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Patterns and processes in the evolutionary history of parrotfishes (Family Labridae)

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Phylogenetic reconstruction of the evolutionary relationships among 61 of the 70 species of the parrotfish genera *Chlorurus* and *Scarus* (Family Labridae) based on mitochondrial and nuclear gene sequences retrieved 15 well-supported clades with mid Pliocene/Pleistocene diversification. Twenty-two reciprocally monophyletic sister-species pairs were identified: 64% were allopatric, and the remainder were sympatric. Age of divergence was similar for allopatric and sympatric species pairs. Sympatric sister pairs displayed greater divergence in morphology, ecology, and sexually dimorphic colour patterns than did allopatric pairs, suggesting that both genetic drift in allopatric species pairs and ecologically adaptive divergence between members of sympatric pairs have played a role in diversification. Basal species typically have small geographical ranges and are restricted to geographically and ecologically peripheral reef habitats. We found little evidence that a single dominant process has driven diversification, nor did we detect a pattern of discrete, sequential stages of diversification in relation to habitat, ecology, and reproductive biology. The evolution of *Chlorurus* and *Scarus* has been complex, involving a number of speciation processes. *Biological Journal of the Linnean Society*, 2012, ••, ••–••.

ADDITIONAL KEYWORDS: adaptive radiation – evolution – sexual selection – speciation – reef fish.

INTRODUCTION

Adaptive radiations are episodes of phenotypic diversification in rapidly multiplying lineages that result in ecologically divergent groups of species (Schluter, 2000). A pervasive theme in the vertebrate literature concerns the concept of radiation as a series of stages, in which diversification proceeds through discrete sequences of phenotypic differentiation (Streelman *et al*., 2002; Streelman & Danley, 2003; Sallan & Friedman, 2012). The most relevant example in this context is the model of radiation by stages in which different vertebrate radiations follow similar evolutionary trajectories, diverging sequentially along axes of habitat, trophic morphology, and communication (Streelman & Danley, 2003). Under this model, the first two stages (ecological) are driven by natural selection, and the third (reproductive) is driven by sexual selection (Streelman & Danley, 2003). One of the primary exemplars for this model of adaptive radiation was the evolution of parrotfishes, comprising a speciose group of fishes confined largely to coral reefs (Streelman *et al*., 2002; Streelman & Danley, 2003). As a result, the processes of sequential diversification under this radiation in stages model have been applied to the evolution of reef fish faunas, especially the Labridae (Smith *et al*., 2008; Kazancioğlu *et al*., 2009). However, recent studies suggest that aspects of this model require reconsideration. First, the concept of sequential action of natural and sexual selection could be profitably re-examined to

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determine whether these processes are contemporaneous and interacting, rather than separate in time (The Marie Curie Speciation Network*, 2012). Second, explicit tests have questioned the concept of radiation in stages (Sallan & Friedman, 2012). Third, the extent to which alternative evolutionary processes such as neutral divergence after geographical isolation have been important in the evolution of parrotfishes requires further examination (Robertson *et al*., 2006). The main aim of the present study was to examine the pattern of species-level divergences in the most speciose clades of parrotfishes aiming to determine the extent to which they conform to a model of adaptive radiation as an explanation for the diversity of this group.

The parrotfishes are a clade of speciose (ten genera, 99 recognized species; Parenti & Randall, (2011) but morphologically uniform (Bellwood, 1994) perciform fishes notable both for their abundance on present day tropical reef systems and their presumed functional importance (Bellwood & Wainwright, 2002).They were previously recognized as a distinct family, the Scaridae, closely related to the Labridae and comprising two sub-familial groups: the scarines and sparisomatines. Phylogenetic analysis (Westneat & Alfaro, 2005) revealed that the parrotfishes are nested within a monophyletic Labridae and are sister to cheiline labrids. Parrotfishes (scarinae) are therefore referred to as scarine labrids in the present study.

The most speciose elements of this taxon, the genera *Scarus*, *Chlorurus*, and *Sparisoma*, display a pattern of recent diversification that is restricted largely to the last 3.5 million years (Robertson *et al*., 2006; Smith *et al*., 2008; Alfaro *et al*., 2009). The distribution of diversity is uneven in terms of taxonomic structure and biogeography. A single genus, *Scarus*, accounts for over 50% of the species, and 70% of all known scarines occur in the Tropical East Pacific and Indo-West Pacific biogeographical regions (Parenti & Randall, 2000, 2011). Parrotfish are relatively uniform in terms of morphology and foraging modes, usually grazing in multi-specific schools over rock and calcareous substrata, (Bellwood & Choat, 1990; Bellwood, 1994). This morphological uniformity contrasts with the complex reproductive biology of parrotfishes; most are protogynous hermaphrodites, with many taxa manifesting distinctive sexually dimorphic colour patterns (Choat & Robertson, 1975; Robertson & Warner, 1978; Kazancioğlu & Alonzo, 2010) and plasticity in somatic growth (Gust, 2004; Munday *et al*., 2004). This combination of taxonomic, ecological, and reproductive characteristics has prompted a number of studies on their evolutionary history, with nine phylogenetic analyses emerging over the last decade.

The evidence for a recent and rapid diversification in scarines (Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009), coupled with the analysis of morphological evolution of the pharyngeal and oral jaws (Price *et al*., 2010), has focused attention on the processes underlying their speciation. Initial studies (Streelman *et al*., 2002) identified a pattern of diversification that represented a classical example of an adaptive radiation, with a signature of natural and sexual selection (Streelman & Danley, 2003). These studies suggested that partitioning by habitat occurred in the deepest evolutionary nodes, with sparisomatinine (primarily *Sparisoma* and *Calotomus*) species being associated with seagrass habitats, and scarinines (*Bolbometopon*, *Cetoscarus*, *Hipposcarus*, *Chlorurus*, and *Scarus*) associated with coral reefs. Trophic diversification was visualized in terms of the development of scraping or excavating feeding modes linked to the fusion of dental plates and jaw articulations that provided flexibility (for scraping) and increased power (for excavating) calcareous reef substrata. Streelman & Danley (2003) further proposed that the dominant element in the more recent diversification of lineages within the scarine clade involved socio-sexual behaviour (male territoriality and breeding systems), and associated colour pattern development, with sexual selection the primary process.

Subsequent studies (Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009; Price *et al*., 2010) analyzed rates of morphological change in the scarine labrids and the extent to which modification of the pharyngeal and oral jaws may be implicated in the diversification of speciose clades of parrotfishes. These studies concluded that that, although changes in the pharyngeal (Alfaro *et al*., 2009) and especially the oral jaws (Price *et al*., 2010) of scarinine parrotfishes occurred at a greater rate than observed in other labrid taxa, and, in the latter case, were correlated with lineage diversification, these changes were not the underlying cause of the cladogenesis that characterizes some groups of parrotfishes. Alfaro *et al*. (2009) and Kazancioğlu *et al*. (2009) concluded that the pattern of increased rates of diversification was better explained by the evolution of extreme dichromatism (and other social and behavioural characters relating to sexual selection) within *Scarus* and *Chlorurus*. This conclusion was consistent with the sequential pattern of diversification proposed by Streelman & Danley (2003).

Previous phylogenetic analyses (approximately 50% of the known species of *Chlorurus* and *Scarus*) focussed on the roles of ecological diversification and sexual selection as factors driving diversification. The role of geographical isolation as a process driving diversification has not been considered in detail. Identification of sister taxa, analysis of geographical

ranges, and estimates of the ages at which divergence occurred are a necessary step in analysis of the patterns associated with speciation in this group. Robertson *et al*. (2006) provided a phylogenetic analysis for the genus *Sparisoma*, retrieving a strong signal of allopatric speciation as the primary agent of diversification in *Sparisoma* and *Nicholsina*. They did not find evidence for sexual selection being a dominant process in the diversification of the group.

The current literature thus poses two contrasting scenarios for the evolutionary diversification of scarine labrids: allopatric speciation (i.e. divergence as a result of the geographical disruption of gene flow) versus adaptive radiation in sympatry. The relative frequency of allopatric versus sympatric distributions of sister species is an important step for understanding speciation in parrotfish genera. Allopatric distribution in sister taxa suggests that speciation has been driven by genetic isolation of geographically distinct populations. The expectation under this scenario would be for relatively minor differences in morphology, ecology, and colour patterns in recently-diverged species pairs. By contrast, sympatric distributions of sister taxa would suggest that natural and/or sexual selection has driven the diversification. In this case, we would predict greater differences in size, morphology, habitat associations, and colour patterns between sister taxa, even in recent divergences. Therefore, we assess patterns of distribution, ecology, and sexual colour dimorphism, as well as evolutionary ages of sister species. This approach would also shed light on the utility of the three-stage model of adaptive radiation proposed by Streelman *et al*. (2002) and Streelman & Danley (2003). We note that, although current sympatric distributions of sister taxa and evidence of ecological and reproductive divergence implies selection on these traits, it does not follow that such species initially diverged in sympatry.

The present study, which represents the first comprehensive species-level analysis of the two most speciose clades of parrotfishes, has two objectives. First, is the development of comprehensive time-calibrated phylogeny sufficient to establish the relationships and spatial distribution of *Scarus* and *Chlorurus* sister-species pairs? If this is the case, it would be possible to determine: (1) whether there is a dominant spatial pattern of allopatry versus sympatry and (2) whether the pattern and magnitude of differences in ecological and reproductive characteristics of sister taxa differs among allopatric versus sympatric sister pairs. Second, given the importance placed on ancestral scarine habitats in previous studies, such a phylogeny would allow the assessment of temporal and spatial patterns of diversification aiming to identify the ancestral habitats of taxa at the base of the tree.

MATERIAL AND METHODS

Taxon sampling

The phylogenetic analysis incorporated 61 species (see Supporting information, Table S1) comprising 16 of the 18 described species of *Chlorurus* and 45 of the 52 described species of *Scarus* (Parenti & Randall, 2011). Samples were obtained by selective spearing and netting, markets, and by exchanges of tissue samples through colleagues and institutions. A further four species (*Bolbometopon muricatum*, *Hipposcarus harid*, *Hipposcarus longiceps*, and *Cetoscarus bicolor*) were included as outgroups.

Laboratory procedures

Total DNA was extracted from tissues using standard salt-chloroform and proteinase K digestion extraction procedures (Sambrook & Russell, 2001). Three markers, two mitochondrial (control region, 16SrRNA) and one nuclear, S7 ribosomal protein gene Intron1 (S7I1), a gene essential for growth (Synetos, Dabeva & Warner, 1992), were sequenced to explore the evolutionary relationships among the parrotfishes (see Supporting information, Table S2). Each 20-μl polymerase chain reaction (PCR) reaction volume contained 2.5 mM Tris-Cl (pH 8.7), 5 mM $KCl(NH_4)_2SO_4$, 200 μM each dNTP, $MgCl_2$ ranging from 1.5 mM to 4 mM, 10 μM each primer, 1 unit of Taq Polymerase (Qiagen) and 10 ng of template DNA. Amplifications followed the same basic cycling protocol: an initial denaturing step of 2 min at 94 °C, followed by 35 cycles, with the first five cycles at 94 °C for 30 s, 30 s at primer specific annealing temperatures (T_a) followed by extensions for 1 min 30 s at 72 °C, with the remaining 30 cycles performed as before but at T_a –2 °C. PCR products were purified by isopropanol precipitation. Two species, *Scarus arabicus* and *Scarus koputea*, routinely amplified two bands of equal size (S7I1) that could not be separated on the agarose gel; therefore, they were cloned before sequencing S7I1. We employed standard cloning procedures in accordance with the manufacturer's instructions (pGEM®-T Easy Vector Systems; Promega). Purified templates were quantified by ultraviolet-visible absorbance (ND-1000 Spectrophotometer; NanoDrop) and sent to Macrogen Inc. (South Korea) for direct sequencing in both directions.

Data compilation

Duplicate sequences were edited using SEQUENCHER, version 4.5 (Gene Codes Corp.), automatically aligned using CLUSTALX (Thompson *et al*., 1997) and finally manually corrected using SE-AL, version 2.0 (http://evolve.zoo.ox.ac.uk) (Rambaut, 1996). Sequences have been deposited in GenBank under accession numbers JX026453–JX026661.

Three specific sequences were downloaded from GenBank: one for the control region: *C. bicolor* (AY324589, I.-S. Chen, unpubl. data); and two for 16S rRNA: *Scarus coelestinus* (AY081083) and *Scarus guacamaia* (AY081085) (Streelman *et al*., 2002). Two of the three genes, the mitochondrial 16SrRNA and the nuclear intron (S7I1), were partitioned into putative stem (conserved 16S = 500 bp, S7I1 = 458 bp) and loop (hypervariable 16S = 105 bp, S7I1 = 194 bp) regions. In total, five separate gene partitions were identified and each region was examined for its best fitting model using MRMODELTEST, version 2.2 and Aikaike information criterion (AIC) (Nylander, 2004; Nylander *et al*., 2004). The five separate gene partitions, each with their specific model, were subsequently concatenated for phylogenetic analysis.

Phylogenetic analysis

Maximum parsimony (MP) analyses were implemented in PAUP* 4.0b10 (Swofford, 1998) using heuristic search methods with 1000 pseudoreplicate bootstraps, tree–bisection–reconnection branch swapping, and random addition of taxa. Two separate heuristic MP runs were performed. First, all sites were treated equally and second, sites were weighted 2 : 1 according to gene partitions; the combined S7I1 (stem and loop, 652 bp) with 16S (stem and loop, 605 bp) sequences were given a weight of 2, and the control region (470 bp) was given a weight of 1. A 50% majority-rule consensus tree was generated from all shortest trees obtained.

Bayesian inference (BI) analyses were implemented in MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001) using CIPRES Portals (Miller *et al*., 2009) (http://www.phylo.org/sub_sections/portal), The analysis of the concatenated data used a partition-mixed model method (pMM) utilizing the identified locus-specific substitution models. Six Bayesian pMM analyses were performed using Markov chain Monte Carlo (MCMC) simulations with four chains of 5 000 000 generations each, sampling trees every 500 generations. Stationarity was reached after 20 000 generations, visualized in TRACER, version 1.5 (Rambaut & Drummond, 2007), and a 50% majority-rule consensus tree was computed using the best 10 000 post burn-in trees from each run. Two outgroups, *C. bicolor* and *B. muricatum*, were used to root resulting trees. The single best tree was selected for molecular dating.

Molecular dating

Fossil records of scarine labrids are limited (Bellwood, 1996; Bellwood, 1997; Bellwood & Wainwright, 2002). The available records include a sparisomatinine parrotfish *Calotomus preisli* (Bellwood & Schultz, 1991) from middle Miocene (approximately 14 Mya) of Austria. The second fossil placed in the late Miocene (approximately 12 Mya) and also reviewed by Bellwood & Schultz (1991) comprised two tooth fragments unique to the genus *Bolbometopon.* This material has been used to age previous parrotfish phylogenies (Streelman *et al*., 2002; Smith *et al*., 2008; Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009).

To estimate ages of diversification for the present *Chlorurus-Scarus* study, we first had to re-examine and calibrate age divergences of the parrotfish phylogeny of Streelman *et al*. (2002) (henceforth referred to as Streelman's data or topology). Streelman's sequence data were obtained from GenBank (accession numbers AY081063–AY081133 and AY081211). Additional to Streelman's data, sequences of two pomacentrids [accession numbers TMO-4c4: U70326 and U70355 (Streelman & Karl, 1997); 12S: AF285920 and AF285927 (Tang, 2001); 16S: AF112577 (Farias *et al*., 1999) and AF119402 (Bernardi & Crane, 1999); cytochrome *b*: AF119399 (Bernardi & Crane, 1999) and AY208553 (Quenouille, Bermingham & Planes, 2004)] and two cichlids [accession numbers TMO-4c4: U70347 and U70357 (Streelman & Karl, 1997); 16S: AF112584 (Farias *et al*., 1999) and AF112637 (Farias, Orti & Meyer, 2000); cytochrome *b*: AF370631 and AF370632 (Farias *et al*., 2001)] were included. The latter four species were used to: (1) root the phylogeny and, more importantly, (2) calibrate the base of the best tree because two fossil pomacentrids, *Palaeopomacentrus orphae* (Bellwood & Sorbini, 1996) and *Lorenzichthys olihan* (Bellwood, 1999), were described from the lower middle Eocene at Monte Bolca, Italy. Both fossil pomacentrids have one common feature (synapomorphy) with modern pomacentrids: two-anal fin spines in supernumerary association (Bellwood, 1999). Using Streelman's data with four additional outgroup taxa, we reconstructed a modified Streelman parrotfish topology by BI employing a pMM method as described above. The resulting best-inferred tree of Streelman's data (Newick format available from S. Klanten) was then used to estimate divergence times. It is this modified Streelman parrotfish topology with age estimates (available from S. Klanten) that served as a basis to select a range of ages to date our comprehensive *Chlorurus-Scarus* phylogeny.

Initial calibrations of our *Chlorurus-Scarus* phylogeny were performed in r8s (Sanderson, 2004), selecting five dates (12, 14, 16, 18, and 20 Mya) derived from the modified Streelman's aged topology (see above) and henceforth referred to as legacy ages. In addition, an internal age of 3.5 Mya for the trans-isthmian *Scarus hoefleri–perrico* sister pair was included. We employed a log PL algorithm (Sander-

son, 2002) with an appropriate smoothing factor to generate separate chronograms. These chronograms were each subsequently used as starting trees for five separate analyses in BEAST, version 1.6 (Drummond & Rambaut, 2007). BEAST estimates ages using a Bayesian MCMC algorithm by sampling trees. The advantage of this algorithm is that it simultaneously estimates branch lengths, all possible topologies, substitution models, and ages based on fossil calibrations. Furthermore, BEAST allows a relaxed clock to estimate rates independently on different branches, either from an uncorrelated exponential distribution or uncorrelated lognormal distribution (UCLD). Five separate legacy calibrations (12, 14, 16, 18, and 20 Mya) were performed each using 5×10^6 MCMC generations with the Yule speciation process (pure birth model) and an UCLD rate model. The following priors were employed after rigorous testing: legacy ages (tmrca) with normal distribution and 1.25 SD, a set internal age of 3.5 Mya with an SD of 0.5; lognormal priors for ucld.mean, meanRate, covariance, and exponential priors for yule.birthRate and ucld.stdev. All resulting files were inspected in TRACER, version 1.5 (Rambaut & Drummond, 2007) to determine that all runs converged and obtained effective sample size values > 200. Resulting tree files were summarized in TREEANNOTATOR with a specified tree and visualized in FIGTREE, version 1.3.1 (Rambaut, 2009) (http://tree.bio.ed.ac.uk/software/figtree/) to illustrate the 95% highest posterior density intervals (HPDs) on selected nodes.

Distributional analysis

Individual species distribution maps were generated through the IUCN Red Listing process (see Supporting information, Table S3). Range size estimates for individual species were obtained through measurement of the area (km^2) occupied by each species as shown on the distribution maps using IMAGE TOOL (see Supporting information, Table S3). Two recent analyses of the biogeography of reef-associated biota (Spalding *et al*., 2007; Briggs & Bowen, 2012) were used to classify distribution patterns. The partitioning of tropical marine environments (warm oceans) into the Indo-West Pacific, East Pacific, Western Atlantic, and Eastern Atlantic (Briggs & Bowen, 2012) accommodated the major biogeographical patterns in the present study. *Sensu* Spalding *et al*. (2007), the Indo-West Pacific region was partitioned as the Western Indo-Pacific (Red Sea and Gulf of Aden to western Sumatra), Central Indo-Pacific (Gulf of Tonkin to Fiji), and the Eastern Indo-Pacific (Hawaii to Marquesas). The Tropical East Pacific extends from the Gulf of California in the north and to northern Peru in the south (Robertson & Cramer, 2009). The Tropical Atlantic was partitioned as the north-west Atlantic (primarily the Caribbean) and the south-west Atlantic (Brazil), and West Africa and the Gulf of Guinea (Spalding *et al*., 2007). The archipelagos at the junction of the Indian and Pacific oceans were identified as the Coral Triangle Region (Veron, 1995; Allen, 2008).

General habitat categories were based on association of reefs with three types of landmasses: continental coasts, high islands, and oceanic islands. The classification is based on distance from continental shores and island size because exposure to terrestrial influences such as run-off may have profound influences on the scarine fauna (Russ, 1984a, b; Mellin *et al*., 2010). High islands were considered to have a minimum area of 150 km^2 with vegetation cover and the potential for run-off and sedimentation (Arnberger & Arnberger, 2001). Oceanic islands had land areas < 150 km^2 with a distance > 200 km from the nearest landmass. For these islands, the dominant influences were exposure to open ocean conditions and reduced impact of terrestrial run-off and sedimentation. The habitat structure of the central Indo-Pacific has been strongly influenced by geologically-recent variations in sea level, including the inundation of the Sunda shelf, resulting in the creation of vast new habitat areas (Coller, 2009; Crandall *et al*., 2012). This represents a recent and novel habitat for parrotfishes, although occupancy appears to be restricted to only a few species. Accordingly, percentage occupancy of the Sunda Shelf habitat was estimated for Indo-Pacific species (see Supporting information, Table S3).

Analysis of distributional allopatry and sympatry was restricted to reciprocally-monophyletic sister species as identified from the phylogeny of 61 species. The method used to estimate the degree of distributional sympatry among sister species followed that previously used by Barraclough & Vogler (2000) and Quenouille *et al*. (2011). The degree of sympatry in sister species was defined as the percentage of overlap between the range of the more restricted species with its more widespread sister. Spatial sympatry in scarine sister species varied to a greater degree than that recorded in the above studies. For example, Quenouille *et al*. (2011) recorded sympatry indices as being consistently > 0.95 in various reef fish taxa. However, the present study identified the lower bound of sympatric overlap as 0.41, whereas allopatric taxa had a spatial overlap of < 10% (0–9.1). Chesser & Zink (1994) also recorded greater variation in the degree of spatial overlap than Quenouille *et al*. (2011) when identifying a speciation mode. For example, in five taxa in which allopatric speciation was identified, they found that distributional overlap varied from 0.07 to 0.197. For the purposes of the

present study, we defined allopatry as < 10% overlap, and sympatry as > 10% overlap, which is consistent with the classification of Quenouille *et al*. (2011).

Morphological analysis

Species of *Chlorurus* and *Scarus* exhibit important functional differences in the structure of oral and pharyngeal jaws (Bellwood & Choat, 1990; Wainwright *et al*., 2004; Price *et al*., 2010). These differences are reflected in external morphology, especially the dimensions of the dental plates and the relative proportions of the head. Head and jaw dimensions are associated with different feeding and foraging modes Bellwood & Choat (1990). Six morphological variables based on the analyses of Bellwood, (1994) and Bellwood & Choat, (1990) were obtained from digital image measurements of five individuals of each of the 61 species, using IMAGE TOOL (http://compdent.uthscsa.edu/dig/itdesc.html). In addition, we estimated body size and dimensions that have been shown to influence swimming speed and foraging modes in reef fishes (Fulton & Bellwood, 2005).

The majority of images were obtained from specimens, although, for a number of larger, rarer species, underwater images of living individuals were used. Measurement biases associated with parallax precluded a landmark-based analysis of this data set. The variables measured were fork length, body depth, head length, maximum cheek depth, snout profile, and dental plate exposure. These measurements were used to estimate the means for each species. Because length measurements could not be obtained from images of living individuals, fork length is the mean of the three to seven largest specimens where fork length could be accessed. Three meristic variables of major importance in separating scarine species, median predorsal scales, number of cheek scales, and pectoral rays, were also counted. The resulting data matrix was analyzed using hierarchical cluster analysis of euclidian distance and average linkage in PRIMER (http://www.primer-e.com/). Descriptions of colour patterns were obtained from digital images of living and newly-collected specimens taken in the field.

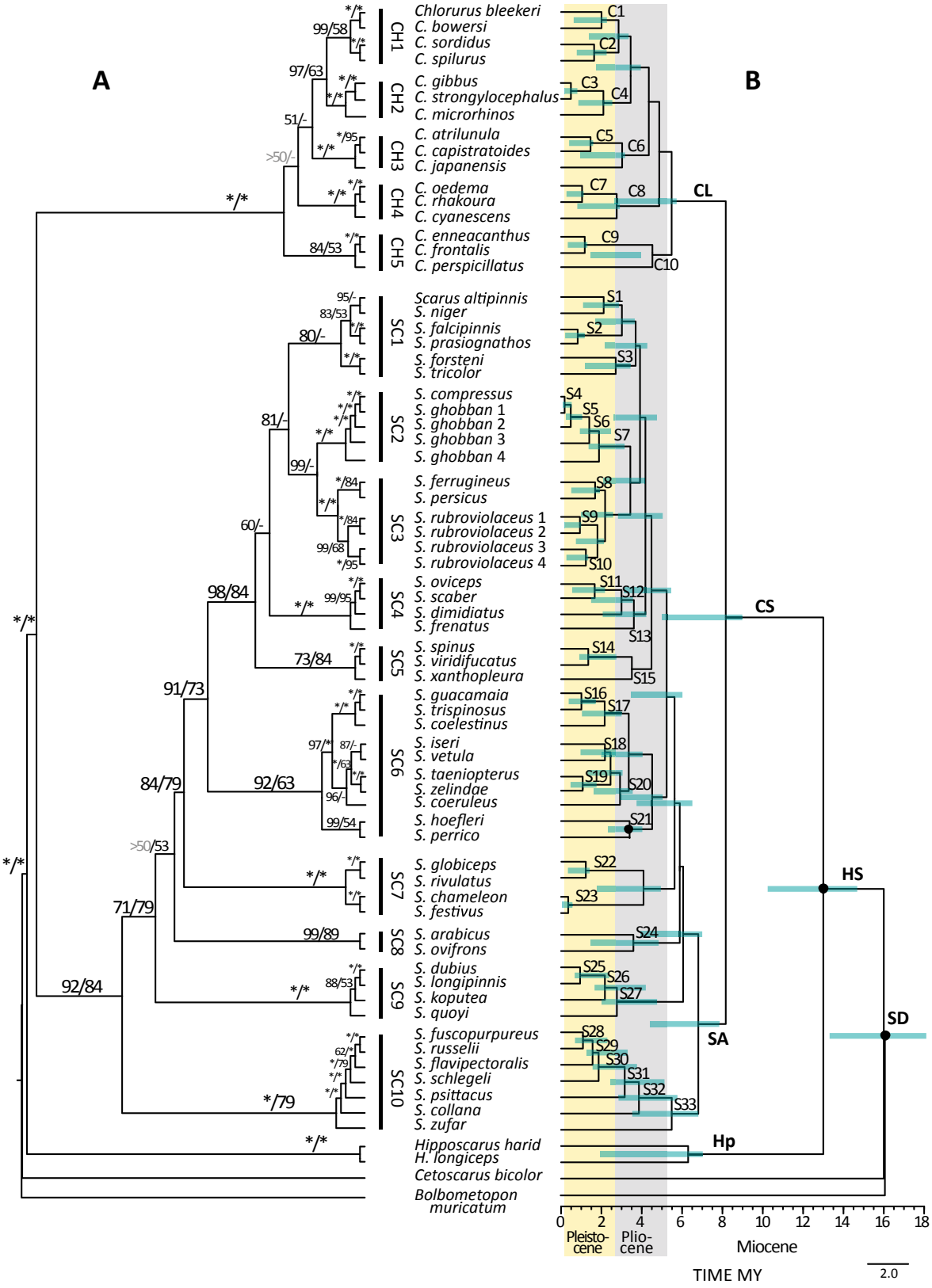
RESULTS

Phylogenetic inference

We examined 1727 bp of sequences, of which approximately 40% was parsimony-informative. The two mitochondrial markers, 16S rRNA and control region, had 605 and 470 bp, respectively, and the nuclear S7I1 contributed a further 652 bp with (14%, 63%, and 57% parsimony-informative sites, respectively). Five gene-specific models (based on AIC) were used for Bayesian partition analysis: 16S stem required a SYM + I + G model, 16S loop required a GTR + G model, the control region (CR) required a HKY + G model, and finally the S7I1 stem and loop both required a HKY + G model. The model selection for Bayesian pMM analysis only requires a general 'form' of the model (Nylander *et al*., 2004) because the Markov chain integrates uncertainties of the parameter values. Therefore, four of the five gene partitions (16S loop, CR, S7I1 stem and loop) had an unequal base frequency, whereas the 16S stem partition's base frequency was set to fixed = equal. Stationarity of the Bayesian analyses was reached after 20 000 generations in all six runs, visualized in TRACER, version b1.5 (Rambaut & Drummond, 2007) and the 50% majority rule consensus tree topology was no different from the best trees of each run (best tree lnL = –21 652.052) with very high posterior probabilities (Fig. 1A). Both MP and Bayesian analyses produced the same tree topology. We therefore included only the support for each retrieved node (Fig. 1A).

In all trees, *Hipposcarus* was sister to *Chlorurus* and *Scarus*. The Bayesian analysis was consistent with previous studies with respect to confirming the monophyly of *Chlorurus* and *Scarus* with strong support (Fig. 1A). Analysis of the 45 species sampled from the genus *Scarus* identified ten clades, with the most basal, SC10, (Fig. 1A) consisting of a complex

Figure 1. Inferred phylogeny of parrotfish genera *Chlorurus*, *Scarus*, and *Hipposcarus* (scarinine labrids) based on a comprehensive taxon sampling (63 out of 72 extant species) and two known outgroup species, *Bolbometapon muricatum* and *Cetoscarus bicolor*, obtained by Bayesian and maximum parsimony (MP) analyses for three loci (16S, control region, S7I1). A, topology of the best Bayesian tree (consensus of 10 000 trees) with posterior probabilities (%) and bootstrap-support (> 50%) of MP (1000 bootstrap replicates) are indicated, asterisks (*), representing 100% posterior probability/bootstrap support respectively. Numbers following species complexes of *Scarus ghobban*, and *Scarus rubroviolaceus* represent the regions: 1 Eastern Pacific (EP), 2 Great Barrier Reef (GBR), 3 Seychelles (SY), 4 Oman (OM). Clade names(CH, SC) are indicated representing five *Chlorurus* and ten *Scarus* clades. B, chronogram based on BEAST Markov chain Monte Carlo runs with 95% highest posterior density interval (HPD) in million years (Myr). Black circles indicate nodes that were constrained, tmrca (SD) at 16 Myr, tmrca (HS) at 13 Myr and the trans-isthmian sister pair tmrca(hoefleri/*perrico*) at 3.5 Myr. Nodes are numbered (for mean age and HPD range estimates, see Table 1): C1–C10 for *Chlorurus* and S1–S33 for *Scarus*.


A
B
CH1
CH2
CH3
CH4
CH5
SC1
SC2
SC3
SC4
SC5
SC6
SC7
SC8
SC9
SC10
Chlorurus bleekeri
C. bowersi
C. sordidus
C. spilurus
C. gibbus
C. strongylocephalus
C. microrhinos
C. atrilunula
C. capistratoides
C. japanensis
C. oedema
C. rhakoura
C. cyanescens
C. enneacanthus
C. frontalis
C. perspicillatus
Scarus altipinnis
S. niger
S. falcipinnis
S. prasiognathos
S. forsteni
S. tricolor
S. compressus
S. ghobban 1
S. ghobban 2
S. ghobban 3
S. ghobban 4
S. ferrugineus
S. persicus
S. rubroviolaceus 1
S. rubroviolaceus 2
S. rubroviolaceus 3
S. rubroviolaceus 4
S. oviceps
S. scaber
S. dimidiatus
S. frenatus
S. spinus
S. viridifucatus
S. xanthopleura
S. guacamaia
S. trispinosus
S. coelestinus
S. iseri
S. vetula
S. taeniopterus
S. zelindae
S. coeruleus
S. hoefleri
S. perrico
S. globiceps
S. rivulatus
S. chameleon
S. festivus
S. arabicus
S. ovifrons
S. dubius
S. longipinnis
S. koputea
S. quoyi
S. fuscopurpureus
S. russelii
S. flavipectoralis
S. schlegeli
S. psittacus
S. collana
S. zufar
Hipposcarus harid
H. longiceps
Cetoscarus bicolor
Bolbometopon muricatum
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
CL
S1
S2
S3
S4
S5
S6
S7
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S9
S10
S11
S12
S13
CS
S14
S15
S16
S17
S18
S19
S20
S21
S22
S23
HS
S24
S25
S26
S27
SD
S28
S29
S30
S31
S32
S33
SA
Hp
0
2
4
6
8
10
12
14
16
18
Pleisto-cene
Plio-cene
Miocene
TIME MY
2.0

grouping of widely distributed and endemic species. Clade SC6 was of interest because it contained all the tropical Atlantic representatives of the genus plus a single distinctive East Pacific species, *S. perrico*. This clade was distinct from SC2, which contained the widespread taxon *Scarus ghobban* and its east Pacific sister species *Scarus compressus*. Clade SC2 was sister to another widespread species, *Scarus rubroviolaceus*, SC3. Ongoing work on this taxon suggests that it is a species complex (Fitzpatrick *et al*., 2011). The most derived clade SC1 consisted of species strongly associated with well-developed crest and front reef habitats. Within the genus *Chlorurus*, the analysis retrieved five clades consisting largely of species with either Pacific or Indian Ocean distributions. In total, we retrieved 22 strongly supported pairs of sister species, with six pairs in *Chlorurus* and the remaining 16 pairs in *Scarus*.

The chronogram (BEAST MCMC analysis; Fig. 1B) used a similar dating procedure to that employed in previous studies (Smith *et al*., 2008; Alfaro *et al*., 2009) but employed different markers. Moreover, Alfaro *et al*. (2009) used additional fossils and direct dating. All studies reported similar conclusions that parrotfishes are a relatively young group. Our results show that the genera *Hipposcarus*, *Scarus*, and *Chlorurus* are of late Miocene (Messinian) age, and that the ages of the 15 clades of *Scarus* and *Chlorurus* range from very late Messinian (5.48 Mya) to mid/late Pleistocene (0.17 Mya) (Table 1).

Short branch lengths and relatively poor resolution of some internal nodes suggested a period of rapid diversification of these genera in the late Miocene/early Pliocene. Age estimates for *Scarus* and *Chlorurus* are provided in Table 1. For *Scarus*, the mean age of 6.8 Mya (95% HPD 4.4–7.8) was older than that of *Chlorurus*, 5.5 Mya (95% HPD 2.6–5.7), suggesting a more recent diversification of the latter genus. The tropical Atlantic clade SC6 had an estimated mean age of 4.52 Mya, which is older than the divergence of the east Pacific populations of *S. ghobban* (mean age, 0.17 Mya) and *S. rubroviolaceus* (mean age, 0.93 Mya). *Scarus perrico* (East Pacific endemic) was sister to the endemic eastern Atlantic *S. hoefleri*, with a mean divergence age of 3.4 Mya. The oldest divergence estimates were found in species at the base of the tree that were also endemics with peripheral distributions. These included *Scarus zufar* (mean age, 5.5 Mya), *S. arabicus* (mean age, 3.6 Mya) from the Omani coast and Gulf of Aden, *S. hoefleri* and *S. perrico* (mean age, 3.4 Mya) from the eastern Atlantic and eastern Pacific, respectively, and *Chlorurus perspicillatus* (mean age, 4.5 Mya), endemic to the Hawaiian Islands. Species endemic to the Red Sea *Chlorurus gibbus* and *Scarus collana* showed different mean divergence estimates of 0.49 and 3.86 Mya, respectively, suggesting a pattern of repeated speciation in this area.

The phylogenetic analysis presented here retrieved a topology and chronology consistent with that of three previous studies (Smith *et al*., 2008; Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009). Although the mean ages estimated in the present study for *Scarus* and *Chlorurus* were slightly older than in these previous studies (6.8 versus 6 and 5.48 versus 4 Mya), they lay within the 95% HPD interval of Alfaro *et al*. (2009).

Distribution patterns

The 61 species analyzed were distributed across the Indo-West Pacific, East Pacific, and Atlantic Ocean regions (Fig. 2A, B, C, D, E, F, G, H, I, J, K, L, M, N, see also Supporting information, Table S3). Fifty-two of the 61 species (36 *Scarus* and 16 *Chlorurus*) were from the Indo-West Pacific and Tropical East Pacific regions. The Pacific Ocean (East to West Pacific) supported 34 species, the Indian Ocean (including the Red Sea) supported 26 species, and the Atlantic supported nine species. Most distributions were concentrated in the central region of the Indo-Pacific or in the Caribbean reef system of the tropical western Atlantic. Peripheral regions, including the northwest Indian Ocean and the Red Sea, Hawaii, the Marquesas and the tropical East Pacific in the broader Indo-Pacific, and East Africa, and Brazil in the Atlantic, were depauperate. The genus *Chlorurus* was absent from both the East Pacific and the Atlantic. Most species occurred within regions bounded by 33° latitude North and South, with only nine species (seven *Scarus* and two *Chlorurus*) colonizing regions beyond this.

Species varied considerably in range size (0.0975–18.968×10^6 km^2), with a strong positive skew for each genus (Fig. 3; see also Supporting information, Table S3). The mean range size for all species was $4.945 \pm 0.64 \times 10^6$ km^2, with *Scarus* at $5.28 \pm 0.77 \times 10^6$ km^2 and *Chlorurus* at $4.01 \pm 1.09 \times 10^6$ km^2. Species in the tropical Atlantic had smaller range sizes ($1.98 \pm 0.33 \times 10^6$ km^2). The majority (57%) of species had relatively small range sizes, of $< 3.0 \times 10^6$ km^2. Two species, *S. rubroviolaceus* and *S. ghobban*, had ranges that spanned the tropical Indo-West Pacific and Eastern Pacific regions (26 000–28 000 km of longitude), with three others, *Scarus niger*, *Scarus frenatus*, and *Scarus psittacus*, exhibiting longitudinal ranges > 15 000 km (Fig. 2). Ten species were endemic to small (< 15% of the mean range size) areas of the Western, Central, and Eastern Indo-Pacific, and the Tropical East Pacific (*sensu* Spalding *et al*., 2007). Five of these (*C. gibbus*, *S. arabicus*, *S. collana*, *Scarus persicus*, *S. zufar*) were restricted to the north-west Indian ocean (Red Sea, Gulf of Aden, and Oman), two (*S. compressus*,

Table 1. Age estimates for *Chlorurus* and *Scarus* species, sister-species pairs, and 15 identified clades

Node	Description	Mean age (Myr)	95% HPD (Myr)
SD	*Bolbometopon – Cetoscarus*	16.0	13.30–18.1
HS	*Hipposcarus* + *Scarus* + *Chlorurus*	13.0	10.24–14.68
CS	*Scarus* + *Chlorurus*	8.16	4.99–8.99
SA	*Scarus*	6.8	4.43–7.84
Hp	*Hipposcarus*	6.3	1.94–7.03
CL	*Chlorurus*	5.48	2.58–5.68
	Chlorurus sister species / clades		
C1	*Chlorurus bleekeri – bowersi*	2.0	0.62–2.27
C2	*Chlorurus sordidus – spilurus*	1.64	0.78–2.26
Clade	CH1	2.85	1.40–3.35
C3	*Chlorurus gibbus – strongylocephalus*	0.49	0.17–0.81
C4	*Chlorurus microrhinos* (clade CH2)	2.1	0.86–2.54
C5	*Chlorurus atrilunula – capistratoides*	1.46	0.23–1.42
C6	*Chlorurus japanensis* (clade CH3)	3.03	0.82–3.03
C7	*Chlorurus oedema – rhakoura*	1.04	0.15–1.0
C8	*Chlorurus cyanescens* (clade CH4)	2.76	0.75–2.86
C9	*Chlorurus enneacanthus – frontalis*	1.17	0.18–1.16
C10	*Chlorurus perspicillatus* (clade CH5)	4.53	1.33–3.81
	Scarus sister species / clades		
S1	*Scarus altipinnis – niger*	2.11	1.09–2.87
S2	*Scarus falcipinnis – prasiognathos*	0.82	0.20–1.17
S3	*Scarus forsteni – tricolor*	2.71	1.18–3.44
Clade	SC1	3.7	2.18–4.28
S4	*Scarus compressus – ghobban* (1)	0.17	0.07–0.53
S5	*Scarus ghobban* (2)	0.48	0.27–1.05
S6	*Scarus ghobban* (3)	1.4	0.93–2.48
S7	*Scarus ghobban* (4) (clade SC2)	1.88	1.36–3.15
S8	*Scarus ferrugineus – persicus*	1.69	0.51–1.93
S9	*Scarus rubroviolaceus* (1) – *rubroviolaceus* (2)	0.93	0.17–0.99
S10	*Scarus rubroviolaceus* (3) – *rubroviolaceus* (4)	1.23	0.26–1.34
Clade	SC3	2.18	0.98–2.58
S11	*Scarus oviceps – scaber*	1.67	0.55–2.18
S12	*Scarus dimidiatus*	3.0	1.49–3.56
S13	*Scarus frenatus* (clade SC4)	3.61	2.06–4.23
S14	*Scarus spinus – viridifucatus*	1.35	0.90–2.74
S15	*Scarus xanthopleura* * (clade SC5)	3.51	N A^{-1}
S16	*Scarus guacamaia – trispinosus*	1.01	0.38–1.72
S17	*Scarus coelestinus*	2.16	1.03–3.0
S18	*Scarus iseri – vetula*	2.17	0.96–2.69
S19	*Scarus taeniopterus – zelindae*	1.07	0.47–1.76
S20	*Scarus coeruleus*	2.92	1.62–3.55
S21	*Scarus hoefleri – perrico* (trans-isthmian pair)	3.4	2.33–4.03
Clade	SC6	4.52	2.89–5.04
S22	*Scarus globiceps – rivulatus*	1.23	0.34–1.42
S23	*Scarus chameleon – festivus*	0.35	0.06–0.56
Clade	SC7	4.09	1.76–4.95
S24	*Scarus arabicus – ovifrons* (clade SC8)	3.59	1.45–4.84
S25	*Scarus dubius – longipinnis*	0.94	0.67–2.39
S26	*Scarus koputea*	2.17	1.66–4.2
S27	*Scarus quoyi* (clade SC9)	2.77	2.01–4.76
S28	*Scarus fuscopurpureus – russelii*	1.08	0.69–2.3
S29	*Scarus flavipectoralis*	1.56	1.26–3.29
S30	*Scarus schlegeli*	1.86	1.56–3.76
S31	*Scarus psittacus*	3.15	2.44–5.13
S32	*Scarus collana*	3.86	2.85–5.77
S33	*Scarus zufar* (clade SC10)	5.48	3.53–6.81

Mean age estimates are shown in million years (Myr) with 95% highest posterior density interval (HPD) from BEAST Markov chain Monte Carlo analysis.

The tmrca (SD) prior of the root base was set to 16 Mya with SD of 1.25, tmrca (HS) set to 13 MY with SD 1.25 and a known vicariance barrier of the trans-isthmian sister taxa tmrca (*S. hoefleri/perrico*) at 3.5 Myr with SD 0.5. 1, eastern Pacific (EP); 2, Great Barrier Reef (GBR); 3, Seychelles (SY); 4, Oman (OM). *Sister species to *S. xanthopleura*, *Scarus caudofasciatus* (IO) not sampled.

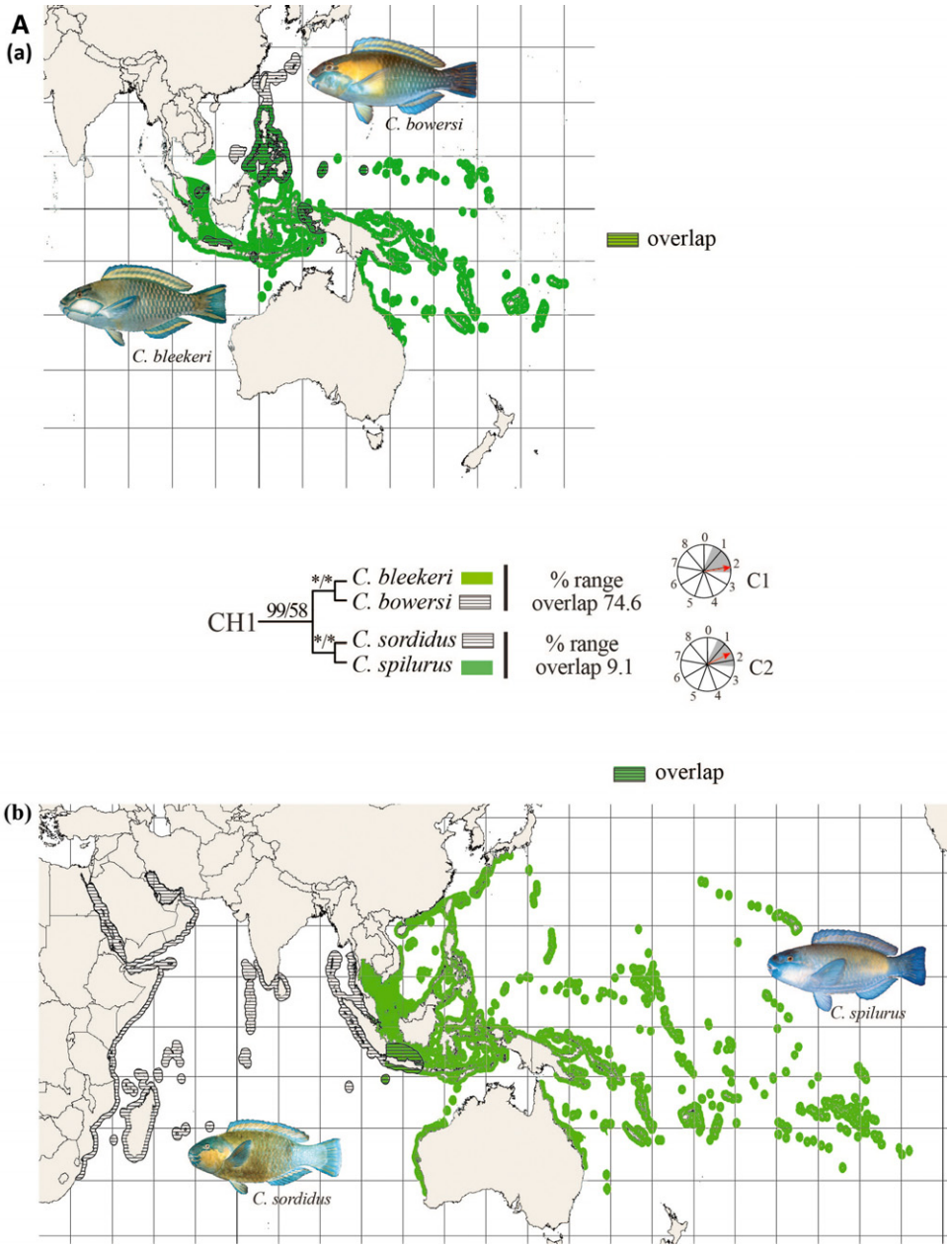


Figure 2. Figure 2 A–N. Distributions, range overlap, and ages of divergence in five clades (Ch1-CH5) of Chlorurus (Fig 2A–E) and ten clades (SC1-SC10)of Scarus Fig 2F–N). Clade structure and support from Fig. 1. Time dials indicate mean age (red arrow) with 95% highest posterior density interval (HPD) (grey area) for each clade.

S. perrico) to the Tropical East Pacific. Six of these species (*S. arabicus*, *S. persicus*, *S. zufar*, *S. koputea*, *S. compressus*, *S. perrico*) occurred in marginal coral reef habitats. These ten species showed limited variation in range size ($0.097–0.760 \times 10^6$ km^2) but a wider range of mean age of diversification (0.17–5.48 mya). This reflects the fact that the peripheral endemics comprised two groups, basal lineages containing *S. zufar*, *S. arabicus*, *S. collana*, *S. perrico*, and *C. perspicillatus* and those belonging to more recently diverged clades, (*S. compressus*, *Scarus dubius*, *S. persicus*, *S. koputea*, and *C. gibbus*) (Table 1).

The tropical Atlantic fauna consisted of a central Caribbean group (*S. guacamaia*, *Scarus coelestinis*, *Scarus coeruleus*, *Scarus vetula*, *Scarus taeniopterus*, *Scarus iseri*), two species (*Scarus trispinosus* and *Scarus zelindae*) in Brazil, and a solitary species (*S. hoefleri*) endemic to West Africa. *Scarus trispinosus* and *S. zelindae* are of recent origin, whereas *S. hoefleri* was basal in the tropical Atlantic clade.

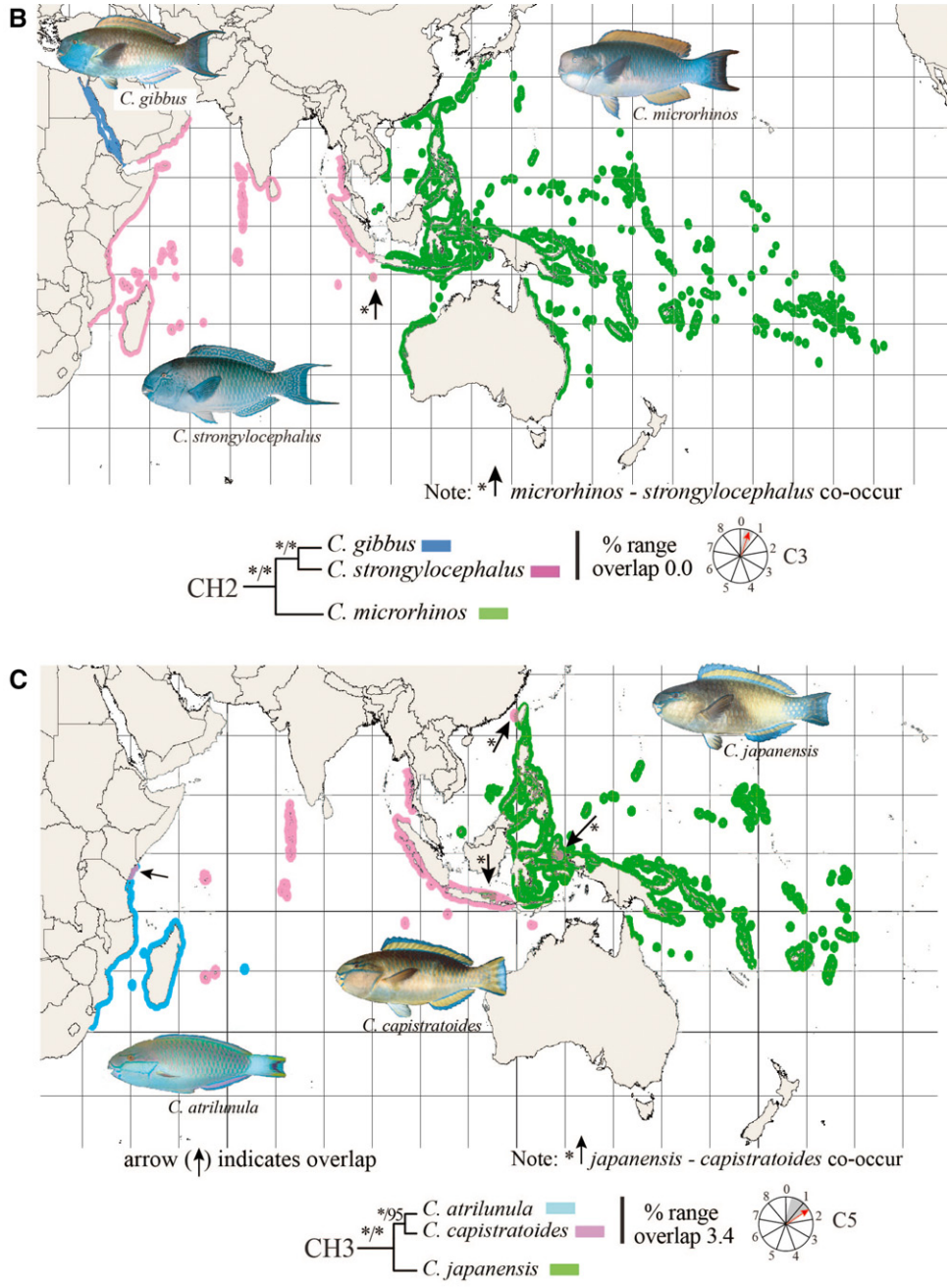


Figure 2. *Continued*

Mapping species distributions revealed variable patterns of spatial overlap among the different clades within each genus. For *Chlorurus*, five of six sister-species pairs had allopatric distributions (Fig. 2B, C, D, E), with only one pair (*Chlorurus bowersi* and *Chlorurus bleekeri*) (Fig. 2A) showing significant (75%) overlap. Distribution patterns in Indo-Pacific species of *Scarus* were more complex, with some clades (Fig. 2M, SC8, 9) dominated by allopatry, others (Fig. 2L, SC7) by sympatry, and others (Fig. 2F, I, SC1, 4) displaying mixtures of sympatric and allopatric distributions. Tropical Atlantic species formed a single clade in which six of the eight western Atlantic species had sympatric distributions within the north-west Atlantic area (Fig. 2K). The most notable feature of clade SC6 was the distribution of the allopatric species pair *S. hoefleri*, east Atlantic, and *S. perrico*, east Pacific. Among the six pairs of allopatric sister species of *Scarus* in the Indo-Pacific, boundaries were located at the junction of the Pacific

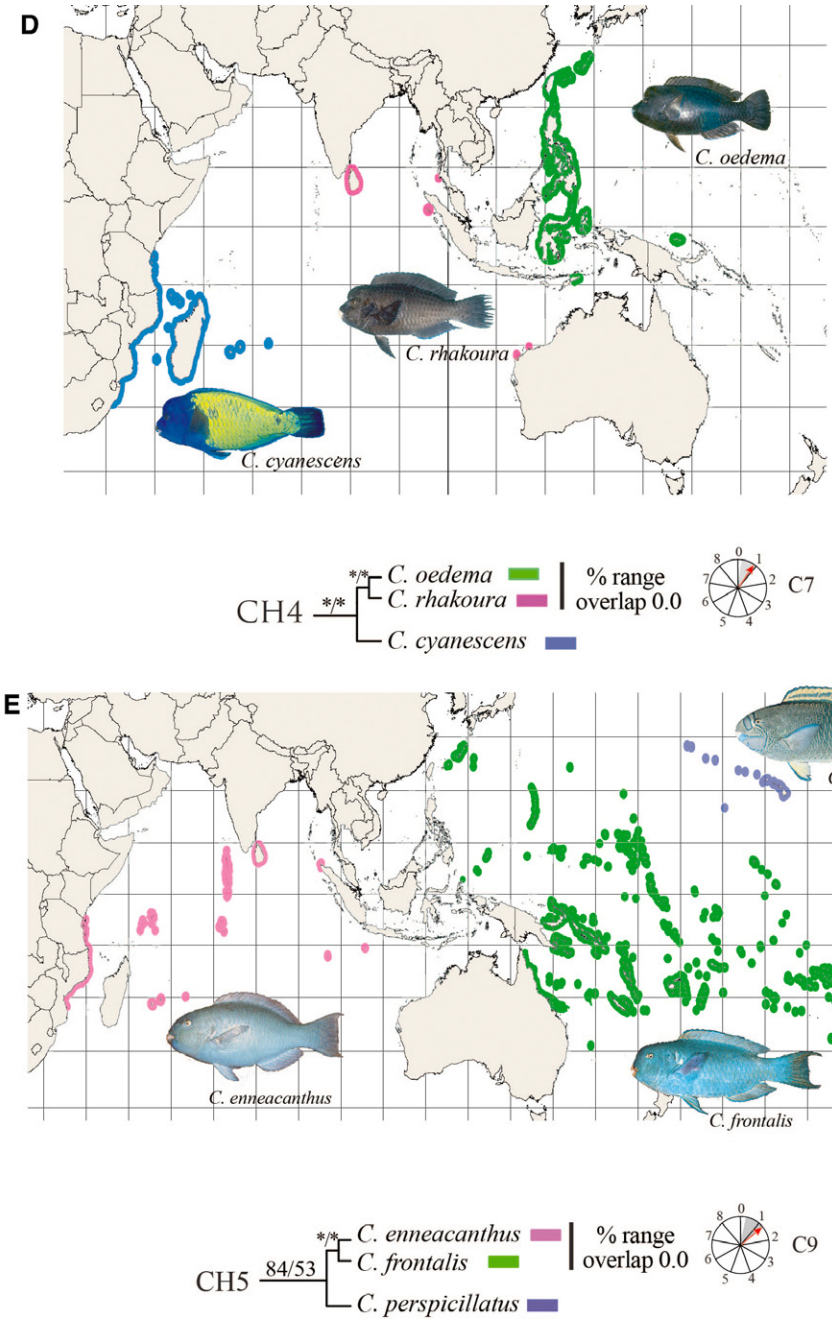
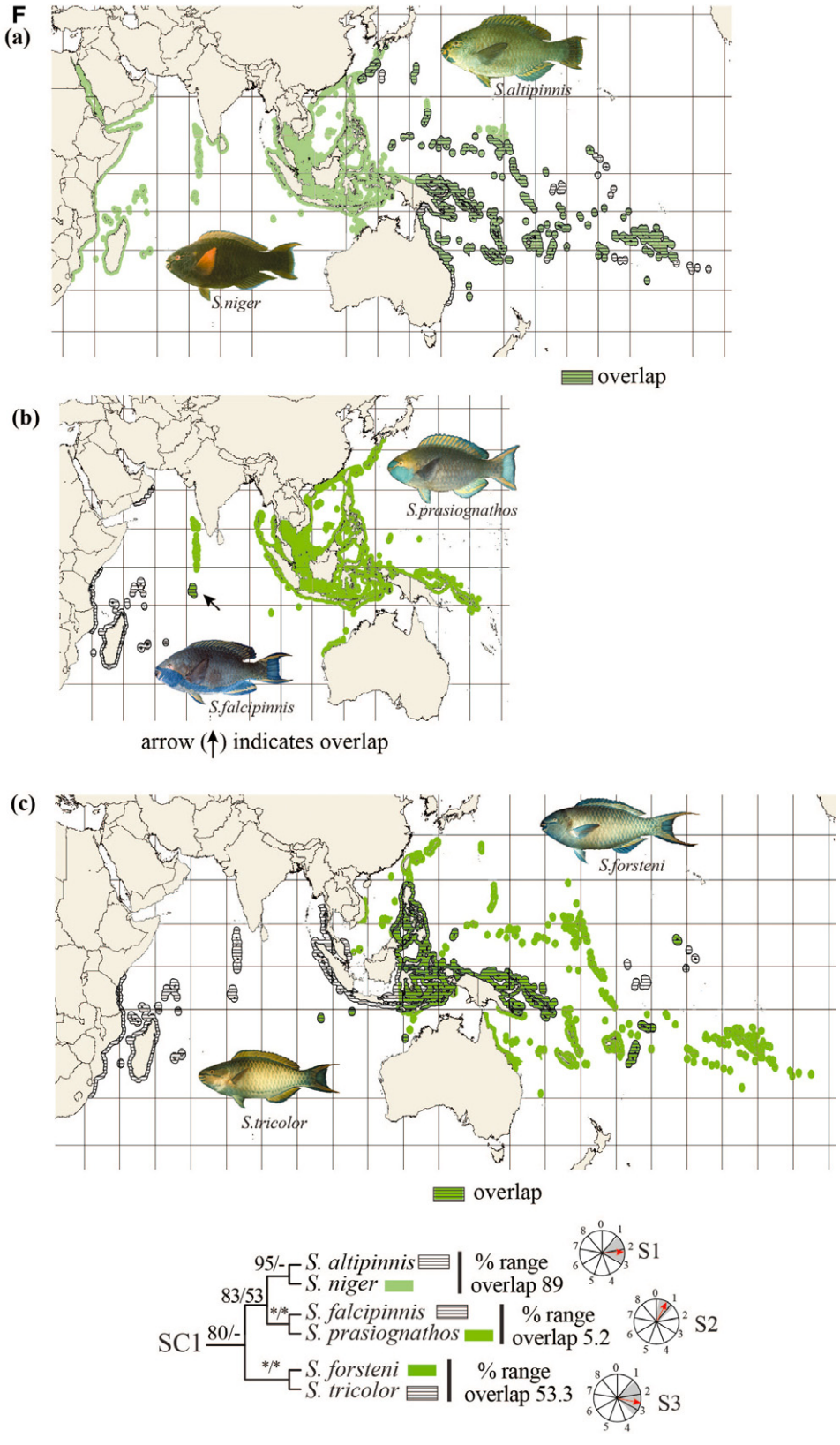


Figure 2. *Continued*

and Indian Oceans ($N = 4$) and between the south-west and north-west Indian Ocean ($N = 1$) and between Hawaii and the Central Pacific ($N = 1$) (Fig. 2). The primary area of overlap between sympatric Indo-Pacific species of both genera was the Coral Triangle at the junction of the Indian and Pacific oceans (four of six cases; Fig. 2) but with examples also in the Eastern Indo-Pacific ($N = 1$), Western Indo-Pacific ($N = 1$), and the Tropical East Pacific ($N = 1$). No locally endemic species of *Chlorurus* or *Scarus* were in the coral triangle area. The main separations in allopatric clades of *Chlorurus* occurred between the Pacific/East Indian Ocean and the central and western Indian Oceans.

Comparisons among sister-species groups

Nodal age estimates for reciprocally monophyletic sister-species pairs (Table 1) indicate the time taken to achieve the distributions shown in Figure 2.



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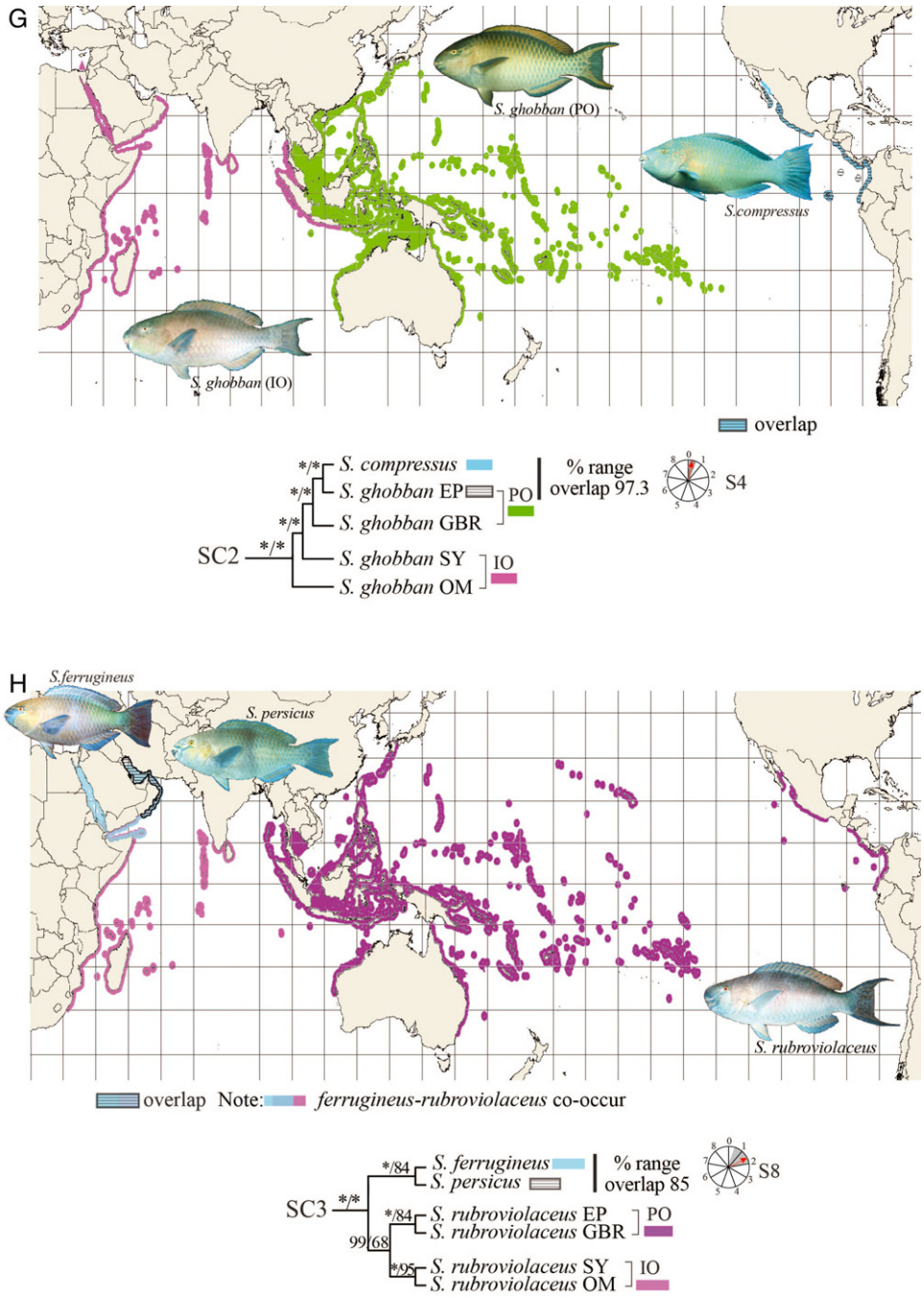


Figure 2. *Continued*

Table 2 identifies the 22 sister species with six *Chlorurus* and 16 *Scarus* (12 Indo-Pacific, four Tropical Atlantic). There were clear distinctions between the mean percentage distributional overlap observed in allopatry (1.9%) and sympatry (79.7%). However, the mean evolutionary age estimates for allopatric (1.48 ± 0.24 Mya) and sympatric sister pairs (1.55 ± 0.32 Mya) could not be separated and the former age range spanned that of the latter (ranges of 2.24 to 1.72 and 1.23 to 1.87 Mya, respectively). Sympatric sister pairs, even including those with very young evolutionary ages, had diverged in range sizes and habitat association (see Supporting information, Table S3).

Among five of the eight sympatric pairs, one pair-member tended to have a much smaller geographical range and colonize habitats on continental and high island shelf systems. In such cases, the other member

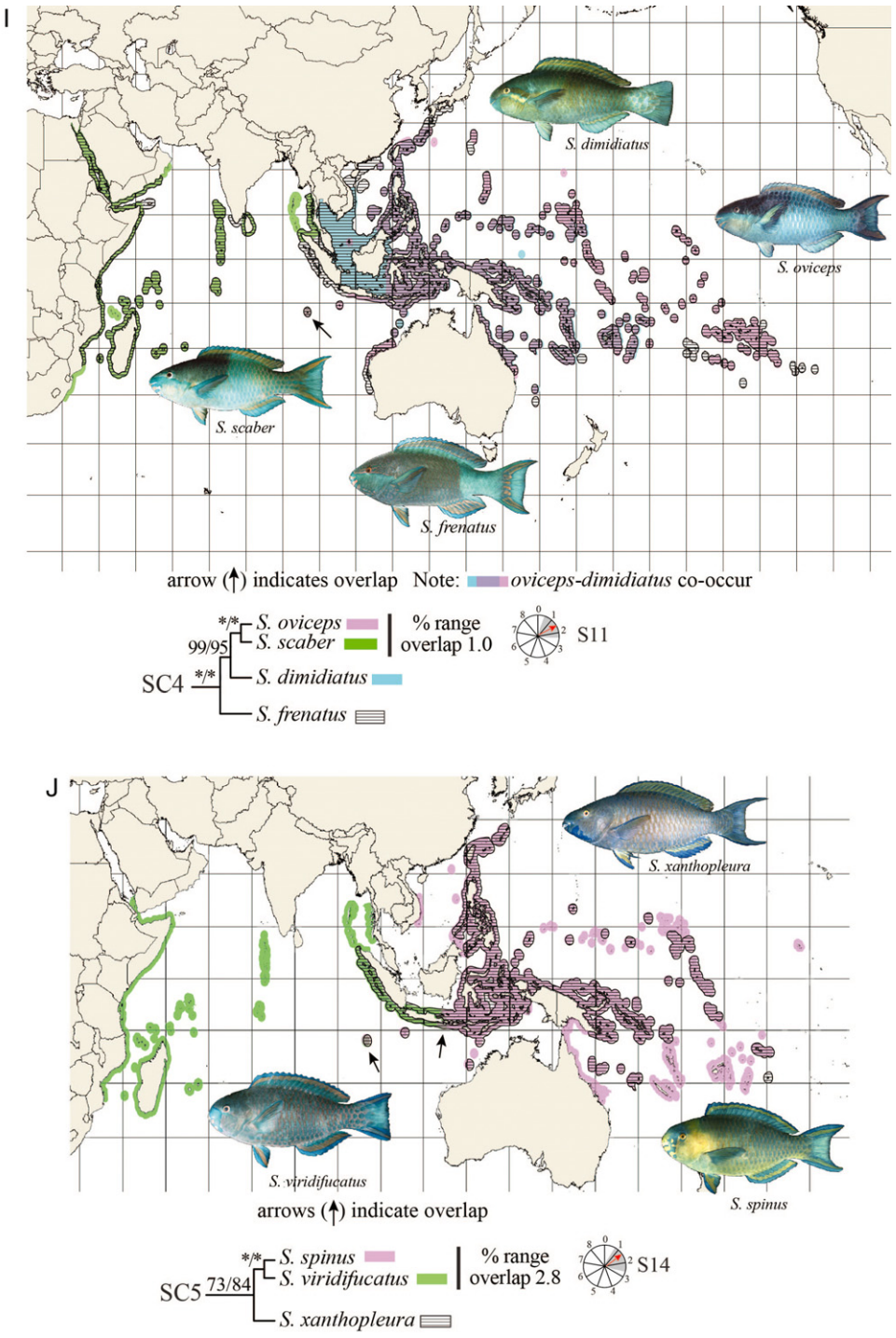


Figure 2. *Continued*

displayed a more extensive distribution over oceanic reef systems, and its range encompassed all or the great majority of the range of the former. Examples include C1 *C. bowersi*/*C. bleekeri* (Fig. 2A), S1 *S. niger*/*Scarus altipinnis* (Fig. 2F), S8 *Scarus ferrugineus*/*S. persicus* (Fig. 2H), S22 *Scarus rivulatus*/*Scarus globiceps*, and S23 *Scarus chameleon*/*Scarus festivus* (Fig. 2L). The differences were most striking where one member of a species pair ranged over the oceanic Island systems of both the Indian and Pacific Oceans (*S. globiceps*/*S. rivulatus*) with *S. rivulatus* being associated with continental margins including the

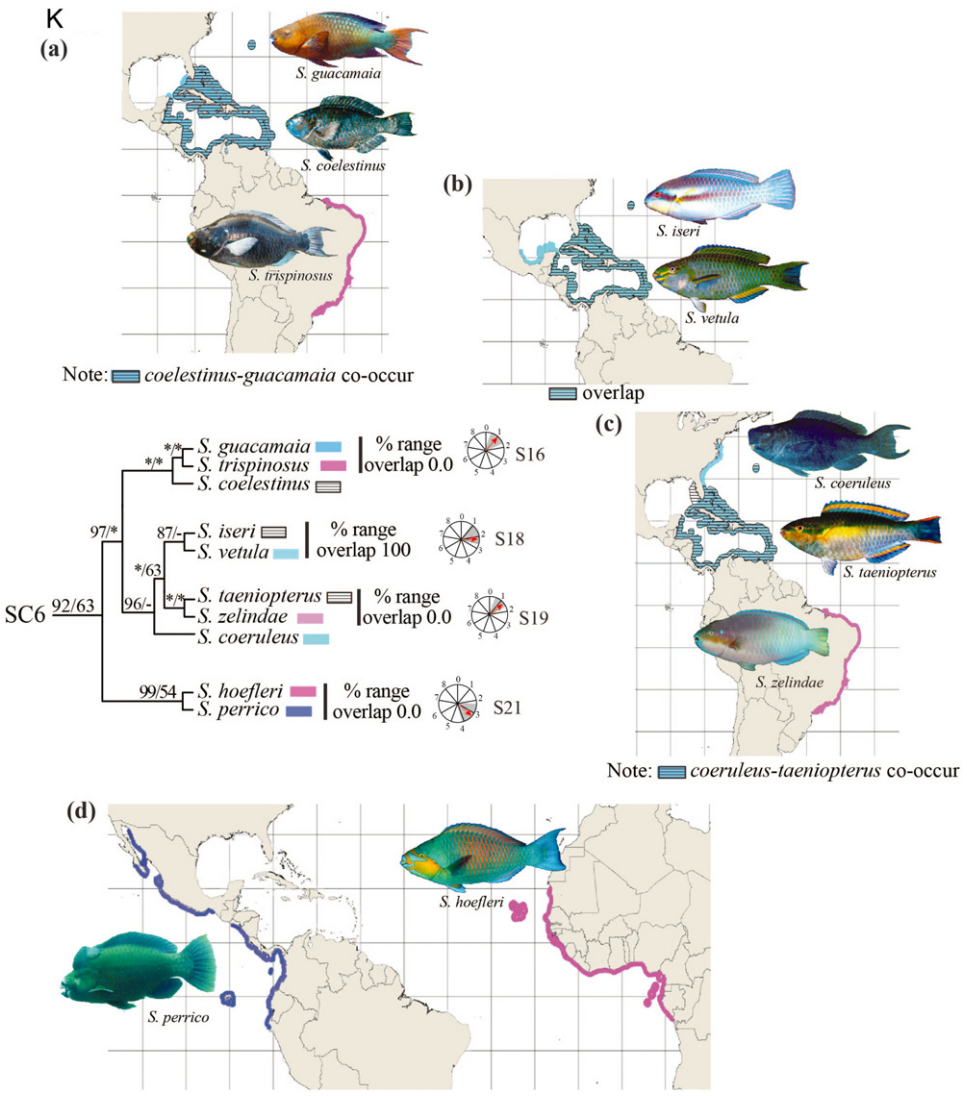


Figure 2. *Continued*

newly inundated Sunda Shelf (see Supporting information, Table S3). The remaining sympatric species (S3, S4, S18; Fig. 2) pairs showed similar range sizes for each member of the pair. In summary, there is some evidence that sympatric sister species, even those of relatively young evolutionary ages, will rapidly diverge in terms of range sizes and habitat characteristics. Although this suggests that sympatric sister pairs may diverge in range size and habitat association, this needs to be confirmed by a more extensive analysis of allopatric species pairs.

Divergence in ecological traits in allopatric versus sympatric groups was evaluated by comparing the pattern of evolutionary relationships in the phylogeny (Fig. 4A) with a dendrogram of the same species based on ecologically relevant morphological and meristic variables. These included head and jaw proportions and body dimensions (Fig. 4B). The molecular and morphological trees were largely congruent at the generic level. However, within each genus, there were groups that were similar to or had diverged from those in the molecular tree. The dendrogram retrieved three clusters with distinctive suites of morphological characters. Cluster 1 consisted of relatively small members of *Scarus* characterized by acute head profiles with reduced cheek depths and partially concealed dental plates. Cluster 2 was dominated by members of the genus *Chlorurus* and the older taxa of *Scarus S. zufar*, *S. arabicus*, and *S. perrico*. These three species displayed morphologies, including obtuse head profiles, increased cheek depth, and large prominent dental plates, similar to those of *Chlorurus*. The third cluster consisted of the largest species of *Scarus* and *Chlorurus*, including the distinctive clade of large excavating

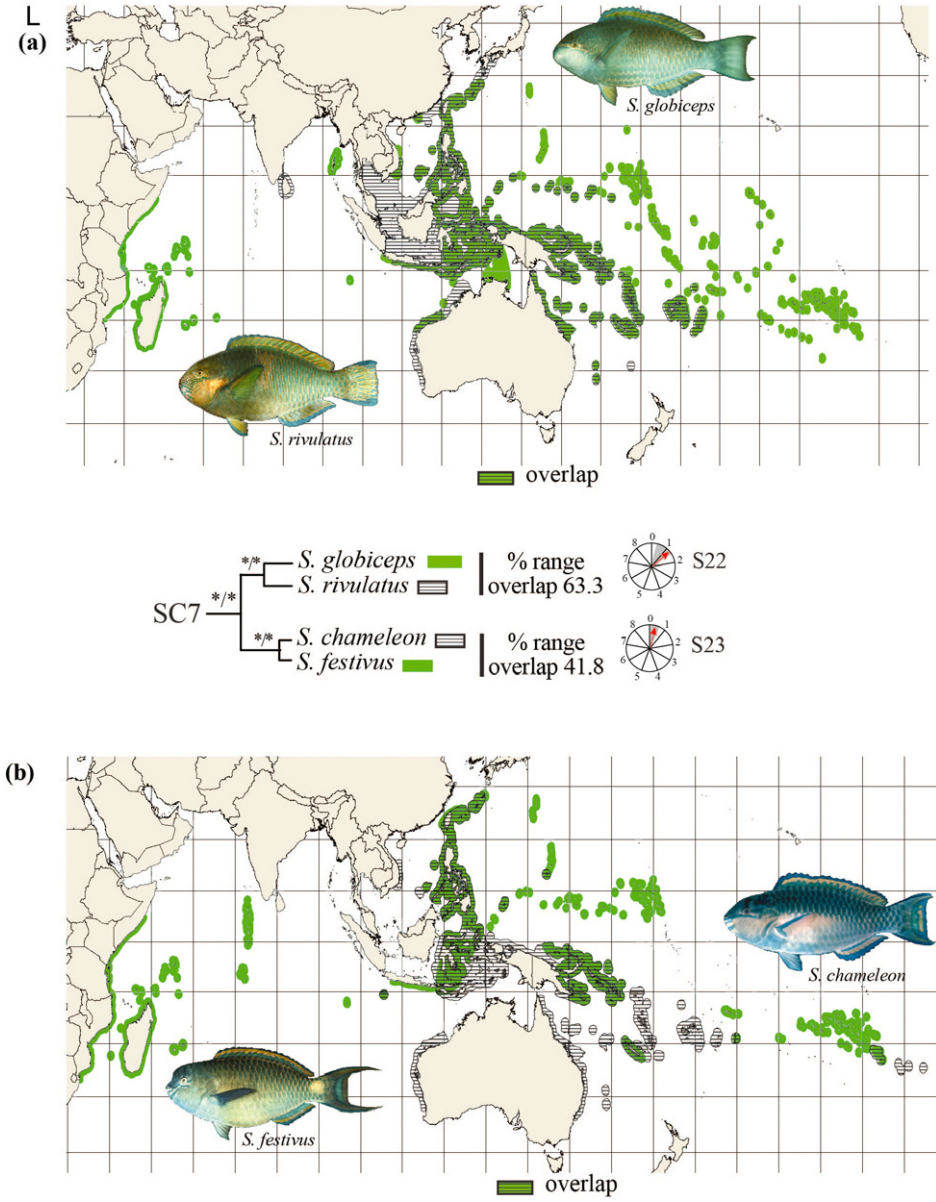
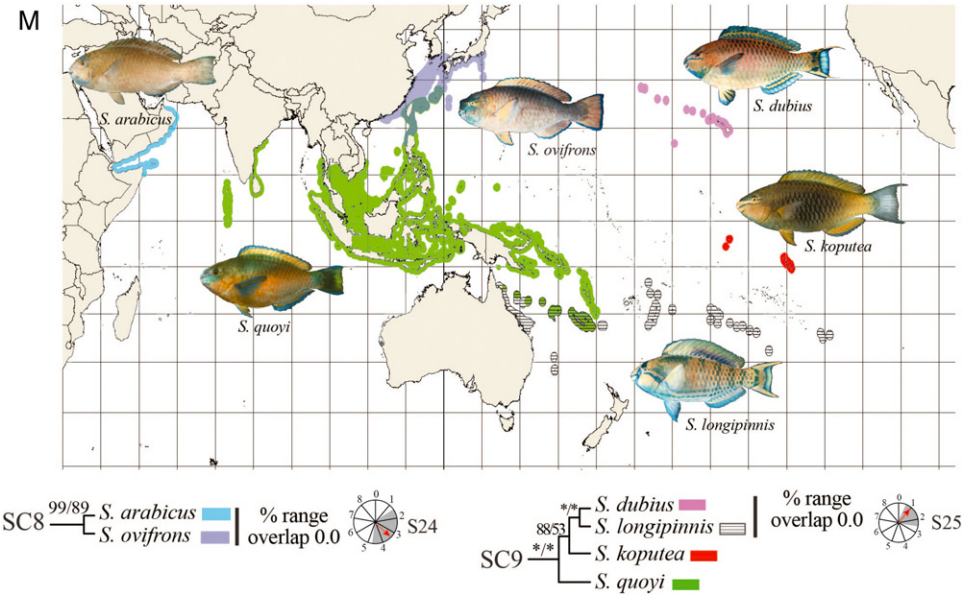


Figure 2. *Continued*

species *Chlorurus microrhinos/strongylocephalus/gibbus*. At a finer scale, large elements of the allopatric clades CH2, CH4, CH5, SC1, and SC10 were mirrored in the morphological dendrogram, whereas the integrity of the sympatric clades (Fig. 4A) was lost with the exception of a single example (*S. persicus/S. ferrugineus*); morphological differentiation occurred in seven of eight sympatric pairs but only in five of 14 allopatric pairs.

We also observed differences in colour patterns between sister species with sympatric versus allopatric distributions. This was notable in the terminal phases including the sympatric pairs *C. bleekeri/C. bowersi* (CH1) and *S. niger/S. altipinnis* (SC1). The former displayed striking differences in the head region of the terminal colour phases (TP) (Fig. 2A). In the *S. niger/S. altipinnis* pair, the differences were even more pronounced, and involved changes to the whole body colour pattern (Fig. 2F). In allopatric members of this same clade (i.e. *S. altipinnis/Scarus prasiognathus/S. falcipinnis*), the differences were the result of modifications to a basic pattern, rather than the development of fundamentally different patterns. This is shown more explicitly in SC10, where colour patterns in the allopatric clade members *Scarus schlegeli/Scarus russelii/*



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Scarus fuscopurpureus display only minor modifications to the basic terminal colour phase. The main features of colour pattern differentiation in sympatric and allopatric sister species are shown in Figure 5. Examples of terminal phase differentiation in sympatric sister species are shown in Figure 5 I (1A–3B). The greatest differences occur in the head region, and clearly illustrate the contrasts with the TP phase of allopatric sister taxa in Figure 5 I (4A–5B). The diagrams are scaled to size to illustrate the differences in the body size trait for sympatric sister taxa.

Differences between sympatric sister-species pairs and allopatric sister-species pairs were also apparent in the initial colour phase patterns (Fig. 5 II, 6A–7C). Figure 5 III (8A, B) illustrates the possible loss of an entire colour phase, as seen in tropical Atlantic sympatric species.

DISCUSSION

Our phylogenetic reconstruction provides a comprehensive analysis of the evolutionary relationships of the genera *Scarus* and *Chlorurus*. Although previous studies included a relatively small number of species (31 versus 61) (Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009), such analyses retrieved similar patterns of clade structure to those reported in the present study. These included a distinctive clade of Atlantic species, a basal clade of *Scarus* containing *S. schlegeli* and *Scarus flavipectoralis*, and a more recent clade of wide-spread species including *S. altipinnis*, *S. prasiognathus*, and *S. niger*. Increased taxon sampling enabled us to identify pairs of sister species and also local endemics that were critical in reconstructing the probable course of evolutionary events.

Distributional analyses revealed that sister taxa varied from spatially isolated to completely overlapping. In the Indo-Pacific, species boundaries were co-incident with ocean basin boundaries, with species overlap occurring largely in the central region (Coral Triangle). In the tropical Atlantic, geographical breaks occurred between the Caribbean and the Brazilian coast, with sister-species overlap confined to the Caribbean. In all three oceans, species with small range sizes were geographically peripheral and confined to marginal coral reef habitats in the northwestern Indian Ocean, the Eastern Indo-Pacific and the Tropical East Pacific. These were dominated by older lineages and may represent relictual distributions. Timing of divergence suggested that distributional sympatry could be rapidly achieved and was often associated with habitat divergence. Our conclusion that different taxonomic groups of reef species will vary in their evolutionary histories mirrors that of Malay & Paulay (2010). Compared to most other labrid tribes (Barber & Bellwood, 2005; Cowman & Bellwood, 2011; Hodge *et al*., 2012), scarines have a brief evolutionary history, colonizing coral reefs well after the Miocene.

In previous studies (Streelman *et al*., 2002; Streelman & Danley, 2003; Smith *et al*., 2008; Kazancioğlu *et al*., 2009), adaptive radiation driven by both natural and sexual selection was seen as the dominant process driving cladogenesis. By contrast, our results suggest that diversification of *Chlorurus* and *Scarus* involved a number of processes, including a

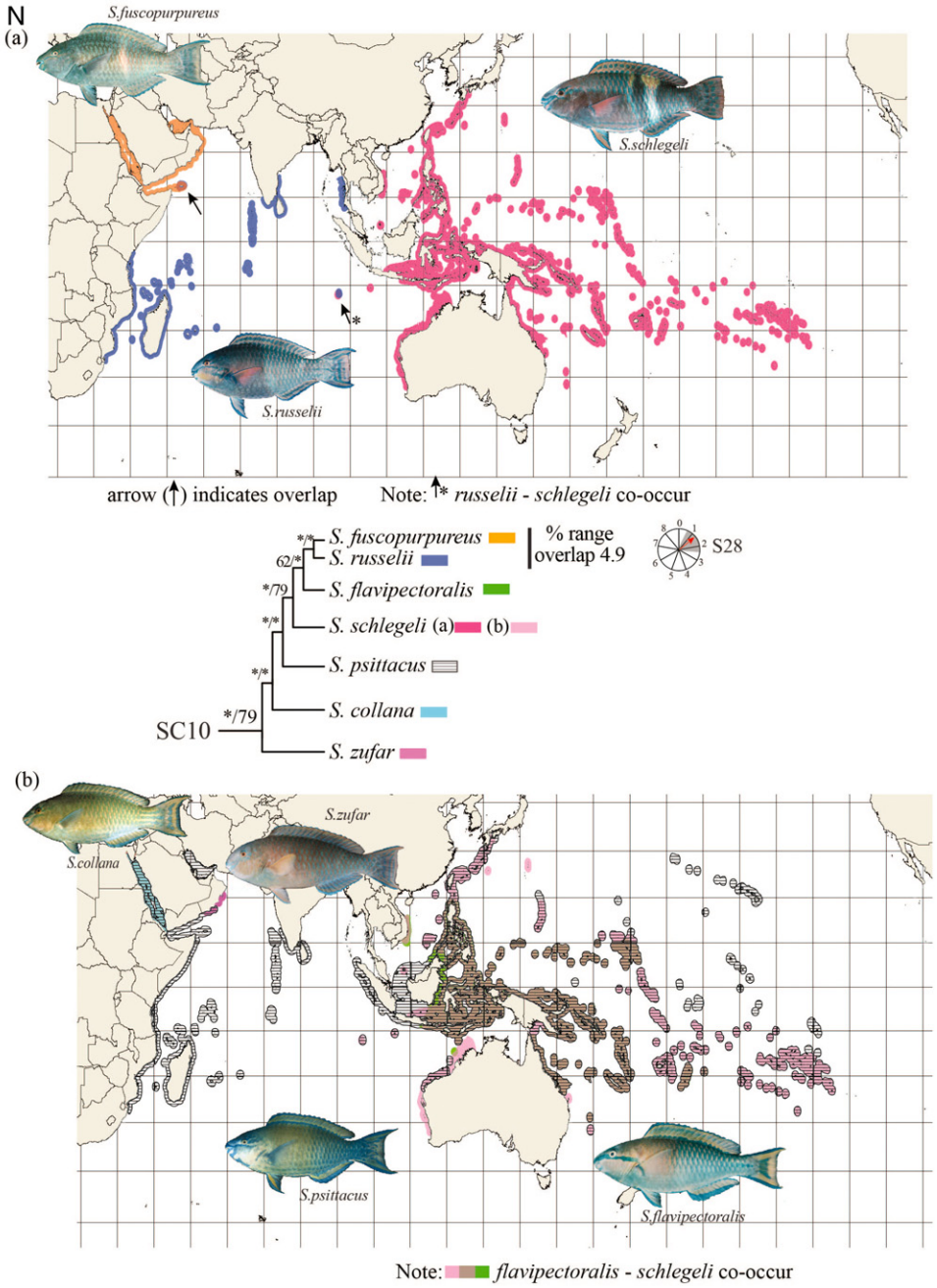

Figure 2. *Continued*

major role for allopatric speciation (i.e. genetic isolation associated with geographical isolation) (Table 2), which mirrors the findings of Robertson *et al*. (2006). This does not imply that natural or sexual selection played no role in allopatric divergences (Sobel *et al*., 2010); rather, we observe that, in contrast to the situation in the majority of sympatric species pairs, we found only minor eco-morphological and colour differences in more than 70% of allopatric species pairs. Moreover, we do not seek to draw a line between allopatry and sympatry because much work remains to be done on the influence of reinforcement both in species pairs that have diverged sympatrically and in pairs that have undergone allopatric divergence and then come into secondary contact.

PHENOTYPIC DIVERSIFICATION IN A COMPLEX TAXON

In comparison to sympatric sister pairs, allopatric species pairs tended to maintain their integrity in the

Table 2. Comparative sister-species distribution, percentage overlap, and nodal age data for *Chlorurus* and *Scarus*

Clade	Node	Sister-species pair	Range size (km^2)	% Overlap	Classification	Mean age (Myr)
CH1						
	C1	*Chlorurus bleekeri*	9 876 807	74.6	Sympatric	2
		Chlorurus bowersi	2 081 582			
	C2	*Chlorurus spilurus*	14 186 253	9.1	Allopatric	1.64
		Chlorurus sordidus	3 469 970			
CH2	C3	*Chlorurus strongylocephalus*	2 699 067	0	Allopatric	0.49
		Chlorurus gibbus	353 841			
CH3	C5	*Chlorurus capistratoides*	1 463 773	3.4	Allopatric	1.46
		Chlorurus atrilunula	769 300			
CH4	C7	*Chlorurus oedema*	2 081 853	0	Allopatric	1.04
		Chlorurus rhakoura	202 584			
CH5	C9	*Chlorurus frontalis*	7 250 285	0	Allopatric	1.17
		Chlorurus enneacanthus	812 265			
SC1	S1	*Scarus altipinnis*	7 145 917	89	Sympatric	2.11
		Scarus niger	15 107 680			
	S2	*Scarus prasiognathus*	7 817 368	5.2	Allopatric	0.82
		Scarus falcipinnis	1 448 781			
	S3	*Scarus forsteni*	9 534 855	53.3	Sympatric	2.71
		Scarus tricolor	6 834 711			
SC2	S4	*Scarus compressus*	675 634	97.3	Sympatric	0.17
		Scarus ghobban	657 250			
SC3	S8	*Scarus ferrugineus*	993 856	85.3	Sympatric	1.69
		Scarus persicus	385 316			
SC4	S11	*Scarus oviceps*	9 383 490	1	Allopatric	1.67
		Scarus scaber	2 976 033			
SC5	S14	*Scarus spinus*	7 358 255	2.8	Allopatric	1.35
		Scarus viridifucatus	2 725 554			
SC7	S22	*Scarus globiceps*	11 363 068	96.2	Sympatric	1.23
		Scarus rivulatus	9 317 924			
	S23	*Scarus festivus*	6 690 884	41.8	Sympatric	0.35
		Scarus chameleon	6 040 865			
SC8	S24	*Scarus ovifrons*	1 319 771	0	Allopatric	3.59
		Scarus arabicus	480 716			
	S25	*Scarus longipinnis*	1 599 827	0	Allopatric	0.94
		Scarus dubius	391 017			
	S28	*Scarus russelii*	1 935 709	4.9	Allopatric	1.08
		Scarus fuscopurpureus	866 127			
SC6	S16	*Chlorurus guacamaia*	2 545 215	0	Allopatric	1.01
		Scarus trispinosus	612 146			
	S18	*Scarus vetula*	2 840 937	100	Sympatric	2.17
		Scarus iseri	2 531 600			
	S19	*Scarus taeniopterus*	2 634 247	0	Allopatric	1.07
		Scarus zelindae	612 146			
	S21	*Scarus hoefleri*	797 547	0	Allopatric	3.4
		Scarus perrico	760 076			

Percentage overlap estimated as the proportion of the species with smaller range size that live within the distribution of the larger range size. (Barraclough & Vogler, 2000) Sympatry > 10% overlap; allopatry < 10% overlap. Mean nodal ages from Table 1.

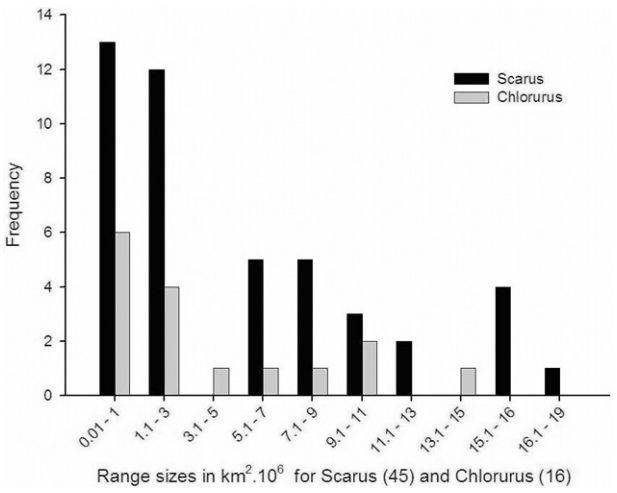

Figure 3. Frequency distribution of range sizes as $km^2 \times 10^6$ for all species by genus: *Scarus* ($N = 45$); Chlorurus ($N = 16$).

cluster analysis based on body dimensions and size, head-shape, and dental plate metrics. This implies ecological similarity among allopatric species pairs, which also showed relatively small differences in colour pattern (Figs 4B, 5). Examples are seen in the *S. schlegeli/russelii/fuscopurpureus* and *Chlorurus microrhinos/strongylocephalus/gibbus* complexes (Fig. 2B, N). Sympatric sister-species pairs displayed greater differences in morphology, size (Fig. 4B), and colour pattern traits (Fig. 5). In addition to extreme differentiation in terminal colour phase (e.g. *S. altipinnis/niger*) (Fig. 2F), the loss of an entire colour phase in each of the co-occurring species as seen in *S. guacamaia/coelestinus* represents another potential mechanism for reproductive differentiation in sympatric sister species.

A further difference in ecological traits among sympatric sister species occurred in the relative size of their geographical distributions and types of reef habitats occupied. The sympatric pair *C. bleekeri/bowersi* (Clade CH1) displayed a 130% difference in their range areas, a reflection of the colonization of western and central Pacific oceanic reefs by *C. bleekeri* (Fig. 2A; see also Supporting information, Table S3). The sister pair *S. festivus/chameleon* (SC 7), with a very recent time of divergence (0.35 Mya), provides an even more striking example. *Scarus festivus* inhabits oceanic reefs of both the Indian and Pacific Oceans, whereas its sister species *S. chameleon* is confined to high island and continental shelf reefs of the Indo-Australian archipelago. A similar pattern is seen in the pair *S. rivulatus/globiceps* (SC 7), which, despite a modest evolutionary age (1.2 Mya), displayed marked differences in longitudinal range, size, and terminal phase colour pattern. *Scarus rivulatus* is one of the few species to successfully colonize the recently inundated and largely non-reefal areas of the Sunda Shelf (see Supporting information, Table S3). This division in habitats between closely-related species is similar to that recorded for clades of the gastropod *Drupella* (Clearmont, Reid & Williams, 2011) and Atlantic labrid fishes (Rocha *et al*., 2005). However, a more comprehensive comparison of range sizes and habitat associations in allopatric species pairs, and which also controls for ocean-basin differences in reef area and habitat structure, is required to fully flesh out and test this comparison. Our data indicate that allopatric and sympatric sister species have similar evolutionary ages and provide little evidence of increased time to sympatry. The relatively low mean values for the evolutionary ages of sympatric pairs may reflect the rapid divergence of sisters in sympatry, or the re-establishment of sympatry after rapid allopatric divergence.

Evolution in the tropical Atlantic involved fewer species, with sympatry as the dominant distributional pattern. This group displayed the greatest disparity in sizes recorded among the species in the present study, with *S. guacamaia* achieving a maximum weight 100-fold greater than in the smallest species, *S. iseri*. However, the two sympatric members of the clade containing *S. guacamaia*, *S. trispinosus* and *S. coelestinus*, (*S. guacamaia* and *S. coelestinus*), are similar in body size and shape, although they differ greatly in colour pattern: bronze and green versus black and blue. The pairs *S. taeniopterus/zelindae* and *S. guacamaia/trispinosus* displayed allopatric distributions, with the former in each case confined to the Caribbean and the latter to Brazil. These recently diverged (1.0 Mya) species pairs each represent the same scenario of geographical isolation, with Amazon outflow constituting a barrier (Rocha & Bowen, 2008) as described for species of *Sparisoma* (Robertson *et al*., 2006). A third allopatric pair, *S. hoefleri/perrico*, diverged 3.4 Mya before the closure of the isthmus.

Colonization of reef habitats and the establishment of present day biogeographical patterns

The chronology of the Indo-Pacific species of *Scarus* offers clues to the origin of the tropical Atlantic assemblage. Given the age of this genus, it is unlikely that the tropical Atlantic fauna has a Tethyan origin. Earlier studies, Bellwood (1994) and Smith *et al*. (2008) suggested that *Scarus* arose in the Indo-Pacific and repeatedly crossed the east Pacific barrier before the closure of the Panamanian Isthmus, implying entry to the Atlantic from the west. Smith *et al*. (2008) identified *S. ghobban* as the likely ancestor of

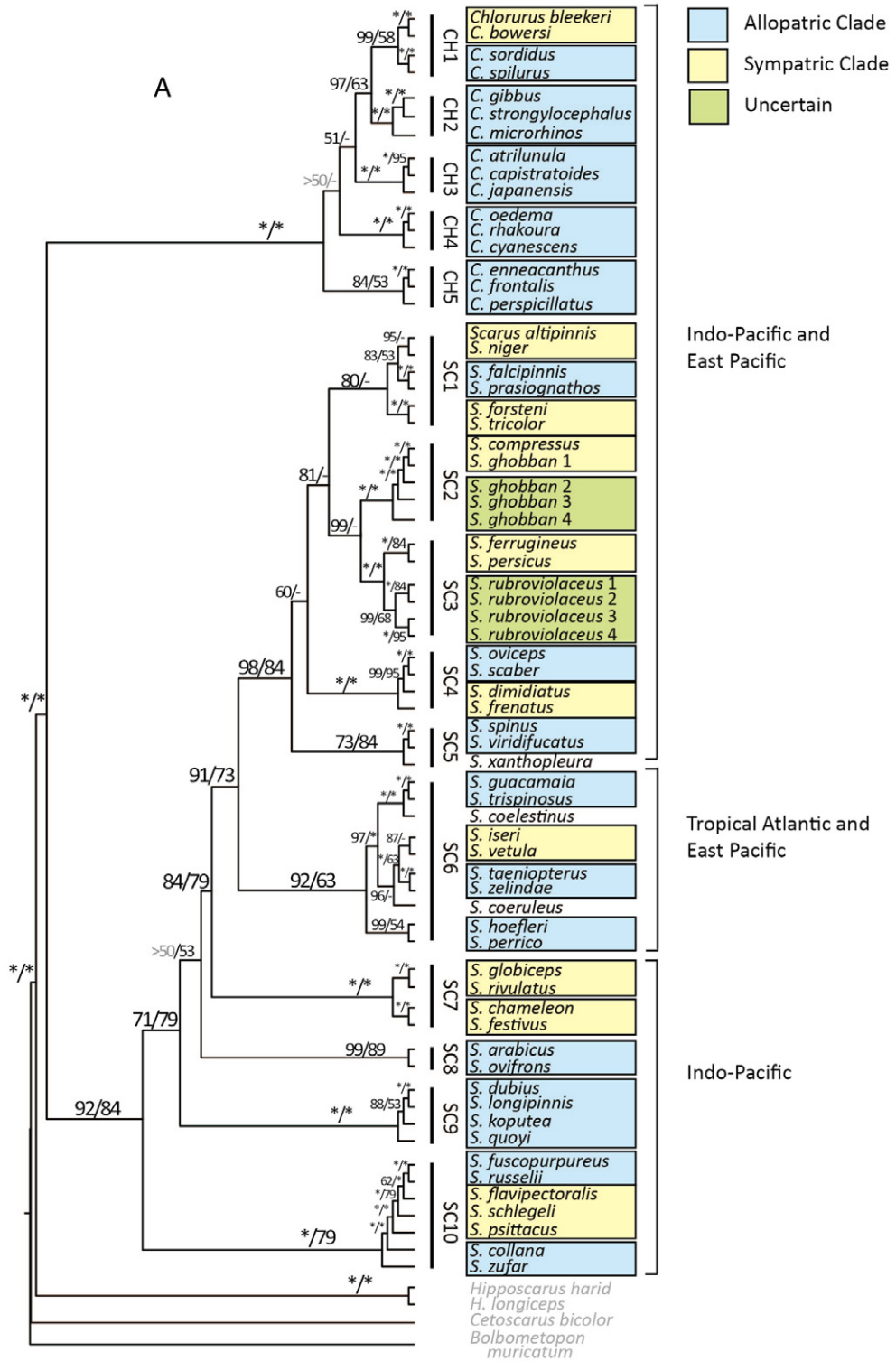

Figure 4. Evolutionary and morphological relationships among the study taxa. A, reconstruction of phylogenetic relationships of *Chlorurus* and *Scarus*. Bayesian and maximum parsimony (MP) analyses for three loci (16S, control region, S7I1) derived from Fig. 1A. B, reconstruction of morphological and meristic relationships of *Chlorurus* and *Scarus* using hierarchical cluster analysis of the means of five morphological and three meristic variables.The analysis uses euclidean distance and average linkage. Sample size; five individuals per species. The inset depicts the three basal *Scarus* species that cluster within *Chlorurus*. Clades are identified on the basis of their distributional relationships.

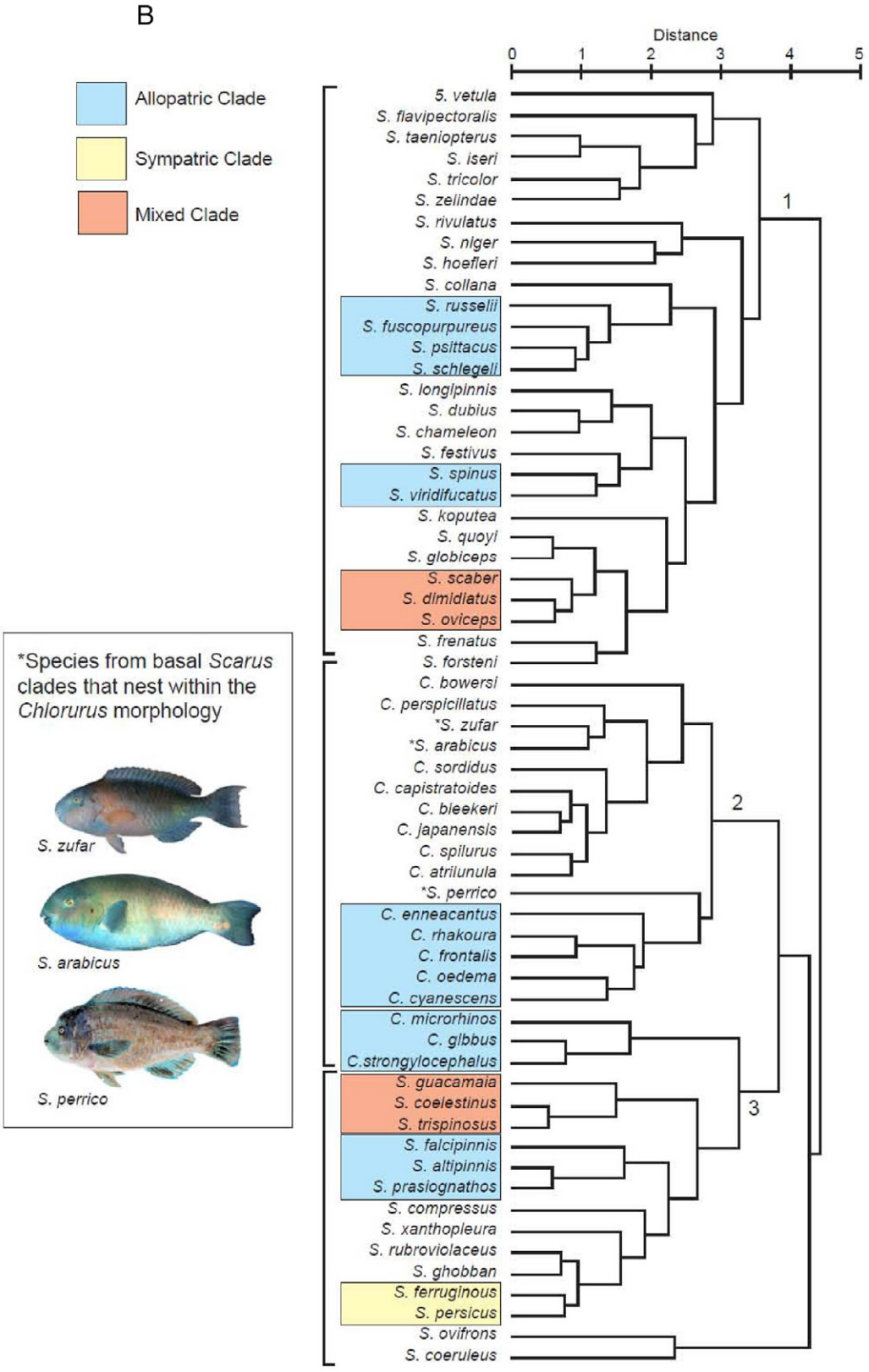

Figure 4. *Continued*

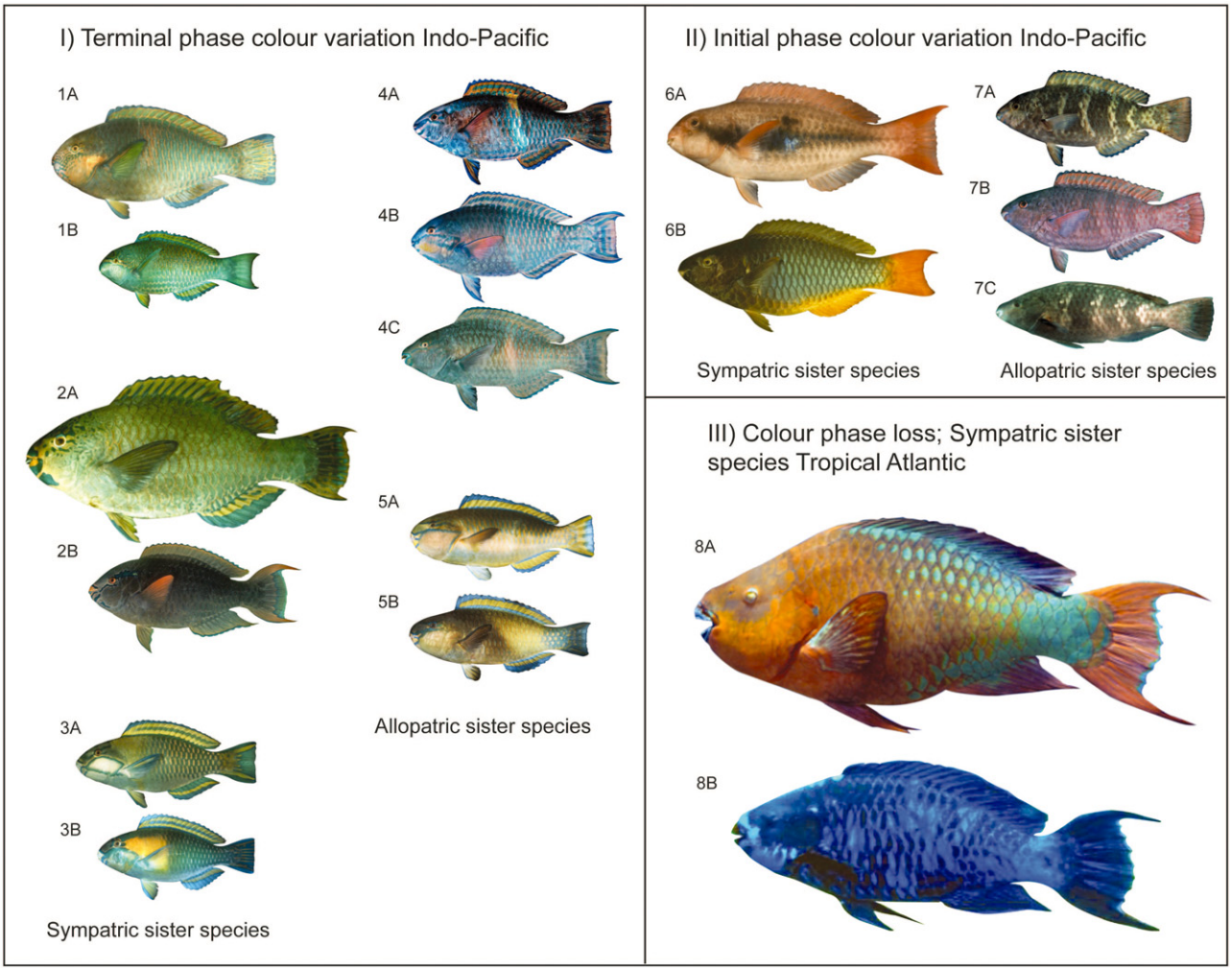

Figure 5. Comparison of colour patterns in sympatric and allopatric sister species showing three patterns of divergence. Images are scaled to mean Fork Length (FL) mm. I, divergence of terminal phase colour patterns in sympatric and allopatric Indo-Pacific species. Sympatric sister species: 1A *Scarus rivulatus*; 1B *Scarus globiceps*; 2A *Scarus altipinnis*; 2B *Scarus niger*; 3A *Chlorurus bleekeri*; 3B *Chlorurus bowersi.* Allopatric sister species: 4A *Scarus schlegeli*; 4B *Scarus russelii*; 4C *Scarus fuscopurpureus*; 5A *Chlorurus capistratoides*; 5B *Chlorurus japanensis.* II, divergence of initial phase colour patterns in sympatric and allopatric Indo-Pacific species. Sympatric sister species: 6A *Scarus forsteni*; 6B *Scarus tricolor.* Allopatric sister species: 7A *Scarus schlegeli*; 7B *Scarus russelii*; 7C *Scarus fuscopurpureus.* III, loss of one colour phase. Sympatric tropical Atlantic species: 8A *Scarus guacamaia*; 8B *Scarus coelestinus.*

the Atlantic fauna. However, the diversification of the widespread clades that include *S. ghobban* and *S. rubroviolaceus*, both of which have colonized the east Pacific, appears to be relatively recent (Lessios & Robertson, 2006; Fitzpatrick *et al*., 2011), implying arrival at the eastern margins of their range well after the closure of the Isthmus. *Scarus compressus* and *S. perrico* provide contrasting chronologies, with the former being an east Pacific sister to *S. ghobban*, and the product of the most recent divergence within the genus (Tables 1, 2). *Scarus perrico*is a relatively ancient East Pacific endemic and sister to the West African endemic *S.hoefleri*. This raises the possibility that an ancestral *Scarus* colonized the Atlantic around southern Africa from the Indian Ocean, migrated through the Atlantic, and passed westwards through the Central American Isthmus to produce *S. perrico*. We cannot exclude the possibility that the common ancestor of the *S. perrico/hoefleri* pair was the product of an earlier diversification of the Indo-Pacific *Scarus* fauna that crossed the east Pacific barrier before the closure of the Isthmus but, given the time frame of Indo-Pacific diversification and easterly migration, this is less likely.

The geographical distribution and divergence times of the endemic species *S. zufar*, *S. collana*, and

S. arabicus (Fig. 2M, N) suggests that the initial diversification of *Scarus* took place in the western margin of the Indo-Pacific, with progressive migration eastwards. This culminated in the arrival in the east Pacific of the colonizing clades of *S. rubroviolaceus* and *S. ghobban* in the mid to late Pleistocene. In addition, diversification of clade SC9 is associated with a progressive eastwards colonization, resulting in a number of central Pacific endemics (e.g. *S. koputea*, *S. dubius*) that are associated with isolated island habitats (Fig. 2N). The provenance of *Chlorurus* is less clear. The location of the endemic *C. perspicillatus* in Hawaii and the greater ages of Pacific clades of *Chlorurus spilurus* relative to *Chlorurus sordidus* (Beck, 2011) suggest a Pacific origin and westerly expansion, with the most recent divergence, *C. gibbus*, arising in the Red Sea.

Both genera contain distinctive endemic species, a number of which are associated with marginal reef habitats at the periphery of the Indo-Pacific and the tropical Atlantic. Fifteen such species with range sizes of $< 0.8 \times 10^6$ km^2 were identified (see Supporting information, Table S3). However, these species displayed a wide range of ages, suggesting that a number of evolutionary processes have acted on peripheral populations. Six species (i.e. *S. zufar*, *S. arabicus*, *S. collana*, *S. perrico*, *S. hoefleri*, and *Chlorurus perspicillatus*) occupied basal positions in their relevant clades, and share a mean evolutionary age of $4.04 \pm 0.34 \times 10^6$ years. All are located in geographically peripheral habitats, with four restricted to ecologically marginal coral reef habitats. This pattern suggests that the ancestral habitats of *Scarus* at least were rocky rather than coral reefs, and that colonization of well developed coral reefs occurred late in the evolutionary history of the clade. At least three of these species, namely *S. perrico* (east Pacific), *S. arabicus*, and *S. zufar* (Oman and the Gulf of Aden), possess robust exposed dental plates, obtuse head profiles, and extensive cheek areas, which are characters that group them morphologically with the excavating genus *Chlorurus* (Fig. 4B). It is not known whether these shared anatomical features result in the same biting mechanics and power as seen in *Chlorurus*, although one interpretation is that these endemics represent an earlier radiation of the genus *Scarus* (Rosenblatt & Hobson, 1969) adapted to rocky reef substrata. A strong association with the fronts and crests of well-developed reef systems dominated by calcium carbonate substrata is more evident in *Chlorurus* and in the most recently diverged clades of *Scarus*.

A second group of restricted range species, *S. compressus*, *S. trispinosus*, *S. zelindae*, *S. dubius*, and *C. gibbus*, appeared very recently, with a mean age of diversification of $0.74 \pm 0.17 \times 10^6$ years. In the case of *S. trispinosus*, *S. zelindae*, *S. dubius*, and *C. gibbus*, this appears to represent divergence after geographical isolation. In taxa such as *S. compressus* the process is unclear. This may represent peripatric speciation from a larger, more widespread population (Pacific populations of *S. ghobban*), or sympatric divergence from a smaller, distinct East pacific population of *S. ghobban*. This example emphasizes the need for additional population genetic scrutiny not only of *S. ghobban*, but also of other widespread species (e.g. *S. niger*, *S. frenatus*, and *S. globiceps*) because these taxa may represent unrecognized species complexes.

Conclusions and future studies

Speciation and the nature of evolutionary processes underlying diversification of sympatric sister taxa

Previous reviews of the evolution of parrotfishes considered them to be exemplars of a model of adaptive radiation in which the component species diverged sequentially along axes of habitat, trophic morphology, and communication (reproduction). By contrast, our analyses revealed examples of contemporaneous patterns of both neutral (drift in allopatry) and adaptive (natural and sexual selection in sympatry) divergence, and did not support the concept of a single dominant sequential process underlying diversification. Moreover, we saw little evidence of a pattern of sequential and independent episodes of natural (i.e. on ecological characters) and sexual selection in parrotfish evolution. We conclude that interactions among ecological and reproductive traits provide a more plausible explanation for species diversification, with the order and intensity of these being influenced by the environmental setting in each case.

For the 64% of the sister species that are allopatric, divergence with genetic drift after isolation is the most plausible process. For sympatric species pairs, the processes are likely to be more variable. If local adaptation interacts with sexual selection then divergence in ecological and reproductive characteristics over relatively limited geographical scales and in the face of gene flow is plausible (van Doorn, Edelaar & Weissing, 2009; Maan & Seehausen, 2011). However, the complex geography of areas where range overlap is recorded would also allow for initial allopatric divergence followed by secondary range expansion to achieve contemporary sympatry. Further work is required to determine the degree to which natural and sexual selection have interacted, and whether divergence has occurred in the presence of gene flow (The Marie Curie Speciation Network*, 2012). From the perspective of our results and conclusions, there are two priorities for future study: determining (1) the extent to which adaptive divergence has played a

role in parrotfish speciation and (2) the relationship between the rate of diversification and the intensity of natural and sexual selection.

Temporal patterns in sister-species diversification

A number of sympatric sister-species pairs (e.g. *S. festivus/chameleon*, *S. globiceps/rivulatus*) (Fig. 2L) displayed evidence of rapid and extensive colonization of Indo-Pacific reef systems over relatively short time periods. A process involving rapid expansion of geographical range size leading to overlap with the range of the sister taxon suggests that re-establishment of sympatry after allopatric divergence (Weir & Price, 2011) occurs rapidly. However, although it is possible to date the divergences of sister species, the evolutionary age of individual taxa is more problematical. Investigation of the alternative hypothesis (i.e. that the taxon with the greater range is in fact older than suggested by age estimates derived from estimates of sister divergences) (Hodge *et al*., 2012) requires coalescence analysis of phylogeographical data (Marko & Hart, 2012). This is a priority for future research. The assumptions that: (1) widespread species such as *S. festivus* have been invariant over extended evolutionary time periods or (2) they cannot achieve widespread distributions within the Indo-Pacific in the time periods indicated, require further testing (Hodge *et al*., 2012).

What processes have driven the rapid diversification and colonization of coral reefs by parrotfishes?

The most striking features of parrotfish evolution concern the apparent complexity of the speciation processes, rapid colonization of the world's tropical reefs, and the retention of relatively conservative morphologies and foraging modes at the same time as exploiting novel ecological opportunities. The structure and geographical organization that characterize present day coral reefs was established during the Miocene (Bellwood & Wainwright, 2002). Unlike other reef fish groups that underwent significantly increased diversification rates during this period (Cowman & Bellwood, 2011), parrotfishes diversified on coral reefs during the Pliocene, approximately 12 Myr after modern reefs were established (Alfaro *et al*., 2009; Kazancioğlu *et al*., 2009). Thus, it is unlikely that parrotfishes were historically important in determining the biological organization of present day reefs. Three aspects of present day parrotfish habitat associations, diets and foraging modes, and distribution patterns, provide a focus for future studies. First, many of the older taxa presently occur in marginal reef habitats; are these representative of early environment of this group? Second, a number of taxa are associated with newly developed non-reef habitats and continental shores exposed by rising sea-levels subsequent to the latest glaciations, as opposed to well developed oceanic reef systems. Is there a temporal sequence in habitat associations with occupancy of fringing reef habitats preceding colonization of well developed and oceanic reef systems? Third, to what extent does the exploitation of a resource base represented by the protein and lipids residing in benthic detrital and bacterial aggregates; the microbial, plant and meiofaunal, and plant assemblages; and living corals (Crossman, Choat & Clements, 2005) contribute to our understanding of the rapid diversification of parrotfishes on tropical reefs. Their role on present coral reefs and the history of this association remains an event of fundamental importance in reef ecology.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Species analyzed, localities, and sample ID.
Table S2. Primer sequences used (two mitochondrial and one nuclear) in the present study. Primer-specific annealing temperatures (T_a) are indicated.
Table S3. Range size (km^2); habitat associations (proportion of range within three categories of reef habitat and the Sunda shelf); NA comprise those species whose distribution does not extend to the vicinity of the Sunda Shelf. Species with ranges that extend over multiple ocean basins are partitioned into Pacific and Indian Ocean distributions.

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Table S1. Species analysed, Localities and Sample ID.

Species	Location and ID Code
Outgroups	
Bolbometopon muricatum (Valenciennes 1840)	Lizard Island, GBR Australia (1951/1952/1953)
Cetoscarus bicolor (Rüppell 1829)	Lizard Island, GBR Australia (573)
Hipposcrus harid (Forsskål 1775)	Seychelles (1608/1609/1610/1611)
Hipposcarus longiceps (Valenciennes 1840)	Lizard Island, GBR Australia (1947/1948)
Chlorurus	
C. atrilunula (Randall & Bruce, 1983)	Sodwana Bay, East Africa (2484/2488)
C. bleekeri (de Beaufort, 1940)	Lizard Island, GBR Australia (1911); Kimbe Bay, Papua New Guinea (3113/3114/3123)
C. bowersi (Snyder, 1909)	Taiwan (2482/3054/3072)
C. capistratoides (Bleeker, 1847)	Amirante Plateau, Seychelles (976/977/978); Christmas Island, Australia (4761/4762/4763/4764)
C. cyanescens (Valenciennes, 1840)	Rodriguez Island, Madagascar (4658/4659)
C. enneacanthus (Lacepède, 1802)	Cocos Keeling Islands, Australia (2686/2687); Christmas Island, Australia (4760)
C. frontalis (Valenciennes, 1840)	Rota Island, Micronesia (345/346); Christmas Island, Australia (4947); Middleton Reef, Australia (4960)
C. gibbus (Rüppell, 1829)	Red Sea, Egypt (4942/4943)
C. japanensis (Bloch, 1789)	Lizard Island, GBR Australia (344); Taiwan (2368/2369)
C. microrhinus (Bleeker, 1854)	Britomart Reef (349), Orpheus Island (351/352), Lizard Island (354/1920/1921/1923/1925), all GBR Australia
C. oedema (Snyder, 1909)	Taiwan (2308/2310/2381/2395/2483)
C. perspicillatus (Steindachner, 1879)	Hawaii Islands, USA (334/335/336)
C. rhakoura (Randall & Anderson, 1997)	Dampier Archipelago, Western Australia (3096/3097/3099)
C. spilurus (Valenciennes, 1840)	Moorea, French Polynesia (310/3978/3990); Bali, Indonesia (332);
C. sordidus (Forsskål, 1775)	Farquhar Island, Seychelles (3409/3411/3413)
C. strongylocephalus (Bleeker, 1854)	Amirante Plateau, Seychelles (875/876/877/881/883/888/889)

Scarus

S. altipinnis (Steindachner, 1879)	Lizard Island, GBR Australia (2845/2846/2847)
S. arabicus (Steindachner, 1902)	Oman (2404/2405/2406/2407/2411)
S. chameleon (Choat & Randall, 1986)	GBR Australia (611); [Taiwan (2366)]
S. coelestinus (Valenciennes, 1840)	Los Roques, Venezuela (276); San Blas, Caribbean Sea (637); (AY081083)
S. coeruleus (Edwards, 1771)	Caribbean Sea (201/202/203/206)
S. collana (Rüppell, 1835)	Red Sea, Egypt (4933/4935)
S. compressus (Osburn & Nichols, 1916)	Panama, East Pacific Ocean (3105/3106/3107)
S. dimidiatus (Bleeker, 1859)	Lizard Island, GBR Australia (1893)
S. dubius (Bennett, 1828)	Hawaii Islands, USA (610/3962)
S. falcipinnis (Playfair, 1868)	Amirante Plateau, Seychelles (970/971); Seychelles (2964)
S. ferrugineus (Forsskål, 1775)	Oman (2430/2431/2495/2496); Masqat Bandar, Oman (4920)
S. festivus (Valenciennes, 1840)	Taiwan (2385/3089); Christmas Island, Australia (4660)
S. flavipectoralis (Schultz, 1958)	GBR, Australia (228/234)
S. forsteni (Bleeker, 1861)	Rota, Micronesia (602/603); Lizard Island, GBR Australia (2803)
S. frenatus (Lacepède, 1802)	Long Island, GBR Australia(42); Seychelles (1481/1482/1483)
S. fuscopurpureus (Klunzinger, 1871)	Oman (2432/2433/2435); Khwar Habalyn, Oman (4898)
S. ghobban EP (Forsskål, 1775)	Panama, East Pacific Ocean (3109/3110); Las Parlas, Panama (1238)
S. ghobban GBR (Forsskål, 1775)	GBR (231); Lizard Island, GBR Australia (5005)
S. ghobban OM (Forsskål, 1775)	Al Halanyatt, Oman (3142/3143); Khwar Ma'ili, Oman (4901)
S. ghobban SY (Forsskål, 1775)	Seychelles (1264/1265/2891/2892/2894)
S. globiceps (Valenciennes, 1840)	GBR Australia (232); Beacon Island, Western Australia (32/43/44)
S. guacamaia (Cuvier, 1829)	Los Roques, Venezuela (271/272/273/2792); Bermuda (2794); (AY081085)
S. *hoefleri* (Steindachner, 1881)	São Tomé and Príncipe, West Africa (315/316); Cape Verde, West Africa (319/320)
S. iseri (Bloch, 1789)	Los Roques, Venezuela (267/268/270)
S. koputea (Randall & Choat, 1980)	Marquesas Islands, French Polynesia (4442/4447)
S. *longipinnis* (Randall & Choat, 1980)	Lizard Island, GBR Australia (1902)
S. niger (Forsskål, 1775)	GBR, Australia (597/598); Seychelles (1243/1460/1461)
S. oviceps (Valenciennes, 1840)	Ningaloo Reef, Western Australia (1080); Lizard Island, GBR Australia (1913/1915)
S. ovifrons (Temminck & Schlegel, 1846)	Taiwan (2294)
S. perrico (Jordan & Gilbert, 1882)	Panama, Eastern Pacific Ocean (3102/3104)
S. persicus (Randall & Bruce, 1983)	Oman (2422/2423/2493/2494/3235/3236); Khwar Ma'ili, Oman (4911)

S. prasiognathus (Valenciennes, 1840)	Seychelles (1811/1812)
S. psittacus (Forsskål, 1775)	Amirante Plateau, Seychelles (913/920/923/928/932)
S. quoyi (Valenciennes, 1840)	Bali, Indonesia (590); Kimbe Bay, Papua New Guinea (3133/3136)
S. rivulatus (Valenciennes, 1840)	Palm Islands, GBR Australia (159/160); GBR Australia (230)
S. rubroviolaceus EP (Bleeker, 1847)	Montusa, Panama (1231/1232); Panama (3108)
S. rubroviolaceus GBR (Bleeker, 1847)	GBR (229), Lizard Island (1955/1956/3358) Australia
S. rubroviolaceus OM (Bleeker, 1847)	Oman (3241/3242); Masqat Banda, Oman (4928)
S. rubroviolaceus SY (Bleeker, 1847)	Seychelles (1285/1286/2946/2949/2951)
S. russelii (Valenciennes, 1840)	Amirante Plateau, Seychelles (856/860/862/865/873)
S. scaber (Valenciennes, 1840)	Amirante Plateau, Seychelles (964/965)
S. schlegeli (Bleeker, 1861)	Britomart Reef, GBR (363/370); Lizard Island, GBR Australia (1888); Rota Micronesia (378/379)
S. spinus (Kner, 1868)	Lizard Island, GBR Australia (1897/1898)
S. taeniopterus (Lesson, 1829)	Los Roques, Venezuela (259/260)
S. tricolor (Bleeker, 1847)	Amirante Plateau, Seychelles (955/956/957)
S. trispinosus (Valenciennes, 1840)	Brazil (323/324/327)
S. vetula (Bloch & Schneider, 1801)	San Blas, Caribbean (634); Bermuda (2729/2732/2733)
S. viridifucatus (Smith, 1956)	Amirante Plateau, Seychelles (972/973/975)
S. xanthopleura (Bleeker, 1853)	Christmas Island, Australia (4662/4663/4664/4665)
S. zelindae (Moura, Figueiredo & Sazima, 2001)	Cabo Frio, Brazil (638/639)
S. zufar (Randall & Hoover, 1995)	Oman (2425/2425/2497); Masqat Banda, Oman (4929)

Table S2. Primer sequences used (two mitochondrial and one nuclear) in this study. Primer-specific annealing temperatures (T_a) are indicated.

Locus (Reference)	Primer name	Primer Sequence	T_a
16S rRNA (Simon et al. 1994)	LR-J-12887 LR-N-13398	5' CCG GTC TGA ACT CAG ATC ACG T 3' 5' CGC CTG TTT ACC AAA AAC AT 3'	51-49-47
Control region (Meyer et al. 1994)	L15995 H16498	5' AAC TCT CAC CCC TAG CTC CCA AAG 3' 5' CCT GAA GTA GGA ACC AGA TG 3'	51-49-47
S7 Intron 1 (Chow & Hazama 1998)	S7I1 F S7I1 R	5' TGG CCT CTT CCT TGG CCG TC 3' 5' AAC TCG TCT GGC TTT TCG CC 3'	50

Table S3. Range size (km^2); Habitat associations (proportion of range within three categories of reef habitat and the Sunda shelf); NA are those species whose distribution does not extend to the vicinity of the Sunda Shelf. Species with ranges that extend over multiple ocean basins are partitioned into Pacific and Indian Ocean distributions.

					Habitat Associations				
Ocean region	Genus	Species	Biogeographic Classification	Range size	Contin-ental	High island	Oceanic Island	Sunda Shelf	Node ages (mya)
Indo-Pacific	*Chlorurus*	*C. bleekeri*	CIP	9876807	3.9	81.1	18.9	7.3	2
		C. bowersi	CIP	2081582	0	88.6	11.33	8.7	2
		C. sordidus	RS WIP	3469970	56.1	27.6	16.3	12.5	2.85
		C. spilurus	CIP EIP	14186253	11.7	55.97	32.3	8.7	2.85
		C. microrhinos	CIP EIP	10677534	6.1	57.8	36.1	3.6	2.1
		C. strongylocephalus	WIP	2699067	32.4	28.6	39	0	0.49
		C. gibbus	RS	353841	100	0	0	NA	0.49
		C. japanensis	CIP EIP	6868024	12.7	68.3	29	1.4	3.03
		C. capistratoides	WIP	1463773	11.4	47.1	41.5	7.6	1.46
		C. atrilunula	WIP	769300	45.7	46.4	7.9	NA	1.46
		C. oedema	CIP	2081853	0	100	0	0	1.04
		C. rhakoura	WIP CIP	202584	81.3	18.7	0	0	1.04
		C. cyanescans	WIP	878583	36	57.9	6.1	NA	2.76
		C. perspicillatus	EIP	359175	0	42.8	47.2	NA	4.43
		C. frontalis	CIP EIP	7250285	4.1	76.1	19.8	0	1.17
		C. enneacanthus	WIP	812265	39.5	18.8	41.6	0	1.17
Indo-Pacific	*Scarus*	*S. niger*	RS WIP CIP EIP	15107680	19.8	58.3	21.9	13.98	2.11
		S. niger	CIP EIP	10991588	2.4	74.4	23.2	17.60	
		S. niger	RS WIP	2727115	66.2	15.3	18.5	4.40	
		S. altipinnis	CIP	7145917	6.5	28.5	65	0.00	2.11
		S. prasiognathus	WIP CIP	7817368	16.5	72.2	11.3	27.02	0.82
		S. prasiognathus	CIP	6336276	10.4	77.9	5.2	30.90	
		S. prasiognathus	WIP	1481092	14.9	47.8	37.3	10.40	
		S falcipinnis	WIP	1448781	19	40.8	40.2	NA	0.82
		S. forsteni	WIP CIP	9534855	6.2	63.6	30.2	2.24	2.71

S. tricolor	WIP CIP	6834711	10.8	69.8	19.4	1.66	2.71
S. tricolor	CIP	4378315	24.7	46.2	29	7.10	
S. tricolor	WIP	2447396	3.1	83.2	14	2.60	
S. ghobban	RS WIP CIP EIP TEP	18967727	26.9	40.9	31.3	11.10	1.88
S. ghobban	CIP EIP	14549926	21.8	46.5	31.7	13.60	0.48
S. ghobban	RS WIP	4417801	45.3	23.4	31.2	2.65	1.4
S. ghobban	TEP	657250	96	4		NA	0.17
S. compressus	TEP	675634	95.8	4.15		NA	0.17
S. ferrugineus	RS WIP	993856	94.6	5.4	0	NA	1.69
S. persicus	WIP	385316	100	0	0	NA	1.69
S. rubroviolaceus	RS WIP CIP EIP TEP	16093331	25.2	38.4	36.4	8.88	
S. rubroviolaceus	CIP EIP	11545740	19.8	40.9	39.3	10.80	
S. rubroviolaceus	RS WIP	4547591	38.7	12.7	48.6	3.60	
S. rubroviolaceus	TEP	661350	94	6		NA	
S. frenatus	RS WIP CIP EIP	16009541	16.5	55.4	28.1	13.20	3.61
S. frenatus	CIP EIP	13248923	56.4	32	11.6	6.30	
S. frenatus	RS WIP	1938317	8.2	60.2	31.6	14.60	
S. dimidiatus	CIP	7442937	3.5	71.3	25.1	25.00	3
S. oviceps	CIP EIP	9383490	5.9	56.6	37.5	0.39	1.67
S. scaber	RS WIP	2976033	44.03	27.3	28.6	0.00	1.67
S. spinus	CIP EIP	7358255	5.8	58.6	35.5	0.00	1.35
S. viridifucatus	RS WIP	2725554	26.9	49.9	23.2	0.00	1.35
S. xanthopleura	WIP CIP EIP	5841269	0	71.6	28.4	2.20	
S. globiceps	WIP CIP EIP	11363068	9.4	60.9	29.6	1.50	1.23
S. globiceps	CIP EIP	9938204	6.7	63.1	29.9	1.70	
S. globiceps	WIP	1424804	26.6	45.5	27.9		
S. rivulatus	WIP CIP	9317924	17.7	71.03	11.1	25.50	1.23
S. festivus	WIP CIP EIP	6690884	5.9	58.3	43.4	0.00	0.35
S. festivus	CIP EIP	5457423		62.1	37.9	0.00	
S. festivus	WIP	1674461	23.6	30.4	46	0.00	
S. chameleon	CIP	6040865	17.5	71.4	11.1	0.00	0.35
S. ovifrons	CIP	1319771	14.6	78.4	7.1	NA	3.59

	S. arabicus	WIP	480716	94.8	7.2	0	NA	3.59
	S. quoyi	WIP CIP	8506840	9.5	87.1	6.8	23.52	2.77
	S. koputea	EIP	224853	0	72.7	27.2	NA	
	S. longipinnis	CIP EIP	1599827	18.8	14.2	67	NA	0.94
	S.dubius	EIP	391017	0	43.1	56.9	NA	0.94
	S. zufar	WIP	97534	100	0	0	NA	5.48
	S. collana	RS	370685	100	0	0	NA	3.86
	S. psittacus	RS WIP CIP EIP	15618825	12.5	54.1	33.4	6.53	3.15
	S. psittacus	CIP EIP	11950004	2.8	60.9	36.3	8.50	
	S. psittacus	RS WIP	3668821	44.1	31.9	24.1	0.00	
	S. schlegeli	CIP EIP	11358546	10.1	56.4	33.4	2.26	1.86
	S. russelli	WIP	1935709	23.7	32.5	36.7	0.00	1.08
	S. fuscopurpureus	RS WIP	866127	96.8	3.2	0	NA	1.08
	S. flavipectoralis	CIP EIP	6041655	1.7	74.4	24.8	5.20	1.56
Tropical Atlantic	*S. perrico*	TEP	760076	91.1	8.9		NA	3.4
	S. hoefleri	WA	797547	70	30		NA	3.4
	S. guacamaia	NWA	2545215	23.3	19	57.7	NA	1.01
	S. trispinosus	SWA	612146	98.7		1.3	NA	1.01
	S. coelestinus	NWA	2531803	22.9	19	58.1	NA	2.16
	S. taeniopterus	NWA	2634247	25.6	18.2	55.7	NA	1.07
	S. zelindae	SWA	612146	98.7		1.3	NA	1.07
	S. iseri	NWA	2531600	22.9	19	58	NA	2.17
	S. vetula	NWA	2840937	25.6	20.4	54.2	NA	2.17
	S. coeruelus	NWA	2761523	29.3	17.7	53.2	NA	2.92

Node ages from Table 2. Biogeographic classifications are from Spalding et al 2007 with the Red Sea partitioned from the Western Indo-Pacific; CIP Central Indo-Pacific, EIP Eastern Indo-Pacific, EIP Eastern Indo-Pacific, TEP Tropical Eastern Pacific. RS Red Sea, NWA North western Atlantic, South Western Atlantic, WA West Africa, Gulf of Guinea.The extent of the distribution for each species was based on point data from collected specimens through scientific expeditions, published journal articles, fisheries data, museum collections information and a distributional data base created and maintained by R. Myers. All distribution records were checked by the IUCN Wrasse specialist group. The species distribution maps were produced using the software ArcView 3.3 and ArcGIS 10. Marine basemaps were created using buffered fixed distances from the shore and bathymetry data. A number of standard basemaps of all possible habitat ranges were created. These basemaps were then used as a guide to clip species-specific distribution maps. For species occurring within a very narrow range or those that are endemic, the ArcView 3.3 software was used to create polygons and shapefiles to represent species distribution range. Maps were

created and plotted by adding a shape (graphics) to a view using the Draw polygon tool in ArcView 3.3. The species maps are available online at http://www.iucnredlist.org/technical-documents/spatial-data, and were generated using a map batcher geoprocessing script in ArcGIS 10.