UNDERSTANDING SPECIES DIVERSITY OF THE AMPHIDROMOUS INDOPACIFIC GOBY GENUS STIPHODON (GOBIIDAE: SICYDIINAE)

A Thesis

by

Yer Lor

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Gobies constitute a great majority of fishes seen in oceanic island fish communities. Of particular interest in these communities are the amphidromous gobies of the subfamily Sicydiinae. Adult gobies spawn upstream in freshwaters from which newly hatched larvae are washed downstream to the sea. These larvae spend anywhere between 91-265 days at sea before returning to freshwater streams. This marine pelagic larval phase is believed to be the main mechanism behind the spatial and temporal dispersal of these species. However, very little life history information is known about these gobies. Males of the genus *Stiphodon* are brightly colored, but females are drab in coloration and pattern. Male coloration is the primary characteristic used to distinguish between these species, however, subtle differences in male coloration, overlapping distributions, as well as a lack of diagnostic morphological characteristics makes it difficult to distinguish species. Historically, most studies have been on identifying and describing species of *Stiphodon* by using morphological and pigmentary characteristics. More recently, molecular systematics and phylogenetic methods have been used to infer species delineations. This study is the most comprehensive phylogenetic analysis of this genus using three nuclear genes to determine species diversity and relationships among species. All nuclear phylogenetic trees support monophyly of the genus and recognize the presence of two clades, one more diverse than the other.

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1. Introduction

The family Gobiidae (Gill and Mooi, 2012; Taillebois *et al.*, 2014) consists of more than 1700 goby species inhabiting brackish, marine, fresh waters (Eschmeyer *et al.*, 2016). Gobies are one of the most diverse group of vertebrates in the world, second only to the Cyprinidae. They greatly contribute to the diversity seen in a number of aquatic habitats, including oceanic island streams. These oceanic island streams are dominated by gobies of the subfamily Sicydiinae. This subfamily contains nine genera of freshwater gobies: *Sicydium* Valenciennes, 1837; *Sicyopterus* Gill, 1860; *Lentipes* Gunther, 1861; *Sicyopus* Gill 1863; *Cotylopus* Guichenot, 1864; *Stiphodon* Weber, 1895; *Parasicydium* Risch, 1980; *Smilosicyopus* Watson, 1999 and *Akihito* Watson, Keith, and Marquet, 2007.

Adult sicydiine gobies can be found in tropical freshwaters in the Caribbean, the Indo-Pacific, West Africa, and Central America where they greatly contribute to the diversity of the freshwater fish communities (Keith and Lord, 2011; Watson, 1995). This particular group of gobies exhibits an amphidromous lifestyle. Amphidromy is a form of diadromy in which larvae, spawned in freshwater, undergo a marine pelagic developmental stage and eventually return upstream as juveniles (Keith *et al.*, 2009; Maeda *et al.*, 2011a; McDowall, 2007). This marine pelagic larval phase varies between 91-265 days in sicydiines and is believed to be essential in explaining the spatial and temporal patterns of dispersal (Keith, 2003; Taillebois *et al*., 2014; Yamasaki and Tachihara, 2006).

Sicydiine gobies share many characteristics that enable the amphidromous life style and allow them to exploit high gradient tropical freshwater streams. These include pelvic fins modified into sucker disks, the soft parts and teeth associated with the jaws and jaws suspensorium modifications (Harrison, 1989; Keith and Lord, 2011; Parenti and Maciolek, 1993; Watson, 1995). These characteristics have facilitated the ability of amphidromous gobies to

ascend waterfalls to exploit high island streams (Keith and Lord, 2011). Many sicydiine gobies are considered rare or endemic to specific regions, but some are wide-ranging. The most widespread sicydiine goby is *Sicyopterus lagocephalus*, ranging across 18,000 km from the western Indian Ocean to the south central Pacific Ocean (Keith *et al*., 2005).

While recent research on sicydiine gobies has included some studies of amphidromy and marine pelagic larval duration (White, 2015; Yamasaki and Tachihara, 2006), phylogeography (Chabarria and Pezold, 2013; Lord *et al.*, 2012), molecular phylogenetics (Keith *et al.*, 2011; Taillebois *et al.*, 2014), and population genetics (Chabarria *et al.*, 2014; Taillebois *et al.*, 2013), most have focused on species descriptions. In the last six years (2010-2016), twenty sicydiines were described. The majority of these new species were described in the genera *Stiphodon* (7 species) and *Sicyopterus* (6 species) (Eschmeyer *et al.*, 2016). Sicydiine taxonomy has been labeled as "chaotic" and "confusing" due to the rapid increase in the number of recognized but morphologically similar species (Watson and Kottelat, 1995; Watson, 1995). Phylogenetic studies using mitochondrial and nuclear genes have recently been used to test hypotheses of sicydiine relationships based upon morphological data and biogeographic patterns (Keith *et al*., 2011; Taillebois *et al.*, 2014). However, these studies have offered little to clarify questions of species validity or diversity. Being focused on maximizing representation across sicydiine genera, the number of species examined within a particular genus has been limited.

The genus *Stiphodon* is comprised of herbivorous gobies that live in open slow and swiftly moving freshwaters (riffles and pools) throughout the Indo-Pacific region from Sri Lanka in the eastern Indian Ocean to French Polynesia (Keith *et al.*, 2009; Maeda, 2014; Watson and Chen, 1998; Watson, 1998, 1996, 1995; Watson *et al.*, 1998) (Figure 2). Adult *Stiphodon* grow to be between 15.5 mm (*S. astilbos*) to 64.0 mm (*S. multisquamus*) in size (Maeda *et al.*, 2015;

Ryan, 1986) and live up to about two years (Yamasaki and Tachihara, 2006). Currently, there are thirty-one recognized species of *Stiphodon* (Eschmeyer *et al.*, 2016). Some species are wide-ranging, i.e., found on more than one archipelago or island (e.g. *S. pelewensis, S. atratus, S. rutilaureus* and *S. ornatus*), while others are endemic to a particular island or archipelago (e.g. *S. annieae, S. discotorquatus, S. niraikanaiensis*, and *S. tuivi*). New species descriptions are still being published such as that of *S. aureofuscus* (Keith *et al.*, 2015a) and *S. palawanensis* (Maeda and Palla, 2015).

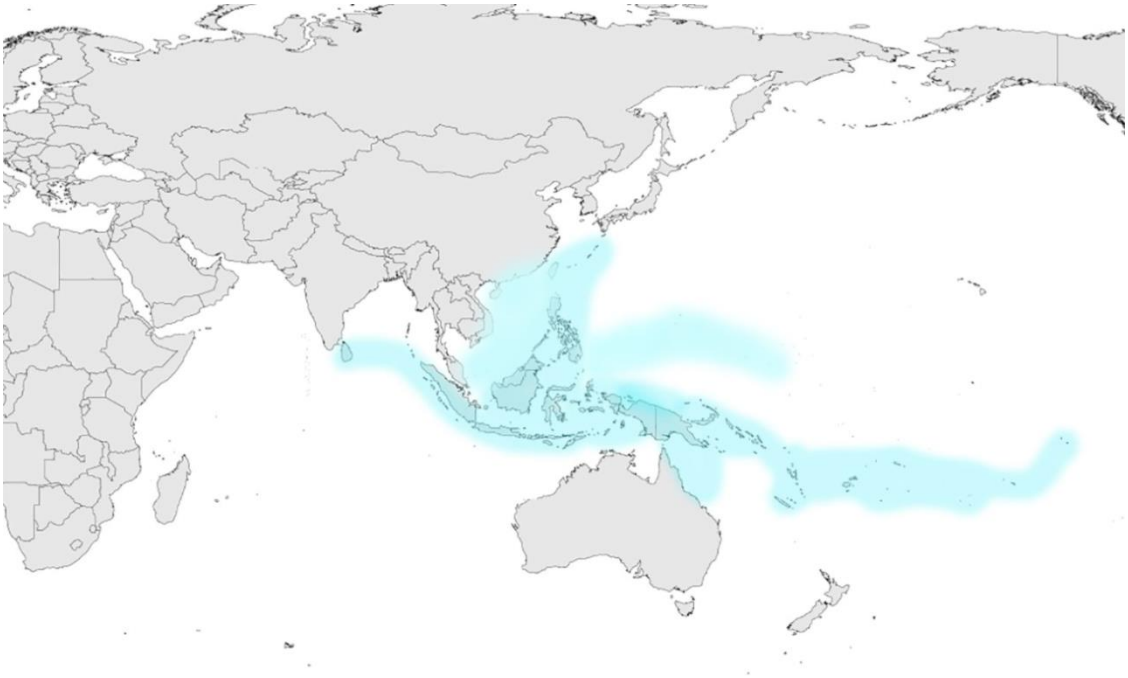

Figure 2. Known *Stiphodon* genus distribution.

Historically, distinguishing *Stiphodon* species has been done by using morphological characters such as the number of teeth in the upper jaw, number of pectoral rays, number and presence or absence of scales in several regions, and the coloration of males (Watson and Kottelat, 1995). Species distinctions can be difficult because of subtle color differences between the males of some species, overlapping ranges of scale and ray counts, poor understanding of

species distributions, and changes in number of tooth and scale counts as specimens grow in size (Maeda *et al.*, 2011a; Maeda *et al.*, 2011b; Maeda *et al.*, 2015). Males also change colors for mating purposes or have been found to have two different color morphs depending on the size of the male as displayed in *S. alcedo*, *S. atropurpureus*, *S. imperiorientis*, *S. niraikanaiensis*, *S. pelewensis* and *S. percnopterygionus* (Maeda *et al.*, 2011a; Maeda *et al.*, 2011b; Maeda, 2014; Nip, 2010). It is important to note that half of the thirty-one recognized species were described based on examining ten or fewer specimens collected from respective type localities; for four species only males were collected for examination and description, so no female descriptions were available for these species. It is not surprising that several *Stiphodon* species previously described have since been determined to be junior synonyms of other *Stiphodon* species, for example, *S. stevensoni* = *S. elegans* (Watson, 1999), *S. olivaceus* = *S. pulchellus* (Maeda *et al.*, 2011b), and most recently *S. aureorostrum* was shown to be a synonym of *S. multisquamus* (Maeda *et al.*, 2015). These synonymies were only based on morphological characteristics.

Attempts to understand the relationships among the nominal species have been few and limited in scope. Several studies (Keith *et al.*, 2011; Taillebois *et al.*, 2014; Watson and Kottelat, 1995) determined that *Stiphodon* was a monophyletic group split into two clades. Species coverage for *Stiphodon* was limited in these studies because they were not examining intrageneric diversity. Watson and Kottelat (1995) did not explicitly list the species they analyzed, but proposed that the genus was divided according to two geographic regions and the number of pectoral rays (Pacific Basin, 14 pectoral rays and western Pacific Basin, 15 or 16 pectoral rays). Of the 57 specimens analyzed by Keith *et al.* (2011), only 14 specimens represented six species of *Stiphodon*. Only four species of *Stiphodon* were included in the 59 specimens analyzed by Taillebois *et al.* (2014). As a result, it is hard to determine and

understand the true diversity of the genus from studies which included four to six species comprised of fewer than twenty specimens.

In a smaller phylogenetic study, the mitochondrial ND5 gene was used to examine the relationship between four *Stiphodon* species (*S. alcedo, S. atropurpureus, S. imperiorientis,* and *S. percnopterygionus*) from the Ryukyu Archipelago, Japan (Maeda *et al.*, 2011a). A neighbor-joining tree indicated that *S. alcedo* and *S. imperiorientis* were the most closely related with *S. atropurpureus* sister to those two species. *S. percnopterygionus* was the most distantly related to the other three species.

The purpose of the present study is to elucidate species diversity and relationships within sicydiine genus *Stiphodon* using three nuclear genes. Bayesian inference and Maximum Likelihood phylogenetic inference methods will be used to determine relationships within the genus and relationships with other species in the Gobiidae. The nuclear data will then be compared to that of the whole mitochondrial genome provided by Ken Maeda, University of Ryukyus. I predict that species diversity as determined in the nuclear phylogeny will be consistent with the diversity described in morphological studies. Without a good understanding of *Stiphodon* diversity, distributions and levels of endemism, it will be difficult to establish scientifically-sound conservation methods as well as a credible phylogeographic hypothesis.

2. Methods

2.1. Specimen collection

Stiphodon specimens were collected from a number of localities throughout the range of the genus –Pohnpei (2007, 2009), Kosrae (2008), Taiwan (2008), Guam (2008), Solomon Islands (2008-2012), Fiji (2008, 2009), Palau (2009), Vanuatu (2011), New Caledonia (2011), Australia

(2011), Moorea (2011), Marquesas Islands, Samoa, and French Polynesia – by members of the Fish Systematics and Conservation Lab at Texas A&M University-Corpus Christi (TAMU-CC), Dr. Kirill Vinnikov, University of Hawaii at Manoa (Marquesas Islands), and Philippe Keith, Muséum National d'Histoire Naturelle, Paris (Samoa and French Polynesia) were included in this study. DNA samples of *Stiphodon* from Iriomote and Okinawa were also provided by Dr. Ken Maeda, University of Ryukyus. Three nuclear genes (interferon regulatory factor 2 (IRF2), recombination activating gene 1 (RAG1) and rhodopsin (Rh) were sequenced for 141 specimens (Supplemental Materials I Table 1). Details of species and corresponding localities are given in Appendix A.

2.2. DNA extraction and PCR amplification

Total genomic DNA was extracted from ethanol-preserved tissues using the Qiagen ®DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) following the manufacturer's instructions. PCR for the IRF2 nuclear gene was conducted using two different sets of primers; the first set of primers was those reported from Taillebois *et al*. (2014): F34 (5'-CARTGGTGCTACCTSTGCGA-3') and R751 (5'-CGTGGTCYTTCCKGAAGCG-3'). The second set of primers was newly designed primers: Stiph-Prot-F1 (5'-TGGGCCATGCTGTGGGAGTT-3') and StiphProt-R1 (5'-ACCGCTTTGGGACGAGTTDNA-3') (this study). RAG1 was amplified using forward primer GnelR1F (5'-GATCTBGAGGAGGACATYRTGG-3') (Tornabene *et al*., 2013) and the reverse primer Rag1Ra (5'- CGGGCRTAGTTCCCRTTCATCCTCAT-3') as reported in Tornabene and Pezold (2011). Primers reported by Chen *et al*. (2003) were used for amplification of Rh: Rh_193F (5'-NTATGAATAYCCTCAGTACTACC-3') and Rh_1039R (5'-

TGCTTGTTCATGCAGATGTAGA-3'). All corresponding primers for each nuclear gene are given in Table 1. All genes were amplified via PCR using Thermo Scientific DreamTaq Green PCR Master Mix (2X) using the following thermal profile: 2 minutes at 94^{o}C, followed by 35-40 cycles of 40 seconds at 94^{o}C, 60 sec at 53-59^{o}C, 90 sec at 72^{o}C, and a single extra extension period of 5 minutes at 72^{o}C. Respective annealing temperature ranges for each gene can be found in Table 1. PCR products were visualized by running 2 μl of each extraction on a 1.5% agarose gel containing SYBER® Green 1 Nucleic Acid Gel Stain. At least two individuals of each species from all available localities were included for sequencing. However, several species only had one species sequenced per locality due to limited tissues available. Sequencing was carried out by Beckman Coulter Genomics (Danvers, Massachusetts), and sequences were aligned using the program Geneious v8.1.3 (http://www.geneious.com, Kearse *et al*., 2012). Eight *Stiphodon* sequences were obtained from GenBank—two *S. hydoreibatus* and two *S. sapphirinus*—for IRF2 (accession numbers: KF668967, KF668968, KF668965, and KF6689656) and Rh (accession numbers: KF669085, KF669086, KF669083, and KF669084) were also included in both the RAxML and MrBayes analyses for comparison and to increase species representation of *Stiphodon*.

2.3. Data analysis and phylogenetic inference

Two phylogenetic inference statistical methods were used to examine species diversity and relationships of *Stiphodon*. The first method Maximum Likelihood (ML) predicts the likelihood, or the probability of the observed data given a model (evolutionary model or substitution model), topology and branch lengths (Felsenstein, 1981; Holder and Lewis, 2003). In other words, the likelihood measures how well the data agrees with the predictions of the

model, tree topology and branch lengths. The ML software program implemented in this study was the Randomized Axcelerated Maximum Likelihood (RAxML) analysis (Stamatakis, 2006). RAxML 7.2.8 was run using the RAxML plugin in Geneious 8.3.1. More specifically, the GTRGAMMA nucleotide model using the rapid bootstrap method and search for the best-scoring ML tree algorithm was tested for each gene and the concatenated dataset *(*Stamatakis *et al.*, 2008). Each analysis ran for 1000 bootstrap replicates and started with a random tree. Each dataset was partitioned as DNA and codon as instructed by the RAxML plugin. *Rhyacicthys aspro* was not successfully amplified for the IRF2 gene, so an empty sequence consisting of N's was made for the purpose of alleviating any missing data issues in the concatenated dataset, however, the same empty placeholder for *Rhyacicthys aspro* was removed prior to the RAxML analysis of the IRF2 gene. The placeholders were also created for the four Genbank sequences (*S. hydoreibatus* and *S. sapphirinus*) for the RAG1 gene for concatenation, but removed prior to the analysis of just the RAG1 gene. Placeholders were created by editing the sequences as a fasta file to replace the dashes with Ns then imported back into Geneious 8.3.1 to check for any errors.

Bootstrapping was used to assess the confidence in phylogenetic analyses, ML specifically in this case. It was used because it can measure the repeatability, accuracy, and type I error rate of the dataset being analyzed (Alfaro *et al.*, 2003; Hillis and Bull, 1993). In bootstrapping random resampling with replacement of the original dataset was done to generate pseudoreplicates which were then subjected to the same phylogenetic searches as the original dataset. Bootstrap support values were calculated to be the proportion of times that a group was sampled in the pseudoreplicates. A 70% or greater bootstrap proportion represented true clades 95% of the time (Hillis and Bull, 1993).

The second phylogenetic inference method used to infer phylogenetic relationships was Bayesian inference (BI). Bayesian inference also uses the likelihood function, but not in the same way as ML does. This phylogenetic inference method is based on the Bayesian Theorem which is the probability of the model given the data. This is also referred to as the posterior probability which is equal to the likelihood function multiplied by the prior probability (what the researcher's knowledge is prior to the analysis) (Holder and Lewis, 2003). Phylogeny was inferred for each gene independently and on a concatenated dataset using Bayesian program MrBayes v3.2.3 (Ronquist *et al*., 2012) and two parallel Metropolis-coupled Markov Chains (MCMC) were ran concurrently. Each gene and concatenated dataset was run for 20,000,000 generations with a sample frequency of 1,000 generations. MrBayes was run via the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal (Miller *et al.*, 2010). Tracer v1.6 (Rambaut *et al.*, 2014) was used to assess the effective sample sizes (ESS) of the MCMC runs to ensure that they were over 200. A consensus tree was constructed of the two MCMC runs after a ten percent burn-in period. The majority rule consensus trees were visualized and edited using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

The best fitting substitution models were determined for each gene and the concatenated dataset using PartitionFinder v1.1.1 (Lanfear *et al*., 2012) (Table 2). PartitionFinder is a good program to use for model selection because it allows the researcher to partition by codon, select phylogenetic analysis (RAxML, MrBayes or BEAST), and select information criterion (AIC, AICc or BIC) (Table 2). All RAxML and MrBayes model selection analyses were run using AICc (Lanfear *et al.*, 2014). The substitution models found in the best schemes file were used for phylogenetic analysis. As previously mentioned, all partitions for RAxML were run using the GTR+G model selection.

Table 1. Nuclear genes primers used in this study.

Primer name	Sequence (5' to 3')	Product	Annealing tempureature (^{o}C)	Reference
F34	CARTGGTGCTACCTSTGCGA	IRF2	57-59	Taillebois *et al.*, 2013
R751	CGTGGTCYTTCCKGAAGCG	IRF2	57-59	Taillebois *et al.*, 2013
StiphProt-F1	TGGGCCATGCTGTGGGAGTT	IRF2	57-59	This study
StiphProt-R1	ACCGCTTTGGGACGAGTT	IRF2	57-59	This study
Rh_193F	NTATGAATAYCCTCAGTACTACC	Rh	53-56	Chen *et al.*, 2003
Rh_1039R	TGCTTGTTCATGCAGATGTAGA	Rh	53-56	Chen *et al.*, 2003
Rag1Ra	CGGGCRTAGTTCCCRTTCATCCTCAT	RAG1	53-56	Tornabene and Pezold, 2011
GnelR1F	GATCTBGAGGAGGACATYRTGG	RAG1	53-56	Tornabene *et al*., 2013

Table 2. Best fitting substitution models for each gene and concatenated dataset for MrBayes.

Dataset	Partitions	Substitution models
IRF2 (Gene1)	Gene1_pos1, Gene1_pos2	GTR+G
	Gene1_pos3	GTR+G
RAG1(Gene2)	Gene2_pos1, Gene2_pos2	HKY+I+G
	Gene2_pos3	GTR+G
Rh (Gene3)	Gene3_pos1	HKY+I+G
	Gene3_pos2	GTR+I+G
	Gene3_pos3	GTR+G
Concatenated	Gene1_pos1	GTR+G
	Gene1_pos2, Gene2_pos2	SYM+I
	Gene1_pos3	SYM+G
	Gene2_pos1, Gene3_pos1	GTR+I+G
	Gene2_pos3	GTR+G
	Gene3_pos2	GTR+I+G
	Gene3_pos3	GTR+G

3. Results

3.1. DNA sequencing

All three genes (IRF2, RAG1, and Rh) were successfully sequenced and resulted in a concatenated dataset consisting of 2,184 base pairs (bp) (666 bp for IRF2, 771 bp for RAG1, and 747 bp for Rh). A total of 132 specimens of *Stiphodon* and thirteen outgroup species were represented. Of the 31 recognized species, 23 species are represented in this study.

3.2. Phylogenetic relationships

Both the phylogenetic trees of the concatenated dataset obtained from RAxML and MrBayes seemed to be congruent. Both showed strong support for the monophyly of *Stiphodon* (Figures 3 and 4, respectively) and split the genus into two clades: one large diverse clade consisting of fifteen species and a much smaller clade consisting of eight species. The bootstrap support values were lower than that of the posterior probabilities.

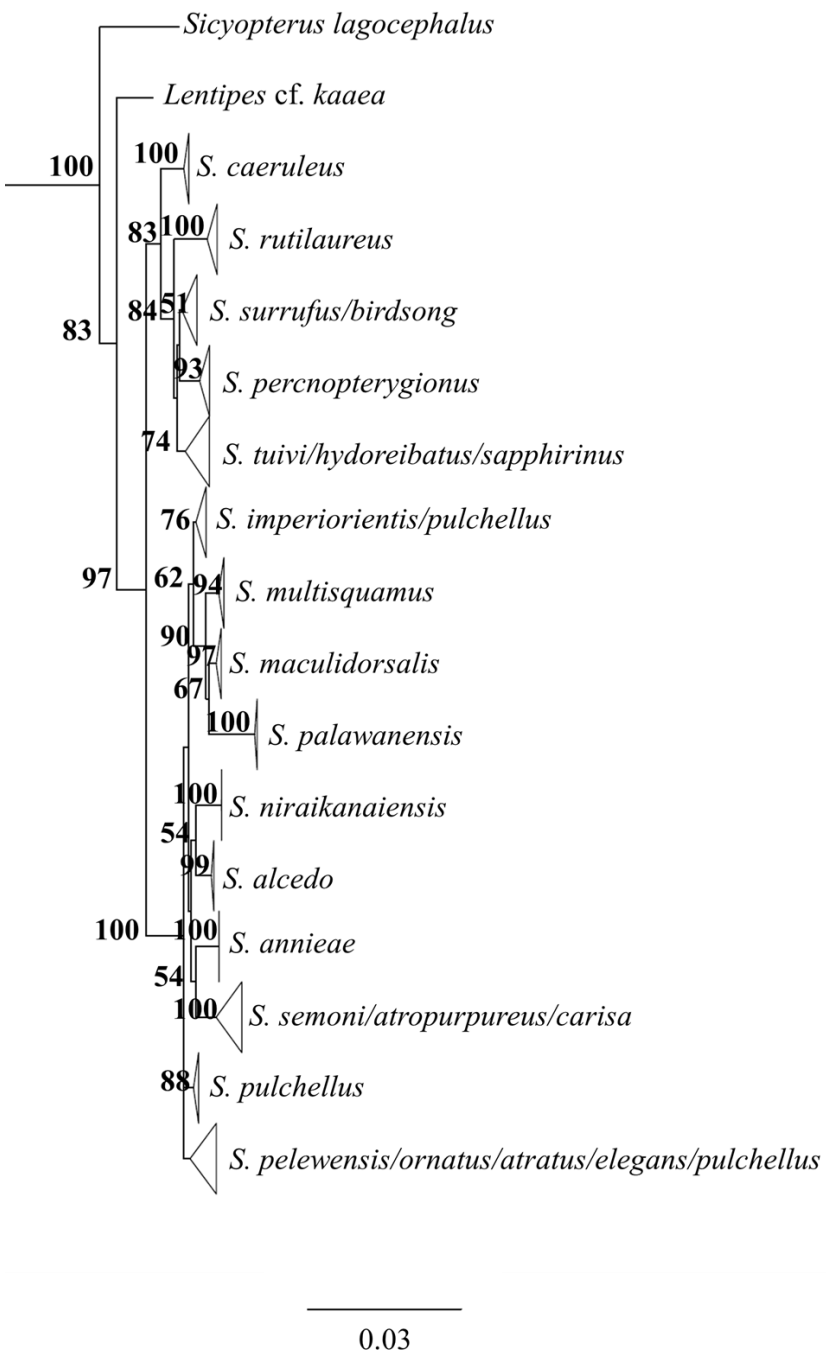

Figure 3. Best-scoring Maximum Likelihood tree showing only sicydiines generated from the RAxML7.2.8 plugin for Geneious 8.3.1 of the concatenated dataset. Only support values greater than 50% are displayed. Support values are in bootstrap support (%). See Supplemental Materials II in Appendix B for the complete best-scoring ML tree.

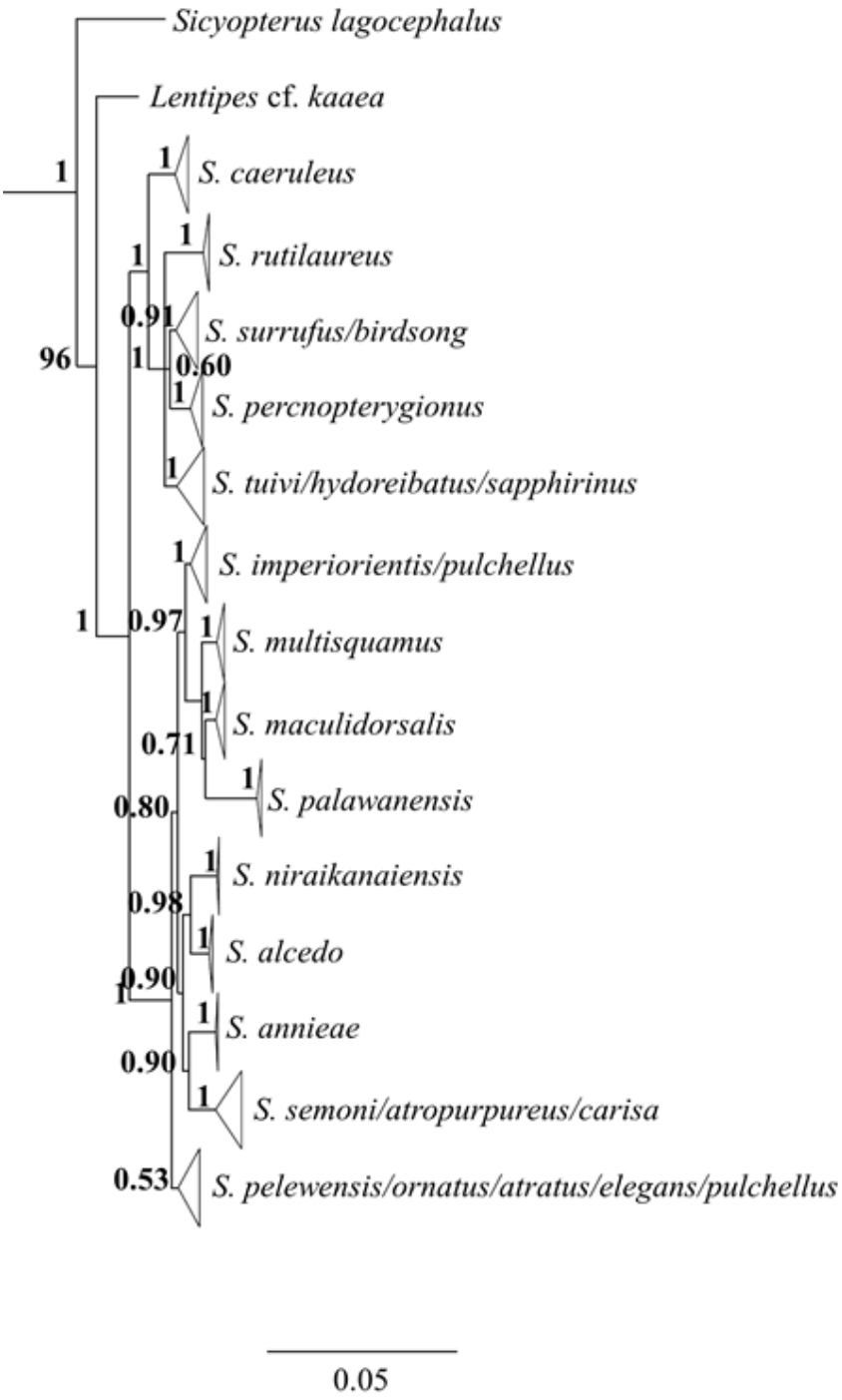

Figure 4. MrBayes majority rule consensus tree of only sicydiines generated using the concatenated dataset ran for 20,000,000 generations and a ten percent burn-in using the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal. Support values are in posterior probabilities. Only posterior probabilities greater than 0.50 are displayed. See Supplemental Materials II in Appendix B for the complete MrBayes majority rule consensus tree.

In the small clade, both phylogenetic trees supported *S. caeruleus, S. rutilaureus, S. percnopterygionus,* and *S. surrufus/birdsong* as monophyletic groups, and *S. caeruleus* was sister to the other four species. This relationship should be analyzed with caution because of the conflicting support values for the sister relationship of *S. rutilaureus* and *S. tuivi/hydoreibatus/sapphirinus* (percent bootstrap support was less than 50% in the best-scoring ML tree, but posterior probability support was 60% in the majority rule consensus tree). *Stiphodon surrufus* and *S. birdsong* were found to be genetically identical in both phylogenetic inference methods. It is also interesting to note that a presumptive *S. pulchellus* female (S127B) did not fall out with the three *S. pulchellus* males in either phylogenetic analysis. Neither tree was able to fully resolve the relationship between *S. tuivi, S. hydoreibatus,* and *S. sapphirinus,* therefore, this grouping will be referred to as the "tuivi" group.

The species relationships were better resolved in the large clade for both phylogenetic analysis methods. *S. pelewensis/ornatus/atratus/elegans/pulchellus* ("pelewensis" group) was sister to two smaller subclades: the first subclade consisted of *S. imperiorientis/pulchellus, S. multisquamus, S. maculidorsalis,* and *S. palawanensis,* and the second subclade consisted of *S. niraikanaiensis, s. alcedo, S. annieae,* and an *S. semoni/atropurpureus/carisa* ("semoni" group). All *S. elegans* species were grouped together within the "pelewensis" group but recognition of that species would make the other species of the group paraphyletic. The first subclade consisted of species the larger body sized *Stiphodon*: *S. imperiorientis/pulchellus, S. multisquamus, S. maculidorsalis,* and *S. palawanensis. Stiphodon maculidorsalis* and *S. palawanensis* were sister species with *S. multisquamus* being sister to these two species. *Stiphodon imperiorientis/pulchellus* in turn was sister to all three of those species. The second subclade consisted of two sister groups: *S. niraikanaiensis* and *S. alcedo* (the only two species with

sixteen pectoral fin rays) and *S. annieae* and *S. semoni/atropurpureus/carisa*. Unlike the majority rule consensus tree, the best-scoring ML tree was unable to resolve the placement of *S. pulchellus* specimens. *Stiphodon pulchellus* specimens were found in three different places in the best-scoring ML tree: as a separate group a polytomy with the "pelewensis" group, within the "pelewensis" group, and with *S. imperiorientis* (Figure 3).

The gene trees were not well resolved for both phylogenetic inference methods, but displayed the similar trends listed above in the concatenated datasets (Appendix B. Supplemental Materials II, Figures 3-8). All gene trees for both RAxML and MrBayes indicated the presence of two clades in *Stiphodon*, but these clades were comprised of polytomies within the two major clades making it hard to determine species relationships using the gene trees. The placement of *S. pulchellus* (127B) was also questionable in all of the gene trees for both RAxML and MrBayes analyses. The best-scoring ML trees were less resolved than the majority rule consensus trees. The IRF2 gene trees were the best resolved of the three gene trees for both methods.

4. Discussion

This is the first comprehensive phylogenetic analysis of species in the genus *Stiphodon* using nuclear genes. Maeda *et al.* (2011a) used the partial mitochondrial NADH dehydrogenase subunit 5 (ND5) gene to construct a neighbor-joining (NJ) tree for four *Stiphodon* species (*S. imperiorientis, S. alcedo, S. atropurpureus,* and *S. percnopterygionus*) from the Ryukyu Archipelago, Japan using *Sicyopterus japonicus* as an outgroup. The nuclear data from this study were consistent with the findings of Maeda *et al.* (2011a) in terms of *S. alcedo* being recovered as a monophyletic species, and *S. percnopterygionus* being sister to the other three *Stiphodon* species. There is disagreement in the relationships between *S. imperiorientis, S. alcedo* and *S.*

atropurpureus with the mitochondrial data presented by Maeda *et al*. (2011b) and the nuclear data presented in this study. The ND5 NJ tree suggested that *S. alcedo* and *S. imperiorientis* were more closely related and that *S. atropurpureus* was sister to these two species. The nuclear data, on the other hand, indicated that *S. alcedo* and *S. atropurpureus* were more closely related and *S. imperiorientis* was sister to these two species.

The two concatenated nuclear trees from RAxML and MrBayes were compared to an unpublished preliminary ML tree of the whole mitochondrial genome provided by Dr. Ken Maeda, University of Ryukyus (unpub. data). Similarities and differences between the mitochondrial and nuclear data were apparent when analyzing the phylogenetic trees. Both nuclear and mitochondrial data supported the monophyly of *Stiphodon* and the division of the genus into two distinct clades, one larger and more diverse than the other, as well as the large clade being split into two subclades. Unlike the nuclear data, the "pelewensis" group was not sister to the two subclades. The first subclade in the large clade for the mitochondrial data consisted of *S. multisquamus, S. palawanensis, S. elegans, S. maculidorsalis, S. imperiorientis, S. pelewensis/ornatus/atratus/pulchellus,* and the second subclade consisted of *S. niraikanaiensis, S. alcedo, S. semoni,* and *S. atropurpureus.* The mitochondrial data disagrees with the placement of species in the first subclade. *S. multisquamus* and *S. palawanensis* were sister species sister to a larger subclade. In the larger subclade, *S. elegans* was a monophyletic group most distantly related to the "pelewensis" group, but *S. imperiorientis* was found to be sister to the "pelewensis" group. *Stiphodon maculidorsalis* was sister to *S. imperiorientis* and the "pelewensis" group, and *S. elegans* was sister to *S. maculidorsalis, S. imperiorientis,* and the "pelewensis" group. There was also disagreement in the two datasets on the placement of *S. elegans*. The nuclear data placed *S. elegans* within the "pelewensis" group, but the mitochondrial

data disagreed and instead suggested that *S. elegans* was sister to the clade consisting of *S. maculidorsalis, S. imperiorientis,* and the "pelewensis" group. Table 3 compares the nominal species statuses between the nDNA and the unpublished mtDNA.

The synonymy of *S. surrufus* and *S. birdsong* as reported in the literature was also supported by both datasets (Keith *et al*., 2015), but the mitochondrial data did not support the synonymy of *S. semoni/atropurpureus* as indicated by the nDNA. Instead, the mtDNA suggested that *S. semoni* and *S. atropurpureus* were actually sister species.

Table 3. Summary of nominal species statuses suggested by nuclear and unpublished preliminary mitochondrial data. Check marks indicate species recognition is supported. Species not included in the analyses are indicated with N/A. Any disagreements by the mitochondrial data analysis with the nuclear data presented in this study are denoted with "Disagrees."

Nominal species	Nuclear DNA	Mitochondrial DNA
Stiphodon alcedo Maeda, Mukai and Tachihara, 2011a	✓	✓
Stiphodon annieae Keith and Hadiaty, 2014	✓	✓
Stiphodon astilbos Ryan, 1986	N/A	N/A
Stiphodon atratus Watson, 1996	*Stiphodon pelewensis* Herre, 1936	*Stiphodon pelewensis* Herre, 1936
Stiphodon atropurpureus Herre, 1927	*Stiphodon semoni* Weber 1895	Disagrees
Stiphodon aureofuscus Keith, Busson, Sauri, Hubert & Hadiaty 2015	N/A	N/A
Stiphodon birdsong Watson, 1996	*Stiphodon surrufus* Watson and Kottelat, 1995	*Stiphodon surrufus* Watson and Kottelat, 1995
Stiphodon caeruleus Parenti and Maciolek, 1993	✓	✓
Stiphodon carisa Watson, 2008	*Stiphodon semoni* Weber, 1895	N/A
Stiphodon discotorquatus Watson, 1995	N/A	N/A
Stiphodon elegans Steindachner, 1879	*Stiphodon pelewensis* Herre, 1936	Disagrees
Stiphodon hydoreibatus Watson, 1999	*Stiphodon tuivi* Watson, 1995	N/A
Stiphodon imperiorientis Watson and Chen, 1998	✓	✓
Stiphodon julieni Keith, Watson and Marquet, 2002	N/A	N/A
Stiphodon kalfatak Keith, Marquet and Watson, 2007	N/A	N/A
Stiphodon larson Watson, 1996	N/A	N/A
Stiphodon maculidorsalis Maeda and Tan, 2013	✓	✓
Stiphodon martenstyni Watson, 1998	N/A	N/A
Stiphodon mele Keith, Marquet and Pouilly, 2009	N/A	N/A
Stiphodon multisquamus Wu and Ni, 1986	✓	✓
Stiphodon niraikanaiensis Maeda, 2013	✓	✓
Stiphodon oatea Keith, Feunteun and Vigneux, 2010	N/A	N/A
Stiphodon ornatus Meinken, 1974	*Stiphodon pelewensis* Herre, 1936	*Stiphodon pelewensis* Herre, 1936
Stiphodon palawanensis Maeda and Palla, 2015	✓	✓

Stiphodon pelewensis Herre, 1936	✓	✓
Stiphodon percnopterygionus Watson and Chen, 1998	✓	✓
Stiphodon pulchellus Herre, 1927	*Stiphodon pelewensis* Herre, 1936	*Stiphodon pelewensis* Herre, 1936
Stiphodon rubromaculatus Keith and Marquet, 2007	N/A	N/A
Stiphodon rutilaureus Watson, 1996	✓	✓
Stiphodon sapphirinus Watson, Keith and Marquet, 2005	*Stiphodon tuivi* Watson, 1995	N/A
Stiphodon semoni Weber 1895	✓	✓
Stiphodon surrufus Watson and Kottelat, 1995	✓	✓
Stiphodon tuivi Watson, 1996	✓	N/A
Stiphodon zebrinus Watson, Allen and Kottelat, 1998	N/A	N/A

Although this was the first comprehensive phylogenetic analysis of the genus *Stiphodon*, monophyly of the genus has been previously confirmed by other morphological and molecular studies. However, the earlier molecular studies were focused on the relationships among sicydiine genera (Keith *et al.*, 2011; Taillebois *et al.*, 2014), or among the Gobioidei (Agorreta *et al.*, 2013; Thacker, 2014, 2009, 2003; Tornabene *et al.*, 2013) with brief notes on *Stiphodon*. Watson and Kottelat (1995) proposed the division of *Stiphodon* into two geographic groupings based on the number of pectoral rays. The species from the central Pacific Basin usually had 14 pectoral rays and those in the western Pacific usually had 15 or 16. They noted that there was a region of overlap in the Philippines, Japan, Palau Islands, Solomon Islands, Bismarck Archipelago, and the northern slopes of New Guinea. The nuclear data presented here do not support the split in *Stiphodon* according to geographic region and the number of pectoral rays because both clades have species that are found in the region of overlap and in both the western Pacific and the central Pacific basins.

In another study, Keith *et al*. (2011) proposed that two groups in *Stiphodon* could be determined based on the number of pectoral fin rays and the size of the adults: *Stiphodon* species with mainly 13-14 pectoral fin rays and of small adult size (generally less than 4 cm standard length) and species with mainly 15-16 rays and of large adult size (generally from 4 to 7 cm standard length). These two groups were named the "sapphirinus group" and "elegans group" and included all of six species of *Stiphodon* for comparison. The findings of this study were generally consistent with these diagnoses but had some exceptions. The eight species in the smaller clade have adults that were generally smaller than 4 cm standard length with the exception of *Stiphodon percnopterygionus* which reaches a length slightly more than 4 cm. In the large clade, adult sizes are generally between 4 to 7 cm with the exception of five species (*S.*

carisa, S. annieae, S. niraikanaiensis, S. ornatus, and *S. pelewensis*) that had adults less than 4 cm standard length. Of the proposed hypotheses by Watson and Kottelat (1995) and Keith *et al*. (2011), the latter is more consistent with the split observed in this study.

Due to the overlap in scale counts (i.e., lateral, transverse forward and transverse reverse series, and predorsal midline scales), these characters alone do not serve as an adequate means of distinguishing among species. The species in the unresolved groups, "tuivi," "semoni," and "pelewensis", all shared similar meristic features (Table 4). The "tuivi" group is made up of blue-colored males that share the same number of pectoral rays (fourteen) and have overlapping premaxillary teeth and scale counts. *Stiphodon hydoreibatus* and *S. sapphirinus* also share a silver-colored second colormorph in the males. Not only do the "semoni" group have the same number of pectoral rays (fifteen) and overlapping premaxillary teeth and scale counts, but they all have olive green-colored males. The species of the "pelewensis" group all had dark vertical bars along the body, fifteen pectoral rays, and overlapping premaxillary teeth and scale counts. In the case of *S. imperiorientis* and *S. pulchellus*, they are not distinct based on meristic counts, but are considered different species because of the color of the male heads and laterally on the body (blue vs olive green, respectively). Previous studies have synonymized *S. birdsong* as *S. surrufus*, but this is the first study to show that these two species are genetically similar.

Table 4. Meristic features of *Stiphodon* species not resolved in nDNA analyses. Counts follow Keith *et al.*, 2015.

Unresolved groups	*Stiphodon* species	Locality	Pectoral rays	Maximum standard length (cm)	Premaxillary tridentic teeth	Lateral series scales	Transverse series reverse scales	Transverse series forward scales	Predorsal midline scales
"tuivi" group	*S. tuivi*	French Polynesia (Marquesas Islands)	14	3.22	34-53	23-39	10-14	12-19	0-14
	S. hydoreibatus	Samoa Archipelago	14	2.66	36-53	23-35	9-10	10-14	1-14
	S. sapphirinus	New Caledonia	14	3.04	31-51	21-35	10-12	7-15	0-12
"semoni" group	*S. semoni*	Australia, Sumatra and Bali, Solomon Islands	15	4.58	33-55	26-38	9-12	9-17	2-13
	S. atropurpueus	Philippines, South China, Taiwan	15	4.26	37-60	30-37	9-11	12-18	5-16
	S. carisa	Sumatra	15	3.99	27-35	27-29	10-11	9-18	5-16
"pelewensis" group	*S. pelewensis*	Palau, Kosrae, Guam, Pohnpei	15	3.99	25-44	33-37	10-11	12-13	6-12
	S. ornatus	Sumatra	15	3.14	24-40	36-39	9-14	14	13-14
	S. atratus	Australia, Halmahera, New Guinea, Guam, Bismarck Archipelago and Admiralty Islands	15	4.87	25-42	32-37	10-11	12-17	9-17
	S, elegans	Society Islands and Tubuai Island, Samoa	15	4.22	24-40	29-39	9-12	11-17	2-13
	S. pulchellus	Philippines	15	5.99	32-56	30-34	10-11	13-17	7-16
	S. imperiorientis	Southern China, Taiwan, Japan	15	5.2	37-50	30-34	9-12	14-17	7-14

Stiphodon literature has suggested that there may be more species descriptions available than actual species. For example, *S. stevensoni* is a junior synonym of *S. elegans* (Watson, 1999), *S. olivaceus* is a junior synonym of *S. pulchellus* (Maeda *et al*., 2011b), and *S. aureorostrum* is a junior synonym of *S. multisquamus* (Maeda *et al*., 2015). Other informal synonymies have been proposed as well: Ebner *et al*. (2011) considered *S. allen* a junior synonym of *S. semoni*, and Maeda *et al*. (2015) made comments about the similarities between *Stiphodon semoni* and *S. atropurpureus*, but determined that more biogeographical studies needed to be done to clarify the relationship between these two species. Keith *et al*. (2015b) proposed three other synonymies in their book Indo-Pacific Sicydiine Gobies: Biodiversity, life traits and conservation: *S. surrufus/birdsong*, *S. pelewensis/atratus/weberi*, and *S. semoni/allen*. They based their synonymy of *S. surrufus/birdsong* on morphology and *S. semoni/allen* was based on comments from Ebner *et al*. (2011), but did not indicate their reason(s) for synonymizing *S. pelewensis/atratus/weberi*. Expanding the nuclear data and unpublished mitochondrial data to include these species would better determine whether molecular studies agree with the synonymies based on morphology.

This idea of fewer species than species descriptions is also supported by the nuclear and mitochondrial data presented in this study. Several groupings from the nuclear dataset indicate that there are potential synonymies yet to be formally proposed (*S. surrufus/birdsong*, *S. semoni/carisa/atropurpureus*, and *S. pelewensis/atratus/ornatus/pulchellus*). Morphological data seem to support synonymizing these species. *Stiphodon surrufus* and *S. birdsong* both usually have a naked predorsal midline and an entirely naked belly (Watson and Kottelat, 1995; Watson, 1996). It is also very interesting to note that *S. semoni*, *S. carisa*, and *S. atropurpureus* all have one unique character in common: a white fatty tissue posterior to the pectoral fin base (Watson and Kottelat, 1995; Watson, 2008, 1996). The function of this fatty tissue is yet to be known.

Stiphodon pulchellus may also be a synonym of *S. pelewensis* because both nuclear and mitochondrial data were not able to distinguish between *S. pelewensis/atratus/ornatus/pulchellus.* In the nuclear analysis, one *S. pulchellus* (S127B) (female) consistently grouped with *S. imperiorientis* while the other three *S. pulchellus* (males) grouped with *S. pelewensis/atratus/ornatus*. There are two possible explanations for such discordance: 1) S127B was a female, so it could have been a misidentification or 2) *S. imperiorientis* and *pulchellus* are more closely related to *S. pelewensis/atratus/ornatus* genetically as suggested by the unpublished mitochondrial data even though the nuclear data does not support the close relationship between *S. imperiorientis* and *S. pelewensis/ornatus/atratus/elegans/pulchellus*. Maeda *et al.* (2011b) indicated that coloration was the only distinguishing feature between *S. pulchellus* and *S. imperiorientis* males. This prompts the question of whether this species was possibly misidentified. The potential misidentification of the female *S. pulchellus* also reiterates the fact that all *Stiphodon* females are drab in coloration and share the same patterns making it hard to distinguish between *Stiphodon* females. This voucher should be reanalyzed to determine species identification. Although we have presented data indicating concordance between morphological and molecular data in this study, discordances were still present. For example, *S. annieae* also shared the same number of pectoral rays (fourteen), maximum adult body size, overlapping premaxillary teeth and scale counts, but it was not genetically related or similar to *S. birdsong* or *S. surrufus* (Keith and Hadiaty, 2014; Watson and Kottelat, 1995; Watson, 1996).

Future research should aim to increase the number of specimens per taxon and locality as well as the number of species representatives for both the outgroups and *Stiphodon*. Also, instead of analyzing nuclear and mitochondrial data separately then make comparisons, both nuclear and

mitochondrial datasets should be analyzed together to reconcile any discordant findings from this study. Studies have cautioned the use of only mitochondrial DNA (mtDNA) loci even though it is a useful tool for studies in phylogenetics and evolves at a faster rate than nuclear genes because empirical studies have found that mtDNA can be misleading about species relationships among closely related species (Edwards *et al*., 2005; Edwards and Bensch, 2009; Fisher-Reid and Wiens, 2011; Rubinoff and Holland, 2005; Shaw, 2002). These studies have also pointed out that the use of multiple nuclear genes was very helpful. Other avenues to explore include the addition of more nuclear genes in favor for the ones that evolve more quickly such as s7 (Chow and Hazama, 1998) or exon-primed-intron-crossing (EPIC) markers (Touriya *et al*., 2003). In the same sense, the addition of nuclear genes in combination with the increase in taxa sampling would also possibly help resolve the species trees.

Instead of using the conventional concatenation method used in this study, another avenue of exploration to infer species phylogenies would be coalescent-based approaches, for example, the multispecies coalescent method (Edwards, 2009). Species estimation using the multispecies coalescent method can be run using *BEAST through the BEAST2 software program (Heled and Drummond, 2010). The *BEAST program allows researchers to simultaneously estimate the gene trees and species tree as well as estimate divergence times and make a time-calibrated species tree.

Other future directions for studying *Stiphodon* would be to conduct a formal systematic review of the genus. All the reviews or summary papers available for this genus have focused on a particular region of the Indo-Pacific: Leyte, Philippines (Watson and Kottelat, 1995), French Polynesia (Watson, 1995), New Guinea region including Australia, the Solomon Islands and Vanuatu (Watson, 1996), Japan and Taiwan (Watson and Chen, 1998), Halmahera and Irian Jaya

(Watson *et al.*, 1998), and, most recently, Western Sumatra (Maeda and Tan, 2013). Having a better idea of the true species diversity of the *Stiphodon* genus will help us better understand species ranges and distributions and lead to hypotheses of phylogeography of these gobies.

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APPENDIX A. SUPPLEMENTAL MATERIALS I

Table 1. Specimens organized according to taxonomy and locality.

Family	Genus	Species	Locality
Butidae	*Butis*	*butis*	Solomon Islands
	Ophiocara	*porocephalus*	Kosrae
Eleotridae	*Eleotris*	*fusca*	Pohnpei
	Kribia	sp.	Guinea
Gobiidae (Gobiinae)	*Exyrias*	*puntang*	Palau
	Gobiosoma	*bosc*	Florida
Gobiidae (Gobionellinae)	*Awaous*	*ocellaris*	Ambon
	Gnatholepis	*thompsoni*	Puerto Rico
Gobiidae (Oxudercinae)	*Periophthalmus*	*argentilineatus*	Iriomote
Gobiidae (Sicydiinae)	*Lentipes*	cf. *kaaea*	Ambon
	Sicyopterus	*lagocephalus*	Fiji
	Stiphodon	*alcedo*	Okinawa
	Stiphodon	*alcedo*	Okinawa
	Stiphodon	*alcedo*	Okinawa
	Stiphodon	*alcedo*	Okinawa
	Stiphodon	*annieae*	Ambon
	Stiphodon	*annieae*	Ambon
	Stiphodon	*annieae*	Ambon
	Stiphodon	*atratus*	Australia
	Stiphodon	*atratus*	Australia
	Stiphodon	*atratus*	New Caledonia
	Stiphodon	*atratus*	New Caledonia
	Stiphodon	*atratus*	New Caledonia
	Stiphodon	*atratus*	Solomon Islands
	Stiphodon	*atratus*	Solomon Islands
	Stiphodon	*atratus*	Ambon
	Stiphodon	*atratus*	Ambon
	Stiphodon	*atratus*	Solomon Islands
	Stiphodon	*atropurpureus*	Okinawa
	Stiphodon	*atropurpureus*	Okinawa
	Stiphodon	*atropurpureus*	Okinawa
	Stiphodon	*atropurpureus*	Sumatra
	Stiphodon	*atropurpureus*	Sumatra Aquarium
	Stiphodon	*birdsong*	Solomon Islands

Stiphodon	*birdsong*	Solomon Islands
Stiphodon	*birdsong*	Solomon Islands
Stiphodon	*birdsong*	Solomon Islands
Stiphodon	*caeruleus*	Kosrae
Stiphodon	*caeruleus*	Kosrae
Stiphodon	*caeruleus*	Kosrae
Stiphodon	*caeruleus*	Pohnpei
Stiphodon	*caeruleus*	Pohnpei
Stiphodon	*carisa*	Indonesia
Stiphodon	*carisa*	Singapore Aquarium
Stiphodon	*carisa*	Singapore Aquarium
Stiphodon	*carisa*	Sumatra
Stiphodon	*carisa*	Sumatra
Stiphodon	*carisa*	Sumatra
Stiphodon	*elegans*	Moorea
Stiphodon	*elegans*	Moorea
Stiphodon	*elegans*	Moorea
Stiphodon	*elegans*	Moorea
Stiphodon	*elegans*	Raiatea
Stiphodon	*elegans*	Tahiti
Stiphodon	*elegans*	Tahiti
Stiphodon	*hydoreibatus**	Futuna
Stiphodon	*hydoreibatus**	Futuna
Stiphodon	*imperiorientis*	Okinawa
Stiphodon	*imperiorientis*	Okinawa
Stiphodon	*imperiorientis*	Okinawa
Stiphodon	*maculidorsalis*	Petshop
Stiphodon	*maculidorsalis*	Petshop
Stiphodon	*maculidorsalis*	Petshop
Stiphodon	*multisquamus*	Okinawa
Stiphodon	*multisquamus*	Okinawa
Stiphodon	*multisquamus*	Okinawa
Stiphodon	*multisquamus*	Okinawa
Stiphodon	*niraikanaiensis*	Okinawa
Stiphodon	*niraikanaiensis*	Okinawa
Stiphodon	*niraikanaiensis*	Okinawa
Stiphodon	*ornatus*	Sumatra
Stiphodon	*ornatus*	Petshop
Stiphodon	*ornatus*	Singapore

		Aquarium
Stiphodon	*ornatus*	Singapore Aquarium
Stiphodon	*ornatus*	Petshop
Stiphodon	*ornatus*	Malaysia Aquarium
Stiphodon	*palawanensis*	Palawan
Stiphodon	*palawanensis*	Palawan
Stiphodon	*palawanensis*	Palawan
Stiphodon	*palawanensis*	Palawan
Stiphodon	*pelewensis*	Guam
Stiphodon	*pelewensis*	Guam
Stiphodon	*pelewensis*	Kosrae
Stiphodon	*pelewensis*	Kosrae
Stiphodon	*pelewensis*	Palau
Stiphodon	*pelewensis*	Palau
Stiphodon	*pelewensis*	Pohnpei
Stiphodon	*pelewensis*	Pohnpei
Stiphodon	*pelewensis*	Pohnpei
Stiphodon	*pelewensis*	Pohnpei
Stiphodon	*percnopterygionus*	Iriomote
Stiphodon	*percnopterygionus*	Iriomote
Stiphodon	*percnopterygionus*	Okinawa
Stiphodon	*percnopterygionus*	Okinawa
Stiphodon	*percnopterygionus*	Okinawa
Stiphodon	*percnopterygionus*	Okinawa
Stiphodon	*percnopterygionus*	Palau
Stiphodon	*percnopterygionus*	Taiwan
Stiphodon	*percnopterygionus*	Taiwan
Stiphodon	*percnopterygionus*	Taiwan
Stiphodon	*percnopterygionus*	Taiwan
Stiphodon	*pulchellus*	Palawan
Stiphodon	*pulchellus*	Palawan
Stiphodon	*pulchellus*	Palawan
Stiphodon	*pulchellus*	Palawan
Stiphodon	*rutilaureus*	New Caledonia
Stiphodon	*rutilaureus*	Solomon Islands
Stiphodon	*rutilaureus*	Solomon Islands
Stiphodon	*rutilaureus*	Solomon Islands
Stiphodon	*rutilaureus*	Solomon Islands
Stiphodon	*rutilaureus*	Solomon Islands
Stiphodon	*rutilaureus*	Solomon Islands

	Stiphodon	*rutilaureus*	Solomon Islands
	Stiphodon	*rutilaureus*	Vanuatu
	Stiphodon	*rutilaureus*	Vanuatu
	Stiphodon	*rutilaureus*	Vanuatu
	Stiphodon	*rutilaureus*	Vanuatu
	Stiphodon	*sapphirinus**	Cook
	Stiphodon	*sapphirinus**	Cook
	Stiphodon	*semoni*	West Papua Aquarium
	Stiphodon	*semoni*	Ambon
	Stiphodon	*semoni*	Ambon
	Stiphodon	*semoni*	Solomon Islands
	Stiphodon	*semoni*	Solomon Islands
	Stiphodon	*semoni*	Solomon Islands
	Stiphodon	*semoni*	Solomon Islands
	Stiphodon	*surrufus*	Palau
	Stiphodon	*surrufus*	Palau
	Stiphodon	*surrufus*	Palau
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Nuku Hiva
	Stiphodon	*tuivi*	Ua Huka
	Stiphodon	*tuivi*	Ua Huka
	Stiphodon	*tuivi*	Ua Huka
	Stiphodon	*tuivi*	Ua Huka
	Stiphodon	*tuivi*	Ua Huka
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
	Stiphodon	*tuivi*	Ua Pou
Odontobutidae	*Perccottus*	*glenii*	Kosrae
Rhyacicthyidae	*Rhyacicthys*	*aspro*	Mongolia

*Denotes sequences obtained via GenBank from Keith *et al*., 2011.

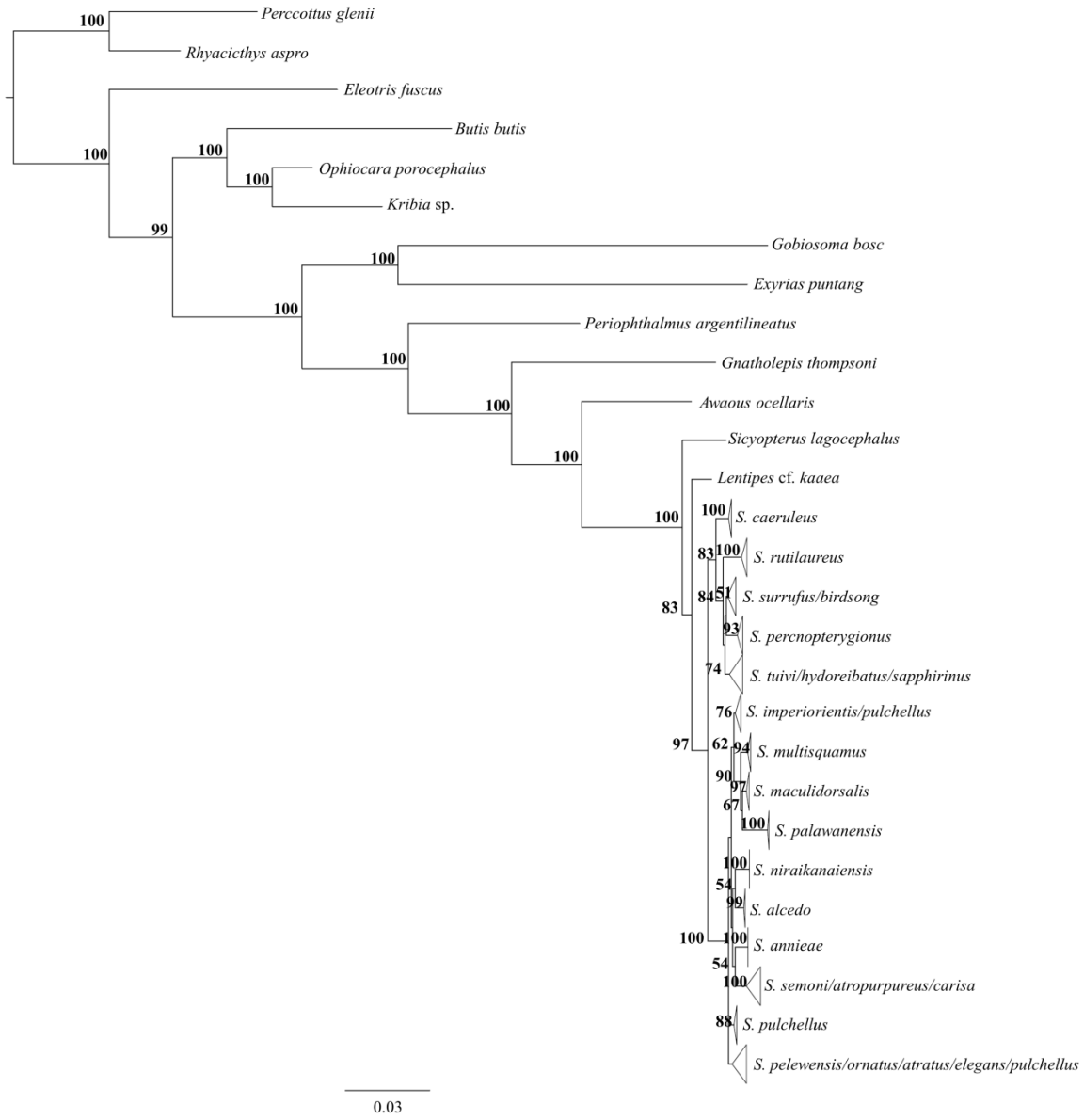


Figure 1. Complete best-scoring Maximum Likelihood tree generated from the RAxML7.2.8 plugin for Geneious 8.3.1 of the concatenated dataset. Only support values greater than 50% are displayed. Support values are in bootstrap support (%).

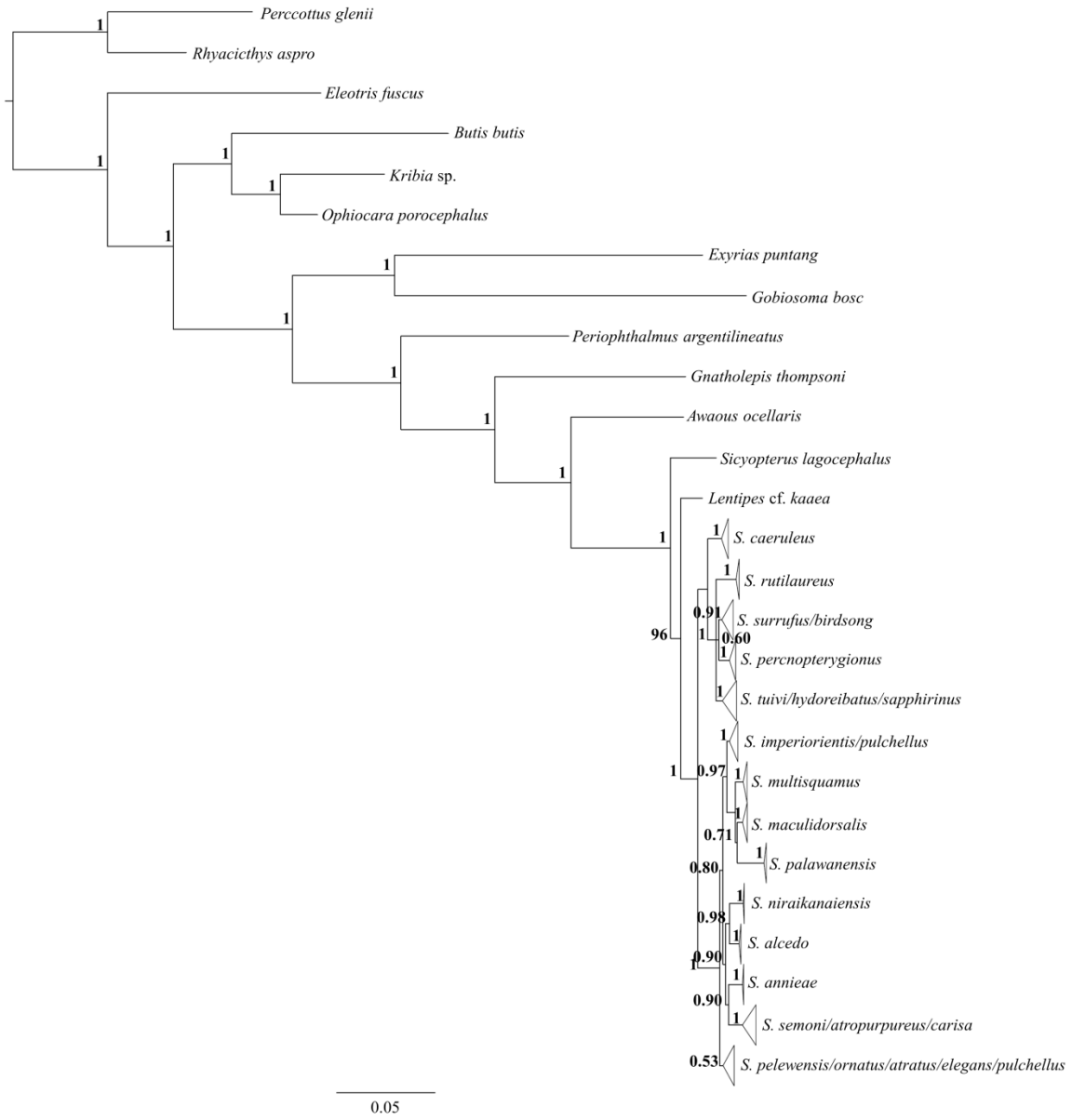


Figure 2A. Complete MrBayes majority rule consensus tree of the concatenated dataset ran for 20,000,000 generations and a ten percent burn-in using the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal. Support values are in posterior probabilities. Only posterior probabilities greater than 0.50 are displayed.

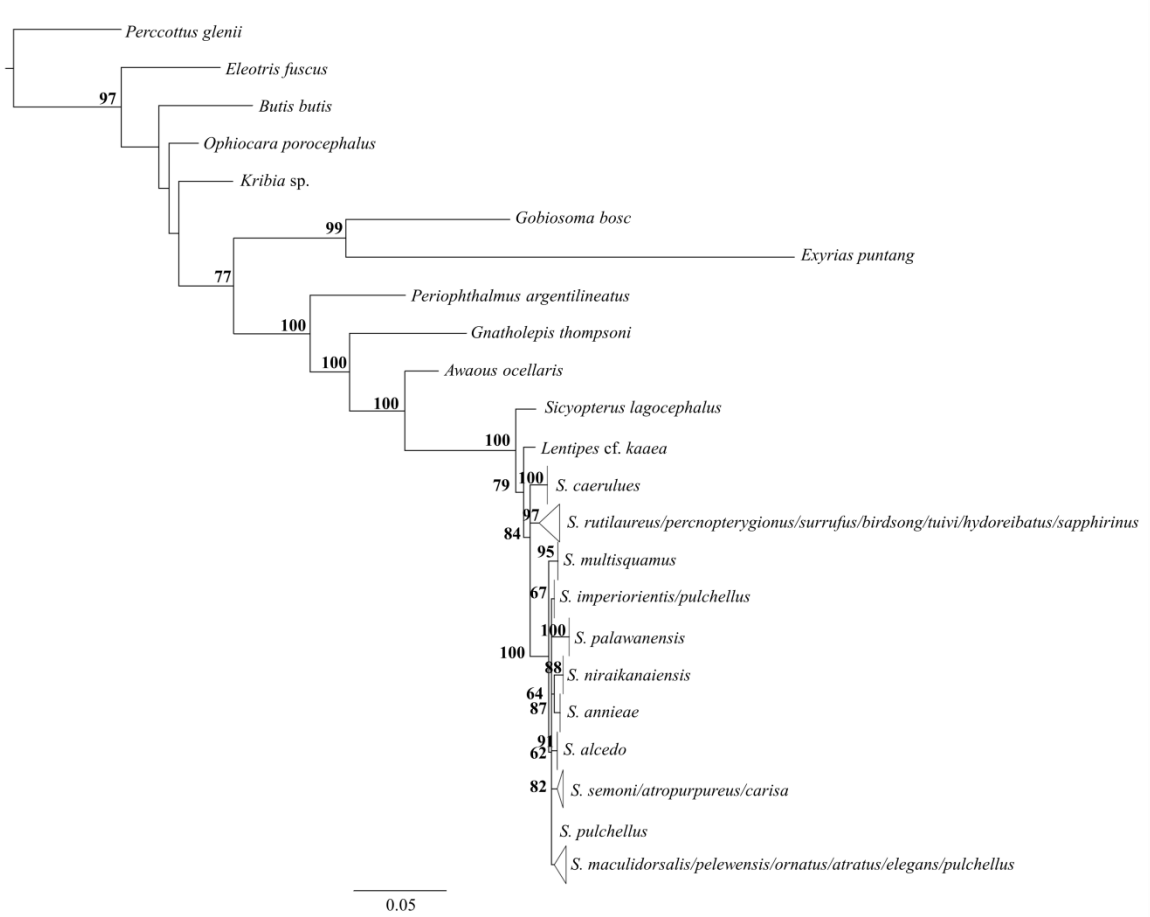


Figure 3. Best-scoring Maximum Likelihood tree generated from the RAxML7.2.8 plugin for Geneious 8.3.1 of the IRF2 gene. Only support values greater than 50% are displayed. Support values are in bootstrap support (%).

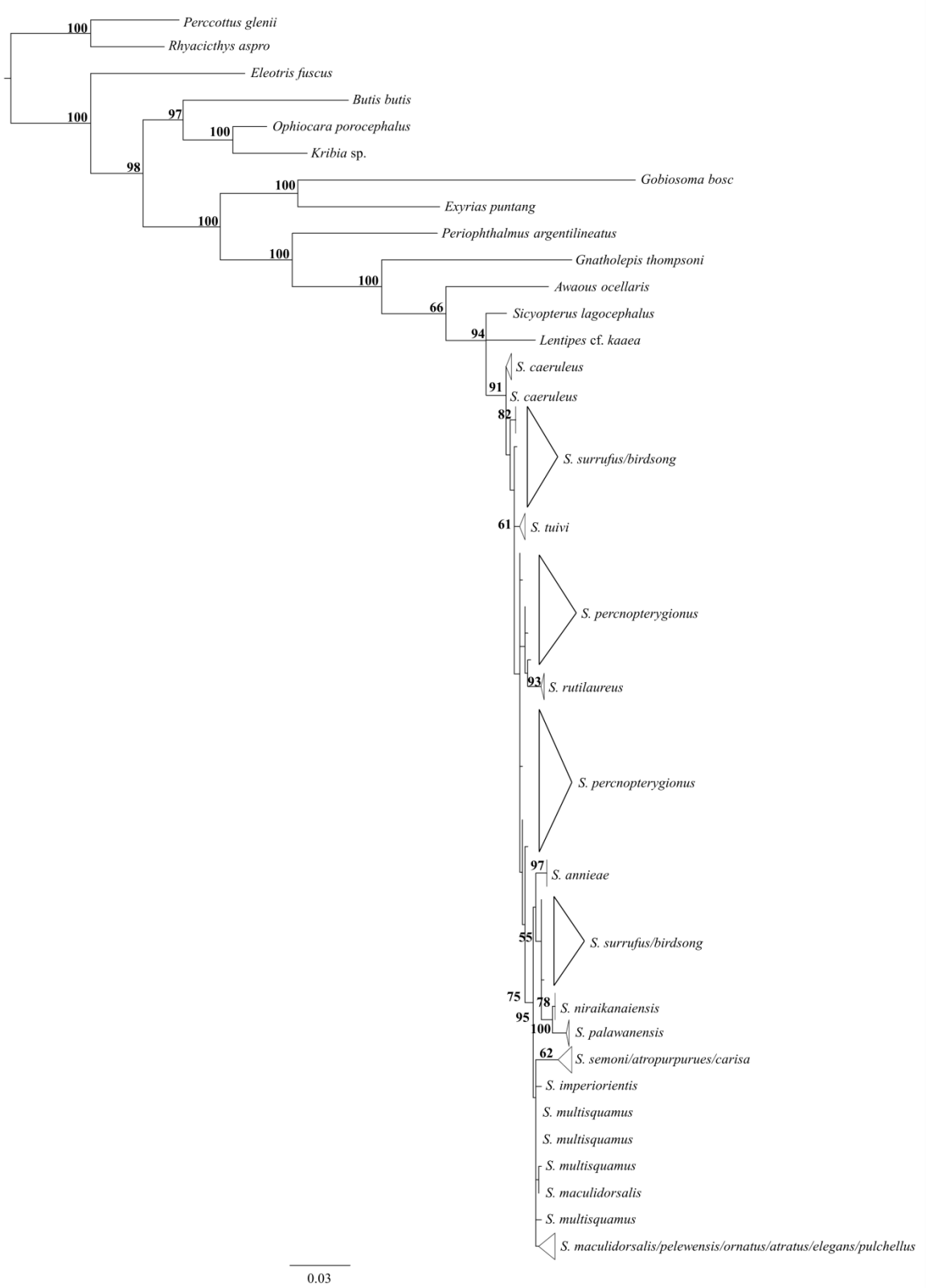


Figure 4. Best-scoring Maximum Likelihood tree generated from the RAxML7.2.8 plugin for Geneious 8.3.1 of the RAG1 gene. Only support values greater than 50% are displayed. Support values are in bootstrap support (%).

A

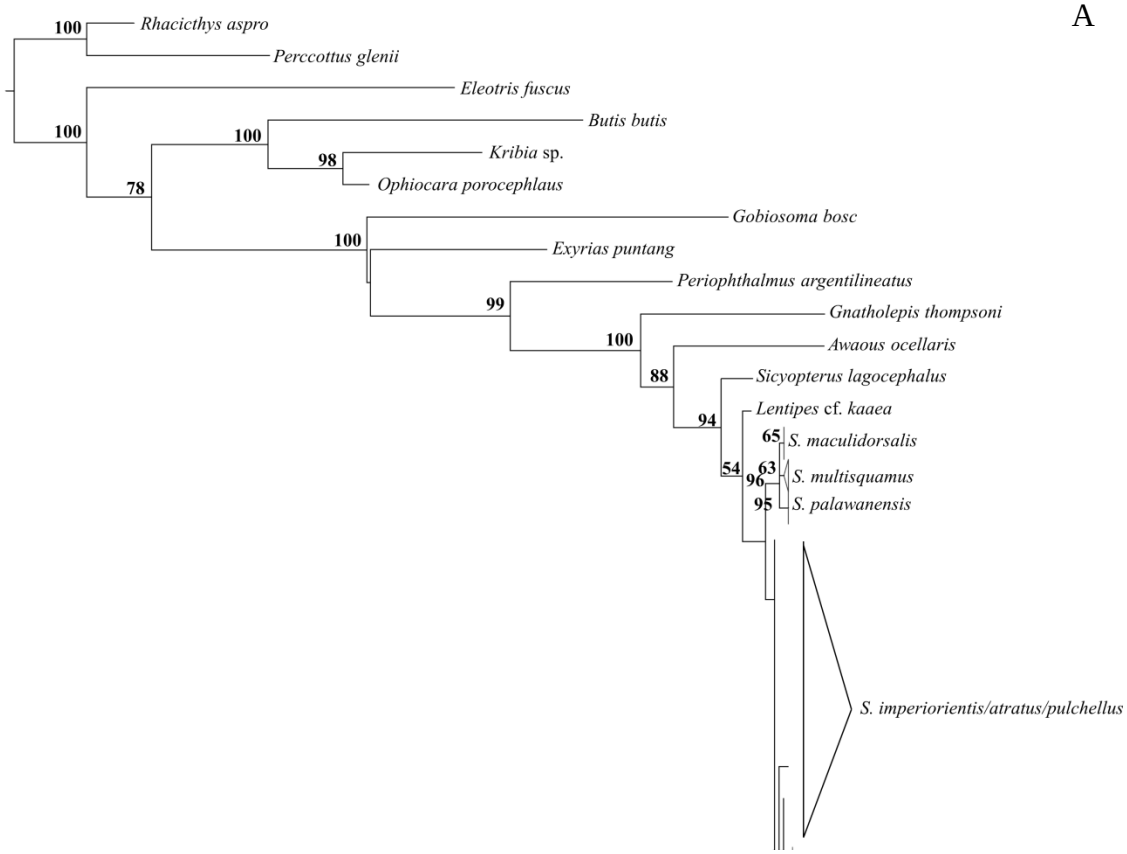
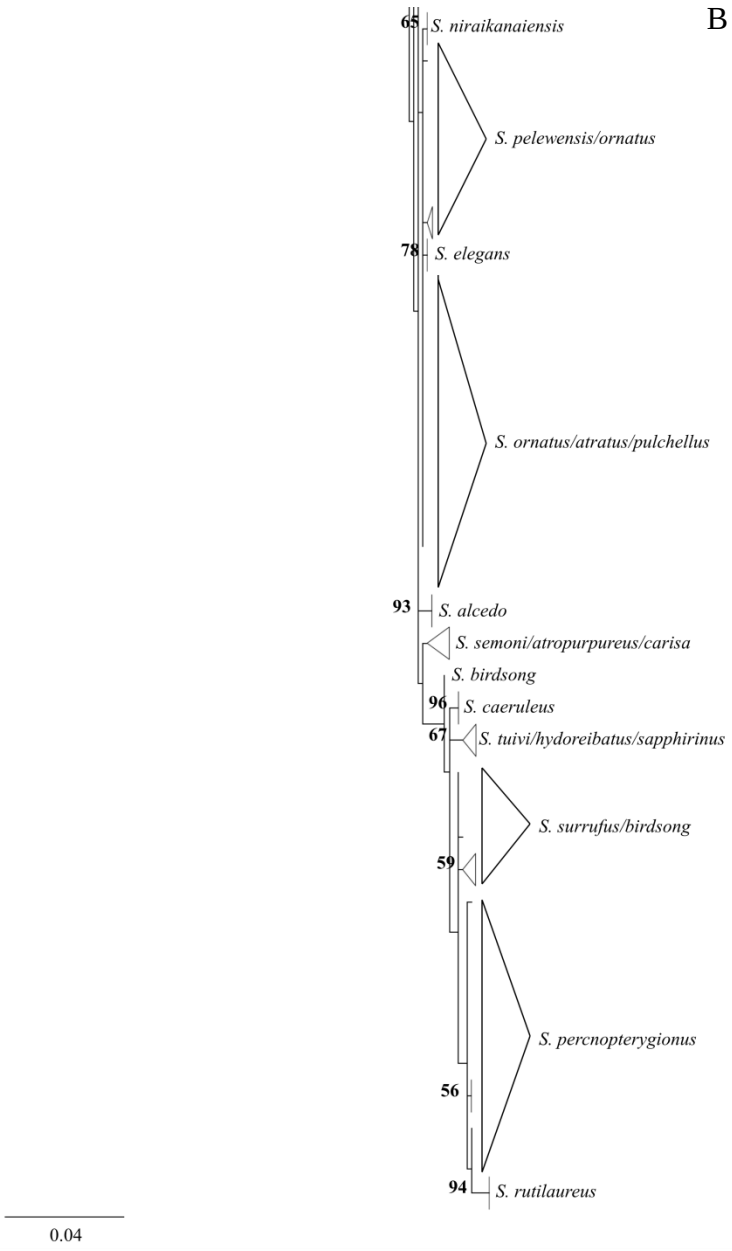


Figure 5A and 5B (below). Best-scoring Maximum Likelihood tree generated from the RAxML7.2.8 plugin for Geneious 8.3.1 of the Rh gene. Only support values greater than 50% are displayed. Support values are in bootstrap support (%).


B
65
S. niraikanaiensis
S. pelewensis/ornatus
78
S. elegans
S. ornatus/atratus/pulchellus
93
S. alcedo
S. semoni/atropurpureus/carisa
S. birdsong
96
S. caeruleus
67
S. tuivi/hydoreibatus/sapphirinus
S. surrufus/birdsong
59
S. percnopterygionus
56
94
S. rutilaureus
0.04

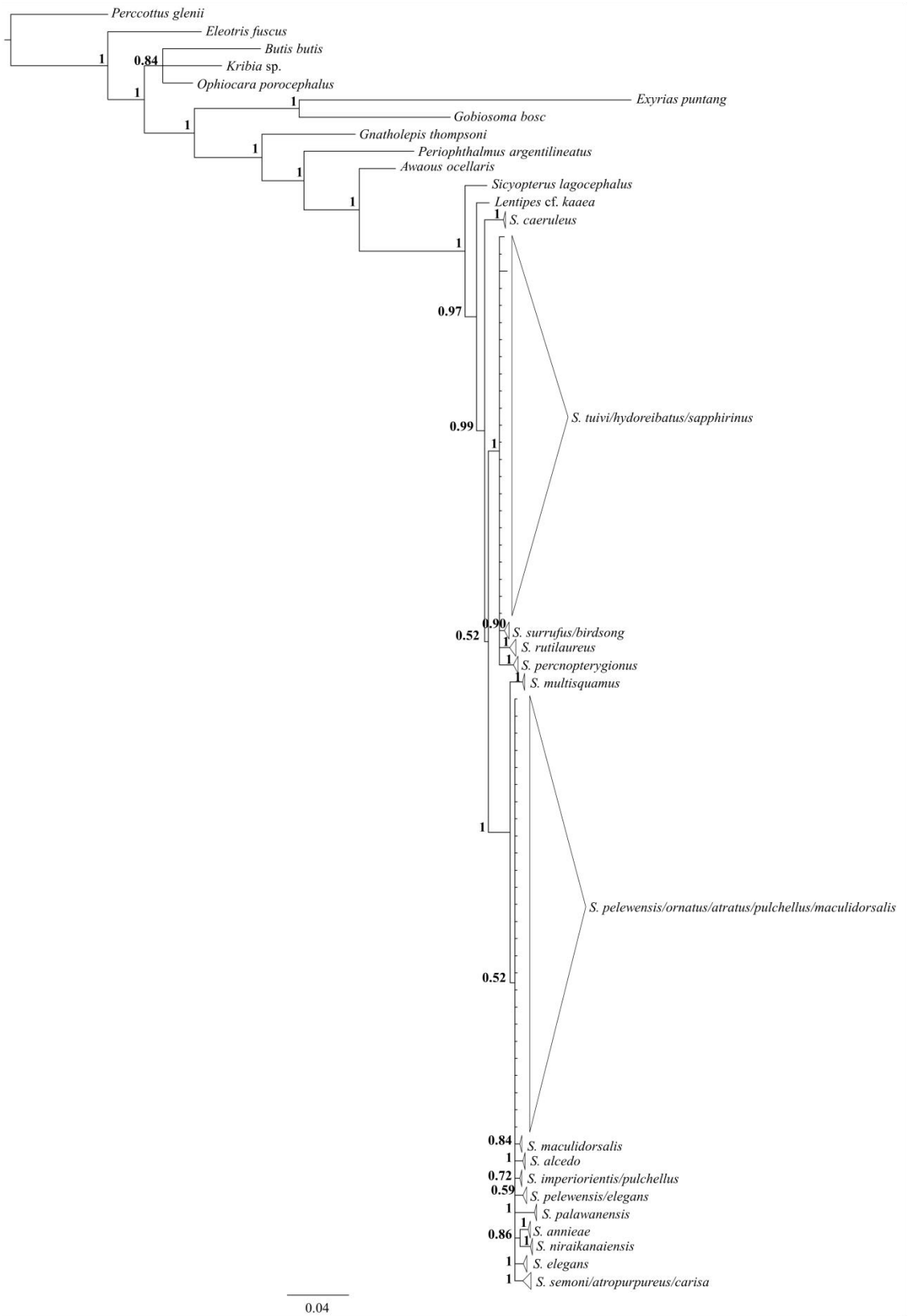


Figure 6. Bayesian phylogeny of the IRF2 gene. Only support values greater than 0.50 are displayed. Support values are posterior probabilities.

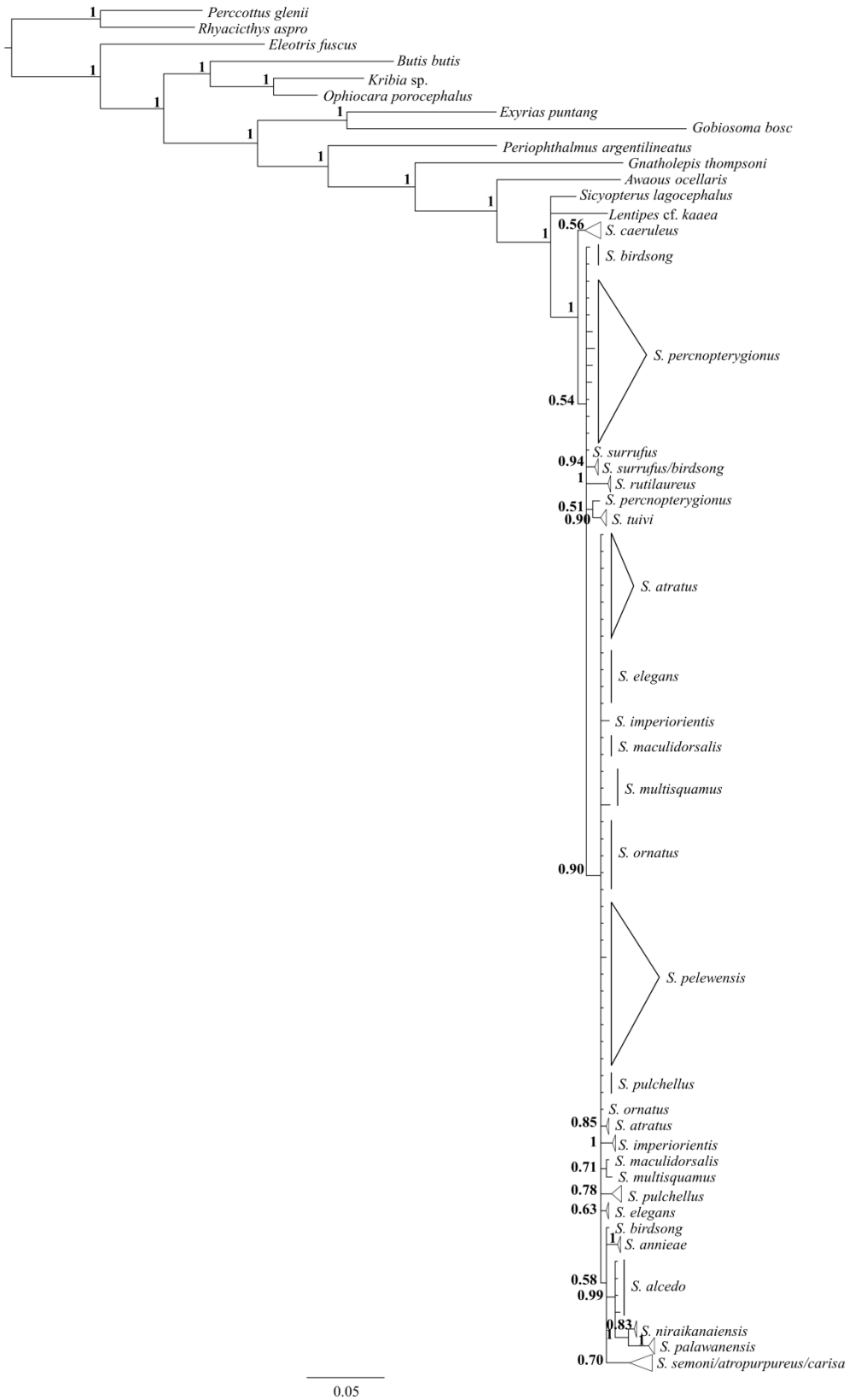


Figure 7. Bayesian phylogeny of the RAG1 gene. Only support values greater than 0.50 are displayed. Support values are posterior probabilities.

A

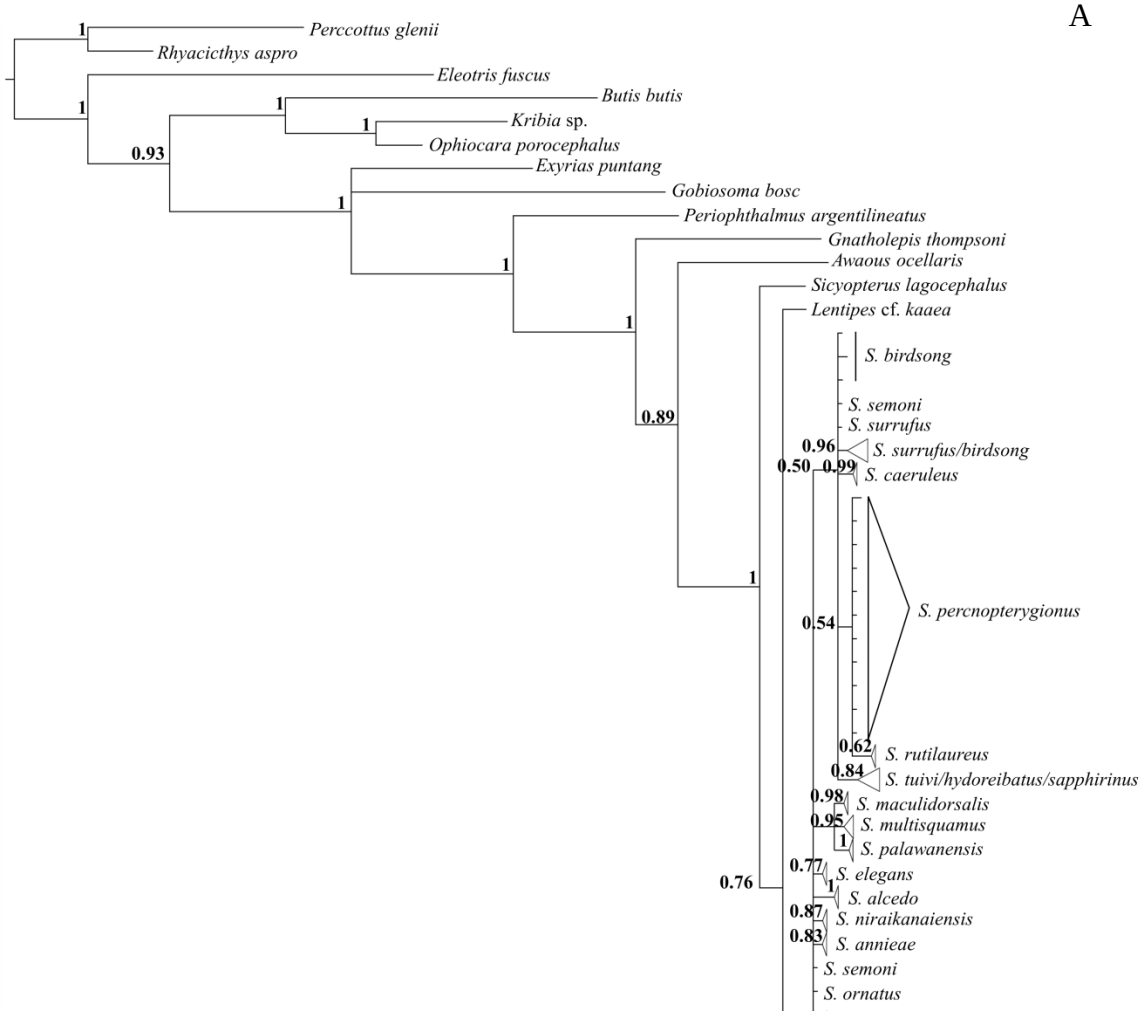
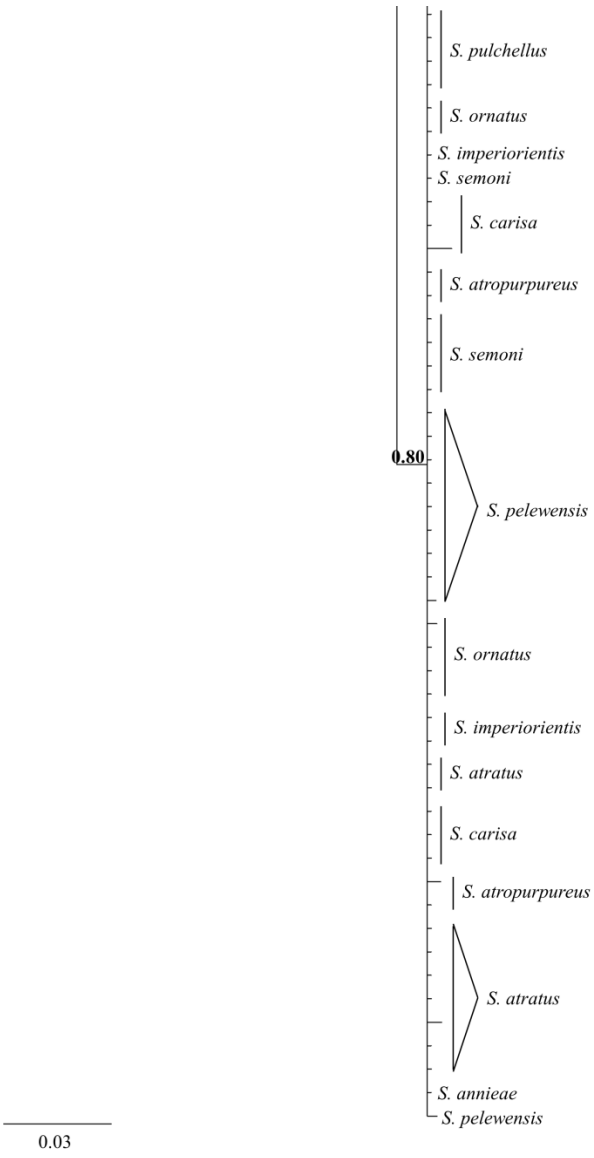


Figure 8A and 8B (below. Bayesian phylogeny of the Rh gene. Only support values greater than 0.50 are displayed. Support values are in posterior probabilities.

B


S. pulchellus
S. ornatus
S. imperiorientis
S. semoni
S. carisa
S. atropurpureus
S. semoni
0.80
S. pelewensis
S. ornatus
S. imperiorientis
S. atratus
S. carisa
S. atropurpureus
S. atratus
S. annieae
S. pelewensis
0.03