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Phylogeography of the sergeants Abudefduf sexfasciatus and A. vaigiensis reveals complex introgression patterns between two widespread and sympatric Indo-West Pacific reef fishes

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#### Abstract

On evolutionary time scales, sea level oscillations lead to recurrent spatio-temporal variation in species distribution and population connectivity. In this situation, applying classical concepts of biogeography is challenging yet necessary to understand the mechanisms underlying biodiversity in highly diverse marine ecosystems such as coral reefs. We aimed at studying the outcomes of such complex biogeographical dynamics on reproductive isolation by sampling populations across a wide spatial range of a species-rich fish genus: the sergeants (Pomacentridae: Abudefduff. We generated a mutli-locus data set that included ten species from 32 Indo-West Pacific localities. We observed a pattern of mito-nuclear discordance in two of the most common and widely distributed species: Abudefduf sexfasciatus and $A$. vaigiensis. The results showed three regional sublineages (Indian Ocean, Coral Triangle region, western Pacific) in $A$. sexfasciatus ( $0.6-1.5 \%$ divergence at locus cytb). The other species, $A$. vaigiensis consisted of three distinct lineages ( $\mathrm{A}, \mathrm{B}$, and C ) whose geographic ranges overlapped ( $9 \%$ divergence at locus $c y t b$ ). Although $A$. vaigiensis lineage A and $A$. sexfasciatus were found to be distinct based on nuclear information, $A$. vaigiensis lineage A was nested within $A$. sexfasciatus in the mitochondrial gene tree. Abudefduf sexfasciatus from the Coral Triangle region and $A$. vaigiensis lineage A were not differentiated from each other at the mitochondrial locus. We then used coalescent-based simulation to characterize a spatially widespread, weak gene flow between the two species. These fishes may be considered as candidates of choice to investigate the complexity of the discrepancies between phenotypic and genetic evolution among sibling species.


## Introduction

Evolutionary processes that lead to reproductive isolation between populations and/or species can be temporally and spatially dynamic. For instance, closely related and previously allopatric species that come into contact may interbreed and form hybrid zones (Barton \& Hewitt, 1985; 1989; Mallet, 2005; Price et al. 2008). Hybrid zones have been intensively studied in terrestrial systems (Harrison, 1993) but have been considered as rare in the sea until recently. However, the number of studies documenting marine hybrid zones has increased within the last decade (DiBattista et al. 2015; Montanari et al. 2012; 2014; 2016 and references therein for examples in fishes).

Characterizing spatial and temporal patterns of inter-specific gene flow in marine systems may have been more difficult than in terrestrial or riverine organisms because marine populations are generally more connected and are expected to experience higher levels of gene flow. In this context, inter-specific gene flow may give rise to complex patterns of hybridization (Fraïsse et al. 2014; Le Moan et al. 2016), not only along the edges of species' ranges, but virtually everywhere their distributions overlap, sometimes resulting in mosaic-like patterns of hybridization (Hobbs et al. 2009; Hobbs \& Allen, 2014). These particularities challenge the classical vision of the mechanisms underlying species formation and/or their integrity in spite of potential gene flow. Yet, it is of primary importance to investigate patterns of biodiversity in such marine ecosystems with complex and highly dynamic histories (see Bowen et al. 2013). Moreover, the role of hybridization in biodiversity is still debated, as it has been considered either as source of evolutionary novelty or as a homogenising process and a potential threat for biological diversity (Seehausen, 2004; Abbott et al. 2013).

The tropical Indo-West Pacific is the largest and most speciose marine biogeographic region (Crandall \& Riginos, 2014). Out of the 3919 reef fish species that occur in the Indo-West Pacific, $69.1 \%$ are widely distributed from the western Indian Ocean to the western Pacific (Allen, 2008; Briggs \& Bowen, 2012; 2013). Marine populations of the western Pacific and the Indian ocean have undergone a succession of stages of geographic isolation on either side of the Indo-Pacific barrier, and subsequent secondary contact (Gaither \& Rocha, 2013) due to cyclic sea-level oscillations (Voris, 2000; Siddall et al. 2003; Naish et al. 2009). This has considerably but also differently impacted the phylogeography of species in this region but also led to very different outcomes. While some species exhibit a strong Indian $v$ s. Pacific phylogeographic structure, others show an apparent lack of differentiation as well as a range of intermediate situations (Gaither \& Rocha, 2013; Bowen et al. 2016; Borsa et al. 2016). Even closely related congeneric species display strikingly different patterns of spatial genetic differentiation. Gaither et al. (2010) found a lack of genetic differentiation across the whole Indo-Pacific in the snapper Lutjanus kasmira but high levels of structure at all geographical scales in the closely related species: L. fulvus. These results are similar to the ones reported by DiBattista et al. (2012) between the undifferentiated Chaetodon meyeri and the structured populations of C. ornatissimus: two sister species of butterflyfishes. Horne and van Herwerden (2013) documented weak genetic differentiation at mitochondrial markers between Naso bexacanthus and N. caesisus whereas these two sister species of unicornfishes were distinct at nuclear loci. This variability indicates that evolutionary and ecological dynamics in these organisms are species-specific. It is even possibly observable at the intra-specific level (Carpenter et al. 2011; Borsa et al. 2016).

Highly diverse and widely distributed animal groups such as the damselfishes (Pomacentridae) provide an opportunity to investigate the different factors involved in generating and maintaining spatial patterns of phenotypic and genetic diversity and differentiation in our oceans. This family comprises 399 valid species in the Indo-Pacific (Eschmeyer et al. 2016). Previous phylogenetic studies have documented the systematics of the Pomacentridae family (e.g. Quenouille et al. 2004; Cooper et al. 2009). Other biogeographic studies have investigated species boundaries at lower taxonomic levels in this family but only a few of them highlighted cases of cross-species hybridization (but see Litsios \& Salamin, 2014 for a noticeable exception in clownfishes) suggesting that this phenomenon could be rare in Pomacentridae compared to other reef fishes from different families including Acanthuridae, Chaetodontidae, Labridae or Pomacanthidae (Montanari et al. 2016).

Within the Pomacentridae, the genus Abudefduf (sergeants) comprises Indo-West Pacific species that are generally widely distributed and locally abundant (Froese \& Pauly 2015). The evolutionary history of this genus is complex, with the possible existence of cryptic species in Abudefduf saxatilis and $A$. vaigiensis that have not been confirmed (Quenouille et al. 2004; 2011) as well as blurred boundaries between $A$. vaigiensis and $A$. sexfasciatus. In a DNA barcoding survey based on the cytochrome oxidase c subunit 1 gene (COI), Hubert et al. (2012) found that out of 668 coral reef fish species sampled in both Indian and Pacific oceans (Madagascar, Réunion and French Polynesia), only three pairs did not show clear inter-specific boundaries, possibly because of mitochondrial introgression or because of the presence of different morphotypes in one of these species. One of these pairs was $A$. sexfasciatus (Lacépède, 1801) and A. vaigiensis (Quoy \& Gaimard, 1825). A case of hybridization involving the Hawaiian endemic $A$. abdominalis and the recently introduced $A$. vaigiensis was also documented (Coleman et al. 2014). This study reported that hybridization is likely to be favoured by human activities, raised conservation issues for $A$. abdominalis, and demonstrated that the species barrier may quickly collapse after secondary contact in these fishes. Altogether, these results led us to consider the Indo-West Pacific species in the genus Abudefduf as candidates to investigate species formation in allopatry, secondary contact, hybridization and the mechanisms by which species integrity is maintained in parapatry or in sympatry, despite opportunity for inter-specific gene flow at broad geographic scales.

In this study, we first investigated the consistency between the phenotype-based taxonomy and the molecular systematics of species in the genus $A b u d e f d u f$. By incorporating several individuals from different populations in each species, we conducted multi-locus phylogenetic analyses including 11 (out of 12) sergeant species occurring in the Indo-West Pacific. We tested for the existence of cryptic lineages in A. vaigiensis. Based on an extensive geographic sampling, we then evaluated patterns of intra- and inter-specific differentiation to look for genetic structure, identify geographic barriers to gene flow and highlight potential hybridization. Finally, we investigated the magnitude, direction and locus-dependence of inter-specific gene flow between $A$. sexfasciatus and $A$. vaigiensis, to propose an evolutionary scenario to explain patterns of diversity and differentiation in these two widely sympatric Indo-West Pacific species.

## Materials and methods

## Collection of specimens

We examined a total of 488 Abudefduf spp. specimens collected between 2006 and 2015 from 32 localities across the tropical Indo-West Pacific (Fig. 1A; sampling details in Supplementary Table S1). Fishes were caught using nets during snorkelling sessions, by angling, or bought on local markets. We used colour patterns (number and colour of lateral stripes, presence of bars or spot in the caudal region, presence of yellow coloration in the dorsal region and flanks) to unambiguously identify the specimens to species. For each specimen, a muscle-tissue or fin-clip sample was taken, then preserved in $95 \%$ ethanol and stored at $-20^{\circ} \mathrm{C}$ in the Marine Biodiversity and Phylogenomics laboratory at the Institute of Oceanography, National Taiwan University (NTU). When possible, voucher specimens were photographed, then formaldehyde-fixed and deposited at the NTU Museum (NTUM), Taipei.

## Molecular procedures

Total genomic DNA was extracted from fin or muscle tissues using the automated LabTurbo 48 Compact System extractor (Taigene Biosciences Corp., Taipei) and LGD 480-500 DNA-extraction kits (Taigene Biosciences Corp.) following the manufacturer's protocol. Amplification of the complete cytochrome $b$ (cytb) gene together with a short fragment of the adjacent tRNA-Thr was done by polymerase chain reaction (PCR). DNA fragments at two nuclear loci were also PCR-amplified: a partial sequence of an exon of the Recombination Activating gene 1 ( $\mathrm{R} A G 1$ ) and a fragment of an intron of the Dystrophin gene $(D y s t)$. Primer sequences and additional
details are provided in Supplementary Table S2. PCRs were done in $25 \mu \mathrm{~L}$ reaction volume, containing $\sim 1-2 \mu \mathrm{~L}$ DNA template, 0.2 mM of each dNTP, $2 \mathrm{mM} \mathrm{MgCl} 2,0.4 \mu \mathrm{M}$ of each primer and 0.5 U GoTaq Flexi DNA polymerase (Promega, Madison WI, U.S.A.) in $5 \mu \mathrm{~L} 5 \mathrm{X}$ manufacturer's buffer plus $12.9 \mu \mathrm{~L}$ sterile distilled water. The thermal profile of the PCR was as follows: initial denaturation at $95^{\circ} \mathrm{C}$ for 4 min , followed by 36 cycles of denaturation at $95^{\circ} \mathrm{C}$ for 40 s , locus-specific annealing temperature for 40 s , elongation at $72^{\circ} \mathrm{C}$ for a locusspecific time, and a final elongation at $72^{\circ} \mathrm{C}$ for 7 min . The PCR products were then checked on $1 \%$ agarose gel, purified using the AMPure magnetic bead cleanup protocol (Agencourt Bioscience Corp., Beverly MA, U.S.A.), and sequenced in both directions by Sanger's method on an ABI 3730 analyzer (Applied Biosystems, Foster City CA, U.S.A) at the Centre of Biotechnology (National Taiwan University, Taipei).

## Phylogenetic inference

We sequenced the amplified fragments at the mitochondrial locus cytb on the full sample set and those at the nuclear loci, RAG1 and Dyst on taxonomically and spatially representative subsets. Chromatograms were examined using the viewing and editing features of GENEIOUS R v. 6.1.8 (Kearse et al. 2012). For each gene, sequences were first automatically aligned using the GENEIOUS homemade alignment method with default values. The alignments were subsequently checked by eye and adjusted manually when necessary. Indels were treated as missing data. All the sequences were deposited in GenBank under accession numbers KU553479 to KU554420 (Supplementary Table S1). We built two matrices of sequences: a mitochondrial one (cytb) and a nuclear one which consisted of the concatenated RAG1 and Dyst sequences. The RAG1 and Dyst markers were also considered separately. For single-gene tree reconstructions, we included all $A b u d e f d u f$ sequences available from GenBank in order to verify their phylogenetic position.

For each alignment, we determined the best partition scheme and the corresponding models of nucleotide substitution that best fit the data using PARTITIONFINDER v. 1.1.1 (Lanfear et al. 2012). Coding sequences (cytb and RAG1) were partitioned according to codon positions (first, second and third position). In PARTITIONFINDER, likelihood scores for all the models implemented in the phylogenetic reconstruction program MRBAYES v. 3.2.6 (Ronquist et al. 2012) were computed and we selected the best-fit models according to the Bayesian Information Criterion associated with the 'greedy search algorithm' option. We then conducted phylogenetic analyses based on maximum likelihood and Bayesian methods using the programs, RAxML v. 8 (Stamatakis, 2014) and MRBAYES, respectively. For Bayesian inference, two independent runs (each with four Markov chains) were made simultaneously for 10 millions of generations and sampled every 1000 generations. We then evaluated the convergence within and across runs. The first $25 \%$ iterations were discarded as burn-in and the remainder was used to generate a $50 \%$ majority rule consensus tree. For Maximum Likelihood analyses, the ML search was run simultaneously with an automatic bootstrapping procedure ('autoMRE' criteria) on each gene partition and following the GTRCAT approximation. Computations were run online through the Cypress Science Gateway portal (www.phylo.org; Miller et al. 2010).

## Genetic diversity, population differentiation and demography

We first focused on mitochondrial sequences (cytb) to compute parameters characterizing the genetic diversity of $A$. sexfasciatus and $A$. vaigiensis lineage A populations, including the number of polymorphic sites per sequence (S), the nucleotide diversity ( $\pi$, Tajima, 1989), and the haplotype diversity ( $H_{d}$; Nei, 1987). To investigate spatial restriction in gene flow, genetic structure was assessed according to the AMOVA framework (Excoffier et al. 1992). We investigated the proportion of genetic variance explained by the differences among regions (considered as groups): Indian Ocean/Coral Triangle region/western Pacific ( $F_{\mathrm{CT}}$ ), and among localities within regions $\left(F_{S C}\right)$ in each of these two species. The AMOVA has been primarily designed for the intra-specific level. However, the degree of inter-specific genetic differentiation at the mitochondrial locus between Coral Triangle region populations of $A$. sexfasciatus and $A$. vaigiensis lineage A was low compared to what it was at the intra-
specific level between regional subgroups of $A$. sexfasciatus (see Results). Therefore, we ran a second AMOVA to obtain a comparative quantification of the genetic differentiation between the two species (considered as groups, $F_{C T}$ ), at the mitochondrial locus, in the Coral Triangle region localities where they cooccured in our sampling (i.e. around Taiwan) and between these localities within each species ( $F_{\mathrm{SC}}$ ).

Departure from the mutation/drift equilibrium was estimated with Tajima's (1993) D and Fu's (1997) Fs. These two indices were initially conceived to detect deviation from selective neutrality but have been also shown to be sensitive to modifications in demographical dynamics. Population genetics analyses were done in Arlequin v. 3.5 (Excoffier et al. 2005). To visualise with more detail the genetic differentiation between A. sexfasciatus and A. vaigiensis lineage A, we reconstructed minimum-spanning mitochondrial haplotype networks using PopART (University of Otago, http://popart.otago.ac.nz). We checked for shared haplotypes between these two species.

The analyses related to genetic diversity were done on each nuclear marker separately (RAG1 and Dyst). The most likely haplotypes were first determined using the algorithm PHASE (Stephens \& Donelly, 2001) implemented in DNASP v. 5.10.1 (Librado \& Rozas, 2009). To optimize haplotype inference without introducing false-positives, the PHASE thresholds were lowered to 0.6 as recommended in Garrick et al. (2010). For each of the two nuclear loci, we ran three replicate runs and changed the seed value each time.

## Inter-specific gene flow

Information obtained from the above analyses led us to characterize the joint effect of time and gene flow on the divergence between the weakly differentiated populations of the Coral Triangle region of $A$. sexfasciatus and A. vaigiensis A. We used the Bayesian coalescent-based estimation implemented in IMa2 (Hey \& Nielsen 2007; Hey 2010) to run this analysis on two populations. The first population consisted of the pooled samples of $A$. vaigiensis A , excluding the two genetically differentiated populations of the Maldives and Red Sea (see Results), while the other population consisted of the pooled samples of $A$. sexfasciatus from the Coral Triangle region (i.e. excluding the genetically differentiated Indian Ocean localities and Moruroa). The 'isolation-with-migration' model implemented in IMa2 assumes that the two populations (hereafter populations 1 and 2 ) of effective size $\theta_{1}$ and $\theta_{2}$ diverged from an ancestral population (of effective size $\theta_{\mathrm{A}}$ ) at time t and then exchanged genes at rates $m_{1}$ (from population 1 to population 2 ) and $m_{2}$ (from population 2 to population 1). The foregoing six parameters are scaled by mutation rate and therefore need to be converted to derive biologically realistic demographic estimates. Here, 2.N.m corresponds to the number of effective migrants with $N$ being the effective population size or the number of diploid individuals and $m$ being the mutation rate (Wright 1931).

The IMa2 analysis was basically run on the three locus data set. Additional partitions (mitochondrial / nuclear) were also considered. For the mitochondrial gene, the mutation rate was set to $1.3 \times 10^{-8}$ mutations per site per year (DiBattista et al. 2013) under the HKY model (Hasegawa et al. 1985) and an appropriate inheritance scalar of 0.25 . For the nuclear genes, we first used IMgc (Woerner et al. 2007) to do a 'four-gamete test' in order to detect and exclude potentially recombining blocks within nuclear loci. The mutation rate was set to $9.7 \times 10^{-10}$ for the autosomal exon RAG1 (Lessios 2008) and $7.5 \times 10^{-9}$ for the autosomal intron Dyst, following Eytan \& Hellberg (2010), under the Infinite-sites model (Kimura 1969). We assumed an inheritance scalar of 1 for both nuclear loci. Preliminary tests were run to characterize prior ranges that encompassed the entire distribution of each parameter estimates for use in subsequent runs. We then ran additional replicate runs with refined priors distributions and different random number seeds. Parameter estimates were congruent across these runs. A last series of runs consisting of 25 to 100 million steps (sampled every 1000 steps) with 0.5 million burn-in steps was carried out to obtain the final estimates. Convergence was checked by visually inspecting the trend lines of all parameters. To assess whether the complete model with gene flow was better supported than the model without gene flow, we tested whether simpler demographic models (with no gene flow) fitted the data. To do so, we followed the nested model approach in the 'load-tree mode' available in IMa2 according to the procedure described in Hey \& Nielsen (2007).

## Results

## Description of sequence datasets

A total of 942 sequences were generated in the course of this study ( 484 at locus cytb, 224 at locus RAG1 and 234 at locus Dyst, see details in Supplementary Table S1, Supporting information). Once additional sequences retrieved from GenBank had been included, the mitochondrial data set consisted of 522 sytb gene sequences aligned over 1161 bp . The nuclear data set consisted of 246 sequences at locus RAG1, aligned over 1456 bp and 234 sequences at locus Dyst aligned over 1211 bp . The two nuclear sequence data sets were concatenated into a single matrix of 204 individuals x 2667 nucleotide sites.

## Mitochondrial gene-based phylogeny

At the mitochondrial locus, six of the species surveyed (Abudefduf bengalensis, $A$. saxatilis, $A$. septemfasciatus, $A$. sordidus, $A$. sparoides and $A$. taurus) were monophyletic and presented low levels of intraspecific divergence (Fig. 2A). In contrast, $A$. vaigiensis was polyphyletic so we coined the three lineages sampled in this study A, B and C. Abudefuf sexfasciatus was paraphyletic with respect to $A$. vaigiensis A (Figs 1B, 2 and S1, Supporting information). Abudefuf vaigiensis A was the most abundant and widely distributed in our total A. vaigiensis samples; its representatives were spread within the clade that also contains the haplotypes from western Pacific $A$. sexfasciatus (Fig. 2A). Abudefuf vaigiensis B consisted of two geographically separated sublineages with one containing the samples collected from Taiping Island in the South China Sea, from Little Liuqiu Island in the Taiwan strait, West Papua in the Coral Triangle and from Guam Island in the West Pacific and the other one consisting of a unique individual from the western Indian Ocean. Abudefduf vaigiensis B was far less abundant than $A$. vaigiensis A with only six individuals of 217 A. vaigiensis sampled. The monophyletic $A$. vaigiensis C was recorded only from the Coral Triangle (with five individuals from the Bali Strait and six individuals from Caohagan Island in the Cebu Sea) (Fig. 1B). A fourth lineage within A. vaigiensis was represented by a single cytb gene sequence from Christmas Island (GenBank: AY208557; Quenouille et al. 2004).

The $A$. sexfasciatus samples included in the mitochondrial gene analysis can be subdivided into three phylogeographic or regional sublineages. One of them included all the individuals from the western Indian Ocean. A second one included all 12 individuals from Moruroa, one individual from New Caledonia, and one individual from West Papua. A third and the largest one, comprised the 124 remaining A. sexfasciatus from the Coral Triangle and the western Pacific Ocean, along with all 204 A. vaigiensis A individuals.

## Nuclear gene-based phylogeny

The nuclear gene-based phylogenetic tree also supported the monophyly of most Abudefduf species surveyed and showed little evidence of intraspecific divergence in most of the species that were sampled from both the Indian and Pacific Oceans. An exception was $A$. septemfasciatus for which a phylogeographic break (Indian vs. western Pacific partition) was observed (Figs 2B and S1, Supporting information). Interestingly, twelve of the 20 phenotypically identified $A$. vaigiensis individuals from the Maldives fell into the $A$. sexfasciatus Indian Ocean clade at both nuclear loci. Three distinct lineages ( $\mathrm{A}, \mathrm{B}, \mathrm{C}$ ) were also observed in $A$. vaigiensis in the nuclear gene-based trees. In contrast with the mitochondrial tree, the samples from previously assigned $A$. vaigiensis A exclusive of the twelve Maldivian $A$. vaigiensis individuals mentioned above formed a monophyletic group which is completely separated from $A$. sexfasciatus (Fig. 2B). In $A$. sexfasciatus, nuclear markers confirmed the phylogeographic pattern as revealed from the results of mitochondrial gene analysis by dividing the samples into three regional sublineages: one from the western Indian Ocean, one from the Coral Triangle region and one from the western Pacific Ocean. There was only weak intraspecific variation within $A$. vaigiensis A across the whole Indo-West Pacific. The fast-evolving intronic marker ( $D y s t$ ) provided better phylogenetic resolution than
the exonic one (RAG1). RAG1 failed to fully resolve the terminal part of the tree, whereas Dyst did and even revealed an intraspecific structure with a distinct lineage including the Red Sea population in A. vaigiensis A (Fig. S1, Supporting information). The placement of $A$. saxatilis indicated that $A$. sexfasciatus and $A$. vaigiensis are not sister species which contrasts with the proximity we found between $A$. sexfaciatus and $A$. vaigiensis A in the mitochondrial gene tree.

## Genetic diversity,population genetic structure and demography

Abudefduf sexfasciatus and $A$. vaigiensis lineage A displayed different patterns of intra-specific genetic diversity and differentiation at the mitochondrial locus. In $A$. sexfasciatus, we found a substantially reduced genetic diversity in the remote atoll of Moruroa where all 12 individuals sampled shared a unique cytb haplotype (Table 1). In both species, populations from several localities displayed significant negative Tajima's $D$ and Fu's $F_{S}$ values. This trend was more obvious in $A$. vaigiensis lineage A than in $A$. sexfasciatus. It was observed, to a lesser extent, at locus RAG1 but not at locus Dyst (Supplementary Table S3A; 3B).

The overall estimates of genetic differentiation at the mitochondrial locus (cytb) in $A$. sexfasciatus was $F_{\mathrm{ST}}=$ $0.786\left(P<10^{-4}\right)$. Most of the genetic differentiation occurred between regions: Indian Ocean, Coral Triangle region and western Pacific ( $F_{C T}=0.669 ; P=0.03$ ). Genetic differentiation was also considerable between localities within regions ( $F_{\mathrm{SC}}=0.351 ; P<10-4$, Table 2 ). Within A. vaigiensis lineage A , there was only negligible differentiation among populations ( $F_{\mathrm{ST}}=0.020, P=0.77 ; F_{\mathrm{SC}}=-0.001, P=0.95$ ) even though it was significant between oceans with $F_{C T}=0.033, P=0.02$ (Table 2). Based on the AMOVA, the degree of genetic differentiation was nonsignifcant $\left(F_{C T}=0.283, P=0.10\right)$ between $A$. sexfasciatus and $A$. vaigiensis lineage A where the two morpho-species were found in sympatry ( $F_{\mathrm{SC}}=-0.009 ; P=0.58$ and $F_{\mathrm{ST}}=0.276 ; P<10^{-4}$ ) at this locus. This confirmed that genetic differentiation was lower between the two species in the Coral Triangle region than within $A$. sexfasciatus across regions.

Three mitochondrial haplotypes, of 137 in total, were shared by $A$. sexfasciatus and $A$. vaigiensis A individuals (Fig. 3A). One of these three haplotypes was common and it characterized several individuals from the two species and from different localities across the Indo-West Pacific. The two other haplotypes were less common in at least one of the two species. Overall, based on mitochondrial haplotypes, the differentiation between $A$. sexfasciatus and $A$. vaigiensis A was weak in the Coral Triangle region. The mitochondrial haplotype network confirmed that Indian Ocean and western Pacific haplotypes were relatively distinct from the Coral Triangle region ones (with 13 and 6 mutations, respectively; i.e. $1.4 \%$ and $0.6 \%$ of pairwise sequence divergence between groups). One individual of $A$. sexfasciatus from West Papua and one from New Caledonia had haplotypes similar to those found in Moruroa, whereas the other haplotypes present at these localities had affinities with those sampled in the Coral Triangle region. At the nuclear loci, several individuals from the Maldives formally identified as A . vaigiensis according to colour pattern possessed alleles characteristic of $A$. sexfasciatus (Fig. 3B, C). Elsewhere, the two species were clearly distinct at locus Dyst with some haplotypes from the Red Sea at an intermediate position between $A$. vaigiensis A and $A$. sexfasciatus (Fig. 3C).

## Coalescence and inter-specific gene flow

Bayesian coalescent-based analyses highlighted contrasting patterns in the timing of divergence and gene flow between Coral Triangle region populations of $A$. sexfasciatus and $A$. vaigiensis A (Table 3; Figs 4 and S 2 , Supporting information). Splitting time $(t)$ between the two species based on both mitochondrial and nuclear information was found to be recent: 0.5 Myrs. Further examination suggested that $t$ (as well as the times of the most recent common ancestor, tMRCA) were likely to be much younger at the mitochondrial locus than at nuclear loci ( $<0.2 \mathrm{vs}$. $\geq 0.9 \mathrm{Myrs}$, respectively). The effective migration rates between the two species ( $5 \times 10^{-7}$ ind./year, Table 3) were low but non-null. This result was corroborated by the model comparison procedure
according to which the complete model (allowing gene flow) better fit the data than the model without gene flow (Supplementary Table S4, Supporting information).

According to the shapes of the relative likelihood distributions, the overall effective migration rate could be marker-dependent. Interspecific gene flow was predominantly driven by mitochondrial gene flow, whereas nuclear gene flow was negligible (Figs 3 and S2, Supporting information). Based on effective migration rate values, we also noted that the estimates are consistent with a slight asymmetry in gene flow between the two species. Although gene flow should be considered as weak in both directions, it seems it preferentially occur from $A$. vaigiensis A to $A$. sexfasciatus (Table 3; Supplementary Table S4). Inferred effective population sizes were high in both species (on the order of 104 or 105 individuals), but the estimates differed according to the locus considered.

## Discussion

In this study, we reported mito-nuclear discordance in two sergeant fishes widely distributed in the Indo-West Pacific which we ascribed to interspecific gene flow. We relied upon a geographic sampling spread across the Indo-Pacific barrier and including peripheral localities (the Mozambique Channel, the Red Sea, Moruroa) and used three genetic markers assumed to evolve at different paces, to span different time frames of the evolutionary history of this system. The phylogenetic results enabled us to characterize the polyphyly of $A$. vaigiensis thus confirming its existence of cryptic diversity (see Quenouille et al. 2011). We drew the spatial distribution of our identified genetic lineages. Our results also showed contrasting spatial patterns of intraspecific differentiation between the phylogeographically structured $A$. sexfasciatus and the more homogeneous A. vaigiensis A. An examination of mito-nuclear patterns of variation then enabled us to characterize complete lineage sorting between the two species at the fast-evolving nuclear intronic locus (Dyst). This contrasted with a very likely incomplete lineage sorting at the nuclear exonic locus ( $\mathrm{R} A G 1$ ) for which the whole terminal part of the phylogenetic tree we inferred was poorly resolved. We also found evidence of weak but nonnegligible interspecific gene flow at the mitochondrial (cytb) locus.

## Systematics of the sergeant fishes

Most species in the genus $A b u d e f d u f$ were monophyletic except $A$. vaigiensis (polyphyletic) and $A$. sexfasciatus (paraphyletic). We found that $A$. vaigiensis actually corresponds to at least three phenotypically similar but genetically divergent lineages (A, B and C; Fig. 2A,B). A previous study by Quenouille et al. (2011) reported three A. vaigiensis taxa ( $\mathrm{A}, \mathrm{B}$ and C ), two of which ( A and B, not C ) match the ones characterized in this study. However, Quenouille et al. (2011) did not further discuss this result that was based on the distinctiveness of mitochondrial cytochrome b and ATP synthase 8 and 6 gene sequences obtained from two single individuals (sequenced and deposited in GenBank by Quenouille et al. 2004). In this study, we confirmed the existence of $A$. vaigiensis A and B taxa as we can unambiguously assign several individuals to each of them based on both mitochondrial and nuclear information. Cryptic diversity in reef fishes often concerns populations whose geographic distributions do not overlap, or weakly so (Taylor \& Helberg 2005; Drew et al. 2010; Leray et al. 2010). In our study, this was the case of $A$. sexfasciatus which showed three regional sublineages (Indian Ocean / Coral Triangle region / western Pacific). The degree of genetic differentiation among these sublineages (up to $1.5 \%$ of divergence between the Indian Ocean sublineage and the two others at $y y t b$ ) was sufficiently high to potentially assign them the rank of subspecies. In contrast, the three cryptic lineages uncovered in $A$. vaigiensis were substantially different from each other for both mitochondrial and nuclear genes but co-occurred widely (i.e. 8.8 to $9 \%$ of divergence between lineages A, B and C at cytb). Abudefduf vaigiensis A was the most frequently sampled and occurred throughout the Indo-West Pacific. Abudefduf vaigiensis B was geographically widespread too but seemed to be relatively uncommon. The cytb gene sequence of a single individual from Guam sequenced by Quenouille et al. (2004) (GenBank AY208561) clustered with A. vaigiensis B haplotypes (Fig. 2A). Abudefduf
vaigiensis C was here reported for the first time and is distinct from the cryptic taxon " C " reported in Quenouille et al. (2011) based on a single sample from Christmas Island that was sequenced at the cytochrome $b$ and the ATP synthase 8 and 6 gene loci (GB Accessions: AY208557 and AY208418, respectively). The geographic distribution of $A$. vaigiensis C is possibly limited to the Coral Triangle. Our results thus revealed cryptic diversity in $A$. vaigiensis and indicated that the number of species was underestimated in the genus $A b u d e f u f$. Altogether, these results on $A$. sexfasciatus and $A$. vaigiensis call for further investigations on the systematics and taxonomy of the genus, to be conducted with more samples and more nuclear gene markers.

## Phylogeography of $A$. sexfasciatus and $A$. vaigiensis lineage $A$

Among the Abudefduf species sampled in both the Indian Ocean and the Pacific Ocean, A. vaigiensis A showed nonsignificant population structure distribution-wide at the mitochondrial locus ( $F_{\mathrm{ST}}=0.020, P=0.78$ ). Genetic homogeneity across the entire Indo-West Pacific has been reported in other reef fishes (Klanten et al. 2007; Horne et al. 2008; Reece et al. 2010, 2011). Such broad scale homogeneity suggests high levels of contemporary intraspecific gene flow or insufficient time to observe divergence. In contrast, substantial levels of intraspecific genetic structure were observed among regional populations as well as within regions in $A$. sexfasciatus. The genetic discontinuities (Indian Ocean/Pacific Ocean) matched the well-known Indo-Pacific barrier (Gaither \& Rocha 2013; Borsa et al. 2016). Geographic structure also reflected the isolation of peripheral insular populations, a pattern that has been documented for reef fishes from Hawaii (Eble et al. 2011; Gaither et al. 2014), the Society Islands (Liu et al. 2014) and the Marquesas (Planes \& Fauvelot 2002; Gaither et al. 2010). Genetic diversity at the mitochondrial locus was extremely low in the Moruroa population of $A$. sexfasciatus, a pattern previously observed for populations from isolated peripheral habitats (Liu et al. 2014). The A. sexfasciatus population from Moruroa was monomorphic at the cytb locus while populations elsewhere had high gene and nucleotide diversities. Remote populations may be particularly sensitive to bottleneck events due to relatively low effective population sizes and/or lack of migrants from external genetic pool that could efficiently maintain genetic diversity.

We hypothesize that populations of $A$. vaigiensis A diverged from their most recent common ancestor more recently than that of $A$. sexfasciatus. Stronger negative Tajima's $D$ and Fu's $F_{\mathrm{S}}$ values in $A$. vaigiensis A support recent population demographic expansion in this species. However, in contrast with the overall lack of genetic structure at loci cytb and RAG1, the Red Sea population of $A$. vaigiensis A showed evidence of differentiation at the fast-evolving intronic locus Dyst, suggesting a more complex demographic history and a role of geographic isolation in the peripheral populations of this species too.

We documented contrasting mito-nuclear phylogenetic patterns between the two closely related (but not sister) species, $A$. sexfasciatus and $A$. vaigiensis A . These two species are genetically distinct based on the nuclear information but $A$. vaigiensis A and the populations of $A$. sexfasciatus from the Coral Triangle region were represented by the same mitochondrial lineage. We clearly identified distinct regional lineages in $A$. sexfasciatus but at the same time found shared mitochondrial haplotypes between the sublineage of $A$. sexfasciatus from the Coral Triangle region and $A$. vaigiensis A. This strongly supports the hypothesis of gene flow over that of incomplete lineage sorting.

## Proposed evolutionary scenario

The comparative analysis of the patterns of genetic diversity and differentiation within and between $A$. sexfasciatus and $A$. vaigiensis lineage A led us to propose the following evolutionary scenario (Fig. 5). The split between $A$. sexfasciatus and $A$. vaigiensis A would be ancient enough to be unambiguously characterized at a relatively fast-evolving nuclear intronic locus such as Dyst. A lack of resolution associated with weak phylogenetic signal at locus RAG1 suggests incomplete lineage sorting at this slower-evolving nuclear exon. At these nuclear loci, the estimates of splitting times and tMRCA are consistent with the existence of the last
common ancestor between the two species in the late Miocene (between 10 and 5 My B.P.; Litsios et al. 2012; Frédérich et al. 2013) or perhaps, even more recently according to our coalescent-based estimates.

In the Coral Triangle region, the non-significant level of divergence $\left(F_{C T}=0.283, P=0.10\right)$ between $A$. sexfasciatus and $A$. vaigiensis lineage $A$ at the mitochondrial locus (cytb) contrasted with the consensus pattern observed at nuclear loci. The estimated splitting time at locus cytb was at least about ten times younger than the one at nuclear genes. The signal that we detected as well as evidence of shared haplotypes is thus consistent with gene flow after secondary contact following at least one initial stage of divergence between the two species. This scenario has been previously invoked to explain the sympatry of sibling species in other reef fishes from various families [e.g. snappers (Lutjanidae): Gaither et al. 2010; wrasses (Labridae): Choat et al. 2012 or damselfishes (Pomacentridae): Quenouille et al. 2011] in which cross-species hybridization sometimes occurs (e.g. Hobbs et al. 2009; DiBattista et al. 2015). This does not rule out more local ecological and biological factors to explain the genetic introgression between $A$. sexfasciatus and $A$. vaigiensis lineage A in part of their range.

## Causes of inter-specific gene flow

In sympatric and closely related reef fish species, traits associated with sexual selection, for example coloration in hamlets, Hypoplectrus spp. (Puebla et al. 2007) or pygmy angelfishes, Centropyge spp. (DiBattista et al. 2012; Bowen et al. 2013; and references therein) or natural selection (Rocha et al. 2005; Bowen et al. 2013 and references therein), generally act as species recognition signals and impede hybridization. In some situations however, divergence may be too recent to have led to complete pre- or postzygotic isolation. In the genus Abudefduf, Coleman et al. (2014) proposed that similarity in colour patterns might be one of the factors responsible for the failure of species recognition and for the subsequent hybridization between A. abdominalis and A. vaigiensis after a recent introduction of the former species to Hawaii. In our case, however, colour pattern differences are obvious between $A$. sexfasciatus and $A$. vaigiensis A , whereas they are not between lineages $\mathrm{A}, \mathrm{B}$ and C of $A$. vaigiensis. Yet, there was no evidence of gene flow between the three cryptic lineages of $A$. vaigiensis ( $\mathrm{A}, \mathrm{B}$ and C ), whereas we found evidence of it between $A$. sexfasciatus and $A$. vaigiensis A. Evidence of interspecific gene flow between $A$. sexfasciatus and $A$. vaigiensis A in part of their range thus suggests that factors other than coloration may have allowed hybridization.

Abudefduf sexfasciatus and A. vaigiensis A often co-occur in strict sympatry and seem to display similar ecological preferences (Aguilar-Medrano \& Barber 2016; personal field observations). Although the niches of the two species broadly overlap, A. vaigiensis which is benthopelagic could be more generalist than $A$. sexfasciatus which is pelagic. Several ecological factors have been invoked as catalysers of hybridization and their putative effects have been discussed by Coleman et al. (2014) for the two Abudefduf species that occur in Hawaii (see also Yaakub et al. 2006; Montanari et al. 2012, 2014, 2016). Two species that form heterospecific social groups and have synchronized spawning seasons may experience external fertilization accidentally involving gametes of both species. However, this hypothesis is unlikely in damselfishes which lay demersal eggs (Gainsford et al. 2015) as do A. sexfasciatus and A. vaigiensis. Relative differences in population density may also favour hybridization. In the field, we often observed local differences in the ratio of $A$. sexfasciatus / A. vaigiensis, but the dominant species was not always the same depending on the locality. Our genetic results further suggest that interspecific gene flow predominantly concerns mitochondrial genes and could be asymmetric: it was slightly higher from $A$. vaigiensis A to $A$. sexfasciatus. As mitochondrial gene inheritance in fishes is assumed to occur through females, this result would be consistent with interspecific mating predominantly involving females of $A$. vaigiensis A and males of $A$. sexfasciatus. An accurate characterization of hybrids is still required to elucidate the biological mechanisms underpinning hybridization in this system, given that putative hybrids seem to be rare. We noted that most of the $A$. vaigiensis A individuals that shared mitochondrial haplotypes with $A$. sexfasciatus (but displayed nuclear characteristics of $A$. vaigiensis) were juveniles from particular insular localities. This could be consistent with hybrids having lower survival rate.

Only one Indian Ocean locality (Maldives) showed adult individuals with unambiguous A. vaigiensis phenotypes associated with $A$. sexfasciatus genotypes at both nuclear and mitochondrial loci. Deep introgression could explain such a situation.

## Conclusive remarks

Sergeants provide an opportunity to further investigate how hybridization and its evolutionary causes work at a trans-oceanic scale; in widely sympatric species and with apparent similar ecological niches. Between $A$. sexfasciatus and $A$. vaigiensis A , inter-specific gene flow occurs not only at spatially restricted hotspots but virtually across the whole western Pacific. Unlike discontinuous island-dwelling systems in which each sampling localities can be considered as a distinct replicate, this configuration could be particularly suitable to better understand what mechanisms prevent species from collapsing in the face of gene flow across unrivalled large scale continuums.

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## Authors' contributions

W.J.C. led the project including the search of funding support for completing this study. W.J.C. and P.B. contributed to the conception, design of the work, led the fieldwork and collected the samples. J.A.M.B. participated to fieldwork, generated most of the molecular data, did the analyses and wrote the manuscript. J.A.M.B., P.B. and W.J.C. interpreted the results. P.B. and W.J.C. revised successive versions of the manuscript.

## Data accessibility

The nucleotide sequences weredeposited in GenBank, with accession numbers KU553479 to KU554420.

Table 1 Genetic diversity and results of neutrality test at the mitochondrial locus for $A$. sexfasciatus and $A$. vaigiensis across sample localities and regions. Localities arranged from west to east. $N$ sample size; $S$ number of polymorphic sites; $H_{d}$ haplotype diversity; $\pi$ nucleotide diversity; $S D$ standard deviation, estimated by bootstrap resampling. Values of Tajima's (1989) $D$ and Fu's (1997) $F_{\text {S }}$ were considered statistically significant $\left(^{*}\right.$ ) when $P<$ 0.05 and $P<0.02$, respectively.

| Species or lineage, Region, Locality | $N$ | S | $H_{\mathrm{d}} \pm S D$ | $\pi \pm S D$ | D | $F_{\text {S }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A. sexfasciatus |  |  |  |  |  |  |
| Indian Ocean |  |  |  |  |  |  |
| Glorieuses | 6 | 8 | $0.93 \pm 0.12$ | $0.0025 \pm 0.0018$ | -1.07 | -1.37 |
| Mauritius | 15 | 17 | $0.94 \pm 0.04$ | $0.0024 \pm 0.0015$ | -1.86* | -4.43* |
| Coral Triangle Region |  |  |  |  |  |  |
| Dongsha | 49 | 23 | $0.86 \pm 0.03$ | $0.0016 \pm 0.0010$ | -2.06* | -12.37* |
| Northern Taiwan | 19 | 12 | $0.80 \pm 0.08$ | $0.0015 \pm 0.0010$ | -1.85* | -4.25* |
| Southern Taiwan | 14 | 6 | $0.60 \pm 0.15$ | $0.0008 \pm 0.0007$ | -1.73* | -3.03* |
| Eastern Taiwan | 13 | 5 | $0.88 \pm 0.06$ | $0.0016 \pm 0.0011$ | -0.22 | -2.59 |
| Virac, Philippines | 4 | 6 | $1.00 \pm 0.18$ | $0.0027 \pm 0.0021$ | -0.31 | -1.16 |
| West Papua | 3 | 11 | $1.00 \pm 0.27$ | $0.0063 \pm 0.0051$ | 0.00 | 0.81 |
| Pacific Ocean |  |  |  |  |  |  |
| New Caledonia | 24 | 21 | $0.84 \pm 0.06$ | $0.0022 \pm 0.0014$ | -2.14* | -5.01* |
| Moruroa | 12 | 0 | $0.00 \pm 0.00$ | $0.0000 \pm 0.0000$ | 0.00 | $N A$ |
| A. vaigiensis lineage $A$ |  |  |  |  |  |  |
| Indian Ocean |  |  |  |  |  |  |
| Eilat, Red Sea | 8 | 13 | $0.96 \pm 0.08$ | $0.0033 \pm 0.0021$ | -1.24 | -2.60 |
| Mozambique Channela ${ }^{\text {a }}$ | 6 | 9 | $0.93 \pm 0.12$ | $0.0030 \pm 0.0021$ | -0.72 | -1.01 |
| Maldives | 21 | 24 | $0.95 \pm 0.04$ | $0.0026 \pm 0.0016$ | -2.08* | -14.28* |
| Coral Triangle Region |  |  |  |  |  |  |
| Hainan | 21 | 21 | $0.97 \pm 0.03$ | $0.0028 \pm 0.0017$ | -1.68* | -11.29* |
| Taiping | 8 | 13 | $0.96 \pm 0.08$ | $0.0038 \pm 0.0024$ | -0.66 | -2.24 |
| Bali | 17 | 22 | $0.97 \pm 0.03$ | $0.0035 \pm 0.0021$ | -1.46 | -8.14* |
| Dongsha | 2 | 4 | $1.00 \pm 0.50$ | $0.0034 \pm 0.0039$ | 0.00 | 1.39 |
| Little Liuqiu ${ }^{\text {b }}$ | 10 | 16 | $0.93 \pm 0.08$ | $0.0037 \pm 0.0023$ | -1.08 | -2.39 |
| Southern Taiwan | 34 | 33 | $0.96 \pm 0.02$ | $0.0038 \pm 0.0021$ | -1.63* | -11.10* |
| Northern Taiwan | 15 | 21 | $0.98 \pm 0.03$ | $0.0037 \pm 0.0022$ | -1.37 | -7.53* |
| Eastern Taiwan | 25 | 25 | $0.96 \pm 0.02$ | $0.0035 \pm 0.0020$ | -1.41 | -8.87* |
| PNG ${ }^{\text {c }}$ | 33 | 31 | $0.97 \pm 0.02$ | $0.0037 \pm 0.0021$ | -1.56* | -11.71* |
| A. vaigiensis lineage $B$ |  |  |  |  |  |  |
| (all localities) | 6 | 44 | $0.93 \pm 0.12$ | $0.0128 \pm 0.0077$ | -1.46* | 1.48 |
| A. vaigiensis lineage $C$ |  |  |  |  |  |  |
| (all localities) | 11 | 10 | $0.89 \pm 0.09$ | $0.0017 \pm 0.0012$ | -1.81* | -4.55* |

[^0]Table 2 Summary of the AMOVA of $A$. sexfasciatus and $A$. vaigiensis lineage A. Sampling localities were nested into regions, at the mitochondrial locus. Percentage of total genetic variation as well as the corresponding fixation indices are given.

| Species or lineage, <br> Source of variation | Percentage of variation | Fixation index |
| :--- | :--- | :--- |
| A. sexfasciatus | 66.9 | $F_{\mathrm{CT}}=0.669^{*}$ |
| Among regions | 11.6 | $F_{\mathrm{SC}}=0.351^{*}$ |
| Among populations within regions | 21.4 | $F_{\mathrm{ST}}=0.786^{*}$ |
| Among populations across regions | 3.3 | $F_{\mathrm{CT}}=0.033$ |
| A. vaigiensis lineage A | $F_{\mathrm{SC}}=-0.001$ |  |
| Among regions | $F_{\mathrm{ST}}=0.020$ |  |
| Among populations within regions | 98.0 |  |

Table 3 Demographic parameter estimates derived from the IMa2 analysis for $A$. sexfasciatus and $A$. vaigiensis lineage A from the Coral Triangle region. For each partition, the following parameters were estimated: mutation rate $(\mu)$; length of alignment ( $($; effective population size [calculated as $(\theta / 4 . \mu . h)$, with $\theta$ being the effective population size in the model] in $A$. sexfasciatus $\left(N_{1}\right)$, in $A$. vaigiensis lineage $\mathrm{A}\left(N_{2}\right)$ and in the ancestral population $\left(N_{\mathrm{a}}\right)$; splitting time $(t)$; time to most recent common ancestor [ $t \mathrm{MRCA}$, calculated as $(t / \mu . \delta]$; and relative migration rates from $A$. sexfasciatus to $A$. vaigiensis lineage $A\left(\mathrm{~m}_{1}\right)$ and reciprocally $\left(\mathrm{m}_{2}\right)$ (with $\mathrm{m}=m . \mu . l$ being the mutation rate in the coalescent).

| Marker | $\mu^{*}$ | $l$ | $N_{1}$ | $N_{2}$ | $N a$ | $t$ | $t M R C A$ | $\mathrm{~m}_{1}$ | $\mathrm{~m}_{2}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Dyst | $7.50 \times 10^{-9}$ | 1040 | 54487 | 28846 | 176282 | 6198718 | 961538 | $2.34 \times 10^{-8}$ | $2.34 \times 10^{-8}$ |
| RAG1 | $9.70 \times 10^{-10}$ | 985 | 562562 | 1295201 | 91580 | 889633 | 2616568 | $4.78 \times 10^{-9}$ | $4.78 \times 10^{-9}$ |
| 2 nuclear loci | $2.70 \times 10^{-9}$ | 2025 | 89255 | 116718 | 514933 | 10518372 | $N A$ | $2.73 \times 10^{-8}$ | $2.73 \times 10^{-8}$ |
| cyt $b$ | $1.30 \times 10^{-8}$ | 1161 | 1347976 | 2733055 | 66670 | 122772 | 163983 | $2.94 \times 10^{-7}$ | $3.55 \times 10^{-7}$ |
| All loci | $4.00 \times 10^{-9}$ | 3186 | 120753 | 197327 | 874719 | 514425 | $N A$ | $4.90 \times 10^{-7}$ | $4.90 \times 10^{-7}$ |

[^1]
## Captions to figures

Figure 1 Map of the Indo-West Pacific, with sampling localities and sample sizes. (A) Sampling localities for Abudefduf spp. (B) Sampling localities for $A$. vaigiensis and $A$. sexfasciatus. Circle surface (red: $A$. sexfasciatus; blue: A. vaigiensis) proportional to sample size. Pie charts give the proportion of each lineage under $A$. vaigiensis (see inset) in a sample. Additional information provided in Table 1 and in Supplementary Table S1.

Fig. 2 Phylogenetic trees of $A b u d e f d u f$ spp. Tree topology corresponds to the best ML tree. Node supports are indicated by bootstrap values (when $>50 \%$ ); white circles indicate nodes whose Bayesian probability was $>90 \%$. Sequences generated in the course of this study are in red for Abudefduf sexfasciatus, in blue for Abudefduf vaigiensis, with different tones for the cryptic groups (A, B or C), or in black (other Abudefduf species); sequences retrieved from GenBank are in grey. When nodes were collapsed for readability purpose, the number of sequences included in a cluster and their geographical origin are mentioned. (A) From mitochondrial sequences (cytb locus). Homologous sequences from Oreochromis niloticus (GenBank NC_013663) and Amphiprion ocellaris (GenBank AP006017) were used as outgroup. Similiaparma lurida (previously Abudefduf luridus) is considered as a separate genus (Cooper et al. 2014). (B) From concatenated (RAG1+ Dyst) nuclear sequences.

Fig. 3 Minimum-spanning parsimony networks for Abudefduf sexfasciatus (in red) and Abudefduf vaigiensis A (in blue). Hatches depicts the number of mutations; circle surface is proportional to sample size; tones of red (A. sexfasciatus) differ according to geographic origin. (A) Haplotypes at the cytb locus. (B), haplotypes of the nuclear exon RAG1. (C) haplotypes of the nuclear intron Dyst.

Fig. 4 Abudefduf sexfasciatus and $A$. vaigiensis lineage A. relative likelihood distributions for three demographic parameters estimated using IMa2. Row A concerns the nuclear data set (RAG1+Dyst) ; row B , the mitochondrial data set (cytb); and row C, the three-locus data set (cytb+RAG1+Dyst). Full line: splitting time ( $t$ ); dashed line: time to most recent common ancestor ( $t \mathrm{MRCA}$ ) in million years before present $(\mathrm{BP})$; black: effective migration rate (m) from $A$. sexfasciatus to $A$. vaigiensis lineage $A$; grey: effective migration rate from $A$. vaigiensis lineage $A$ to $A$. sexfasciatus Migration rates expressed in individuals per million year. Effective population size ( Ne , in millions of individuals) for $A$. sexfasciatus (in black), A. vaigiensis lineage A (in grey) and for the ancestral population (black dashed line).

Fig. 5 Schematic overview of the proposed evolutionary scenario. In the species tree depicting the split between Coral Triangle region populations of $A$. sexfasciatus and $A$. vaigiensis A , the mitochondrial locus (cytb) exhibit inter-specific gene flow. Among the nuclear loci, lineage sorting is complete at the intron Dyst but incomplete at the exon RAG1.


A 100 Figure 2 - Bertrand, Borsa, Chen
similiparma lurida - Madeira (x2) declivifrons (AY208549) - Panama A. taurus - Western Atlantic (x2) notatus (AY208552) - Indonesia A. sordidus - Indo-West Pacific ( $\times 24$ ) 101 A. septemfasciatus - Indo-West Pacific (x52)
A vaiglensis - Glorieuses A. vaigiensis - Taiping A. vaigiensis - West Papua O A. vaigiensis (AY208561) - Guam 100 A. vaigiensis - Little Liuqiu A. vaigiensis - West Papua vaigiensis - West Papua
A. sparoides - Western Indian Ocean (x22)
A. whitleyi (AY208562) - Eastern Australia
A. whitleyi - New Caledonia (x3)

A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Bali
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan

| A. vaigiensis - Bali |
| :--- |
| A. vaigiensis - Bali |
| A. vaigiensis - Bali |
| A. vaigiensis - Caohagan |$\quad$ A. vaigiensis lineage C

A. margariteus - Réunion (x3)
A. vaigiensis (AY208557) - Christmas

A. sparoides - Indian Ocean (x3)
A. whitleyi - New Caledonia (x3)
A. Iorenzi - Kota Kinabalu (x4)
A. bengalensis - West Pacific (x5)
A. vaigiensis - Glorieuses Is.
A. vaigiensis - West Papua
A. vaigiensis - Taiping
A. vaigiensis - West Papua
A. septemfasciatus - Pacific Ocean (x3)
A. septemfasciatus - Indian Oqean (x6)
 A. bengalensis (AY208547) - Australia A. bengalensis - West Pacific (x15) A. troschelii (AY208560) - Panama
A. vaigiensis - Bali
A. vaigiensis - Bali
A. vaigiensis - Bali
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Caohagan
A. vaigiensis - Bali
 A. vaigiensis - Caohagan A. vaigiensis - Bali A. vaigiensis - Caohagan A. vaigiensis - Bali A. vaigiensis lineage $C$



100 (A I 91 (AYominalis (AY208546) - Coral triangle (x6)
A. sexfasciatus - New Caledonia (x2), Moruroa (x9) A. Iorenzi - Coral triangle (x6) A. sexfasciatus - Western Indian Ocean (x23) A. sexfasciatus - New Caledonia 7 A. sexfasciatus - West Papua A. sexfasciatus - Moruroa (x12) ${ }^{1}$ A. vaigiensis - Indo-West Pacific (x204) !
A. vaigiensis - Maldives (x12)
ensis - Indo-West Pacific (x85) A. saxatilis - Puerto Rico (x2)


Figure 3 - Bertrand, Borsa, Chen



Figure 5 - Bertrand, Borsa, Chen


Supplementary material for "Phylogeography of the sergeants Abudefduf sexfasciatus and A. vaigiensis reveals complex introgression patterns between two widespread and sympatric Indo-West Pacific reef fishes"

Joris A. M. Bertrand, Philippe Borsa and Wei-Jen Chen
Supplementary Tables S1-S4 and Supplementary Figures S1 and S2 here appended.

Supplementary Table S1 Abudefdufspp. individuals sampled for the present study. Each row represents an individual identified to species,for whichsampling location (region, locality and geographic coordinates), individual number, main collector and when available, GenBank accession number at three loci (cytb, RAG1 and Dyst). $N$, total number of individuals sequenced at a locus

| Individual no. | Species | Sampling details |  |  |  |  | GenBank no. |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Region | Locality | Lat. | Long. | Coll. | cytb | RAG1 | Dyst |
| WJC1514 | A. bengalensis | Coral Triangle | East Taiwan | 24.94 | 121.89 | WJC | KU553479 | KU554197 | KU553963 |
| WJC1515 | A. bengalensis | Coral Triangle | East Taiwan | 24.94 | 121.89 | WJC | KU553480 | KU554198 | KU553964 |
| WJC1728 | A. bengalensis | Coral Triangle | Van Dom | 21.13 | 107.45 | WJC | KU553481 | KU554199 | KU553965 |
| WJC4314 | A. bengalensis | Coral Triangle | South Taiwan | 120.71 | 22.01 | WJC | KU553482 | - | - |
| WJC4315 | A. bengalensis | Coral Triangle | South Taiwan | 120.71 | 22.01 | WJC | KU553483 | - | - |
| WJC4316 | A. bengalensis | Coral Triangle | South Taiwan | 120.71 | 22.01 | WJC | KU553484 | - | - |
| WJC5381 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553485 | KU554200 | KU553966 |
| WJC5382 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553486 | - | KU553967 |
| WJC5383 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553487 | KU554201 | KU553968 |
| WJC5384 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553488 | - | KU553969 |
| WJC5385 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553489 | - | - |
| WJC5386 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553490 | - | - |
| WJC5387 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | - | - | - |
| WJC5388 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | - | - | - |
| WJC5389 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553491 | - | - |
| WJC5390 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553492 | - | - |
| WJC5391 | A. bengalensis | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553493 | - | - |
| WJC5375 | A. lorenzi | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553494 | KU554202 | KU553970 |
| WJC5376 | A. lorenæi | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553495 | KU554203 | KU553971 |
| WJC5377 | A. lorenəi | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553496 | KU554204 | KU553972 |
| WJC5378 | A. lorenri | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | - | KU554205 | KU553973 |
| WJC5379 | A. lorenzi | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553497 | - | - |
| WJC5380 | A. lorenzi | Coral Triangle | Kota Kinabalu | 115.99 | 5.96 | WJC | KU553498 | - | - |
| WJC67 | A. saxatilis | Atlantic Ocean | Puerto Rico | 18.48 | -66.13 | KC | KU553499 | KU554206 | KU553974 |
| WJC84 | A. saxatilis | Atlantic Ocean | Puerto Rico | 18.48 | -66.13 | KC | KU553500 | KU554207 | KU553975 |
| EPS159 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553501 | KU554208 | KU553976 |
| EPS160 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553502 | - | - |
| EPS214 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553503 | - | - |
| EPS215 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553504 | - | - |
| EPS216 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553505 | - | - |
| EPS217 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553506 | - | - |
| EPS218 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553507 | - | - |
| EPS221 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553508 | KU554209 | KU553977 |
| EPS222 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553509 | KU554210 | KU553978 |
| EPS223 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553510 | KU554211 | KU553979 |
| EPS224 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553511 | - | - |
| EPS225 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553512 | - | - |
| EPS226 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553513 | - | - |
| EPS227 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553514 | - | - |
| EPS228 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553515 | - | - |
| EPS229 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553516 | - | - |
| EPS232 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553517 | - | - |
| EPS234 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553518 | - | - |
| EPS235 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553519 | - | - |
| EPS236 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553520 | - | - |
| EPS238 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553521 | - | - |
| EPS239 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553522 | - | - |
| EPS240 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553523 | - | - |
| EPS241 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553524 | - | - |
| EPS285 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553525 | - | - |
| EPS286 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553526 | - | - |
| EPS287 | A. septemfasciatus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553527 | - | - |
| EPS445 | A. septemfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553528 | - | - |
| EPS446 | A. septemfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553529 | - | - |
| EPS447 | A. septemfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553530 | - | - |
| EPS448 | A. septemfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553531 | - | - |
| EPS449 | A. septemfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553532 | - | - |
| WJC806 | A. septemfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553533 | KU554212 | KU553980 |
| WJC807 | A. septemfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553534 | KU554213 | - |
| WJC1265 | A. septemfasciatus | Pacific Ocean | New Caledonia | -20.93 | 165.40 | WJC | KU553535 | KU554214 | KU553981 |
| WJC1415 | A. septemfasciatus | Coral Triangle | East Taiwan | 23.24 | 121.41 | WJC | KU553536 | KU554215 | KU553982 |
| WJC1426 | A. septemfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553537 | KU554216 | - |
| WJC3447 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553538 | KU554217 | - |
| WJC3448 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553539 | KU554218 | KU553983 |
| WJC3449 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553540 | KU554219 | KU553984 |
| WJC3450 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553541 | KU554220 | - |


| WJC3451 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553542 | KU554221 | - |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| WJC3452 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553543 | KU554222 | - |
| WJC3453 | A. septemfasciatus | Indian Ocean | Aceh | 5.55 | 95.32 | WJC | KU553544 | KU554223 | - |
| WJC4282 | A. septemfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553545 | - | - |
| WJC4283 | A. septemfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553546 | - | - |
| WJC4284 | A. septemfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553547 | - | - |
| WJC4326 | A. septemfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553548 | - | - |
| WJC4794 | A. septemfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553549 | - | - |
| WJC4795 | A. septemfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553550 | - | - |
| EPS426 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553604 | KU554241 | KU553995 |
| EPS427 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553605 | KU554242 | KU553996 |
| EPS428 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553606 | KU554243 | KU553997 |
| EPS429 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553607 | KU554244 | KU553998 |
| EPS430 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553608 | KU554245 | KU553999 |
| EPS431 | A. sexfasciatus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553609 | KU554246 | KU554000 |
| EPS469 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553610 | - | KU554001 |
| EPS470 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553611 | - | KU554002 |
| EPS471 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553612 | - | - |
| EPS472 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553613 | - | KU554003 |
| EPS473 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553614 | - | - |
| EPS474 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553615 | - | KU554004 |
| EPS475 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553616 | - | - |
| EPS476 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553617 | - | - |
| EPS477 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553618 | - | KU554005 |
| EPS478 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553619 | - | - |
| EPS479 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553620 | - | KU554006 |
| EPS480 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553621 | - | - |
| EPS481 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553622 | - | - |
| EPS482 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553623 | - | - |
| EPS483 | A. sexfasciatus | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553624 | - | - |
| WJC707 | A. sexfasciatus | Coral Triangle | Virac | 13.58 | 124.23 | HH | KU553625 | KU554247 | KU554007 |
| WJC708 | A. sexfasciatus | Coral Triangle | Virac | 13.58 | 124.23 | HH | KU553626 | KU554248 | KU554008 |
| WJC709 | A. sexfasciatus | Coral Triangle | Virac | 13.58 | 124.23 | HH | KU553627 | KU554249 | KU554009 |
| WJC710 | A. sexfasciatus | Coral Triangle | Virac | 13.58 | 124.23 | HH | KU553628 | KU554250 | KU554010 |
| WJC715 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553629 | KU554251 | KU554011 |
| WJC716 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553630 | KU554252 | KU554012 |
| WJC717 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553631 | KU554253 | KU554013 |
| WJC718 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553632 | KU554254 | KU554014 |
| WJC719 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553633 | KU554255 | KU554015 |
| WJC720 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553634 | KU554256 | KU554016 |
| WJC721 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553635 | KU554257 | KU554017 |
| WJC845 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553636 | KU554258 | KU554018 |
| WJC846 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553637 | KU554259 | KU554019 |
| WJC847 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553638 | KU554260 | KU554020 |
| WJC848 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553639 | KU554261 | KU554021 |
| WJC922 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553640 | KU554262 | KU554022 |
| WJC923 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553641 | - | - |
| WJC924 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553642 |  |  |
| WJC925 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553643 | KU554263 | KU554023 |
| WJC926 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553644 | KU554264 | KU554024 |
| WJC927 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553645 | KU554265 | - |
| WJC928 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553646 | KU554266 | KU554025 |
| WJC929 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553647 | - | KU554026 |
| WJC930 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553648 | KU554267 | KU554027 |
| WJC931 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553649 | KU554268 | KU554028 |
| WJC955 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553650 | KU554269 | KU554029 |
| WJC1266 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | KU553651 | - | - |
| WJC1267 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | KU553652 | - | - |
| WJC1268 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | - | - | - |
| WJC1269 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | KU553653 | - | - |
| WJC1270 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | KU553654 | - | - |
| WJC1271 | A. sexfasciatus | Pacific Ocean | New Caledonia | -20.35 | 163.93 | PB | KU553655 | - | - |
| WJC1272 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553656 | KU554270 | KU554030 |
| WJC1273 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553657 | KU554271 | KU554031 |
| WJC1274 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553658 | KU554272 | KU554032 |
| WJC1275 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553659 | KU554273 | KU554033 |
| WJC1276 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553660 | KU554274 | KU554034 |
| WJC1277 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553661 | - | - |
| WJC1278 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553662 | - | - |
| WJC1279 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553663 | - | - |
| WJC1280 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553664 | - | - |
| WJC1281 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553665 | - | - |
| WJC1282 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553666 | - | - |
| WJC1283 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553667 | - | - |
| WJC1284 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553668 | - | - |
| WJC1285 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553669 | - | - |
| WJC1286 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553670 | - | - |


| WJC1287 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553671 | - | - |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| WJC1288 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553672 | - | - |
| WJC1289 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553673 | - | - |
| WJC1290 | A. sexfasciatus | Pacific Ocean | New Caledonia | -22.32 | 166.47 | PB | KU553674 | - | - |
| WJC1616 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553675 | KU554275 | KU554035 |
| WJC1617 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | wJC | KU553676 | KU554276 | KU554036 |
| WJC1618 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553677 | KU554277 | KU554037 |
| WJC2251 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553678 | - | - |
| WJC2283 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553679 | KU554278 | KU554038 |
| WJC2284 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553680 | - | - |
| WJC2285 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553681 | - | - |
| WJC2286 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553682 | - | - |
| WJC2287 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553683 | - | - |
| WJC2288 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553684 | - | - |
| WJC2289 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553685 | - | - |
| WJC2290 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | wJC | KU553686 | - | - |
| WJC2295 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553687 | KU554279 | KU554039 |
| WJC2296 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553688 | KU554280 | KU554040 |
| WJC2297 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553689 | - | - |
| WJC2298 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553690 | - |  |
| WJC2299 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553691 | - | - |
| WJC2300 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553692 | - |  |
| WJC2301 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553693 | KU554281 | KU554041 |
| WJC2302 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553694 | KU554282 | KU554042 |
| WJC2303 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | wJC | KU553695 | - | KU554043 |
| WJC2304 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553696 | - | - |
| WJC2305 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553697 | - |  |
| WJC2306 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553698 | - |  |
| WJC2307 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553699 | - |  |
| WJC2308 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553700 | - |  |
| WJC2309 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553701 | - |  |
| WJC2310 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553702 | - |  |
| WJC2573 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553703 | - |  |
| WJC2574 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553704 | - | KU554044 |
| WJC2575 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553705 | KU554283 | KU554045 |
| WJC2582 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553706 | KU554284 | KU554046 |
| WJC2583 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553707 | KU554285 | KU554047 |
| WJC2584 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553708 | KU554286 | KU554048 |
| WJC2585 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553709 | KU554287 | KU554049 |
| WJC2586 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553710 | KU554288 | KU554050 |
| WJC2587 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553711 | KU554289 | KU554051 |
| WJC2588 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553712 | - |  |
| WJC2589 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553713 | - | KU554052 |
| WJC2590 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553714 | KU554290 | KU554053 |
| WJC2591 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553715 | KU554291 | KU554054 |
| WJC2592 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553716 | KU554292 | KU554055 |
| WJC2593 | A. sexfasciatus | Pacific Ocean | Moruroa | -21.85 | -138.88 | PB, MK | KU553717 | - | - |
| WJC2686 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553718 | - | - |
| WJC2687 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553719 | - | - |
| WJC2714 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553720 | - | - |
| WJC2715 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553721 | - | - |
| WJC2716 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553722 | - | - |
| WJC2717 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553723 | - | - |
| WJC2718 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553724 | - | - |
| WJC2719 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553725 | - |  |
| WJC2720 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553726 | - | - |
| WJC2721 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553727 | - | - |
| WJC2742 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553728 | - | - |
| WJC4131 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553729 | KU554293 | KU554056 |
| WJC4133 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | wJC | KU553730 | - | KU554057 |
| WJC4134 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553731 | - |  |
| WJC4135 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553732 | - | - |
| WJC4136 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553733 | - | - |
| WJC4137 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553734 | - | - |
| WJC4138 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553735 | - | - |
| WJC4139 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553736 | - | - |
| WJC4140 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553737 | - | - |
| WJC4141 | A. sexfasciatus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553738 | - | - |
| WJC4195 | A. sexfasciatus | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553739 | KU554294 | KU554058 |
| WJC4197 | A. sexfasciatus | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553740 | KU554295 | KU554059 |
| WJC4198 | A. sexfasciatus | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553741 | KU554296 | KU554060 |
| WJC4200 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553742 | - | KU554061 |
| WJC4201 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553743 | - | KU554062 |
| WJC4202 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553744 | - | - |
| WJC4203 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553745 | - | - |
| WJC4204 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553746 | - | KU554063 |
| WJC4205 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553747 | KU554297 | KU554064 |


| WJC4206 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553748 | KU554298 | KU554065 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| WJC4207 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553749 | KU554299 | KU554066 |
| WJC4208 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553750 | KU554300 | KU554067 |
| WJC4209 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553751 | KU554301 | KU554068 |
| WJC4210 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553752 | KU554302 | KU554069 |
| WJC4211 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553753 | KU554303 | KU554070 |
| WJC4212 | A. sexfasciatus | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553754 | KU554304 | KU554071 |
| WJC4317 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553755 | - | - |
| WJC4320 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553756 | - | - |
| WJC4323 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553757 | - | - |
| WJC4324 | A. sexfasciatus | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553758 | - | - |
| WJC4366 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553759 | - | - |
| WJC4367 | A. sexfasciatus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553760 | - | - |
| WJC4796 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553761 | - | - |
| WJC4797 | A. sexfasciatus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553762 | - | - |
| EPS51 | A. sordidus | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553551 | KU554224 | - |
| EPS52 | A. sordidus | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553552 | KU554225 | - |
| EPS157 | A. sordidus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553553 | KU554226 | - |
| EPS237 | A. sordidus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553554 | - | - |
| EPS283 | A. sordidus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553555 | KU554227 | - |
| EPS284 | A. sordidus | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553556 | KU554228 | - |
| EPS442 | A. sordidus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553557 | - | - |
| EPS443 | A. sordidus | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553558 | - | - |
| WJC1260 | A. sordidus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553559 | KU554229 | KU553985 |
| WJC1261 | A. sordidus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553560 | KU554230 | KU553986 |
| WJC1410 | A. sordidus | Coral Triangle | East Taiwan | 23.24 | 121.41 | WJC | KU553561 | - | - |
| WJC1411 | A. sordidus | Coral Triangle | East Taiwan | 23.24 | 121.41 | WJC | KU553562 | - | - |
| WJC1425 | A. sordidus | Coral Triangle | East Taiwan | 23.24 | 121.41 | WJC | KU553563 | KU554231 | - |
| WJC4125 | A. sordidus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553564 | - | - |
| WC4155 | A. sordidus | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553565 | - | - |
| WJC4368 | A. sordidus | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553566 | - | - |
| WJC4789 | A. sordidus | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553567 | - | - |
| WJC4960 | A. sordidus | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553568 | KU554232 | - |
| EPS204 | A. sparoides | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553569 | KU554233 | KU553987 |
| EPS255 | A. sparoides | Indian Ocean | Juan de Nova | -17.03 | 42.72 | PB, WJC | KU553570 | KU554234 | KU553988 |
| EPS424 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553571 | KU554235 | KU553989 |
| EPS425 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553572 | KU554236 | KU553990 |
| EPS450 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553573 | - | - |
| EPS451 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553574 | - | - |
| EPS452 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553575 | - | - |
| EPS453 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553576 | - | - |
| EPS454 | A. sparoides | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553577 | - | - |
| EPS484 | A. sparoides | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553578 | - | - |
| EPS485 | A. sparoides | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553579 | - | - |
| EPS486 | A. sparoides | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553580 | - | - |
| EPS487 | A. sparoides | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553581 | - | - |
| EPS488 | A. sparoides | Indian Ocean | Mauritius | -20.12 | 57.45 | PB | KU553582 | - | - |
| WJC85 | A. taurus | Atlantic Ocean | Puerto Rico | -66.13 | 18.48 | KC | KU553583 | KU554237 | KU553991 |
| EPS15 | A. vaigiensis $A$ | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553763 | KU554305 | KU554072 |
| EPS16 | A. vaigiensis $A$ | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553764 | KU554306 | KU554073 |
| EPS17 | A. vaigiensis $A$ | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553765 | KU554307 | KU554074 |
| EPS18 | A. vaigiensis $A$ | Indian Ocean | Europa | -22.37 | 40.37 | PB, WJC | KU553766 | KU554308 | KU554075 |
| EPS342 | A. vaigiensis $A$ | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553767 | KU554309 | KU554076 |
| EPS343 | A. vaigiensis $A$ | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553768 | KU554310 | KU554077 |
| PNG24 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553769 | - | - |
| PNG313 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553770 | - | - |
| PNG610 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553771 | KU554311 | KU554078 |
| PNG611 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553772 | KU554312 | KU554079 |
| PNG612 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553773 | KU554313 | KU554080 |
| PNG613 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553774 | KU554314 | KU554081 |
| PNG614 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553775 | KU554315 | KU554082 |
| PNG615 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553776 | KU554316 | KU554083 |
| PNG616 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553777 | KU554317 | KU554084 |
| PNG617 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553778 | KU554318 | KU554085 |
| PNG618 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553779 | KU554319 | KU554086 |
| PNG619 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553780 | KU554320 | KU554087 |
| PNG620 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553781 | - | - |
| PNG621 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553782 | - | - |
| PNG622 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553783 | - | - |
| PNG623 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553784 | - | - |
| PNG624 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553785 | - | - |
| PNG625 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553786 | - | - |
| PNG626 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553787 | - | - |
| PNG627 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553788 | - | - |
| PNG628 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553789 | - | - |
| PNG629 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553790 | - | - |
| PNG630 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553791 | - | - |


| PNG631 | A. vaigiensis $A$ | Coral Triangle | PNG | -5.22 | 145.80 | WJC | KU553792 | - | - |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PNG2150 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553793 | KU554321 | KU554088 |
| PNG2151 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553794 | KU554322 | KU554089 |
| PNG2152 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553795 | KU554323 | KU554090 |
| PNG2153 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553796 | KU554324 | KU554091 |
| PNG2154 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553797 |  |  |
| PNG2155 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553798 | KU554325 | KU554092 |
| PNG2156 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553799 | KU554326 | KU554093 |
| PNG2157 | A. vaigiensis $A$ | Coral Triangle | PNG | -9.83 | 151.51 | WJC | KU553800 | KU554327 | KU554094 |
| PNG2685 | A. vaigiensis $A$ | Coral Triangle | PNG | -1.84 | 150.85 | WJC | KU553801 | KU554328 | KU554095 |
| WJC711 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553802 | KU554329 | KU554096 |
| WJC712 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553803 | KU554330 | KU554097 |
| WJC713 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553804 | KU554331 | KU554098 |
| WJC714 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.02 | 122.00 | WJC | KU553805 | KU554332 | KU554099 |
| WJC849 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553806 | KU554333 | KU554100 |
| WJC850 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553807 | KU554334 | KU554101 |
| WJC851 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553808 | KU554335 | KU554102 |
| WJC852 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553809 | KU554336 | KU554103 |
| WJC853 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553810 | KU554337 | KU554104 |
| WJC854 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553811 | KU554338 | KU554105 |
| WJC956 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553812 | - | - |
| WJC957 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553813 |  |  |
| WJC958 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553814 | - |  |
| WJC959 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553815 | - |  |
| WJC960 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553816 |  |  |
| WJC961 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553817 | KU554339 | KU554106 |
| WJC1069 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553818 | KU554340 | KU554107 |
| WJC1373 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553819 | KU554341 | KU554108 |
| WJC1374 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553820 | KU554342 | KU554109 |
| WJC1412 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553821 | KU554343 | KU554110 |
| WJC1413 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553822 | KU554344 | KU554111 |
| WJC1414 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553823 | KU554345 | KU554112 |
| WJC1427 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553824 | KU554346 | KU554113 |
| WJC1428 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553825 | KU554347 | KU554114 |
| WJC1429 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553826 | KU554348 | KU554115 |
| WJC1430 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 23.48 | 121.51 | WJC | KU553827 | KU554349 | KU554116 |
| WJC1437 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553828 |  |  |
| WJC1438 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553829 |  |  |
| WJC1439 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553830 |  |  |
| WJC1440 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553831 | KU554350 | KU554117 |
| WJC1441 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553832 | - |  |
| WJC1442 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553833 | - |  |
| WJC1443 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553834 | - |  |
| WJC1444 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553835 | - |  |
| WJC1445 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553836 | KU554351 | KU554118 |
| WJC1446 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553837 | - | - |
| WJC1447 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553838 | KU554352 | KU554119 |
| WJC1448 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553839 | - | - |
| WJC1547 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553840 | KU554353 | KU554120 |
| WJC1548 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553841 |  |  |
| WJC1549 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553842 | - |  |
| WJC1550 | A. vaigiensis $A$ | Coral Triangle | East Taiwan | 22.75 | 121.15 | WJC | KU553843 | - |  |
| WJC1612 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553844 | KU554354 | KU554121 |
| WJC1613 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553845 | KU554355 | KU554122 |
| WJC1614 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553846 | KU554356 | KU554123 |
| WJC1615 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553847 | KU554357 | KU554124 |
| WJC1802 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553848 | KU554358 | KU554125 |
| WJC1803 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553849 | - |  |
| WJC1804 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553850 | KU554359 | KU554126 |
| WJC1805 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553851 | KU554360 | KU554127 |
| WJC1806 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553852 | - |  |
| WJC2333 | A. vaigiensis $A$ | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553853 | KU554361 | KU554128 |
| WJC2954 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553854 | KU554362 | KU554129 |
| WJC2955 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553855 | KU554363 | KU554130 |
| WJC2956 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553856 | KU554364 | KU554131 |
| WJC2957 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553857 | KU554365 | KU554132 |
| WJC2958 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553858 | KU554366 | KU554133 |
| WJC2959 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553859 | KU554367 | KU554134 |
| WJC2960 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553860 | KU554368 | KU554135 |
| WJC4132 | A. vaigiensis $A$ | Coral Triangle | Dongsha | 20.72 | 116.70 | WJC | KU553861 | - | KU554136 |
| WJC4234 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553862 | KU554369 | KU554137 |
| WJC4235 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553863 | KU554370 | KU554138 |
| WJC4236 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553864 | KU554371 | KU554139 |
| WJC4237 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553865 | - | KU554140 |
| WJC4238 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553866 | KU554372 | KU554141 |
| WJC4239 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553867 | KU554373 | KU554142 |
| WJC4240 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553868 | KU554374 | KU554143 |


| WJC4241 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553869 | KU554375 | KU554144 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| WJC4242 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553870 | KU554376 | KU554145 |
| WJC4243 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553871 | KU554377 | - |
| WJC4244 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553872 | KU554378 | KU554146 |
| WJC4245 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553873 | KU554379 | KU554147 |
| WJC4246 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553874 | KU554380 | KU554148 |
| WJC4247 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553875 | KU554381 | KU554149 |
| WJC4248 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553876 | KU554382 | KU554150 |
| WJC4249 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU533877 | KU554383 | - |
| WJC4250 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553878 | KU554384 | KU554151 |
| WJC4251 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553879 | KU554385 | KU554152 |
| WJC4252 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553880 | KU554386 | KU554153 |
| WJC4253 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553881 | KU554387 | KU554154 |
| WJC4254 | A. vaigiensis $A$ | Indian Ocean | Maldives | 4.17 | 73.51 | JDB | KU553882 | KU554388 | KU554155 |
| WJC4285 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553883 | - | - |
| WJC4286 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553884 | - | - |
| WJC4287 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553885 | - | - |
| WJC4288 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553886 | - | - |
| WJC4289 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553887 | - | - |
| WJC4290 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553888 | - | - |
| WJC4291 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553889 | - | - |
| WJC4292 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.01 | 120.71 | WJC | KU553890 | - | - |
| WJC4305 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553891 | - | - |
| WJC4306 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553892 | - |  |
| WJC4307 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553893 | - | - |
| WJC4308 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553894 | - | - |
| WJC4309 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553895 | - | - |
| WJC4310 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | wJC | KU553896 | - | - |
| WJC4311 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | wJC | KU553897 | - | - |
| WJC4312 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553898 | - | - |
| WJC4313 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553899 | - | - |
| WJC4325 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553900 | - | - |
| WJC4348 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553901 | - | - |
| WJC4349 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553902 | - | - |
| WJC4350 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553903 | - |  |
| WJC4351 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553904 | - | - |
| WJC4352 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553905 | - | - |
| WJC4353 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 21.98 | 120.75 | WJC | KU553906 | - | - |
| WJC4369 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | wJC | KU553907 | - | - |
| WJC4515 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553908 | - | - |
| WJC4516 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553909 | - | - |
| WJC4517 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | wJC | KU553910 | - | - |
| WJC4518 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | wJC | KU553911 | - | - |
| WJC4519 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553912 | - | - |
| WJC4520 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553913 | - | - |
| WJC4521 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553914 | - | - |
| WJC4522 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553915 | - | - |
| WJC4523 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | WJC | KU553916 | - | - |
| WJC4524 | A. vaigiensis $A$ | Coral Triangle | North Taiwan | 25.16 | 121.76 | wJC | KU553917 | - | - |
| WJC4773 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553918 | - | - |
| WJC4774 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553919 | - | - |
| WJC4775 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553920 | - | - |
| WJC4776 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553921 | - | - |
| WJC4777 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553922 | - | - |
| WJC4778 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553923 | - | - |
| WJC4779 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553924 | - | - |
| WJC4780 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553925 | - | - |
| WJC4781 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553926 | - | - |
| WJC4782 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553927 | - | - |
| WJC4783 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553928 | - | - |
| WJC4784 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553929 | - | - |
| WJC4785 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553930 | - | - |
| WJC4786 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553931 | - | - |
| WJC4787 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | WJC | KU553932 | - | - |
| WJC4788 | A. vaigiensis $A$ | Coral Triangle | Hainan | 18.36 | 109.77 | wJC | KU553933 | - | - |
| WJC4790 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553934 | - | - |
| WJC4792 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | wJC | KU553935 | - | - |
| WJC4793 | A. vaigiensis $A$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | wJC | KU553936 | - | - |
| WJC4841 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553937 | KU554389 | KU554156 |
| WJC4842 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553938 | KU554390 | KU554157 |
| WJC4843 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553939 | KU554391 | KU554158 |
| WJC4844 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553940 | KU554392 | KU554159 |
| WJC4845 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553941 | KU554393 | KU554160 |
| WJC4847 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553942 | KU554394 | KU554161 |
| WJC4848 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553943 | KU554395 | KU554162 |
| WJC4849 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553944 | KU554396 | KU554163 |
| WJC4850 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553945 | KU554397 | KU554164 |


| WJC4853 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553946 | KU554398 | KU554165 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| WJC4855 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553947 | KU554399 | KU554166 |
| WJC4856 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553948 | - | KU554167 |
| WJC4857 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553949 | KU554400 | KU554168 |
| WJC4858 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553950 | KU554401 | KU554169 |
| WJC4859 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553951 | KU554402 | KU554170 |
| WJC4860 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553952 | KU554403 | KU554171 |
| WJC4861 | A. vaigiensis $A$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553953 | KU554404 | KU554172 |
| WJC4946 | A. vaigiensis $A$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553954 | - | KU554173 |
| WJC5227 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553955 | - | KU554174 |
| WJC5228 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553956 | - | KU554175 |
| WJC5229 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553957 | - | KU554176 |
| WJC5230 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553958 | - | KU554177 |
| WJC5231 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553959 | - | KU554178 |
| WJC5232 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553960 | - | KU554179 |
| WJC5233 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553961 | - | KU554180 |
| WJC5234 | A. vaigiensis $A$ | Red Sea | Eilat | 29.52 | 34.93 | TGR | KU553962 | - | KU554181 |
| EPS432 | A. vaigiensis $B$ | Indian Ocean | Glorieuses | -11.53 | 47.33 | PB, WJC | KU553587 | KU554405 | KU554182 |
| WC2961 | A. vaigiensis $B$ | Coral Triangle | Taiping | 10.38 | 114.37 | WJC | KU553588 | KU554406 | KU554183 |
| WJC4193 | A. vaigiensis $B$ | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553589 | KU554407 | - |
| WJC4194 | A. vaigiensis $B$ | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553590 | KU554408 | KU554184 |
| WJC4196 | A. vaigiensis $B$ | Coral Triangle | West Papua | 0.43 | 130.82 | PB | KU553591 | KU554409 | KU554185 |
| WJC4791 | A. vaigiensis $B$ | Coral Triangle | South Taiwan | 22.35 | 120.37 | WJC | KU553592 | KU554410 | - |
| WJC1291 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553593 | KU554411 | KU554186 |
| WJC1292 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553594 | KU554412 | KU554187 |
| WJC1293 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553595 | KU554413 | KU554188 |
| WJC1294 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553596 | KU554414 | KU554189 |
| WJC1295 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553597 | KU554415 | KU554190 |
| WJC1296 | A. vaigiensis $C$ | Coral Triangle | Caohagan | 10.20 | 124.02 | FG | KU553598 | KU554416 | KU554191 |
| WJC4840 | A. vaigiensis $C$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553599 | KU554417 | KU554192 |
| WJC4846 | A. vaigiensis $C$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553600 | KU554418 | KU554193 |
| WJC4851 | A. vaigiensis $C$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553601 | KU554419 | KU554194 |
| WJC4852 | A. vaigiensis $C$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553602 | - | KU554195 |
| WJC4854 | A. vaigiensis $C$ | Coral Triangle | Bali | -8.08 | 114.42 | SB | KU553603 | KU554420 | KU554196 |
| WJC1262 | A. whitleyi | Pacific | New Caledonia | -22.32 | 166.47 | PB | KU553584 | KU554238 | KU553992 |
| WJC1263 | A. whitleyi | Pacific | New Caledonia | -22.32 | 166.47 | PB | KU553585 | KU554239 | KU553993 |
| WJC1264 | A. whitleyi | Pacific | New Caledonia | -22.32 | 166.47 | PB | KU553586 | KU554240 | KU553994 |
| N | - | - | - | - |  | - | 484 | 224 | 234 |

Collectors' names abbreviations: SB, S. Bahri; PB, P. Borsa; WJC, W-J Chen; JDB, J. DiBattista; KC, K. Conway; FG, F. Giancarlo; HH, H.-C. Ho; MK, M. Kulbicki; TGR, T. Gurevich Raguso.

Supplementary Table S2 Locus names, PCR primers used, amplification conditions, resulting sequence length and evolution models at the three loci scored in this study

| Locus | PCR name ( $\mathrm{F} / \mathrm{R}$ ) | Primer sequence | Source | Annealing temperature | Sequence length (bp) | Evolution model by codon position |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  | 1 | 2 | 3 |
| Cytochrome $b$ | $\begin{aligned} & \text { 14695-F / } \\ & 15969-\mathrm{R} \end{aligned}$ | 5'-AAGCCACCGTTGTTATTCAACTA-3'/ <br> 5'-ATCCTAGCTTTGGGAGTTAGGG-3' | WJC, unpubl. | $55^{\circ} \mathrm{C}$ | 1161 | K80+I+G | HKY+I | GTR+G |
| RAG1 | $\begin{aligned} & 2533-\mathrm{F} / \\ & 4061-\mathrm{R} \end{aligned}$ | $\begin{aligned} & \text { 5'-CTGAGCTGCAGTCAGTACCATAAGATGT-3' } \\ & \text { /-'AATACTTGGAGGTGTAGAGCCAGT-3 } \end{aligned}$ | López et al., 2004 / <br> Chen et al., 2007 | $54^{\circ} \mathrm{C}$ | 1456 | K80+I | K80+I | HKY+G |
| Dystrophin | Dyst-MCE1-F / <br> Dyst-MCE1-R | 5'-TTACAGCAGACCAACAGTGAGAA-3' / <br> 5'-GAAGTTAACCACGTTGACCTGAG-3' | WJC, unpubl. | $55^{\circ} \mathrm{C}$ | 1211 | HKY | - | - |

## References

Chen W-J, Ruiz-Carus R, Ortí G (2007) Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. Journal of Fish Biology,70 (sb), 202-218.
López JA, Chen W-J, Ortí G (2004) Esociform phylogeny. Copeia, 2004, 449-464.

Supplementary Table S3A Genetic diversity and results of neutrality test derived from $A$. sexfasciatus and $A$. vaigiensis lineage A at nuclear locus RAG1 across sample localities and regions. N sample size (number of sequences); $S$ number of polymorphic sites; $H_{d}$ haplotype diversity; $\pi$ nucleotide diversity; $S D$ standard deviation, estimated by bootstrap resampling. Values of Tajima's (1989) $D$ and Fu's (1997) $F_{S}$ wereconsidered statistically significant $\left(^{*}\right)$ when $P<0.05$ and $P<0.02$, respectively

| Species, region, Locality | $N$ | S | $H_{\mathrm{d}} \pm \mathrm{SD}$ | $\pi \pm$ SD | Tajima's $D$ | Fu's Fs |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A. sexfasciatus, Indian Ocean |  |  |  |  |  |  |
| Glorieuses | 6 | 11 | $0.73 \pm 0.16$ | $0.0045 \pm 0.0029$ | 2.15 | 3.64 |
| Mauritius | NA | NA | NA | NA | NA | NA |
| A. sexfasciatus, Coral Triangle Region |  |  |  |  |  |  |
| Coral Triangle | 6 | 10 | $1.00 \pm 0.96$ | $0.0029 \pm 0.0022$ | 0.67 | -2.21 |
| North Taiwan | 16 | 15 | $0.96 \pm 0.04$ | $0.0028 \pm 0.0016$ | -0.39 | -5.23* |
| South Taiwan | 10 | 9 | $0.93 \pm 0.08$ | $0.0024 \pm 0.0015$ | 0.42 | -3.05 |
| East Taiwan | 6 | 8 | $1.00 \pm 0.10$ | $0.0024 \pm 0.0017$ | 0.05 | -2.90* |
| Dongsha | 2 | 0 | $0.00 \pm 0.00$ | $0.0000 \pm 0.0000$ | 0 | NA |
| A. sexfasciatus, Pacific Ocean |  |  |  |  |  |  |
| New Caledonia | 10 | 7 | $0.53 \pm 0.18$ | $0.0012 \pm 0.0009$ | -1.31 | 0.25 |
| Moruroa | 14 | 7 | $0.81 \pm 0.07$ | $0.0017 \pm 0.0011$ | 0.34 | -0.48 |
| A. vaigiensis $A$, Indian Ocean |  |  |  |  |  |  |
| MozambiqueChannel | 12 | 10 | $1.00 \pm 0.03$ | $0.0022 \pm 0.0014$ | -0.10 | -10.80* |
| Maldives | 18 | 18 | $0.95 \pm 0.04$ | $0.0035 \pm 0.0020$ | -0.11 | -4.51 |
| A. vaigiensis $A$, Coral Triangle Region |  |  |  |  |  |  |
| PNG | 26 | 16 | $0.90 \pm 0.04$ | $0.0020 \pm 0.0012$ | -1.07 | -3.96 |
| North Taiwan | 6 | 6 | $0.93 \pm 0.12$ | $0.0017 \pm 0.0013$ | -0.35 | -1.67 |
| South Taiwan | 20 | 8 | $0.85 \pm 0.05$ | $0.0011 \pm 0.0008$ | -0.75 | -3.36* |
| East Taiwan | 22 | 17 | $0.95 \pm 0.03$ | $0.0021 \pm 0.0012$ | -1.30 | -6.02* |
| Hainan | 6 | 9 | $0.93 \pm 0.12$ | $0.0025 \pm 0.0017$ | -0.52 | -0.94 |
| Dongsha | 2 | 1 | $1.00 \pm 0.50$ | $0.0007 \pm 0.0010$ | 0 | 0 |
| Taiping | 8 | 9 | $0.93 \pm 0.08$ | $0.0018 \pm 0.0012$ | -1.22 | -1.95 |
| Bali | 22 | 16 | $0.98 \pm 0.02$ | $0.0023 \pm 0,0014$ | -0.86 | -17.52* |

Supplementary Table S3B Genetic diversity and results of neutrality test derived from $A$. sexfasciatus and $A$. vaigiensis lineage A at nuclear locus $D y s t$ across sample localities and regions. $N$ sample size (number of sequences); $S$ number of polymorphic sites; $H_{d}$ haplotype diversity; $\pi$ nucleotide diversity; $S D$ standard deviation, estimated by bootstrap resampling. Values of Tajima's (1989) $D$ and Fu's (1997) F $F_{\text {s }}$ were considered statistically significant (*) when $P<0.05$ and $P<0.02$, respectively

| Species, Region, Locality | N | S | $H_{\mathrm{d}} \pm \mathrm{SD}$ | $\pi \pm$ SD | Tajima's $D$ | Fu's Fs |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A. sexfasciatus, Indian Ocean | 24 | 5 | $0.44 \pm 0.12$ | $0.0008 \pm 0.0006$ | -1.19 | -1.50 |
| Coral Triangle region |  |  |  |  |  |  |
| North Taiwan | 28 | 1 | $0.49 \pm 0.05$ | $0.0005 \pm 0.0005$ | 1.48 | 1.58 |
| South Taiwan | 16 | 2 | $0.51 \pm 0.13$ | $0.0005 \pm 0.0005$ | -0.19 | -0.18 |
| East Taiwan | 22 | 3 | $0.54 \pm 0.09$ | $0.0006 \pm 0.0005$ | -0.71 | -1.08 |
| West Papua | 6 | 1 | $0.33 \pm 022$ | $0.0003 \pm 0.0004$ | -0.93 | 0 |
| Dongsha | 18 | 2 | $0.45 \pm 0.12$ | $0.0005 \pm 0.0005$ | -0.44 | -0.38 |
| Pacific Ocean |  |  |  |  |  |  |
| New Caledonia | 8 | 92 | $0.82 \pm 0.10$ | $0.0383 \pm 0.0213$ | 0.71 | 9.54* |
| Moruroa | 20 | 4 | $0.53 \pm 0.04$ | $0.0022 \pm 0.0014$ | 2.51* | 5.56* |
| A. vaigiensis lineage A |  |  |  |  |  |  |
| Indian Ocean |  |  |  |  |  |  |
| Eilat (Red Sea) | 16 | 1 | $0.13 \pm 0.11$ | $0.0001 \pm 0.0002$ | -1.16 | -0.70 |
| MozambiqueChannel | 12 | 1 | $0.41 \pm 0.13$ | $0.0004 \pm 0.0004$ | 0.54 | 0.73 |
| Maldives | 38 | 36 | $0.61 \pm 0.05$ | $0.0155 \pm 0.0079$ | 2.35* | 19.75* |
| Coral Triangle Region |  |  |  |  |  |  |
| PNG | 36 | 4 | $0.67 \pm 0.07$ | $0.0008 \pm 0.0007$ | -0.33 | -1.97 |
| North Taiwan | 8 | 1 | $0.25 \pm 0.18$ | $0.0002 \pm 0.0003$ | -1.05 | -0.18 |
| South Taiwan | 24 | 4 | $0.65 \pm 0.08$ | $0.0008 \pm 0.0006$ | -0.73 | -1.51 |
| East Taiwan | 24 | 2 | $0.47 \pm 0.11$ | $0.0005 \pm 0.0005$ | -0.07 | -0.02 |
| Hainan | 6 | 5 | $0.53 \pm 0.17$ | $0.0026 \pm 0.0019$ | 0 | NA |
| Dongsha | 4 | 1 | $0.50 \pm 0.27$ | $0.0005 \pm 0.0006$ | -0.61 | 0.17 |
| Taiping | 16 | 6 | $0.76 \pm 0.06$ | $0.0025 \pm 0.0016$ | -0.19 | 2.13 |
| Bali | 32 | 2 | $0.55 \pm 0.08$ | $0.0006 \pm 0.0005$ | 0.47 | 0.52 |

Supplementary Table S4 Output from the nested-model comparison procedure of IMa2. Numbersin first column correspond to the following models: 1 full (allowing all the parameters to vary); 2: equal migration rates; 3: coalescent migration rate is zero from population 1 to 2 (A. sexfasciatus to $A$. vaigiensis lineage A ); 4: coalescent migration rate is zero from population 2 to 1 (A. vaigiensis lineage A to $A$. sexfasciatus); and 5: coalescent migration rate is zero. Values in between brackets are fixed under the model considered. From left to right, the different columns report the $\log$-likelihood of the model: $\log (\mathrm{P})$, the number of varying parameters in the model: \#terms, the number of degrees of freedom: df , the log-likelihood ratio statistics: 2LLR, the effective sample size (ESS), the estimates for effective population sizes of the ancestral population $\left(\theta_{\mathrm{a}}\right)$, population $1\left(A\right.$. sexfasciatus, $\left.\theta_{1}\right)$ and population $2\left(A\right.$. vaigiensis lineage $\left.\mathrm{A}, \theta_{2}\right)$, the effective migration rates from population 1 to population 2 (from $A$. sexfasciatus to $A$. vaigiensis lineage $\mathrm{A}, m_{1>2}$ ) and from population 2 to population 1 (from $A$. vaigiensis lineage A to $A$. sexfasciatus, $m_{2>1}$ ) and the statistical significance of the model

| Model | $\log (\mathrm{P})$ | \#terms | df | 2LLR | ESS | $\theta_{\text {a }}$ | $\theta_{1}$ | $\theta_{2}$ | $m_{1>2}$ | $m_{2>1}$ | $P$-value |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | -2,473 | 5 | - | - | 1.309 | 6.3572 | 9.2907 | 0.0117 | 0.00960 | 0.07270 | NA |
| 2 | -3,626 | 4 | 1 | 2.305 | 1678 | 6.3426 | 10.238 | 36.435 | 0.04660 | [0.04660] | 0.129 |
| 3 | -4,084 | 4 | 1* | 3.223 | 169.4 | 6.8252 | 9.7426 | 38.493 | [0.00000] | 0.075900 | 0.036 |
| 4 | -15,54 | 4 | 1* | 26.14 | 133.4 | 5.9223 | 10.356 | 46.515 | 0.08590 | [0.00000] | 0.000 |
| 5 | -41,61 | 3 | 2* | 78.27 | 1.000 | 4.6473 | 11.025 | 18.515 | [0.00000] | [0.00000] | 0.000 |

[^2]Supplementary Figure S1 Phylogenetic trees inferred for Abudefduf spp. based on nuclear loci (RAG1 and Dyst). Tree topology corresponds to the best maximum likelihood (ML) tree. When $>50$, node supports is indicated by bootstrap values;white circles show nodes whose Bayesian posterior probability was $>90 \%$. Abudeffduf sexfasciatus sequences generated in this study are in red, $A$. vaigiensis sequences are in different tones of blue depending on the lineage ( $\mathrm{A}, \mathrm{B}$ or C ), and sequences from other Abudefduf species are in black; sequences retrieved from GenBank are in grey. When nodes were collapsed for readability purpose, the number of sequences included in each cluster as well as their geographical origin were reported. (A) ML tree of RAG1 gene sequences. The homologous sequence in Amphiprion ocellaris (GenBank AY208631) was used as an outgroup. Similiparma lurida (previously Abudefduf luridus) is considered as a separate genus (Cooper et al., 2014). The $A$. whitleyi sequence from Lizard Is. (GenBank FJ616626) highlighted in orange may have been misidentified. (B) ML tree of Dyst gene sequences.

Supplementary Figure S2 Abudefduf sexfasciatus and $A$. vaigiensis lineage A, relative likelihood distributions at each of demographic parameters inferred from IMa2 analysis. Left column: splitting time ( $t$, full line) and $\nexists \mathrm{MRCA}$ (dashed line, when available) in million years before present (Myrs BP). Central column: effective migration rates ( $m$ ) from $A$. sexfasciatus to $A$. vaigiensis (in black) and from $A$. vaigiensis lineage A to $A$. sexfasciatus (in grey) in individuals per million year (ind./Myr). Right column: effective population size ( Ne ) in millions of individuals (ind.) for $A$. sexfasciatus ( $N_{1}$, in black), A. vaigiensis lineage A ( $N_{2}$, in grey) and the ancestral population ( $N_{\mathrm{A}}$, dashed black line). (A) From RAG1 gene sequences and (B) from Dyst gene sequences.





(Myrs B.P.)


Effective migration rate
(ind./Myr)


Ne
(x10 ${ }^{6}$ ind.)


[^0]:    a 'Mozambique Channel' groups samples from Europa, Juan de Nova and Glorieuses Is.;
    ${ }^{\mathrm{b}}$ Little Liuqiu is a small island off the southwestern coast of Taiwan;
    c 'PNG' groups samples from Madang, Kavieng and the Louisiade archipelago at the eastern extremity of New Guinea.

[^1]:    * When several loci were involved, the average mutation rate was calculated as the geometric mean of the individual mutation rates

[^2]:    * test distribution of 2LLR is a mixture

