

POPULATION STRUCTURE OF THE ASIAN AMPHIDROMOUS SICYDIINAE GOBY, *STIPHODON PERCNOPTERYGIONUS*, INFERRED FROM MITOCHONDRIAL COI SEQUENCES, WITH COMMENTS ON LARVAL DISPERSAL IN THE NORTHWEST PACIFIC OCEAN

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AMPHIDROMY
SICYDIINAE
STIPHODON
LARVAL DISPERSAL
POPULATION STRUCTURE

ABSTRACT. – *Stiphodon percnopterygionus* (Gobiidae: Sicydiinae) is an amphidromous goby distributed mainly in the northwest Pacific Ocean, from Taiwan to the south of Japan. This study evaluated the population genetic structure of *S. percnopterygionus* using partial cytochrome oxidase I (COI). A total of 496 base pairs were sequenced for 88 individuals from six localities. Twenty-eight haplotypes were recovered. Diversity indices were $h = 0.709 \pm 0.003$ and $\pi = 0.05 \pm 0.035$ and are what is currently found for Sicydiinae gobies. All results lead to the conclusion that *S. percnopterygionus* population is expanding. Indeed, the haplotype network displayed a star-like pattern and the mismatch distribution analysis results indicate a recent demographic expansion. AMOVA results and the haplotype network indicate the absence of population structure within the distribution area sampled. The lack of population structure is probably due to the amphidromous life cycle. *S. percnopterygionus* larvae have a long marine dispersal phase (99 ± 16 days), allowing them to disperse far from their emission location, especially in the northwest Pacific Ocean, as larvae are probably transported by the Kuroshio Current.

INTRODUCTION

Tropical island streams are colonised by amphidromous gobies belonging to the Sicydiinae sub-family and they are the major contributors to fish biodiversity. There are eight known genera: *Akihito* Watson, Keith & Marquet, 2007; *Lentipes* Günther, 1861; *Cotylopus* Guichenot, 1864; *Sicyopterus* Gill, 1860; *Sicyopus* Gill, 1863; *Smilosicyopus* Gill, 1863; *Stiphodon* Weber, 1895 and *Sicydium* Valenciennes, 1837, totalling nearly 110 species distributed in the Indo-Pacific, West Africa, Central American and Caribbean areas (Keith & Lord 2011a, Taillebois *et al.* 2014). These gobies are especially adapted to life on small volcanic islands; they colonise steep rapid streams in altitude by clinging to rock surfaces and climbing over waterfalls using their pelvic sucker (modified pelvic fins), pectoral fins and mouth (Schoenfuss & Blob 2003, Keith & Lord 2011b). *Sicyopterus* and *Stiphodon* are the most diverse genera with about 25-30 valid species each. Most *Stiphodon* species are in the western and southern Pacific Ocean and also colonise the Indo-Pacific barrier islands, with possibly a species in Sri Lanka (Watson *et al.* 2000, Keith & Lord 2011a). Most Sicydiinae species

are endemic to archipelagos or even one island (Radtko & Kinzie 1996, Lord *et al.* 2012). Only a few species are able to colonise entire oceans or even both the Indian and Pacific oceans like *Sicyopterus lagocephalus* (Lord *et al.* 2012).

Sicydiinae life cycle is adapted to the distinctive conditions in these habitats, *i.e.* subjected to important hydrological and seasonal variations and poor in nutrients. The adults live and reproduce in freshwater. As soon as they hatch, larvae drift downstream to the ocean where they undergo larval development while having a planktopelagic lifestyle (Ellien *et al.* 2011, Keith & Lord 2011b, Iida *et al.* 2013). After a usually long marine phase, post-larvae return to rivers, and as soon as they enter freshwater, they undergo an important metamorphosis (Keith *et al.* 2008, Taillebois *et al.* 2011) allowing them to migrate upstream and colonise the adult habitat. It has been suggested that the marine stage is likely to be a key element to the dispersal and to the distribution of these species (McDowall 2003, 2007, Keith *et al.* 2008, Keith & Lord 2011b, Lord *et al.* 2012). Freshwater species with a diadromous life cycle and marine species with a pelagic larval stage exhibit a higher dispersal ability and lower interpopula-

tion structure than strictly freshwater species or marine species lacking a pelagic larval stage (Allibone & Wallis 1993, Doherty *et al.* 1995). The pelagic larval duration (PLD) can thus be considered in many cases as a proxy to the dispersal ability of larvae, which should be closely linked to the population structure of these species (Victor & Wellington 2000, Bernardi *et al.* 2003, Lord *et al.* 2012). There is an increasing effort made towards the understanding of the biology and ecology of Sicydiinae gobies as they show interesting features: they show high levels of endemism (Lord *et al.* 2012) with high species richness (Keith *et al.* 2011).

A few recent studies have worked on identifying the pelagic larval duration of Sicydiinae species and in some cases the PLD results have been coupled to population structure analyses. If we look at all the PLD studies, we can conclude that the PLD of amphidromous Sicydiinae roughly ranges from 60 to 160 days (Radtke *et al.* 2001, Yamasaki *et al.* 2007, Shen & Tzeng 2008, Lord *et al.* 2010, Taillebois *et al.* 2012), meaning that even for species restricted to one island the PLD is long, greater than that generally found for reef fish (maximum PLD of about 30 days) (Victor & Wellington 2000). The genetic studies done on Sicydiinae showed that species endemic to a small geographic region show no population structure, as expected considering the long PLDs (Fitzimmons *et al.* 1990, Zink *et al.* 1996, Watanabe *et al.* 2006, Lord *et al.* 2012, Taillebois *et al.* 2013). But for more broadly distributed species, barriers to dispersal have been uncovered, revealed by strong population structure on either side of the Indo-Pacific Barrier (Lord *et al.* 2012) and on either side of the Torres Strait (Taillebois *et al.* 2013).

Despite the fact that *Stiphodon* species are the most abundant, life history studies have only been published for two of them: the PLD is known for *Stiphodon percnopterygionus* (Yamasaki *et al.* 2007) and the genetic structure and demographic history has been studied for *Stiphodon caeruleus*, endemic to Micronesia (Chabarría *et al.* 2014). This paper will focus on the Asian species, *Stiphodon percnopterygionus* Watson & Chen, 1998, otherwise known as the stream jewel goby. *Stiphodon percnopterygionus* is widely distributed in the northwestern Pacific. It is known from Japanese main islands (Honshu, Sikoku, and

Kyushu), the Ryukyu Archipelago, Ogasawara Islands, eastern and southern Taiwan, Lanyu Island, China, Guam and Palau (Watson & Chen 1998, Sasaki *et al.* 2001, Nip 2010, Nakabo 2013). It is abundant in the Ryukyu Archipelago and Taiwan, but very rare in other regions.

The aim of this paper is to assess a preliminary population structure of *Stiphodon percnopterygionus*, in the Asian part of its distribution using the partial mitochondrial marker, cytochrome oxidase 1 (COI) and thus to infer larval dispersal, especially in the zone of the Kuroshio current, which could potentially act as a barrier to dispersal between the western and eastern parts of the distribution.

MATERIALS & METHODS

Sampling and molecular analyses: A total of 88 specimens were collected in the Ryukyu Islands (Amami, Ishigaki, Iriomote and Okinawa), on Ogasawara Island and in Taiwan (Table I, Fig. 1).

The specimens were preserved in 95 % ethanol. Total DNA was extracted from a fin clip using the DNeasy Tissue Kit (Qiagen ©) following the manufacturer's instructions. A fragment of the Cytochrome oxidase I (COI) mitochondrial gene was amplified using primers FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward *et al.* 2005).

All PCRs were performed on Biometra thermocyclers in a 25 μ l volume of 5 % of DMSO, 5 μ g of bovine serum albumin, 300 μ M of each dNTP, 0.3 μ M of Taq DNA polymerase from Qiagen, 2.5 μ l of the corresponding buffer, and 1.7 pM of each of the two primers. After a 2-minute denaturation at 94°C, the PCR ran 50 cycles of 25 seconds at 94 °C, 25 seconds at 52 °C and 1 minute at 72 °C, with a 3-minute terminal elongation.

The PCR products were used as the template DNA for cycle sequencing reactions performed using Dye Terminator Cycle Sequencing FS Ready Reaction Kits (Applied Biosystems) and were run on an ABI 377 DNA Sequencer (Applied Biosystems). The forward primer FishF1 and the reverse primer FishR1 were used for the direct cycle sequencing reaction. All sequences were obtained in both directions and checked manually against

Table I. – Diversity indices for each sampling location and for all localities for *Stiphodon percnopterygionus*: number of samples (n); number of haplotypes (Nh); haplotype diversity (*h*); nucleotide diversity (π); segregating sites (S); number of pairwise differences (k).

Locality	n	Nh	<i>h</i>	π	S	k
Amami	8	3	0.464 \pm 0.200	0.0015 \pm 0.0014	3	0.750 \pm 0.614
Iriomote	8	4	0.643 \pm 0.184	0.0015 \pm 0.0014	3	0.750 \pm 0.614
Ishigaki	16	8	0.700 \pm 0.127	0.0027 \pm 0.0020	10	1.358 \pm 0.885
Ogasawara	7	4	0.714 \pm 0.181	0.0031 \pm 0.0024	4	1.524 \pm 1.033
Okinawa	28	10	0.696 \pm 0.088	0.0023 \pm 0.0017	11	1.145 \pm 0.088
Taiwan	21	12	0.860 \pm 0.071	0.0036 \pm 0.0024	10	1.771 \pm 1.068
Total	88	28	0.709 \pm 0.054	0.05 \pm 0.035	26	1.306 \pm 0.824

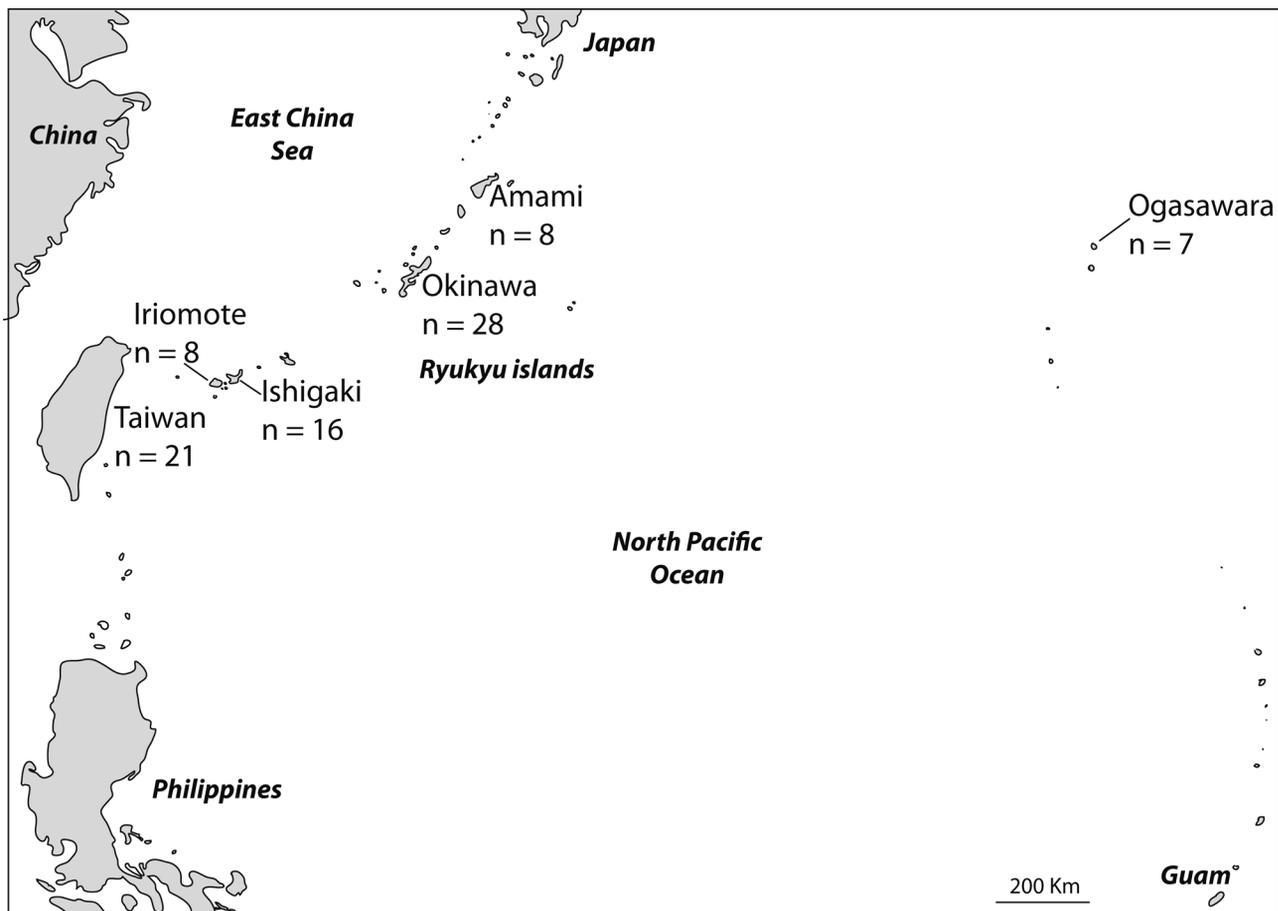


Fig. 1. – Sampling locations and number of specimens collected at each location.

their chromatogram using Sequencher (Gene Codes Corporation). They were aligned by hand using Bioedit (Hall 1999) with the criteria listed by Barriol (1994).

Molecular diversity: For each location, diversity indices such as the number of haplotypes, the number of variable positions, the haplotype diversity (h) and the nucleotide diversity (π) were estimated with Arlequin 3 software (Excoffier *et al.* 2005). The relationship among all observed haplotypes was inferred by median joining haplotype networks, drawn from the haplotype data using Network v.4.5.02 (Bandlet *et al.* 1999; fluxus-engineering.com). We used default parameters implemented in Network.

Levels of genetic structure between different locations were estimated by F_{ST} statistics (Weir & Cokerham 1984), using the estimator implemented in Arlequin; it integrates both the haplotype frequencies and the pairwise nucleotide differences. Distributions of the F_{ST} statistic under the null hypothesis of genetic homogeneity through localities were obtained from 10,000 random permutations of specimens among localities and used to test the significance of values calculated from real data.

Population demographics: Neutrality (equilibrium) was assessed by calculating Fu's F_s (Fu & Li 1993) as Fu's F_s is more robust regarding small samples. Fu's F_s gives an estimate

of the population demographics; a negative value indicates an overabundance of low frequency haplotypes, a possible signature of recent demographic expansion or of directional selection. Significance was tested using 10,000 coalescent simulated from the observed number of haplotypes in Arlequin. The distribution of the number of pairwise differences between haplotypes based on computed inter-haplotype distances (mismatch distributions) were also calculated (Avise 2004).

Mismatch distribution was calculated under Arlequin which provides an estimate of the mutational parameters ($\theta = 2\mu N$) θ_0 , θ_i and Thau (τ) which are values that respectively represent the effective population size at the time of the most common ancestor, the current effective population size and the time needed to go from θ_0 to θ_i (age of expansion) (Rogers & Harpending 1992, Schneider & Excoffier 1999). This distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but is usually unimodal in populations having passed through recent demographic expansion (Slatkin & Hudson 1991, Rogers & Harpending 1992). The validity of this stepwise expansion model is tested using a parametric bootstrap approach. 1000 simulations (number of random samples) under a coalescent algorithm modified from Hudson (1990) were done using the calculated parameters, to define the parameters' 99 % confidence intervals and the sum of squared derivations of these parameters, SSD_{obs} . The same calculation is done on simulated

parameters, SSD_{sim} . The p-value of the test is then approximated by:

$p\text{-value} = SSD_{sim} \text{ larger or equal to } SSD_{obs} / \text{Number of random samples}$

Non-significant values for SSD mean that the observed data do not deviate from that expected under an expansion model. The raggedness index is also calculated (Harpending 1994), similarly to the SSD. The raggedness index takes larger values for multimodal distributions found in a stationary population than for unimodal and smoother distributions typical of expanding populations. Non-significant raggedness index also indicate population expansion.

Observed mismatch distributions were plotted along with two demographic models, one being at the mutation-drift equilibrium (constant population size) using the nucleotidic diver-

sity as an estimation of θ (Rozas & Rozas 1995) and the other under a demographic expansion model in DnaSP v.5 (Librado *et al.* 2009).

RESULTS

Genetic diversity and population structure

A total of 496 base pairs of the COI mitochondrial gene were sequenced for the 88 individuals of *S. percnopetrygionus*. There were a total of 26 variable sites (5.24 % of 496 bp). According to these variable sites, a total of 28 haplotypes were found for these 88 individuals. Both the overall haplotype diversity and the nucleotide diversity

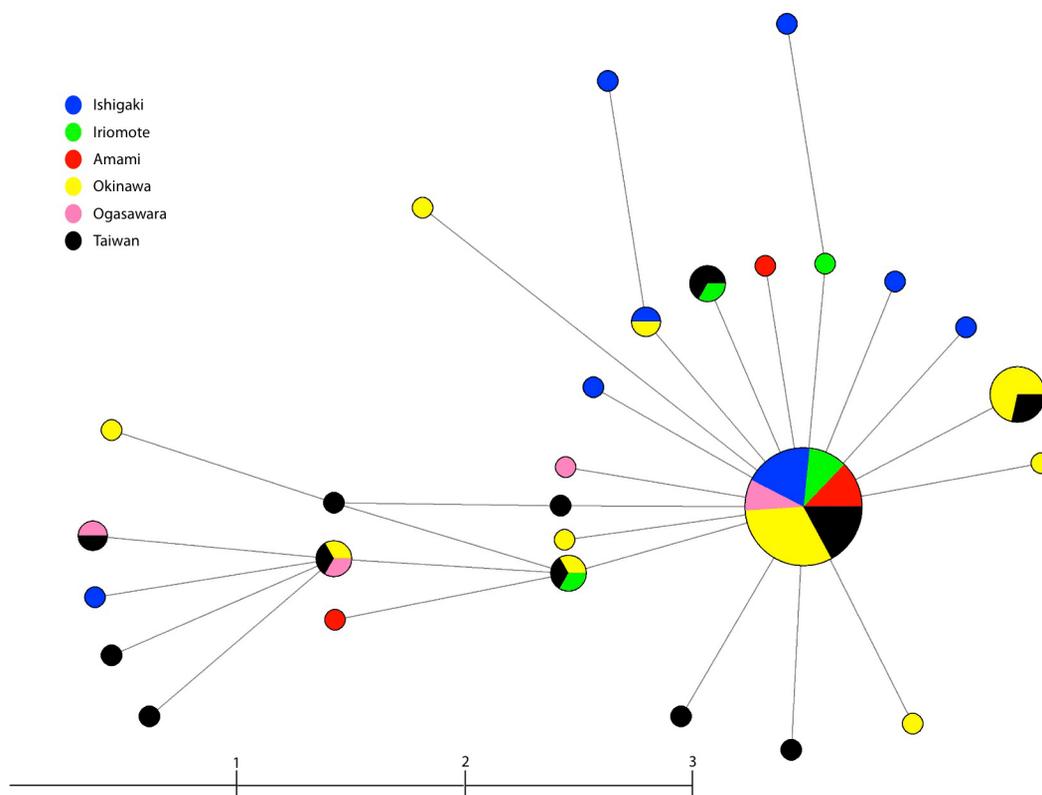


Fig. 2. – Median joining network. Each circle represents a haplotype; circle size indicates the proportion of individuals. The scale bar gives the number of mutational steps. Each color represents a locality.

Table II. – AMOVA results for *Stiphodon percnopetrygionus*. F_{ST} values are under diagonal, p-values are above diagonal.

FST						
	Amami	Iriomote	Ishigaki	Ogasawara	Okinawa	Taiwan
Amami		0.99 ± 0.003	0.82 ± 0.034	0.31 ± 0.036	0.58 ± 0.049	0.41 ± 0.059
Iriomote	-0.04348		0.96 ± 0.014	0.25 ± 0.038	0.59 ± 0.065	0.57 ± 0.059
Ishigaki	-0.02388	-0.03814		0.23 ± 0.041	0.18 ± 0.045	0.08 ± 0.016
Ogasawara	0.02745	0.02745	0.02707		0.05 ± 0.014	0.97 ± 0.012
Okinawa	-0.01003	-0.01003	0.01520	0.05368		0.12 ± 0.031
Taiwan	0.00124	-0.01477	0.02924	-0.05439	0.02313	

are high ($h = 0.709 \pm 0.003$; $\pi = 0.05 \pm 0.035$) (Table I). The values for the haplotype diversity for each site varied from 0.46 to 0.86. The median joining haplotype network showed that there are shared haplotypes in all 6 localities suggesting no evidence of genetic structure within these localities (Fig. 2). This median joining network is supported by the fact that pairwise F_{ST} values were very low and insignificant among all localities (Table II), once again showing no evidence of a genetic structure between the sampled *S. percnopterygionus* in its distribution area.

Population demographics

Fu's F_s values are all negative and significant except for Amami and Ogasawara (both small samples, respectively $n = 8$ and $n = 7$) (Table III).

As no structure was detected between all the locations sampled, it is only pertinent to discuss these statistical simulations on the total number of samples, and the overall Fu's F_s is both negative and significant.

The difference between the observed mismatch distribution and the simulated mismatch under a demographic expansion model is insignificant for all the samples (SSD p -value = 0.69). The observed mismatch distribution for the totality of the samples is unimodal (peak at 2 muta-

tional steps). The observed mismatch distribution is very close to the distribution for a demographic expansion model (Fig. 3). Population expansion is also supported by the raggedness index for which the overall value is low (0.037), which is typical of a unimodal distribution, and insignificant (p -value = 0.84), indicative of a good fit of the data to a model of population expansion. Furthermore, this is supported by the haplotype network, as a star like pattern is generally associated to a colonization scenario followed by a recent expansion.

DISCUSSION

Freshwater species with a diadromous life cycle and marine species with a pelagic larval stage exhibit higher dispersal ability and lower interpopulation structure than strictly freshwater species or marine species lacking a pelagic larval stage (Allibone & Wallis 1993, Doherty *et al.* 1995). Sicydiinae gobies, via their amphidromous life cycle, have the ability to disperse over long distances as larvae, one of the reasons being their long larval duration. The pelagic larval duration (PLD) can thus be considered in many cases as a proxy to dispersal ability and it should be closely linked to the population structure of these spe-

Table III. – Estimated demographic parameters for *Stiphodon percnopterygionus* population at each sampling location and for the total sample. Raggedness index (rg); sum of squared derivations (SSD); * corresponds to a p -value ≤ 0.05 ; ** corresponds to a p -value ≤ 0.01 .

	Tau	Tetha 0	Tetha 1	rg	rg p-value	SSD	SSD p-value	Fu's F_s
Amami	2.210	0	0.95	0.130	0.75	0.0100	0.65	-0.31
Iriomote	0.960	0	∞	0.220	0.45	0.0340	0.25	*-1.83
Ishigaki	1.330	0.360	4.03	0.025	1.00	0.0010	1.00	*-4.39
Ogasawara	2.580	0.004	3.21	0.045	1.00	0.0110	0.70	-0.65
Okinawa	1.110	0.021	∞	0.710	0.50	0.0030	0.75	*-6.35
Taiwan	1.840	0	∞	0.060	0.25	0.0040	0.40	*-8.28
Total sample	1.068	0.238	∞	0.037	0.84	0.0017	0.69	** -28.61

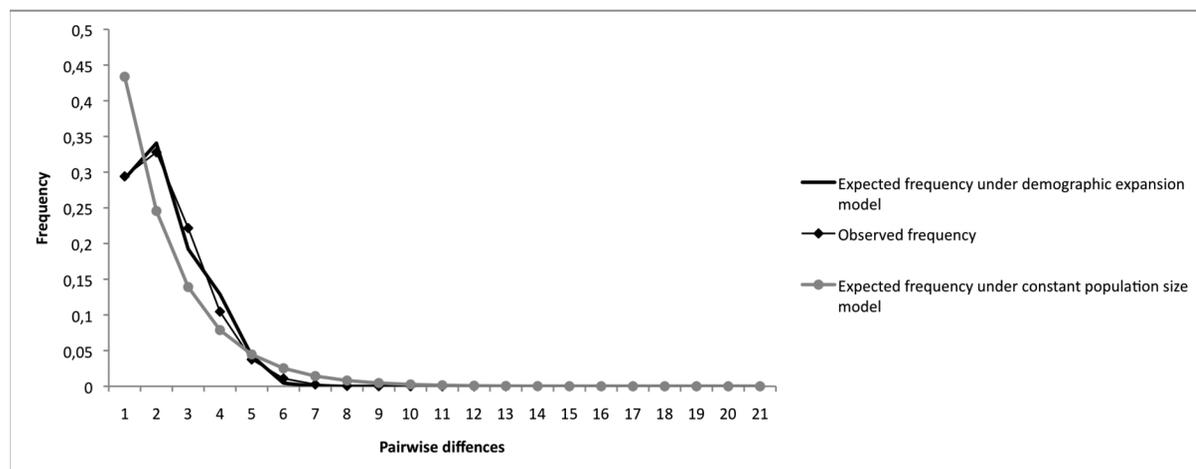


Fig. 3. – Overall mismatch distribution analysis for *Stiphodon percnopterygionus* population sampled in all locations.

cies (Victor & Wellington 2000, Bernardi *et al.* 2003, Lord *et al.* 2012). The molecular approach is an effective method for studying connectivity between populations. There are few molecular studies on amphidromous gobies, involving species more or less widely distributed in their geographic range to uncover the degree of connectivity within each species' distribution area.

The present work showed that *Stiphodon percnopterygionus* has a relatively high haplotype diversity ($h = 0.709 \pm 0.003$). High haplotype diversity is commonly found for Sicydiinae gobies; Watanabe *et al.* (2006) found 74 haplotypes for 77 *Sicyopterus japonicus* specimens. Other authors found the same results for amphidromous species from Hawaii (Fitzimons *et al.* 1990, Zink *et al.* 1996, Chubb *et al.* 1998). For *Sicyopterus sarasini* and *Sicyopterus aiensis*, respectively endemic to New Caledonia

and Vanuatu, for *Sicyopus zosterophorum* and *Smilosicyopus fehlmanni* both widely distributed across the Central West Pacific, haplotype diversities ranged from 0.9 to 0.99 (Lord *et al.* 2012, Taillebois *et al.* 2013). Grant & Bowen (1998) suggested that high levels of both haplotype and nucleotide diversities are indicative of either a long stable evolutionary history or secondary contact among differentiated lineages.

The median joining haplotype network for *Stiphodon percnopterygionus* had a star shaped structure with one predominant haplotype shared in all sampled locations. The presence of shared haplotypes at distant locations suggests that there is gene flow. This may indicate that gene flow between distant populations has occurred on a relatively recent evolutionary time scale (Horne *et al.* 2008). This is consistent with phylogenetic results show-

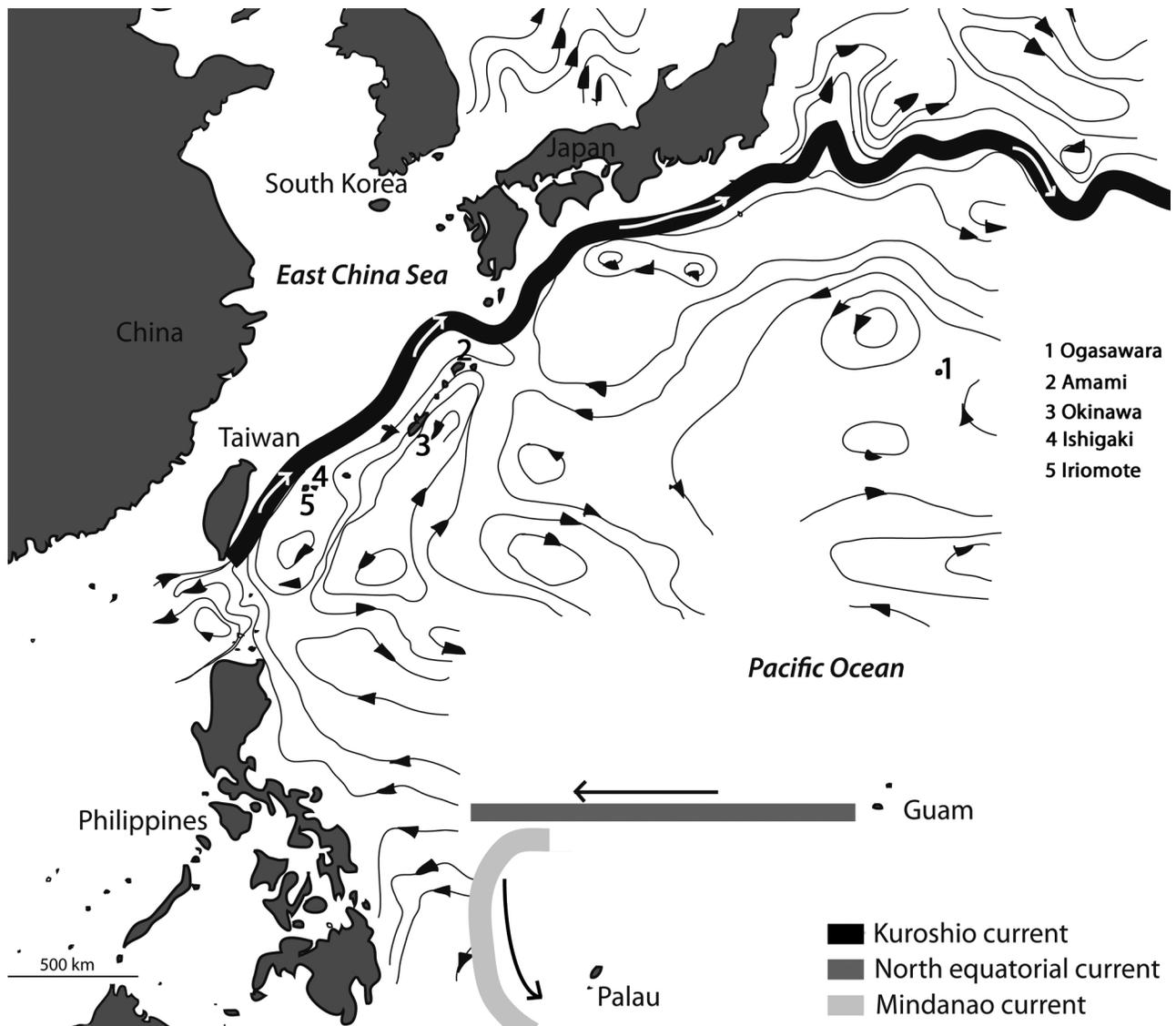


Fig. 4. – Major ocean currents in the northwest Pacific Ocean. The Kuroshio Current emerges from the north of the Philippines and flows along the east coast of Taiwan, in the Ruykyu archipelago and along the east coast of the Japanese mainland (modified from Barkley 1970).

ing that Sicydiinae species have recently evolved and that the number of species has remarkably increased only 2.5–5.8 Myrs ago (Keith *et al.* 2011). Many haplotypes, differing by one or two mutational steps, radiate from this central one and this star-like pattern is concordant with a recent expansion (Grant & Bowen 1998, Chabarría *et al.* 2014). The demographic analysis is congruent with previous facts. Mean Fu's F_s are negative and statistically significant, indicating expanding populations. The mismatch distribution is unimodal and fits the expected frequencies under an expanding population model (supported by both SSD and raggedness index). The dispersal pattern for this species, as for other Sicydiinae species, seems to enable *S. percnopterygionus* to disperse all around potential habitats that may be suitable in its distribution area from Japanese islands to Taiwan, Guam and Palau.

Stiphodon percnopterygionus is not the only Sicydiinae goby inhabiting this Asian area. Other species co-occur such as *Sicyopterus japonicus*. *Sicyopterus japonicus* is the only tropical and temperate Sicydiinae goby; its distribution ranges from Taiwan to central eastern coast of Japan. In their study of the mitochondrial control region (Dloop), Watanabe *et al.* (2006) found no evidence of genetic structure throughout the species distribution range, as we found on *S. percnopterygionus*. The distribution range of *Sicyopterus japonicus* and *Stiphodon percnopterygionus* partly overlap. *S. percnopterygionus* is abundant in the Ryukyu Archipelago and Taiwan, but rather rare in other regions. Recently, this species was recorded from the northern range, *i.e.* Shikoku and Honshu, but the occurrences are local and populations are very small (Shibuya & Takahashi 1998, Kitahara 2012, Nakao & Hirashima 2012). Northernmost record is from a stream in the Izu Peninsula of Honshu, Shizuoka Prefecture, central Japan, where the water temperature keeps around 20 degrees during the winter due to the existence of a hot spring (Kitahara 2012). It is also very rare (only 1 or 2 individuals were discovered) in Guangdong, China (Nip 2010). The distribution of these Sicydiinae gobies in this area may be related to the long larval period allowing long distance dispersal and to the presence of the north-flowing Kuroshio Current on the West side of the north Pacific Ocean (Barkley 1970). This northward current emerges from the south of the Philippines and flows along the east coast of Taiwan, in the Ruykyu archipelago and along the east coast of the Japanese mainland (Fig. 4). This current probably transports larvae of amphidromous fish and facilitates the colonisation of streams on the islands along the current (Watanabe *et al.* 2006, Iida *et al.* 2010, Maeda *et al.* 2011). The Kuroshio Current allows occasional migrants from islands in the south at its origin, such as *Stiphodon alcedo* recently discovered in the Ryukyu Archipelago but in low abundance (Maeda *et al.* 2011). The low abundance for this species in this area is probably due to competition with congeners such as *Stiphodon percnopterygionus*.

The rarity of *S. percnopterygionus* in Guam and Palau is probably due to the same type of mechanism; this species is abundant in Taiwan and the Japanese Islands, along the path of the Kuroshio current, and it can sporadically migrate further than its main distribution area, especially since this species also has a long pelagic larval duration of 99 ± 16 days (Yamasaki *et al.* 2007). Indeed with such a long PLD, it is possible that some of the larvae may be caught in currents other than the main Kuroshio Current via different gyres, and carried to the south towards Guam (Fig. 4). The north equatorial current breaks south into the Mindanao current, which can carry larvae towards Palau. These dispersal routes are probably not frequent, explaining the very low abundance of *S. percnopterygionus* in Guam and Palau. Alternatively, *S. percnopterygionus* may also be present in unexplored areas further south, explaining the sporadic dispersal to Palau and Guam.

The main advantage in being amphidromous is the colonisation of new favourable habitats (McDowall 2010). Indeed, island streams are unstable and represent an environmental extreme, due to seasonal hydrological and climatic variations, but also, at deeper time scales, islands have continuously appeared and disappeared due to volcanic activity, glacial periods impacting sea level and also due to erosion and subsidence of islands. Sicydiinae gobies have the ability to stay at sea for long periods during their larval stage, enabling them to find suitable habitats to colonise. The success in the colonisation of tropical island streams is linked to the long larval marine phase, but the processes leading to many endemic species, to the co-occurrence of several congeneric species in the same streams and the processes shaping the current distribution of Sicydiinae species remain unknown. The lack of population structure commonly found for Sicydiinae species, as we found for *S. percnopterygionus*, denotes their strong potential to find and colonise suitable habitats, but each species is differently confronted to limitations in their capacity to colonise habitats always further. The strength and direction of marine currents, the duration of the marine phase, habitat preferences, interspecific competition or even the biogeographic history represent some factors that could influence the dispersal ability, the species distribution area and the evolution of the group. These factors, shaping species boundaries are not yet fully understood, and need to be studied for conservation purposes. In future studies, areas at the boundaries of the species distribution, such as Guam and Palau, need to be thoroughly sampled to see whether there are barriers to the dispersal of *S. percnopterygionus* explaining its low abundance in these areas. Conservation and management plans will only be pertinent when the population connectivity is known for the entire distribution range.

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