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Does your lip stick? Evolutionary aspects of the mouth morphology of the Indo-Pacific clinging goby of the *Sicyopterus* genus (Teleostei: Gobioidei: Sicydiinae) based on mitogenome phylogeny

Clara Lord[1] | Laure Bellec[2] | Agnès Dettaï[3] | Céline Bonillo[4] | Philippe Keith[1]

[1]Unité Biologie des organismes et écosystèmes aquatiques (BOREA), Sorbonne Université, Muséum national d'Histoire naturelle, Université de Caen Normandie, CNRS, IRD, CP26, Université des Antilles, Paris, France

[2]IFREMER, Centre Brest, REM/EEP/LEP, ZI de la Pointe du Diable, Plouzané, France

[3]Institut Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, École Pratique des Hautes Études, CNRS, CP30, Sorbonne Université, Paris, France

[4]Département Systématique et Évolution, UMS 2700 "Outils et Méthodes de la Systématique Intégrative" MNHN-CNRS, Service de Systématique Moléculaire, Muséum national d'Histoire naturelle, CP26, Paris cedex 05, France

Correspondence
Clara Lord, Unité Biologie des organismes et écosystèmes aquatiques (BOREA), Sorbonne Université, Muséum national d'Histoire naturelle, Université de Caen Normandie, Université des Antilles, CNRS, IRD, CP26, 57 rue Cuvier 75005 Paris, France.
Email: claralord@mnhn.fr

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Contributing authors: Clara Lord (claralord@mnhn.fr); Laure Bellec (laure.bellec@ifremer.fr); Agnès Dettaï (agnes.dettai@mnhn.fr); Céline Bonillo (bonillo@mnhn.fr); Philippe Keith (philippe.keith@mnhn.fr)

Abstract

Sicydiinae gobies have an amphidromous life cycle. Adults grow, feed, and reproduce in rivers, while larvae have a marine dispersal phase. Larvae recruit back to rivers and settle in upstream habitats. Within the Sicydiinae subfamily, the *Sicyopterus* genus, one of the most diverse (24 species), is distributed in the tropical islands of the Indo-Pacific. One of the characters used to determine *Sicyopterus* species is the upper lip morphology, which can be either smooth, crenulated, or with papillae, and with (2 or 3) or without clefts. The mouth is used as a secondary locomotor organ along with the pelvic sucker. It is thus strongly related to the climbing ability of species and is of major importance for the upstream migration and the colonization of insular freshwater systems. The mouth also has an important role in the feeding mechanism of these herbivorous species. In this paper, we have established a molecular phylogeny of the genus based on the 13 mitochondrial protein-coding genes to discuss the relationship between 18 *Sicyopterus* species. There is a well-supported dichotomy in the molecular phylogeny of the *Sicyopterus* genus and this separation into two clades is also morphologically visible, with the distinction of species with three clefts and species with 0 or 2 clefts on the upper lip. The mouth morphology can thus be separated with regard to the molecular phylogeny obtained. The evolution of the mouth morphology is discussed in terms of the adaptation of the *Sicyopterus* genus to settlement and life in tropical insular river systems.

KEYWORDS

mitogenome, mouth morphology, phylogeny, Sicydiinae, *Sicyopterus*

1 | INTRODUCTION

In the Indo-Pacific area, river systems are colonized by freshwater gobies, belonging mainly to the Sicydiinae subfamily, with a life cycle adapted to the conditions in these distinctive habitats, which are, particularly in islands, young oligotrophic rivers subject to extreme climatic and hydrological seasonal variation. These fish species spawn in freshwaters, the free embryos drift downstream to the sea where they undergo a planktonic phase, before returning to rivers to grow and reproduce (Keith, 2003; McDowall, 1997); hence, they are called amphidromous (McDowall, 1988, 1997, 2004). Twenty years ago, there was only scant knowledge of the practical details of their biological cycle and the parameters leading to this evolution in amphidromous gobies, but it has improved with each passing year. These gobies contribute most to the diversity of fish communities in the Indo-Pacific and have the highest levels of endemism (Keith, 2003; Keith & Lord, 2011a, 2011b; Keith, Lord, & Maeda, 2015).

Ninety percent of the tropical freshwater gobies are distributed in the Indo-Pacific area, and only 10% occur in the Atlantic and Caribbean regions. This subfamily has traditionally been united by the presence of a sucker formed by the fusion of the pelvic fins, which adheres entirely to the belly of the fish (Keith & Lord, 2011b). Molecular phylogenies (Keith et al., 2011; Taillebois et al., 2014) of the Sicydiinae based on samples from the Indo-Pacific area and the Caribbean Sea demonstrated the monophyly of the subfamily. Based on morphological and DNA sequence data (mitochondrial: *16S rRNA*, *COI*, and *Cytb* genes; nuclear: *rhodopsin* and *IRF2PB1* genes, totaling 3,545 nucleotides), there are 8 known genera: *Sicydium* Valenciennes, 1837; *Sicyopterus* Gill, 1860; *Lentipes* Günther, 1861; *Sicyopus* Gill, 1863; *Cotylopus* Guichenot, 1863; *Stiphodon* Weber, 1895; *Smilosicyopus* Watson, 1999; and *Akihito* Watson, Keith & Marquet, 2007 (Keith et al., 2015; Taillebois et al., 2014).

Sicyopterus and *Stiphodon* are the two most diverse genera with, respectively, 24 (Table 1) and 30 species (Keith et al., 2015; Unpublished data). They are distributed in the Indo-Pacific from the Western Indian Ocean to the Eastern Pacific one (Keith et al., 2015; Lord, Brun, Hautecœur, & Keith, 2010). Among the 24 known *Sicyopterus* species, *S. lagocephalus* Pallas, 1770, which is the most widespread Sicydiinae (Lord et al., 2012), represents a model species for amphidromous gobies in terms of the study of life-history traits, biology, and physiology (Ellien et al., 2011; Ellien, Werner, & Keith, 2016; Keith et al., 2008; Lord et al., 2010, 2012; Taillebois et al., 2011). 19 other *Sicyopterus* are

TABLE 1 Known *Sicyopterus* species and their distribution (LE: local endemic; WP: Western Pacific; PNG: Papua New Guinea; FP: French Polynesia) (Keith et al., 2015; unpublished data)

	Known species	Upper lip morphology	Distribution
1	*Sicyopterus aiensis* Keith, Marquet & Watson, 2004	Smooth, 3 clefts	LE—Vanuatu
2	*Sicyopterus calliochromus* Keith, Allen & Lord, 2012	Crenulated, 2 clefts	LE—Papua Province, Indonesia
3	*Sicyopterus cynocephalus* (Valenciennes, 1837)	Smooth, 3 clefts	WP—Indonesia, PNG, Philippines, Solomon, Australian wet tropics
4	*Sicyopterus erythropterus* Keith, Allen & Lord, 2012	Smooth, 3 clefts	LE—Papua Province, Indonesia
5	*Sicyopterus eudentatus* Parenti & Maciolek, 1993	Crenulated, 3 clefts	LE—Micronesia
6	*Sicyopterus fasciatus* (Day, 1874)	Smooth, 3 clefts	LE—Burma
7	*Sicyopterus franouxi* (Pellegrin, 1935)	Crenulated, 3 clefts	LE—Madagascar
8	*Sicyopterus griseus* (Day, 1877)	Papillae, 0 cleft	LE—India, Sri Lanka
9	*Sicyopterus japonicus* (Tanaka, 1909)	Smooth, 3 clefts	Taiwan, Japan
10	*Sicyopterus lagocephalus* (Pallas, 1770)	Smooth, 3 clefts	Indo-Pacific
11	*Sicyopterus lengguru* Keith, Lord & Hadiaty, 2012	Smooth, 3 clefts	LE—Papua Province, Indonesia
12	*Sicyopterus lividus* Parenti & Maciolek, 1993	Papillae, 2 clefts	LE—Micronesia
13	*Sicyopterus longifilis* de Beaufort, 1912	Crenulated, 2 clefts	WP—Indonesia, PNG, Philippines, Solomon
14	*Sicyopterus marquesensis* Fowler, 1932	Crenulated, 3 clefts	LE—Marquesas Islands
15	*Sicyopterus microcephalus* (Bleeker, 1855)	Papillae, 0 cleft	WP—Indonesia, Andaman (?), Timor, Philippines
16	*Sicyopterus ocellaris* Keith, Allen & Lord, 2012	Smooth, 3 clefts	LE—PNG
17	*Sicyopterus parvei* (Bleeker, 1853)	Smooth, 3 clefts	LE—Indonesia
18	*Sicyopterus pugnans* (Ogilvie-Grant, 1884)	Papillae, 2 clefts	LE—Samoa, Society Islands (FP)
19	*Sicyopterus punctissimus* Sparks & Nelson, 2004	Smooth, 3 clefts	LE—Madagascar
20	*Sicyopterus rapa* Parenti & Maciolek, 1996	Crenulated, 3 clefts	LE—Rapa Island
21	*Sicyopterus sarasini* Weber & de Beaufort, 1915	Smooth, 3 clefts	LE—New Caledonia
22	*Sicyopterus squamosissimus* Keith et al., 2015	Crenulated, 2 clefts	LE—South Sumatra, West Java
23	*Sicyopterus stimpsoni* (Gill, 1860)	Smooth, 3 clefts	LE—Hawaii
24	*Sicyopterus stiphodonoides* Keith, Allen & Lord, 2012	Papillae, 0 cleft	LE—Solomon, PNG

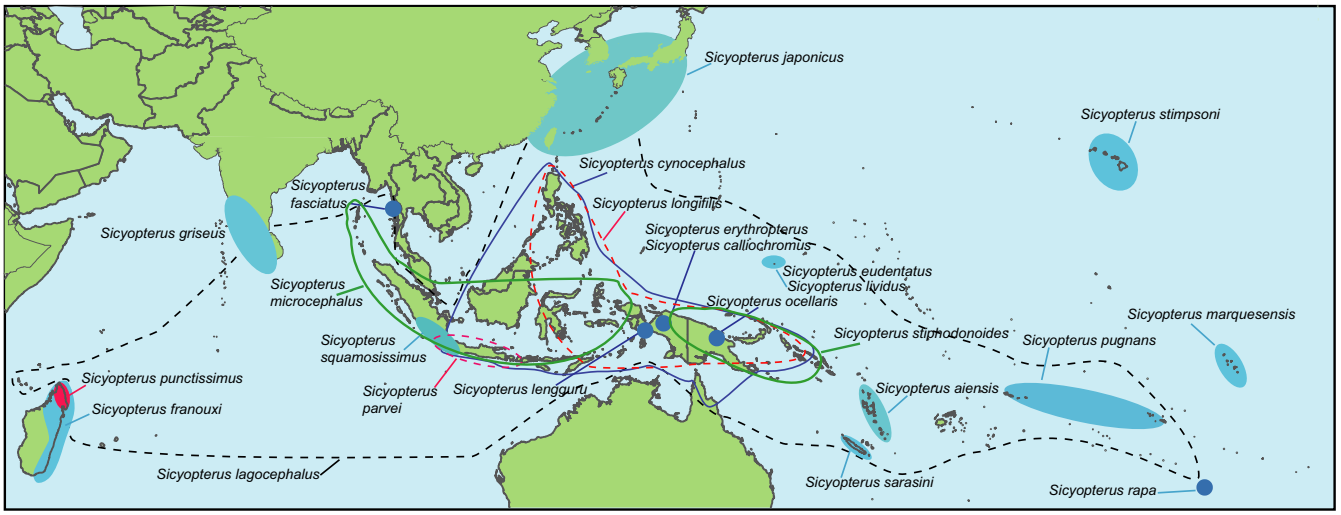

FIGURE 1 Map of the distribution of the 24 known *Sicyopterus* species in the Indo-Pacific

local endemics with a very restricted distribution area, illustrating the high level of endemism for these Sicydiinae gobies (Keith et al., 2015). Nearly all the endemic species live in sympatry with at least one other *Sicyopterus* species endemic or not, and they are found from the lower to the upper reaches of rivers (Keith et al., 2015) (Figure 1). Furthermore, *Sicyopterus* species have strong patrimonial and economical values as the postlarvae are fished while recruiting back in estuaries. At certain times of the year, the biomass of fish larvae recruiting and migrating upstream is so great that they become a major source of food for local human populations in the Indo-Pacific area (Réunion Island, Vanuatu, French Polynesia, Philippines, etc.) (Hoareau, Lecomte-Finiger, Grondin, Conand, & Berrebi, 2007; Manacop, 1953).

In the *Sicyopterus* genus, the ascending process on the premaxilla is broad at the dorsal tip, the tongue is fused to the floor of the mouth, and it has numerous large tricuspid premaxillary teeth in both sexes. The morphology of the mouth is variable and is often used in taxonomy to discriminate the species (Keith & Lord, 2011b; Keith et al., 2015). Indeed, three main groups are distinguished: The first one has three clefts on the upper lip, two midlateral ones, and one anteriorly; the second group only has two midlateral clefts on the upper lip; and the third group has no clefts. Furthermore, the border of the upper lip, whether it has clefts or not, can be either smooth, crenulated, or with papillae (Table 1). Both the teeth and the morphology of the lip are of particular importance in this genus as it is correlated to the feeding (Keith & Lord, 2011b) and climbing behaviors. Indeed, the mouth, the teeth, as well as the digestive system are adapted to a benthic herbivorous feeding mode, and the tricuspid premaxillary teeth are adapted for scraping diatoms growing on rock surfaces. *Sicyopterus* species maintain "gardens" of low-growing periphyton in swift water on the upper surfaces of large pebbles and boulders. These conspicuous patches of diatoms represent a food source and the area for the initiation of stereotypical social behavior, including territoriality and courtship (Barbeyron, Lefrançois, Monti, Keith, & Lord, 2017; Fitzsimons, McRae, Schoenfuss, & Nishimoto, 2003). *Sicyopterus* is also able to climb over waterfalls by using alternately its pelvic suction cup and its lips: as the oral disk attaches to the substrate, it expands to almost twice its resting area, after which the posterior body is pulled upwards; once the pelvic disk attaches, the oral disk releases and the anterior body advances. The mouth is thus used as a secondary locomotor organ (Schoenfuss & Blob, 2003).

For just over 15 years, the complete mitochondrial genome (mitogenome) has been used to resolve the phylogenetic relationships in Teleostean (Miya & Nishida, 2015). The use of the mitogenome has often successfully resolved problematic phylogenies. In addition, in many cases, phylogenies based on the analysis of nuclear genes and those based on mitogenomes are congruent (Campbell, Lopez, Sado, & Miya, 2013; Li et al., 2009). Until now, only two mitogenomes have been published for *Sicyopterus* species (Chiang, Chen, Lin, Chang, & Ju, 2013; Chiang, Chen, Lin, Hsiao, & Ju, 2013), that is, for the two most studied species, *S. lagocephalus* and *S. japonicus* (Tanaka, 1909). Sicydiinae gobies diversified only recently (around 4 million years ago) (Keith et al., 2011), with species emerging from the central-west Pacific. Keith, Galewski, Cattaneo-Berrebi, Hoareau, and Berrebi (2005) have previously studied the relationship between species but they studied it between only seven species of *Sicyopterus*, based on partial *Cytochrome b* sequences.

The aim of this paper was to resolve the phylogenetic relationships between *Sicyopterus* species, based on the 13 protein-coding genes of the mitochondrial genome and to look into the evolution of the mouth morphology. Furthermore, it is to improve our knowledge on the colonization processes of tropical insular water systems by amphidromous species, and their success in such extreme environments in the light of molecular phylogenetics and mouth morphology.

2 | MATERIALS AND METHODS

2.1 | Sample collection

A total of 54 *Sicyopterus* specimens, representing 18 species out of the 24 known species according to the work of Keith et al. (2015) and our unpublished data, were used for the present work (Table 2). Fish

TABLE 2 Sampling of *Sicyopterus* specimens throughout the Indo-Pacific tropical islands, representing 18 species out of the 24 known species. The table includes out-groups used for the phylogenetic reconstruction. All the specimens for which the sample number starts by "Aqua" come from an aquarium wholesaler. GenBank accession numbers in bold were generated in the present study

			Mitogenome
Species	**Sampling location**	**Sample number**	**GenBank accession number**
Sicyopterus aiensis	Vanuatu	9A	**MK426281**
Sicyopterus aiensis	Vanuatu	ai225	**MK496934**
Sicyopterus cynocephalus	Solomon Islands	12031	**MK496936**
Sicyopterus cynocephalus	Solomon Islands	6924	**MK496935**
Sicyopterus eudentatus	Micronesia	1	**MK496937**
Sicyopterus eudentatus	Micronesia	eudbrian	**MK496940**
Sicyopterus eudentatus	Micronesia	13	**MK496938**
Sicyopterus eudentatus	Micronesia	166883	**MK496939**
Sicyopterus franouxi	Madagascar	SfB	**MK496941**
Sicyopterus franouxi	Madagascar	SfC	**MK496942**
Sicyopterus franouxi	Madagascar	SfD	**MK496943**
Sicyopterus japonicus	Japan	NC_018826.1	NC_018826.1
Sicyopterus japonicus	Japan	15	**MK496944**
Sicyopterus japonicus	Japan	16	**MK496945**
Sicyopterus lagocephalus	Solomon Islands	12057	**MK496946**
Sicyopterus lagocephalus	Papua	BSP3	**MK496947**
Sicyopterus lagocephalus	Vanuatu	LP8	**MK496948**
Sicyopterus lagocephalus	Asia	NC_022838.1	NC_022838.1
Sicyopterus lengguru	Papua	G1	**MK496949**
Sicyopterus lividus	Micronesia	12	**MK496950**
Sicyopterus lividus	Micronesia	5228	**MK496951**
Sicyopterus lividus	Micronesia	5242	**MK496952**
Sicyopterus lividus	Micronesia	5243	**MK496953**
Sicyopterus longifilis	Indonesia	AquaIndo1	**MK496958**
Sicyopterus longifilis	Indonesia	AquaIndo2	**MK496959**
Sicyopterus longifilis	Indonesia	Aqua6920	**MK496956**
Sicyopterus longifilis	Indonesia	Aqua6921	**MK496957**
Sicyopterus longifilis	Philippines	2	**MK496954**
Sicyopterus longifilis	Philippines	2A	**MK496955**
Sicyopterus marquesensis	Marquesas Islands	5	**MK496960**
Sicyopterus marquesensis	Marquesas Islands	5A	**MK496961**
Sicyopterus microcephalus	Indonesia	Aqua1006	**MK496964**
Sicyopterus microcephalus	Indonesia	Aqua1001	**MK496963**
Sicyopterus microcephalus	Philippines	14	**MK496962**
Sicyopterus parvei	Indonesia	Aqua1004	**MK496965**
Sicyopterus parvei	Indonesia	Aqua1005	**MK496966**
Sicyopterus pugnans	Society Islands	pug1A	**MK496971**
Sicyopterus pugnans	Society Islands	pug1B	**MK496972**
Sicyopterus pugnans	Society Islands	pug1C	**MK496973**
Sicyopterus punctissimus	Madagascar	3	**MK496974**
Sicyopterus punctissimus	Madagascar	3A	**MK496975**

(Continues)

TABLE 2 (Continued)

Species	Sampling location	Sample number	Mitogenome GenBank accession number
Sicyopterus sarasini	New Caledonia	sar8A	**MK496976**
Sicyopterus sarasini	New Caledonia	sar53	**MK496980**
Sicyopterus sarasini	New Caledonia	sar51	**MK496978**
Sicyopterus sarasini	New Caledonia	sar23	**MK496977**
Sicyopterus sarasini	New Caledonia	sar52	**MK496979**
Sicyopterus squamosissimus	Sumatra	Aqua11919	**MK496981**
Sicyopterus squamosissimus	Sumatra	Aqua11921	**MK496982**
Sicyopterus stimpsoni	Hawaii	4507	**MK496983**
Sicyopterus stimpsoni	Hawaii	4508	**MK496984**
Sicyopterus stimpsoni	Hawaii	4509	**MK496985**
Sicyopterus stiphodonoides	Solomon Islands	DB09-972	**MK496988**
Sicyopterus stiphodonoides	Solomon Islands	6953	**MK496986**
Sicyopterus stiphodonoides	Solomon Islands	6954	**MK496987**
Total *Sicyopterus* = 54			

Out-group	Sampling location	Sample number	Mitogenome GenBank accession number
Stiphodon pelewensis	Indonesia	Aqua5409	**MK496968**
Stiphodon pelewensis	Vanuatu	atra3	**MK496967**
Stiphodon tuivi	Marquesas Islands	5477	**MK496969**
Stiphodon tuivi	Marquesas Islands	5479	**MK496970**
Rhinogobius brunneus	Asia	NC_028435.1	NC_028435.1
Redigobius bikolanus	Asia	NC_029320.1	NC_029320.1
Total number of specimens = 60			

were collected from freshwater streams of islands in the Indian and Pacific oceans, thus in the entire distribution area of the *Sicyopterus* genus. Individuals were sampled using a DEKA 3000 electrofishing system (Gerätebau). Fish were sampled on the entire stream, from the lower part to the higher reaches, as defined by Keith, Marquet, Gerbeaux, Vigneux, and Lord (2013). According to the Annex IV of the Directive 2010/63/EU, fish were either euthanized using an overdose of clove essential oil (10%), or a piece of fin was taken while the fish was anaesthetized. In the case of anaesthetization, the fish was then awakened in clear water before it was released. Entire fish or fin clips were stored and preserved in 95% alcohol for molecular genetic analysis. To complete our sampling, an aquarium wholesaler provided specimens from Asia.

2.2 | DNA extraction and mitogenome amplification

Pectoral fin tissue was used to extract total genomic DNA from the 56 individuals (52 *Sicyopterus* and 4 *Stiphodon* as out-groups) using the Macherey & Nagel NucleoSpin® Tissue kits following the manufacturer's instructions on an Eppendorf epMotion 5075.

In the study, the complete mitochondrial genome was sequenced for all of the specimens (Table 2). We obtained the mitogenome using a protocol established by Hinsinger et al. (2015): they developed a framework for the sequencing and multiplexing of mitogenomes on NGS (next-generation sequencing) platforms that implements (I) a universal long-range PCR-based amplification technique, (II) a two-level multiplexing approach (i.e., divergence-based and specific tag indexing), and (III) a dedicated demultiplexing and assembling script from an Ion Torrent sequencing platform.

The mitogenome was amplified with three overlapping fragments, called MT1, MT2, and MT3, with three pairs of primers (Table 3). A Hot Start LongAmp® Taq DNA Polymerase (New England Biolabs)-modified protocol was used. The amplification of the three fragments was performed by PCR in a final 18 µl volume including 5X LongAmp Taq Reaction Buffer, 0.4 ng/µl bovine serum albumin, 3.5% DMSO, 300 nM of each primer, 300 µM of dNTPs, and 1 unit of LongAmp Taq polymerase. After an initial denaturation of 30 s at 94°C, the DNA was amplified through 45 cycles of 20 s at 94°C, 30 s at 62.5°C, and 15 min at 65°C, with a terminal elongation for 15 min at 65°C (Hinsinger et al., 2015) on a Biometra

TABLE 3 Primers used for the amplification of the mitogenome in three overlapping fragments of about 7,000 base pairs each (MT1, MT2, and MT3)

Primer name	Sequence (5'>3')	Fragment amplified
12SL1091 (Kocher et al., 1989)	AAACTGGGATTAGATACCCCACTAT	MT1
R7061 (Hinsinger et al., 2015)	GGGTTATGTGGCTGGCTTGAAAC	
F5231 (Hinsinger et al., 2015)	TAGATGGGAAGGCTTCGATCCTACA	MT2
R11944 (Hinsinger et al., 2015)	CATAGCTTTTACTTGGATTTGCACCA	
F11910 (Hinsinger et al., 2015)	CAGCTCATCCATTGGTCTTAGGAAC	MT3
12SH1478 (Kocher et al., 1989)	TGACTGCAGAGGGTGACGGGCGGTGTGT	

thermocycler. The length of each fragment amplified (MT1, MT2, and MT3) is about 7,000 bp.

Data processing and sequence assembly were done in Geneious 8.1.5 (Kearse et al., 2012); the mitogenome for each specimen was annotated using MitoAnnotator (Iwasaki et al., 2013). All the sequences were aligned with MAFFT Alignment (Katoh, Misawa, Kuma, & Miyata, 2002) (implemented in Geneious). The percentage of identity between sequences and the number of differing bases were calculated on Geneious 8.1.5. The alignment was then processed in Gblocks© v0.91b (Castresana, 2000) in order to remove gaps, with the options for a less stringent selection, that is, allowing smaller final blocks, allowing gap positions within the final blocks, and allowing less strict flanking positions.

2.3 | Phylogenetic reconstruction

A phylogenetic tree based on the thirteen concatenated genes was performed using Bayesian inference (MrBayes v.3.2; Ronquist et al., 2012). The best-fitting models of evolution were computed in PartitionFinder v1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012). The analysis was undertaken using the three-codon positions for each gene as a partition (Table 4) and was run for 10 million generations, sampling every 250 generations with two independent runs to access convergence. Run convergence was checked using TRACER v.1.6.0 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Trees were summarized using the 50% majority rule method after discarding the first 25% of the sample as burnin and visualized using FigTree v.1.4.2 (Rambaut, 2012). Two species of *Stiphodon*, and for which the mitogenome was obtained via the method described above, and two other gobioids (*Rhinogobius* and *Redigobius*) found in GenBank database were used as out-groups.

3 | RESULTS

3.1 | Mitogenome analysis

We obtained mitogenomes for 52 *Sicyopterus* specimens, corresponding to 18 species. Two mitogenomes were available on GenBank (one *S. lagocephalus* and one *S. japonicus*), totaling 54 mitogenomes for 18 species (Table 2). The complete mitochondrial genome was found to be around 16,500 bp for each individual (Table 5). The structural organization of the mitogenome for each specimen consists of 2 rRNA genes, 22 tRNA genes, 13 protein-coding genes, and a control region (for abbreviations of genes, see Table 4). All the protein-coding genes are coded on the H strand apart from the ND6 gene (Figure 2). The mean percentage of divergence between all 54 complete mitochondrial genome is 7% with 12,386 identical sites over the 16,500 bp. We noticed that the 22 tRNA genes were highly conserved between species, with often <2% divergence between the most divergent species (Table 6A). The 22 tRNA genes and the other non-coding regions, the rRNA genes and the control region, were discarded from the data set, and only the 13 protein-coding genes were included in the phylogenetic reconstruction, representing 11,589 bp. After alignment of the 54 concatenated sequences, the mean percentage of divergence between all *Sicyopterus* sequences is 8.2% (as opposed to 7% for the complete mitochondrial genome) (Table 5). The maximum percentage of divergence between two sequences is 10.88% (between *Sicyopterus longifilis* and *Sicyopterus japonicus*) with about 1,260 differing nucleotides. The minimum percentage of divergence between two species is 0.88% (*S. cynocephalus* and *S. aiensis*). Some sequences between two individuals of the same species show no difference. For each protein-coding gene, the minimum and maximum interspecific divergence percentage was calculated (Table 6B). Of the 13 protein-coding genes, the most divergent ones code for the *NADH dehydrogenase subunits*. Indeed, the *ND6*, *ND2*, and *ND4*, respectively, show mean divergence

TABLE 4 Models selected by codon partition for each of the 13 mitochondrial protein-coding genes for the phylogenetic reconstruction

Codon position on each genes	Model selected
*ND1*_1; *ND1*_2; *ND1*_3; *ND2*_1; *ND2*_2; *ND2*_3; *COI*_1; *COII*_1; *ATP8*_1; *ATP8*_2; *ATP6*_1; *ATP6*_2; *ATP6*_3; *COIII*_1; *ND3*_1; *ND3*_2; *ND4L*_1; *ND4L*_2; *ND4L*_3; *ND4*_1; *ND4*_2; *ND4*_3; *ND5*_1; *ND5*_2; *ND5*_3; *ND6*_3; *Cytb*_1; *Cytb*_3	GTR + I + G
*COI*_3; *COII*_3; *ATP8*_3; *COIII*_3; *ND3*_3; *ND6*_1	GTR + G
*COI*_2; *COII*_2; *COIII*_2; *Cytb*_2	HKY + I + G
*ND6*_2	F81 + G

Abbreviations: ATP6, ATP synthase membrane subunit 6; ATP8, ATP synthase membrane subunit 8; *COI*, *cytochrome c oxidase subunit 1; COII*, *cytochrome c oxidase subunit 2; COIII*, *cytochrome c oxidase subunit 3; Cytb*, *cytochrome b*. Gene_1, codon position 1; Gene_2, codon position 2; Gene_3, codon position 3; ND, NADH dehydrogenase subunits 1, 2, 3, 4, 4L, 5, 6.

TABLE 5 Mean statistics on the complete mitochondrial genome and on the 13 concatenated protein-coding genes for the 54 *Sicyopterus* mitogenome sequences (bp = base pairs; sd = standard deviation)

54 *Sicyopterus* mitogenomes	Mean length (bp)	Minimum length (bp)	Maximum length (bp)	Number of identical sites	Pairwise % of divergence	%GC
13 protein-coding genes	11,584.2 (sd. 10.1)	11,556	11,589	8,306	8.2	45%
Complete mitogenome	16,501.2 (sd. 3.6)	16,495	16,514	12,386	7	44.7%

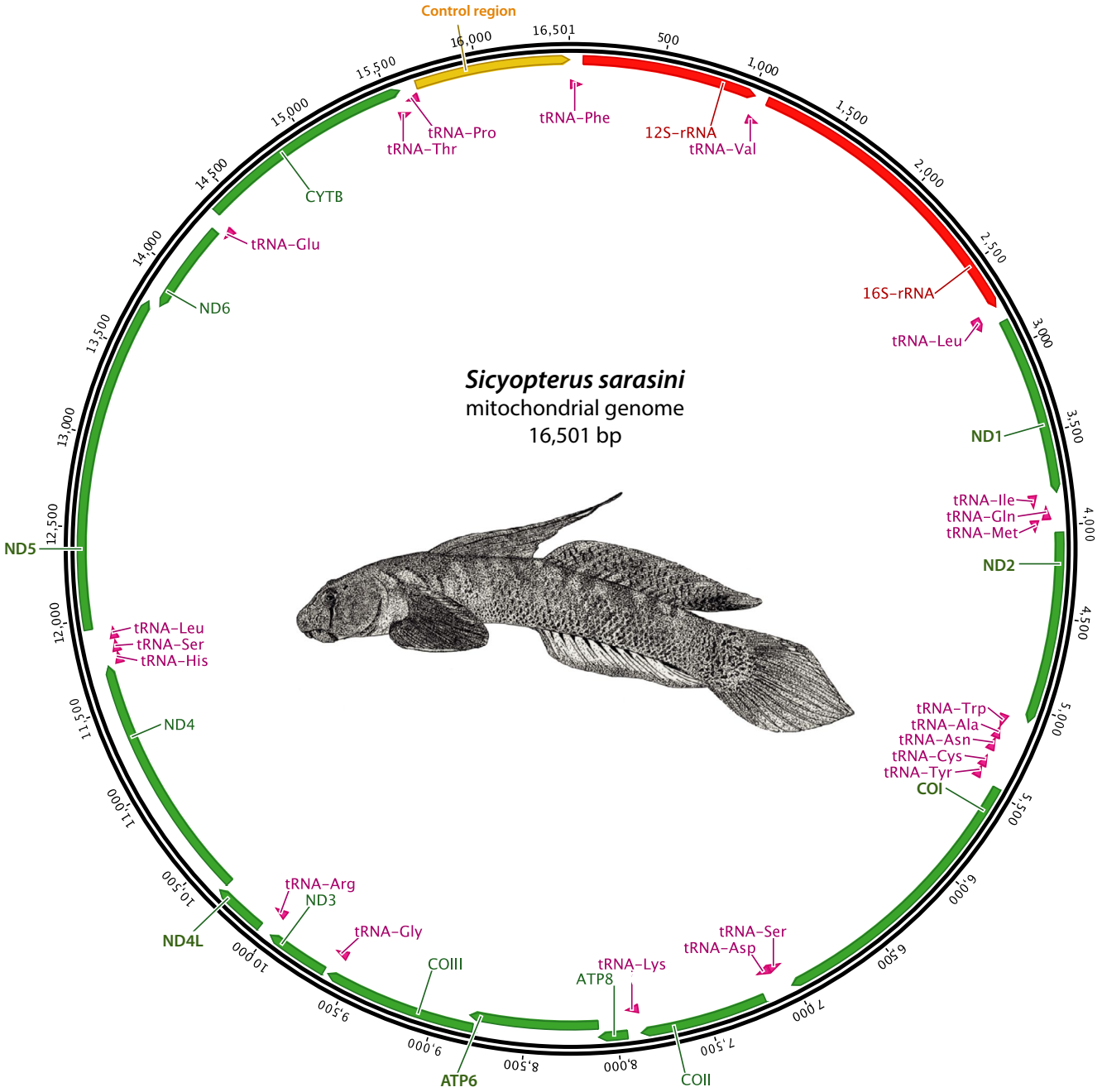

FIGURE 2 Mitogenome map for *Sicyopterus sarasini* (16,501 bp) as an example to show the order of the 13 protein-coding genes (green), the two rRNA genes (*12S* and *16S*) (red), the 22 tRNA genes (pink), and the position of the control region (yellow). The first position is set at the *tRNA-Phe*. Arrows show the coding direction either on the H strand (all coding genes apart from *ND6*) or the L strand (Drawing by C. Lord; Lord & Keith, 2008)

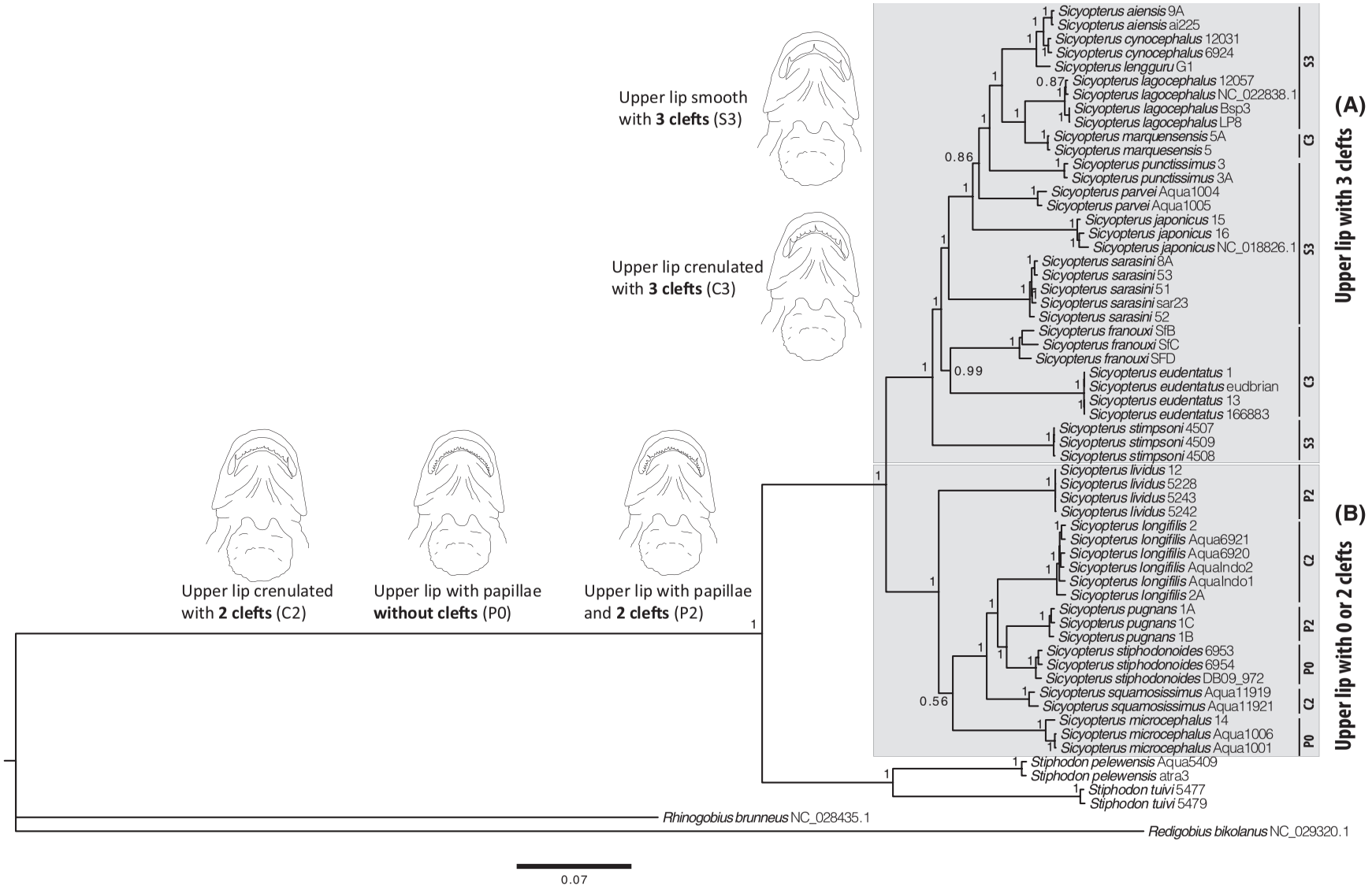

FIGURE 3 Molecular phylogeny inferred by Bayesian reconstruction of 18 *Sicyopterus* species (54 specimens) based on the 13 mitochondrial protein-coding genes (11,589 bp). Posterior probabilities are given at each node. Drawings represent a ventral view of the head, with a particular interest for the morphology of the upper lip. For each *Sicyopterus* species in the phylogenetic tree, the lip morphology is reported on the right (S3 = upper lip smooth with three clefts; C3 = upper lip crenulated with three clefts; C2 = upper lip crenulated with two clefts; P2 = upper lip with papillae and two clefts; P0 = upper lip with papillae and no cleft). Clade A includes all species with three clefts; clade B includes all species with two or no clefts

TABLE 6 (A) Length, direction, and mean percentage of divergence for each non-coding sequence over the 54 *Sicyopterus* mitogenome sequences. (B) Length, direction, mean divergence percentage, minimum intraspecific divergence percentage and minimum and maximum interspecific divergence percentage for each of the 13 protein-coding mitochondrial genes

(A)

Non-coding	Length (pb)	Direction	Mean % divergence
tRNA-Phe	68	Forward	2.2
tRNA-Val	72	Forward	2.5
tRNA-Leu	75	Forward	1.6
tRNA-Ile	72	Forward	3.8
tRNA-Gln	71	Reverse	0
tRNA-Met	69	Forward	1.9
tRNA-Trp	71	Forward	1.6
tRNA-Ala	69	Reverse	0.4
tRNA-Asn	73	Reverse	0.2
tRNA-Cys	66	Reverse	3.5
tRNA-Tyr	71	Reverse	2.1
tRNA-Ser	71	Reverse	0.3
tRNA-Asp	72	Forward	3.4
tRNA-Lys	75	Forward	2
tRNA-Gly	72	Forward	2.4
tRNA-Arg	69	Forward	1
tRNA-His	69	Forward	2.7
tRNA-Ser	70	Forward	1.9
tRNA-Leu	73	Forward	0
tRNA-Glu	69	Reverse	0.6
tRNA-Thr	72	Forward	2.5
tRNA-Pro	70	Reverse	1.6
12S-rRNA	960	Forward	2.6
16S-rRNA	1,717	Forward	3.9
Control region	836–846	Forward	10.8

(B)

Coding gene	Length (bp)	Direction	Mean % divergence	Min intraspecific % divergence	Min interspecific % divergence	Maximum % divergence
ND1	975	Forward	9.4	0	1.13	13.95
ND2	1,047	Forward	10.2	0	1.72	14.8
COI	1,554	Forward	6.5	0	0.77	9.46
COII	699	Forward	4.2	0	0	6.29
ATP8	165	Forward	3.7	0	0.61	7.27
ATP6	684–717	Forward	9.6	0	0.42	13.6
COIII	840	Forward	6.4	0	0.83	9.4
ND3	351	Forward	8	0	1.17	12.82
ND4L	297	Forward	7.8	0	1.35	12.46
ND4	1,386	Forward	9.8	0	0.87	13.42
ND5	1,839	Forward	8.5	0	1.25	12.34
ND6	522–531	Reverse	10.8	0	1.45	16.06
Cytb	1,197	Forward	8	0	1.17	11.36

percentages of 10.8%, 10.2%, and 9.8% (with a maximum interspecific divergence percentage of 16.06, 14.8, and 13.42). After the *ATPase 8* (3.7%) and the *cytochrome c oxidase II* (4.2%), the *cytochrome c oxidase I* is the least variable of the 13 protein-coding genes, with a mean divergence percentage of 6.5% (Table 6B).

The phylogenetic analysis was undertaken on the 60 protein-coding gene sequence alignment (Table 2; see fasta file as Supporting Information). The phylogenetic tree obtained by Bayesian inference and based on the 13 protein-coding genes (11,589 bp) is divided into two well-supported clades (A & B) with a high posterior probability (PP) value (PP = 1), separated from the out-groups, the other Sicydiinae *Stiphodon*, and the other two gobioidei (Figure 3). All the nodes are strongly supported, even the most basal ones. With this reconstruction based on the 13 protein-coding genes, the species are well separated in their gene sequences and, as the deep nodes are well supported, we can also apprehend interspecific relationships.

3.2 | Mouth morphology versus DNA sequence data

There is a clear and well-supported dichotomy into two clades (A & B), which is also morphologically visible, with the distinction of species with three clefts (A) and species with 0 or 2 clefts (B) on the upper lip. Clade A is composed of 12 species presenting three clefts on the upper lip (one median cleft and two midlateral ones) (Figure 3), that is, *S. aiensis*, *S. cynocephalus*, *S. lengguru*, *S. lagocephalus*, *S. marquesensis*, *S. punctissimus*, *S. parvei*, *S. japonicus*, *S. sarasini*, *S. franouxi*, *S. eudentatus*, and *S. stimpsoni*, the latter being in basal position for this clade. All the species of the clade A are differentiated and well supported by PP values, and the relationship between the species is well supported.

Clade B is composed of six species with either two midlateral clefts on the upper lip or no clefts on the upper lip, that is, *S. lividus*, *S. longifilis*, *S. pugnans*, *S. stiphodonoides*, *S. squamosissimus*, and *S. microcephalus* (Figure 3). In this clade, all the species are well differentiated and well supported by PP values (apart from one basal node, PP = 0.56, giving an uncertainty as to the position of *S. microcephalus* within this clade).

4 | DISCUSSION

4.1 | Mitogenome phylogenetic reconstruction

Mitochondrial markers (*COI*, *Cytb*…) are frequently used to reconstruct teleostean intra- and interspecific relationships. For 30 years, the mitochondrial genome has indeed been the most frequently used marker to study animal molecular diversity (Galtier, Nabholz, Glémin, & Hurst, 2009) because it presents several advantages. It is easy to amplify as the mitogenome exists in several copies within a cell, and mitochondrial DNA shows a high degree of mutation. This high variability is useful to obtain information on the evolutionary history of lineages over a short period of time (Galtier et al., 2009). However, the use of only one marker, or even a partial sequence, is now considered insufficient (Dowton, Meiklejohn, Cameron, & Wallman, 2014).

The use of the mitogenome brings robust results, and it is compatible with most of the markers already published (Miya & Nishida, 2015). For several years now, next-generation sequencing techniques have been developed, reducing costs and improving sequencing output (Hinsinger et al., 2015). In teleostean molecular phylogenetic reconstruction, protein-coding genes are the ones usually used to assess the relationship between different groups or different species; the non-coding regions are not as used as they are often not informative (Miya & Nishida, 2000; Peng, He, Wang, Wang, & Diogo, 2006; Zardoya & Meyer, 1996). Miya and Nishida (2000) demonstrated that nucleotide sequences from the 13 concatenated protein-coding plus the stem region of the tRNA genes were most able to reproduce the phylogeny of teleosts, unlike individual genes. Furthermore, Inoue, Miya, Tsukamoto, and Nishida (2003) worked on the relationships of actinopterygians using 12 of the 13 protein-coding genes and the stem region of tRNA genes, and they found that their topology exhibited congruence with a hypothesis based on nuclear markers, showing the strong potential of using the mitogenome to reconstruct teleost phylogenetic relationships.

The relationship between *Sicyopterus* species has been studied previously based on partial *cytochrome b* sequences, but only seven *Sicyopterus* species were included in the study (Keith et al., 2005). Based on 58 Sicydiinae mitogenomes (52 *Sicyopterus* obtained in this study; two *Sicyopterus* from GenBank database; and four *Stiphodon* obtained in this study used as out-groups), we used the 13 protein-coding genes to study the organization of the *Sicyopterus* genus. We thus obtained, for the first time, mitogenomes for 18 species out of the 24 known species. In our case, the tRNA genes were of no use because of the high percentage of conservation between species, so we chose to discard them from the analysis. This is probably due to the fact that the Sicydiinae subfamily is young, and the radiation of the different genera and species occurred only about 4 Myrs ago (Keith et al., 2011). By discarding the non-coding regions, we enhanced the informative power of the data by 1.2% (from 7% of mean divergence percentage for the complete mitochondrial genome to 8.2% for the 13 protein-coding genes).

After analysis of the 13 protein-coding genes, we discovered that genes coding for *NADH dehydrogenase subunits* (*ND* genes) were far more informative than, for example, the gene coding for the *cytochrome c oxidase I* (*COI*). Indeed, the *COI* is the 10th most variable gene out of 13. DNA barcoding uses short genetic sequences as a way to identify species; usually, it uses a short genetic marker of mitochondrial genome (Blaxter, 2003); two mitochondrial genes were selected to resolve closely related species of the animal kingdom, namely *COI* (Hebert, Ratnasingham, & Waard, 2003; Savolainen, Cowan, Vogler, Roderick, & Lane, 2005) and *cytochrome b* (Lekshmi & Soni, 2007). DNA barcoding is an effective tool for species identification, but we show here it is not always informative enough to determine the interspecific relationships, especially in the case of taxonomic groups that have undergone recent speciation processes. Indeed, in the case of Sicydiinae gobies, for which the radiation likely took place only 4 million years ago (Keith

et al., 2011), genetic mitochondrial markers such as *ND6* or *ND2* would be more appropriate to determine interspecific relationships.

The Bayesian phylogenetic reconstruction showed the monophyly of the *Sicyopterus* genus, as previously shown in previous Sicydiinae phylogenies (Keith et al., 2005, 2011; Taillebois et al., 2014). Species differentiation is well supported, and both the basal and the terminal nodes are well supported (PP = 1), giving information as to the relationship between species.

The mitogenome phylogenetic reconstruction recovered two well-supported clades (PP = 1). One composed of 12 species (clade A) and one composed of six species (clade B). Both clades A and B have a simultaneous appearance. In clade A, *S. stimpsoni* (endemic to Hawaii) has a basal position. Among the most recent species of clade A, we find the widely spread *S. lagocephalus*, sharing a sister relationship with *S. marquesensis* (endemic to the Marquesas Islands) and the clade including *S. aiensis* (endemic to Vanuatu), a sister relationship which has already been recovered by Keith et al. (2005) in their phylogenetic study based on *cytochrome b*.

Within clade A, the case of the subclade composed of *S. aiensis*, *S. cynocephalus*, and *S. lengguru* must however be discussed. Although they show a divergence of around 1% over 11,589 bp, *S. aiensis*, *S. cynocephalus*, and *S. lengguru* are separated and supported by high PP values (PP = 1). This study shows that the use of the mitogenome as opposed to just one partial mitochondrial gene is more powerful in terms of phylogenetic signal (Teacher, André, Merila, & Wheat, 2012), as these three species, which have separated recently during the Sicydiinae radiation, can be clearly distinguished based on their DNA sequences (Keith et al., 2011). The position of *S. lengguru* within this molecular phylogeny might be challenged by the fact that we only have one specimen. Additional specimens should be added to validate, or not, its position within the clade. For all the other species, the mean divergence percentage over the 13 protein-coding genes is between around 4% (*S. lagocephalus* versus *S. aiensis*) and nearly 11% for the most distant species (*S. longifilis* versus *S. eudentatus*).

4.2 | Evolution of the mouth morphology

Our molecular phylogeny reflects the mouth morphology, as clades A and B can also be separated according to this morphology. Clade A is represented by species presenting three clefts on the upper lip and clade B by species without or with two clefts on the upper lip. In the phylogeny by Keith et al. (2005), this dichotomy could also have been seen, but they had too few species to discuss that aspect. Indeed, they included in their study only seven species and only one with two clefts, which had a well-supported basal position as opposed to the six other species, which all have three clefts.

Apart from the clefts, the morphology of the lips can also vary from one species to another; species with three clefts have either smooth lips or crenulated upper lips; species with two clefts have either a crenulated upper lip or with papillae. Species with no cleft have an upper lip with papillae (Figure 3). So crenulated upper lips are found both in clades A and B, whereas smooth lips are only found in clade A and papillae are only found in clade B. In other words, the absence or presence and number of clefts and the presence of papillae can be used as characters to classify the different species in the two different clades, whereas the crenulated upper lip character could be an evolutionary convergence between the two clades.

4.3 | A mouth for climbing

The mouth is of great importance in the *Sicyopterus* genus for the success of the upstream migration. Indeed, *Sicyopterus* species, and more generally Sicydiinae gobies, have an extraordinary climbing ability. The strongly effective pelvic suction cup and well-developed pectoral fins, combined with the use of the mouth as a secondary sucker, allow Sicydiinae gobies to rapidly access the upper reaches of the river above waterfalls (Keith, 2003). Studies on climbing performances of the *Sicyopterus* genus were done on the Hawaiian species, *S. stimpsoni*, which have a smooth upper lip with three clefts (Figure 3, clade A); this species "inches up" vertical surfaces by alternately attaching oral and pelvic suckers to the substrate (Schoenfuss & Blob, 2003). As the oral disk attaches to the substrate, it expands to almost twice its resting area (and this is facilitated by the presence of the three clefts) after which the posterior body is pulled upwards; once the pelvic disk attaches, the oral disk releases and the anterior body advances. The mouth is thus used as a secondary locomotor organ (Keith et al., 2015). As opposed to the climbing technique used by *S. stimpsoni* (inching up), *Sicydium punctatum* (also with smooth upper lip and three clefts) climbs by using substantial axial fin movement (Kawano, Bridges, Schoenfuss, Maie, & Blob, 2013), like *Lentipes concolor* (smooth upper lip, no cleft) (*Sicydium* and *Lentipes* genera both belong to the Sicydiinae subfamily). This latter climbing behavior is referred to as "powerburst climbing" (Schoenfuss & Blob, 2003). Bouts of powerburst climbing by *L. concolor* begin in or near direct water flow and are initiated by a single, rapid adduction of the pectoral fins. Kawano et al. (2013) noted that *S. stimpsoni* and *S. punctatum* showed different selection patterns due to their different climbing behavior. Stronger selection was noted for *S. punctatum*, as its climbing style requires more movements of the fins and body axis than *S. stimpsoni*, and because powerburst climbers must detach their pelvic sucker from the substrate in order to propel their body (Blob et al., 2008). *S. stimpsoni*, an "inching" climber, is constantly attached to the substrate due to the alternate use of oral and pelvic suckers (Schoenfuss & Blob, 2003). An interesting next step for our study would be to quantify the climbing performance and behavior of other *Sicyopterus* species with the same and different mouth morphologies (two clefts, no cleft, crenulated, papillae, etc.) to assess how variation in mouth morphology may contribute to variation in climbing biomechanics and capabilities, and species' altitudinal zonation observed within the rivers (see further, "A mouth for feeding").

4.4 | A mouth for feeding

Sicydiinae gobies climb in altitude to find suitable territories to settle and their herbivorous or omnivorous feeding modes allow them

to exploit the richest source of food in these distinctive habitats. *Sicyopterus* species are all herbivorous, scraping algae off rock surfaces, using their tricuspid teeth and their upper lip nearly as soon as they enter the river after their dispersal at sea (Keith et al., 2015). Seven days after the recruitment in freshwater, *S. japonicus* shows a single row of closely set tricuspid teeth along the entire length of each upper jaw (Sahara, Moriyama, Iida, & Watanabe, 2016). These teeth have a unique feature of pedicellate attachment enhancing the ability of individual functional tooth to move closely over irregularities in the rock surfaces during the scraping of algae (Sahara, Moriyama, Iida, & Watanabe, 2013). All *Sicyopterus* species have the same type of teeth, that is, tricuspid teeth on the premaxillary, except *S. lividus*, which has bicuspid teeth on the premaxillary (Keith et al., 2015).

The development of the benthic algal community begins with motile species of diatoms and short tuft-like algal colonies (Julius, Blob, & Schoenfuss, 2005; Tuji, 2000). In *S. stimpsoni* gut contents, the presence of short algae and diatoms indicates that they only feed off rock surfaces and that the algal succession is continually reinitiated. *S. stimpsoni* (Fitzimons et al., 2003) and *S. punctatum* (Barbeyron et al., 2017) maintain "gardens" by continuously grazing the same patch of rock, the territory, thus maintaining their preferred species. In Guadeloupe rivers, two *Sicydium* species co-occur in the same rivers: *Sicydium punctatum* and *Sicydium plumieri*. It has recently been shown that these two species have a different diet, with *S. punctatum* preferring pedunculate diatom species and *S. plumieri* feeding on ribbon-shaped diatoms (Monti et al., 2018). Both species have smooth upper lips and three clefts, but their teeth are different. *S. plumieri* has strong unicuspid teeth, and *S. punctatum* has more fragile tricuspid teeth (Watson, 2000). Although their trophic niches partially overlap, these results suggest that closely related sympatric species show some level of specialization in their feeding behavior.

The differences in feeding behavior is of particular interest when we know that, in the Western Pacific, several *Sicyopterus* species live in sympatry in the same rivers (Figure 1). Species zonation can be observed as some species can be found all along the river, only from the lower to middle courses or only in the upper reaches; but different species of the same genera can also have an overlapping distribution (Keith & Lord, 2011a). In some areas, no less than three species of *Sicyopterus* may be found in the same river, such as *Sicyopterus lagocephalus*, *Sicyopterus cynocephalus*, and *Sicyopterus stiphodonoides* (Poitete River, Kolobangara, Solomon Islands, Keith & Lord pers.obs). *S. stiphodonoides'* upper lip has no cleft and has papillae, while the upper lip of the other two species is smooth with three clefts. *S. franouxi* and *S. punctissumus* co-occur in streams from Madagascar; although both have three clefts, *S. franouxi* has a crenulated upper lip while *S. punctissimus* has a smooth upper lip. In Micronesia, *S. eudentatus* (two clefts with papillae on the upper lip) and *S. lividus* (three clefts with a crenulated upper lip), both endemic species, are found thriving in the same rivers (Figure1; Table 1). Mechanistically, feeding involves a cyclical protrusion of the premaxilla to scrape diatoms from the substrate. The presence of clefts, whether there are 2 or 3, may be an advantage for the lip to adhere better to the substrate while scraping but also to help the oral sucker to come loose at each cycle. The difference in lip morphology may also play a role in the microalgal selection, potentially contributing to non-overlapping trophic niches for co-occurring species within the same reach of a river. It would be interesting to study the feeding behavior of Sicydiinae species with different mouth morphologies, to see whether having 0, 2, or 3 clefts can change the capacity to feed on short or pedunculate diatom species for example.

4.5 | Climbing and feeding: similar mechanisms involved

To climb waterfalls, the oral sucker is cyclically protruded and attached to the climbing surface; to feed, the premaxilla is cyclically protruded to scrape diatoms from the substrate. The current data cannot resolve whether oral movements for climbing were co-opted from feeding or feeding movements co-opted from climbing. However, similarities between feeding and climbing kinematics in *S. stimpsoni*, for example, are consistent with evidence of exaptation with modifications, between these behaviors (Cullen, Maie, Schoenfuss, & Blob, 2013).

Longitudinal species' zonation within a river could reflect differences in both feeding behavior and climbing abilities due to mechanical differences among mouth morphologies. The oral sucker applies its greatest force at maximal expansion (Blob et al., 2007), and an upper lip presenting clefts will have a greater expansion potential compared to a lip devoid of clefts. Generally, species with three clefts climb higher (Keith et al., 2015; pers. obs). Therefore, a greater number of clefts may confer advantages for climbing and feeding behaviors. Out of the 24 known species of *Sicyopterus*, there are 21 species presenting clefts while only three have no clefts (Keith et al., 2005). The presence of clefts is thus likely to be an adaptation to the benthic-feeding mode and to the settlement in different parts of rivers by the climbing behavior. The lip morphology may facilitate life in sympatry, allowing species to colonize different habitats. Species with different lip morphology may be able to graze different algal species from rock surfaces, but they also might have different climbing abilities. Although *Sicyopterus* species are faced with similar environmental conditions (short and steep fast-flowing rivers), the responses generated phenotypic diversity (Blackledge & Gillespie, 2004; Eroukhanoff et al., 2009) such as different mouth morphologies.

4.6 | Upper lip ornaments: evolutionary novelties?

Endemic *Sicyopterus* species emerged during the Pliocene period and preceded *S. lagocephalus* (three clefts, smooth upper lip) radiation (Keith et al., 2005). Both clades A and B in the mitogenome phylogenetic reconstruction have a simultaneous appearance, so it is not possible to determine an ancestral state with this phylogeny. Other Sicydiinae genera have different mouth morphologies. For instance, *Sicyopus*, *Smilosicyopus*, *Stiphodon*, *Cotylopus* (Keith, Hoareau, & Bosc, 2007), and *Akihito* never exhibit clefts nor papillae or crenulated upper lips (Keith et al., 2015). *Lentipes* species

sometimes have a very small median cleft but more often no cleft at all with a smooth upper lip (Keith et al., 2015). Finally, the *Sicydium* genera, which has a sister relationship with *Sicyopterus*, can exhibit three clefts on the smooth upper lip or crenulated upper lip with one median cleft (Harrison, Miller, & Pezold, 2008). As *Sicyopterus* and *Sicydium* share a sister relationship, it is not surprising to find the same type of mouth morphologies, but there are no *Sicydium* species without clefts. In previous phylogenies of Sicydiinae gobies, *Stiphodon* or *Cotylopus* recover a basal position, placing *Sicyopterus* and *Sicydium* as more derived taxa (Keith et al., 2011). The smooth, cleft-free upper lip may be regarded as an ancestral state for Sicydiinae gobies, and the appearance of clefts or any other ornament of the upper lip may be regarded as a derived character, *that is*, the appearance of 2–3 clefts in the *Sicyopterus* genus might then be a derived character, and the presence of a clade with three clefts and one with two seems to be an evolutionary convergence. Additional studies are needed to assess whether the presence of clefts is indeed evolutionary novelties, rather than ancestral retention, resulting from an adaptation to the colonization of short, steep, and fast-flowing rivers, or an adaptation to feeding in environments poor in nutrients and to sympatric life.

5 | CONCLUSION

In this paper, 18 *Sicyopterus* species described with morphological characters were genetically confirmed for the first time, based on 13 mitochondrial protein-coding genes. The phylogenetic reconstruction based on mitogenome data allowed the distinction of the 18 species based on their gene sequences, even for recent speciation events, and it also allowed the resolution of interspecific relationships. Hence, two well-supported clades were recovered with a strong correlation to the mouth morphology of *Sicyopterus* species. We thus found a group with three clefts on the upper lip and one group with two or no clefts. The morphology of the mouth is of great importance in the *Sicyopterus* genus, as it is used for feeding and as a secondary sucker for climbing. Many Sicydiinae gobies live in sympatry, with often several species of the same genus inhabiting the same rivers. For *Sicyopterus* species, the diversity in mouth morphologies has played no small role in their ability to successfully colonize and inhabit environmentally challenging tropical island rivers. Colonization of island riverine systems with steep waterfalls is facilitated by *Sicyopterus*' exceptional climbing capabilities. Exploitation of rich diatomaceous and algal food sources in nutrient-poor environments is possible because of *Sicyopterus*' benthic herbivorous feeding mode. Differential niche occupancy may in part be due to *Sicyopterus*' capacity to feed on different algal communities. Further, the search for food in upper reaches has been thought to play a key role in the upstream migration of amphidromous species (Gross, Coleman, & McDowall, 1988). The order in the emergence of the climbing and grazing mechanisms remains unknown, but they are closely linked, as it is well illustrated in the *Sicyopterus* genus. The study of the various mechanisms leading to the slight differences between the different species in terms of climbing abilities and habitat preferences, and enabling them to co-occur, remains to be done. As a perspective to this work, one of the aims would be to include the six *Sicyopterus* species missing in our data set. It would also be interesting to undertake the same analysis on *Sicyopterus*'s sister genus, *Sicydium* and to study the evolutionary convergence between those two groups in terms of mouth morphology and its role in climbing efficiency and feeding specialization.

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AUTHOR CONTRIBUTIONS

Clara Lord conceived and designed the study, acquired the data, extracted and amplified the data, analyzed and interpreted the data, and drafted the article. As an English–French bilingual, the manuscript is submitted in grammatically correct English. Laure Bellec contributed to the analysis and interpreted the data. Agnès Dettaï and Céline Bonillo helped in the acquisition of the data and performed DNA sequencing using the Ion Torrent at the "Service de Systématique Moléculaire" of the MNHN. Philippe Keith conceived and designed the study, acquired the data, critically revised the article for intellectual content and approved the final version of the manuscript to be published.

ORCID

Clara Lord https://orcid.org/0000-0003-1520-9977

REFERENCES

Barbeyron, C., Lefrançois, E., Monti, D., Keith, P., & Lord, C. (2017). Gardening behaviour of *Sicydium punctatum* (Teleostei: Gobioidei: Sicydiinae): *In vitro* experiments in the context of chlordecone pollution in Guadeloupe Island rivers. *Cybium: International Journal of Ichthyology*, *41*(2), 85–92.

Blackledge, T. A., & Gillespie, R. G. (2004). Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. *Proceedings of the Natural Academy of Sciences of the United States of America*, *101*, 16228–16233. https://doi.org/10.1073/pnas.0407395101

Blaxter, M. (2003). Molecular systematics: Counting angels with DNA. *Nature*, *421*, 122–124. https://doi.org/10.1038/421122a

Blob, R. W., Bridges, W. C., Patcek, M. B., Maie, T., Cediel, R., Bertolas, M. M., … Shoenfuss, H. (2008). Morphological selection in an extreme flow environment: Body shape and waterfall-climbing success in the Hawaiian stream fish *Sicyopterus stimpsoni*. *Integrative and Comparative Biology*, *48*(6), 734–749. https://doi.org/10.1093/icb/icn086

Blob, R. W., Wright, K. M., Becker, M., Maie, T., Iverson, T. J., Julius, M. L., & Schoenfuss, H. L. (2007). Ontogenetic change in novel functions: Waterfall climbing in adult Hawaiian gobiid fishes. *Journal of Zoology*, *273*, 200–209. https://doi.org/10.1111/j.1469-7998.2007.00315.x

Campbell, M. A., Lopez, J. A., Sado, T., & Miya, M. (2013). Pike and salmon as sister taxa: Detailed intraclade resolution and divergence time estimation of Esociformes + Salmoniformes based on whole mitochondrial genome sequences. *Gene*, *530*, 57–65. https://doi.org/10.1016/j.gene.2013.07.068

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chiang, T.-Y., Chen, I.-S., Lin, H. D., Chang, W. B., & Ju, Y. M. (2013). Complete mitochondrial genome of *Sicyopterus japonicus* (Perciformes, Gobiidae). *Mitochondrial DNA*, *24*(3), 191–193. https://doi.org/10.3109/19401736.2012.744980

Chiang, T.-Y., Chen, I.-S., Lin, H. D., Hsiao, S. T., & Ju, Y. M. (2013). Complete mitochondrial genome of the amphidromous, red-tailed goby *Sicyopterus lagocephalus* (Pallas) (Teleostei, Gobiidae). *Mitochondrial DNA*, *26*(5), 670–671. https://doi.org/10.3109/19401736.2013.840600

Cullen, J. A., Maie, T., Schoenfuss, H. L., & Blob, R. W. (2013). Evolutionary novelty versus exaptation: Oral kinematics in feeding versus climbing in the waterfall-climbing Hawaiian goby *Sicyopterus stimpsoni*. *PLoS ONE*, *8*, e53274. https://doi.org/10.1371/journal.pone.0053274

Dowton, M., Meiklejohn, K., Cameron, S. L., & Wallman, J. (2014). A preliminary framework for DNA barcoding, incorporating the multispecies coalescent. *Systematic Biology*, *63*, 639–644. https://doi.org/10.1093/sysbio/syu028

Ellien, C., Valade, P., Bosmans, J., Taillebois, L., Teichert, N., & Keith, P. (2011). Influence of salinity on larval development of *Sicyopterus lagocephalus* (Pallas, 1770) (Gobioidei). *Cybium: International Journal of Ichthyology*, *35*(4), 381–390.

Ellien, C., Werner, U., & Keith, P. (2016). Morphological changes during the transition from freshwater to sea water in an amphidromous goby, *Sicyopterus lagocephalus* (Pallas 1770) (Teleostei). *Ecology of Freshwater Fish*, *25*, 48–59. https://doi.org/10.1111/eff.12190

Eroukhmanoff, F., Hargeby, A., Arnberg, N. N., Hellgren, O., Bensch, S., & Svensson, E. I. (2009). Parallelism and historical contingency during rapid ecotype divergence in an isopod. *Journal of Evolutionary Biology*, *22*, 1098–1110. https://doi.org/10.1111/j.1420-9101.2009.01723.x

Fitzsimons, J. M., McRae, M. G., Schoenfuss, H. L., & Nishimoto, R. T. (2003). Gardening behavior in the amphidromous Hawaiian fish *Sicyopterus stimpsoni* (Osteicthyes: Gobiiidae). *Ichthyological Exploration of Freshwaters*, *14*, 185–191.

Galtier, N., Nabholz, B., Glémin, S., & Hurst, G. D. D. (2009). Mitochondrial DNA as a marker of molecular diversity: A re-appraisal. *Molecular Ecology*, *18*, 4541–4550. https://doi.org/10.1111/j.1365-294X.2009.04380.x

Gross, M. R., Coleman, R. M., & McDowall, R. M. (1988). Aquatic productivity and the evolution of diadromous fish migration. *Science*, *239*, 1291–1293. https://doi.org/10.1126/science.239.4845.1291

Harrison, I. J., Miller, P. J., & Pezold, F. (2008). Gobiidae. In M. Stiassny, G. G. Teugels, & C. D. Hopkins (Eds.), *The fresh and brackish water fishes of lower guinea, west-central africa* (pp. 491–544). Paris:IRD Publications Scientifiques du Muséum. Faune et Flore Tropicale 42(2).

Hebert, P. D. N., Ratnasingham, S., & De Waard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B*, *270*, S96–S99. https://doi.org/10.1098/rsbl.2003.0025

Hinsinger, D. D., Debruyne, R., Thomas, M., Denys, G. P. J., Mennesson, M., Utge, J., & Dettaï, A. (2015). Fishing for barcodes in the Torrent: From COI to complete mitogenomes on NGS platforms. *DNA Barcodes*, *3*, 170–186. https://doi.org/10.1515/dna-2015-0019

Hoareau, T., Lecomte-Finiger, R., Grondin, H.-P., Conand, C., & Berrebi, P. (2007). Oceanic larval life of La Réunion ''bichiques'' amphidromous gobiid post-larvae. *Marine Ecology Progress Series*, *333*, 303–308. https://doi.org/10.3354/meps333303

Inoue, J. G., Miya, M., Tsukamoto, K., & Nishida, M. (2003). Basal actinopterygian relationships: A mitogenomic perspective on the phylogeny of the ''ancient fish''. *Molecular Phylogenetics and Evolution*, *26*, 110–120. https://doi.org/10.1016/S1055-7903(02)00331-7

Iwasaki, W., Fukunaga, T., Isagozawa, R., Yamada, K., Maeda, Y., Satoh, T. P., … Nishida, M. (2013). MitoFish and MitoAnnotator: A mitochondrial genome database of fish with accurate and automatic annotation pipeline. *Molecular Biology and Evolution*, *30*, 2531–2540. https://doi.org/10.1093/molbev/mst141

Julius, M. L., Blob, R. W., & Schoenfuss, H. L. (2005). The survival of *Sicyopterus stimpsoni*, an endemic amphidromous Hawaiian gobiid fish, relies on the hydrological cycles of streams: Evidence from changes in algal composition of diet through growth stages of fish. *Aquatic Ecology*, *39*, 473–484.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*, 3059–3066. https://doi.org/10.1093/nar/gkf436

Kawano, S. M., Bridges, W. C., Schoenfuss, H. L., Maie, T., & Blob, R. W. (2013). Differences in locomotor behavior correspond to different patterns of linear and nonlinear morphological selection in two species of waterfall-climbing gobiid fishes. *Evolutionary Ecology*, *27*, 949–969.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keith, P. (2003). Biology and ecology of amphidromous Gobiidae in the Indo-Pacific and the Caribbean regions. *Journal of Fish Biology*, *63*, 831–847.

Keith, P., Galewski, T., Cattaneo-Berrebi, G., Hoareau, T., & Berrebi, P. (2005). Ubiquity of *Sicyopterus lagocephalus* (Teleostei: Gobioidei) and phylogeography of the genus *Sicyopterus* in the Indo-Pacific area inferred from mitochondrial cytochrome *b* gene. *Molecular Phylogenetics and Evolution*, *37*, 721–732. https://doi.org/10.1016/j.ympev.2005.07.023

Keith, P., Hoareau, T., & Bosc, P. (2007). The genus *Cotylopus* (Teleostei: Gobioidei) endemic to the rivers of islands of the Indian Ocean with description of a new species from Mayotte (Comoros). *Journal of Natural History*, *39*(17), 1395–1406. https://doi.org/10.1080/00222930400007456

Keith, P., Hoareau, T., Lord, C., Ah-Yane, O., Gimmonneau, G., Robinet, T., & Valade, P. (2008). Characterisation of postlarval to juvenile stages, metamorphosis, and recruitment of an amphidromous goby, *Sicyopterus lagocephalus* (Pallas 1767) (Teleostei: Gobiidae: Sicydiinae). *Marine and Freshwater Research*, *59*, 876–889. https://doi.org/10.1071/mf08116

Keith, P., & Lord, C. (2011a). Tropical freshwater gobies: Amphidromy as a life cycle. In R. A. Patzner, J. L. Van Tassell, M. Kovacic, & B. G. Kapoor (Eds.), *The Biology of Gobies* (pp. 119–128). St. Helier, Jersey: Science Publishers.

Keith, P., & Lord, C. (2011b). Systematics of Sicydiinae. In R. A. Patzner, J. L. Van Tassell, M. Kovacic, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 243–277). St. Helier, Jersey: Science Publishers.

Keith, P., Lord, C., Lorion, J., Watanabe, S., Tsukamoto, K., Cruaud, C., … Dettaï, A. (2011). Phylogeny and biogeography of Sicydiinae (Teleostei: Gobioidei) inferred from mitochondrial and nuclear genes. *Marine Biology*, *158*, 311–326. https://doi.org/10.1007/s00227-010-1560-z

Keith, P., Lord, C., & Maeda, K. (2015). *Indo-Pacific Sicydiinae Gobies. Biodiversity, life traits and conservation* (p. 256). Paris, France: Société Française d'Ichtyologie (Ed).

Keith, P., Marquet, G., Gerbeaux, P., Vigneux, E., & Lord, C. (2013). *Poissons et crustacés d'eau douce de Polynésie* (p. 282). Paris, France: Société Française d'Ichtyologie (Ed).

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the Natural Academy of Sciences of the United States of America*, *86*, 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695–1701. https://doi.org/10.1093/molbev/mss020

Lekshmi, R. S., & Soni, K. B. (2007). Barcoding of life. *Millennium Zoology*, *8*(1), 44–46.

Li, B., Dettaï, A., Cruaud, C., Couloux, A., Desoutter-Meniger, M., & Lecointre, G. (2009). RNF213, a new nuclear marker for acanthomorph phylogeny. *Molecular Phylogenetics and Evolution*, *50*, 345–363. https://doi.org/10.1016/j.ympev.2008.11.013

Lord, C., Brun, C., Hautecœur, M., & Keith, P. (2010). Insights on endemism: Comparison of the marine larval duration estimated by otolith microstructural analysis of three amphidromous *Sicyopterus* species (Gobiidae: Sicydiinae) from Vanuatu and New Caledonia. *Ecology of Freshwater Fish*, *19*, 26–38. https://doi.org/10.1111/j.1600-0633.2009.00386.x

Lord, C., & Keith, P. (2008). Threatened fishes of the world: *Sicyopterus sarasini* Weber & de Beaufort, 1915 (Gobiidae). *Environmental Biology of Fishes*, *83*, 169–170. https://doi.org/10.1007/s10641-007-9311-9

Lord, C., Lorion, J., Dettaï, A., Watanabe, S., Tsukamoto, K., Cruaud, C., & Keith, P. (2012). From endemism to widespread distribution: Phylogeography of three amphidromous *Sicyopterus* species (Teleostei: Gobioidei: Sicydiinae). *Marine Ecology Progress Series*, *455*, 269–285. https://doi.org/10.3354/meps09617

Manacop, R. (1953). The life story and habits of the goby, *Sicyopterus extraneus*, Gobiidae with an account of the gobyfry fishery of Cagayan River, oriental misamis. *Philippines Journal of Fisheries*, *2*, 1–58.

McDowall, R. M. (1988). *Diadromy in fishes: Migrations between freshwater and marine environments*. London, UK: Croom Helm.

McDowall, R. M. (1997). Is there such a thing as amphidromy? *Micronesica*, *30*(1), 3–14.

McDowall, R. M. (2004). Ancestry and amphidromy in island freshwater fish fauna. *Fish and Fisheries*, *5*, 75–85.

Miya, M., & Nishida, M. (2000). Use of mitogenomic information in teleostean molecular phylogenetics: A tree-based exploration under the maximum-parsimony optimality criterion. *Molecular Phylogenetics and Evolution*, *17*, 437–455. https://doi.org/10.1006/mpev.2000.0839

Miya, M., & Nishida, M. (2015). The mitogenomic contributions to molecular phylogenetics and evolution of fishes: A 15-year retrospect. *Ichtyological Research*, *62*(1), 29–71. https://doi.org/10.1007/s10228-014-0440-9

Monti, D., Lefrançois, E., Lord, C., Mortillaro, J.-M., Lopez, P. J., & Keith, P. (2018). Selectivity on epilithic diatom consumption for two tropical sympatric gobies: Sicydium punctatum (Perugia 1986) and Sicydium plumieri (Bloch 1786). *Cybium: International Journal of Ichthyology*, *42*(4), 365–373.

Peng, Z., He, S., Wang, J., Wang, W., & Diogo, R. (2006). Mitochondrial molecular clocks and the origin of the major otocephalan clades (Pisces: Teleostei): A new insight. *Gene*, *370*, 113–124. https://doi.org/10.1016/j.gene.2005.11.024

Rambaut, A. (2012). *Figtree 1.4.0*. Retrieved from http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Sahara, N., Moriyama, K., Iida, M., & Watanabe, S. (2013). Unique features of pedicellate attachment of the upper jaw teeth in the adult gobiid fish *Sicyopterus japonicus* (Teleostei, Gobiidae): Morphological and structural characteristics and development. *Journal of Morphology*, *274*(5), 512–524. https://doi.org/10.1002/jmor.20111

Sahara, N., Moriyama, K., Iida, M., & Watanabe, S. (2016). Time and order of eruption of first functional teeth in the upper jaw of post-larval life of *Sicyopterus japonicus* (Gobiidae:Sicydiinae) during cranial metamorphosis at the time of river recruitment. *Archives of Oral Biology*, *66*, 8–14.

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., & Lane, R. (2005). Towards writing the encyclopaedia of life: An introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*, 1805–1811. https://doi.org/10.1098/rstb.2005.1730

Schoenfuss, H. L., & Blob, R. W. (2003). Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): Vertical propulsion at the aquatic-terrestrial interface. *Journal of Zoology*, *261*, 191–205. https://doi.org/10.1017/S0952836903004102

Taillebois, L., Castelin, M., Lord, C., Chabarria, R., Dettaï, A., & Keith, P. (2014). New Sicydiinae phylogeny (Teleostei: Gobioidei) inferred from mitochondrial and nuclear genes: Insights on evolutionary history and geographical ranges. *Molecular Phylogenetics and Evolution*, *70*, 260–271. https://doi.org/10.1016/j.ympev.2013.09.026

Taillebois, L., Keith, P., Valade, P., Torres, P., Baloche, S., Dufour, S., & Rousseau, K. (2011). Involvement of thyroid hormones in the control of larval metamorphosis in *Sicyopterus lagocephalus* (Teleostei: Gobioidei) at the time of river recruitment. *General and Comparative Endocrinology*, *173*, 281–288. https://doi.org/10.1016/j.ygcen.2011.06.008

Teacher, A. G., André, C., Merila, J., & Wheat, C. W. (2012). Whole mitochondrial genome scan for population structure and selection in the Atlantic herring. *BMC Evolutionary Biology*, *12*, 248. https://doi.org/10.1186/1471-2148-12-248

Tuji, A. (2000). Observation of developmental processes in loosely attached diatom (Bacillariophyceae) communities. *Phycological Research*, *48*, 75–84. https://doi.org/10.1111/j.1440-1835.2000.tb00199.x

Watson, R. E. (2000). *Sicydium* from the Dominican Republic with description of a new species. *Stuttgarter Beiträge Zur Naturkunde*, *A*, 1–31.

Zardoya, R., & Meyer, A. (1996). The complete nucleotide sequence of the mitochondrial genome of the lungfish (*Protopterus dolloi*) supports its phylogenetic position as a close relative of land vertebrates. *Genetics*, *142*, 1249–1263.

SUPPORTING INFORMATION

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