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Distribuição geográfica promovendo a divergência de padrões de cores em peixes recifais

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Thiago Matheus Jantsch Fiuza

Distribuição geográfica promovendo a divergência de padrões de cores em peixes recifais

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Este trabalho é dedicado para a dona Romana e a tia Gioconda

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“Nothing is more dangerous than a dogmatic worldview - nothing more constraining, more blinding to innovation, more destructive of openness to novelty.”

Stephen Jay Gould

RESUMO

Recifes de corais estão entre os ecossistemas mais diversos do planeta mesmo representando uma pequena porção dos oceanos. As espécies encontradas nesses ambientes exibem uma grande variedade de padrões de cores, dentre elas os peixes. Os peixes recifais, como conhecemos hoje em dia, são geologicamente recentes e suas principais funções ecológicas foram estabelecidas há cerca de 20 milhões de anos. Desde o Mioceno até os dias de hoje, as linhagens de peixes recifais ainda estão se diversificando, mas aparentemente a única novidade evolutiva são as variações em padrões de cores. Pesquisas demonstram que em certos grupos de espécies aparentadas que vivem em simpatria tendem a ser mais diferentes uma das outras do que espécies aparentadas que vivem em alopatria. Aqui testamos a hipótese de que há maior diferença entre os padrões de cores de espécies filogeneticamente aparentadas que vivem em simpatria em comparação com aquelas que vivem em alopatria. Para isso selecionamos oito clados (totalizando 47 espécies) dentro de cinco famílias características de peixes recifais (Grammatidae, Pomacanthidae, Lutjanidae, Labridae e Pseudochromidae). Os clados estudados foram selecionados por terem uma composição mista de espécies simpátricas e alopátricas. Nós então compilamos fotografias para cada espécie e usamos softwares de análise de imagens para quantificar diferenças nas cores e padrões. Nós utilizamos dois métodos complementares (*Colordistance* e *Patternize*) para avaliar a variação presente entre cores dentro desses grupos de peixes, também como nos padrões de cor. Para sete dos oito clados testados, as médias dos índices de Colordistance e Patternize foram substancialmente maiores (i.e. indicando uma maior diferença nos padrões de cores) em espécies simpátricas em

comparação com espécies alopátricas. As médias totais dos índices tanto para Colordistance quanto para Patternize também foram maiores em pares de espécies simpátricas comparadas às espécies alopátricas. Esses resultados corroboram nossa hipótese da influência da distribuição geográfica das espécies na divergência de padrões de cores. Esse padrão observado pode ser resultado de um ou mais dos seguintes fenômenos biológicos: reforço de barreiras pré-zigóticas, deslocamento de caractere reprodutivo e fatores de genéticos conhecidos como atributos mágicos “magic traits”. Além disso, os resultados demonstram que esses fenômenos ocorrem amplamente em diferentes famílias de peixes recifais. Mas as comparações entre espécies irmãs e não-irmãs também demonstraram que um maior tempo de divergência entre as espécies faz com que as espécies não-irmãs apresentem uma média de dissimilaridade, nos dois índices utilizados, maior do que as espécies irmãs independente da distribuição geográfica.

Palavras-chave: Evolução, Ecologia, Coexistência, Simpatria, Alopatria, Deslocamento de caractere reprodutivo, Reforço, Atributos mágicos, Acasalamento preferencial,

ABSTRACT

Coral reefs are one of the most diverse ecosystems found on Earth, even though they comprise only a small fraction of the ocean. Marine fish species found on coral reefs display a wide variety of colours and patterns. Most of the reef fishes we recognize today are geologically recent, and their main ecological functions were developed around 20 Ma. From the late Miocene on, many common reef fish lineages have diversified strongly, however the only novelty seems to be variation within their color patterns. Research has shown that certain groups of related species that live in sympatry tend to look more different to each other than related species that live in allopatry. Here we test the hypothesis that greater differences of colour patterns in phylogenetically related reef fishes would be observed on those living in sympatry compared to those that live in allopatry. We selected eight clades (totaling 47 species) within five characteristic reef fish families (Grammatidae, Pomacanthidae, Lutjanidae, Labridae and Pseudochromidae). Clades were selected if they had a mixed composition of sympatric and allopatric species. We then compiled pictures for each species, and used advanced image analyzing software to quantify differences in both coloration and pattern. We used two complimentary approaches (*Colordistance* and *Patternize*) to assess the variation present between the colours found within these groups of fish, as well as their patterns. For all but one of the clades tested, the mean Colordistance and Patternize scores were substantially higher (i.e. indicating greater difference in color patterns) in sympatric species then those of allopatric species (within the same clade). The total average dissimilarity score for sympatric species was also higher than allopatric species, both on Patternize

and Colordistance. Non-sister species pairs had a higher score, on both metrics used, when compared to sister species. These results corroborate our hypothesis of geographic influence on colour patterns. This pattern could be a result of one or more of the following: reinforcement, reproductive character displacement or magic traits. Moreover, results show that these phenomena seem to occur in widely different reef fish families. However, the comparison between sister and non-sister species showed that the divergence time also influences the dissimilarity of colour patterns among related species, as non-sister species had a higher dissimilarity score for both metrics used, despite their geographical distribution.

Keywords: Evolution, Ecology, Coexistence, Sympatry, Allopatry, Reproductive character displacement, Reinforcement, Magic traits, Assortative mating.

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INTRODUÇÃO GERAL

Recifes de coral são uma área pequena, correspondendo a menos de 0.1% da cobertura dos oceanos (SPALDING; RAVILIOUS; GREEN, 2001). Os peixes que vivem associados a ambientes recifais são conhecidos pela sua alta diversidade de espécies (mais de 6.500 espécies; KULBICKI et al., 2013), de comportamentos, de morfologias e de padrões de cores (KUITER; DEBELIUS, 2006; SALE, 2002). Os peixes constituem o grupo de vertebrados com o maior número de pigmentos celulares (SALIS et al., 2019). Recifes de coral como se conhece hoje começaram a surgir nos últimos 50 milhões de anos, quando surgiram os primeiros corais modernos como os do gênero *Acropora* (BELLWOOD; WAINWRIGHT, 2002). Logo após o surgimento dos corais, aparececem no registro fóssil várias famílias de peixes recifais que existem ainda hoje. Os gêneros atuais de peixes surgiram já no Mioceno, cerca de 15 milhões de anos atrás (BELLWOOD; WAINWRIGHT, 2002). Nessa época os recifes de coral já eram parecidos com os de hoje em dia, no entanto não existia a riqueza de espécies que há atualmente (BELLWOOD; GOATLEY; BELLWOOD, 2017). A maior taxa diversificação dos peixes recifais é produto dos últimos cinco milhões de anos, ou seja, grande parte das espécies atuais é resultado de processos de especiação que aconteceram durante Plioceno e Pleistoceno (BELLWOOD; GOATLEY; BELLWOOD, 2017; BELLWOOD; WAINWRIGHT, 2002; MCMILLAN; WEIGT; PALUMBI, 1999). As flutuações de nível do mar que ocorreram durante as glaciações do Pleistoceno foram um dos promotores dessa radiação (GAITHER; ROCHA, 2013; LUDT; ROCHA, 2015). Ao diminuir o nível do mar a área de ilhas e continentes aumentava criando barreiras entre populações antigas e promovendo a especiação por

vicariância (GAITHER; ROCHA, 2013). Contudo fatores ecológicos também aparentam influenciar essa radiação recente dos peixes recifais. Bellwood et al. (2017) afirmam em seu trabalho que apesar de os peixes continuarem diversificando nos últimos cinco milhões de anos, a única novidade aparenta ter sido apenas o surgimento de novos padrões de cores.

Cores são uma característica biológica vitalmente importante relacionada à sobrevivência individual e reprodução. Entre suas funções básicas estão camuflagem, aposematismo, escolha de parceiros, comunicação social, termoregulação e até mesmo prevenir parasitismo (CUTHILL et al., 2017). Por possuírem águas claras que permitem ampla visualização, a cor dos organismos é sugerida como de grande importância nos ambientes recifais. Entretanto, estudos sobre a ecologia visual de peixes recifais demonstraram que suas colorações podem não ser tão aparentes para eles e realizam uma função dupla de camuflagem e comunicação (MARSHALL, 2000). Outros estudos demonstram que as combinações mais comuns são complementares (uma cor reflete uma região do espectro em que a outra não reflete), sendo a combinação de cores mais comum é azul e amarelo, pois ela realiza bem essa função dupla (MARSHALL, 2000).

Alterações nos padrões de cores podem ocorrer ao longo dos estágios ontogenéticos em peixes recifais, assim evitando a agressão intraespecífica dos indivíduos mais velhos com os mais novos, e permitindo a coexistência em um mesmo habitat, o que é conhecido como camuflagem de espécie (FRICKE, 1980). Mudanças no padrão de cor durante o processo de especiação poderiam possibilitar a convivência entre espécies irmãs, ao diminuir comportamentos como de reprodução e agressão entre as espécies, não se

reconhecendo como coespecíficos. A diferença entre padrões de cores também poderia estar criando barreiras reprodutivas, uma vez que as espécies irmãs não vejam mais uma a outra como possíveis parceiros reprodutivos. Diversos trabalhos levantam a questão do papel da coloração no processo de especiação em peixes recifais e por consequência na radiação recente do grupo (BOWEN et al., 2013; CHOAT et al., 2012; PUEBLA et al., 2007).

Essas mudanças de cores em simpatria poderiam ser interpretadas com base na teoria clássica do deslocamento de caractere. O deslocamento de caractere é um fenômeno biológico que pressupõe que quando duas populações de espécies aparentadas, previamente isoladas, entram em contato novamente, elas tendem a divergir para evitar uma competição por recursos, em geral os caracteres deslocados são morfológicos, ecológicos, comportamentais ou fisiológicos (BROWN; WILSON, 1956). Um dos exemplos mais clássico de deslocamento de caractere é dos tentilhões de Darwin, do gênero *Geospiza.* Essas espécies, endêmicas de Galápagos, estão distribuídas de forma desigual pelas ilhas desse arquipélago, sendo que em ilhas nas quais duas ou mais espécies co-ocorrem, as caraterísticas morfológicas dos bicos diferem. Através dessa diferenciação, as espécies conseguem evitar a competição por um mesmo tipo de semente, particionando os recursos disponíveis (GRANT; GRANT, 2006). Contudo, nas ilhas onde só uma das espécies está presente, elas tendem a ter um bico de tamanho intermediário, o que possibilita um forrageio mais diversificado (LACK, 1947). MacArthur e Wilson (1967) questionaram a universalidade desse fenômeno, porém, diversos exemplos de deslocamento de caractere têm sido relatados na literatura, para mamíferos (SIMBERLOFF et al., 2000), aves (FJELDSA, 1983), répteis

(MELVILLE, 2002), anfíbios (AMÉZQUITA et al., 2006), peixes (ROBINSON; WILSON, 1994), insetos (TYNKKYNEN; RANTALA; SUHONEN, 2004) e até plantas (GLEESON, 1981).

Brown e Wilson (1956) afirmam em seu estudo que espécies simpátricas, proximamente aparentadas, tenderão a ser mais diferentes uma das outras do que espécies alopátricas. Em um trabalho com peixes papagaio foi observado esse padrão na coloração de algumas espécies irmãs (CHOAT et al., 2012). Contudo, no caso de peixes recifais é difícil conceber como a diferenciação do padrão de cor em espécies que vivem em simpatria evitaria a competição por um recurso e também qual recurso seria esse que poderia ser compartilhado. Eventos de deslocamento de caractere em complexos de espécies miméticas exemplificam mudanças de cores em espécies simpátricas (PFENNIG; KIKUCHI, 2012), porém não encontramos nenhum exemplo desse fenômeno ocorrendo em peixes recifais na literatura. Talvez no caso de espécies que dependem profundamente do seu padrão de cor para a camuflagem, como os cavalos-marinhos-pigmeus (*Hippocampus "*bargibanti" group) em que cada espécie desse grupo geralmente apresentam uma alta especificidade pelo substrato o qual vive associado (*e.g.* gorgônias, hidróides, macroalgas) (LOURIE; KUITER, 2008; LOURIE; RANDALL, 2003) e potencialmente estariam competindo pelo substrato no qual se camuflam.

Sabendo que peixes recifais utilizam padrões de cores para o reconhecimento intraespecífico e também na escolha de parceiros reprodutivos (MARSHALL, et al., 2019; SALIS et al., 2018) esse padrão observado estaria mais relacionado a uma variação da teoria de deslocamento de caractere, o *deslocamento de caractere reprodutivo*. Visto que a teoria original de

deslocamento de caractere pressupõe que o caractere deslocado estaria sob seleção divergente pra evitar competição por um recurso em comum (BROWN; WILSON, 1956). Butlin (1989) define *deslocamento de caractere reprodutivo* como o processo pelo qual barreiras pré-zigóticas são fortalecidas entre táxons já isolados completamente por barreiras pós-zigóticas (*e.g.* inviabilidade ou esterilidade dos híbridos). Howard (1992) define como *reforço de barreiras pré-zigóticas* como o processo que cria essas barreiras pra previnir hibridização. Então *deslocamento de caractere reprodutivo* seria o resultado desse *reforço,* isto é, o padrão de maior diferença em um atributo isolador [*isolating trait*] (*e.g.* caractere morfológico, sistema de sinalização ou a habilidade de discriminar coespecíficos) em áreas de simpatria entre táxons aparentados do que em alopatria. Contudo visto que padrões de cores exercem uma função dupla de comunicação e camuflagem talvez eles sejam um atributo mágico. O termo atributo mágico (*magic trait*) é definido por: um atributo morfológico ou comportamental que está vinculado à escolha de parceiros e por isso acredita-se que sejam responsáveis por acelerar o processo de especiação (SERVEDIO et al., 2011).

Padrões de cores poderiam estar atuando em processos genéticos conhecidos como “atributos mágicos” (PUEBLA et al., 2007). Todavia McMillan e colaboradores (1999) alertam para se ter cautela ao assumir a função dos padrões de cores no reconhecimento específico ou de parceiros, afirmando que, caso ocorram, mudanças genéticas podem aumentar e se manter. Barreiras pré-zigóticas tem tendência de acontecer de forma mais rápida em espécies simpátricas (COYNE; ORR, 1997), e caso os padrões de cores estiverem relacionados a este processo, eles podem estar sob pressão

evolutiva divergente em espécies aparentadas que vivem em simpatria. As barreiras pré-zigóticas são suscetíveis a serem aprimoradas pela seleção natural por reduzirem o desperdício de gametas com a produção de híbridos estéreis ou inviáveis (DOBZHANSKY, 1940; MAYR, 1963).

Portanto hipotetizamos que espécies aparentadas que vivem em simpatria devem sofrer pressão evolutiva para apresentarem padrões de cores bem distintos. Assim elas evitariam um desperdício de energia com a produção de híbridos estéreis ou inviáveis que não carregariam seus genes para futuras gerações. Por outro lado espécies irmãs que vivem em alopatria também podem apresentar padrões bem diferentes, no entanto não teriam a mesma pressão que espécies simpátricas têm para se diferenciar. Para testar a nossa hipótese compilamos 47 espécies de peixes recifais contidos em oito clados monofiléticos diferentes, pertencentes a cinco famílias conspícuas de ambientes recifais (Labridae, Lutjanidae, Pomacanthidae, Grammatidae e Pseudochromidae). Os clados selecionados são compostos por um misto de espécies simpátricas e alopátricas e foram selecionados com base em filogenias publicadas, com exceção do clado da família Pseudochromis que não possui filogenia, contudo pudemos incluir todo o gênero *Pictichromis* formando assim um clado monofilético. Com as fotografias das espécies desses clados fizemos comparações utilizando os pacotes de Colordistance e Patternize no R que fornecem índices de dissimilaridade entre duas imagens. Usamos também os dados de distribuição das espécies para definir quais eram simpátricas ou alopátricas. Com os índices de dissimilaridade fizemos comparações entre espécies alopátricas e simpátricas e também comparamos

espécies irmãs com espécies não-irmãs para verificar a influência do grau de parentesco nas diferenças de padrões de cores

Objetivos

Comparar os padrões de coloração de espécies (proximamente aparentadas) alopátricas e simpátricas; Testar se há uma prevalência de maior diferença nos padrões de cores em espécies simpátricas de peixes recifais. Para alcançar esses objetivos vou:

1. Analisar as filogenias e a distribuição das espécies irmãs para classificá-las como simpátricas ou alopátricas;
2. Utilizar os pacotes Colordistance e Patternize no R para quantificar a diferença nos padrões de cores entre espécies irmãs.

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Capítulo Único

“Geographic distribution driving colour pattern divergence in reef fishes”

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Geographic distribution driving colour pattern divergence in reef fishes

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Running-title: Geographic distribution driving colour pattern divergence in reef fishes

Abstract

Aim: Evaluate divergence in colour patterns among closely related sympatric and allopatric reef fish species.

Location: Global.

Taxon: 57 reef fish species distributed in eight clades (*Sparisoma, Bodianus, Halichoeres, Thalassoma, Gramma, Pictichromis, Lutjanus* and *Centropyge*).

Methods: We analysed differences in colouration and colour patterns among species using two packages in R: Colordistance and Patternize. We performed Mann-Whitney U tests to confirm the differences between allopatric species and sympatric species and also with sister-species and non-sister species. To test the differences among clades we ran a Kruskal-Wallis test.

Results: We found significant effects of phylogeny and geography on both colour dissimilarity indices. Sister species and allopatric species showed the lowest indices values, compared with non-sisters and sympatric species, respectively. Of the eight clades we tested, seven of them supported our initial hypothesis that sympatric species tend to have a greater divergence in their colour patterns. There was not a significant difference among the clades we tested.

Main conclusions: We demonstrated a greater difference of colour patterns of sympatric species of reef fish compared with alopatric pairwise comparisons. This indicates that colour patterns differences seems to allow for the coexistence of reef fish species in the same clades. However, these results are also influenced by the level of species relatedness. Non-sister species

presenting higher divergence in their colour mirroring their deeper divergence times, compared with the recently separated sister species, still carrying some colour resemblance.

Keywords: Evolution, Ecology, Coexistence, Sympatry, Allopatry, Reproductive character displacement, Reinforcement, Magic traits, Assortative mating

1 | Introduction

Coral reefs cover less than 0.1% of the ocean's surface (Spalding, M.D.; Ravilious, C.; Green, 2001). Despite this relatively small area, they are an ecosystem in which several marine taxa (*e.g.* Porifera, Cnidaria, Crustacea, Mollusca, Chordata) (Reaka-Kudla, 1997) reach their highest species richness. Fishes that are associated to coral reef environments are well known for their exceptional diversity in species (over 6.500 species; Kulbicki et al. 2013), morphologies and colour patterns (Bellwood and Wainwright 2002; Kuiter and Debelius 2006).

Modern coral reefs arose within the last 50 million years, coinciding with the recent domination of branching corals, especially those within the genus *Acropora* (Bellwood & Wainwright, 2002). It is during this time that most modern reef fish families begin to appear in the fossil record, establishing most of the phylogenetic and functional diversity seen on reefs today (Bellwood et al., 2017). The current genera of reef fishes were already established during the Miocene, at around 15 million years ago, and since then very few new ecological functions appeared (Bellwood et al., 2017; Bellwood & Wainwright, 2002). During this time, coral reefs had all ecological functions in place, but back then they did not have all the species richness of today (Bellwood *et al*., 2017).

The current diversity of reef fish species is partially a product of an increased in diversification in the last 5 million years mostly due to allopatric speciation processes that occurred during the Pliocene and Pleistocene (Bellwood et al., 2017; Bellwood & Wainwright, 2002; Ludt & Rocha, 2015; McMillan et al., 1999). Sea level fluctuations that occurred during glaciations in the Pleistocene

are one of the key drivers of this radiation (Gaither & Rocha, 2013; Ludt & Rocha, 2015). During times of low sea levels (due to increased glacial freezing at the poles), there is a greater amount of exposed land area that creates barriers to dispersal between populations. This division of populations by hard barriers is the main cause of vicariance speciation (Gaither & Rocha, 2013). A study by Quenouille et al. (2011) showed that most of speciation events within marine environments are allopatric in nature, and most cases of sympatric speciation (species that diverged within the same area) are due to secondary contact (*i.e.* when populations previously isolated expand their ranges into one another). However, have demonstrated that sympatric speciation does also occur throughout reef environments (Bowen et al., 2013b; Rocha et al., 2005).

For species to coexist, they need to overcome the constraints of limiting similarity (MacArthur & Levins, 1967). One frequent observation is the phenomenon of character displacement. Character displacement occurs when two previously isolated populations of closely related species start to coexist and diverge in various features to avoid competition for resources. Most frequently the characters displaced are morphological, ecological, behavioral or physiological (Brown & Wilson, 1956).

Bellwood et al (2017) suggested that coloration was the only novelty in the recent diversification of reef fishes, perhaps this could be a product of Character displacement. Colours are a biological character vitally important to individual survival and reproduction. Among its basic functions are camouflage, aposematism (i.e. warning colours), mate choice, social communication, thermoregulation and reducing parasitism (Cuthill *et al*., 2017). Studies on the visual ecology of reef fishes have demonstrated that their colorations serve a

dual function of camouflage and communication (Marshall, 2000). So colour patterns in reef fish besides their adaptive functions could have been an evolutionary pathway that allowed for their further diversification allowing for the coexistence of closely related reef fish.

We expect that this pattern of greater dissimilarity in colour pattern of related species is widespread in reef fishes from different families and from different biogeographic regions. To test this hypothesis, we quantified colour dissimilarity in eight clades of reef fishes from five different families (Labridae, Grammatidae, Lutjanidae, Pomacanthidae and Pseudochromidae) to test if colour patterns are more different in closely related sympatric species than in allopatric related ones. We also aimed to understand the influence of species relatedness (i.e. belonging to a certain clade and being sister species or non-sister species) on the geographical pattern of colour difference.

2 | Materials and Methods

2.1 | Data Collection

We selected eight monophyletic groups possessing at least three species that were a mix of both sympatric (occurring in the same geographic range) and allopatric species (occurring in separate geographic range). Clades were included if there were representative photos available of individuals in a lateral position clearly displaying the complete side of the fish. The clades selected based on these criteria were: *Sparisoma* 'aurofrenatum group' (Robertson et al. 2006); *Bodianus* 'rufus group' (da Motta-Neto et al., 2020; Santini et al., 2016); *Centropyge* 'acanthops complex' (Gaither et al., 2014); Gramma (Duarte, 2017); *Lutjanus* 'bengalensis group' (Frédérich & Santini, 2017); *Halichoeres* 'garnoti group' (Wainwright et al., 2018); *Thalassoma* 'lunare group' (Bernardi et al.,

2004). The clade Pictichromis did not have published phylogeny, however, we were able to include the whole genus of such species which guaranteed a monophyletic group. Clades were selected from all major biogeographic realms to ensure a widespread representation of species (Kulbicki et al., 2013).

Images of the species analyzed herein were sourced from an internal lab image database in addition to online repositories such as Flickr. Furthermore, some were requested directly from underwater photographers. For species that have sexually dimorphic colorations (*i.e.* the coloration differs depending on the sex of individual), the images chosen were of adult terminal males, since these males are sexually selected for by females during reproduction. None of the species included herein are known to display nuptial coloration (coloration shown only during courtship) and hidden UV patterns (colour patterns with ultra-violet spectrum, not visible to regular cameras) thereby removing this potential confounding effect. The geographic distribution data for each species were downloaded from IUCN Red list website (IUCN, 2019). For species in which range data was not available from the IUCN, shapefiles of the distribution were created using QuantumGis software (QGis Development Team 2019) based on presence/absence records from Global Biodiversity Information Facility (GBIF.org, 2019).

2.2 | Image processing

All images were processed to ensure they were comparable in the analyses. First, each fish image had the background removed using the selection tool in Adobe Photoshop. Once completed, images were resized to 1000x750 pixels with a resolution of 300 dpi (dots per inch). The colour levels were corrected by selecting a black area of the picture, the pupil in most cases, with the

eyedropper tool (Fig 1a). Then the cropped out pictures of fish were exported to Adobe Illustrator and the Image Trace feature was applied (Fig 1b). Image trace creates a simplified, vector image containing only the number of colors that the user specifies. The number of colours selected depended on each species and the number of colours that created a reliable and simplified representation of the colours on the original picture. This technique was utilized to overcome the limitations due to picture quality since images used in this project were from several different sources. After using image trace, the pictures were then imported back into Adobe Photoshop.

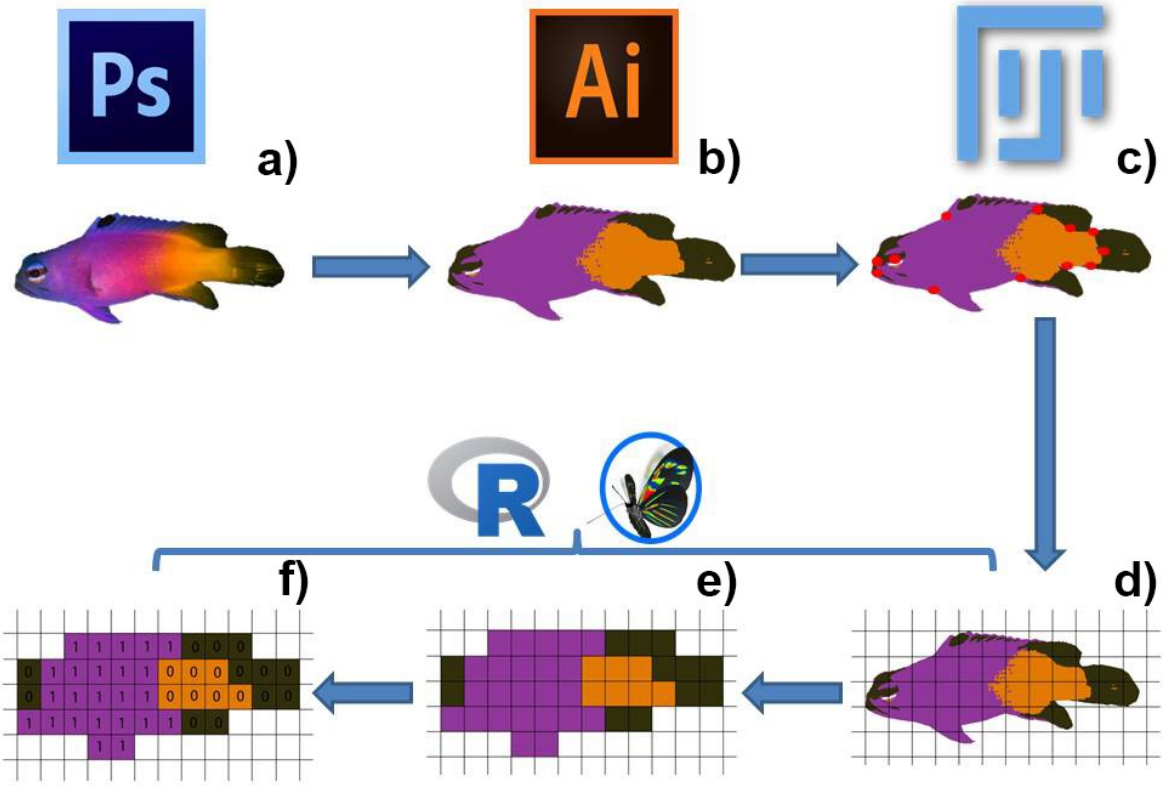

Figure 1 - A schematic simplified representation of the process to use patternize.

The same colours that were found in different species of the same clade were averaged out by selecting a sample of 25x25 pixels of such colour on

each species that it was present, the samples were placed together on a different file and then were selected and applied the Average Blur Filter on Photoshop. Using the Color Range command on the file being processed, we were able to select each separate colour found on the image and isolate them in separate layers. The layers were then blended using the Color Overlay option in which we used The Hex Triplet code of the average RGB value of each colour selected. We selected a different set of five colours for the *Gramma*, *Centropyge*, *Lutjanus* and *Bodianus* clade. For the *Sparisoma* clade we selected eight colours and nine colours for the *Halichoeres* clade. The clade with most colours selected was also the one with the most species, the *Thalassoma* clade with 10 colours found throughout the nine species. The *Pictichromis* clade was the one with the least colours, only two (yellow and purple) although it was the second in species number with eight species. Examples of one species of each clade are on supplementary files Figure S.1.

2.3 | Measuring coloration difference using Colordistance

Colordistance (Weller & Westneat, 2019) is a library in R (R Development Core Team 2017) that provides a measurement of the difference in coloration found between images. The more different the colours found in images, the greater the difference generated by colordistance. Colordistance uses two methods, k-clustering (simplifying the image to a few, average colours) or histogram binning (measuring the raw colours present). We used the default, which is the histogram binning method. This method works by splitting the three-dimensional colour space axes (Red, Green and Blue) into smaller equal-area bins (Figure 2c). The colour of each pixel from the image (which contains a Red, Green and Blue value) is then plotted into the RGB space and

the amounts of pixels that fall within each bin are counted. For example, if an image is mostly red in colour, the majority of its pixels will fall within the bin that contains red colours, and this bin will then have the greatest percentage of pixels. We chose to split each axis in two sections creating 8 bins in total. Therefore, all possible colours fall into one of eight colour categories. The numbers of pixels allocated in each bin create histograms which are utilized to measure the difference between the colours present in each image (Fig. 2d). Colordistance has multiple metrics to calculate similarity and differences between the histogram generated for each image. We chose to use Earth Movers distance since it is the default and recommended metric in Colordistance. Its output is a matrix of pairwise comparisons for every photo within a clade and their values range from 0 (most similar) to 1 (most different). We rescaled all pairwise-comparison values to a range in which the highest value within each clade was changed to 1 and corrected the other values accordingly. This was done so results could be compared across the different clades studied. While useful, the results of Colordistance only account for differences in the relative amount of each colour and do not contain any information about how these colours are arranged spatially, i.e. their pattern.

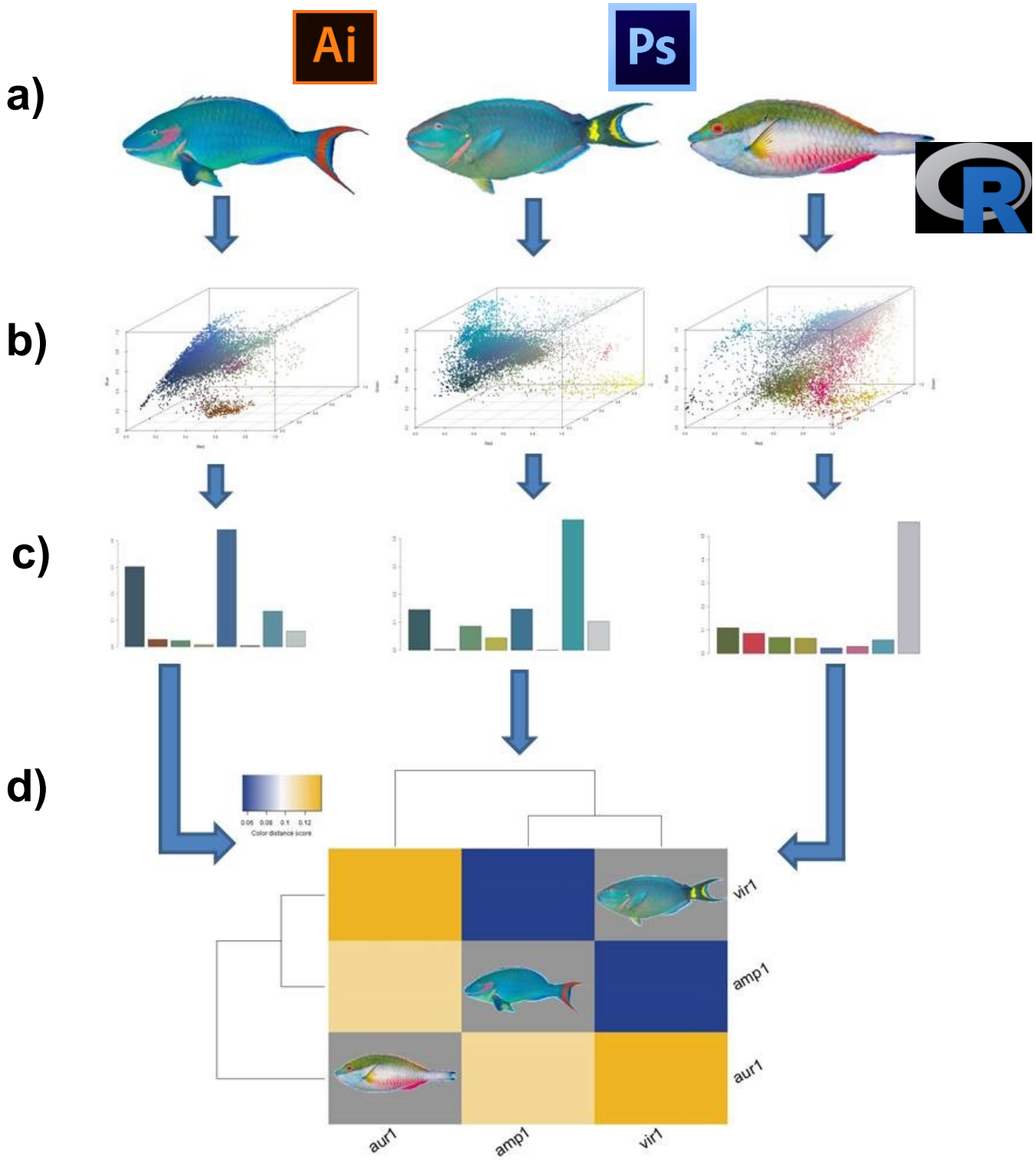

Figure 2 - Schematic figure showing the steps to obtaining the result from Colordistance. Just like the images for Patternize, the ones used on Colordistance also are processed on Adobe Photoshop and Illustrator.

2.4 | Measuring coloration difference using Patternize

Patternize (Van Belleghem et al., 2018) is an R library that was also used as it provides a direct method for comparing differences in colour patterns. It differs from Colordistance in that it also accounts for the position of the colour within the picture, thus providing an evaluation of the pattern. We used an adapted version of the methods in Hemingson et al. (2019). Since patterns are inherently spatial in nature, all images need to be aligned to be accurately compared and we used Fiji to set landmarks (Schindelin et al., 2012) (Fig. 1c). For example, if we are interested in comparing the patterns on the dorsal fins within a group of fish, then the dorsal fin of each fish's image needs to be in the same exact location. Therefore, landmarks were placed in anatomical positions that could be easily identified in all species from the same clade (Fig. S.2 - 9). These anatomical landmarks are used by patternize to align images prior to the pattern analysis which was done separately for every image analyzed. We also had to add a sample of 25x25 pixels of all the colours present on the species of each clade because Patternize would not run the analysis if colour of species A is not present in the image of species B. The sample square was done separately for each of the clades (since each clade analyzed had different colours) and were placed in the same location for each image preventing it from influencing the analysis. The background colour of most clades were white, however, species that had white as a primary component in their coloration were placed on a solid background colour that was not present on their colour pattern. The *Sparisoma* clade had its background changed to black (Fig. S.1a) and the *Halichoeres* clade had red as its background colour (Fig. S. 1d). Regardless of the colour, the background was accounted for and removed from

every clade's analysis. Due to the small sample size in the *Sparisoma* "aurofrenatum" and *Bodianus* "rufus" clade, a dummy species was created to ensure the colour analysis would run (Fig. S. 10). This dummy species was an outline of one of the species in that clade coloured with equal portions of each colour present in that group of species. Then for each analysis the program would align all the images by the landmarks and record the colours we set one by one.

Patternize was set to split the image into 250,000 blocks of equal size as a matrix. With the images aligned, it records if the selected colour range was present in each block following a specific pathway through the body of the fish. According to the resolution selected to run the analysis the block would vary in size, we always set the resolution to 300 dpi, depending to the size more than one value of colour could fall on the block to which it would be averaged out (Fig. 1d-e). If the colour was present in a specific block on species A but absent in the same block for species B, then we know that species A has that colour and species B lacks that colour in the same morphological location (Fig 1e). This procedure can only analyze one colour at a time, so each colour within a clade was run separately (e.g. *Thalassoma* had 10 colours, so 10 runs [one for each colour] and a final run for the background; 11 in total). In each run, a presence for the colour being analyzed was registered as a 1, and absence as 0 (Fig. 1f). This yielded a matrix of presence/absence data. This matrix was then converted to a unique letter identifier that represented each colour (*e.g.* b for black, w for white, etc.). Very infrequently, a block would fall in the division of two colours calculating as a different colour in the analysis. To overcome this issue, a new category was created as the colour 'other' that was not considered

in the analysis. All colour matrices were then added yielding a final matrix containing the presence of all colours analyzed. In this matrix, the 250,000 columns are the blocks in which the colour was recorded and the rows are the species present within a specific clade. We used Gower´s dissimilarity measure because it is compatible with categorical data (i.e. our colours: yellow, red, blue, etc). Like colordistance, patternize generates a matrix of pairwise-comparisons between all the species within a clade where the values range from 0 (identical) to 1 (completely different). As was done with the Colordistance scores, the resulting values in patternize were also standardized. For the final result representation, Patternize provided a PCoA (principal coordinate analysis) that groups the species with the most similar colour patterns and places the most different looking species as far apart as possible. It also provided a PCoA for each individual colour and heat maps for the presence of each colour that was analyzed.

2.5 | Distribution Analysis

The amount of range overlap and the range symmetry between all species within each clade were calculated according to the procedures utilized by Barraclough and Vogler (2000). Range overlap is defined as the area of overlap between two species divided by the area of the species with the smaller range. The resulting value extends from 0 (no overlap) to 1 (complete overlap of the smaller-ranged species within the larger-ranged species). We used the geographical distribution overlap results to assess if the species compared are allopatric or sympatric. The range symmetry value of two species is obtained by dividing the smaller distribution area by the total area of both species. This metric takes on values from 0 to 0.5. When a species-pair’s ranges are similar

in size, the value is closer to 0.5. When the pairs ranges are very different in size, this value is closer to 0. Symmetry results are presented on supplementary material (Table S1).

2.6 | Statistical analysis

We performed Mann-Whitney U test to compare allopatric and sympatric distributions and also sister species pairs and non-sister species pairs for both Colordistance and Patternize scores. We also ran Kruskal-Wallis tests with the Colordistance and Patternize scores to see if there is difference among the clades. We use Pairwise Wilcoxon Rank Sum Test to check the differences between the clades.

3 | Results

The eight clades investigated totaled 57 species and 115 comparisons between species, 49 between sympatric species and 66 between allopatric species. Results from two clades are detailed below as examples (the others are given as supplementary figures S1 – S6). A complete table with the results of Distribution overlaps, Range symmetry, Colordistance and Patternize scores (raw and standardized) are provided in supplementary Table 1.

As an example of the results we obtained we are going to present two clades we tested the *Halichoeres* “garnoti” group (Fig. 2) and the *Lutjanus* “bengalensis” group (Fig. 3). The composite image with results of the other clades we tested are provided in supplementary material (Fig. S. 12 – 17). The *Halichoeres* clade has five species with a distinction of species endemic to the Brazilian Province (*H. brasiliensis and H. dimidiatus*) and the Caribbean (*H.*

cyanocephalus and H. garnoti) with an exception of *H. radiatus* that is found throughout the Caribbean but is also found in oceanic islands off the Brazilian coast. This clade had differing highest values for the comparisons of Colordistance and Patternize. The highest score for Colordistance was between *H. brasiliensis* and *H. dimidiatus*, which are sympatric and the highest score for Patternize was among the allopatric species pair, *H. brasiliensis* and *H. cyanocephalus*. The lowest scoring species pair was the same for Colordistance and Patternize, *H. cyanocephalus* and *H. dimidiatus* with 0.13 and 0.43 respectively, followed by *H. brasiliensis* and *H. dimidatus* with 0.40 and 0.64. The overall score for Colordistance was 0.62 for allopatric species pairs and 0.91 for sympatric species pairs. Allopatric species pairs also scored lower with 0.75 in Patternize, in contrast of 0.88 for sympatric comparisons. The lowest scoring pairs were both grouped but at opposite sides of the *x*-axis but at a similar distance from *H. garnoti* which was at the opposite side of *y*-axis.

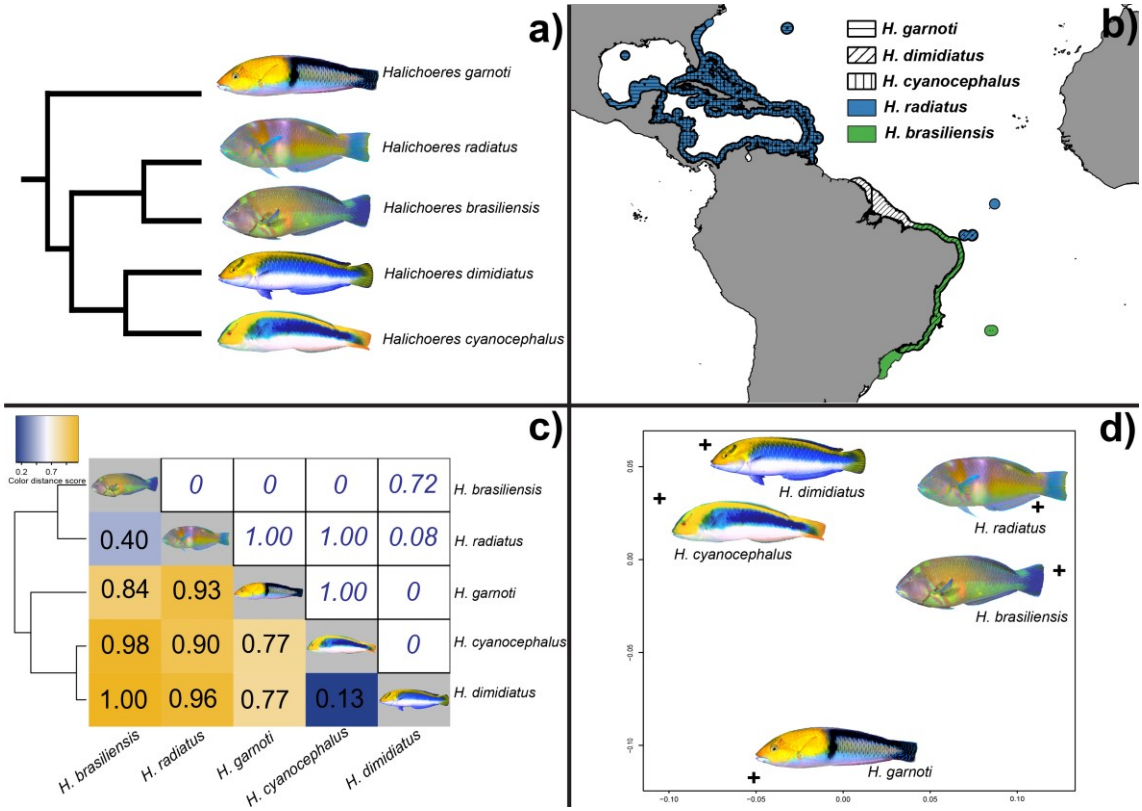

Figure 3 - *Halichoeres* 'garnoti' group: **a)** Phylogeny adapted from Wainwright et al. (2018); **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

The *Lutjanus* "bengalensis" group (Fig. 3) is wide-spread on the Indo-pacific but five of its six species are found on the western Indo-pacific, with *L. viridis* being the only species found within this group at the Tropical Eastern Pacific. This clade scored lower on the allopatric comparisons than on the sympatric ones as group, it scored 0.41 for allopatry and 0.62 for sympatry on the Colordistance scale and for the Patternize scores it had 0.51 for allopatry and 0.67 for sympatry. The highest scores for a pair comparison were the same for Colordistance and Patternize scores being the comparison between the sympatric *L. bengalensis* and *L. boutton.* The lowest scoring species pair, the allopatric pair *L. notatus* and *L. quinquilineatus,* was also the same for

Colordistance and Patternize, with 0.08 and 0.29 respectively. The pair with the lowest score got grouped together at the center of the PCA, with *L. viridis* being the closet species to them and the other were evenly placed farther away from this central clustering.

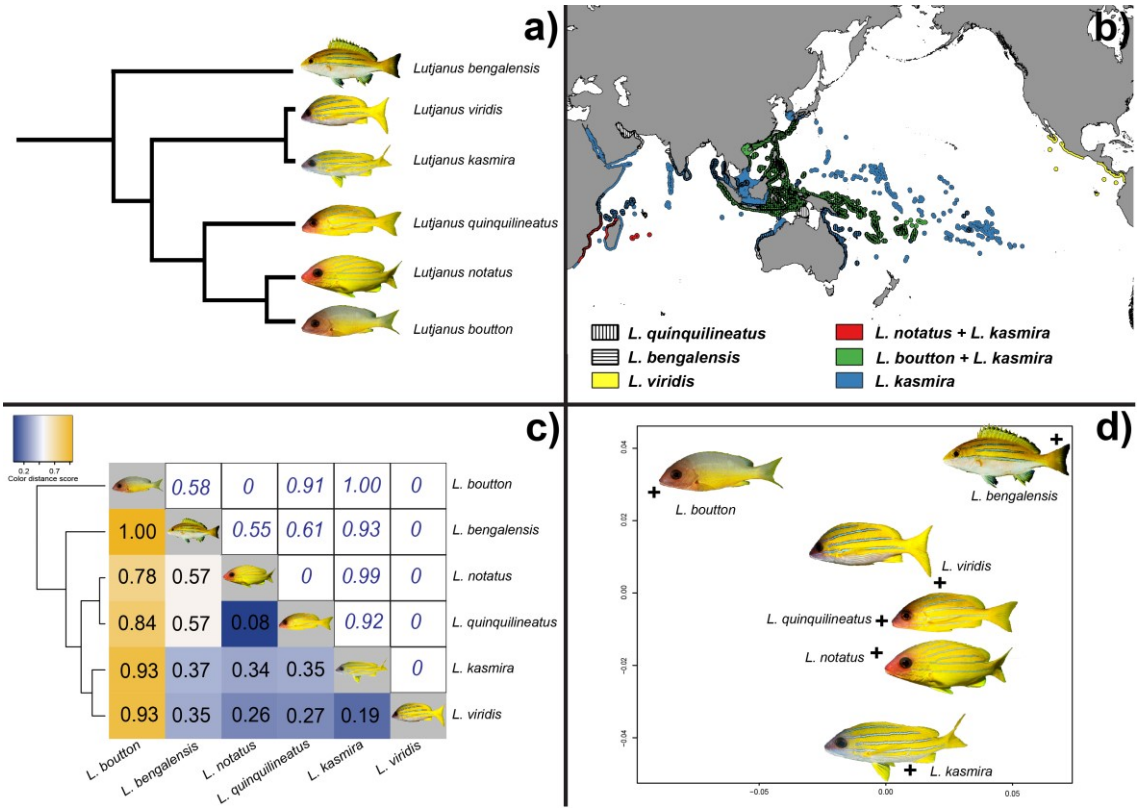

Figure 4 - *Lutjanus* 'bengalensis' group: **a)** Phylogeny adapted from Frédérich and Santini (2017); **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

Taken together, our "geographical" prediction was supported in seven out of eight studied cases (see Table 1 for the means of species pairs comparisons in each clade when in allopatry and sympatry). On Figure 5a the general mean score of the Colordistance comparisons for allopatric species was 0.49, which was significantly different (p=0.005) from the sympatric species that scored 0.65. For Patternize (Fig. 5b) the difference in average score was also

significant (p=0.005), the score for allopatry was 0.66 and 0.78 for sympatry. When comparing the average of sister species (a proxy of recent diversification; Fig. 5c Colordistance = 0.32; Fig. 5d Patternize = 0.56) with non-sister species (a proxy of greater phylogenetic distance; 0.59 and 0.74 respectively) we got a significant difference between them both for Colordistance score (p=0.002) and Patternize (p=0.005). Colordistance scores for each of the clades tested were not significantly different (df = 7, p-value = 0.1705) (Fig. 5e). The Patternize score for clades was significant (df = 7, p-value = 0.01244), however when we did Pairwise Wilcoxon Rank Sum Tests and none of the comparisons among the clades were significant (all p<0.2).

Table 1 - Means of species pairs scores each clade when in allopatry and sympatry for Colordistance and Patternize. **Pictichromis* genus numbers are shown with* and without the microendemics** (see description of Fig. S.16 for details). Y = yes, N = no.

	Colordistance		**Patternize**		**Supports**
Clade	**Allopatry**	**Sympatry**	**Allopatry**	**Sympatry**	**prediction**
Sparisoma	0.69	0.82	0.84	0.99	Y
Bodianus	0.62	1.00	0.84	0.92	Y
Gramma	0.44	0.71	0.58	0.83	Y
Halichoeres	0.62	0.91	0.75	0.88	Y
Centropyge	0.46	0.46	0.65	0.53	N
Lutjanus	0.41	0.62	0.51	0.67	Y
*Pictichromis**	0.40	0.45	0.56	0.55	Y/N
*Pictichromis***	0.38	0.82	0.48	0.79	Y
Thalassoma	0.50	0.54	0.72	0.80	Y
Total mean**	**0.49**	**0.65**	**0.66**	**0.78**	**Y**

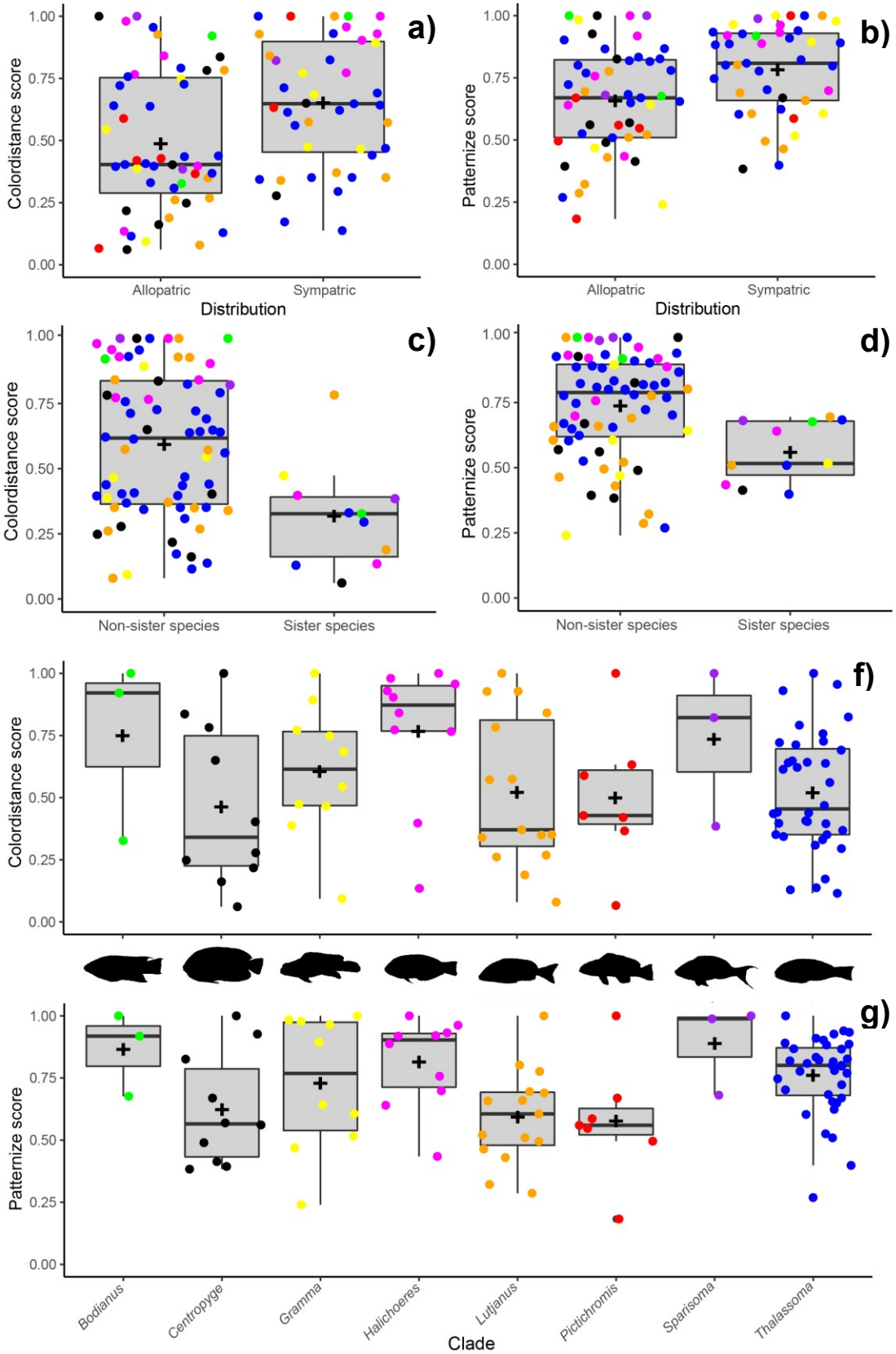

Figure 5 - a) Colordistance scores for the geographical comparison. The black cross sign represents the average. Colors on the dots represent each clade tested: Green – *Bodianus*, Black – *Centropyge*, Yellow – *Gramma*, Magenta – *Halichoeres*, Orange – *Lutjanus*, Purple – *Sparisoma*, Blue – *Thalassoma*; **b)** Patternize scores for the geographical comparison; **c)** Colordistance scores for the comparison of sister species and non-sister species; **d)** Patternize scores for the comparison of sister species and non-sister species; **e)** Colordistance scores grouped by clade; **f)** Patternize scores grouped by clade.

4 | Discussion

The total average scores of sympatric species were higher than the scores of allopatric species both for Colordistance and Patternize. When looking at each clade separately, of the eight clades tested, the average scores of seven clades supported our prediction that closely related species living in sympatry tend to have a greater dissimilarity in colour patterns. In all eight clades we analyzed, the highest scoring pairs (i.e. the most different in coloration) for both Colordistance and Patternize were sympatric species and all the lowest scoring comparisons were between allopatric species on both analysis. However, we found a stronger signal of phylogenetic relatedness. Non-sister species have greater dissimilarity scores, on both metrics used, as a longer time since diversification provides more opportunities for them to diverge in colouration.

Even though we noticed that sympatric species had a greater dissimilarity score than allopatric species pairs of both sister species and non-sister species, sympatric sister species had a lower score than allopatric non-sister species. Evidencing that more distantly related species pairs associated with a sympatric distribution have the greater tendency to look different. In other words, a stronger signal could be detected on non-sister species that had more time to diverge compared to recently diverged sister species.

This pattern of sympatric species displaying a higher dissimilarity in colour patterns than allopatric ones has been described for reef fishes (Hemingson et al., 2019; Puebla et al., 2007; Salis et al., 2018; Tavera & Wainwright, 2019). In contrast with these previous studies that had focused on

a specific group of reef fishes, we aimed to present how these patterns could evoque more general mechanisms that could have played a role in the current diversity of reef fish of different clades/ families. As we had mentioned before that these could be an example of character displacement, however it is hard to envisage how classic character displacement would be acting on the colour patterns of reef fish, what could be the resource under competition and how the differentiation on the colour pattern would release them from the competition. We could not find examples of these phenomenon happening on reef fishes but there was one study that exemplified on freshwater fish how complexes of sympatric mimetic species differentiate on their colour patterns under character displacement (Pfennig & Kikuchi, 2012). Perhaps on the case of species that depend heavily on their colour pattern for camouflage, like the pigmy seahorses (*Hippocampus* spp.) that each species in this group generally present a high specificity to the host species they live associated (*e.g.* gorgonians, hydroids, macroalgae) (Lourie & Kuiter, 2008; Lourie & Randall, 2003) and are potentially competing for the host they use to camouflage themselves.

But this pattern of increased dissimilarity in colour patterns of sympatric species observed could be explained by a few theories but they are somewhat controversial as basic concepts are debated and different authors adopt different definitions to fundamental terms. Reinforcement, Reproductive character displacement (RCD) and magic traits could apply to the explanation of some of these patterns observed in our results and in other reef fishes. Since coloration is an important part of species recognition, and thus mate recognition systems, we could expect closely related sympatric species to show greater divergence in their colour patterns than closely related allopatric species due to

“reinforcement” processes resulting in RCD. Howard (1993) defines reinforcement as the strengthening of prezygotic barriers that help to prevent hybridization. Often, hybridization yields infertile, nonviable offspring; preventing the continuation of a lineage in those individuals (Dobzhansky, 1940; Mayr, 1963). Therefore RCD often results in more pronounced differences on *isolating traits* (*e.g.* morphological character, signaling mechanism or ability to distinguish conspecifics; *i.e.* mating trait) of closely related species in areas of sympatry than in allopatry. A pattern of RCD is a product of mate recognition, which sympatric species of closely related species have deviated in mate recognition more than allopatric populations (Higgie et al., 2000).

Nonetheless it is well known that RCD can be the result of other processes apart from reinforcement, and also that reinforcement can happen without leading up to RCD (*e.g.,* when population ranges are completely sympatric) (Servedio, 2004). Several alternative hypotheses have to be ruled out, which can be very hard to prove that reinforcement has occurred (Coyne & Orr, 2004; Noor, 1999). Another complication that makes it hard to identify the cause of the observed patterns on reef fish is that reinforcement does not always leave its signature, RCD (the relationship between reinforcement and RCD, and dispute over the definition of the latter, is reviewed in Howard 1993) (Servedio, 2004).

Another explanation for the greater difference in colour pattern of sympatric related species is Magic traits. The term *magic trait* is defined as: a morphological or behavior trait that is linked to assortative mating. Therefore it is believed that magic traits would accelerate the speciation process (Servedio *et al*., 2011). This is the proposed explanation for the differentiation of the sympatric species of Hamlets of the genus *Hypoplectrus* (Puebla et al., 2007).

Also a recent review on this topic stated that the colour patterns in reef fish can indeed be regarded as magic traits (Salis et al., 2019). But it seems that this could be the case for species that speciated in sympatry but we cannot be sure that this would apply for all reef fish as other factors can promote the diversification of colour patterns on reef fish. Another constraint in this explanation is that it has been proposed that for magic traits to happen, male and female should have the same mating cue, in our case colour patterns (Maan & Seehausen, 2010). But some of the species we analyzed in this paper present sexual dimorphism and if the species in question have sexual dimorphism, often it is the males that are under sexual selection by females (Hoskin & Higgie, 2010)

These differences in mating traits started by species interactions may be expanded by sexual selection, inducing increased separation among populations (Noor, 1999; Schluter, 2001). For African Cichlids, it has been proposed the possibility that colour diversification, and speciation, may occur in these fishes as a side effect of sexual selection and that male coloration may be among the first traits to diverge in closely related species (Deutsch, 1997). Maan and Sefc (2013) provide a compendium with several examples of colour driven speciation in Cichlid fishes from Africa and in the Americas as well. But studies focusing on these roles for reef fish are still incipient. Also, in some cases has been suggested that colour pattern differences among sympatric species were to reduce aggressive behavior towards similar looking species (Salis et al., 2018; Seehausen & Schluter, 2004) similar to what happens in ontogenetic colour differences (Fricke, 1980; Salis et al., 2018). In African cichlids it has been shown that differentiation of coloration between males of

one species reduces the aggression between them and provides a novelty preferred by females and then through sexual selection promotes divergent selection (Seehausen & Schluter, 2004).

If sympatric species overlap in mating signals there could be a selection for reducing signal interference (Chek et al., 2003; Cooley et al., 2006; Taper & Case, 1992). This phenomenon has been called noisy neighbors and aids RCD (Howard, 1993; Noor, 1999). This selection can occur between non related species because signal interference can operate in any sympatric species (Hoskin & Higgie, 2010). In our allopatric species comparisons, they could retain similar colour patterns to their related species or not. So, there was no consistency in the differences among allopatric closely related species in our results.

Because different mechanisms can influence the genotype of isolated populations such as drift, genetic flow, natural selection, RCD speciation due to signal interference (Hoskin & Higgie, 2010) and thus the emergence of new colour patterns in allopatry. Species interactions may generate in isolated populations the emergence of allopatric species with different colorations as they may be under selection for the reduction of signal interference (*i.e.* Colour patterns) with different species interactions in each population and with limited gene flow between them (Hoskin & Higgie, 2010). This is an example of allopatric RCD speciation because a mating trait is diverging from its original state to accommodate species interactions in a section of its range, making them differentiate their mating trait from their own species and to the species it is coexisting. Perhaps this could be the case between the species pair *L.boutton-notatus,* as they are not sister species but still diverge greatly on their

colour patterns.–Similarly, parapatric divergence may happen where a species interacts with different species over its range, or interacts with a single species in part of its range.

Hoskin and Higgie (2010) address that environmentally complex regions (*e.g.* New Guinea, Central / South America, and the heathlands of South Africa and south-western Australia) provide an especially conducive scenario because the amount of variation in physical features (e.g. topography, soils, moisture) is typically reflected in species with small and patchy distributions, and high species turnover (Buckley & Jetz, 2008). Coincidentally the areas with high levels of species richness for marine species, especially reef organisms, are similar to the ones Hoskin and Higgie suggested, being the Indo-Australian Archipelago in which New Guinea is included and for the Atlantic Ocean it is the Caribbean, surrounded by South and Central America. Reefs also have another factor that can influence on this complexity is that they vary greatly on the zones found within the reef (*e.g.* reef flat, reef front, lagoon, reef crest). Thus, providing the complexity that results in the mosaic of species interactions among populations of species and serves as a powerful engine of diversification (Hoskin & Higgie, 2010). In places with high diversity such as those, a feedback process can take place where diversity then creates an increasingly conducive scenario for further diversification, causing radiations of similar species (Hoskin and Higgie 2010). An issue is to know to which account divergence among populations is driven by adaptation to environments differing in species interactions vs. underlying habitat, or both. Situations where mating traits are the main character under divergence are insightful in this regard (Hoskin & Higgie, 2010). Reproductive Character Displacement speciation may have been

driven by community rearrangements combined with environmental disturbances, such as the glaciation and global climate changes during the Quaternary (Hoskin & Higgie, 2010). This has been proposed to several fish and other marine taxa (Floeter et al., 2008; Gaither & Rocha, 2013; Ludt & Rocha, 2015). These periods saw great alterations on distributions, population connectivity and community structures, which exposed populations to selective pressures from novel and heterogeneous communities (McPeek & Gavrilets, 2006). Selective pressures on mating traits experienced by a particular population can be changed by range expansions, introductions of new species, or removal of species from a community.

We suggest that colour assortative mating can be one of the reasons for the coexistence of related species of reef fish and that colour based speciation seems to be widespread across regions and reef fish species of different families and the aforementioned theoretical work on speciation seems to be applicable to at least some of the modern reef fish. Although, it was deemed that the effects of colour patterns on speciation were unlikely, new evidence shows that it has a role in sympatric speciation events and it can be fast. With a few studies stating the importance of colours as Magic traits in speciation of reef fishes (Puebla et al., 2007; Salis et al., 2019). Even if sympatric species actually produce viable hybrids and they start reproducing preferentially among themselves they can diverge enough to become a new species (Melo et al., 2009). Although, it requires much more work (e.g. genetic analysis, reproductive behavior, distribution analysis, visual ecology) for every single comparison to determine if they are results from RCD, Reinforcement, Magic traits or even a combination of those. Anyways, it seems reasonable to assume that part of the

great diversity of colour patterns in reef fish exemplifies cases of colour driven speciation and that colour differentiation on previous isolated species can favour coexistence. Although a stronger signal could be detected on non-sister species that had more time to diverge compared to recently diverged sister species.

However for other groups of fish, different mate recognition systems such as acoustical signals (Bowen et al., 2013a; Tricas & Boyle, 2014), olfactory (Boyle & Tricas, 2014), fluorescence light (Michiels et al., 2008) and even electrical signals (Hopkins & Bass, 1981) may be more important and thus act on the disruptive selection of mating traits combined with assortative mating and consequently on mating trait driven speciation. We only will be able to see the whole picture if results are in a well-resolved biogeographic setting where the relationship and history among populations is known (Hoskin & Higgie, 2010). Perhaps these could be some of the explanations of the great diversity of colour patterns, and consequently reef fish species, seen today in reef environments as clear water allow for a great diversity of behaviours regarding communication and sexual selection. Because of this, an increase in water turbidity can be a serious anthropogenic threat that could cause a loss of species as it has been proposed to African cichlids (Seehausen et al., 1997).

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Supporting information

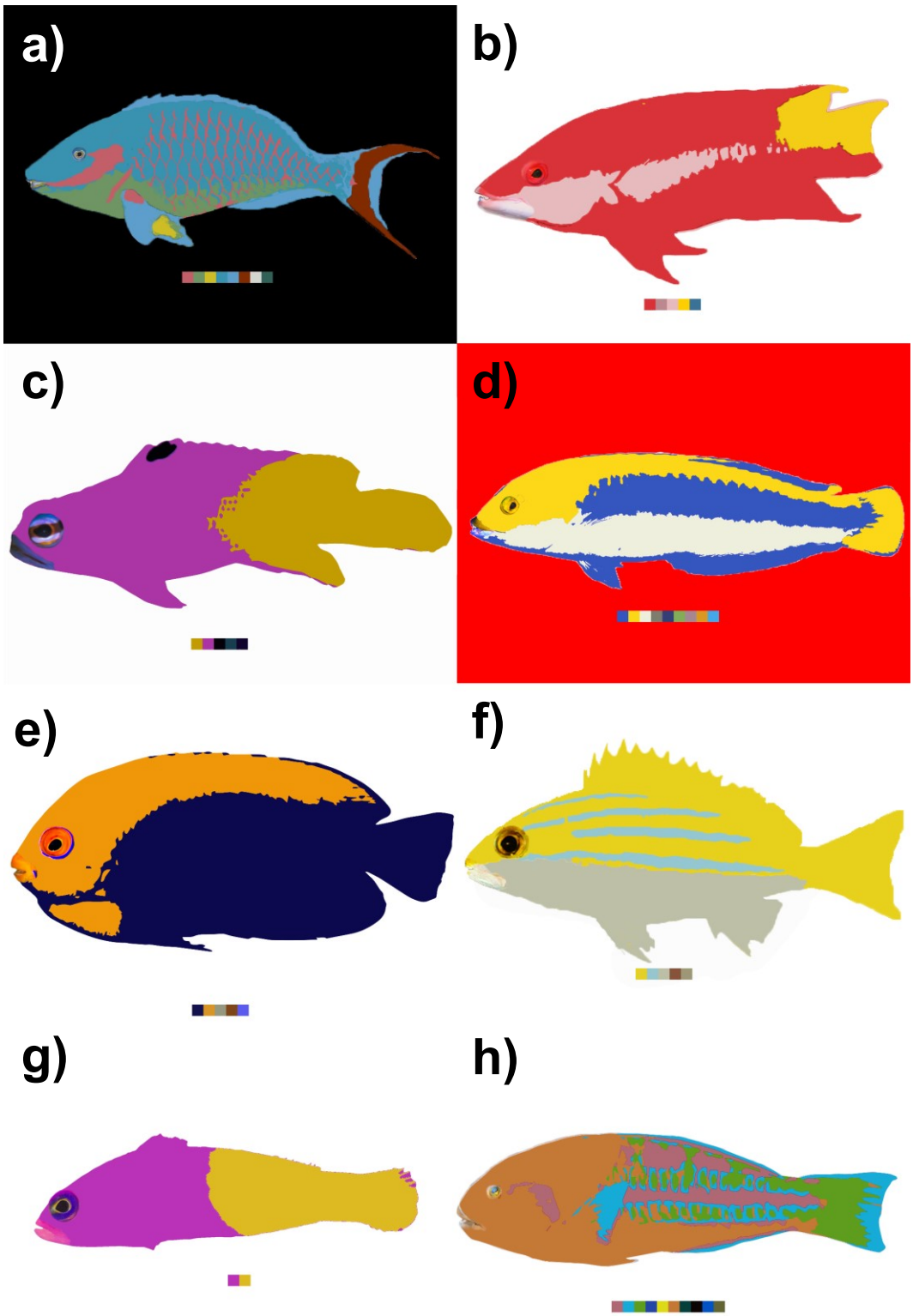

Fig. S. 1 – An example from each clade of the images used to run the Patternize analysis, on the Colordistance the images did not have the colour samples at the bottom. a) *Sparisoma amplum*; b) *Bodianus pulchellus*; c) *Gramma brasiliensis*; d) *Halichoeres dimidatus*; e) *Centropyge aurantonotus*; f) *Lutjanus bengalensis*; g) *Pictichromis coralensis*; h) *Thalassoma trilobatum*.

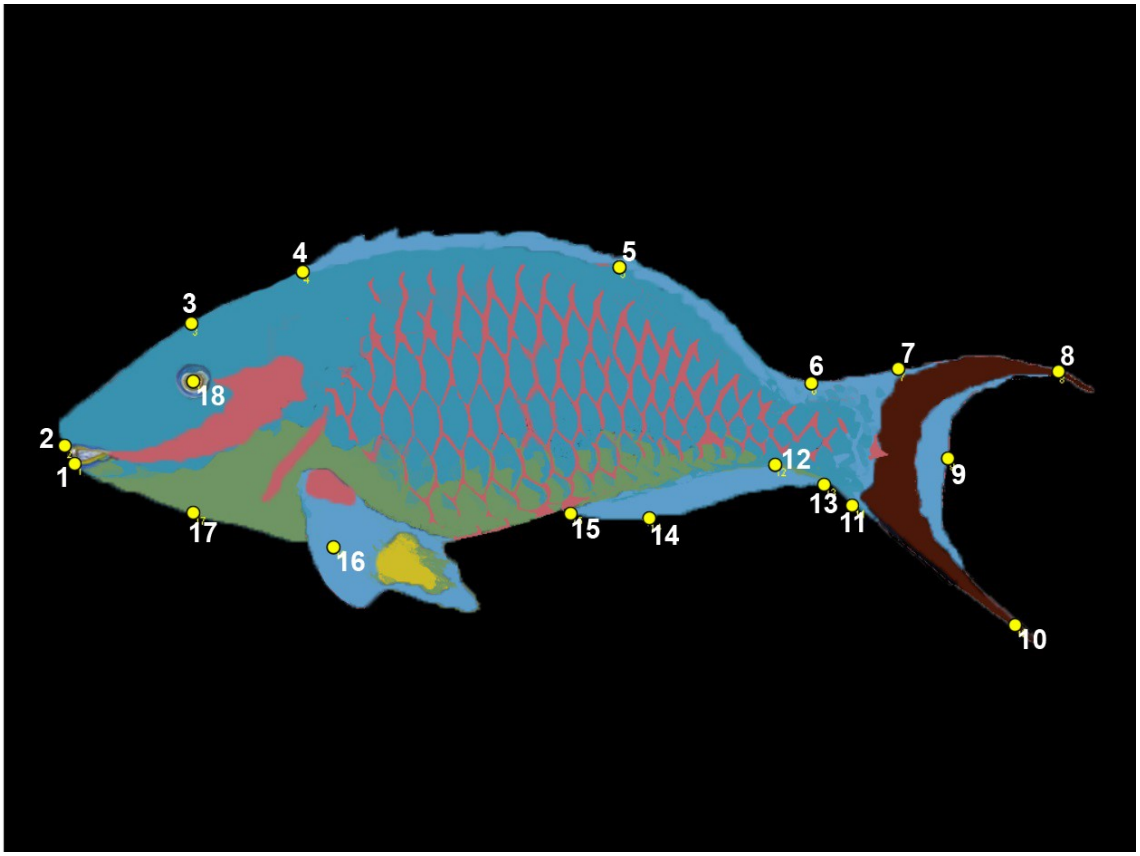

Fig. S. 2 - Landmark positions for the *Sparisoma* clade.

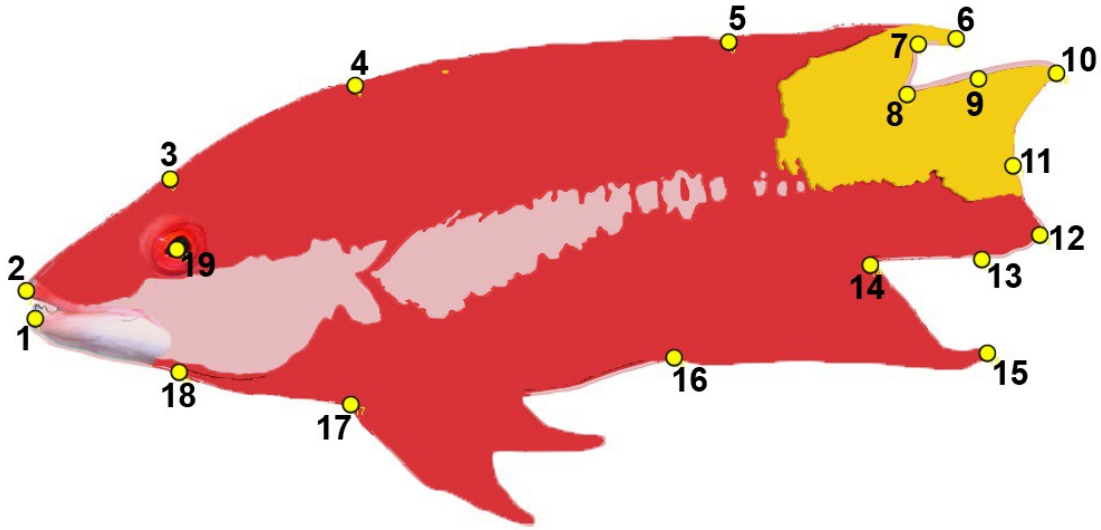

Fig. S. 3 - Landmark positions for the *Bodianus* clade.

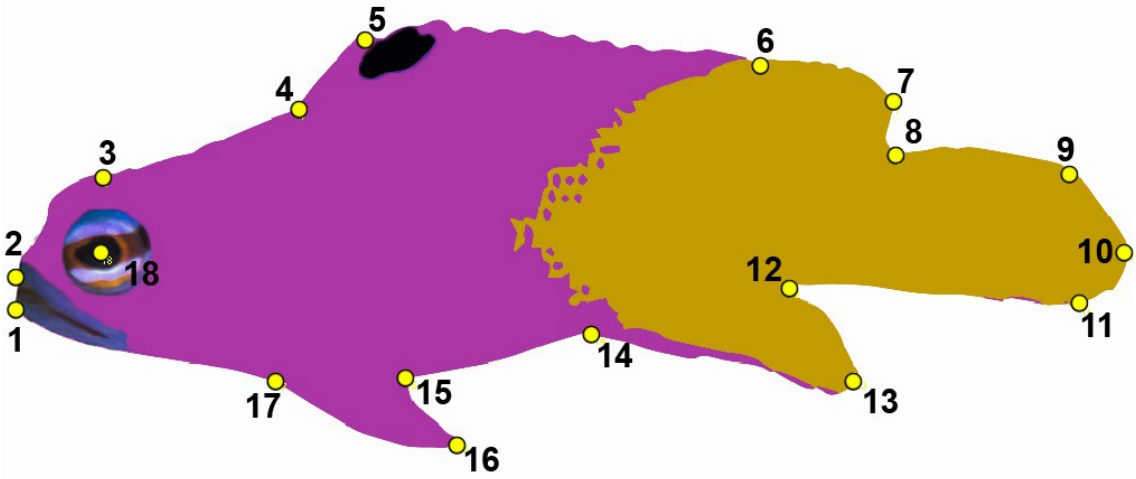

Fig. S. 4 - Landmark positions for the *Gramma* clade.

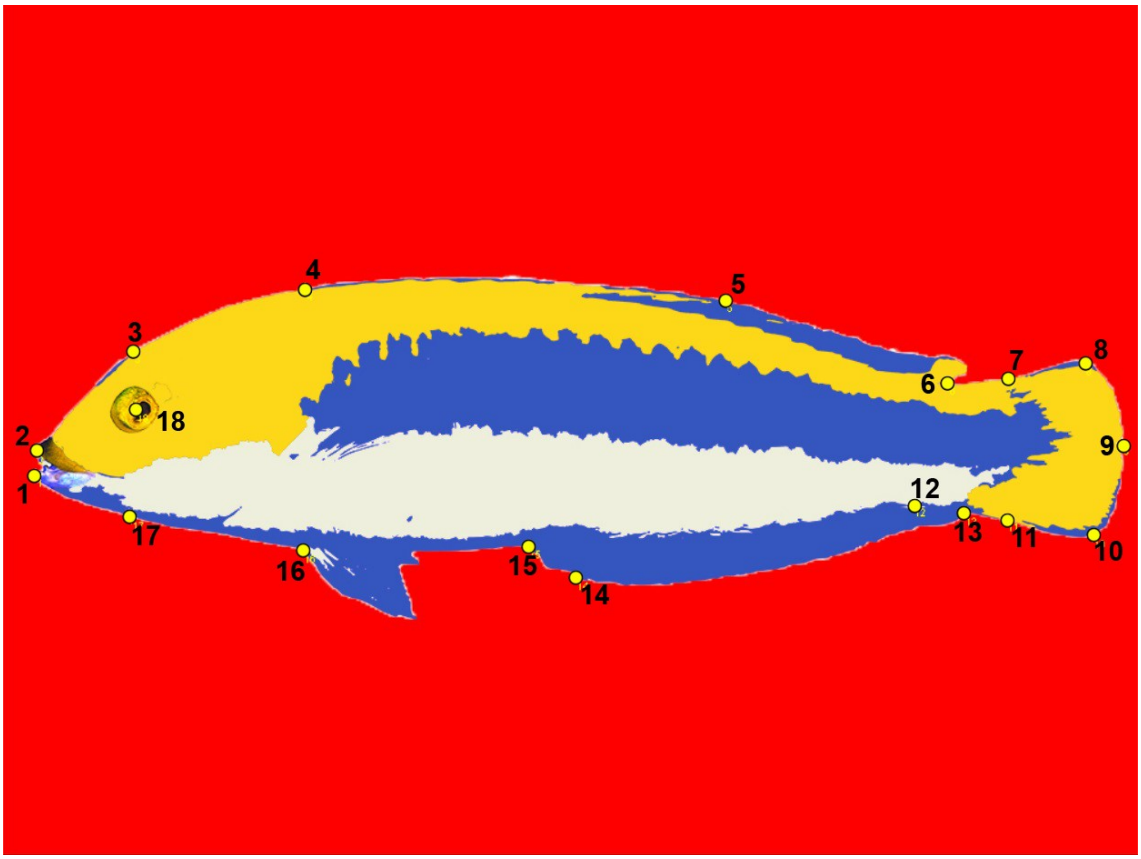

Fig. S. 5 - Landmark positions for the *Halichoeres* clade.

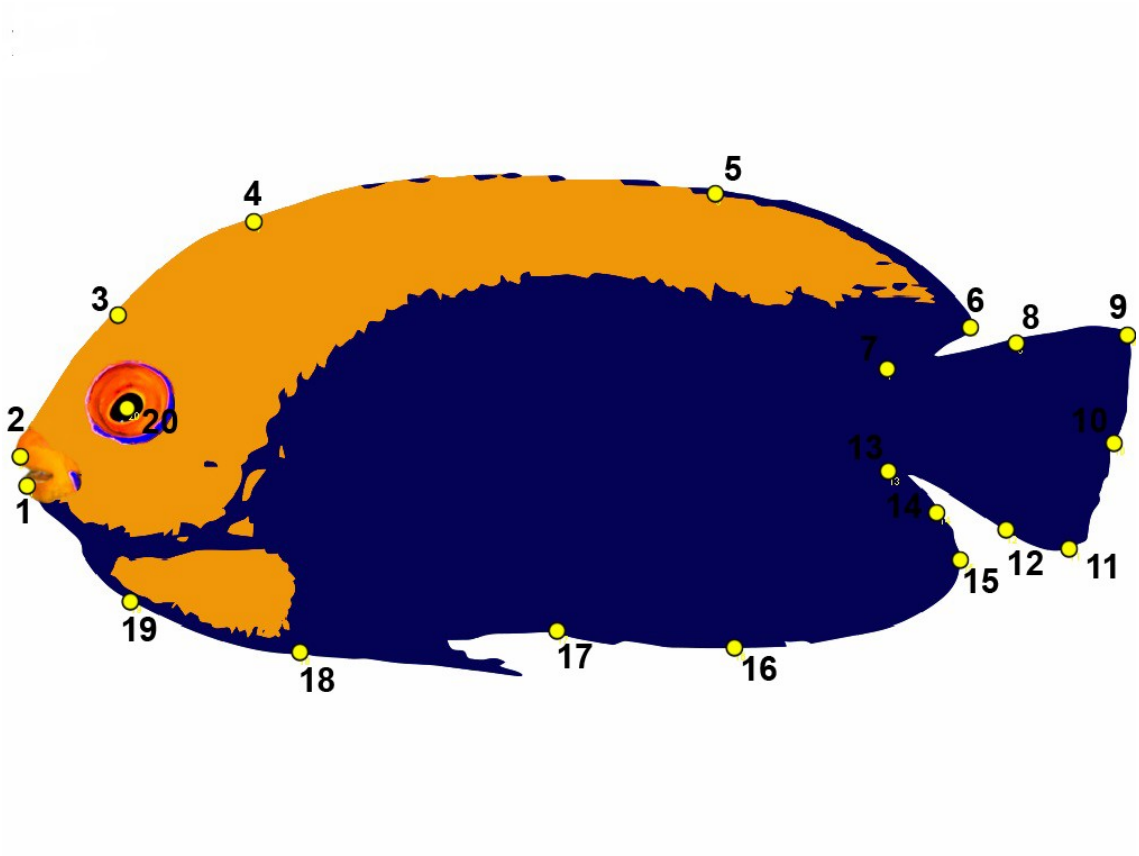

Fig. S. 6 - Landmark positions for the *Centropyge* clade.

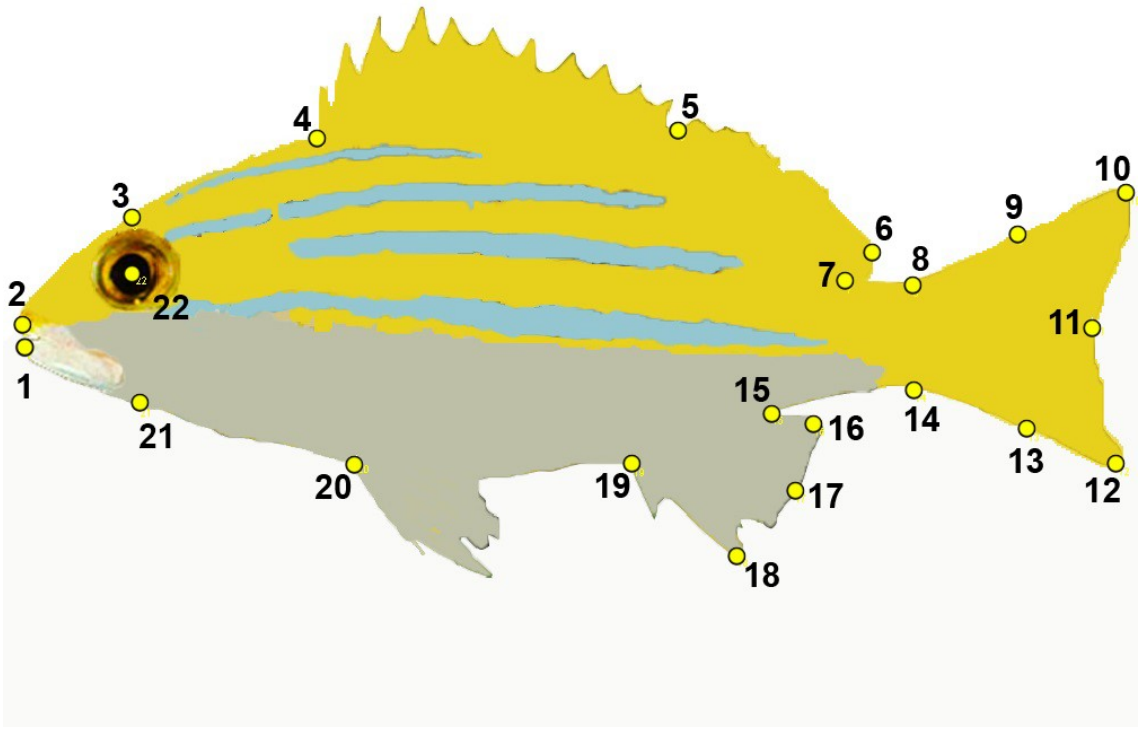

Fig. S. 7 - Landmark positions for the *Lutjanus* clade.

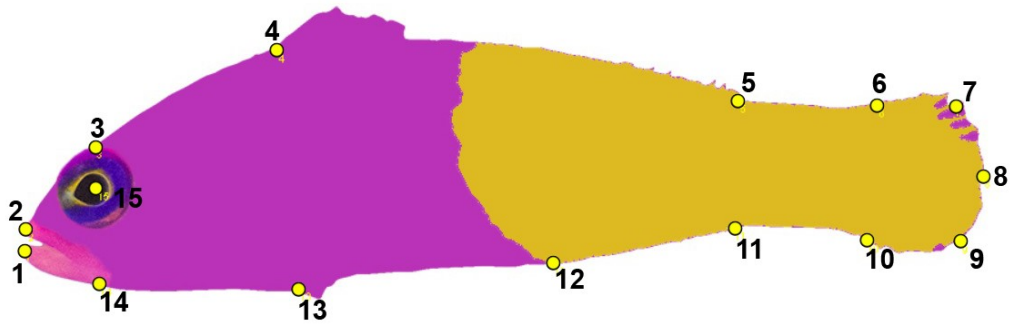

Fig. S. 8 - Landmark positions for the *Pictichromis* genus.

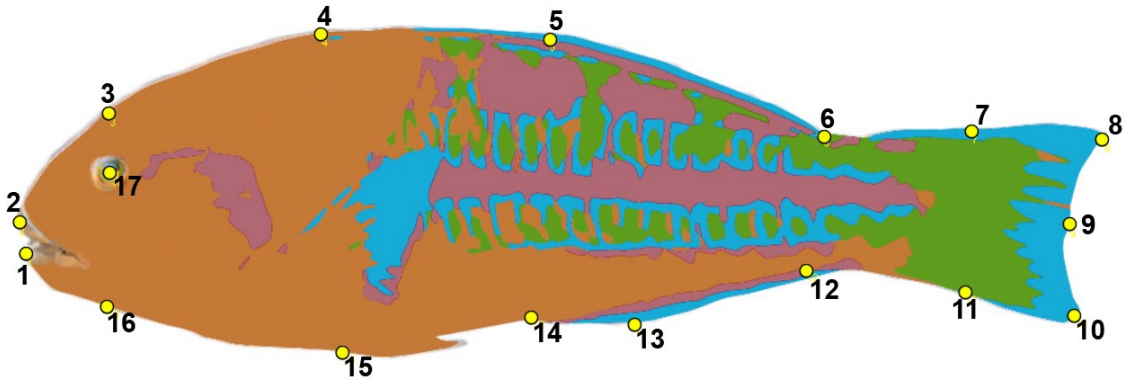

Fig. S. 9 - Landmark positions for the *Thalassoma* clade.

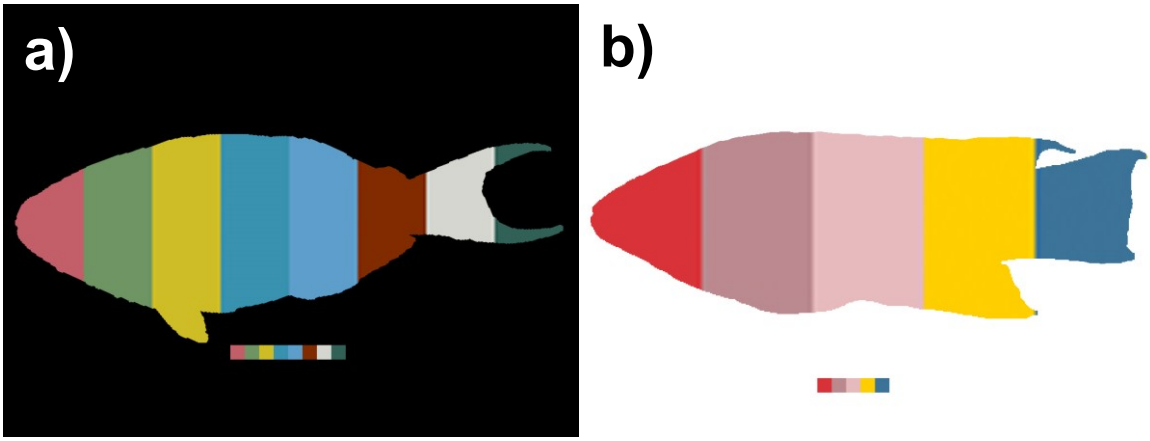

Fig. S. 10 – Dummy species used to run Patternize: a) *S. viride* used as outline for the *Sparisoma* clade; b) *B. rufus* used as outline for the *Bodianus* clade.

Supplementary results

For the *Sparisoma* "aurofrenatum" group (Fig. S. 11) we had three comparisons, two between allopatric species and one for sympatric species. The species pair *Sparisoma aurofrenatum* and *S.viride* with a 100% overlap in distribution did not have the highest scores from Colordistance and Patternize, with 0.82 and 0.99 respectively. The highest score was between *S. amplum* and *S. aurofrenatum* which are allopatric species (highest score is always set to 1.00). The allopatric comparison between *S. amplum* and *S. viride* had the lowest scores (Colordistance = 0.38; Patternize = 0.68). Averaging the allopatric comparisons scores they had scores of 0.69 for Colordistance and 0.84 for Patternize which are smaller than the respective sympatric scores of 0.82 and 0.99. On the PCA from Patternize, *S. amplum* and *S. viride* are grouped close together and *S. aurofrenatum* is placed on the opposite side of the *x*-axis.

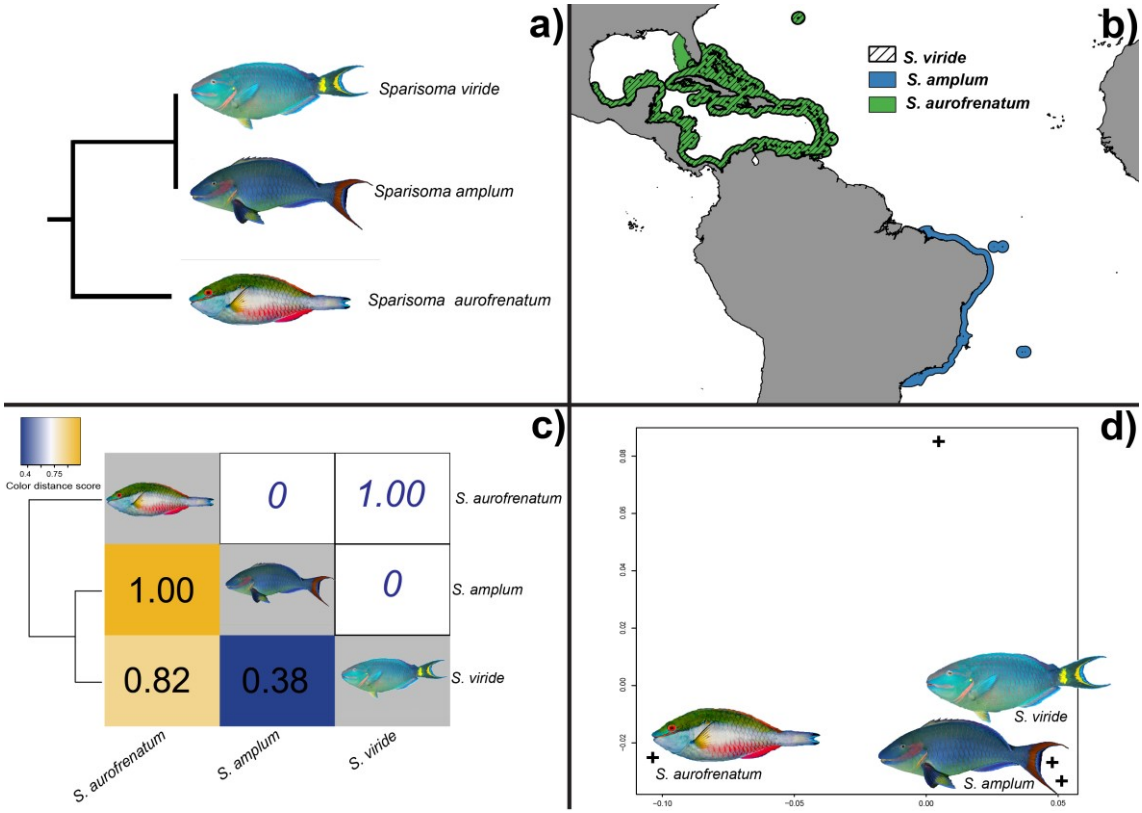

Fig. S. 11 – Sparisoma *"aurofrenatum" group:* ***a)*** *Phylogeny adapted from Robertson et al. (2006);* ***b)*** *Species geographical distribution;* ***c)*** *Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix;* ***d)*** *PCA based on the Patternize scores. The unlabeled cross on the top of the figure represents the dummy species used to run the analysis, as it needs at least four species to be able to run.*

The other clade we tested with only three species is the *Bodianus* "rufus" group (Fig. S. 12). It also had an overall greater scores for the sympatric species compared to the allopatric ones with Colordistance scores of 0.62 for allopatric species and 1.00 for sympatric and Patternize scores of 0.84 for allopatry and 0.92 for sympatry. The highest Colordistance score was between the sympatric species pair of *Bodianus pulchellus* and *B. rufus* yet the highest Patternize score was between *B. insularis* and *B. rufus* which are allopatric. The Patternize PCA shows that *B. insularis* and *B. pulchellus* are placed closer together far from *B. rufus.*

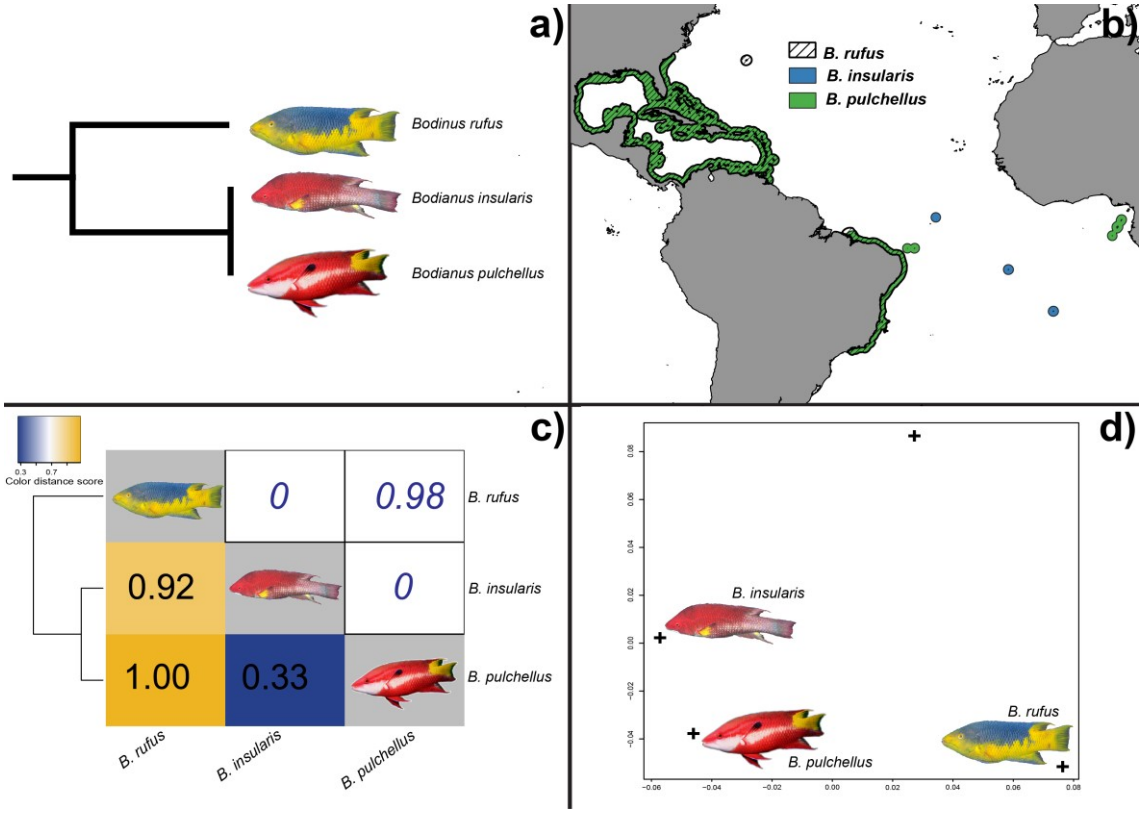

Fig. S. 12 – *Bodianus* "rufus" clade. a) Phylogeny adapted from Santini et al. (2016) and da Motta-Neto et al., (2020); **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores

The *Gramma* clade has five species (Fig. S. 13), one of them (*Gramma brasiliensis*) is endemic to Brazil and the other four are all sympatric with varying percentages of distribution overlap at the Caribbean Sea. The average scores of sympatric comparisons are 0.71 for Colordistance and 0.83 for Patternize. The averages among allopatric species were lower for both Colordistance scores with 0.44 and 0.58 for Patternize. For Colordistance the highest score was between *G. dejongi* and *G. linki*, and for the Patternize score it was with *G. linki* and *G. loreto. G. brasiliensis* and *G. loreto* are allopatric species and had the lowest score for Colordistance (0.09) and Patternize (0.24). On the PCA, *G. brasiliensis* and *G. loreto* are grouped close together, *G. dejongi* and *G. melacara* are also located near the same value on the *x*-axis but

on opposite sides of the *y*-axis. Yet *G. linki* is located far from the other four species.

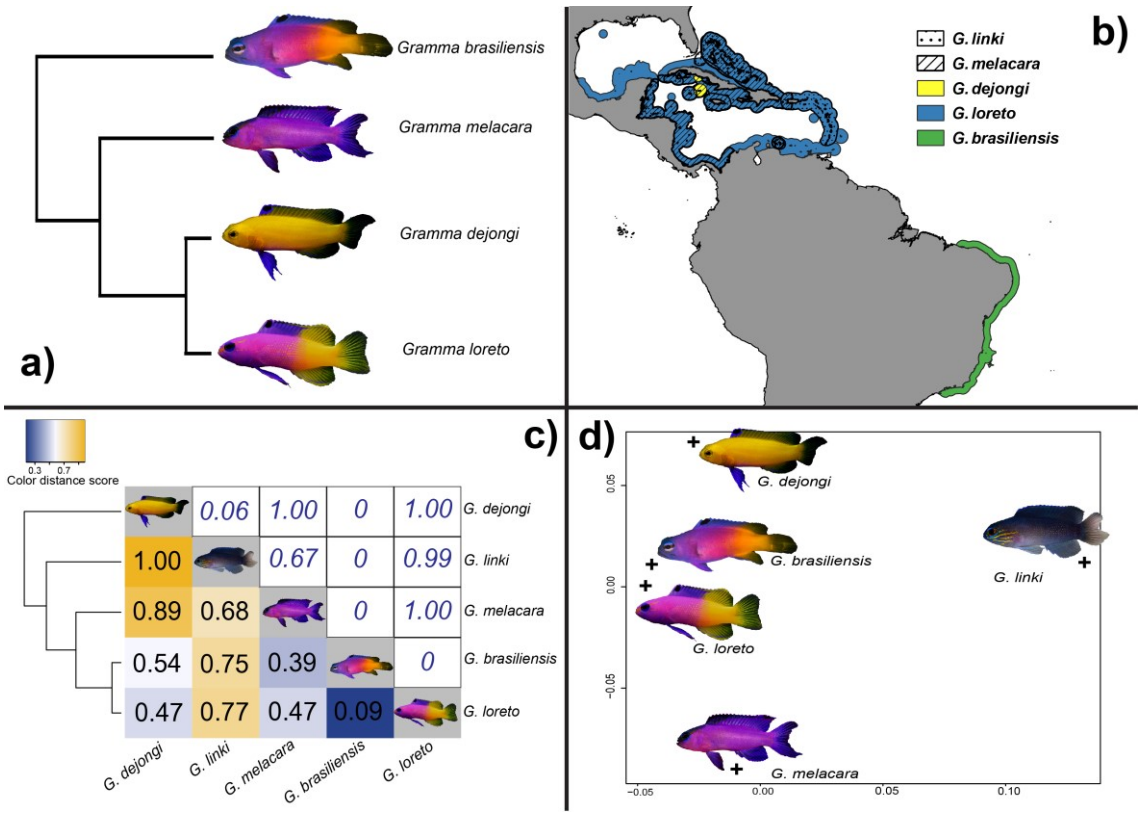

Fig. S. 13 - *Gramma* 'brasiliensis' group: **a)** Phylogeny adapted from (Duarte, 2017), G. linki did not had any available DNA sequence in GenBank; **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

The most widespread clade (Fig. S. 14), *Centropyge* "acanthops" complex, with species from the Western Atlantic, Mid Atlantic Ridge, Eastern Atlantic, Western Indian all the way to the Hawaiian Archipelago. Its group score was the same for allopatric and sympatric comparisons in the Colordistance score with 0.46, although the Patternize score had a higher score for allopatric comparisons (0.65) than for sympatric (0.53). Of the 10 comparisons, only two were of sympatric species. The highest score was between the allopatric species pair of *C. argi* and *C. fisheri.* The allopatric species, *C. aurantonotus* and *C. resplendens*, scored the lowest value on

Colordistance with 0.06. The Patternize lowest value (0.38) was between *C. argi* and *C. aurantonotus* which is a sympatric pair but is closely followed by the allopatric pair *C. acanthops* and *C. aurantonotus* with 0.39 as the score. On the PCA analysis the score results do not seem well represented, with the closest species being *C. aurantonotus* and *C. acanthops* and with the exception of *C. fisheri* that is located on the far right of the *x*-axis all the other species are located towards the left side of the *x*-axis and evenly spread through the *y*-axis.

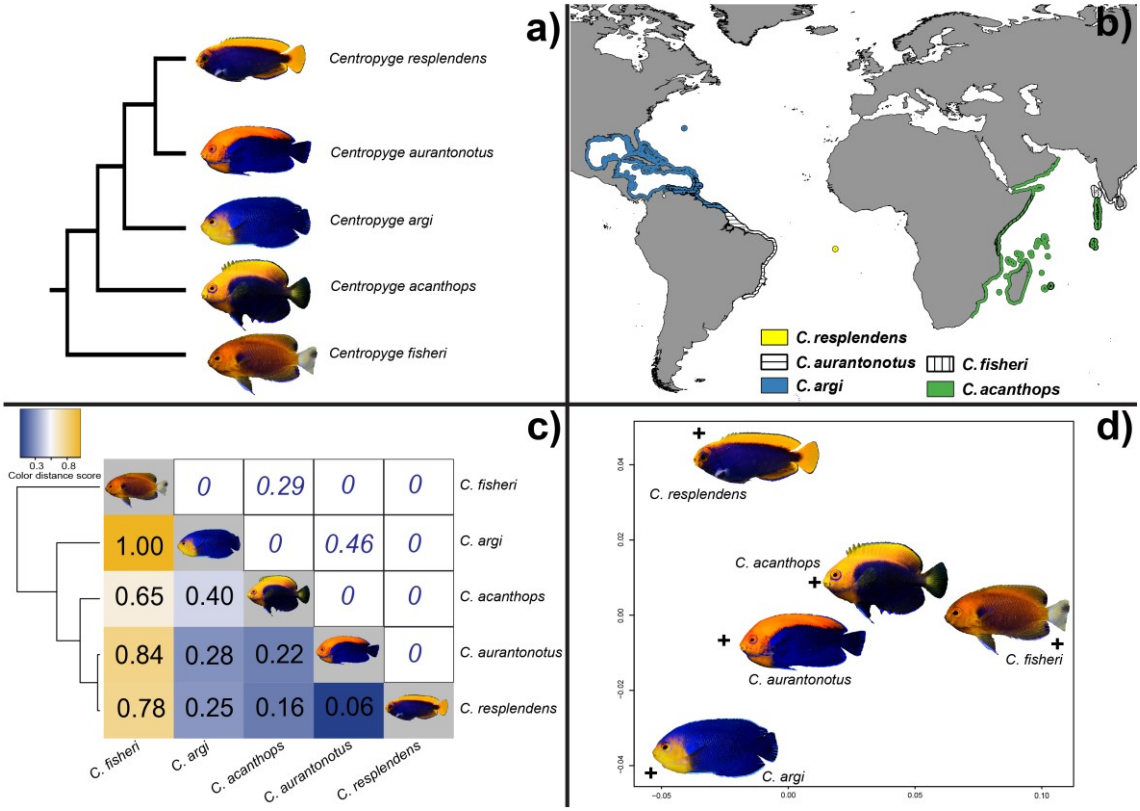

Fig. S. 14 - *Centropyge* 'acanthops complex'; **a)** Phylogeny adapted from Gaither et al. (2014); **b)** Species geographical distribution, *C. fisheri* distribution extends all the way to Hawaii but it is not shown here; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

This was the clade (Fig. S. 15) without phylogeny but we were able to include the whole genus *Pictichromis* so we had a monophyletic group that we could evaluate. The same species pair, the sympatric *P. diadema* and *P. porphyrea*, had the highest score for both Colordistance and Patternize. Two

species pair had the lowest value for Colordistance, *P. aurifrons* and *P. caitlinae* scored 0.06 as did *P. dinar* and *P. paccagnellorum.* They were closely followed by *P. coralensis* and *P. paccagnellorum* that scored 0.07 on Colordistance. For Patternize there was a similar outcome with one of the same pairs, *P. dinar* and *P. paccagnellorum,* and a different species pair, *P. coralensis* and *P. dinar*, that both scored 0.15, the lowest score on Patternize for this clade. But as in Colordistance comparisons, there was also a result with a very close value from one of the pairs that scored the lowest value on Colordistance, *P. aurifrons* and *P. caitlinae*, which scored 0.16 on the Patternize analysis. Taking all species from the clade into account the overall average was 0.4 for allopatric species and 0.45 for sympatric species on Colordistance. Yet on Patternize, with all the species the allopatric score was 0.56 and 0.55 for the sympatric species. We also tested this group without the microendemics that are within the range of other species (*P. aurifrons, P. caitlinae, P. dinar* and *P. ephippiata*). Using only the species with bigger distribution areas (*P. coralensis, P. diadema, P. paccagnellorum* and *P. porphyrea*) the difference between allopatric and sympatric increased. For Colordistance the allopatric average was 0.38 and 0.82 for sympatric. For allopatry the Patternize score average was 0.48 and 0.79 for sympatry. The PCA grouped the species trio *P. coralensis, P. dinar* and *P. paccagnellorum* and placed them at the right side of the *x*-axis and the bottom of the *y*-axis. Also near the bottom of the *y*-axis, on the left side of the *x*-axis are *P. porphyrea* and *P. ephippiata*, close to one another but not forming a distinctive grouping. On the left side of the *x*-axis, closer to the top of the *y*-axis is a cluster formed by *P. aurifrons* and *P. caitlinae*. Isolated at the upper right corner is *P. diadema*.

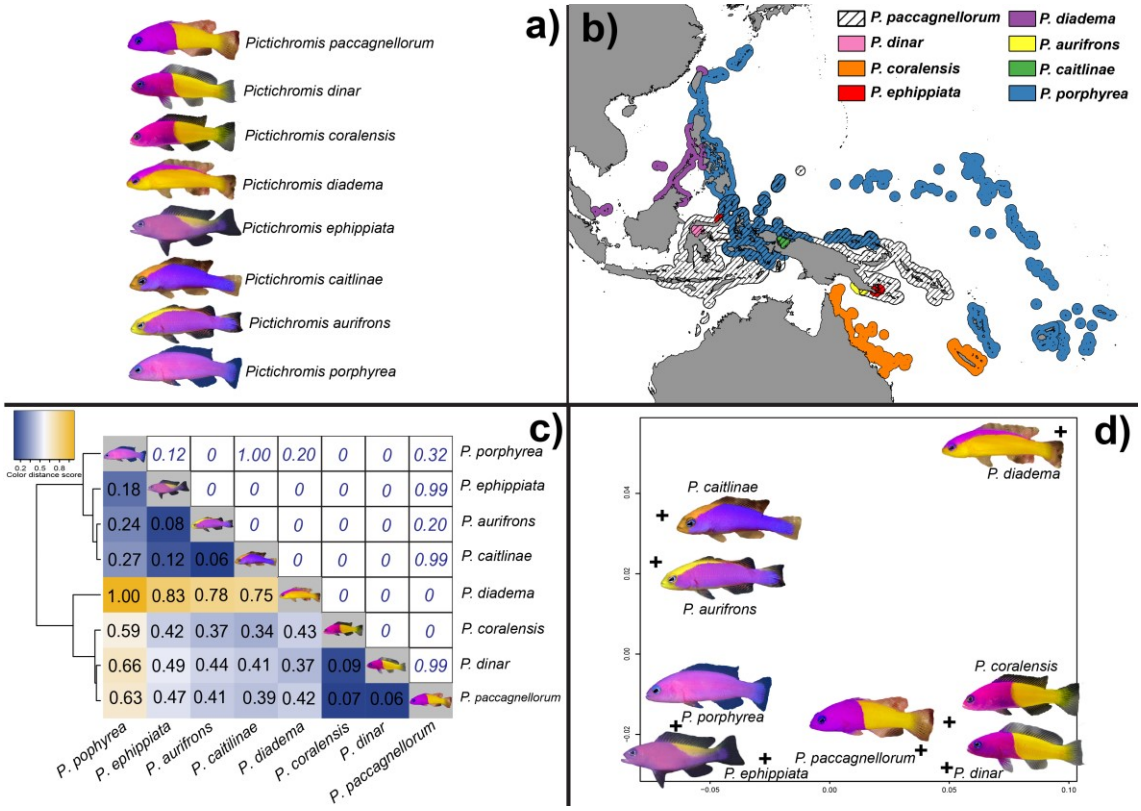

Fig. S. 15 - *Pictichromis* genus: **a)** All species present on the genus; **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

The biggest clade tested with ten species was *Thalassoma* "lunare" group (Fig. S. 16) and it scored as a whole in Colordistance 0.5 for allopatric comparisons and 0.54 for sympatric comparisons. The averages for the total group in Patternize were 0.72 for allopatry and 0.8 for sympatry. It is a wide spread group with species from Eastern African coast all the way to the American western coast. With one particular species, *T. purpureum*, having a distribution from the South African coast, up to the Red Sea and across the whole Indian and Pacific Ocean to the Tropical Eastern Pacific. It is sympatric to all but one species, *T. virens*. Several of this *Thalasoma* species co-occur at and around the Indo-Australian Archipelago, but a few species (*T. lucasanum,*

T. robertsoni and *T. virens*) are endemic to the Tropical Eastern Pacific. The other two species with more restricted distributions are *T. loxum* endemic to the Oman Coast and *T. cupido* that occurs only from Taiwan to South Korea, around Southern Japan and on a few islands nearby. This clade had different highest scoring pairs for Colordistance and Patternize. On the Colordistance analysis the highest scoring pair was *T. robertsoni* and *T. virens*, a sympatric pair. On Patternize the highest scoring species were the sympatric *T. cupido* and *T. lunare.* The allopatric species pair *T. lucasanum* and *T. robertsoni* had the lowest scoring species pair on Colordistance (*T. cupido* and *T. loxum* with 0.13) as well as the third (*T. amblycephalum* and *T. cupido* with 0.14) had a contrasting result on Patternize scoring 0.68 and 0.75 respectively. *Thalassoma lucasanum* and *T. robertsoni* formed the closest grouping on the PCA on the upper left corner and *T. amblycephalum* is not too far off to the right. Also on the top closer to the middle of the *x*-axis are *T. loxum* and *T. cupido* close to each other as are the other pair on the far right side of the *x*-axis, *T. purpureum* and *T. virens* but none of this pairs are as tight as *T. lucasanum* and *T. robertsoni.* More isolated from the other is *T. trilobatum* on the center of the graph and *T. lunare* which is the farthest from all other species on the bottom of the *y*-axis on the middle of the *x*-axis.

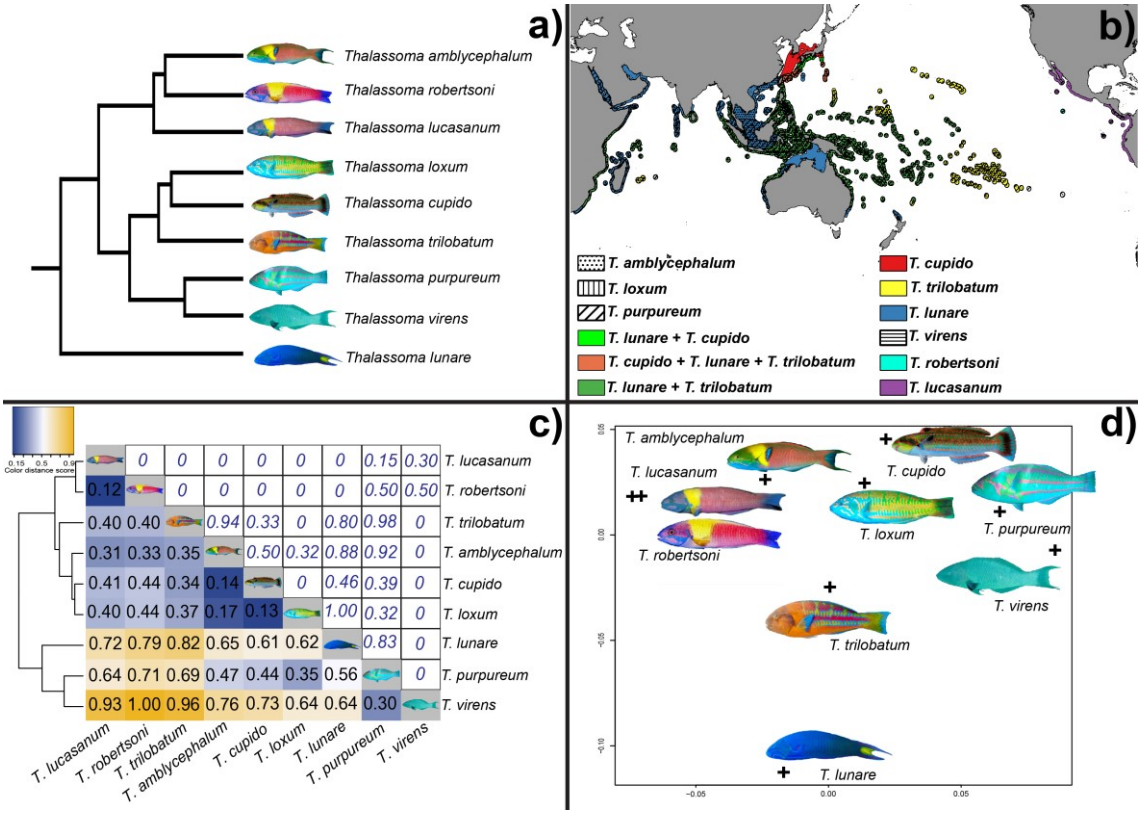

Fig. S. 16 - *Thalassoma* 'lunare' group: **a)** Phylogeny adapted from Bernardi et al. (2004); **b)** Species geographical distribution; **c)** Colordistance score matrix and cluster on the lower left. Percent distribution overlap on the top right side of the matrix; **d)** PCA based on the Patternize scores.

Table S. 1 – Table with all the distribution analysis and dissimilarity scores from Colordistance and Patternize.

Clade	Species A	Species B	Overlap	Range Symmetry	Colordistance	Colordistance normalized	Paternize	Patternize normalized
Sparisoma	*S. amplum*	*S. aurofrenatum*	0	0.19	0.35	1	0.16	1
Sparisoma	*S. amplum*	*S. viride*	0	0.19	0.14	0.38	0.11	0.68
Sparisoma	*S. aurofrenatum*	*S. viride*	1	0.49	0.29	0.82	0.16	0.99
Bodianus	*B. insularis*	*B. pulchellus*	0	0.03	0.19	0.33	0.10	0.68
Bodianus	*B. insularis*	*B. rufus*	0	0.03	0.52	0.92	0.15	1
Bodianus	*B. pulchellus*	*B. rufus*	0.98	0.49	0.57	1	0.14	0.92
Gramma	*G. brasiliensis*	*G. dejongi*	0	0.09	0.48	0.54	0.08	0.47
Gramma	*G. brasiliensis*	*G. linki*	0	0.30	0.66	0.75	0.18	0.98
Gramma	*G. brasiliensis*	*G. loreto*	0	0.14	0.08	0.09	0.04	0.24
Gramma	*G. brasiliensis*	*G. melacara*	0	0.22	0.34	0.39	0.12	0.64
Gramma	*G. dejongi*	*G. linki*	0.06	0.04	0.88	1	0.17	0.96
Gramma	*G. dejongi*	*G. loreto*	1	0.02	0.42	0.47	0.09	0.52
Gramma	*G. dejongi*	*G. melacara*	1	0.03	0.79	0.89	0.16	0.89
Gramma	*G. linki*	*G. loreto*	0.99	0.27	0.68	0.77	0.18	1
Gramma	*G. linki*	*G. melacara*	0.67	0.40	0.60	0.68	0.18	0.98
Gramma	*G. loreto*	*G. melacara*	1	0.36	0.41	0.47	0.11	0.61
Halichoeres	*H. brasiliensis*	*H. cyanocephalus*	0	0.20	0.47	0.98	0.25	1
Halichoeres	*H. brasiliensis*	*H. dimidiatus*	0.72	0.41	0.48	1	0.23	0.93
Halichoeres	*H. brasiliensis*	*H. garnoti*	0	0.18	0.40	0.84	0.23	0.92
Halichoeres	*H. brasiliensis*	*H. radiatus*	0	0.17	0.19	0.40	0.16	0.64
Halichoeres	*H. cyanocephalus*	*H. dimidiatus*	0	0.26	0.06	0.13	0.11	0.43
Halichoeres	*H. cyanocephalus*	*H. garnoti*	1	0.46	0.37	0.77	0.17	0.70
Halichoeres	*H. cyanocephalus*	*H. radiatus*	1	0.45	0.43	0.90	0.24	0.96
Halichoeres	*H. dimidiatus*	*H. garnoti*	0	0.23	0.37	0.77	0.19	0.76
Halichoeres	*H. dimidiatus*	*H. radiatus*	0.08	0.22	0.46	0.96	0.22	0.89
Halichoeres	*H. garnoti*	*H. radiatus*	1	0.49	0.44	0.93	0.23	0.92

Centropyge	*C. acanthops*	*C. argi*	0	0.45	0.31	0.40	0.09	0.57
Centropyge	*C. acanthops*	*C. aurantonotus*	0	0.33	0.17	0.22	0.06	0.39
Centropyge	*C. acanthops*	*C. fisheri*	0.29	0.13	0.50	0.65	0.11	0.67
Centropyge	*C. acanthops*	*C. resplendens*	0	0.01	0.12	0.16	0.08	0.49
Centropyge	*C. argi*	*C. aurantonotus*	0.46	0.29	0.21	0.28	0.06	0.38
Centropyge	*C. argi*	*C. fisheri*	0	0.16	0.77	1	0.16	1
Centropyge	*C. argi*	*C. resplendens*	0	0.01	0.19	0.25	0.09	0.56
Centropyge	*C. aurantonotus*	*C. fisheri*	0	0.07	0.65	0.84	0.14	0.83
Centropyge	*C. aurantonotus*	*C. resplendens*	0	0.03	0.05	0.06	0.07	0.41
Centropyge	*C. fisheri*	*C. resplendens*	0	0	0.60	0.78	0.15	0.93
Lutjanus	*L. bengalensis*	*L. boutton*	0.58	0.32	0.42	1	0.16	1
Lutjanus	*L. bengalensis*	*L. kasmira*	0.93	0.16	0.15	0.37	0.11	0.69
Lutjanus	*L. bengalensis*	*L. notatus*	0.55	0.12	0.24	0.57	0.11	0.66
Lutjanus	*L. bengalensis*	*L. quinquilineatus*	0.61	0.26	0.24	0.57	0.10	0.61
Lutjanus	*L. bengalensis*	*L. viridis*	0	0.15	0.15	0.35	0.08	0.52
Lutjanus	*L. boutton*	*L. kasmira*	1	0.29	0.39	0.93	0.13	0.80
Lutjanus	*L. boutton*	*L. notatus*	0	0.06	0.33	0.78	0.11	0.70
Lutjanus	*L. boutton*	*L. quinquilineatus*	0.91	0.43	0.35	0.84	0.11	0.66
Lutjanus	*L. boutton*	*L. viridis*	0	0.08	0.39	0.93	0.12	0.78
Lutjanus	*L. kasmira*	*L. notatus*	0.99	0.03	0.14	0.34	0.08	0.50
Lutjanus	*L. kasmira*	*L. quinquilineatus*	0.92	0.35	0.15	0.35	0.07	0.46
Lutjanus	*L. kasmira*	*L. viridis*	0	0.03	0.08	0.19	0.08	0.51
Lutjanus	*L. notatus*	*L. quinquilineatus*	0	0.05	0.03	0.08	0.05	0.29
Lutjanus	*L. notatus*	*L. viridis*	0	0.44	0.11	0.26	0.07	0.43
Lutjanus	*L. quinquilineatus*	*L. viridis*	0	0.06	0.11	0.27	0.05	0.32
Pictichromis	*P. aurifrons*	*P. caitlinae*	0	0.38	0.04	0.06	0.03	0.16
Pictichromis	*P. aurifrons*	*P. coralensis*	0	0.07	0.24	0.37	0.13	0.73
Pictichromis	*P. aurifrons*	*P. diadema*	0	0.12	0.50	0.78	0.17	0.97
Pictichromis	*P. aurifrons*	*P. dinar*	0	0.37	0.28	0.44	0.13	0.75

Pictichromis	*P. aurifrons*	*P. ephippiata*	0	0.45	0.05	0.08	0.07	0.39
Pictichromis	*P. aurifrons*	*P. paccagnellorum*	0.20	0.02	0.27	0.41	0.12	0.69
Pictichromis	*P. aurifrons*	*P. porphyrea*	0	0.01	0.15	0.24	0.05	0.30
Pictichromis	*P. caitlinae*	*P. coralensis*	0	0.04	0.22	0.34	0.13	0.74
Pictichromis	*P. caitlinae*	*P. diadema*	0	0.07	0.49	0.75	0.17	0.94
Pictichromis	*P. caitlinae*	*P. dinar*	0	0.49	0.27	0.41	0.14	0.76
Pictichromis	*P. caitlinae*	*P. ephippiata*	0	0.42	0.08	0.12	0.08	0.43
Pictichromis	*P. caitlinae*	*P. paccagnellorum*	0.99	0.01	0.25	0.39	0.12	0.70
Pictichromis	*P. caitlinae*	*P. porphyrea*	1	0	0.18	0.27	0.05	0.31
Pictichromis	*P. coralensis*	*P. diadema*	0	0.37	0.28	0.43	0.09	0.50
Pictichromis	*P. coralensis*	*P. dinar*	0	0.04	0.06	0.09	0.03	0.15
Pictichromis	*P. coralensis*	*P. ephippiata*	0	0.06	0.27	0.42	0.08	0.44
Pictichromis	*P. coralensis*	*P. paccagnellorum*	0	0.18	0.04	0.07	0.03	0.18
Pictichromis	*P. coralensis*	*P. porphyrea*	0	0.07	0.38	0.59	0.12	0.67
Pictichromis	*P. diadema*	*P. dinar*	0	0.07	0.24	0.37	0.10	0.55
Pictichromis	*P. diadema*	*P. ephippiata*	0	0.10	0.54	0.83	0.15	0.84
Pictichromis	*P. diadema*	*P. paccagnellorum*	0	0.11	0.27	0.42	0.10	0.56
Pictichromis	*P. diadema*	*P. porphyrea*	0.20	0.04	0.65	1	0.18	1
Pictichromis	*P. dinar*	*P. ephippiata*	0	0.42	0.32	0.49	0.08	0.45
Pictichromis	*P. dinar*	*P. paccagnellorum*	0.99	0.01	0.04	0.06	0.03	0.15
Pictichromis	*P. dinar*	*P. porphyrea*	0	0	0.43	0.66	0.12	0.65
Pictichromis	*P. ephippiata*	*P. paccagnellorum*	0.99	0.01	0.30	0.47	0.07	0.40
Pictichromis	*P. ephippiata*	*P. porphyrea*	0.12	0	0.12	0.18	0.05	0.29
Pictichromis	*P. paccagnellorum*	*P. porphyrea*	0.32	0.26	0.41	0.63	0.10	0.59
Thalassoma	*T. amblycephalum*	*T. cupido*	0.50	0.07	0.10	0.14	0.13	0.75
Thalassoma	*T. amblycephalum*	*T. loxum*	0.32	0.01	0.13	0.17	0.11	0.60
Thalassoma	*T. amblycephalum*	*T. lucasanum*	0	0.05	0.23	0.31	0.09	0.53
Thalassoma	*T. amblycephalum*	*T. lunare*	0.88	0.49	0.48	0.65	0.16	0.88
Thalassoma	*T. amblycephalum*	*T. purpureum*	0.92	0.49	0.35	0.47	0.12	0.70

Thalassoma	*T. amblycephalum*	*T. robertsoni*	0	0	0.25	0.33	0.09	0.51
Thalassoma	*T. amblycephalum*	*T. trilobatum*	0.94	0.44	0.26	0.35	0.14	0.80
Thalassoma	*T. amblycephalum*	*T. virens*	0	0.01	0.57	0.76	0.13	0.72
Thalassoma	*T. cupido*	*T. loxum*	0	0.16	0.10	0.13	0.12	0.68
Thalassoma	*T. cupido*	*T. lucasanum*	0	0.43	0.30	0.41	0.15	0.82
Thalassoma	*T. cupido*	*T. lunare*	0.46	0.07	0.46	0.61	0.18	1
Thalassoma	*T. cupido*	*T. purpureum*	0.39	0.07	0.33	0.44	0.14	0.78
Thalassoma	*T. cupido*	*T. robertsoni*	0	0.04	0.33	0.44	0.15	0.83
Thalassoma	*T. cupido*	*T. trilobatum*	0.33	0.09	0.26	0.34	0.16	0.89
Thalassoma	*T. cupido*	*T. virens*	0	0.10	0.54	0.73	0.14	0.82
Thalassoma	*T. loxum*	*T. lucasanum*	0	0.20	0.30	0.40	0.12	0.65
Thalassoma	*T. loxum*	*T. lunare*	1	0.01	0.47	0.62	0.16	0.89
Thalassoma	*T. loxum*	*T. purpureum*	0.32	0.01	0.26	0.35	0.11	0.62
Thalassoma	*T. loxum*	*T. robertsoni*	0	0.20	0.33	0.44	0.12	0.67
Thalassoma	*T. loxum*	*T. trilobatum*	0	0.02	0.28	0.37	0.14	0.77
Thalassoma	*T. loxum*	*T. virens*	0	0.38	0.48	0.64	0.12	0.65
Thalassoma	*T. lucasanum*	*T. lunare*	0	0.05	0.54	0.72	0.15	0.87
Thalassoma	*T. lucasanum*	*T. purpureum*	0.15	0.05	0.48	0.64	0.14	0.81
Thalassoma	*T. lucasanum*	*T. robertsoni*	0	0.06	0.09	0.12	0.05	0.27
Thalassoma	*T. lucasanum*	*T. trilobatum*	0	0.07	0.30	0.40	0.14	0.78
Thalassoma	*T. lucasanum*	*T. virens*	0.30	0.13	0.70	0.93	0.16	0.93
Thalassoma	*T. lunare*	*T. purpureum*	0.83	0.50	0.42	0.56	0.17	0.93
Thalassoma	*T. lunare*	*T. robertsoni*	0	0	0.59	0.79	0.15	0.87
Thalassoma	*T. lunare*	*T. trilobatum*	0.80	0.44	0.62	0.82	0.16	0.91
Thalassoma	*T. lunare*	*T. virens*	0	0.01	0.48	0.64	0.16	0.90
Thalassoma	*T. purpureum*	*T. robertsoni*	0.50	0	0.53	0.71	0.15	0.82
Thalassoma	*T. purpureum*	*T. trilobatum*	0.98	0.44	0.52	0.69	0.14	0.80
Thalassoma	*T. purpureum*	*T. virens*	0.20	0.01	0.22	0.30	0.07	0.40
Thalassoma	*T. robertsoni*	*T. trilobatum*	0	0	0.30	0.40	0.14	0.80

Thalassoma	*T. robertsoni*	*T. virens*	0.50	0.29	0.75	1	0.17	0.94
Thalassoma	*T. trilobatum*	*T. virens*	0	0.01	0.71	0.96	0.15	0.83