513	2.119	2.401	2.976	4.209	2.401	2.201	2.768	2.229	0.513
TA163	3.405	2.145	2.166	4.167	1.940	2.166	4.344	1.932	2.399	3.678	1.139	1.780	3.339	1.139	1.113	3.272	3.789	2.399
TA164	3.420	1.940	2.107	4.174	1.892	2.107	4.418	1.722	2.331	3.593	0.672	1.344	3.125	0.672	0.569	3.042	3.785	2.331
TA165	3.226	1.932	1.940	3.998	1.722	1.940	4.174	1.731	2.166	3.485	1.219	1.884	3.470	1.219	1.139	3.226	3.593	2.166
TA176	0.257	2.072	1.848	1.457	1.899	1.848	1.631	2.132	1.832	0.856	3.693	4.195	4.594	3.693	3.530	2.625	0.770	1.832
TA177	0.682	2.375	2.043	1.603	2.072	2.043	1.587	2.412	2.046	1.364	3.872	4.412	4.796	3.872	3.726	2.974	1.065	2.046
TA180	0.257	2.220	1.982	1.317	2.093	1.982	1.465	2.333	1.899	0.573	4.048	4.553	5.027	4.048	3.866	2.945	0.257	1.899
TA181	0.257	2.220	1.982	1.317	2.093	1.982	1.465	2.333	1.899	0.573	4.048	4.553	5.027	4.048	3.866	2.945	0.257	1.899

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA182	1.465	3.183	2.908	0.573	2.984	2.908	0.257	3.262	2.853	1.760	4.717	5.222	5.720	4.717	4.563	3.932	1.470	2.853
TA187	3.405	2.145	2.166	4.167	1.940	2.166	4.344	1.932	2.399	3.678	1.139	1.780	3.339	1.139	1.113	3.272	3.789	2.399
TA189	3.253	1.722	1.892	4.014	1.689	1.892	4.260	1.516	2.107	3.405	0.843	1.502	3.275	0.843	0.672	3.005	3.598	2.107
TA190	3.420	1.940	2.107	4.174	1.892	2.107	4.418	1.722	2.331	3.593	0.672	1.344	3.125	0.672	0.569	3.042	3.785	2.331
TA192	3.405	2.145	2.166	4.167	1.940	2.166	4.344	1.932	2.399	3.678	1.139	1.780	3.339	1.139	1.113	3.272	3.789	2.399
TA193	3.866	2.331	2.611	4.585	2.401	2.611	4.881	2.107	2.829	3.979	0.000	0.672	2.713	0.000	0.257	3.027	4.238	2.829
TA194	3.866	2.331	2.611	4.585	2.401	2.611	4.881	2.107	2.829	3.979	0.000	0.672	2.713	0.000	0.257	3.027	4.238	2.829
TA195	3.598	2.166	2.331	4.344	2.107	2.331	4.585	1.940	2.560	3.789	0.569	1.219	2.988	0.569	0.573	3.100	3.979	2.560
TA197	3.420	1.940	2.107	4.174	1.892	2.107	4.418	1.722	2.331	3.593	0.672	1.344	3.125	0.672	0.569	3.042	3.785	2.331
TA198	3.785	2.399	2.560	4.521	2.331	2.560	4.760	2.166	2.795	3.992	0.573	1.139	2.869	0.573	0.682	3.178	4.181	2.795
TA199	3.253	1.722	1.892	4.014	1.689	1.892	4.260	1.516	2.107	3.405	0.843	1.502	3.275	0.843	0.672	3.005	3.598	2.107
TA201	4.413	4.584	4.799	4.995	4.640	4.799	5.327	4.424	4.967	4.542	3.930	3.849	1.899	3.930	3.995	2.449	4.836	4.967
TA203	4.448	4.247	4.594	4.798	4.467	4.594	5.244	4.116	4.732	4.381	4.066	3.898	2.749	4.066	4.087	2.354	4.796	4.732
TA204	3.713	2.646	2.910	4.038	2.711	2.910	4.380	2.438	3.118	3.839	1.708	1.794	2.919	1.708	1.747	3.008	4.116	3.118
TA209	3.188	3.529	3.200	3.480	3.159	3.200	3.362	3.501	3.261	3.615	4.758	5.243	5.789	4.758	4.681	4.349	3.404	3.261
TA210	3.145	3.010	2.742	3.914	2.696	2.742	3.898	2.978	2.812	3.480	4.311	4.771	5.280	4.311	4.225	3.732	3.362	2.812
TA211	3.145	3.010	2.742	3.914	2.696	2.742	3.898	2.978	2.812	3.480	4.311	4.771	5.280	4.311	4.225	3.732	3.362	2.812
TA212	3.450	2.656	2.528	4.297	2.340	2.528	4.383	2.490	2.727	3.816	2.980	3.394	4.031	2.980	2.968	3.264	3.826	2.727
TA213	3.063	2.978	2.696	3.823	2.673	2.696	3.798	2.968	2.742	3.376	4.410	4.881	5.427	4.410	4.311	3.783	3.245	2.742

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA047	TA048	TA050	TA051	TA053	TA056	TA059	TA060	TA061	TA063	TA067	TA069	TA070	TA071	TA072	TA073	TA074	TA075
TA214	3.145	3.010	2.742	3.914	2.696	2.742	3.898	2.978	2.812	3.480	4.311	4.771	5.280	4.311	4.225	3.732	3.362	2.812
TA215	3.362	3.137	2.902	4.138	2.812	2.902	4.138	3.064	3.012	3.732	4.153	4.587	5.013	4.153	4.096	3.682	3.639	3.012
TA216	2.869	3.881	3.995	3.434	3.955	3.995	3.755	3.848	4.050	2.852	4.628	4.806	3.785	4.628	4.554	1.899	3.125	4.050
TA217	3.245	3.064	2.812	4.020	2.742	2.812	4.011	3.010	2.902	3.599	4.225	4.673	5.142	4.225	4.153	3.698	3.494	2.902
TA218	3.245	3.064	2.812	4.020	2.742	2.812	4.011	3.010	2.902	3.599	4.225	4.673	5.142	4.225	4.153	3.698	3.494	2.902
TA219	4.514	4.270	4.423	3.907	4.425	4.423	4.244	4.279	4.436	4.380	5.125	5.277	6.154	5.125	5.026	5.014	4.610	4.436
TA220	5.859	5.816	5.774	5.547	5.602	5.774	5.629	5.650	5.953	6.238	4.982	5.122	5.546	4.982	5.079	6.087	6.258	5.953
TA221	0.513	2.333	2.093	1.317	2.229	2.093	1.445	2.468	1.982	0.569	4.238	4.743	5.248	4.238	4.048	3.124	0.000	1.982
TA222	3.693	2.107	2.401	4.418	2.201	2.401	4.717	1.892	2.611	3.785	0.257	0.843	2.852	0.257	0.000	2.958	4.048	2.611
TA223	3.420	1.940	2.107	4.174	1.892	2.107	4.418	1.722	2.331	3.593	0.672	1.344	3.125	0.672	0.569	3.042	3.785	2.331
TA224	3.598	2.166	2.331	4.344	2.107	2.331	4.585	1.940	2.560	3.789	0.569	1.219	2.988	0.569	0.573	3.100	3.979	2.560
TA225	3.693	2.107	2.401	4.418	2.201	2.401	4.717	1.892	2.611	3.785	0.257	0.843	2.852	0.257	0.000	2.958	4.048	2.611
TA226	1.167	1.945	1.718	1.952	1.714	1.718	2.096	1.957	1.761	1.546	3.110	3.658	4.310	3.110	2.950	2.832	1.516	1.761
TA227	5.848	5.913	5.816	5.521	5.650	5.816	5.547	5.755	5.989	6.270	5.198	5.398	5.848	5.198	5.286	6.274	6.238	5.989
TA228	5.893	6.062	5.913	5.555	5.755	5.913	5.521	5.913	6.078	6.353	5.465	5.718	6.188	5.465	5.543	6.506	6.270	6.078
TA229	3.598	2.166	2.331	4.344	2.107	2.331	4.585	1.940	2.560	3.789	0.569	1.219	2.988	0.569	0.573	3.100	3.979	2.560
TA230	3.883	2.711	3.066	4.189	2.890	3.066	4.584	2.521	3.253	3.911	1.804	1.744	2.885	1.804	1.822	2.964	4.253	3.253
TA234	3.481	3.012	2.892	4.276	2.766	2.892	4.357	2.902	3.034	3.796	3.789	4.153	4.504	3.789	3.752	3.321	3.800	3.034
TA235	3.494	3.229	3.012	4.269	2.902	3.012	4.276	3.137	3.139	3.877	4.096	4.514	4.894	4.096	4.054	3.684	3.796	3.139

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA076																		
TA077	2.132																	
TA078	0.573	1.982																
TA079	0.513	2.333	0.672															
TA080	2.132	0.000	1.982	2.333														
TA082	3.212	2.812	3.362	3.481	2.812													
TA083	2.938	3.159	3.188	3.149	3.159	1.529												
TA084	0.672	1.848	0.513	1.049	1.848	3.145	3.044											
TA086	2.547	2.538	2.432	2.945	2.538	3.021	3.660	2.029										
TA087	2.072	0.257	1.899	2.220	0.257	2.902	3.200	1.832	2.674									
TA088	2.093	0.682	2.119	2.384	0.682	2.513	2.847	1.891	2.493	0.856								
TA089	0.000	2.132	0.573	0.513	2.132	3.212	2.938	0.672	2.547	2.072	2.093							
TA090	3.145	3.010	3.376	3.362	3.010	0.672	1.317	3.220	3.394	3.064	2.673	3.145						
TA091	5.666	5.650	5.848	6.057	5.650	5.808	5.179	5.478	5.720	5.816	5.277	5.666	5.988					
TA092	5.661	5.755	5.893	6.041	5.755	5.812	5.105	5.537	5.875	5.913	5.340	5.661	5.932	0.569				
TA093	4.650	5.117	4.939	4.910	5.117	5.370	4.305	4.722	5.718	5.201	4.740	4.650	5.310	2.320	1.996			
TA094	2.132	0.000	1.982	2.333	0.000	2.812	3.159	1.848	2.538	0.257	0.682	2.132	3.010	5.650	5.755	5.117		
TA095	6.555	6.690	6.628	7.005	6.690	6.607	6.366	6.181	5.611	6.887	6.405	6.555	6.960	2.988	3.339	4.845	6.690	

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA096	3.866	2.331	3.785	4.238	2.331	3.912	4.490	3.420	2.981	2.560	2.201	3.866	4.311	4.896	5.122	5.269	2.331	5.630
TA097	3.693	2.107	3.598	4.048	2.107	3.843	4.400	3.253	2.923	2.331	2.014	3.693	4.225	4.982	5.198	5.250	2.107	5.771
TA098	1.426	2.679	1.367	1.599	2.679	3.823	3.220	1.317	2.971	2.656	2.715	1.426	3.866	4.880	4.937	3.935	2.679	5.757
TA099	4.994	4.209	4.808	5.406	4.209	5.013	5.789	4.385	2.782	4.413	4.206	4.994	5.556	5.848	6.188	6.789	4.209	4.982
TA107	5.666	5.650	5.848	6.057	5.650	5.808	5.179	5.478	5.720	5.816	5.277	5.666	5.988	0.000	0.569	2.320	5.650	2.988
TA108	5.478	5.605	5.712	5.848	5.605	5.702	4.954	5.369	5.784	5.755	5.197	5.478	5.808	0.672	0.257	1.751	5.605	3.470
TA109	4.468	3.750	4.113	4.519	3.750	4.516	4.295	4.105	4.635	3.730	4.145	4.468	4.851	6.118	6.390	5.879	3.750	6.843
TA110	4.838	4.436	4.610	4.966	4.436	4.901	4.322	4.514	5.106	4.464	4.636	4.838	5.203	4.986	5.270	4.881	4.436	5.850
TA111	5.118	4.177	4.787	5.300	4.177	4.762	4.776	4.626	4.588	4.244	4.480	5.118	5.243	5.700	6.070	6.047	4.177	6.013
TA112	6.278	5.768	5.904	6.505	5.768	6.041	6.317	5.687	4.907	5.859	6.055	6.278	6.602	6.935	7.364	7.697	5.768	5.953
TA113	5.044	4.124	4.700	5.203	4.124	4.764	4.751	4.566	4.611	4.177	4.454	5.044	5.227	5.822	6.180	6.077	4.124	6.191
TA114	5.509	5.141	5.117	5.687	5.141	5.463	5.630	4.958	4.447	5.200	5.468	5.509	5.971	6.775	7.155	7.224	5.141	6.118
TA115	5.904	5.446	5.565	6.157	5.446	5.593	5.844	5.321	4.520	5.550	5.686	5.904	6.153	6.464	6.879	7.228	5.446	5.500
TA116	6.505	5.961	6.157	6.763	5.961	6.153	6.464	5.904	5.006	6.072	6.207	6.505	6.733	6.833	7.276	7.741	5.961	5.700
TA117	5.565	5.169	5.267	5.844	5.169	5.170	5.391	4.994	4.177	5.285	5.353	5.565	5.726	6.011	6.410	6.776	5.169	5.072
TA118	5.653	5.553	5.423	5.930	5.553	5.496	5.327	5.157	4.740	5.661	5.664	5.653	5.966	5.251	5.644	6.005	5.553	4.408
TA120	5.848	5.913	6.079	6.238	5.913	5.930	5.264	5.712	5.976	6.078	5.491	5.848	6.064	0.573	0.257	2.243	5.913	3.223
TA121	5.133	5.328	5.369	5.478	5.328	5.513	4.680	5.054	5.631	5.462	4.938	5.133	5.588	1.049	0.770	1.274	5.328	3.772

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA122	3.785	2.399	3.789	4.181	2.399	3.745	4.282	3.405	3.027	2.636	2.107	3.785	4.097	4.646	4.823	4.969	2.399	5.517
TA123	5.105	4.869	5.304	5.547	4.869	4.954	4.610	4.880	4.852	5.067	4.425	5.105	5.179	1.317	1.465	2.782	4.869	3.088
TA153	3.593	3.780	3.957	3.787	3.780	1.708	1.691	3.823	4.159	3.824	3.335	3.593	1.113	6.124	5.967	5.237	3.780	7.287
TA154	4.761	4.425	4.514	4.838	4.425	4.982	4.356	4.476	5.222	4.423	4.669	4.761	5.243	5.333	5.588	5.031	4.425	6.271
TA155	4.519	3.726	4.185	4.626	3.726	4.395	4.229	4.113	4.474	3.741	4.074	4.519	4.779	5.795	6.091	5.727	3.726	6.437
TA156	4.519	3.726	4.185	4.626	3.726	4.395	4.229	4.113	4.474	3.741	4.074	4.519	4.779	5.795	6.091	5.727	3.726	6.437
TA157	4.113	3.459	3.812	4.185	3.459	4.085	3.769	3.784	4.398	3.447	3.788	4.113	4.395	5.712	5.953	5.391	3.459	6.569
TA158	4.141	3.447	3.851	4.244	3.447	4.019	3.732	3.789	4.314	3.454	3.750	4.141	4.356	5.541	5.795	5.309	3.447	6.361
TA159	5.848	5.913	6.079	6.238	5.913	5.930	5.264	5.712	5.976	6.078	5.491	5.848	6.064	0.573	0.257	2.243	5.913	3.223
TA160	5.303	5.462	5.537	5.661	5.462	5.602	4.813	5.207	5.702	5.605	5.062	5.303	5.693	0.843	0.513	1.509	5.462	3.615
TA161	2.593	3.849	2.538	2.852	3.849	4.120	4.246	2.327	1.982	3.891	3.833	2.593	4.279	6.281	6.369	5.946	3.849	5.925
TA162	1.982	0.573	1.992	2.229	0.573	2.574	2.857	1.821	2.593	0.682	0.257	1.982	2.696	5.435	5.491	4.809	0.573	6.595
TA163	3.405	2.145	3.485	3.789	2.145	3.488	3.927	3.124	3.042	2.367	1.722	3.405	3.745	4.626	4.729	4.662	2.145	5.736
TA164	3.420	1.940	3.405	3.785	1.940	3.596	4.090	3.058	2.909	2.166	1.689	3.420	3.912	4.824	4.982	4.928	1.940	5.801
TA165	3.226	1.932	3.299	3.593	1.932	3.427	3.840	2.961	3.005	2.145	1.516	3.226	3.662	4.730	4.824	4.654	1.932	5.886
TA176	0.257	2.072	0.682	0.770	2.072	3.101	2.861	0.569	2.365	2.043	1.982	0.257	3.063	5.479	5.478	4.536	2.072	6.333
TA177	0.682	2.375	1.234	1.065	2.375	3.058	2.717	1.113	2.625	2.364	2.132	0.682	2.933	5.353	5.297	4.305	2.375	6.314
TA180	0.257	2.220	0.569	0.257	2.220	3.339	3.035	0.843	2.741	2.132	2.229	0.257	3.245	5.859	5.848	4.775	2.220	6.779
TA181	0.257	2.220	0.569	0.257	2.220	3.339	3.035	0.843	2.741	2.132	2.229	0.257	3.245	5.859	5.848	4.775	2.220	6.779

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA182	1.465	3.183	1.757	1.470	3.183	3.964	3.101	1.860	3.691	3.123	3.079	1.465	3.798	5.183	5.105	3.746	3.183	6.383
TA187	3.405	2.145	3.485	3.789	2.145	3.488	3.927	3.124	3.042	2.367	1.722	3.405	3.745	4.626	4.729	4.662	2.145	5.736
TA189	3.253	1.722	3.226	3.598	1.722	3.547	4.016	2.903	2.882	1.940	1.503	3.253	3.843	4.931	5.079	4.928	1.722	5.955
TA190	3.420	1.940	3.405	3.785	1.940	3.596	4.090	3.058	2.909	2.166	1.689	3.420	3.912	4.824	4.982	4.928	1.940	5.801
TA192	3.405	2.145	3.485	3.789	2.145	3.488	3.927	3.124	3.042	2.367	1.722	3.405	3.745	4.626	4.729	4.662	2.145	5.736
TA193	3.866	2.331	3.785	4.238	2.331	3.912	4.490	3.420	2.981	2.560	2.201	3.866	4.311	4.896	5.122	5.269	2.331	5.630
TA194	3.866	2.331	3.785	4.238	2.331	3.912	4.490	3.420	2.981	2.560	2.201	3.866	4.311	4.896	5.122	5.269	2.331	5.630
TA195	3.598	2.166	3.593	3.979	2.166	3.662	4.179	3.226	2.958	2.399	1.892	3.598	3.997	4.729	4.896	4.942	2.166	5.655
TA197	3.420	1.940	3.405	3.785	1.940	3.596	4.090	3.058	2.909	2.166	1.689	3.420	3.912	4.824	4.982	4.928	1.940	5.801
TA198	3.785	2.399	3.789	4.181	2.399	3.745	4.282	3.405	3.027	2.636	2.107	3.785	4.097	4.646	4.823	4.969	2.399	5.517
TA199	3.253	1.722	3.226	3.598	1.722	3.547	4.016	2.903	2.882	1.940	1.503	3.253	3.843	4.931	5.079	4.928	1.722	5.955
TA201	4.413	4.584	4.321	4.836	4.584	5.084	5.544	3.891	2.429	4.753	4.490	4.413	5.496	5.595	5.847	6.285	4.584	4.380
TA203	4.448	4.247	4.193	4.796	4.247	4.426	4.826	3.840	2.616	4.389	4.350	4.448	4.965	5.617	5.957	6.279	4.247	4.610
TA204	3.713	2.646	3.627	4.116	2.646	3.630	3.814	3.224	2.964	2.863	2.521	3.713	4.075	3.718	4.021	4.273	2.646	4.440
TA209	3.188	3.529	3.514	3.404	3.529	1.791	0.573	3.363	3.978	3.576	3.139	3.188	1.445	5.156	5.024	4.197	3.529	6.436
TA210	3.145	3.010	3.376	3.362	3.010	0.672	1.317	3.220	3.394	3.064	2.673	3.145	0.000	5.988	5.932	5.310	3.010	6.960
TA211	3.145	3.010	3.376	3.362	3.010	0.672	1.317	3.220	3.394	3.064	2.673	3.145	0.000	5.988	5.932	5.310	3.010	6.960
TA212	3.450	2.656	3.632	3.826	2.656	1.328	2.248	3.293	2.910	2.835	2.167	3.450	1.689	5.097	5.118	4.970	2.656	5.979
TA213	3.063	2.978	3.289	3.245	2.978	0.843	1.317	3.171	3.460	3.010	2.675	3.063	0.257	6.124	6.064	5.366	2.978	7.131

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA076	TA077	TA078	TA079	TA080	TA082	TA083	TA084	TA086	TA087	TA088	TA089	TA090	TA091	TA092	TA093	TA094	TA095
TA214	3.145	3.010	3.376	3.362	3.010	0.672	1.317	3.220	3.394	3.064	2.673	3.145	0.000	5.988	5.932	5.310	3.010	6.960
TA215	3.362	3.137	3.599	3.639	3.137	0.573	1.457	3.376	3.318	3.229	2.742	3.362	0.513	5.741	5.693	5.233	3.137	6.634
TA216	2.869	3.881	2.713	3.125	3.881	4.287	4.497	2.493	1.891	3.930	3.932	2.869	4.519	6.369	6.506	6.180	3.881	5.859
TA217	3.245	3.064	3.480	3.494	3.064	0.569	1.365	3.289	3.347	3.137	2.696	3.245	0.257	5.860	5.808	5.265	3.064	6.794
TA218	3.245	3.064	3.480	3.494	3.064	0.569	1.365	3.289	3.347	3.137	2.696	3.245	0.257	5.860	5.808	5.265	3.064	6.794
TA219	4.514	4.270	4.329	4.610	4.270	4.676	3.934	4.273	5.086	4.276	4.442	4.514	4.901	4.938	5.156	4.536	4.270	6.033
TA220	5.859	5.816	6.041	6.258	5.816	5.932	5.341	5.661	5.830	5.989	5.435	5.859	6.124	0.257	0.672	2.549	5.816	2.869
TA221	0.513	2.333	0.672	0.000	2.333	3.481	3.149	1.049	2.945	2.220	2.384	0.513	3.362	6.057	6.041	4.910	2.333	7.005
TA222	3.693	2.107	3.598	4.048	2.107	3.843	4.400	3.253	2.923	2.331	2.014	3.693	4.225	4.982	5.198	5.250	2.107	5.771
TA223	3.420	1.940	3.405	3.785	1.940	3.596	4.090	3.058	2.909	2.166	1.689	3.420	3.912	4.824	4.982	4.928	1.940	5.801
TA224	3.598	2.166	3.593	3.979	2.166	3.662	4.179	3.226	2.958	2.399	1.892	3.598	3.997	4.729	4.896	4.942	2.166	5.655
TA225	3.693	2.107	3.598	4.048	2.107	3.843	4.400	3.253	2.923	2.331	2.014	3.693	4.225	4.982	5.198	5.250	2.107	5.771
TA226	1.167	1.945	1.385	1.516	1.945	3.552	3.411	1.169	2.562	1.967	1.748	1.167	3.555	5.127	5.131	4.286	1.945	6.145
TA227	5.848	5.913	6.079	6.238	5.913	5.930	5.264	5.712	5.976	6.078	5.491	5.848	6.064	0.573	0.257	2.243	5.913	3.223
TA228	5.893	6.062	6.169	6.270	6.062	5.983	5.248	5.818	6.172	6.218	5.605	5.893	6.057	1.113	0.573	2.054	6.062	3.632
TA229	3.598	2.166	3.593	3.979	2.166	3.662	4.179	3.226	2.958	2.399	1.892	3.598	3.997	4.729	4.896	4.942	2.166	5.655
TA230	3.883	2.711	3.713	4.253	2.711	3.889	4.128	3.342	3.029	2.910	2.726	3.883	4.367	4.109	4.451	4.690	2.711	4.652
TA234	3.481	3.012	3.639	3.800	3.012	0.513	1.764	3.362	3.000	3.139	2.660	3.481	1.049	5.588	5.602	5.331	3.012	6.294
TA235	3.494	3.229	3.732	3.796	3.229	0.682	1.587	3.480	3.310	3.339	2.812	3.494	0.770	5.631	5.588	5.213	3.229	6.480

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA096																		
TA097	0.257																	
TA098	4.014	3.865																
TA099	2.869	2.988	4.971															
TA107	4.896	4.982	4.880	5.848														
TA108	5.058	5.122	4.740	6.186	0.672													
TA109	4.742	4.612	3.822	5.578	6.118	6.237												
TA110	5.059	4.978	3.851	5.881	4.986	5.123	1.643											
TA111	4.418	4.362	4.423	4.810	5.700	5.970	1.509	1.860										
TA112	5.687	5.688	5.613	4.488	6.935	7.315	3.772	4.021	2.768									
TA113	4.488	4.418	4.352	4.939	5.822	6.070	1.274	1.791	0.257	2.869								
TA114	5.453	5.412	4.844	4.612	6.775	7.073	2.988	3.404	2.341	1.049	2.365							
TA115	5.362	5.369	5.239	4.212	6.464	6.833	3.613	3.755	2.593	0.573	2.713	1.027						
TA116	5.701	5.725	5.844	4.362	6.833	7.245	4.116	4.252	2.988	0.513	3.125	1.509	0.672					
TA117	5.080	5.095	4.904	4.000	6.011	6.365	3.541	3.563	2.538	1.147	2.674	1.290	0.573	1.139				
TA118	5.531	5.545	4.714	4.800	5.251	5.594	3.616	3.006	2.760	2.183	2.887	2.112	1.760	2.176	1.445			
TA120	5.198	5.286	5.138	6.200	0.573	0.513	6.550	5.426	6.180	7.420	6.298	7.245	6.935	7.315	6.464	5.705		
TA121	4.967	5.006	4.367	6.215	1.049	0.513	5.953	4.856	5.797	7.245	5.879	6.935	6.768	7.211	6.307	5.529	1.027	

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA122	0.573	0.682	3.998	3.123	4.646	4.762	5.024	5.239	4.744	6.049	4.816	5.798	5.687	6.051	5.362	5.731	4.896	4.680
TA123	3.885	4.001	4.592	4.924	1.317	1.529	5.934	5.094	5.465	6.668	5.598	6.523	6.179	6.552	5.705	5.243	1.445	1.764
TA153	4.988	4.915	4.368	6.340	6.124	5.844	5.779	5.962	6.208	7.594	6.195	6.967	7.121	7.708	6.663	6.771	6.097	5.627
TA154	5.254	5.151	3.789	6.154	5.333	5.426	1.470	0.513	2.088	4.252	1.960	3.547	4.019	4.529	3.857	3.352	5.758	5.123
TA155	4.494	4.387	3.847	5.249	5.795	5.953	0.513	1.470	1.049	3.470	0.843	2.768	3.275	3.772	3.174	3.255	6.237	5.700
TA156	4.494	4.387	3.847	5.249	5.795	5.953	0.513	1.470	1.049	3.470	0.843	2.768	3.275	3.772	3.174	3.255	6.237	5.700
TA157	4.516	4.388	3.472	5.497	5.712	5.795	0.573	1.461	1.686	4.007	1.502	3.223	3.772	4.316	3.613	3.593	6.118	5.500
TA158	4.388	4.271	3.486	5.333	5.541	5.643	0.682	1.367	1.502	3.869	1.344	3.123	3.615	4.157	3.439	3.417	5.953	5.365
TA159	5.198	5.286	5.138	6.200	0.573	0.513	6.550	5.426	6.180	7.420	6.298	7.245	6.935	7.315	6.464	5.705	0.000	1.027
TA160	5.006	5.058	4.550	6.196	0.843	0.257	6.091	4.985	5.879	7.276	5.970	7.000	6.796	7.224	6.331	5.556	0.770	0.257
TA161	4.715	4.637	2.824	4.195	6.281	6.254	5.366	5.666	5.565	5.489	5.549	4.897	5.118	5.647	4.787	5.049	6.493	6.050
TA162	2.401	2.201	2.655	4.387	5.435	5.340	4.101	4.641	4.520	6.126	4.480	5.505	5.768	6.297	5.446	5.753	5.650	5.062
TA163	1.139	1.113	3.742	3.632	4.626	4.646	5.106	5.310	5.001	6.433	5.051	6.092	6.051	6.464	5.701	5.994	4.824	4.520
TA164	0.672	0.569	3.690	3.339	4.824	4.896	4.775	5.081	4.638	6.051	4.684	5.720	5.701	6.095	5.389	5.758	5.079	4.762
TA165	1.219	1.139	3.598	3.744	4.730	4.729	4.998	5.244	4.964	6.443	5.001	6.065	6.067	6.494	5.725	6.017	4.931	4.576
TA176	3.693	3.530	1.403	4.796	5.479	5.303	4.465	4.793	5.044	6.177	4.982	5.437	5.791	6.387	5.438	5.528	5.661	4.972
TA177	3.872	3.726	1.691	5.054	5.353	5.122	4.869	5.084	5.427	6.590	5.376	5.870	6.177	6.777	5.791	5.817	5.479	4.794
TA180	4.048	3.866	1.493	5.198	5.859	5.661	4.486	4.896	5.203	6.387	5.118	5.593	6.026	6.630	5.701	5.787	6.041	5.303
TA181	4.048	3.866	1.493	5.198	5.859	5.661	4.486	4.896	5.203	6.387	5.118	5.593	6.026	6.630	5.701	5.787	6.041	5.303

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA182	4.717	4.563	1.219	5.929	5.183	4.890	4.664	4.626	5.417	6.733	5.335	5.930	6.338	6.963	5.973	5.701	5.324	4.472
TA187	1.139	1.113	3.742	3.632	4.626	4.646	5.106	5.310	5.001	6.433	5.051	6.092	6.051	6.464	5.701	5.994	4.824	4.520
TA189	0.843	0.672	3.554	3.470	4.931	4.982	4.667	5.020	4.605	6.067	4.638	5.698	5.725	6.133	5.421	5.788	5.188	4.823
TA190	0.672	0.569	3.690	3.339	4.824	4.896	4.775	5.081	4.638	6.051	4.684	5.720	5.701	6.095	5.389	5.758	5.079	4.762
TA192	1.139	1.113	3.742	3.632	4.626	4.646	5.106	5.310	5.001	6.433	5.051	6.092	6.051	6.464	5.701	5.994	4.824	4.520
TA193	0.000	0.257	4.014	2.869	4.896	5.058	4.742	5.059	4.418	5.687	4.488	5.453	5.362	5.701	5.080	5.531	5.198	4.967
TA194	0.000	0.257	4.014	2.869	4.896	5.058	4.742	5.059	4.418	5.687	4.488	5.453	5.362	5.701	5.080	5.531	5.198	4.967
TA195	0.569	0.573	3.839	3.223	4.729	4.823	4.894	5.154	4.684	6.045	4.744	5.753	5.688	6.067	5.369	5.739	4.982	4.714
TA197	0.672	0.569	3.690	3.339	4.824	4.896	4.775	5.081	4.638	6.051	4.684	5.720	5.701	6.095	5.389	5.758	5.079	4.762
TA198	0.573	0.682	3.998	3.123	4.646	4.762	5.024	5.239	4.744	6.049	4.816	5.798	5.687	6.051	5.362	5.731	4.896	4.680
TA199	0.843	0.672	3.554	3.470	4.931	4.982	4.667	5.020	4.605	6.067	4.638	5.698	5.725	6.133	5.421	5.788	5.188	4.823
TA201	3.930	3.995	4.302	2.072	5.595	5.830	6.000	6.073	5.490	4.903	5.587	4.839	4.541	4.826	4.227	4.667	5.876	5.829
TA203	4.066	4.087	4.028	2.617	5.617	5.911	4.101	4.272	3.413	2.582	3.518	2.461	2.156	2.571	1.813	2.589	6.014	5.851
TA204	1.708	1.747	3.344	3.063	3.718	3.947	3.737	3.640	3.251	4.658	3.356	4.403	4.256	4.660	3.897	4.043	4.109	3.848
TA209	4.758	4.681	3.520	6.099	5.156	4.878	4.833	4.764	5.287	6.805	5.271	6.142	6.317	6.932	5.844	5.726	5.179	4.615
TA210	4.311	4.225	3.866	5.556	5.988	5.808	4.851	5.203	5.243	6.602	5.227	5.971	6.153	6.733	5.726	5.966	6.064	5.588
TA211	4.311	4.225	3.866	5.556	5.988	5.808	4.851	5.203	5.243	6.602	5.227	5.971	6.153	6.733	5.726	5.966	6.064	5.588
TA212	2.980	2.968	3.985	4.362	5.097	5.040	4.884	5.107	4.860	6.183	4.910	5.758	5.720	6.218	5.279	5.600	5.207	4.921
TA213	4.410	4.311	3.817	5.690	6.124	5.932	4.809	5.202	5.273	6.663	5.243	6.000	6.224	6.812	5.809	6.046	6.204	5.693

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA096	TA097	TA098	TA099	TA107	TA108	TA109	TA110	TA111	TA112	TA113	TA114	TA115	TA116	TA117	TA118	TA120	TA121
TA214	4.311	4.225	3.866	5.556	5.988	5.808	4.851	5.203	5.243	6.602	5.227	5.971	6.153	6.733	5.726	5.966	6.064	5.588
TA215	4.153	4.096	4.012	5.317	5.741	5.588	4.976	5.243	5.222	6.508	5.231	5.944	6.041	6.602	5.593	5.837	5.808	5.407
TA216	4.628	4.554	2.977	3.866	6.369	6.399	5.180	5.534	5.296	5.044	5.285	4.486	4.700	5.203	4.404	4.760	6.623	6.209
TA217	4.225	4.153	3.931	5.432	5.860	5.693	4.907	5.216	5.227	6.550	5.222	5.952	6.092	6.663	5.654	5.897	5.932	5.492
TA218	4.225	4.153	3.931	5.432	5.860	5.693	4.907	5.216	5.227	6.550	5.222	5.952	6.092	6.663	5.654	5.897	5.932	5.492
TA219	5.125	5.026	3.544	6.142	4.938	4.986	1.800	0.672	2.382	4.545	2.287	3.846	4.252	4.791	4.019	3.439	5.333	4.670
TA220	4.982	5.079	5.090	5.866	0.257	0.843	6.290	5.156	5.822	7.000	5.953	6.874	6.528	6.879	6.074	5.322	0.569	1.274
TA221	4.238	4.048	1.599	5.406	6.057	5.848	4.519	4.966	5.300	6.505	5.203	5.687	6.157	6.763	5.844	5.930	6.238	5.478
TA222	0.257	0.000	3.865	2.988	4.982	5.122	4.612	4.978	4.362	5.688	4.418	5.412	5.369	5.725	5.095	5.545	5.286	5.006
TA223	0.672	0.569	3.690	3.339	4.824	4.896	4.775	5.081	4.638	6.051	4.684	5.720	5.701	6.095	5.389	5.758	5.079	4.762
TA224	0.569	0.573	3.839	3.223	4.729	4.823	4.894	5.154	4.684	6.045	4.744	5.753	5.688	6.067	5.369	5.739	4.982	4.714
TA225	0.257	0.000	3.865	2.988	4.982	5.122	4.612	4.978	4.362	5.688	4.418	5.412	5.369	5.725	5.095	5.545	5.286	5.006
TA226	3.110	2.950	1.789	4.541	5.127	4.963	4.826	5.074	5.266	6.499	5.226	5.857	6.121	6.668	5.774	5.861	5.305	4.652
TA227	5.198	5.286	5.138	6.200	0.573	0.513	6.550	5.426	6.180	7.420	6.298	7.245	6.935	7.315	6.464	5.705	0.000	1.027
TA228	5.465	5.543	5.248	6.567	1.113	0.682	6.848	5.739	6.568	7.860	6.675	7.640	7.364	7.769	6.879	6.117	0.569	1.065
TA229	0.569	0.573	3.839	3.223	4.729	4.823	4.894	5.154	4.684	6.045	4.744	5.753	5.688	6.067	5.369	5.739	4.982	4.714
TA230	1.804	1.822	3.461	2.919	4.109	4.376	3.447	3.472	2.873	4.256	2.979	4.022	3.897	4.275	3.594	3.842	4.538	4.271
TA234	3.789	3.752	4.020	4.786	5.588	5.513	4.691	4.982	4.779	5.971	4.809	5.470	5.505	6.041	5.060	5.392	5.702	5.366
TA235	4.096	4.054	4.108	5.212	5.631	5.492	5.056	5.281	5.231	6.476	5.252	5.948	6.000	6.550	5.543	5.789	5.693	5.333

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA122																		
TA123	3.568																	
TA153	4.673	5.346																
TA154	5.441	5.445	5.999															
TA155	4.775	5.588	5.717	1.465														
TA156	4.775	5.588	5.717	1.465	0.000													
TA157	4.742	5.516	5.285	1.317	0.672	0.672												
TA158	4.612	5.333	5.252	1.317	0.569	0.569	0.257											
TA159	4.896	1.445	6.097	5.758	6.237	6.237	6.118	5.953										
TA160	4.714	1.631	5.731	5.270	5.822	5.822	5.643	5.500	0.770									
TA161	4.714	5.694	4.809	5.702	5.301	5.301	5.134	5.101	6.493	6.147								
TA162	2.331	4.620	3.354	4.646	4.062	4.062	3.750	3.730	5.650	5.197	3.848							
TA163	0.672	3.563	4.225	5.474	4.901	4.901	4.775	4.667	4.824	4.576	4.609	1.940						
TA164	0.513	3.814	4.514	5.239	4.571	4.571	4.494	4.387	5.079	4.823	4.554	1.892	0.573					
TA165	0.843	3.705	4.153	5.386	4.816	4.816	4.667	4.571	4.931	4.646	4.542	1.722	0.257	0.569				
TA176	3.598	4.890	3.520	4.744	4.486	4.486	4.101	4.113	5.661	5.133	2.493	1.899	3.226	3.253	3.058			
TA177	3.693	4.751	3.220	5.052	4.874	4.874	4.465	4.468	5.479	4.954	2.688	2.072	3.253	3.379	3.098	0.573		
TA180	3.979	5.324	3.682	4.793	4.566	4.566	4.141	4.185	6.041	5.478	2.713	2.093	3.593	3.598	3.405	0.513	0.856	
TA181	3.979	5.324	3.682	4.793	4.566	4.566	4.141	4.185	6.041	5.478	2.713	2.093	3.593	3.598	3.405	0.513	0.856	0.000

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA182	4.585	4.937	4.012	4.519	4.739	4.739	4.254	4.296	5.324	4.678	3.321	2.984	4.174	4.260	4.014	1.529	1.457	1.445
TA187	0.672	3.563	4.225	5.474	4.901	4.901	4.775	4.667	4.824	4.576	4.609	1.940	0.000	0.573	0.257	3.226	3.253	3.593
TA189	0.770	3.956	4.455	5.154	4.488	4.488	4.387	4.293	5.188	4.896	4.494	1.689	0.682	0.257	0.573	3.098	3.241	3.420
TA190	0.513	3.814	4.514	5.239	4.571	4.571	4.494	4.387	5.079	4.823	4.554	1.892	0.573	0.000	0.569	3.253	3.379	3.598
TA192	0.672	3.563	4.225	5.474	4.901	4.901	4.775	4.667	4.824	4.576	4.609	1.940	0.000	0.573	0.257	3.226	3.253	3.593
TA193	0.573	3.885	4.988	5.254	4.494	4.494	4.516	4.388	5.198	5.006	4.715	2.401	1.139	0.672	1.219	3.693	3.872	4.048
TA194	0.573	3.885	4.988	5.254	4.494	4.494	4.516	4.388	5.198	5.006	4.715	2.401	1.139	0.672	1.219	3.693	3.872	4.048
TA195	0.257	3.684	4.587	5.335	4.667	4.667	4.612	4.494	4.982	4.762	4.628	2.107	0.569	0.257	0.672	3.420	3.530	3.785
TA197	0.513	3.814	4.514	5.239	4.571	4.571	4.494	4.387	5.079	4.823	4.554	1.892	0.573	0.000	0.569	3.253	3.379	3.598
TA198	0.000	3.568	4.673	5.441	4.775	4.775	4.742	4.612	4.896	4.714	4.714	2.331	0.672	0.513	0.843	3.598	3.693	3.979
TA199	0.770	3.956	4.455	5.154	4.488	4.488	4.387	4.293	5.188	4.896	4.494	1.689	0.682	0.257	0.573	3.098	3.241	3.420
TA201	3.995	4.844	6.109	6.309	5.727	5.727	5.838	5.700	5.876	5.824	2.829	4.640	4.243	4.121	4.318	4.209	4.387	4.622
TA203	4.294	5.060	5.849	4.525	3.784	3.784	4.038	3.880	6.014	5.875	3.292	4.467	4.603	4.332	4.635	4.286	4.615	4.618
TA204	1.804	2.928	4.795	3.924	3.396	3.396	3.447	3.267	4.109	3.890	4.403	2.711	2.091	1.875	2.152	3.523	3.711	3.911
TA209	4.490	4.587	1.403	4.809	4.759	4.759	4.295	4.255	5.179	4.741	4.491	3.159	4.090	4.324	4.016	3.106	2.861	3.288
TA210	4.097	5.179	1.113	5.243	4.779	4.779	4.395	4.356	6.064	5.693	4.279	2.696	3.745	3.912	3.662	3.063	2.933	3.245
TA211	4.097	5.179	1.113	5.243	4.779	4.779	4.395	4.356	6.064	5.693	4.279	2.696	3.745	3.912	3.662	3.063	2.933	3.245
TA212	2.702	4.059	2.278	5.275	4.673	4.673	4.455	4.341	5.207	4.974	4.365	2.340	2.466	2.673	2.476	3.276	3.190	3.634
TA213	4.209	5.341	1.147	5.216	4.764	4.764	4.356	4.331	6.204	5.808	4.286	2.673	3.842	3.997	3.745	3.001	2.881	3.145

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA122	TA123	TA153	TA154	TA155	TA156	TA157	TA158	TA159	TA160	TA161	TA162	TA163	TA164	TA165	TA176	TA177	TA180
TA214	4.097	5.179	1.113	5.243	4.779	4.779	4.395	4.356	6.064	5.693	4.279	2.696	3.745	3.912	3.662	3.063	2.933	3.245
TA215	3.912	4.878	1.219	5.332	4.851	4.851	4.516	4.448	5.808	5.492	4.310	2.812	3.596	3.789	3.547	3.245	3.101	3.494
TA216	4.689	5.784	5.145	5.583	5.101	5.101	4.998	4.958	6.623	6.299	0.569	3.955	4.661	4.542	4.601	2.768	3.043	2.988
TA217	3.997	5.024	1.139	5.281	4.809	4.809	4.448	4.395	5.932	5.588	4.287	2.742	3.662	3.843	3.596	3.145	3.007	3.362
TA218	3.997	5.024	1.139	5.281	4.809	4.809	4.448	4.395	5.932	5.588	4.287	2.742	3.662	3.843	3.596	3.145	3.007	3.362
TA219	5.254	5.061	5.592	0.573	1.757	1.757	1.470	1.445	5.333	4.824	5.553	4.425	5.239	5.059	5.154	4.488	4.744	4.555
TA220	4.729	1.317	6.256	5.516	5.953	5.953	5.889	5.712	0.569	1.049	6.411	5.602	4.730	4.931	4.845	5.666	5.538	6.057
TA221	4.181	5.547	3.787	4.838	4.626	4.626	4.185	4.244	6.238	5.661	2.852	2.229	3.789	3.785	3.593	0.770	1.065	0.257
TA222	0.682	4.001	4.915	5.151	4.387	4.387	4.388	4.271	5.286	5.058	4.637	2.201	1.113	0.569	1.139	3.530	3.726	3.866
TA223	0.513	3.814	4.514	5.239	4.571	4.571	4.494	4.387	5.079	4.823	4.554	1.892	0.573	0.000	0.569	3.253	3.379	3.598
TA224	0.257	3.684	4.587	5.335	4.667	4.667	4.612	4.494	4.982	4.762	4.628	2.107	0.569	0.257	0.672	3.420	3.530	3.785
TA225	0.682	4.001	4.915	5.151	4.387	4.387	4.388	4.271	5.286	5.058	4.637	2.201	1.113	0.569	1.139	3.530	3.726	3.866
TA226	2.970	4.471	3.942	5.068	4.804	4.804	4.474	4.463	5.305	4.803	3.025	1.714	2.556	2.621	2.384	1.044	1.123	1.328
TA227	4.896	1.445	6.097	5.758	6.237	6.237	6.118	5.953	0.000	0.770	6.493	5.650	4.824	5.079	4.931	5.661	5.479	6.041
TA228	5.122	1.757	5.988	6.044	6.559	6.559	6.390	6.237	0.569	0.856	6.623	5.755	4.982	5.286	5.079	5.712	5.478	6.079
TA229	0.257	3.684	4.587	5.335	4.667	4.667	4.612	4.494	4.982	4.762	4.628	2.107	0.569	0.257	0.672	3.420	3.530	3.785
TA230	2.062	3.407	5.167	3.751	3.097	3.097	3.234	3.052	4.538	4.316	4.480	2.890	2.426	2.091	2.464	3.711	3.974	4.064
TA234	3.596	4.680	1.884	5.114	4.516	4.516	4.261	4.166	5.702	5.434	4.198	2.766	3.385	3.517	3.362	3.339	3.278	3.635
TA235	3.843	4.741	1.344	5.394	4.907	4.907	4.597	4.516	5.693	5.407	4.348	2.902	3.547	3.752	3.517	3.362	3.212	3.639

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA181	TA182	TA187	TA189	TA190	TA192	TA193	TA194	TA195	TA197	TA198	TA199	TA201	TA203	TA204	TA209	TA210	TA211	TA212
TA182	1.445																		
TA187	3.593	4.174																	
TA189	3.420	4.111	0.682																
TA190	3.598	4.260	0.573	0.257															
TA192	3.593	4.174	0.000	0.682	0.573														
TA193	4.048	4.717	1.139	0.843	0.672	1.139													
TA194	4.048	4.717	1.139	0.843	0.672	1.139	0.000												
TA195	3.785	4.418	0.569	0.513	0.257	0.569	0.569	0.569											
TA197	3.598	4.260	0.573	0.257	0.000	0.573	0.672	0.672	0.257										
TA198	3.979	4.585	0.672	0.770	0.513	0.672	0.573	0.573	0.257	0.513									
TA199	3.420	4.111	0.682	0.000	0.257	0.682	0.843	0.843	0.513	0.257	0.770								
TA201	4.622	5.134	4.243	4.206	4.121	4.243	3.930	3.930	4.050	4.121	3.995	4.206							
TA203	4.618	5.082	4.603	4.374	4.332	4.603	4.066	4.066	4.305	4.332	4.294	4.374	2.488						
TA204	3.911	4.189	2.091	1.961	1.875	2.091	1.708	1.708	1.822	1.875	1.804	1.961	3.715	3.196					
TA209	3.288	3.245	4.090	4.262	4.324	4.090	4.758	4.758	4.400	4.324	4.490	4.262	5.779	5.230	4.128				
TA210	3.245	3.798	3.745	3.843	3.912	3.745	4.311	4.311	3.997	3.912	4.097	3.843	5.496	4.965	4.075	1.445			
TA211	3.245	3.798	3.745	3.843	3.912	3.745	4.311	4.311	3.997	3.912	4.097	3.843	5.496	4.965	4.075	1.445	0.000		
TA212	3.634	4.217	2.466	2.696	2.673	2.466	2.980	2.980	2.675	2.673	2.702	2.696	4.660	4.380	2.908	2.372	1.689	1.689	
TA213	3.145	3.714	3.842	3.912	3.997	3.842	4.410	4.410	4.097	3.997	4.209	3.912	5.614	5.062	4.188	1.470	0.257	0.257	1.892

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA181	TA182	TA187	TA189	TA190	TA192	TA193	TA194	TA195	TA197	TA198	TA199	TA201	TA203	TA204	TA209	TA210	TA211	TA212
TA214	3.245	3.798	3.745	3.843	3.912	3.745	4.311	4.311	3.997	3.912	4.097	3.843	5.496	4.965	4.075	1.445	0.000	0.000	1.689
TA215	3.494	4.011	3.596	3.752	3.789	3.596	4.153	4.153	3.843	3.789	3.912	3.752	5.289	4.807	3.889	1.529	0.513	0.513	1.339
TA216	2.988	3.644	4.661	4.489	4.542	4.661	4.628	4.628	4.609	4.542	4.689	4.489	2.560	2.887	4.300	4.791	4.519	4.519	4.504
TA217	3.362	3.898	3.662	3.789	3.843	3.662	4.225	4.225	3.912	3.843	3.997	3.789	5.387	4.880	3.975	1.465	0.257	0.257	1.503
TA218	3.362	3.898	3.662	3.789	3.843	3.662	4.225	4.225	3.912	3.843	3.997	3.789	5.387	4.880	3.975	1.465	0.257	0.257	1.503
TA219	4.555	4.185	5.239	4.978	5.059	5.239	5.125	5.125	5.151	5.059	5.254	4.978	6.216	4.551	3.751	4.356	4.901	4.901	4.957
TA220	6.057	5.405	4.730	5.048	4.931	4.730	4.982	4.982	4.824	4.931	4.729	5.048	5.630	5.682	3.822	5.313	6.124	6.124	5.193
TA221	0.257	1.470	3.789	3.598	3.785	3.789	4.238	4.238	3.979	3.785	4.181	3.598	4.836	4.796	4.116	3.404	3.362	3.362	3.826
TA222	3.866	4.563	1.113	0.672	0.569	1.113	0.257	0.257	0.573	0.569	0.682	0.672	3.995	4.087	1.747	4.681	4.225	4.225	2.968
TA223	3.598	4.260	0.573	0.257	0.000	0.573	0.672	0.672	0.257	0.000	0.513	0.257	4.121	4.332	1.875	4.324	3.912	3.912	2.673
TA224	3.785	4.418	0.569	0.513	0.257	0.569	0.569	0.569	0.000	0.257	0.257	0.513	4.050	4.305	1.822	4.400	3.997	3.997	2.675
TA225	3.866	4.563	1.113	0.672	0.569	1.113	0.257	0.257	0.573	0.569	0.682	0.672	3.995	4.087	1.747	4.681	4.225	4.225	2.968
TA226	1.328	1.966	2.556	2.468	2.621	2.556	3.110	3.110	2.789	2.621	2.970	2.468	4.116	4.542	3.207	3.596	3.555	3.555	3.339
TA227	6.041	5.324	4.824	5.188	5.079	4.824	5.198	5.198	4.982	5.079	4.896	5.188	5.876	6.014	4.109	5.179	6.064	6.064	5.207
TA228	6.079	5.304	4.982	5.384	5.286	4.982	5.465	5.465	5.198	5.286	5.122	5.384	6.165	6.379	4.451	5.105	6.057	6.057	5.283
TA229	3.785	4.418	0.569	0.513	0.257	0.569	0.569	0.569	0.000	0.257	0.257	0.513	4.050	4.305	1.822	4.400	3.997	3.997	2.675
TA230	4.064	4.410	2.426	2.152	2.091	2.426	1.804	1.804	2.061	2.091	2.062	2.152	3.736	2.996	0.573	4.494	4.367	4.367	3.272
TA234	3.635	4.214	3.385	3.505	3.517	3.385	3.789	3.789	3.547	3.517	3.596	3.505	4.899	4.293	3.477	1.960	1.049	1.049	1.044
TA235	3.639	4.138	3.547	3.733	3.752	3.547	4.096	4.096	3.789	3.752	3.843	3.733	5.201	4.747	3.820	1.631	0.770	0.770	1.209

Table S5.4 continued - Pairwise morphological distance between *Mugil* individuals from the Tropical Southwestern Atlantic marine province, calculated through Euclidian distance using the scores of multiple factor analysis.

	TA213	TA214	TA215	TA216	TA217	TA218	TA219	TA220	TA221	TA222	TA223	TA224	TA225	TA226	TA227	TA228	TA229	TA230	TA234
TA214	0.257																		
TA215	0.770	0.513																	
TA216	4.533	4.519	4.534																
TA217	0.513	0.257	0.257	4.519															
TA218	0.513	0.257	0.257	4.519	0.000														
TA219	4.880	4.901	4.982	5.484	4.935	4.935													
TA220	6.268	6.124	5.860	6.493	5.988	5.988	5.128												
TA221	3.245	3.362	3.639	3.125	3.494	3.494	4.610	6.258											
TA222	4.311	4.225	4.096	4.554	4.153	4.153	5.026	5.079	4.048										
TA223	3.997	3.912	3.789	4.542	3.843	3.843	5.059	4.931	3.785	0.569									
TA224	4.097	3.997	3.843	4.609	3.912	3.912	5.151	4.824	3.979	0.573	0.257								
TA225	4.311	4.225	4.096	4.554	4.153	4.153	5.026	5.079	4.048	0.000	0.569	0.573							
TA226	3.531	3.555	3.658	3.248	3.598	3.598	4.813	5.307	1.516	2.950	2.621	2.789	2.950						
TA227	6.204	6.064	5.808	6.623	5.932	5.932	5.333	0.569	6.238	5.286	5.079	4.982	5.286	5.305					
TA228	6.193	6.057	5.812	6.798	5.930	5.930	5.588	1.139	6.270	5.543	5.286	5.198	5.543	5.364	0.569				
TA229	4.097	3.997	3.843	4.609	3.912	3.912	5.151	4.824	3.979	0.573	0.257	0.000	0.573	2.789	4.982	5.198			
TA230	4.465	4.367	4.212	4.311	4.282	4.282	3.660	4.211	4.253	1.822	2.091	2.061	1.822	3.436	4.538	4.910	2.061		
TA234	1.274	1.049	0.672	4.348	0.843	0.843	4.802	5.693	3.800	3.752	3.517	3.547	3.752	3.707	5.702	5.768	3.547	3.766	
TA235	1.027	0.770	0.257	4.564	0.513	0.513	5.042	5.741	3.796	4.054	3.752	3.789	4.054	3.735	5.693	5.702	3.789	4.156	0.569

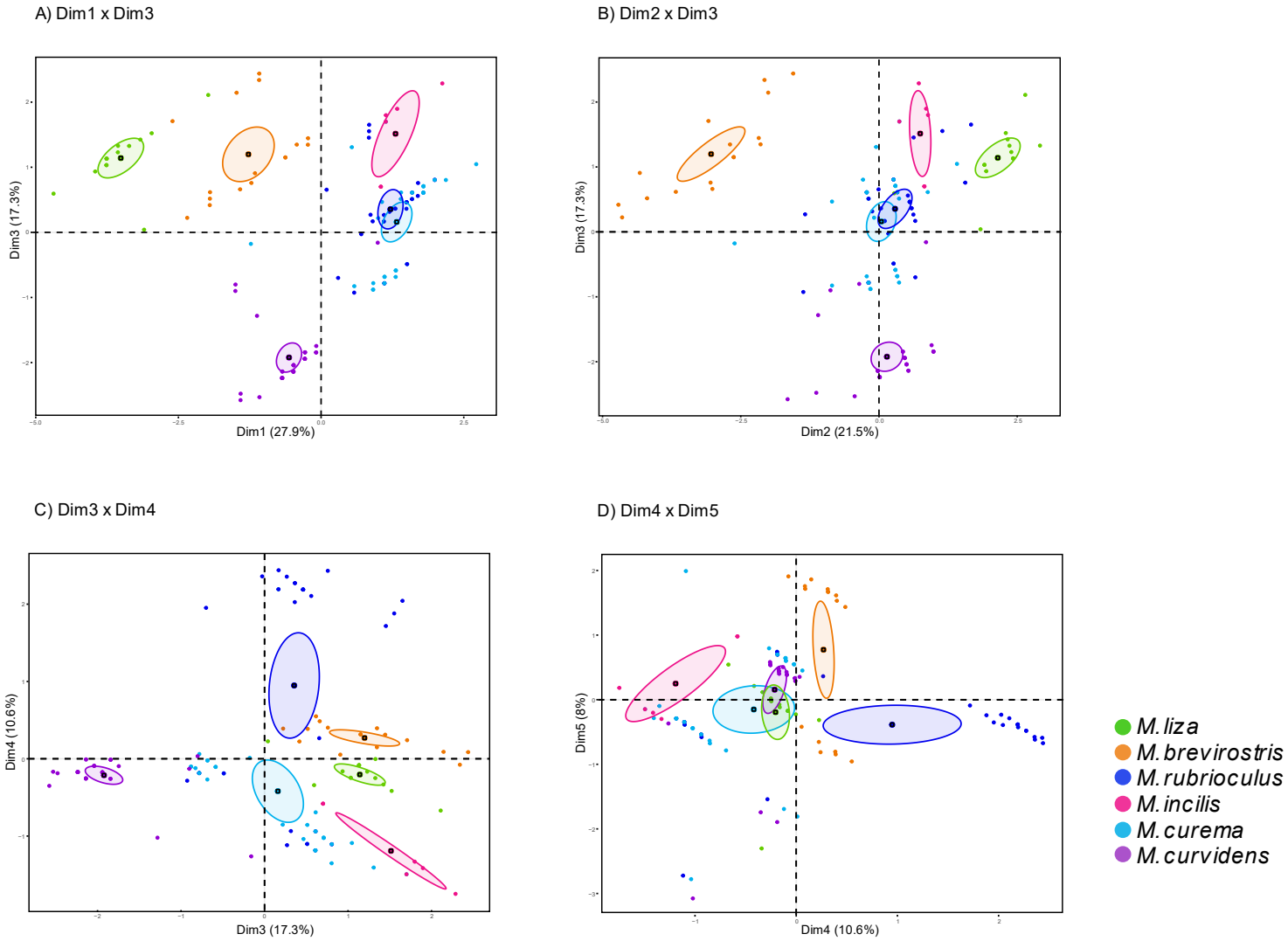

Figure S5.1 - Plot of dimensions 1x3, 2x3, 3x4 and 4x5 of the Multiple factor analysis (MFA). Ellipses represent 95% confidence level.

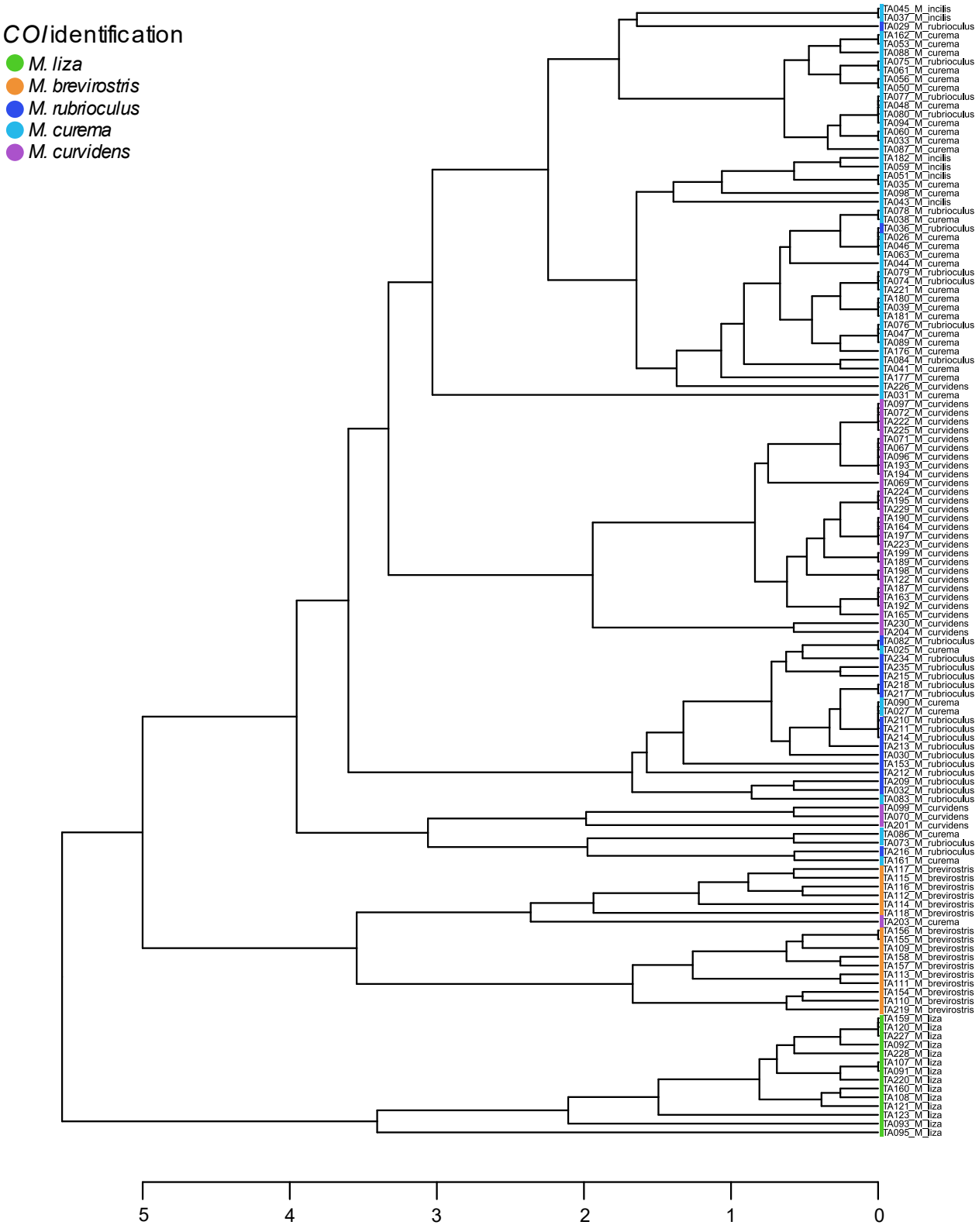

Figure S5.2 - Dendrogram based on Euclidian morphological distance matrix (Table S5.3) calculated using the scores of MFA dimensions. Labels are based on morphological identification and the colors are based on COI identification.

Table S6.1 - Information of *Mugil* individuals from Tropical Southwestern Atlantic marine province.

Voucher	ID	Morphologic ID	COI ID	COI Genbank access number	Locality
MUFAL2166	TA025	*Mugil curema*	*Mugil curema*	MK837973	Santo Antonio
MUFAL2170	TA029	*Mugil rubrioculus*	*Mugil rubrioculus*	MK837976	Santo Antonio
MUFAL2171	TA030	*Mugil rubrioculus*	*Mugil rubrioculus*	MK837977	Santo Antonio
MUFAL2173	TA032	*Mugil rubrioculus*	*Mugil rubrioculus*	MK837979	Santo Antonio
MUFAL2176	TA035	*Mugil curema*	*Mugil curema*	MK837981	Santo Antonio
MUFAL2177	TA036	*Mugil rubrioculus*	*Mugil rubrioculus*	MK837982	Santo Antonio
MUFAL2185	TA044	*Mugil curema*	*Mugil curema*	MK837988	Santo Antonio
MUFAL2186	TA045	*Mugil incilis*	*Mugil curema*	MK837989	Santo Antonio
MUFAL2189	TA048	*Mugil curema*	*Mugil curema*	MK837992	Santo Antonio
MUFAL2197	TA056	*Mugil curema*	*Mugil curema*	MK837996	Santo Antonio
MUFAL2201	TA060	*Mugil curema*	*Mugil curema*	MK837998	Santo Antonio
MUFAL2202	TA061	*Mugil curema*	*Mugil curema*	MK837999	Santo Antonio
MUFAL2208	TA067	*Mugil curvidens*	*Mugil curvidens*	MK838001	Santo Antonio
MUFAL2210	TA069	*Mugil curvidens*	*Mugil curvidens*	MK838002	Santo Antonio
MUFAL2211	TA070	*Mugil curvidens*	*Mugil curvidens*	MK838003	Santo Antonio
MUFAL2212	TA071	*Mugil curvidens*	*Mugil curvidens*	MK838004	Santo Antonio
MUFAL2213	TA072	*Mugil curvidens*	*Mugil curvidens*	MK838005	Santo Antonio
MUFAL2215	TA074	*Mugil rubrioculus*	*Mugil curema*	MK838007	Santo Antonio
MUFAL2216	TA075	*Mugil rubrioculus*	*Mugil curema*	MK838008	Santo Antonio
MUFAL2218	TA077	*Mugil rubrioculus*	*Mugil curema*	MK838010	Santo Antonio
MUFAL2223	TA082	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838014	Santo Antonio
MUFAL2225	TA084	*Mugil rubrioculus*	*Mugil curema*	MK838016	Santo Antonio
MUFAL2227	TA086	*Mugil curema*	*Mugil curema*	MK838017	Santo Antonio
MUFAL2228	TA087	*Mugil curema*	*Mugil curema*	MK838018	Santo Antonio
MUFAL2231	TA090	*Mugil curema*	*Mugil curema*	MK838021	Santo Antonio
MUFAL2232	TA091	*Mugil liza*	*Mugil liza*	MK838022	Santo Antonio
MUFAL2233	TA092	*Mugil liza*	*Mugil liza*	MK838023	Santo Antonio
MUFAL2234	TA093	*Mugil liza*	*Mugil liza*	MK838024	Santo Antonio
MUFAL2236	TA095	*Mugil liza*	*Mugil liza*	MK838026	Santo Antonio
MUFAL2237	TA096	*Mugil curvidens*	*Mugil curvidens*	MK838027	Santo Antonio
MUFAL2238	TA097	*Mugil curvidens*	*Mugil curvidens*	MK838028	Santo Antonio
MUFAL2239	TA098	*Mugil curema*	*Mugil curema*	MK838029	Santo Antonio
MUFAL2240	TA099	*Mugil curvidens*	*Mugil curvidens*	MK838030	Santo Antonio
MUFAL2248	TA107	*Mugil liza*	*Mugil liza*	MK838031	Santo Antonio
MUFAL2249	TA108	*Mugil liza*	*Mugil liza*	MK838032	Santo Antonio
MUFAL2250	TA109	*Mugil brevirostris*	*Mugil brevirostris*	MK838033	Santo Antonio
MUFAL2251	TA110	*Mugil brevirostris*	*Mugil brevirostris*	MK838034	Santo Antonio
MUFAL2252	TA111	*Mugil brevirostris*	*Mugil brevirostris*	MK838035	Santo Antonio
MUFAL2253	TA112	*Mugil brevirostris*	*Mugil brevirostris*	MK838036	Santo Antonio
MUFAL2254	TA113	*Mugil brevirostris*	*Mugil brevirostris*	MK838037	Santo Antonio
MUFAL2255	TA114	*Mugil brevirostris*	*Mugil brevirostris*	MK838038	Santo Antonio
MUFAL2256	TA115	*Mugil brevirostris*	*Mugil brevirostris*	MK838039	Santo Antonio
MUFAL2257	TA116	*Mugil brevirostris*	*Mugil brevirostris*	MK838040	Santo Antonio
MUFAL2258	TA117	*Mugil brevirostris*	*Mugil brevirostris*	MK838041	Santo Antonio
MUFAL2259	TA118	*Mugil brevirostris*	*Mugil brevirostris*	MK838042	Santo Antonio
MUFAL2261	TA120	*Mugil liza*	*Mugil liza*	MK838043	Santo Antonio

MUFAL2262	TA121	*Mugil liza*	*Mugil liza*	MK838044	Santo Antonio
MUFAL2263	TA122	*Mugil curvidens*	*Mugil curvidens*	MK838045	Santo Antonio
MUFAL2264	TA123	*Mugil liza*	*Mugil liza*	MK838046	Santo Antonio
MUFAL2295	TA154	*Mugil brevirostris*	*Mugil brevirostris*	MK388724	Santo Antonio
MUFAL2296	TA155	*Mugil brevirostris*	*Mugil brevirostris*	MK838048	Santo Antonio
MUFAL2297	TA156	*Mugil brevirostris*	*Mugil brevirostris*	MK838049	Santo Antonio
MUFAL2298	TA157	*Mugil brevirostris*	*Mugil brevirostris*	MK838050	Santo Antonio
MUFAL2299	TA158	*Mugil brevirostris*	*Mugil brevirostris*	MK838051	Santo Antonio
MUFAL2300	TA159	*Mugil liza*	*Mugil liza*	MK838052	Santo Antonio
MUFAL2301	TA160	*Mugil liza*	*Mugil liza*	MK838053	Santo Antonio
MUFAL2304	TA163	*Mugil curvidens*	*Mugil curvidens*	MK838056	Manguaba
MUFAL2306	TA165	*Mugil curvidens*	*Mugil curvidens*	MK838058	Manguaba
MUFAL2124	TA182	*Mugil incilis*	*Mugil curema*	MK838063	Santo Antonio
MUFAL2318	TA187	*Mugil curvidens*	*Mugil curvidens*	MK838064	Manguaba
MUFAL2320	TA189	*Mugil curvidens*	*Mugil curvidens*	MK838065	Manguaba
MUFAL2321	TA190	*Mugil curvidens*	*Mugil curvidens*	MK838066	Manguaba
MUFAL2323	TA192	*Mugil curvidens*	*Mugil curvidens*	MK838067	Manguaba
MUFAL2324	TA193	*Mugil curvidens*	*Mugil curvidens*	MK838068	Manguaba
MUFAL2325	TA194	*Mugil curvidens*	*Mugil curvidens*	MK838069	Manguaba
MUFAL2326	TA195	*Mugil curvidens*	*Mugil curvidens*	MK838070	Manguaba
MUFAL2328	TA197	*Mugil curvidens*	*Mugil curvidens*	MK838071	Manguaba
MUFAL2329	TA198	*Mugil curvidens*	*Mugil curvidens*	MK838072	Manguaba
MUFAL2330	TA199	*Mugil curvidens*	*Mugil curvidens*	MK838073	Manguaba
MUFAL2332	TA201	*Mugil curvidens*	*Mugil curvidens*	MK838074	Manguaba
MUFAL2334	TA203	*Mugil curema*	*Mugil curvidens*	MK838075	Manguaba
MUFAL2335	TA204	*Mugil curvidens*	*Mugil curvidens*	MK838076	Manguaba
MUFAL2340	TA209	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838077	Santo Antonio
MUFAL2341	TA210	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838078	Santo Antonio
MUFAL2342	TA211	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838079	Santo Antonio
MUFAL2343	TA212	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838080	Santo Antonio
MUFAL2344	TA213	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838081	Santo Antonio
MUFAL2345	TA214	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838082	Santo Antonio
MUFAL2346	TA215	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838083	Santo Antonio
MUFAL2347	TA216	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838084	Santo Antonio
MUFAL2348	TA217	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838085	Santo Antonio
MUFAL2349	TA218	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838086	Santo Antonio
MUFAL2350	TA219	*Mugil brevirostris*	*Mugil brevirostris*	MK838087	Santo Antonio
MUFAL2351	TA220	*Mugil liza*	*Mugil liza*	MK838088	Santo Antonio
MUFAL2353	TA222	*Mugil curvidens*	*Mugil curvidens*	MK838090	Santo Antonio
MUFAL2354	TA223	*Mugil curvidens*	*Mugil curvidens*	MK838091	Santo Antonio
MUFAL2355	TA224	*Mugil curvidens*	*Mugil curvidens*	MK838092	Santo Antonio
MUFAL2356	TA225	*Mugil curvidens*	*Mugil curvidens*	MK838093	Santo Antonio
MUFAL2358	TA227	*Mugil liza*	*Mugil liza*	MK838095	Santo Antonio
MUFAL2359	TA228	*Mugil liza*	*Mugil liza*	MK838096	Santo Antonio
MUFAL2360	TA229	*Mugil curvidens*	*Mugil curvidens*	MK838097	Santo Antonio
MUFAL2361	TA230	*Mugil curvidens*	*Mugil curvidens*	MK838098	Santo Antonio
MUFAL2365	TA234	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838099	Santo Antonio
MUFAL2366	TA235	*Mugil rubrioculus*	*Mugil rubrioculus*	MK838100	Santo Antonio

Table S6.2 - Genetic distance matrix of a subsample of *Mugil* individuals to test the pair of enzyme with higher reproducibility.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. M.brevirostris_TA113_rep1	0.000															
2. M.brevirostris_TA113_rep2	0.002	0.000														
3. M.brevirostris_TA156_rep1	0.032	0.033	0.000													
4. M.brevirostris_TA156_rep2	0.032	0.033	0.003	0.000												
5. M.rubrioculus_TA030_rep1	0.524	0.525	0.527	0.527	0.000											
6. M.rubrioculus_TA030_rep2	0.524	0.525	0.528	0.527	0.003	0.000										
7. M.rubrioculus_TA212_rep1	0.524	0.525	0.528	0.528	0.026	0.025	0.000									
8. M.rubrioculus_TA212_rep2	0.522	0.524	0.526	0.527	0.026	0.025	0.002	0.000								
9. M.curvidens_TA224_rep1	0.649	0.648	0.647	0.648	0.636	0.636	0.635	0.635	0.000							
10. M.curvidens_TA224_rep2	0.648	0.647	0.647	0.647	0.635	0.634	0.635	0.634	0.003	0.000						
11. M.curvidens_TA193_rep1	0.651	0.650	0.648	0.650	0.638	0.637	0.637	0.637	0.102	0.102	0.000					
12. M.curvidens_TA193_rep2	0.651	0.650	0.649	0.650	0.636	0.636	0.636	0.636	0.101	0.102	0.003	0.000				
13. M.curvidens_TA071_rep1	0.652	0.651	0.651	0.653	0.638	0.637	0.637	0.638	0.099	0.099	0.106	0.106	0.000			
14. M.curvidens_TA071_rep2	0.654	0.653	0.652	0.653	0.639	0.637	0.638	0.638	0.099	0.099	0.105	0.106	0.002	0.000		
15. M.liza_TA093_rep1	0.720	0.719	0.724	0.723	0.680	0.680	0.679	0.678	0.751	0.753	0.748	0.752	0.753	0.756	0.000	
16. M.liza_TA093_rep2	0.721	0.720	0.724	0.723	0.679	0.680	0.679	0.678	0.750	0.752	0.748	0.751	0.753	0.755	0.002	0.000
PstI-SphI																

Table S6.2 continued - Genetic distance matrix of a subsample of *Mugil* individuals to test the pair of enzyme with higher reproducibility.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. M.brevirostris_TA113_rep1	0.000															
2. M.brevirostris_TA113_rep2	0.004	0.000														
3. M.brevirostris_TA156_rep1	0.028	0.027	0.000													
4. M.brevirostris_TA156_rep2	0.028	0.027	0.005	0.000												
5. M.rubrioculus_TA030_rep1	0.533	0.531	0.537	0.535	0.000											
6. M.rubrioculus_TA030_rep2	0.527	0.525	0.533	0.529	0.005	0.000										
7. M.rubrioculus_TA212_rep1	0.533	0.531	0.537	0.534	0.024	0.024	0.000									
8. M.rubrioculus_TA212_rep2	0.526	0.524	0.531	0.527	0.025	0.024	0.006	0.000								
9. M.curvidens_TA224_rep1	0.667	0.666	0.667	0.669	0.601	0.599	0.601	0.598	0.000							
10. M.curvidens_TA224_rep2	0.666	0.667	0.667	0.669	0.599	0.597	0.599	0.597	0.007	0.000						
11. M.curvidens_TA193_rep1	0.669	0.667	0.667	0.669	0.606	0.596	0.605	0.597	0.056	0.052	0.000					
12. M.curvidens_TA193_rep2	0.666	0.663	0.666	0.665	0.605	0.596	0.604	0.594	0.054	0.049	0.010	0.000				
13. M.curvidens_TA071_rep1	0.670	0.670	0.671	0.672	0.597	0.598	0.598	0.597	0.050	0.048	0.056	0.051	0.000			
14. M.curvidens_TA071_rep2	0.666	0.665	0.666	0.666	0.596	0.596	0.597	0.595	0.049	0.047	0.056	0.049	0.006	0.000		
15. M.liza_TA093_rep1	0.744	0.746	0.746	0.747	0.674	0.674	0.670	0.673	0.714	0.715	0.729	0.716	0.714	0.718	0.000	
16. M.liza_TA093_rep2	0.745	0.745	0.746	0.749	0.672	0.672	0.668	0.673	0.714	0.714	0.729	0.716	0.714	0.719	0.006	0.000
PstI-HpaII																

Table S6.3 - Dataset of *Mugil* individuals from Tropical Southwestern Atlantic marine province used in each analysis, showing the number of base pairs (bp) and SNPs.

Dataset	Number of bp	Number of SNPs	Analyses	Result
comparative data sets				
6sp_0MD	987	987	PCoA, STRUCTURE, F_{ST}, RAxML, SNAPP	Main
6sp_20MD	3,508	3,508	PCoA, RAxML	Suppl Info
6sp_40MD	7,762	7,762	PCoA, RAxML	Suppl Info
5sp_0MD (without *M. liza*)	2,332	2,332	STRUCTURE	Suppl Info
species specific data sets				
brevirostris_0MD	756	756	δaδi	Main
curema_0MD	918	918	δaδi	Main
curvidens_0MD	3,240	3,240	STRUCTURE, δaδi	Suppl Info/Main
liza_0MD	555	555	δaδi	Main
rubrioculus_0MD	1,389	1,389	δaδi	Main
data set with invariable sites				
0MD_inva	82,148	1,293	He, Ho, θ, π, Tajima's D	Main

Table S6.4 - Genetic differentiation (F_{ST}) of *Mugil* species estimated with 6sp_0MD (987 SNPs).

	1	2	3	4	5	6
1. *M. liza*						
2. *M. brevirostris*	0.951					
3. *M. rubrioculus*	0.940	0.855				
4. *M. incilis*	0.936	0.939	0.907			
5. *M. curema*	0.944	0.933	0.909	0.025		
6. *M. curvidens*_SA	0.941	0.922	0.891	0.891	0.890	
7. *M. curvidens*_MB	0.947	0.931	0.902	0.911	0.902	0.005

SA – individuals sampled at Santo Antonio estuary;
MB – individuals sampled at Manguaba estuary.

Table S6.5. Akaike's information criterion (AIC) and AIC weights for the four demographic models tested by δaδi for *Mugil* sp. from Tropical Southwestern Atlantic marine province.

	Neutral		**Two epoch**		**Bottlegrowth**		**Three epoch**	
	AIC	**AIC weights**	**AIC**	**AIC weights**	**AIC**	**AIC weights**	**AIC**	**AIC weights**
M. liza	120.06	3.50217E-06	96.26	0.515752904	97.24	0.315963842	98.5	0.168279752
M. brevirostris	217.48	2.81859E-25	119.76	0.00046737	105.04	0.734629095	107.08	0.264903535
M. rubrioculus	266.14	4.89218E-34	113.58	0.656879278	115.5	0.251514403	117.52	0.091606319
M. curema	157.65	9.30775E-12	109.28	0.296658336	108.18	0.514183957	110.18	0.189157707
M. curvidens	456.29	1.69796E-52	371.18	5.14432E-34	217.88	1	375.18	6.96208E-35

Table S6.6 - Demographic parameter estimates and standard deviation for the best fitted model for each species of *Mugil* from Tropical Southwestern Atlantic marine province.

Species	**Model**	**Ne(1)**	**Ne(2)**	**T(1)**
M. liza	Two epoch	2.743 ± 0.845	not applicable	0.135 ± 0.067
M. brevirostris	Bottlegrowth	0.021 ± 0.012	10.46 ± 1.527	0.127 ± 0.028
M. rubrioculus	Two epoch	3.99 ± 0.747	not applicable	0.161 ± 0.036
M. curema	Bottlegrowth	0.18 ± 0.041	6.92 ± 4.382	0.168 ± 0.047
M. curvidens	Bottlegrowth	0.0304 ± 0.002	9.978 ± 1.994	0.044 ± 0.002

Ne(1) - First change in population size related to the ancestral;
Ne(2) - Second change in population size related to the ancestral (just applicable in bottlegrowth model);
T(1) - Estimated time when the change happened in generations related to a reference effective population size.

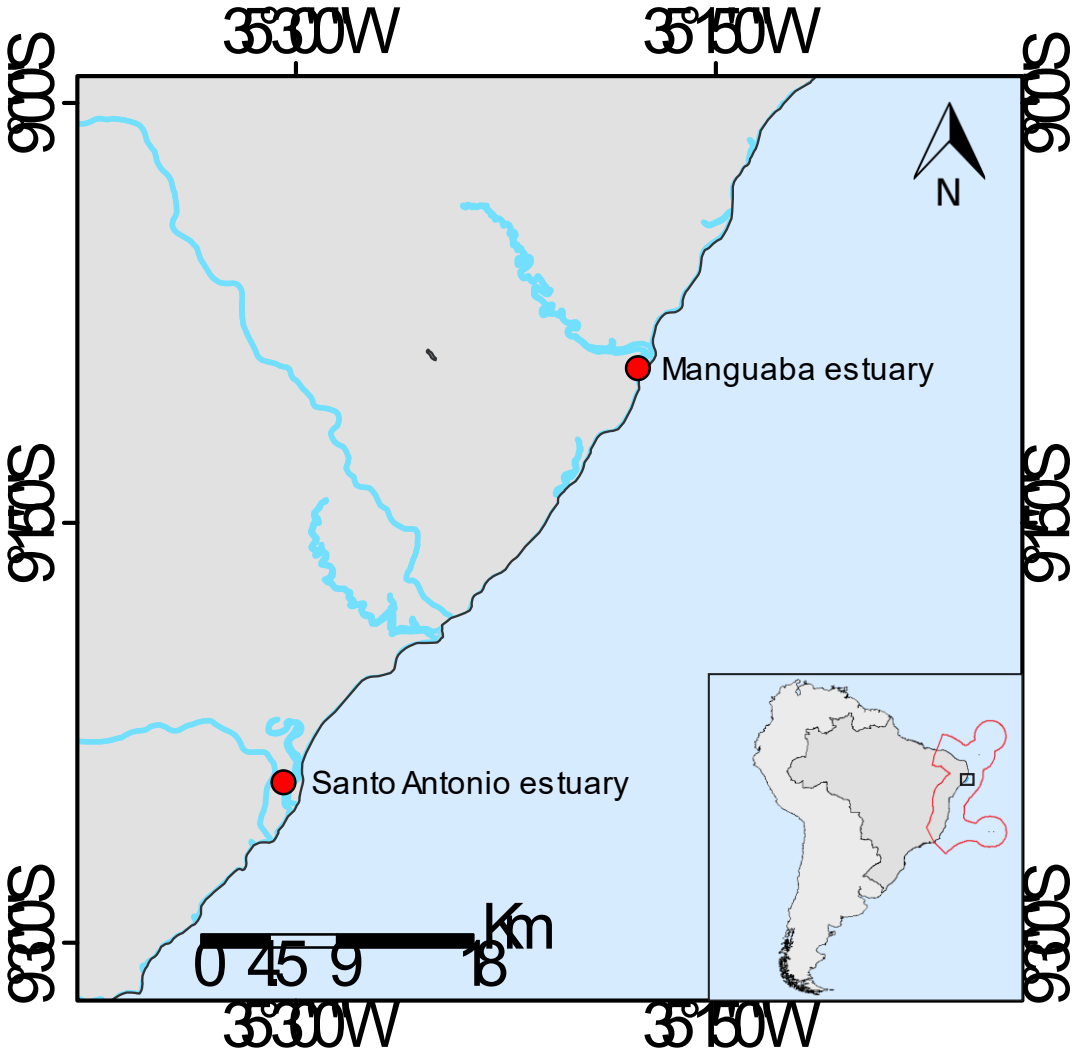

Figure S6.1. Sampling sites (red dots). Tropical Southwestern Atlantic marine province is highlighted in inset map.

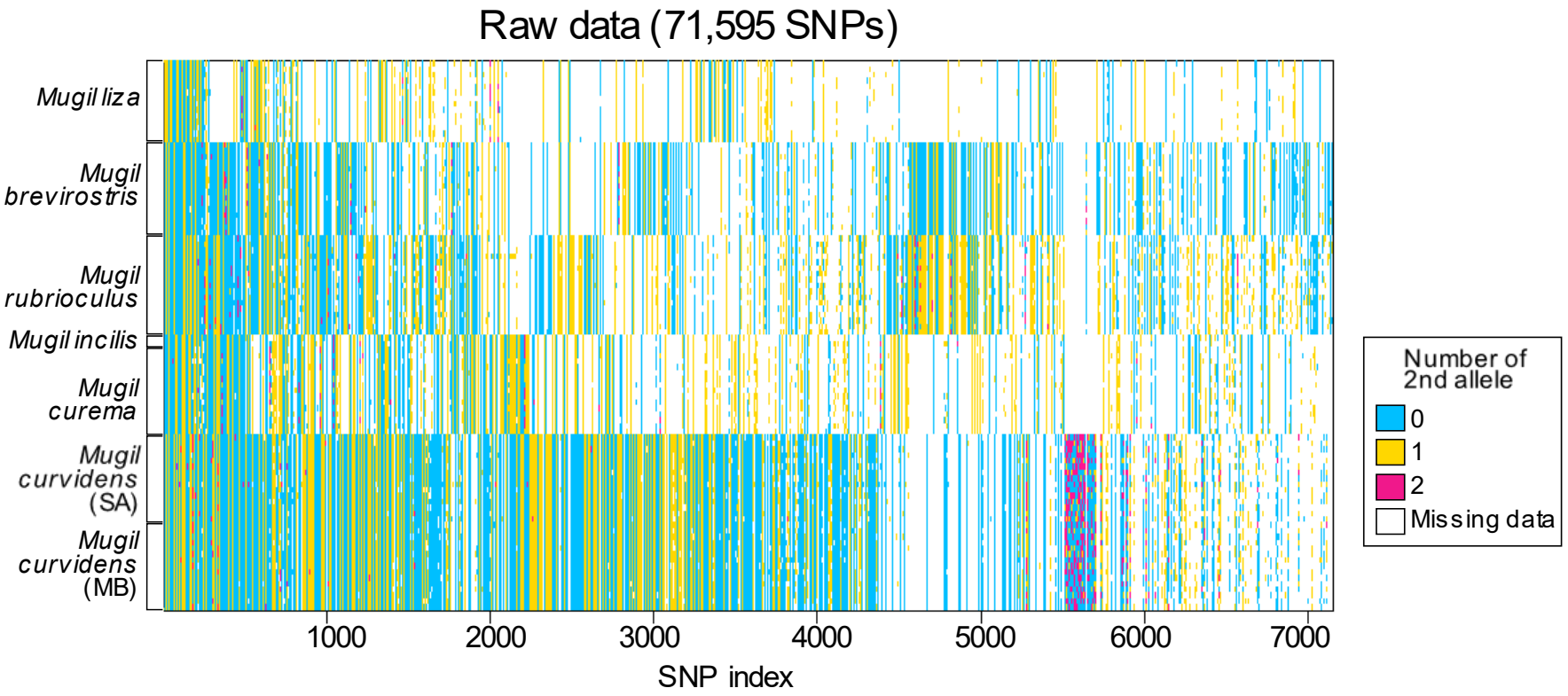

Figure S6.2 – *DART-seq* raw data. Lines represent the 94 *Mugil* individuals, and columns represent the 71,595 SNPs before filtering. SA – individuals sampled at Santo Antonio river; MB – individuals sampled at Manguaba river.

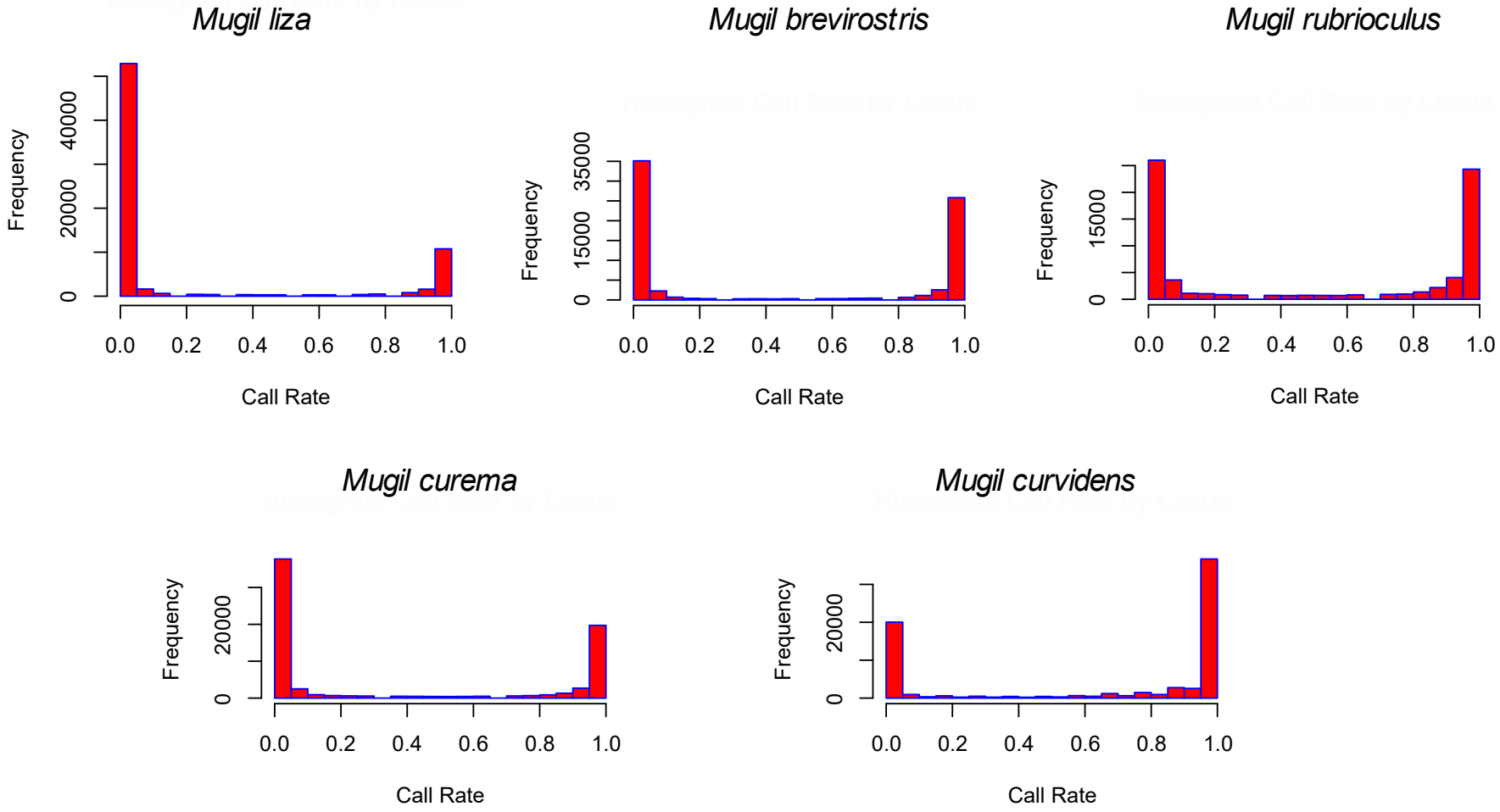

Figure S6.3 - Histogram of call rate of all loci in each *Mugil* species. The bi-modal distributions show that loci are either always or never called across all individuals of the same species.

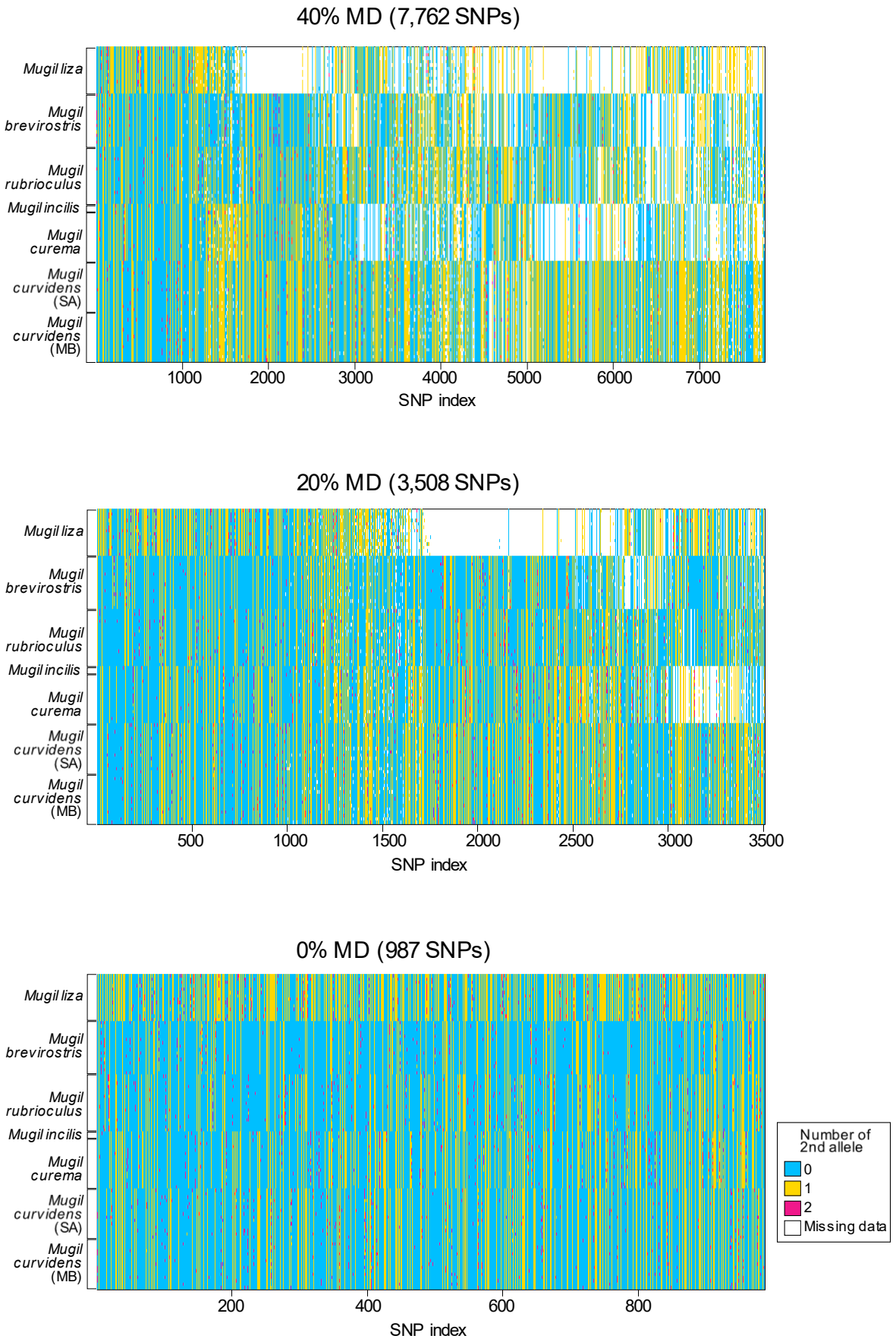

Figure S6.4 - SNPs after filtering considering three levels of maximum missing data (MD) per locus. From the top to the bottom: 40% missing data resulted in 7,762 SNPs, 20% missing data resulted in 3,508 SNPs, and 0% missing data resulted in 987 SNPs. SA – individuals sampled at Santo Antonio river; MB – individuals sampled at Manguaba river.

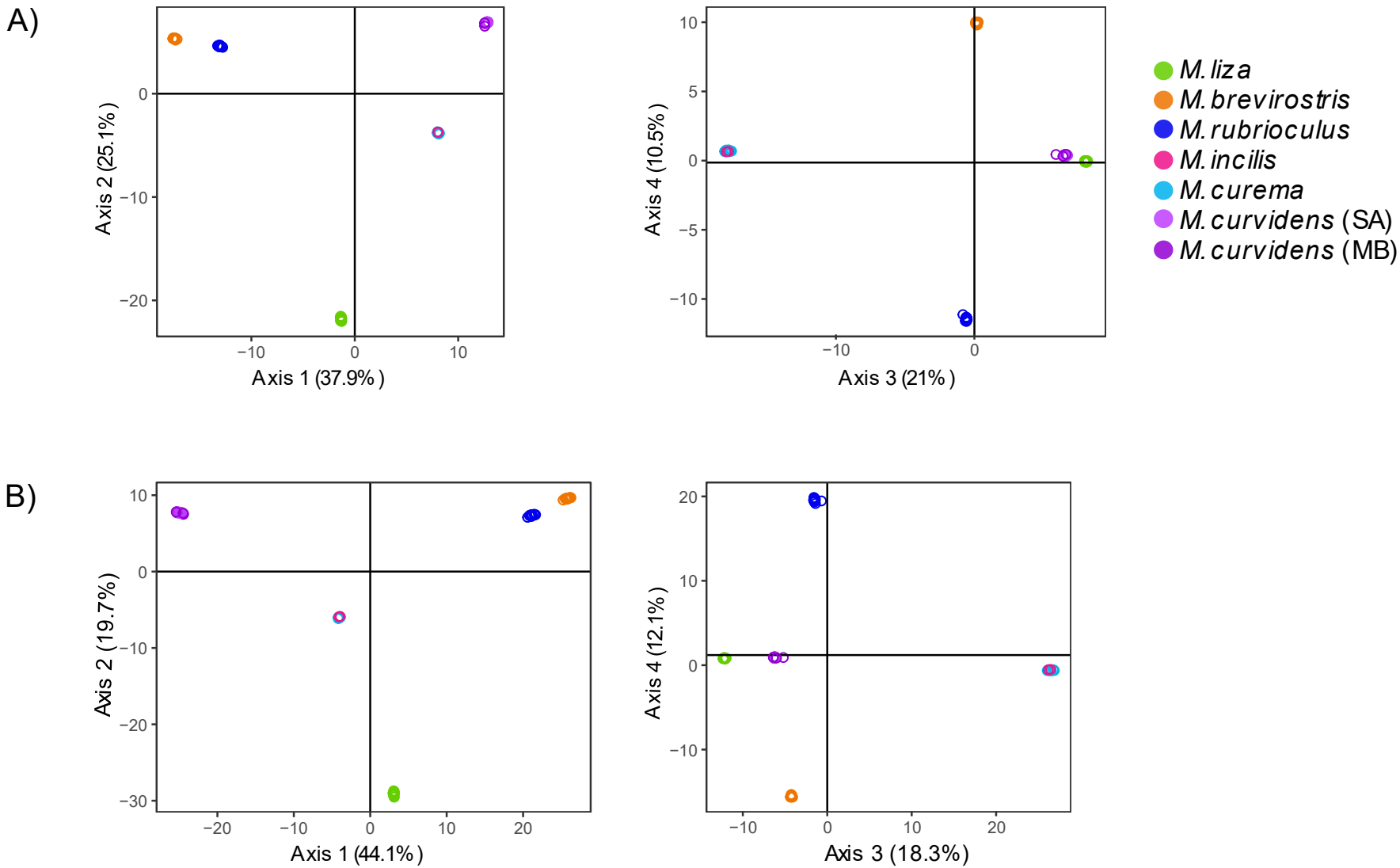

Figure S6.5 - PCoA analysis considering a maximum of 20 and 40% of missing data.

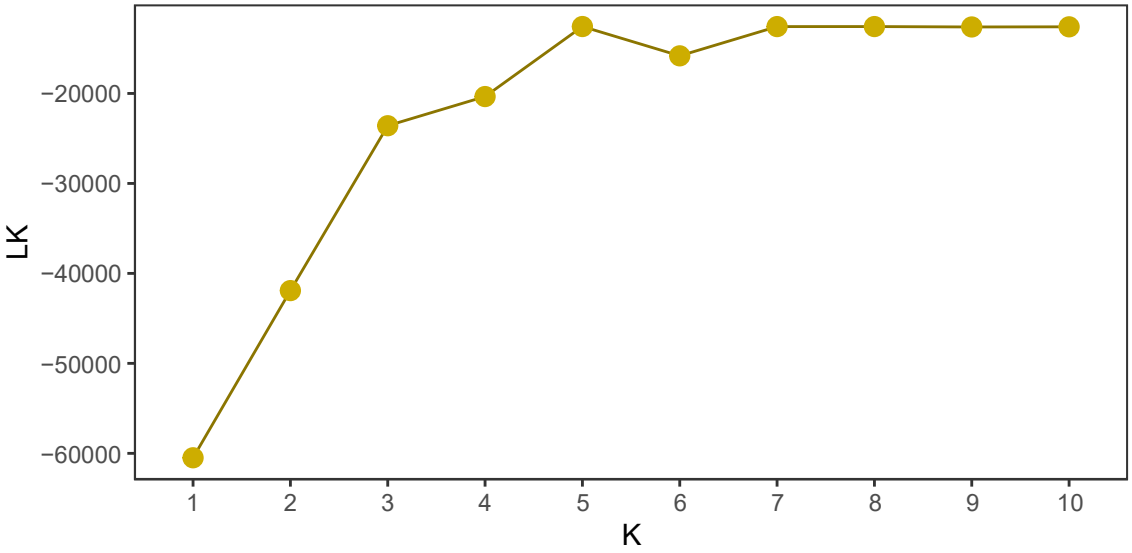

Figure S6.6 - Log-likelihood plot of Structure analysis using the dataset with 0% missing data (987 SNPs). The Log-likelihoods were highly consistent across all replicates, showing a peak at K=5.

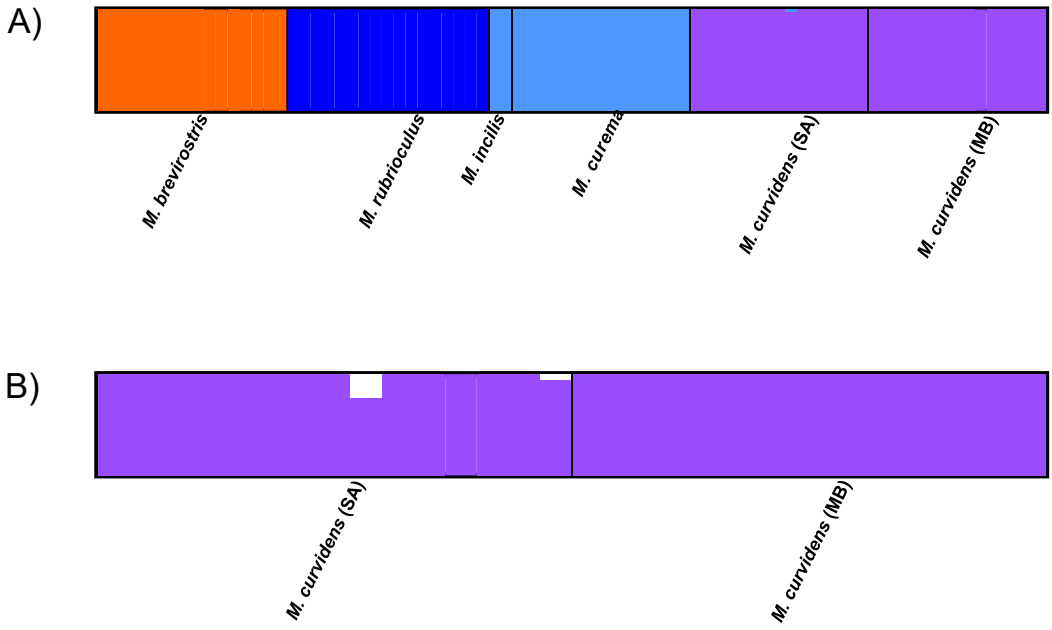

Figure S6.7 - Structure analyses considering two partitions of the data. A) Analysis performed with 80 *Mugil* individuals (excluding *M. liza* lineage) and a dataset with 0% of missing data (2,332 SNPs). It is evidenced the presence of four homogeneous lineages, where the individuals morphologically identified as *M. incilis* belong to the same evolutionary lineages as *M. curema* individuals. B) Analysis performed only with 30 individuals *M. curvidens* individuals sampled at two different rivers (Santo Antonio, SA, and Manguaba, MB) considering 0% of missing data (3,240 SNPs). There is no sign of population genetic structure between the two rivers at K=2.

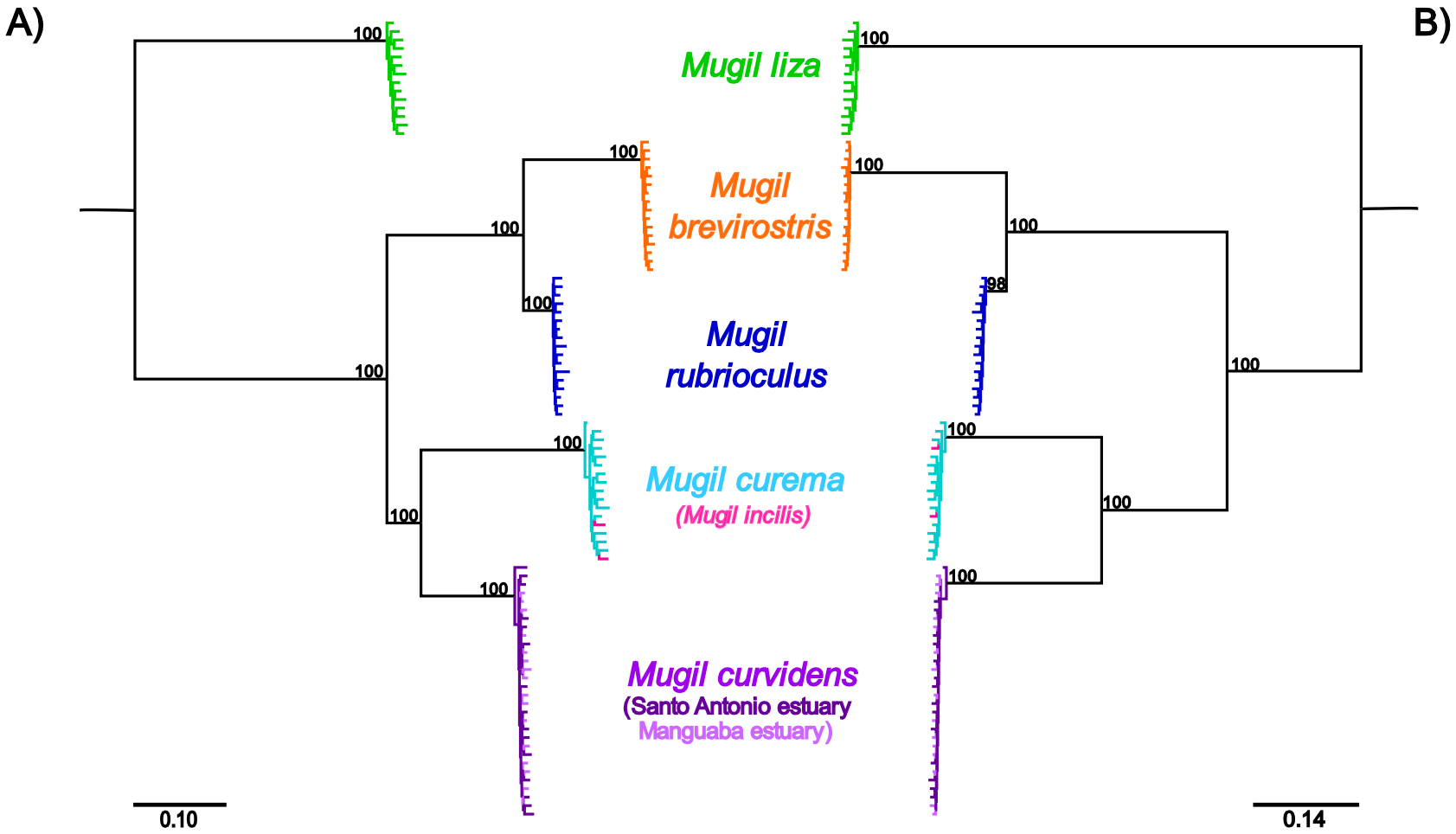

Figure S6.8 - Maximum likelihood phylogenetic tree performed with 1,000 bootstrap replicates (bs) and GTRGAMMA model. The majority consensus tree is represented for a dataset considering a maximim of 20% (left) and 40% of missing data (right). Numbers near the nodes represent node support bs.

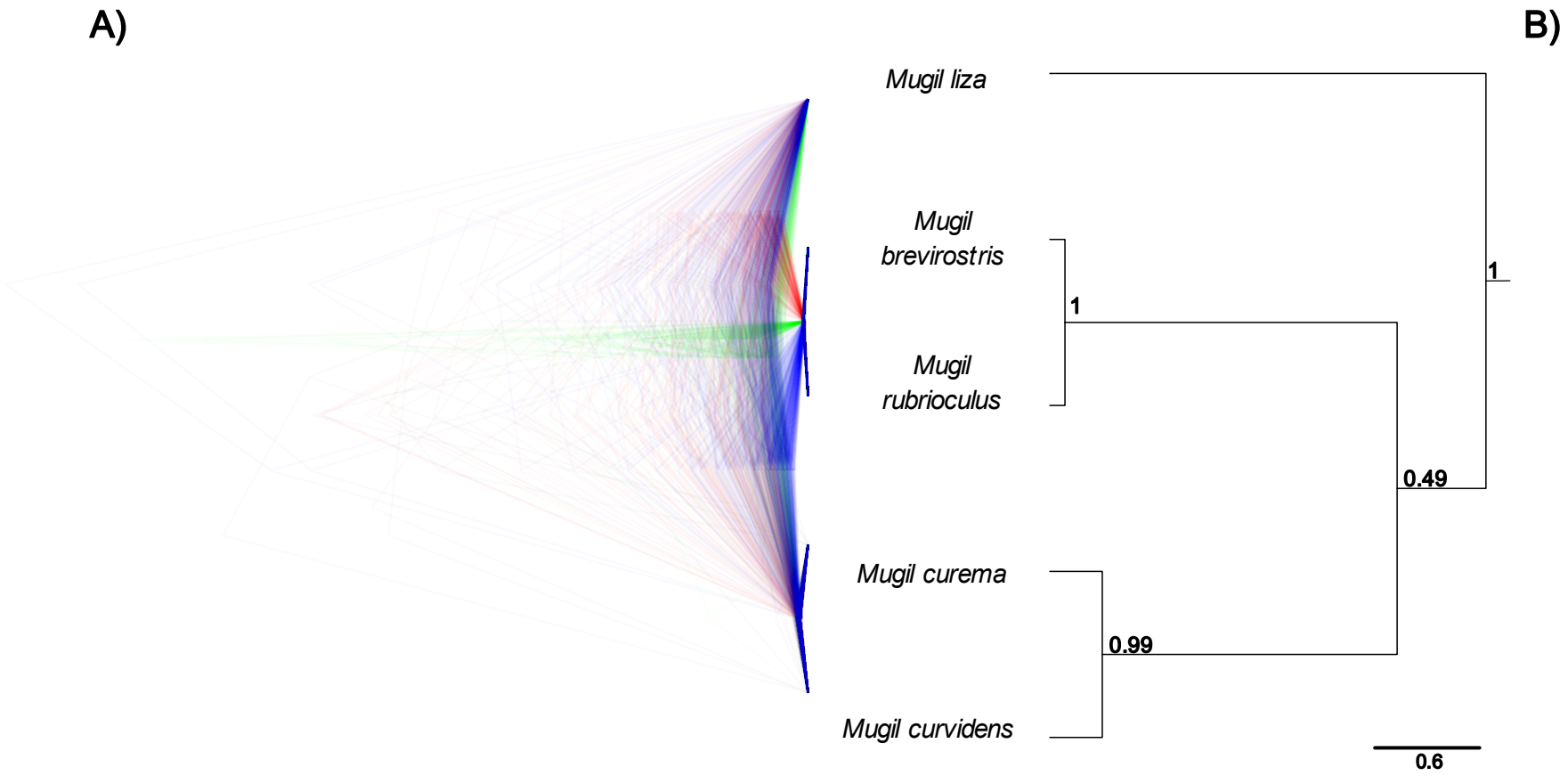

Figure S6.9 - Bayesian phylogenetic analysis performed with the dataset with 0% missing data (987 SNPs), using SNAPP. The cloudogram on the left represents the topologies estimated for all independent SNPS, with the most common topology in blue, and alternative topologies in green and red. The maximum credibility tree on the right shows a consensus species tree, showing the posterior probabilities values at the nodes.

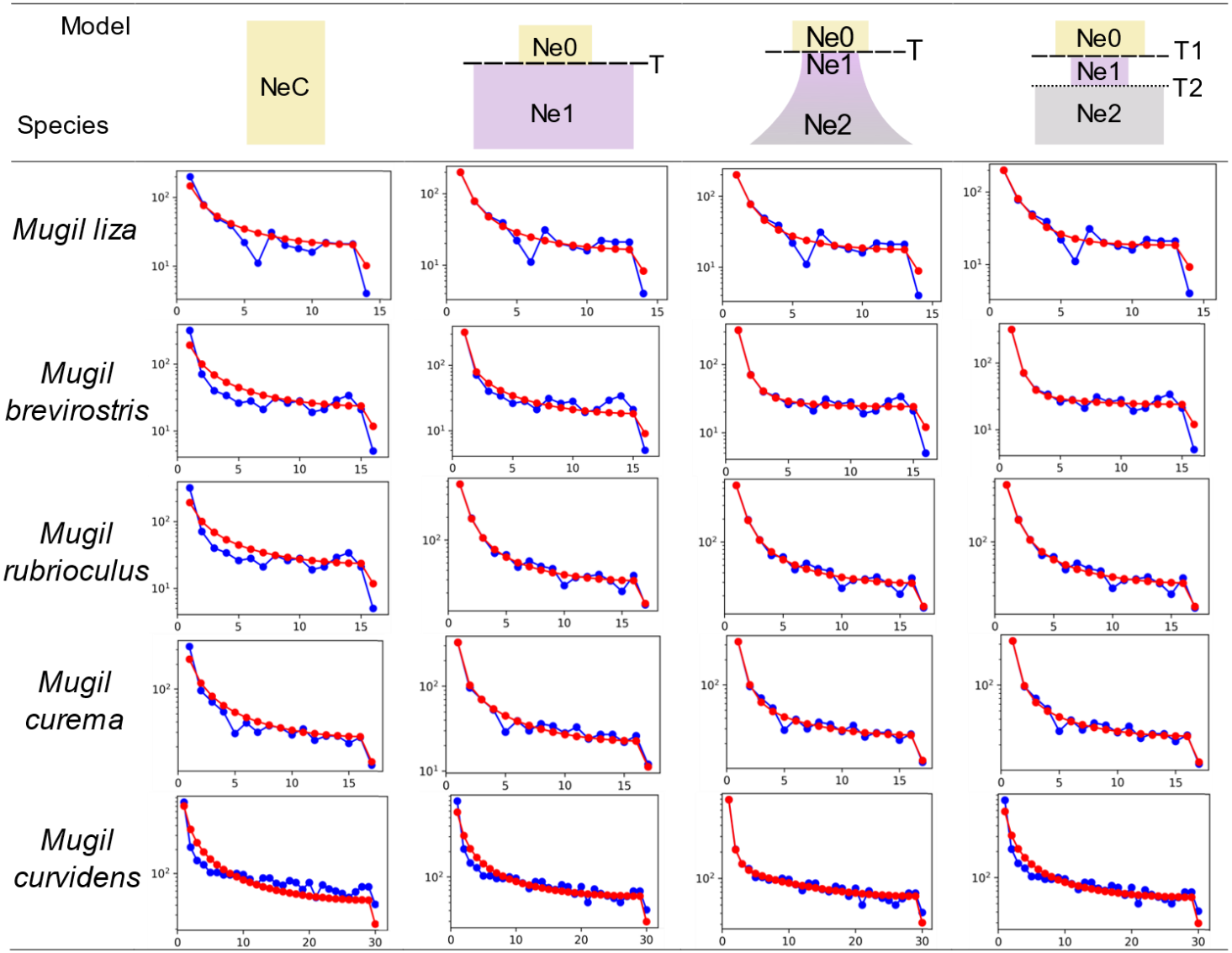

Figure S6.10. Observed (blue) and simulated (red) site frequency spectra for each species, considering 4 demographic models.