From endemism to widespread distribution: phylogeography of three amphidromous *Sicyopterus* species (Teleostei: Gobioidei: Sicydiinae)

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ABSTRACT: Island freshwater habitats are colonised by amphidromous fish, which display an oceanic larval phase while the rest of their life cycle takes place in rivers. In the present study we evaluated the population structure of the widespread *Sicyopterus lagocephalus* (Gobioidei) over its Indo-Pacific distribution range, which encompasses the island chain (Indonesia – Papua New Guinea – Malaysia) known as the Indo-Pacific Barrier (IPB). Additional analysis of 2 endemic species, *S. aiensis* (Vanuatu) and *S. sarasini* (New Caledonia), living in sympatry with *S. lagocephalus*, represented comparative material and was used as the basis for an assessment of endemism and dispersal. Mitochondrial cytochrome *b* sequence data were obtained for 332 *S. lagocephalus*, 210 *S. aiensis* and 87 *S. sarasini* across each species' range. Haplotype networks and *F*-statistics were used to determine patterns of population structure. A discrete phylogeographic diffusion model under a time-scaled coalescent tree prior was used to assess the history of the spatial expansion of *S. lagocephalus* across its wide spatial distribution. *S. lagocephalus* demonstrates high population structure across the IPB. It also displays a strong structure between Tahiti and all other locations sampled. No other population structure was identified in the entire western Pacific. Phylogenetic reconstruction and coalescence analysis indicate that the oldest population originated in the western Pacific, from which the eastern Pacific and the Indian Oceans were colonised. For the 2 endemic species, no genetic structure was identified across their respective ranges. From the genetic results associated with known elements of the life history of these species, we improved our understanding of the simultaneous existence of geographically close endemic species and a widespread species.

KEY WORDS: *Sicyopterus* · Amphidromy · Pelagic larval duration · Biogeographical barrier · Endemism · Indo-Pacific · Cytochrome *b* · Population structure

INTRODUCTION

Within the open ocean, tropical insular marine, terrestrial and freshwater environments usually represent highly isolated and fragmented habitats. The colonisation of tropical insular rivers, subject to extreme climatic and hydrologic seasonal variations (McDowall 2007, Crandall et al. 2009), requires specific adaptations of the life cycle of species occurring in these distinctive habitats. Diadromy is among the evolutionary answers to the instability of freshwater habitats. Spending part of their life cycle at sea,

diadromous species are capable of escaping drought or cyclonic flood events while colonising new environments via oceanic dispersal. This oceanic dispersal of freshwater organisms represents an essential element in the persistence and structuring of populations at both the local (stream, river, archipelago) and regional scales (Clobert et al. 2003, Keith 2003).

Amphidromous species (both vertebrate and invertebrate organisms) represent most of island freshwater macrofaunal biodiversity (Keith 2003, McDowall 2007, Keith & Lord 2011a). The adults grow, feed and reproduce in rivers. After hatching, larvae drift downstream to the sea, where they undergo larval development (Shen & Tzeng 2002, 2008, Crandall et al. 2009, Lord et al. 2010). After this marine dispersal stage, the post-larvae come back to rivers; they recruit in estuaries and from there colonise the adult freshwater habitat. Freshwater species with an amphidromous life cycle and marine species with a pelagic larval stage exhibit higher dispersal abilities and potentially a lower degree of population structure than strictly freshwater species or marine species of low vagility (Allibone & Wallis 1993, Doherty et al. 1995, Cook et al. 2009, Crandall et al. 2009). Adults of amphidromous species never return to sea after they recruit back to rivers (Radtke & Kinzie 1996, Keith 2003, McDowall 2009). Species persistence in these island freshwater systems partially depends on colonisation or recolonisation events. This is influenced by the proximity of environments, species dispersal capacities, island biogeography and the presence of favourable oceanic currents (Murphy & Cowan 2007, McDowall 2007, Crandall et al. 2009).

Amphidromous species are known in many taxa throughout the Indo-Pacific area, including gastropods (Cook et al. 2009, Crandall et al. 2009), decapod crustaceans (Dennenmoser et al. 2010) and fish in the families Eleotridae and Gobiidae (Keith & Lord 2011a,b). Among gobies, the subfamily Sicydiinae is the most diverse with about 8 to 10 genera and more than 110 species (Keith et al. 2011). The genus *Sicyopterus*, comprising about 25 species, is the most diverse among sicydiine gobies (Keith et al. 2004, Keith & Lord 2011a). In amphidromous gobies, some species have extended pelagic larval durations (PLDs), and others have shorter ones (Lord et al. 2010). These gobies are therefore biological models particularly suitable for studying the relationship between PLD, spatial population genetic structure and species distribution with respect to biogeography (McDowall 2003, 2007, Keith et al. 2008, Iida et al. 2009, Lord et al. 2010).

In this study, we first focussed on the most widespread species, *Sicyopterus lagocephalus* (Pallas, 1767). This species is found from the western Indian Ocean (Comoros Islands, Mascarene Islands) through Sri Lanka and Indonesia, and into the western and eastern Pacific Ocean (including Micro- and Macronesia, New Caledonia, and French Polynesia) and from Japan to northeastern Australia (Watson et al. 2000, Keith et al. 2005). We then focussed on 2 other species, *S. aiensis* Keith, Watson & Marquet, 2004 and *S. sarasini* Weber & de Beaufort, 1915, which are endemic species respectively from Vanuatu and New Caledonia, and live in sympatry with *S. lagocephalus*. *S. lagocephalus* is the most recently diverged species of the genus (Keith et al. 2005), and it has acquired specific life history traits such as its extensive PLD (Lord et al. 2010), allowing it to colonise the entire Indo-Pacific. While *S. lagocephalus* is heavily exploited for human consumption, *S. aiensis* and *S. sarasini* are both rare and fragile species (Lord & Keith 2008).

Oceans are partitioned into biogeographical provinces, which are recognised by habitat discontinuities (Briggs 1974, Gaither et al. 2010). Physical entities separate these biogeographical provinces, and these barriers have been intermittently shaping species distribution and intraspecific population structure over time (due e.g. to sea level fluctuations, island emersion and submergence). These barriers mainly act on species displaying dispersal potential through the marine environment as larvae, juveniles or adults (Gaither et al. 2010, 2011). This is, for example, the case for many fish (Briggs 1974, Planes & Fauvelot 2002, Bay et al. 2004, Craig et al. 2007, Gaither et al. 2010, 2011, Winters et al. 2010) and invertebrates (Lavery et al. 1995, 1996, Lessios et al. 2001, 2003, Barber et al. 2002) across the Indo-Malay archipelago, widely recognised as the Indo-Pacific Barrier (IPB; an intermittent barrier of throughflow current and chain of islands, depending on sea level fluctuations over the geological time scale) that separates the Indian and the Pacific Oceans. However, some marine organisms lack any genetic structure across the IPB (Bowen et al. 2001, Lessios & Robertson 2006, Horne et al. 2008), suggesting different evolutionary histories and dispersal capabilities between organisms.

The aim of this study was to understand the differences in the distribution of 3 *Sicyopterus* species belonging to the same Gobiidae subfamily (Sicydiinae) by exploring their population structure and their evolutionary history, using a new phylo-

geographic approach analysing mitochondrial DNA sequence data. Comprehensive sampling throughout the widespread species' range (i.e. *S. lagocephalus*), comprising Pacific central localities and localities from western and eastern distribution boundaries, was conducted to examine its population structure in the Indo-Pacific area and to assess whether the pattern of genetic structure was concordant with known biogeographical boundaries. This comprehensive sampling was also aimed at studying the evolutionary history of this species, bringing new insights into its structure in the western Pacific. Finally, results for the 2 endemic species (species with a restricted distribution) were compared to those obtained for *S. lagocephalus*, and their meaning in terms of endemism was examined in the light of ecological data such as PLD and larval behaviour.

MATERIALS AND METHODS

Sampling

We sampled 332 *Sicyopterus lagocephalus* specimens across its range. Specimens were collected in the Western Pacific (New Caledonia; Santo, Maewo and Malekula Islands in Vanuatu; Okinawa; Guam; Fiji; Samoa), from Tahiti in French Polynesia, and from the Indian Ocean (Comoros and Reunion Islands) (Fig. 1, Table 1). In addition, 87 *S. sarasini* specimens were caught in New Caledonia from 3 different locations (mid-western coast, south-western coast and Côte Oubliée), and 210 *S. aiensis* specimens were caught from 3 different islands of the Vanuatu archipelago, namely Santo (Tasmate and Penaorou — western coast, Peavot — eastern coast), Maewo and Malakula. Sampling details are summarised in Table 1.

All specimens were collected between 2006 and 2008. They were mostly adults sampled by electrofishing (Portable Dekka 3000 electric device), using a large hand net. Specimens collected at Reunion Island by traditional fishermen were newly recruited post-larvae and were caught using large funnel-shaped baskets. For all 3 species, either entire specimens were caught, or a piece of fin was clipped off the live specimen that was then released back into the river. All specimens were anaesthetised in a 10% clove oil solution prior to fin clipping or were killed by an overdose of this anaesthetic. All samples were kept in 95% alcohol and stored at –4°C.

Laboratory procedures

Total genomic DNA was extracted using the NucPrep™6100 Nucleic Acid Prep Station machine in accordance with the manufacturer's instructions. DNA solution (80 µl) was collected from each specimen and kept frozen at –20°C. A fragment of cytochrome *b* (cyt*b*) was amplified using the primers CytbF217-5'TCG AAA YAT ACA TGC CAA TGG3' and CytbR1043-5'GAA GTA YAG GAA GGA YGC AAT TT3', which were specifically designed for this study. All PCRs were performed on Biometra thermocyclers in a total 25 µl volume of 5% DMSO, 5 µg bovine serum albumin, 300 µM of each dNTP, 0.3 µM *Taq* DNA polymerase (Qiagen), 2.5 µl of the corresponding buffer and 1.7 pM of each of the 2 primers. After a 2 min denaturation at 94°C, the PCR ran 50 cycles of 25 s at 94°C, 25 s at 52°C and 1 min at 72°C, with a 3 min terminal elongation.

Purification and sequencing of PCR products were performed at Genoscope (www.genoscope.cns.fr/) using the forward and reverse PCR primers. Chromatograms were assembled and edited using Sequencher 4.1.4 (Gene Codes Corporation) and then aligned using Bioedit (Hall 1999).

Data analysis

For each of the 3 species, and for each locality, diversity indices such as the number of haplotypes, the number of variable positions, haplotype diversity (*h*) and the mean number of pairwise differences (π) were estimated with Arlequin v.3.1 software (Excoffier et al. 2005). Within species, the relationships among haplotypes from distinct localities were inferred from haplotype networks built using the median joining method implemented in Network 4.5.02 (Bandelt et al. 1999; www.fluxus-engineering .com). Haplotype diversity was high for all 3 species, so we used the 'frequency > 1' criterion to simplify the networks as their reading was otherwise unwieldy. The 'frequency > 1' criterion simplifies networks by ignoring sequences which are unique in the dataset. Default parameters were used and a post-processing parsimony calculation was run in order to eliminate unnecessary median vectors and links. Levels of genetic structure among localities were estimated by the ϕ_{ST} statistic (Weir & Cockerham 1984), using its estimator implemented in Arlequin, which integrates both the haplotype frequencies and the pairwise nucleotide diversities between haplotypes. Distributions of the ϕ_{ST} statistic under the
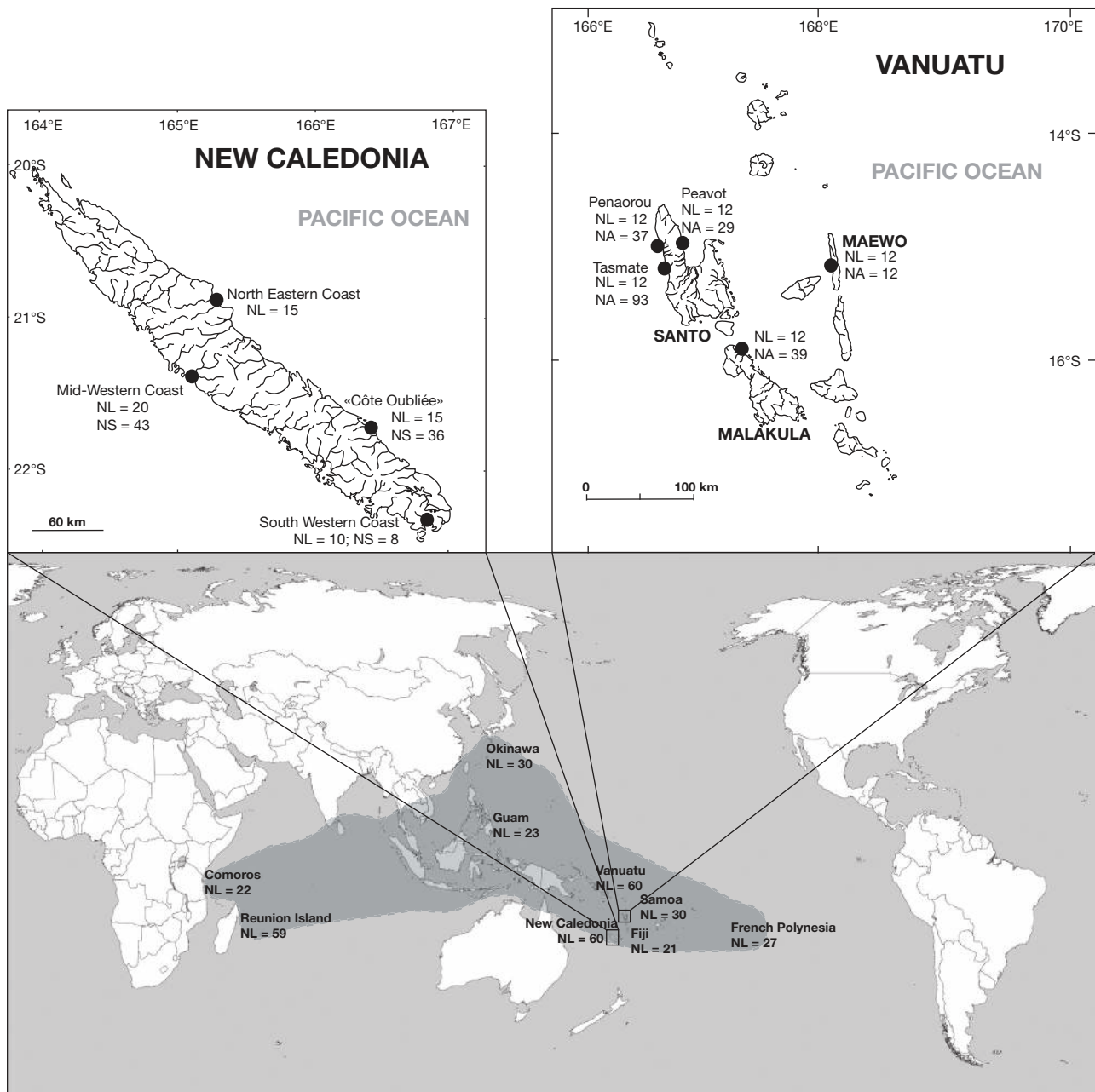
Fig. 1. *Sicyopterus* spp. Sampling locations for *S. lagocephalus* (NL: number of specimens), *S. sarasini* (NS: number of specimens) and *S. aiensis* (NA: number of specimens), in New Caledonia, Vanuatu and in the Indo-Pacific area. The shaded area indicates the distribution of *S. lagocephalus*

null hypothesis of genetic homogeneity between localities were obtained from 10 000 random permutations of specimens among localities and used to test the significance of values calculated from real data. The Mantel correlation test implemented in Arlequin (1000 permutations) was performed between genetic and geographic distance across the whole sampling area to evaluate the possibility that the structure found resulted from isolation by distance. Fu's F_s (Fu

1997) was calculated to test departure from mutation-drift equilibrium. Significance was tested using 10 000 coalescent trees simulated from the observed number of haplotypes in Arlequin.

To assess the origin of *Sicyopterus lagocephalus*, we used the phylogeographic model developed by Lemey et al. (2009), as implemented in Beast v.1.5.4 (Drummond & Rambaut 2007). In this model, transitions among localities are set under a discrete state Markov

Table 1. *Sicyopterus* spp. Diversity indices for *S. lagocephalus*, *S. sarasini* and *S. aiensis* for cytochrome *b* sequences. Sample location (see Fig. 1), number of individuals (n), number of haplotypes (haps), haplotype diversity (h), mean number of pairwise differences (π) and Fu's F_s. Significant values are in **bold**, $\alpha = 0.05$

Region	Archipelago	Island or location	S. lagocephalus					S. sarasini					S. aiensis				
			n	No. of haps	h	π	F_s	n	No. of haps	h	π	F_s	n	No. of haps	h	π	F_s
Western Pacific Ocean	New Caledonia	NE coast	15	14	0.99	5.64	**−8.14**	43	29	0.97	5.86	**−17.28**					
		MW coast	20	20	1	4.50	**−20.65**	8	8	1	6.29	−3.27					
		SW coast	10	10	1	5.56	**−5.49**	36	26	0.95	5.83	**−15.44**					
		Côte oubliée	15	14	0.99	4.76	**−9.26**										
		Total	60	55	0.99	5.01	**−25.55**	87	53	0.97	4.83	**−9.30**					
	Vanuatu	Santo-Tasmate	12	12	1	5.71	**−7.47**						93	70	0.99	6.39	**−25.09**
		Santo-Penaorou	12	12	1	4.09	**−9.34**						37	27	0.97	5.52	**−17.65**
		Santo-Peavot	12	11	0.98	4.85	**−5.67**						29	24	0.98	5.57	**−14.78**
		Maewo	12	12	1	3.65	**−10.03**						12	11	0.98	5.11	**−5.43**
		Malakula	12	10	0.97	4.74	−3.84						39	36	0.99	6.54	**−25.16**
		Total	60	48	0.99	4.67	**−25.66**						210	125	0.99	6.02	**−17.62**
	Japan	Okinawa	30	27	0.99	5.35	**−24.13**										
	Marianna	Guam	23	19	0.97	4.80	**−12.34**										
	Samoa	Upulu	30	15	0.94	5.65	−2.70										
	Fiji	Viti Levu	21	20	0.99	6.15	**−15.01**										
Polynesia	French Polynesia	Tahiti	27	21	0.97	7.40	**−9.15**										
Western Indian Ocean	Mascareines	Reunion	59	15	0.44	0.94	**−13.54**										
	Comoros	Mohéli, Anjouan	22	11	0.75	3.55	−2.68										
		Total	332	231	0.99	4.82	**−14.53**										

matrix. This matrix is added to a phylogenetic model including a tree prior and a model of nucleotide evolution. The method of Lemey et al. (2009) thus enables ancestral state reconstruction while simultaneously accounting for phylogenetic uncertainty. Here, a coalescent model of constant population size was used as a tree prior, and the heterogeneity of the mutation rate across lineages was set under an uncorrelated log-normal clock. According to Rocha et al. (2005), the cyt*b* mean mutation rate ranges from 1.93 to 2.17% in reef-associated gobies of the genus *Gnatholepis*. This genus is a member of the Gobioidei subfamily Gobionellinae, and its sister group includes Sicydiinae gobies (Thacker 2009). Furthermore, *Gnatholepis* is an inhabitant of tropical environments worldwide (Thacker 2004). The biology of *Gnatholepis* is relatively similar to that of Sicydiinae gobies. They have the common gobioid pattern of benthic egg-laying, nest-guarding by the male, and long larval life that can reach ca. 125 d (Shulman & Bermingham 1995, Thacker 2004, Keith & Lord 2011b). We therefore set this parameter under a normal prior spanning this range (mean 2.05%, standard deviation 6.122 × 10^{-4}%) to obtain a time-scaled phylogeny. A generalized time reversible (GTR) model, with a gamma law (4 categories) and a proportion of invariants (I), was used as a model of nucleotide evolution. Other priors were left at default values. Six independent analyses, starting from distinct coalescent trees, were run over 43 million generations and sampled each 5000 steps. To assess whether marginal posterior distributions of each parameter were properly sampled and whether the independent runs converged, results were analysed with Tracer v.1.4.1 (http://beast.bio.ed.ac.uk/Tracer). After discarding 8 million samples as a burn-in, the 6 runs were pooled and resampled every 30000 steps, resulting in a final sample of 7000 trees. The maximum clade credibility tree was

drawn from these pooled results (option 'target branch lengths'). The posterior probabilities of nodes and ancestral geographical localities were calculated from the rest of the trees. Results of the phylogeographic model are viewed using the Google earth software as an animation retracing events through time. Snapshots of the animation were used to create figures showing important details of the evolution of *S. lagocephalus*.

RESULTS

Sicyopterus lagocephalus in the Indo-Pacific area

Of the 684 base pairs of the cyt*b* region sequenced for the 332 *S. lagocephalus* specimens (GenBank *S. lagocephalus* sequences JX029412–JX029743), 185 (27%) base pairs were variable. Overall haplotype diversity and mean number of pairwise differences were high ($h = 0.99$, $\pi = 4.82$). Their values for the different localities were very similar, except for the Indian Ocean localities ($h = 0.44$, $\pi = 0.94$ for Reunion Island and $h = 0.75$, $\pi = 3.55$ for Comoros), which displayed the lowest values (Table 1).

The median haplotype network suggested the occurrence of 3 main genetic entities: (1) Western Pacific (WP, which includes Okinawa, Guam, Fiji, Samoa, Vanuatu and New Caledonia), (2) Tahiti and (3) Indian Ocean (IO, represented by Comoros and Reunion) (Fig. 2). Results suggested by this haplotype network were supported by fixation indices between sampling locations. Indeed, significant ϕ_{ST} values ranged from 0.3306 to 0.3762 between all WP localities and Tahiti, from 0.2870 to 0.6095 between all WP localities and IO localities, and from 0.4705 to 0.6913 between IO localities and Tahiti (Table 2).

By contrast, the haplotype network did not show any genetic structure within WP (Fig. 2). ϕ_{ST} values were usually not significant, although there was a very low but significant structure between Samoa and 3 locations in the WP: New Caledonia (0.0160), Vanuatu (0.0259) and Okinawa (0.0365) and also between Okinawa and New Caledonia (0.0168).

Within the IO, the ϕ_{ST} value between Comoros and Reunion Island was significant but relatively low (0.0906), and most of the specimens from Reunion and Comoros share the same haplotype, with the lowest haplotype diversity in the sampling (Table 2). Overall ϕ_{ST} for *Sicyopterus lagocephalus* was 0.29 (p < 0.001). The correlation coefficient (r = 0.69) between genetic and geographic distances was significant (p = 0.009) across the whole sampling area, but drastically dropped (0.28) and became insignificant (p = 0.24) after removing the 2 IO localities.

Fu's F_s values were negative for all localities where *Sicyopterus lagocephalus* was sampled. All values were significant, indicating departures from mutation-drift equilibrium, except for Samoa and Comoros (Table 1).

Higher haplotype diversities and lack of distinct clade structure in the WP compared to the IO suggest the WP as the origin for this species. Results obtained from the Bayesian reconstruction of the coalescent tree were consistent with previous analyses (Fig. 3). Indeed, haplotypes within the WP were mixed while the genetic structure evidenced between WP, IO and Tahiti was recovered. The root state posterior probability plot (Fig. 3) shows that the Bayesian results also suggest that the most recent common ancestor of *Sicyopterus lagocephalus* appeared in the WP although the current dataset precluded determining from which locality of the WP (Reunion: 1.5%; Comoros: 3.9%; Tahiti: 7.3%; Samoa: 13.8%; Guam: 13.6%; Okinawa: 15.1%; Fiji: 14.7%; Vanuatu: 14.5%; New Caledonia: 15.6%). Two main clades, strongly supported by posterior probability values, including only specimens from Tahiti or IO suggest that these 2 localities were first colonised in approximately the same period (IO: 240 000 yr, 95% highest

Table 2. *Sicyopterus lagocephalus* in the Indo-Pacific. Pairwise ϕ_{ST} for cytochrome *b* data. Significant values in **bold**

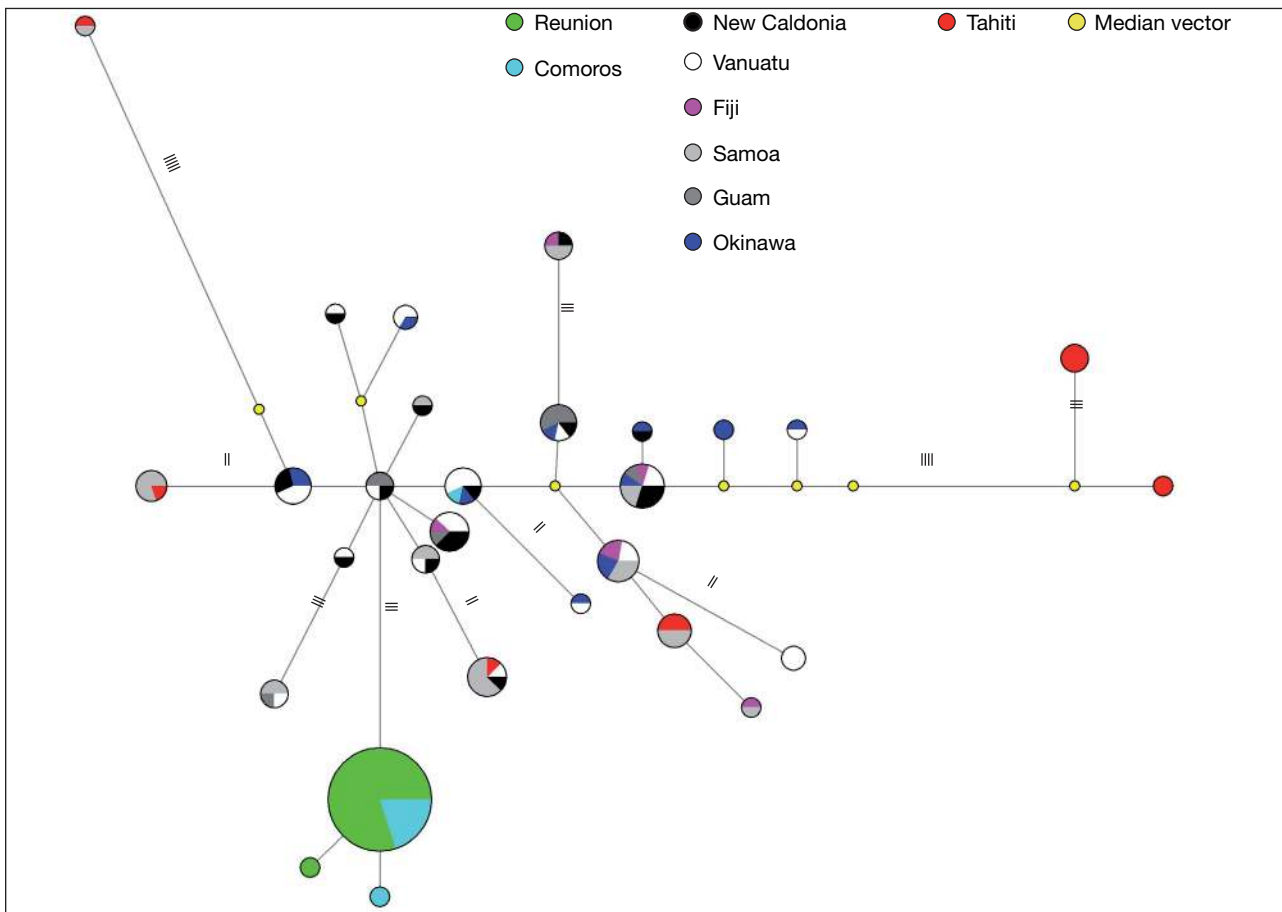|	Reunion	Comoros	Tahiti	Fiji	Guam	Okinawa	Samoa	New Caledonia
Comoros	**0.0906**							
Tahiti	**0.6913**	**0.4705**						
Fiji	**0.5956**	**0.2942**	**0.3306**					
Guam	**0.6095**	**0.3064**	**0.3570**	−0.0088				
Okinawa	**0.5860**	**0.3089**	**0.3319**	−0.0071	0.0123			
Samoa	**0.5700**	**0.3035**	**0.3489**	0.0110	0.0151	**0.0365**		
New Caledonia	**0.5061**	**0.2870**	**0.3718**	0.0032	0.0002	**0.0168**	**0.0160**	
Vanuatu	**0.5358**	**0.3106**	**0.3762**	−0.0049	−0.0094	0.0041	**0.0259**	0.0053

Fig. 2. *Sicyopterus lagocephalus.* Haplotype median joining network for *S. lagocephalus* in the Indo-Pacific. Number of mutations is shown on each branch. Colours correspond to each location and are given above the network

posterior density [HPD: 100 000, 410 000]; Tahiti: 260 000 yr, 95% HPD [120 000, 430 000]; see Fig. 4 at 0.27 Myr). A few specimens from IO and Tahiti also appeared dispersed elsewhere in the tree (Fig. 3).

Figs. 4 & 5 show the phylogeographic analysis under a neutral coalescent model with discrete spatial diffusion (Lemey et al. 2009). The panels in Fig. 4 summarise the temporal dynamics of *Sicyopterus lagocephalus* (see the animation 'output_date.kml', at www.int-res.com/articles/suppl/m455p269_supp/), and the spread of this species over time is visualised in Google Earth (for methods see Lemey et al. 2009). The lines connecting different locations represent branches in the maximum clade credibility tree (Fig. 3) on which state exchanges occur, and circle areas reflect the number of branches maintaining a particular state at that time point. The results show that spatial expansion was initiated in the WP (Fig. 4 at 0.49 Myr) and the species has then expanded into the Eastern Pacific and into the IO. Fig. 5 is a summary of the expansion of *S.*

lagocephalus over time during the last million years; the lines represent the localities between which the movements most likely took place. In Fig. 5, results show that the IO was most likely colonised by individuals from the northwestern Pacific (here represented by samples from Okinawa), as the line linking both oceans is between Okinawa and the Comoros.

Endemics

Of the 707 base pairs sequenced for the 87 *Sicyopterus sarasini* specimens, 63 (9%) were variable. The 87 cyt*b* mitochondrial sequences contained 53 unique haplotypes (GenBank *S. aiensis* sequences JX029202–JX029411). Of the 656 base pairs sequenced for the 210 *S. aiensis* specimens, 105 (16%) were variable positions. The 210 cyt*b* mitochondrial sequences contained 125 unique haplotypes (GenBank *S. sarasini* sequences JX029744–JX029830).
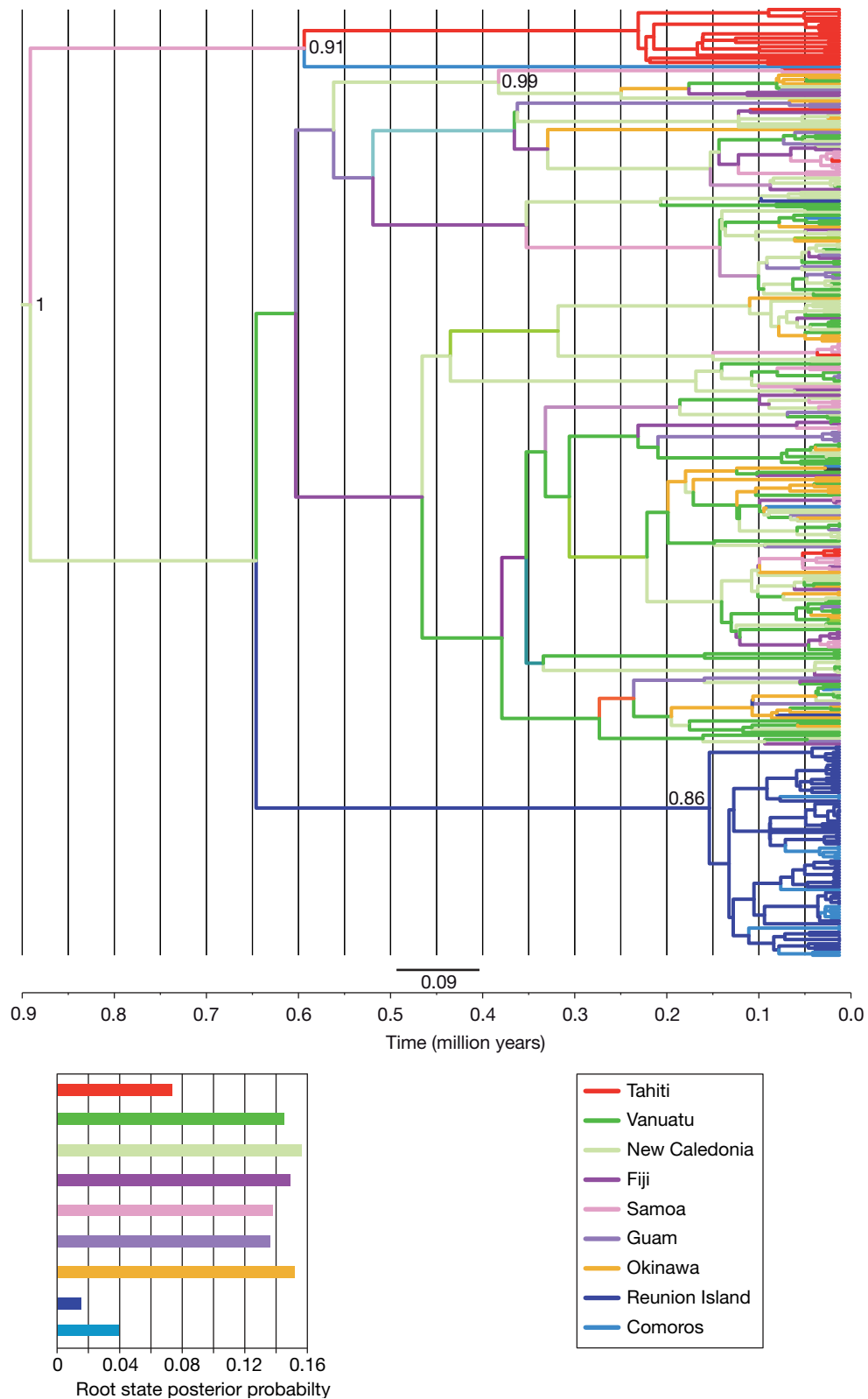
Fig. 3. *Sicyopterus lagocephalus.* Maximum clade credibility (MCC) phylogeny in the Indo-Pacific. Branches are coloured according to their most probable location state of their descendent nodes. The root state posterior probability for each location is given by the histogram. Posterior probability values higher than 0.80 are given on the tree
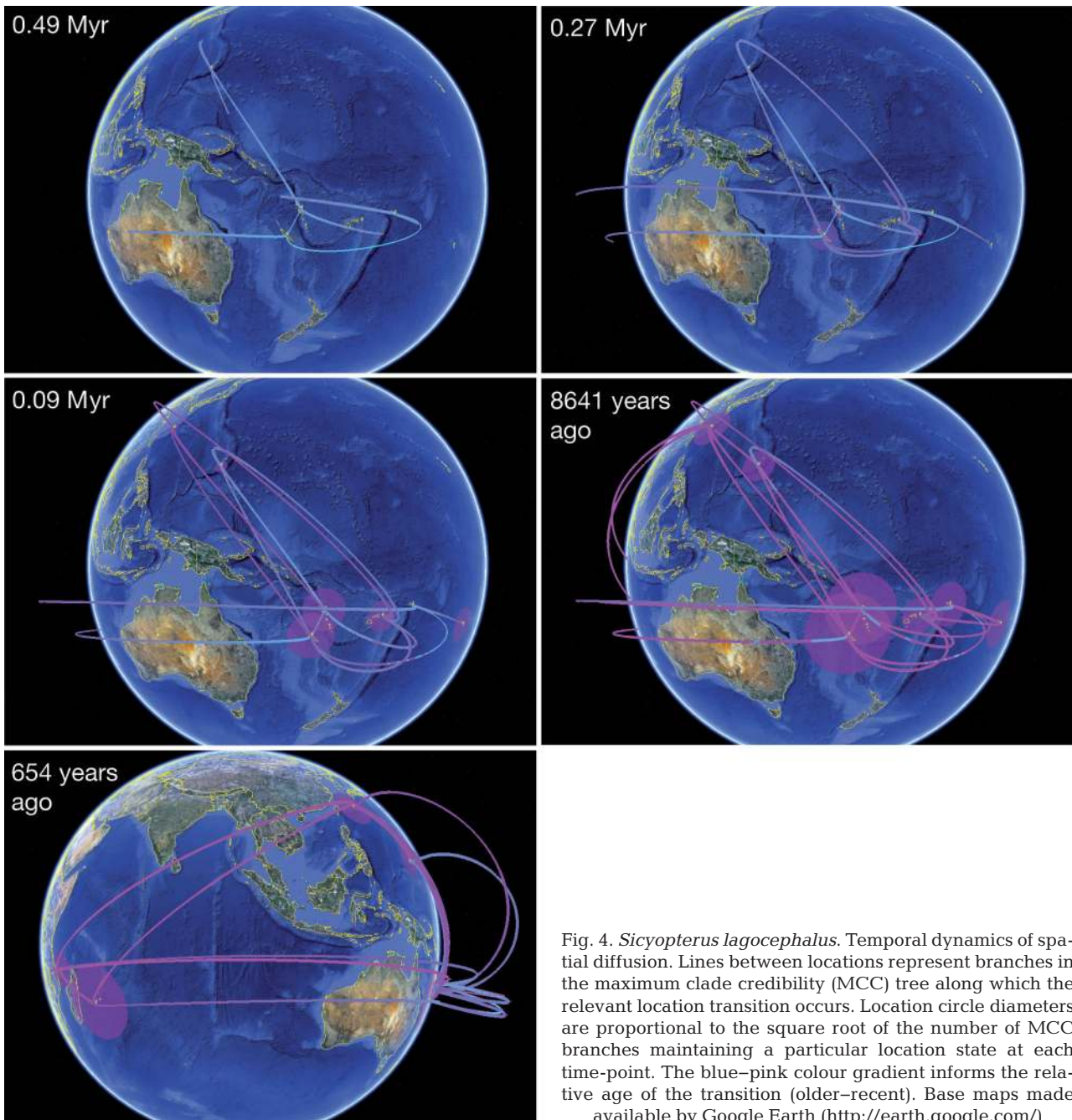
Fig. 4. *Sicyopterus lagocephalus*. Temporal dynamics of spatial diffusion. Lines between locations represent branches in the maximum clade credibility (MCC) tree along which the relevant location transition occurs. Location circle diameters are proportional to the square root of the number of MCC branches maintaining a particular location state at each time-point. The blue–pink colour gradient informs the relative age of the transition (older–recent). Base maps made available by Google Earth (http://earth.google.com/)

For both endemic species, overall haplotype diversity and mean number of pairwise differences were high (Table 1) across collection locations, and the values were similar between species. Fu's F_s values were also all negative and highly significant for all locations, indicating departure from mutation-drift equilibrium (Table 1). The median joining haplotype networks for both species show no evidence of regional structure, as specimens from different locations shared the same haplotypes (Fig. 6a,b). The most frequent haplotypes are shared by individuals sampled in all locations. This result is supported by the non-significance of pairwise ϕ_{ST} values calculated between the localities sampled in New Caledonia for *Sicyopterus sarasini* and in Vanuatu for *S. aiensis* (Tables 3 & 4). Overall ϕ_{ST} was 0.047 (p < 0.001) for *S. sarasini* and −0.003 (p < 0.001) for *S. aiensis*. In New Caledonia, no regional *S. sarasini* population structuring was observed; neither was there for *S. aiensis* in Vanuatu. These results for the 2 endemic species
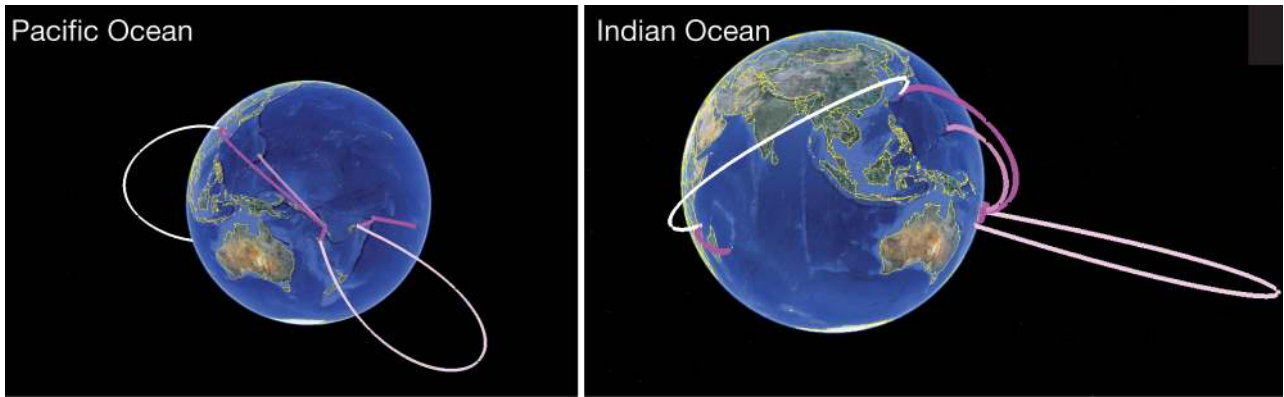
Fig. 5. *Sicyopterus lagocephalus*. Summary of exchanges among localities. Only rates supported by a Bayes Factor > 3 are represented. The colour and thickness of the lines represent the relative strength by which rates are supported; thin white lines and thick pink lines suggest relatively weak and strong support, respectively. Base maps made available by Google Earth (http://earth.google.com/)
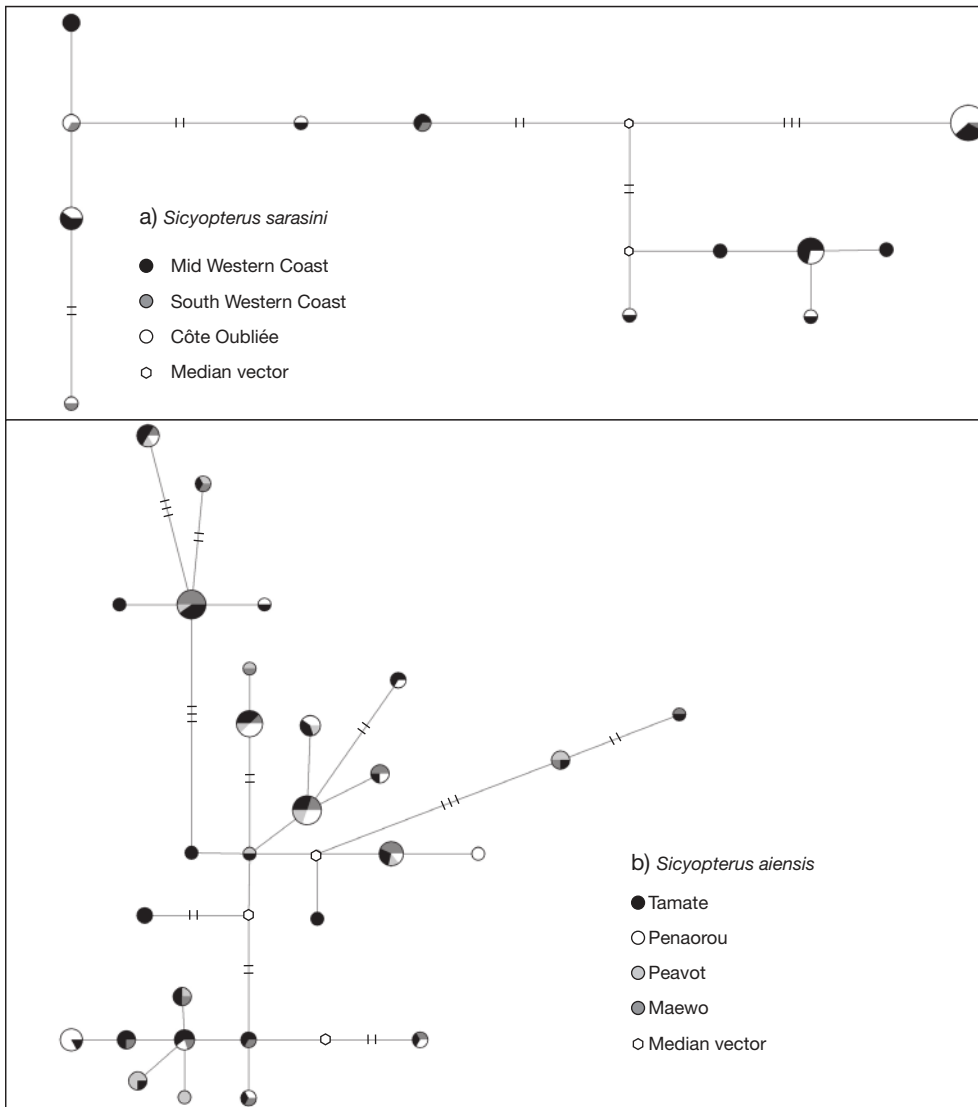

Fig. 6. *Sicyopterus sarasini* and *S. aiensis*. Haplotype median joining network for (a) *S. sarasini* and (b) *S. aiensis.* Number of mutations is shown on each branch. Shades correspond to each location and are given below the network

Table 3. *Sicyopterus lagocephalus* and *S. sarasini* in New Caledonia. Pairwise φ_{ST} values for cytochrome *b* data are below the diagonal for *S. lagocephalus* and above the diagonal for *S. sarasini*. There are no significant values

	North-eastern coast	Mid-western coast	South-western coast	Côte Oubliée
North-eastern coast	–			
Mid-western coast	−0.0041	–	−0.0148	0.0206
South-western coast	−0.0141	−0.0084	–	0.0105
Côte Oubliée	0.0147	−0.0138	0.0022	–

Table 4. *Sicyopterus lagocephalus* and *S. aiensis* in Vanuatu. Pairwise φ_{ST} values for cytochrome *b* data are below the diagonal for *S. lagocephalus* and above the diagonal for *S. aiensis*. Significant values in **bold**

	Tasmate	Penaorou	Peavot	Maewo	Malakula
Tasmate (West Santo)	–	−0.0004	0.0103	−0.0241	−0.0042
Penaorou (West Santo)	0.0436	–	0.0079	−0.0324	−0.0064
Peavot (East Santo)	0.0303	**0.0617**	–	−0.0227	0.0058
Maewo	0.0085	0.0615	0.0033	–	−0.0317
Malakula	0.0227	**0.0647**	−0.0215	−0.0106	–

were comparable to ϕ_{ST} results found for *S. lagocephalus* sampled in Vanuatu and New Caledonia (Tables 3 & 4).

DISCUSSION

Sicyopterus lagocephalus in the Indo-Pacific: role of biogeographical barriers

Our Bayesian reconstruction of *S. lagocephalus* phylogeny shows that the species first appeared and expanded in the WP and that it secondarily colonised the IO and Tahiti. This result is congruent with the findings of Keith et al. (2011) in which the authors placed the appearance of the *Sicyopterus* ancestor in the WP between 2.53 and 5.58 Myr. The WP has become an important centre of species diversification for many marine and terrestrial taxa since the late Cenozoic, because of the emergence of new islands and palaeo-oceanographic re-arrangements (Planes & Galzin 1997). Using information from a broad-scale survey of Indo-Pacific marine fish, Briggs (2005) provided strong support for the theory that the WP has been operating as a centre of origins. In our study, we showed that as for the genus *Sicyopterus*, the spatial expansion of *S. lagocephalus* initiated in the WP (during the last million years; Fig. 4), and the species then expanded in the Eastern Pacific and in the IO during separate colonisation events both around 0.25 Myr. Fig. 5 shows that colonisation of the IO was most

likely initiated by individuals from the north western Pacific (represented here by samples from Okinawa). However, in this study we lack samples from the Indo-Malay archipelago, so it is impossible to know whether colonisation of the IO came from the north-western Pacific or from populations occurring in this unsampled area. *S. lagocephalus* is the most recent species of the genus (Keith et al. 2005), and it has acquired specific life history traits such as its extensive PLD (Lord et al. 2010), allowing it to colonise the entire Indo-Pacific. Analysing samples of *S. lagocephalus* across the entire 18 000 km range showed that high genetic structure only appeared at a very large spatial scale (Lord 2009, this study). Indeed, only low genetic structure was recovered within the entire WP, whereas strong structure appeared between the WP and locations at the extremities of the species distribution area, i.e. the western IO and Polynesia. This result confirms what Dennenmoser et al. (2010) suggested in their study on amphidromous shrimps, that population structure is rare for these species or only expressed over large scales, and that if there is genetic population differentiation, it should be expected near the limits of the species' geographic distribution. This is the first time that such a structure has been revealed for an amphidromous goby on such a scale and with such a large number of samples, as all other population genetics studies that have focused on amphidromous species were done either at local island scales (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Watanabe et al.

2006), at the Pacific Ocean scale (Crandall et al. 2009) or with limited sampling (Hoareau et al. 2012). Strongly negative F_s values indicate non-equilibrium population dynamics that might result from recent population expansions during colonisation events. However, the alternative hypothesis of selective events cannot be rejected (Slatkin & Hudson 1991, Fu 1997, Crandall et al. 2009).

Results for *Sicyopterus lagocephalus* suggest high levels of gene flow across the entire WP, a vast geographic area including the Japanese islands, the Marianna archipelago, Vanuatu, New Caledonia, Fiji and Samoa. However, we observed low but significant structure between Samoa and all other WP locations, except Guam and Fiji, and between New Caledonia and Okinawa. The hypothesis of periodic isolation by oceanic currents cannot be ruled out, but the fact that some locations do not show a genetic structure with all the localities in the WP suggests mechanisms of extinction and recolonisation. These processes dominate in the biogeographical history of stream fauna and particularly for amphidromous species, because of the high instability of habitats and dispersal specificities, and promote non-equilibrium dynamics in population structure (Covich 2006, Cook et al. 2009), therefore creating small-scale patchiness in genetic composition in the WP.

In organisms displaying such highly dispersive larvae, spatial genetic structure is expected either at scales exceeding dispersal abilities, according to an isolation-by-distance model (Planes & Fauvelot 2002), or on both sides of biogeographical barriers to dispersal. And indeed, there was a significant correlation between genetic and geographic distance across the whole sampling area. However, this correlation disappeared when only considering Pacific samples in the analysis, and the genetic homogeneity among localities from the WP, which can be very distant from one another (e.g. Fiji/Guam), presents a striking contrast with the sharp genetic break between Tahiti and close localities from the south WP, such as Samoa or Fiji. These results therefore clearly support the hypothesis that the genetic structure in *Sicyopterus lagocephalus* is explained by factors other than geographic distances. The relative isolation of Tahiti for this species, with respect to the rest of the Pacific distribution area, may be due to a combination of geographical isolation, biogeographical barrier to dispersal or of ecological distinctiveness of the zone. The geomorphology of the Polynesian system is unique as it features very isolated islands without intervening continental plates (Planes 1993). The combination of currents between the South Equator-

ial Current, the South Equatorial Counter Current and the Marquesas Counter Current can explain the population structure observed between Tahiti and the rest of the Pacific. The currents prevailing in this region play an important role in structuring populations of many marine organisms (Planes 1993, Gaither et al. 2010). Planes (1993) found genetic structure within French Polynesia for the widespread convict surgeonfish *Acanthurus triostegus*, and authors usually also observe the isolation of the Marquesas Islands (Planes & Fauvelot 2002, Gaither et al. 2010) as well as specificities in the population structure in Polynesia (Planes 1993).

Crandall et al. (2009) undertook the only other large-scale population genetic study on 2 species of amphidromous gastropods (*Neritina canalis* and *Neripteron dilatatus*) distributed from the Philippines to Eastern Pacific locations. Even if both species show no evidence of genetic structure within WP archipelagos (Vanuatu, Samoa and Fiji), which promotes long-distance dispersal abilities, the authors found a strong genetic structure between WP locations and Polynesia in the amphidromous gastropod *N. canalis*. Westerly currents prevail in this area but the authors uncovered an eastward colonisation event 0.6 Myr of Polynesia from the WP, thanks to periodic oceanic current reversals, allowing occasional eastward colonisation (Lessios & Robertson 2006). Polynesia was then isolated after colonisation, leading to the genetic structure observed for this species. The same mechanism most likely occurred for *Sicyopterus lagocephalus*, with a colonisation event of French Polynesia ca. 0.27 Myr, followed by its isolation.

According to our results, colonisation of the IO occurred around 0.24 Myr, most likely from the north-western Pacific. With its long PLD, averaging 130 d (Lord et al. 2010), *Sicyopterus lagocephalus* was able to colonise the IO using the equatorial current with a stepwise migration, as the species is present in Sri Lanka (Watson et al. 2000, Keith et al. 2011). The Indo-Malayan islands, called the IPB, constitute a strong barrier to dispersal, which likely explains our results as already suggested for reef fish (Lacson & Clark 1995, McMillan & Palumbi 1997, Planes & Fauvelot 2002, Bay et al. 2004, Winters et al. 2010), echinoderms (Williams & Benzie 1998, Lessios et al. 2001), crustaceans (Lavery et al. 1995, 1996, Duda & Palumbi 1999) and also other diadromous species such as *Anguilla marmorata*, a catadromous species therefore displaying a marine larval stage (Minegishi et al. 2008, Watanabe et al. 2008). As many eustatic changes occurred over evolutionary time scales, for instance during the Plio-Pleistocene

(Hantoro 1997, Voris 2000), this barrier has been very intermittent. At least 3 lowering of sea level events (as much as 120 m below present) occurred during the Pleistocene (1.8 Myr to 11 000 yr ago; Voris 2000, Gaither et al. 2010). This geologically intermittent barrier has influenced the biogeography of many marine species (Barber et al. 2002). Moreover, Plio-Pleistocene glacial periods resulted in extended periods of decreased rainfall on Pacific islands, maybe drying out streams in these areas (Crandall et al. 2009), creating a physical barrier much less penetrable by larvae, and altering the stepwise progression of larvae from island to island from the Pacific to the IO. The IO *S. lagocephalus* population was probably isolated during this period, leading to the structure evidenced in this study.

Concerning the IO populations, previous studies based on allozyme polymorphism have shown high gene flow and genetic homogeneity for *Sicyopterus lagocephalus* within the Mascarene Islands (Berrebi et al. 2005). However, their results suggested a composite structure resulting from some allochthonous contribution (Berrebi et al. 2005, Hoareau et al. 2007) but they were unable to conclude where the allo-recruitment came from, although other areas in the IO or locations in the Indonesian region, which is at the junction of Indian and Pacific Oceans, can be inferred. The results in the present study suggest that the western IO is isolated and that larvae may come from the WP and west Indonesian region, but very sporadically. It is probable that larvae cannot currently disperse from the WP to the IO and that exchanges between the 2 oceanic basins are ancient due to sea level fluctuations. Furthermore, the haplotype diversity was much lower in the IO than in the Pacific ($h = 0.44$ to 0.75 compared to 0.99). This difference may be due to smaller effective population size in the IO, reflecting the current isolation of this ocean basin from the rest of the distribution area. The low but significant population structure observed between Comoros and Reunion is most likely due to the presence of *S. lagocephalus* in unprospected localities in the eastern IO. The haplotypes shared between the 2 oceanic basins may either reflect the low level of current gene flow or only ancestral polymorphism, or both. In the western IO, stream restocking is due mainly to self-recruitment at the scale of the Mascarenes and the Comoros, with very little to no allo-recruitment from the Pacific. This result has implications in terms of the conservation of *S. lagocephalus* in the IO, where recruiting post-larvae are massively exploited and where stocks are known to be declining (Delacroix 1987).

Pelagic larval duration, gene flow and endemism

The 2 endemic species studied, namely *Sicyopterus sarasini* and *S. aiensis*, did not display any spatial genetic structure across their distribution areas, respectively New Caledonia and Vanuatu. Both species also displayed departures from neutrality (strongly negative F_s), which might be a sign of population expansion. For all 3 species studied in this work, the genetic diversity estimated from overall haplotype diversity was very high. This result is in accordance with previous studies on amphidromous gobies. Watanabe et al. (2006) found 74 haplotypes for 77 *S. japonicus* specimens, and such diversity has also been observed in other amphidromous species endemic to Hawaii (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998) or from Chile (Dennenmoser et al. 2010).

Sicyopterus endemics, which are older than the widespread *S. lagocephalus* (Keith et al. 2005), have shorter PLDs. Indeed, *S. sarasini* and *S. aiensis* both have a PLD of about 80 d versus 130 to 260 d for *S. lagocephalus* (Lord et al. 2010), and this could explain their restricted distribution area. Dispersal plays a fundamental role in the structuring of populations (Levin 2006). PLD is not necessarily a good proxy of the dispersal abilities of species (Victor & Wellington 2000, Bowen et al. 2006, Weersing & Toonen 2009), but a positive relationship between larval duration and gene flow has been found in some taxa (Doherty et al. 1995, Riginos & Victor 2001) and this also seems to be the case for Sicydiinae. For all endemic Sicydiinae species, the mean PLD is usually around 70 to 80 d (Radtke et al. 1988, 2001, Yamasaki et al. 2007, Shen & Tzeng 2008). These values represent enough time to allow dispersal over very large spatial scales, and explain the genetic homogeneity over the entire distribution range of endemic species (McDowall 2007, Crandall et al. 2009). A lack of genetic structure over large spatial scales is indeed reported for *S. japonicus*, distributed from Taiwan to the central eastern coast of Japan (Watanabe et al. 2006) and in 5 other gobiids, namely *Awaous guamensis*, *Stenogobius hawaiiensis*, *Lentipes concolor*, *Sicyopterus stimpsoni* and *Eleotris sandwicensis*, distributed in the Hawaiian Islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998). Relatively long PLDs are usually reported for all amphidromous organisms. The PLD of laboratory-cultured amphidromous gastropods has been estimated at 40 to 98 d (Kano 2006); the PLD is up to 220 d for laboratory-reared

Palaemonidae decapod crustaceans (Dennenmoser et al. 2010); and otolith analysis of Galaxiidae revealed a 93 to 202 d PLD for these fishes (McDowall et al. 1994). The PLD of *S. lagocephalus* is both very long and plastic. It allows this species to delay metamorphosis until it finds a suitable environment to colonise (Victor 1986, Keith et al. 2008, Valade et al. 2010, Taillebois et al. 2011), and partially explains why it is so broadly distributed. The lack of population structure for island endemic species confirms that dispersal occurs readily on the scale of 100 to 500 km as is predicted by the PLD and confirms the high dispersive potential of larvae, which translates to an adaptation to the colonisation of insular freshwater habitats. But the distribution of endemic species also reflects local selective constraints. Indeed, *S. sarasini* is found only in the south of New Caledonia, and more specifically in rivers on ultramafic substrate (i.e. nickel-rich substrate; Marquet et al. 2003, Lord & Keith 2008). Other endemic Sicydiinae species respond to environmental cues, like *S. japonicus* which only occurs in temperate areas of Japan and Taiwan, its life cycle following seasonal variations (Iida et al. 2009). It is well known that larval behaviour, such as swimming, orientation and sensory abilities, influences larval dispersal trajectories and the colonisation of remote islands (Leis 2007, Murphy & Cowan 2007). Newly hatched Sicydiinae larvae are capable of an active salinity choice: Iida et al. (2010) showed that *S. japonicus* larvae, during downstream migration, have a preference for low salinity haloclines as they provide them with superior swimming endurance. Competent recruiting post-larvae might also be able to choose the fresh water coming from a specific substrate, like salmonids do (Dittman & Quinn 1996). It is likely that *S. sarasini* individuals preferably choose a specific substrate (probably nickel rich) to settle (Lord et al. 2010). Therefore, larvae of endemic species may have a specific behaviour that prevents them from going far at sea, enabling them rather to stay close to the environment to which they are adapted (Murphy & Cowan 2007).

The fact that endemic *Sicyopterus* species respond to environmental specificities is probably more of a key element explaining their distribution than their dispersal capacities, which remain high for amphidromous species in terms of their adaptation to colonisation of insular freshwater systems. By contrast, PLD plasticity, a trait that probably appeared in the most recently diverged species *S. lagocephalus*, allowed it to colonise the entire Indo-Pacific.

Our study provides new and original insight into the genetic connectivity of a widespread species, demonstrating the evidence of past gene flow resulting in the colonisation of the IO and Tahiti from the WP. The genetic structure obtained may be due to the existence of barriers to larval dispersal for this species, barriers that are often recovered for reef organisms. It would be of great interest to include specimens in the west Indonesian and Indian regions in the study, as they represent a transitional zone between the Indian and Pacific Oceans and as they would help clarify the role of the IPB in the structure observed.

Analysis of the 2 endemic species improved our understanding of the complicated link between the evolution of and the distribution of amphidromous species. Low genetic structure and long pelagic larval duration reflect a high dispersal potential for these freshwater species. The marine larval dispersal phase is an adaptation to life in exceedingly unstable freshwater insular systems. Larval retention in freshwater insular species may lead to population extinction (McDowall 2007), whereas long-distance dispersal ensures that suitable habitats are progressively colonised, balancing possible local extinctions. The fact that there are endemic species is certainly the result of a complex combination of historical, biogeographical, environmental and physiological factors.

Our results will help implement conservation and management measures to protect the endemic species and to develop sustainable fisheries (Keith & Marion 2002). Indeed, this study is to be placed in a historical context at a large spatial scale and forms the necessary basis for more precise studies at local scales for conservation purposes. The lack of population structure in island endemics shows that stream fauna population recovery processes probably occur at least at the island scale (Cook et al. 2009). The marine component of larval development and the high dispersal potential of the amphidromous species allow natural restocking to take place. Our molecular studies indicate, as has been suggested before (Covich 2006, McDowall 2007), that amphidromous species will most likely recolonise impacted stream habitats if these are restored (in the context of anthropogenic stream modifications) and if the longitudinal hydrologic connectivity is maintained (Cook et al. 2009). Healthy populations of amphidromous species should be protected as they represent spawners emitting larvae at sea; larvae that are then capable of colonising any suitable habitat far from their emission location.

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