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Patterns of lineage diversification in rabbitfishes

Philippe Borsa^{a,*}, Sarah Lemer^{a,b}, Didier Aurelle^b

^a *Institut de recherche pour le développement, UR 128, Nouméa, New Caledonia*

^b *Dimar (UMR 6540 CNRS / Université de la Méditerranée), Station marine d'Endoume, rue de la Batterie des lions, Marseille, France*

* Corresponding author. Fax: +687 264326.

E-mail address: Philippe.Borsa@noumea.ird.nc (P. Borsa)

Abstract – Fishes of the tropical Indo-Pacific family Siganidae comprise 28 species, characterized by their body proportions and their colour patterns. A mitochondrial phylogeny of 20 Siganidae species was produced to infer their evolutionary history. Three distinct, major clades were found, that also correspond to the early radiation of the family into three major ecological types: fusiform species that also live in schools on the inshore reef flats (*S. canaliculatus*, *S. fuscescens*, *S. luridus*, *S. rivulatus*, *S. spinus*, *S. sutor*); deep-bodied species including brightly coloured ones whose adults live in pairs on the reef front (*S. corallinus*, *S. doliatus*, *S. puellus*, *S. punctatus*, *S. unimaculatus*, *S. virgatus*, *S. vulpinus*), and species that live in small schools in mangroves, estuaries and estuarine lakes (*S. guttatus*, *S. javus*, *S. lineatus*, *S. randalli*, *S. vermiculatus*); and a third clade including a cosmopolitan species, *S. argenteus*, the only species of the family known to possess a pelagic, prejuvenile stage and *S. woodlandi*, a recently described species from New Caledonia and morphologically close to *S. argenteus*. The partition of the genus into two sub-genera, *Lo* (erected for *S. unimaculatus*, *S. vulpinus* and three related species possessing a tubular snout) and *Siganus* (all the other species), had no phylogenetic rationale. The present results indicate that the tubular snout, which apparently results from ecological specialization, is a recent acquisition within the deep-body clade. The Western Indian Ocean endemic *S. sutor* appeared as the sister-species of the Red Sea endemic *S. rivulatus* within a well-supported subclade that also included *S. canaliculatus* and *S. fuscescens*. *S. spinus* did not appear as sister-species to *S. luridus*. *S. lineatus* haplotypes formed a paraphyletic group with *S. guttatus*, and an early isolation of Maldives *S. lineatus* was suggested. Unexpectedly, *S. randalli* did not appear as the sister-species of *S. vermiculatus*, but its haplotypes instead were embedded within the West Pacific *S. lineatus* haplogroup, suggesting recent introgression. Among currently-recognized sister-species with parapatric distribution, *S. doliatus* and *S. virgatus* haplotypes formed a single, unresolved haplogroup, as did *S. unimaculatus* and *S. vulpinus*. The occurrence of two distinct clades within *S. fuscescens* was confirmed.

1. Introduction

Increasingly complex theories are being developed regarding the origin and evolution of marine species in the central Indo-West Pacific region, whose coral reef and lagoon habitats harbour the highest biological diversity of all seas (Woodland, 1983; Palumbi, 1996; Hughes et al., 2002; Barber and Bellwood, 2005; Briggs, 2006). Molecular phylogenies provide the information against which hypotheses on the biogeography of species and scenarios of ecological specialization can be tested.

Rabbitfishes (Siganidae) are typical Indo-Pacific coral-reef fishes, that occupy all types of coastal habitats, from estuaries and mangroves to the reef front, the reef flat, and seaweed mats in the lagoon. Twenty eight nominal species are currently recognized in that family, based on morphology and colour patterns (Woodland, 1990; Randall and Kulbicki, 2005). Fishes of the family Siganidae exhibit uniformity in those characters (i.e. numbers of fin spines and rays, tooth shape, tooth count) which the systematics of fishes usually rely on. The fact that a majority of fishes of the family have bright and unique colour patterns has been exploited for defining species boundaries, but higher-level classification essentially relies on gross body proportions, shape of tail, and length of snout (Woodland, 1990). The family Siganidae in its present description consists of a single genus, *Siganus*, which has been subdivided into sub-genera *Siganus* and *Lo*, the latter to distinguish those five species that possess a prominent, tubular snout (De Beaufort and Chapman, 1951; Woodland, 1990; Randall, 2005). The other species have “pointed, blunt, or tumid snouts” (Woodland 1990). *Lo* was originally erected as a genus by Seale (1906), but Woodland (1990) considered that the differences in snout shape between *Lo* and *Siganus* were not sufficiently clear-cut for being recognized as different genera, arguing that the pointed shape of the snout of *S. corallinus* approaches the condition of *S. (Lo) uspi*, whose snout is the shortest of all 5 *Lo* species. Presumed, closely related (sibling) species of Siganidae have essentially parapatric distributions. Nine such groups of sibling taxa have been recognized (Woodland, 1999), most of which share their common boundaries in the central Indo-West Pacific region. It has been argued that this phenomenon may result from habitat specialization together with competitive exclusion, and be a cause of higher species richness in an area where the diversity of habitats is greater than in other regions of the Indo-Pacific (Woodland, 1983; 1999; Barber and Bellwood, 2005).

Only scarce genetic data are available concerning fishes of the family Siganidae. An allozyme study conducted on ten *Siganus* spp. species allowed phylogenetic inference based on allozyme distances (Lacson and Nelson, 1993). The authors provisionally concluded that a major dichotomy separated deep-bodied and shallow-bodied *Siganus* spp. They observed that the genetic distance between two sibling species with virtually parapatric distribution (*S. randalli* and *S. vermiculatus*) was of the same order as those estimated between geographic populations within a species (Fig. 1). Also, the subgenus *Lo*, represented by *S. vulpinus*, did not appear as a clade distinct from the other species. Instead, *S. vulpinus* paired with *S. punctatus* (Fig. 1), a result that was deemed “unpredicted” by Lacson and Nelson (1993). No test of robustness of the allozyme tree was, nor can be performed from the data that were

then published, hence it is not clear how much reliable its topology was. These authors noticed that *S. argenteus* was the least divergent genetically from outgroup *Naso lituratus* (Acanthuridae), but the topology of the allozyme tree (Fig. 1) apparently contradicted this statement. Other, recent genetic surveys focused on the loss of genetic variation in *S. luridus* and *S. rivulatus* populations subsequent to their crossing of the Suez Canal (Bonhomme et al., 2003; Hassan et al., 2003; Azzurro et al., 2006), and on examining the barcoding potential of a short fragment of the cytochrome *b* gene, that was also used to estimate the degree of genetic differentiation between populations of eight Siganidae species at the scale of the West Pacific (Lemer et al., 2007). Further phylogenetic analysis is necessary to understand the evolution of morphology (i.e. body proportions and snout shape) and life-history (i.e. duration of the pelagic stage) in Siganidae. As these characters are of obvious ecological relevance, they may have been involved in speciation in Siganidae.

Here, we inferred the phylogeny of twenty *Siganus* species from the nucleotide sequences of a 825-bp composite fragment of the mitochondrial DNA, with the following objectives: to test the validity of the current taxonomy of Siganidae, based on Woodland's (1990) extensive revision; to explore the patterns of lineage diversification in that family and eventually relate them to scenarios of ecological specialization; to address the genetic relationships between parapatrically distributed sibling species and to tentatively infer speciation processes.

2. Materials and methods

Siganidae species were separated by Woodland (1990) into "deep-bodied" and "fusiform" species. For the 20 species examined here, the range of variation of the ratio of standard length to body depth was the following: *S. argenteus*, 2.4-3.0; *S. canaliculatus*, 2.4-2.8; *S. corallinus*, 1.7-2.4; *S. doliatus*, 1.8-2.2; *S. fuscescens*, 2.3-2.9; *S. guttatus*, 1.8-2.3; *S. javus*, 2.0-2.3; *S. lineatus*, 1.9-2.2; *S. luridus*, 2.1-2.8; *S. puellus*, 2.3-2.6; *S. punctatus*, 1.9-2.3; *S. randalli*, 2.0-2.2; *S. rivulatus*, 2.6-3.2; *S. spinus*, 2.3-2.8; *S. sutor*, 2.2-2.6; *S. unimaculatus*, 2.1-2.4; *S. vermiculatus*, 1.9-2.2; *S. virgatus*, 1.8-2.3; *S. vulpinus*, 1.9-2.4; *S. woodlandi*, 2.1-2.4 [after Woodland (1990) except *S. woodlandi*: Randall and Kulbicki (2005)]. *S. puellus* tends to show a ratio of standard length to body depth higher than the other deep-bodied species, but nevertheless cannot be considered fusiform, owing to its rather flat, rectangular shape. Some of these species (*S. fuscescens*, *S. luridus*, *S. rivulatus*, *S. spinus*, *S. sutor*), all fusiform, have a drab colour while the other species exhibit bright colours and/or contrasted colour patterns (Woodland, 1990). Most species of the reef front, all brightly coloured, are encountered as pairs (*S. corallinus*, *S. doliatus*, *S. puellus*, *S. punctatus*, *S. vulpinus*) while the other species usually occur as small schools (Randall et al., 1990; Woodland, 1990; Woodland, 2001). Randall and Kulbicki (2005) report that *S. woodlandi* occurs in schools as does *S. argenteus*, partly based on a picture of a school of *S. woodlandi* by Fourmanoir and Laboute (1976). However, one of us (P. B.) has regularly observed pairs of adult *S. woodlandi* on various parts of the reef front around southern New Caledonia at 6-15 m depth. In only one instance did three pairs of *S. woodlandi* appear to forage together, forming an ephemeral, loose school of 6 individuals. *S. argenteus* is the only Siganidae known to spawn pelagic eggs and to possess an extended pelagic,

prejuvenile stage that is analogous to the acronurus stage of Acanthuridae (which also have pelagic eggs) (Woodland, 1990; Leis and Carson-Ewart, 2001). All other Siganidae species except, perhaps, *S. woodlandi* for which no life-history data are available, spawn benthic eggs (Popper et al., 1979; Leis and Richards, 1984) or are believed to (Woodland, 1990), and lack a transitional pelagic stage (Woodland, 1990; Leis and Carson-Ewart, 2001). Four pairs of sibling, parapatric species sensu Woodland (1999) were sampled for the present survey. These were *S. doliatus* / *S. virgatus*, *S. guttatus* / *S. lineatus*, *S. luridus* / *S. spinus* and *S. unimaculatus* / *S. vulpinus*.

Details on the individuals sampled (sampling location and date, voucher specimens) are in Table 1. DNA was extracted from ethanol-preserved fin clips or muscle fragments using either the standard phenol–chloroform extractions with Phase-Lock Gel™ tubes (Eppendorf, Le Pecq, France), or the DNeasy® kit (Qiagen GmbH, Hilden, Germany). Individual DNA extracts were subjected to PCR amplification of two partial mitochondrial genes: a 326-bp fragment of the cytochrome *b* gene, using a pair of degenerate primers (forward: 5'-TCMGAYATCGCCACAGCCTTCTC-3'; reverse: 5'-AGGAAGTGGAAGKGC GAAGAA-3') designed from the alignment of the cytochrome *b* gene sequences of Siganidae available in GENBANK (accession nos. AY190542-AY190555), and an expected 575-bp fragment of the 16s rRNA gene, using the forward and reverse primers (respectively, 5'-CGCCTGTTTATCAAAAACAT-3' and 5'-CCGGTCTGAACTCAGATCACGT-3') of Clements et al. (2003). The amplification reactions were done in 96-well plates with each well containing 20 µL reaction mixture [2 µL template DNA, 2.5 mM MgCl₂, 0.48 mM dNTP mix, 0.5 µM of each primer and 0.5 U *Taq* polymerase (Promega, Madison WI, U.S.A.)] in a Robocycler™ thermocycler (Stratagene, La Jolla CA, U.S.A.). The PCR program consisted of 3 min DNA-denaturation (at 94°C) followed by 35 cycles of 1 min denaturation (94°C) / 1 min annealing (60°C) / 1 min elongation (72°C). The PCR products were shipped to GATC Biotech (Konstanz, Germany), or Macrogen (Seoul, South Korea) for nucleotide sequencing and the sequences were subsequently retrieved from the company's Internet website. Nucleotide sequences were deposited in GENBANK (list in Table 1).

All nucleotide sequences were aligned using BIOEDIT (Hall, 1999). The phylogenetic relationships among species in the genus *Siganus* were inferred from the matrix combining cytochrome *b* and 16S rDNA sequence data using three different algorithms: neighbour-joining (NJ) (Saitou and Nei, 1987), maximum parsimony (MP) (Fitch, 1971), and maximum likelihood (ML) (Felsenstein, 1973). NJ and MP analyses were done using MEGA 3.1 (Kumar et al., 2004). Gaps were treated as deletions in pairwise comparisons of sequences (option "Pairwise-Deletion" in MEGA 3.1). ML analysis was done using PHYML (Guindon and Gascuel, 2003). Three substitution models were considered for analysing the nucleotide differences between haplotypes: Jukes-Cantor (Jukes and Cantor, 1969), Kimura-2 parameter (Kimura, 1980), and Tamura-Nei (Tamura and Nei, 1993). The substitution model for NJ and ML analyses was chosen according to the likelihood approach implemented in

MODELTEST (Posada and Crandall, 1998), version 3.7 (2006); the best fit was provided by a Tamura-Nei model with Γ -distributed rates of substitution per site ($\alpha=0.5$).

3. Results

Mitochondrial-DNA sequence data were obtained on a total of 825 base pairs (bp) contributed by a fragment of the cytochrome *b* gene (300 bp) and a fragment of the 16S rRNA gene (521-522 bp before alignment), for 164 individuals of 20 Siganidae and three Acanthuridae species (GENBANK accession nos. listed in Table 1). For the cytochrome *b* gene fragment, 115 nucleotide sites were variable across the whole dataset (19 first positions, 3 second positions, 93 third positions) resulting in 12 amino-acid substitutions. All 16S rDNA data were easily aligned with 6 single-nucleotide gaps, yielding 87 variable nucleotide sites. The NJ tree obtained from the matrix of pairwise nucleotide distances is presented in Fig. 2. MP analysis of the combined dataset yielded a set of 64 most parsimonious trees of 550 steps, whose topology at the supra-specific level was nearly identical to that of the NJ tree, as was that of the tree produced by ML analysis. The only differences concerned the position of the branch leading to *S. javus* relative to that leading to *S. puellus*, which was poorly resolved in NJ and unresolved with MP and ML analysis, and the position of the node separating *S. vermiculatus* from the *S. guttatus* / *S. lineatus* haplogroup. Percentages of occurrence of a node after bootstrap resampling are given for each NJ, MP, and ML analysis (Fig. 2).

The inner tree topology was characterized by three well-supported and deeply separated ingroup clades of similar lengths (*I-III* on Fig. 2). Clade *I* grouped all deep-bodied species, while Clade *III* grouped all species with fusiform body except *S. argenteus*. *S. argenteus* and *S. woodlandi* (whose ratio of standard length to body depth is intermediate between those of deep-bodied and fusiform species) formed Clade *II*. All species that live in pairs on the reef front, except *S. woodlandi*, were grouped in Clade *I*, but the latter also included species that live in small schools or pairs in turbid habitats such as mangroves, estuarine lakes and estuaries (*S. guttatus*, *S. javus*, *S. lineatus*, *S. vermiculatus*). All drab-coloured species, which also have tumid snout and form large schools, were grouped into Clade *III*.

S. luridus and *S. spinus* appeared to have diverged relatively early from each other (average nucleotide divergence estimate between haplotypes of the two species, $\hat{d} = 0.068$) and did not appear as sister-clades (Fig. 2). *S. rivulatus* and *S. sutor* appeared to be sister-clades (with $\hat{d} = 0.020$). *S. lineatus* haplotypes formed a paraphyletic cluster with *S. guttatus*. The paraphyly of *S. lineatus* haplotypes was caused by the external placement of the Maldives *S. lineatus* haplotype relative to the group formed by *S. guttatus* and those *S. lineatus* haplotypes that were sampled in Sulawesi and New Caledonia (i.e., across the West Pacific) (Fig. 2). *S. guttatus* and West Pacific *S. lineatus* appeared as very recently differentiated sister-clades ($\hat{d} = 0.005$). *S. randalli* haplotypes were embedded within the West Pacific *S. lineatus* haplogroup. *S. doliatus* haplotypes similarly formed a single, common haplogroup with *S. virgatus*, and so did *S. unimaculatus* with *S. vulpinus*. *S. fuscescens* haplotypes clustered into two distinct, although closely related haplogroups ($\hat{d} = 0.009$).

4. Discussion

The present results distinguished three major clades in Siganidae, which had not been identified earlier (Lacson and Nelson, 1993; Lemer et al., 2007). The early separation between deep-bodied and fusiform species indicates that general body shape has a strong phylogenetic component in Siganidae. This separation however was not complete, as Clade II grouped both a fusiform species (*S. argenteus*) and a species with intermediate body depth (*S. woodlandi*). Nevertheless, the caudal fin in the two latter species is strongly forked, unlike in all the other Siganidae species. General body shape thus underwent early diversification in Siganidae and has since then been relatively stable. A similar situation has been described in emperor fishes, *Lethrinus* spp. (Lethrinidae) (Lo Galbo et al., 2003). This situation is not systematic among Teleosts, where body proportions may also change rapidly, like in e.g. *Naso* spp. (Acanthuridae) (Klanten et al., 2004) and Cichlidae (Kassam et al., 2006). The distinction of two basic morphological types in Siganidae is clearly correlated with distinct life-styles. A proportion of the deep-bodied species occur in pairs close to the hard-coral substratum on the reef front, where they can sneak into crevices for feeding and for seeking protection from predators. This in turn favours territoriality and, perhaps, pairing behaviour. Fusiform species conversely occur on reef flats and algal flats and rely on schooling behaviour and swimming speed for escaping predators. Correlatively, the colour of the fusiform species is drab (an advantage in open habitats also frequented by predators), while that of the species of the reef front is usually bright and contrasted (e.g. Woodland, 1990; Randall, 2005).

S. argenteus [also a schooling, fusiform species; Woodland (1990)] was placed together with *S. woodlandi* in a distinct clade that appeared to coalesce simultaneously with the two others. This early trichotomy may reflect the true phylogeny, or conceal two successive dichotomies that the present sequence data were not sufficient to resolve. Given that *S. argenteus* possesses the prejuvenile, pelagic stage that is also present in Acanthuridae, parsimonious reasoning leads to considering the prejuvenile stage as ancestral in Siganidae, in which case the prejuvenile stage would have been lost once, or twice (depending on whether the actual basic divergence was a succession of two dichotomies, or a trichotomy), early in the history of the family. Life-history data for *S. woodlandi*, which are presently lacking, are desirable for a better understanding of the evolution of the prejuvenile stage in Siganidae.

The present results do not support the phylogenetic separation of Siganidae species with tubular snout, heretofore grouped under subgenus *Lo* (Seale, 1906), versus the other species (subgenus *Siganus*), thus confirming Lacson and Nelson's (1993) preliminary conclusions from allozymes. *S. (Lo) unimaculatus* and *S. (Lo) vulpinus* branched within a subclade of Clade I, as sister-species to *S. puellus* and *S. punctatus*, both with slightly elongate snout and placed under subgenus *Siganus*. We hypothesize that the mechanism for the apparition of the tubular snout of *S. unimaculatus*, *S. vulpinus* and related species (namely, *S. magnificus*, *S. niger* and *S. uspi*) is ecological specialization, as a longer snout allows easier access to pockets of algae growing at the dead basis of hard-coral colonies (Woodland, 1990). Albeit moderate

compared to *S. unimaculatus* and *S. vulpinus*, an elongate snout independently evolved in *S. corallinus* (Fig. 2), which is another deep-bodied species typical of the coral-reef front habitat. Therefore, the elongate / tubular snout appears to be a specialized state that arose in deep-bodied species dwelling on the coral reef.

Quoting Woodland (1990), "*S. sutor* is very similar in proportions to *S. canaliculatus* and *S. fuscescens*", but only "field observations of the colours of live fish indicate that it is distinct". According to the present results, *S. sutor* actually appears as the sister-species of *S. rivulatus*. Those two species form a subclade within a strongly supported monophyletic group that also comprises *S. canaliculatus* and *S. fuscescens*. Both *S. canaliculatus* and *S. fuscescens* have an Indo-Pacific distribution, but both are geographically isolated from *S. sutor* and *S. rivulatus* (Woodland, 1990). *S. sutor* is endemic to the western Indian Ocean while until recently the distribution of *S. rivulatus* was confined to the Red Sea (Woodland, 1990). Therefore, it is sensible to assume that the two latter species arose by allopatric speciation, and that they are an offshoot of an ancestral *S. canaliculatus* / *S. fuscescens* form from which their ancestor was geographically isolated.

We examined another pair of presumed sibling species within Clade III, namely *S. luridus* / *S. spinus*. Woodland's (1990; 1999) rationale for placing *S. luridus* and *S. spinus* as sibling species was their similar ratio of total length to body depth, the similar size and stoutness of their fin spines, and their parapatric distribution. The topology of the mitochondrial tree (present results) however does not support the sibling-species hypothesis. The genetic divergence patterns reported here and above thus highlighted the limitations of general morphology for accurately assessing evolutionary relationships at the immediate supra-specific level, at least in fusiform, drab species.

Among the other currently-recognized sister-species with parapatric distribution, *S. doliatus* and *S. virgatus* haplotypes formed a single, unresolved haplogroup, as did *S. unimaculatus* and *S. vulpinus*. Since *S. doliatus* and *S. virgatus* are believed to hybridize in their zone of contact in the Indo-Malay region (Woodland, 1990; Randall, 2005), it is possible that the latter harbours *S. doliatus* mitochondria as a result of introgression. In that case, further sampling of *S. virgatus* would be necessary, ideally outside the zone of overlap to sample 'true' *S. virgatus* mitochondria. Alternatively, the divergence between the two species may be recent, to the point that the sorting of their mitochondrial lineages is still not perceptible. The same rationale applies to the pair *S. unimaculatus* / *S. vulpinus*.

In cases where several haplotypes were sampled in a species, they clustered as distinct haplogroups and intraspecific nucleotide distances were at least about one order of magnitude lower than the distances separating two nominal species, thus validating Woodland's (1990) taxonomy at the species level. Apart from the two pairs of sibling species discussed in the above paragraph, there was one noticeable exception to this general pattern, which concerned the ensemble formed by *S. lineatus*, *S. guttatus* and *S. randalli* haplotypes. Although restricted to a single haplotype, the Maldives sample of *S. lineatus* appears as clearly distinct from all five other *S. lineatus* haplotypes, sampled across the West Pacific. Moreover, the Maldives *S. lineatus* haplotype branches externally to the clade formed by *S. guttatus* and

West-Pacific *S. lineatus*. Considering both the geographic distribution and the patterns of genetic differentiation observed in the *S. guttatus* / *S. lineatus* species complex, a two-step scenario of genetic differentiation may be proposed, with a first event of allopatric differentiation separating a Maldives population from a stock ancestral to both *S. guttatus* and West-Pacific *S. lineatus* and a subsequent, second event of allopatric differentiation between the two latter. *S. guttatus* and West Pacific *S. lineatus* subsequently underwent secondary contact in the Indo-Malay region. Both the pattern of reciprocal monophyly here observed between the two latter entities and the maintaining of two distinct ornamentation types (spotted: *S. guttatus*; lineate: *S. lineatus*) on either side of a narrow zone of contact in the Indo-Malay archipelago (Woodland 1990) despite possible hybridization (Lemer et al., 2007) confirm they have reached the status of biological species. The present results, if confirmed by a larger sample of haplotypes, therefore may challenge the current placement of Maldives *S. lineatus* as conspecific with West Pacific *S. lineatus*. Alternatively, the paraphyly of *S. lineatus* may be only apparent, with West-Pacific *S. lineatus* having been recently introgressed by *S. guttatus* mitochondria while the genomic integrity of Maldives *S. lineatus* was preserved because of its geographical isolation. An indirect way of addressing that question is now to study the population genetics of the *S. guttatus* / *S. lineatus* contact zone, using nuclear-DNA markers.

Both morphology and colour patterns (Woodland, 1990), and allozymes (Lacson and Nelson, 1993; Fig. 1) indicate a close relationship of *S. randalli* with *S. vermiculatus*. Unexpectedly, *S. randalli* haplotypes did not form a sister-clade to those of *S. vermiculatus*, but instead were embedded within the West-Pacific *S. lineatus* haplogroup, suggesting recent introgression of *S. randalli* by *S. lineatus* mitochondria. Although the allozyme study of Lacson and Nelson (1993) unfortunately did not include *S. lineatus* samples, the genetic distance between its close relative *S. guttatus* and *S. vermiculatus* was nearly one order of magnitude larger than the distance between *S. randalli* and *S. vermiculatus*.

Another point deserving further attention is the confirmation of two distinct subclades within *S. fuscescens*. This was earlier hinted by Lemer et al. (2007), based on a much shorter mtDNA fragment and on smaller sample sizes. Here, one subclade appeared to be geographically widely distributed, from the Riau archipelago to New Caledonia, while despite substantial sampling effort the other subclade seemed to be restricted to the South-West Pacific, with haplotypes sampled exclusively in the Solomon Islands and New Caledonia. This phylogeographic pattern may correspond to a former history of allopatric differentiation followed by secondary contact, or, perhaps, to introgression from a yet undescribed, cryptic species within the current taxon *S. fuscescens*. Our suspicion that *S. fuscescens* might consist of two reproductively isolated forms is reinforced by Woodland's (1999) sibylline notice that "further study since the publication of [his 1990] revision suggests that cryptic species may need to be dissected out of *Siganus fuscescens*". The use of nuclear markers [e.g. length-polymorphic introns; Hassan et al. (2002)] to test the null hypothesis of panmixia in populations harbouring the two mitochondrial types is warranted.

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Table 1

Samples of Acanthuridae and Siganidae analysed for the present survey. IRDN : Institut de recherche pour le développement, Nouméa; MNHN: Museum national d'histoire naturelle, Paris; BPBM: Bernice Pauahi Bishop museum, Honolulu.

Species, Sampling location	Sampling date	Voucher specimens	GENBANK accession nos.	
			<i>Cytochrome b</i>	<i>16S rRNA</i>
ACANTHURIDAE				
<i>Acanthurus blochii</i>				
Southern lagoon, New Caledonia	Apr. 2006	IRDN Ablo1, 2 ^a	DQ898027	DQ898095, 096
<i>A. triostegus</i>				
Moruroa, Gambier archipelago	Jul. 2006	MNHN 2007-0003	EF210172	EF210160
<i>Naso unicornis</i>				
Southern lagoon, New Caledonia	Nov. 2006	-	EF210173	EF210162
SIGANIDAE				
<i>Siganus argenteus</i>				
Rodrigues I.	Aug. 2006	-	DQ898028; EF210174	DQ898097; EF210163
Truk, Caroline Is.	Apr. 2006	-	DQ898035	DQ898097
Honiara, Solomon Is.	Aug. 2006	-	DQ898030, 031	DQ898097
Southern lagoon, New Caledonia	Feb. 2005-Mar. 2006	IRDN Sarg 5-8 ^a ; MNHN 2006-1525	DQ898028, 030, 035, 036	DQ898097
Ouvea lagoon, Loyalty Is.	Feb. 2006	-	DQ898036	DQ898098
<i>S. canaliculatus</i>				
Riau archipelago	Nov. 2006	MNHN 2007-0005	EF210175	EF210164
Honiara, Solomon Is.	Aug. 2006	-	EF210176	EF210165
<i>S. corallinus</i>				
Manus I., Bismarck archipelago	Aug. 2006	-	DQ898037	DQ898105
Honiara, Solomon Is.	Aug. 2006	-	DQ898037, EF210177	DQ898105
Southern lagoon, New Caledonia	Feb.-July 2005	MNHN 2006-1526; MNHN 2007-0006	DQ898037	DQ898105
<i>S. doliatus</i>				
Truk, Caroline Is.	Apr. 2006	-	DQ898038	DQ898106
Southern lagoon, New Caledonia	Feb. 2005-Apr. 2006	IRDN Sdol 2-4 ^a ; MNHN 2006-1527; MNHN 2007-0007, 0008	DQ898038, 039	DQ898106, 107
<i>S. fuscescens</i>				
Riau archipelago	Nov. 2006	IRDN Scff 2-5 ^a ; MNHN 2007-0009	EF210179-181	DQ898099; EF210166
Misima I., Louisiades archipelago	Nov. 2006	-	EF210182-184	DQ898100; EF210167, 168
Honiara, Solomon Is.	Aug. 2006	-	DQ898040-042, EF210178	DQ898099, 100
Southern lagoon, New Caledonia	June 2005-Feb. 2006	MNHN 2006-1528, 1529, 1530	DQ898040-045	DQ898099-104
<i>S. guttatus</i>				
Makassar, Sulawesi I.	Sep. 2005	-	DQ898047, 049, 050	DQ898108
<i>S. javus</i>				
Makassar, Sulawesi I.	Sep. 2005	-	DQ898052-054	DQ898109
<i>S. lineatus</i>				
Addu atoll, Maldives archipelago	Aug. 2005	BPBM 40390	DQ898062	DQ898108
Makassar, Sulawesi I.	Sep. 2005	-	DQ898055, 059-061	DQ898108
Honiara, Solomon Is.	Aug. 2006	-	DQ898059	DQ898108
New Caledonia	Apr. 2002-Mar. 2006	IRDN Slin 0, 2 ^a ; MNHN 2006-1531	DQ898055, 059-061	DQ898108
<i>S. luridus</i>				
Lattaqiah, Syria	2002	-	DQ898056-058	DQ898110, 111
Reunion I.	May. 2006	-	EF210185, 186	DQ898110
<i>S. puellus</i>				
Southern lagoon, New Caledonia	Feb.-July 2005	IRDN Spue 2-4 ^a ; MNHN 2006-1532	DQ898063	DQ898112
Truk, Caroline Is.	Apr. 2006	-	DQ898063	DQ898112

<i>S. punctatus</i>				
Southern lagoon, New Caledonia	Feb. 2005-Mar. 2006	IRDN Spun 2-4 ^a ; MNHN 2006-1533; MNHN 2007-0010	DQ898068, 069	DQ898113, 114
Makassar, Sulawesi I.	Sep. 2005	-	DQ898068	DQ898113
Truk, Caroline Is.	Apr. 2006	-	DQ898068	DQ898113
<i>S. randalli</i>				
Honiara, Solomon Is.	Aug. 2006	-	DQ898059; EF210188	DQ898108
Manus I., Bismarck archipelago	Aug. 2006	-	DQ898059	DQ898108
<i>S. rivulatus</i>				
Lattajiah, Syria	2002	-	DQ898075-078	DQ898115, 116
<i>S. spinus</i>				
Manus I., Bismarck archipelago	Aug. 2006	-	DQ898079; EF210189-192	DQ898117; EF210169
Southern lagoon, New Caledonia	Jan. 2003; June 2005	MNHN 2006-1534	DQ898079	DQ898117
Moorea I., Society archipelago	Apr. 2006	MNHN 2006-1535	EF210192	DQ898117
<i>S. sutor</i>				
Rodrigues I.	Aug. 2006	-	EF210193, 194	DQ898115
<i>S. unimaculatus</i>				
Sulu Sea	Nov. 2006	MNHN 2007-0011, 0012	DQ898084, 085	DQ898119; EF210170
<i>S. vermiculatus</i>				
Makassar, Sulawesi I.	Sep. 2005	-	DQ898083	DQ898118
<i>S. virgatus</i>				
Makassar, Sulawesi I.	Nov. 2006	-	DQ898039	EF210171
<i>S. vulpinus</i>				
Southern lagoon, New Caledonia	Feb.-July 2005	IRDN JNC1782; MNHN 2006-1536	DQ898084	DQ898119
Truk, Caroline Is.	Apr. 2006	-	DQ898084	DQ898119
<i>S. woodlandi</i>				
Southern lagoon, New Caledonia	June-July 2005	IRDN Swoo 2 ^a ; MNHN 2006-1537	DQ898091	DQ898120

^a skulls

Captions to figures

Fig. 1. *Siganus* spp. Neighbour-joining tree [MEGA 3.1: Kumar et al. (2004)] built from pairwise Rogers' genetic distances estimated from allelomorph frequencies at 14 enzyme loci [Table 2 of Lacson and Nelson (1993)]. *Naso lituratus* (Acanthuridae) chosen as outgroup [see Tang et al. (1999) for a justification]. Scale bar: 10% Rogers' genetic distance.

Fig. 2. *Siganus* spp haplotypes (*Acanthurus blochii*, *A. triostegus* and *Naso unicornis* haplotypes as outgroup). Neighbour-joining (NJ) phylogeny obtained from the combined nucleotide sequence data set of cytochrome *b* (300 bp) and 16s rRNA (525 bp) gene fragments. Nucleotide distances estimated according to a Tamura-Nei model with Γ -distributed rates of substitution [MEGA 3.1: Kumar et al. (2004)]. Bootstrap resampling scores >50% given as % left to a node [bold: NJ, 1000 resamplings; italics: maximum parsimony (MEGA 3.1), 1000 resamplings; regular: maximum likelihood (PHYML: Guindon and Gascuel [2003]), 100 resamplings]. Geographic origin of samples: Syria (✕); Mascarene archipelago (✚); Addu atoll, Maldives (□); Riau archipelago (Δ); Sulu Sea (▼); Makassar strait, Sulawesi (▲); Truk, Caroline Islands (■); Bismarck and Louisiades archipelagoes (◆); Solomon Islands (❖); New Caledonia (●); Ouvéa, Loyalty Islands (○); Moorea, Society Islands (▽). Behaviour: S, schooling; P, pairing; (references in section 2.). Scale bar: 2% nucleotide distance.



