EVOLUTION OF THE GENUS SICYDIUM (GOBIIDAE:SICYDIINAE)

A Dissertation

by

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

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ABSTRACT

Gobies are one of the most diverse groups of fishes on the planet. Despite their prominence, little is known about their evolution and diversity. Gobies of the genus *Sicydium* are abundant inhabitants of tropical streams in the Atlantic and eastern Pacific basins. The amphidromous life history of *Sicydium* presents challenges in understanding their diversity and the evolutionary relationships among species. Additionally, poor species descriptions, parochial studies, and highly variable morphological characters have resulted in taxonomic uncertainty within the genus. This dissertation presents separate phylogenetic hypotheses of the relationships between species of *Sicydium* based upon morphology and DNA data.

In the first chapter, I present a phylogenetic hypothesis of the relationships among the species of *Sicydium* based upon morphological characters. This chapter shows that changes in jaw morphology are important in the evolution of *Sicydium*. Three clades of *Sicydium* were recovered, however the relationship among these clades was unresolved. Different oral morphology characters were important in determining the relationships between species. This includes morphology of the premaxillary teeth, which has been used as a diagnostic character for species of *Sicydium*. This study presents the first phylogenetic hypothesis based on morphology for the species of *Sicydium.*

The second chapter presents a molecular assessment of species diversity and a molecular phylogenetic hypothesis of *Sicydium* based upon two nuclear and two mtDNA genes. Previous molecular studies that have included *Sicydium* were at the population or subfamily level. Here I present the first molecular hypothesis of the evolutionary relationships among the species of

Sicydium. The analysis recovered two clades of a monophyletic *Sicydium,* with most relationships among species well resolved.

In the third chapter, I present a population level study of an eastern Pacific species, *Sicydium salvini*, Ogilvie-Grant, 1884. This chapter explores the diversity of a species recovered by the previous chapters. This study showed that *S. salvini* inhabits a wide geographic range including areas previously considered to be occupied by *Sicydium multipunctatum*. DNA samples from across the range of this species were used to test for population structure and population expansion. A lack of structure between river populations was found which is most easily explained by the amphidromous life history. The dispersion capabilities of the marine larvae allow free exchange of genes between distant populations. I also show that tectonic activity may explain a historic population expansion for *Sicydium salvini.*

In this dissertation I present different views on the evolution of *Sicydium.* In the first two chapters, I present phylogenetic hypotheses of the species of *Sicydium*. The third chapter shows a population level view of *Sicydium salvini.* These data can be used as a stepping-stone for future work involving the evolution of sicydiine gobies as well as providing new characters for a much needed taxonomic revision of *Sicydium.* It also clarifies population level dynamics by showing high connectivity between stream populations and an increase in population size due to historical environmental events.

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INTRODUCTION

Gobies represent one of the most diverse groups of vertebrates in the world. The number of species in a fish family is second only to the family Cyprinidae. With high morphological diversity we may conclude that gobies have evolved in a wide variety of habitats. They have invaded nearly all marine and freshwater habitats. Members of the subfamily Sicydiinae are dominant in tropical montane island streams and continental rivers with brief coastal plains (Parenti & Maciolek 1993; Keith 2003). The amphidromous life history of sicydiine gobies enables them to invade these oceanic islands. Amphidromy is a life history in which adults live and breed in freshwater, but the larvae develop in the ocean. During spawning, eggs are attached to the undersides of rocks and guarded by males (Keith 2003). Once hatched, the larvae are passively swept out to sea where they live pelagically before returning to freshwater. Upon entering the river estuaries, they undergo a transformation for a period of a few days, then mass migrations of postlarvae re-enter the streams.

Gobies of the genus *Sicydium* inhabit subtropical and tropical streams in the eastern Pacific and Atlantic basins. As long noted by ichthyologists (e.g. Thys Van Den Audenaerde 1967) the taxonomy of *Sicydium* is confusing and chaotic. The number of nominal species is probably greater than the actual number of species because of overlapping morphological characters, striking color variation, and seemingly few diagnostic characters for species. There are 23 nominal species, many of which are poorly described such as *S. plumieri* (based on drawing) and/or known from limited material, such as *S. condotense*, which was described from a single specimen (Regan, 1914). Of those 23 taxa, 18 are currently considered valid (Eschmeyer 2015). Also little is known about the evolutionary history of *Sicydium*; species

relationships have been hypothesized based upon overall appearance of species without formal analysis. The relationship between *Sicydium, Sicyopterus,* and *Parasicydium* is poorly understood with conflicting data from different studies.

The first two chapters address the phylogenetic relationships between species of *Sicydium.* The first chapter attempts to resolve the phylogenetic relationships between species based on morphological data. This is the first study of its kind for *Sicydium* and uses both previously described morphological characters and newly discovered characters to construct a phylogeny of *Sicydium.* Previous studies of *Sicydium* have focused on tooth morphology as a diagnostic tool to distinguish species (Ogilvie-Grant 1884; Watson 2000; Pezold et al. 2006). In addition to tooth morphology, other oral morphology characters plus fin and pigment characters are included in this analysis. A molecular-based phylogenetic analysis of *Sicydium* is presented in the second chapter. Using two mitochondrial DNA and two nuclear DNA genes, this is the first molecular based phylogeny of *Sicydium.* The diversity of species within *Sicydium* is also explored by testing if previously described morphological species correspond to molecular lineages. With this phylogeny, I also offer a hypothesis of the drivers of speciation within *Sicydium.* Additionally a recent molecular analysis of the subfamily Sicydiinae hypothesized that *Sicydium* to be paraphyletic with respect to *Parasicydium* (Taillebois et al. 2014). With these two chapters, the monophyly of *Sicydium* is tested and new information on the relationships between *Parasicydium, Sicydium,* and *Sicyopterus* is presented.

Lastly, the third chapter addresses a population level study to explore the diversity within *Sicydium salvini.* In this chapter I use a mitochondrial gene to test for population structure between rivers across the range of *S. salvini.* Additionally I test the correspondence between genetic and morphological species. *Sicydium mulitpunctatum* was thought to occur from Mexico

to El Salvador and *S. salvini* occurring from Nicaragua to Panama. I will show that there is a single species occurring from Mexico to Panama and that *S. multipunctatum* is a likely synonym of *S. salvini*. I use historical demographic analysis to test population changes and proposed timings of those changes. Previous studies of amphidromous sicydiine gobies have shown a strong influence from the glacial cycles of the Pleistocene (Lord et al. 2012). Here the effects of geologic uplift and glacial cycles on the population genetics and demographics of *S. salvini* are investigated.

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CHAPTER I: A phylogenetic analysis of *Sicydium* (Teleostei: Gobiidae: Sicydiinae): A morphological perspective

Abstract

A phylogenetic analysis of Sicydium (Gobiidae: Sicydiinae) based upon 26 morphological characters is presented. The characters were primarily features of oral morphology. Sicydium was recovered as monophyletic and as sister to Sicyopterus. This realtionship is based on three synapomorphic characters: the presence of lateral clefts of the upper lip, short ascending and articular processes of the premaxilla, and expanded maxillae. This finding is in agreement with previous morphological and molecular studies of the subfamily Sicydiinae. Considered by some to be a synonym of Sicydium, Parasicydium was obtained in a sister group relationship with Sicydium + Sicyopterus. supported by a single synapomorphy(fused H and K oculoscapular canal pores). Within Sicydium three clades of species were obtained based on characters of lip morphology, tooth shape, arrangement and orientation.

1. Introduction

Gobies (Teleostei: Gobiidae) represent one of the most speciose families of vertebrates in the world with over 1700 species (Eschmeyer, 2013). With high morphological diversity, gobies have adapted to a wide variety of habitats, invading nearly all tropical and subtropical marine and freshwater habitat types. Members of the subfamily Sicydiinae are abundant in tropical montane island streams and continental rivers with brief coastal plains (Keith, 2003). While most of the genera reside in the western Pacific and Indian oceans, gobies in the genus *Sicydium* inhabit subtropical and tropical streams in the eastern Pacific and Atlantic basins. Fish community diversity in the insular Caribbean relies heavily upon these herbivorous amphidromous gobies (Keith, 2003). Sicydiine gobies have specializations of their pelvic fin that aid in their rock climbing abilities. The rays of the pelvic fin are thickened and highly branched articulating with the pelvic bones to form a fused pelvic disc (Parenti & Maciolek 1993). Presumably these modifications allow *Sicydium* to adhere firmly to rocks in fast moving waters. Using their strong pelvic disks, they adhere to rocks from which they scrape algae. Hoese (1984) proposed the highly branched and thickened pelvic rays as a synapomorphy for the subfamily Sicydiinae. Like other sicydiine gobies, the species of *Sicydium* are amphidromous. This life history involves adults that live and breed in freshwater and have an oceanic larval stage. This life history allows species to inhabit ranges that are larger than those of typical primary freshwater fishes (e.g. *Sicydium salvini;* Chabarria and Pezold, 2013).

Sicydium was originally erected by Valenciennes in 1837, for the species *Gobius plumieri* Bloch 1786. When Gill (1860) erected the subfamily Sicydiinae, he divided *Sicydium* into two subgenera: *Sicydium* and *Sicyopterus*. This division was based solely on the presence of a fleshy

appendage at the base of the lower jaw in the subgenus *Sicydium* and its absence in *Sicyopterus* (Gill 1860). Although Gill's taxonomy was followed by Bleeker (1874), *Sicyopterus* and *Sicydium* were not adequately and clearly diagnosed for more than a century. Akihito and Meguro (1979) formally distinguished *Sicydium* and *Sicyopterus* using oral morphology, dentition and osteology of the suspensorium (see Table 1).

Table 1 Characters used to distinguish *Sicydium* and *Sicyopterus* (Akihito & Meguro 1979).

	Sicydium	***Sicyopterus***
Lateral Cleft	In the corner of the mouth	Anterior to the corner of the mouth
Maxillary	lower margin at same level of the upper part of the sac containing replacement teeth; anterior portion not protruding	lower margin lower than teeth in upper jaw; anterior part protruding
Swelling between the labial and dentary teeth	present	absent
Dentaries	widely separated at anterior tips	narrowly separated at anterior tips
Labial Teeth	begin at anterior tip of each dentary	beginning behind anterior tip of dentary
Projection of Soft Tissue at the Symphysis	present	absent
Teeth in Lower Jaw	bent laterally away at symphysis; size uniform	not bent laterally at symphysis; largest teeth at symphysis

Parasicydium, a monotypic sicydiine genus, has also been considered a synonym of *Sicydium* by some authors (Parenti & Maciolek, 1993). *Sicydium* and *Parasicydium* do share some similarities, but they can be distinguished based upon lip morphology, squamation and tooth morphology (Harrison, 1993). Harrison (1993) distinguished these two genera based on the presence of lateral clefts in the upper lip in *Sicydium*, and the occurrence of sexually dimorphic premaxillary teeth and the absence of dorsal squamation anterior to the origin of the second dorsal fin in *Parasicydium.* However, in a recent molecular phylogeny of the subfamily Sicydiinae, *Parasicydium* was found to be nested within *Sicydium* (Taillebois et al., 2014). *Sicydium* and *Parasicydium* share the putative synapomorphy of a dorsolateral fleshy lobe on the lower lip (Harrison, 1993).

There are 23 nominal species of *Sicydium* (Table 2). Many of these species have been poorly described, such as *S. plumieri* (based on a drawing) and/or are known from limited material, such as *S. condotense* which was described from a single specimen (Regan, 1914). As long noted by curators and authors (e.g. Thys Van Den Audenaerde, 1967) the taxonomy of *Sicydium* is confusing and chaotic. Because of overlapping morphological character state distributions, striking color variation between individuals of the same species, and seemingly few diagnostic characters for species, the number of nominal species is probably greater than the actual number of species within the genus. Traditional meristic characters alone have been of little help in diagnosing species. One reason for this is that as individuals get larger, the number of scale rows or teeth increases (Pezold et al. 2006).

Table 2 List of the species of *Sicydium.*

Species	**Ocean Basin**	**Valid**
S. adelum, Bussing, 1995	Caribbean	Yes
S. altum, Meek, 1907	Caribbean	Yes
S. antillarum, Ogilvie-Grant, 1884	Caribbean	No
S. brevifile, Ogilvie-Grant, 1884	Eastern Atlantic	Yes
S. buscki, Evermann & Clark, 1906	Caribbean	Yes
S. bustamantei, Greef, 1884	Eastern Atlantic	Yes
S. caguitae Evermann & Marsh, 1899	Caribbean	No
S. cocoensis, Heller & Snoodgrass, 1903	Eastern Pacific	Yes
S. condotense, Regan, 1914	Eastern Pacific	Yes
S. crenilabrum, Harrison, 1993	Eastern Atlantic	Yes
S. fayae, Brock, 1942	Eastern Pacific	No
S. gilberti, Watson, 2000	Caribbean	Yes
S. gymnogaster, Ogilvie-Grant, 1884	Gulf of Mexico	Yes
S. hildebrandi, Eigenmann, 1918	Eastern Pacific	Yes
S. montanum, Hubbs, 1920	Caribbean	No
S. multipunctatum, Regan 1906	Eastern Pacific	No
S. pittieri, Regan, 1907	Eastern Pacific	No
S. plumieri (Bloch, 1786)	Caribbean	Yes
S. punctatum, Perugia, 1896	Caribbean	Yes
S. rosenbergii, Boulenger, 1899	Eastern Pacific	Yes
S. salvini, Ogilvie-Grant, 1884	Eastern Pacific	Yes
S. siragus, Poey, 1860	Caribbean	No
S. vincente, Jordan and Evermann, 1898	Caribbean	No

Systematic studies of *Sicydium* have been few and generally restricted to limited geographical areas (e.g. Harrison, 1993; Pezold et al. 2006; Watson 2000). Of the 23 species that have been described in *Sicydium*, 18 are currently recognized as valid (Eschmeyer, 2015). The morphology of the upper jaw teeth has been long noted to be important in diagnosing species (Brockman, 1965; Ogilvie-Grant, 1884; Watson, 2000). Watson (2000) used tooth morphology to distinguish four species from the Dominican Republic. Pezold at al. (2006) used tooth morphology, number of tooth rows, the presence of clefts in the upper lip and the length of the

jaw to distinguish between West African sicydiine species. Although tooth morphology appears to be an important character, there are species that share the same tooth morphologies. This has lead to further difficulty in diagnosing species.

Unfortunately many authors have not included tooth morphology in species descriptions or, if included, noted the observations were much too generalized to be informative. For example, in the original description of *S. hildebrandi* the premaxillary teeth were described as "truncate" (Eigenmann 1918). More recent studies (Bussing 1996; Watson 2000; Pezold et al. 2006) employed the use of a scanning electron microscope (SEM) to obtain greater detail on the morphology of the upper jaw teeth.

Here I present a systematic study of *Sicydium* based on morphological data. This study included 14 of the 18 currently recognized species of *Sicydium.* *Sicydium rosenbergii* described from Paramba, Ecuador as *Oreogobius rosenbergii* (Boulenger, 1899) and *S. fayae* described from the Tres Marias islands (Brock, 1942) were poorly described and beyond the type specimens no specimens could be properly attributed to these species. Although recognized by Murdy & Hoese (2003) and Van Tassell (2011), *S. montanum* from Venezuela was synonymized with *S. punctatum* by Watson (2000). Because this synonymization is based upon morphological data in a taxonomic revision (Watson, 2000) versus recognition in a species list (Murdy & Hoese 2003; Van Tassell 2011), *S. montanum* was not included in the analysis and is considered a synonym of *S. punctatum*. *Sicydium multipunctatum* was also not included in the analysis because it could not be distinguished from *S. salvini* by Chabarria & Pezold (2013) based upon genetics and examination of type specimens. Three species of *Sicyopterus* and *Parasicydium bandama* were included in the analysis because of the close relationship between these two genera and *Sicydium* (Parenti and Maciolek, 1993; Keith et al. 2011; Taillebois et al., 2014). In

addition *Stiphodon atratus* and *Lentipes concolor* were included as outgroups to help with polarization of characters. The goal of this study was to present a phylogenetic hypothesis on the relationships between the species of *Sicydium.*

2. Materials and Methods

This study focused primarily on oral morphology characters that have been used to distinguish sicydiine genera in previous studies (Akihito & Meguro 1979; Sakai & Nakamura 1979; Harrison 1993; Parenti & Maciolek 1993), as well as characters that have been used to distinguish species of *Sicydium* in previous studies (Harrison 1993; Watson 2000; Pezold et al. 2006). Additional characters came from comparisons between species. Specimens were examined using a Zeiss Stemi DV4 stereoscope. Photographs were taken using a Zeiss SteREO Discovery V20. Some specimens were cleared and double stained following the procedure of Taylor and Van Dyke (1985). Terminology of the lateralis canals and canal pores follows that of Takagi (1957) and Akihito, Hayashi, & Yoshino (1984).

Specimens used for scanning electron microscopy (SEM) of teeth were first dissected. Careful attention was taken to ensure entire sections of teeth were removed intact. Once removed the samples were dehydrated in 99% pure ethanol (EtOH) for 24 hours. Specimens were then removed from the EtOH and vacuum freeze dried to remove any excess liquid. Specimens were then sputtercoated using an Emitech sputtercoater. SEM imaging was performed using a JEOL Neoscope Benchtop SEM.

A total of 26 characters were chosen for this analysis. These characters showed variation among species of *Sicydium* and/or between the genera examined for this study. The characters were coded for analysis for 14 species of *Sicydium,* 3 species of *Sicyopterus*, and one species

each of *Sicyopus, Smilosicyopus, Cotylopus, Lentipes, Stiphodon,* and *Parasicydium* (Table 3). Gobies in the *Stenogobius* group have been shown to be closely related to the sicydiine gobies (Thacker & Roje 2011; Agorreta & Rüber 2012; Tornabene et al. 2013). Therefore *Awaous banana* and *Stenogobius* sp. were used as outgroups for this analysis. When polarity of characters was ambiguous the characters were treated as unordered. Maximum parsimony analysis of the coded morphological data was accomplished using PAUP* 4.0a136 for Macintosh (Swofford, 2002). The analysis was equally weighted with heuristic searches of 500 random addition sequence replicates starting from a random tree with tree bisection and reconnection (TBR) branch swapping. Nonparametric bootstrap analysis was performed with 500 replicates of full heuristic searches using 10 random addition sequences, and TBR branch swapping.

3. Results

3.1 Phylogenetic Analysis

Descriptions of characters, their state distributions and polarities are given below. The maximum parsimony analysis resulted in 4 equally parsimonious cladograms. The strict consensus tree had a length of 46 steps, a consistency index (CI) of 0.61 and a retention index of 0.86 (Fig. 1). The phylogenetic analysis resulted in a monophyletic Sicydiinae. The base of this clade is a polytomy comprising *Sicyopus, Smilosicyopus,* and a well resolved clade containing Cotylopus*, Lentipes, Stiphodon, Sicyopterus, Parasicydium* and *Sicydium.* This clade is supported by two synapomorphies: the presence of setiform teeth (Character 1) and a sac of

replacement teeth in the upper jaw (Character 2). *Sicydium* was found to be monophyletic and obtained in a sister relationship with *Sicyopterus*. *Parasicydium* was found to be distinct from *Sicydium* and obtained in a sister group relationship to the clade comprising *Sicydium* + *Sicyopterus*. Within the *Sicydium* clade, three distinct clades were recovered, however the relationships between the clades were unresolved. The first clade contains *Sicydium punctatum, S. buscki, S. bustamantei, S. gymnogaster,* and *S. crenilabrum* "B". A second clade contained *Sicydium plumieri, S. altum, S. cocoensis, S. hildebrandi, S. brevifile, S. salvini, S. adelum* and *S. gilberti* and a third clade contained of *S. condotense* and *S. crenilabrum "*A".

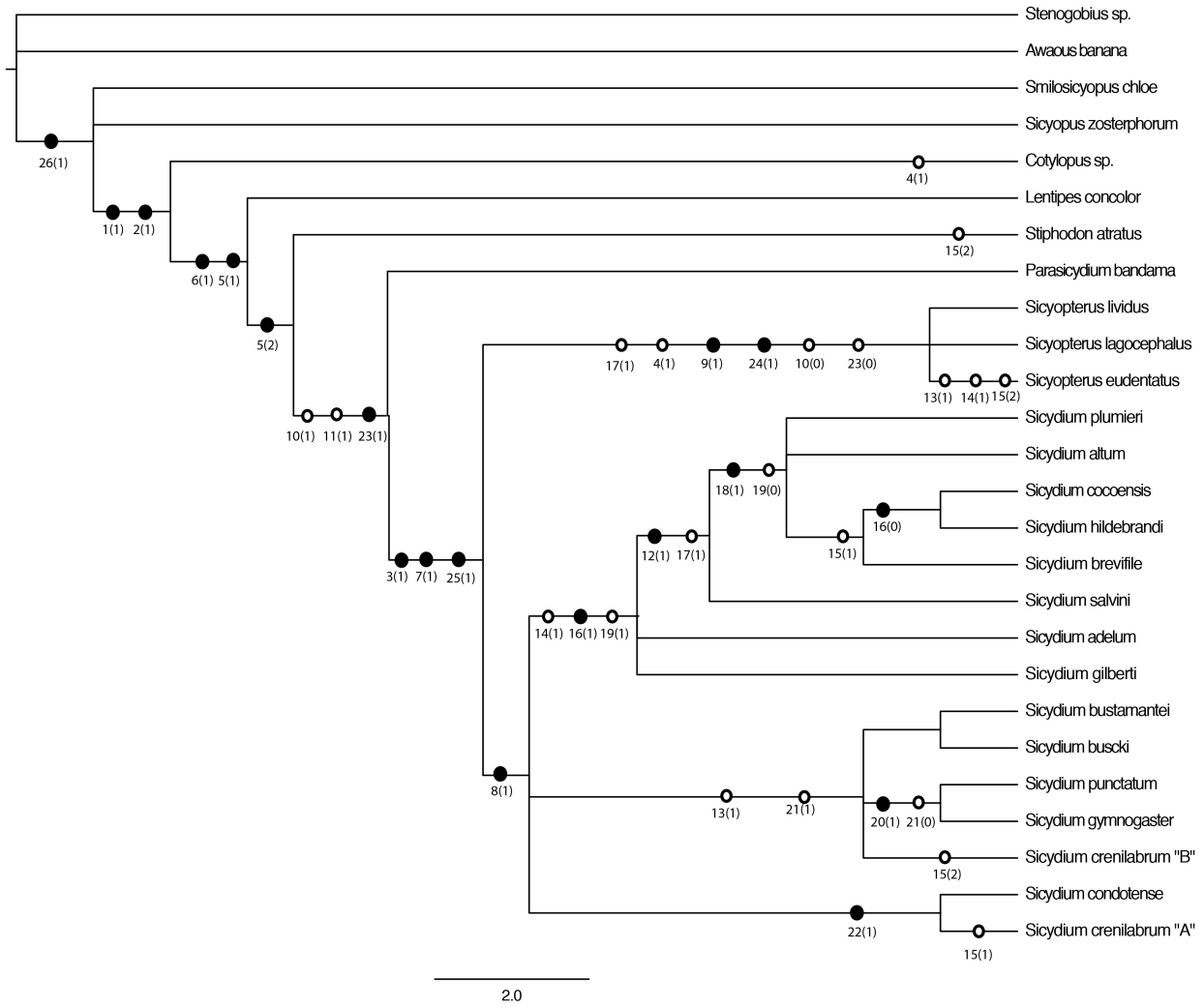

Figure 1. Strict consensus of four most parsimonious trees with character state changes mapped. Character numbers are listed below circles indicating changes. The character state is in parentheses next to character number. Open circles indicate homoplasious characters.

Table 3 Character Matrix used in the phylogenetic analysis**.**

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Stenogobius sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Awaous banana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sicyopus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Smilosicyopus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cotylopus acutipinnis	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lentipes concolor	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Stiphodon atratus	1	1	0	0	2	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1
Parasicydium bandama	1	1	0	0	2	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Sicyopterus lividus	1	1	1	1	2	1	1	0	1	0	1	0	0	?	0	?	1	0	0	0	0	0	0	1	1	1
Sicyopterus lagocephalus	1	1	1	1	2	1	1	0	1	0	1	0	0	0	0	?	1	0	0	0	0	0	0	1	1	1
Sicyopterus eudentatus	1	1	1	1	2	1	1	0	1	0	1	0	1	1	2	0	1	0	0	0	0	0	0	1	1	1
Sicydium plumieri	1	1	1	0	2	1	1	1	0	1	1	1	0	1	0	1	1	1	0	0	0	0	1	0	1	1
Sicydium altum	1	1	1	0	2	1	1	1	0	1	1	1	0	1	0	1	1	1	0	0	0	0	1	0	1	1
Sicydium cocoensis	1	1	1	0	2	1	1	1	0	1	1	1	0	1	1	0	1	1	0	0	0	0	1	0	1	1
Sicydium hildebrandi	1	1	1	0	2	1	1	1	0	1	1	1	0	1	1	0	0	1	0	0	0	0	1	0	1	1
Sicydium brevifile	1	1	1	0	2	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	0	1	0	1	1
Sicydium adelum	1	1	1	0	2	1	1	1	0	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	1
Sicydium gilberti	1	1	1	0	2	1	1	1	0	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	1
Sicydium salvini	1	1	1	0	2	1	1	1	0	1	1	1	0	1	0	1	1	0	1	0	0	0	1	0	1	1
Sicydium bustamantei	1	1	1	0	2	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1
Sicydium buscki	1	1	1	0	2	1	1	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1
Sicydium punctatum	1	1	1	0	2	1	1	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1
Sicydium gymnogaster	1	1	1	0	2	1	1	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1
Sicydium condotense	1	1	1	0	2	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1
Sicydium crenilabrum "A"	1	1	1	0	2	1	1	1	0	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1
Sicydium crenilabrum "B"	1	1	1	0	2	1	1	1	0	1	1	0	1	0	2	0	0	0	0	0	0	0	1	0	1	1

3.2 Sicydium crenilabrum Forms A & B

Upon examination of *S. crenilabrum* specimens, it became apparent that there were two different forms (A&B; Fig. 2). *Sicydium crenilabrum* form A has a crenate upper lip and tricuspid teeth.

Figure 2 Both forms of *Sicydium crenilabrum.* A) Form "A"; B) Form "B". Liberia

This form matches the description of the species and drawing of the holotype (Harrison, 1993: Fig. 6a). Form B of this species has a much larger and wider lip, with extensive crenulations. In addition, tooth morphology differs between the two forms with form A having tricuspid teeth and form B having unicuspid teeth. Form B appears to match the drawing of the paratype by Harrison (1993, Fig. 6b,d). Considering the utility of tooth morphology in diagnosing species,

the vast differences in oral morphology, and differences in pigmentation it is likely that there are two different species currently attributable to *S. crenilabrum.*

3.3 Description of Characters and states

Character 1. Teeth in upper jaw. 0=conical, 1=setiform. Conical teeth are found in the two outgroup taxa, *Stenogobius* and *Awaous.* In the sicydiine genera *Sicyopus* and *Smilosicyopus* the premaxillary teeth are also conical (Sakai & Nakamura 1979; Parenti & Maciolek 1993). This is considered the plesiomorphic state (Parenti and Maciolek, 1993). Setiform teeth, thin and stalk like, are found in the species of the other sicydiine genera: *Cotylopus, Stiphodon, Lentipes, Parasicydium, Sicyopterus* and *Sicydium.*

Character 2. Sac of replacement teeth in the upper jaw. 0=absent (Fig. 3, A), 1=present (Fig. 3, B). Parenti and Maciolek (1993) considered the presence of a sac of replacement teeth to be the derived state among sicydiines. The outgroup taxa lack the sac of replacement teeth. Within sicydiine genera, *Sicyopus* and *Smilosicyopus* lack this storage of replacement teeth on the premaxilla. This sac of replacement teeth, the derived character state, is present in *Sicydium, Sicyopterus, Parasicydium, Cotylopus, Lentipes* and *Stiphodon.* There is variation in the size of this sac, with *Sicydium* and *Sicyopterus* having the most extensive (Fig 3, B).

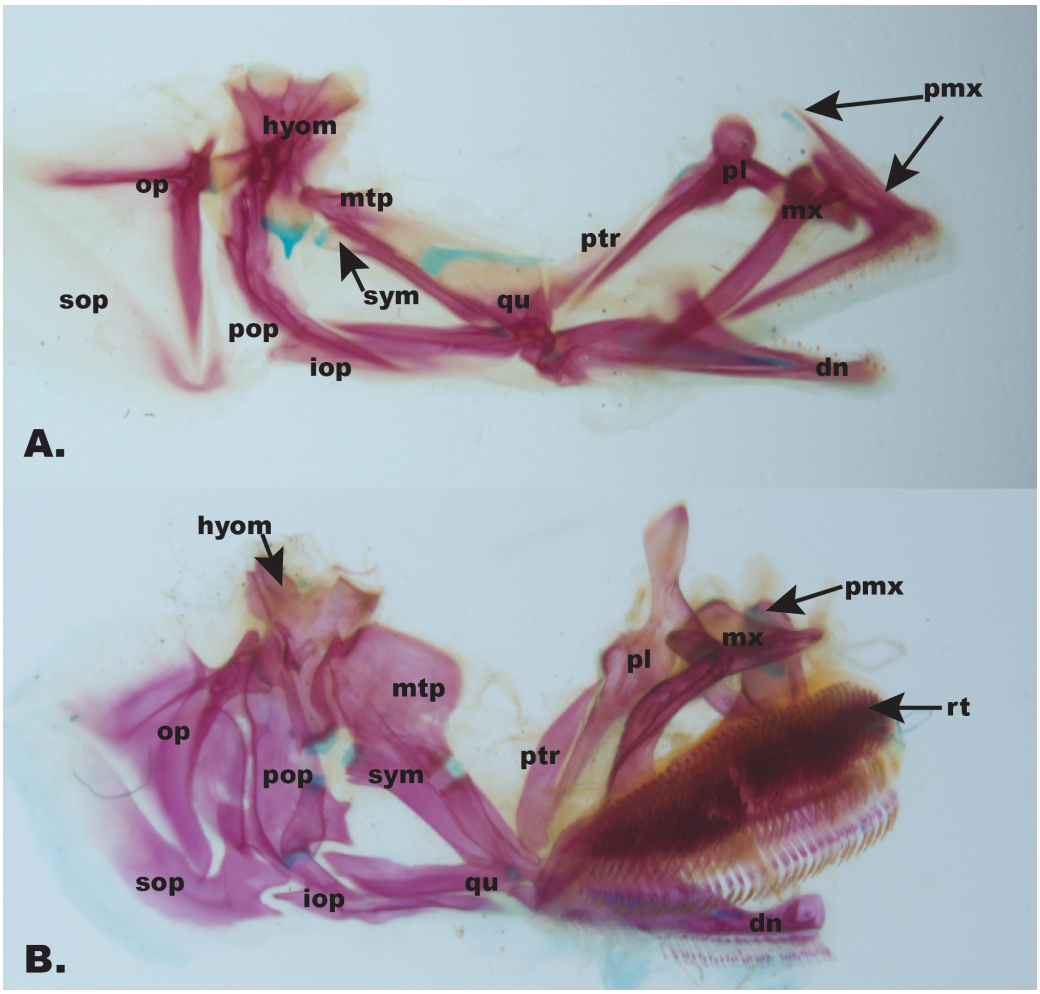

Figure 3 Suspensorium, Lateral view, right side of A) *Awaous banana* B) *Sicydium altum.* List of abbreviations: dn= dentary; hyom= hyomandibular; iop= interopercle; mtp= metapterygoid; mx= Maxilla; pl= palatine; pmx= ascending process of the premaxilla; pop= preopercle; ptr=pterygoid; qu= quadrate; rt= sac of replacement teeth; sop= subopercle ; sym=symplectic.

Character 3. Ascending/Articular Process of Premaxilla. 0=pointed (Fig. 3, A), 1=blunt (Fig. 3, B). The ascending/articular process of *Sicydium* and *Sicyopterus* is short and blunt (Fig. 3, B). Parenti and Maciolek (1993) considered this character state to be derived and a synapomorphy

for *Sicydium* + *Sicyopterus.* In all other sicydiine and outgroup taxa the ascending process of the premaxilla is pointed (Fig. 3, A).

Character 4. Medial gap between left and right premaxillary tooth rows. 0= absent, 1=present. The presence of a medial gap between the left and right premaxillary tooth rows is only found in Sicyopterus (Parenti & Maciolek 1993). Awaous, Stenogobius and in all other members of the Sicydiinae the left and right premaxillary tooth rows are continuous.

Character 5. *Labial teeth.* 0=absent, 1=medial (Fig. 4, A), 2=complete (Fig. 4, B). Labial teeth are absent in *Awaous, Stenogobius, Sicyopus,* and *Smilosicyopus*. In *Stiphodon, Parasicydium, Sicydium* and *Sicyopterus* the labial teeth form a complete row along the labial margin of the lower jaw (Fig. 4, B). *Lentipes* and *Cotylopus* appear to be intermediate, possessing a small row of labial teeth medially on the lower jaw (Fig. 4, A). The presence of labial teeth is inferred to be derived because they are absent in the outgroup.

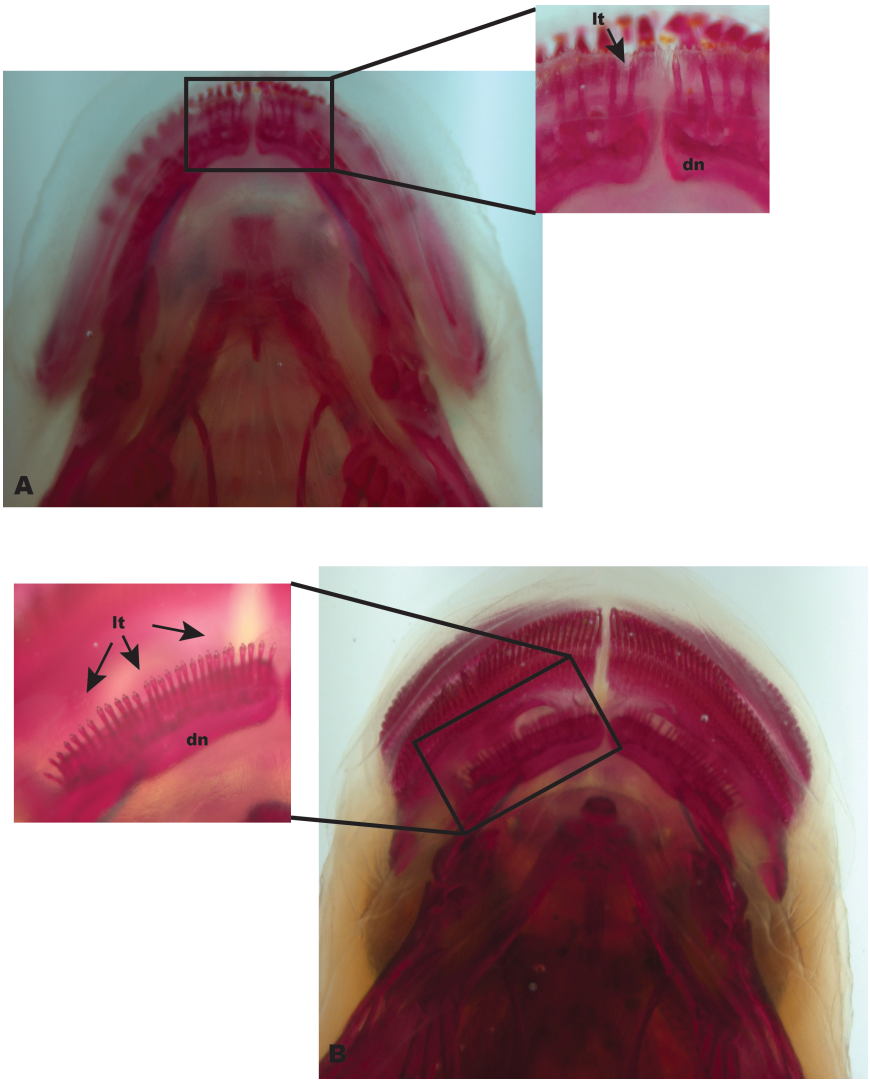

Figure 4 Ventral view of the head of A. *Lentipes concolor* that has labial teeth only on the medial end of the dentaries B. *Sicydium buscki* possesses labial teeth that cover the entire dentary. dn= dentary; lt= Labial teeth

Character 6. Medial Cleft of the upper lip. 0= absent; 1= present (Fig. 5, A). In all outgroup taxa (*Awaous* and *Stenogobius)* the upper lip is complete without a medial cleft, the plesiomorphic condition. The sicydiine genera *Sicyopus, Smilosicyopus,* and *Cotylopus* also lack a medial cleft. The cleft if present in *Lentipes, Stiphodon, Sicyopterus* and *Sicydium* (Fig. 5, A).

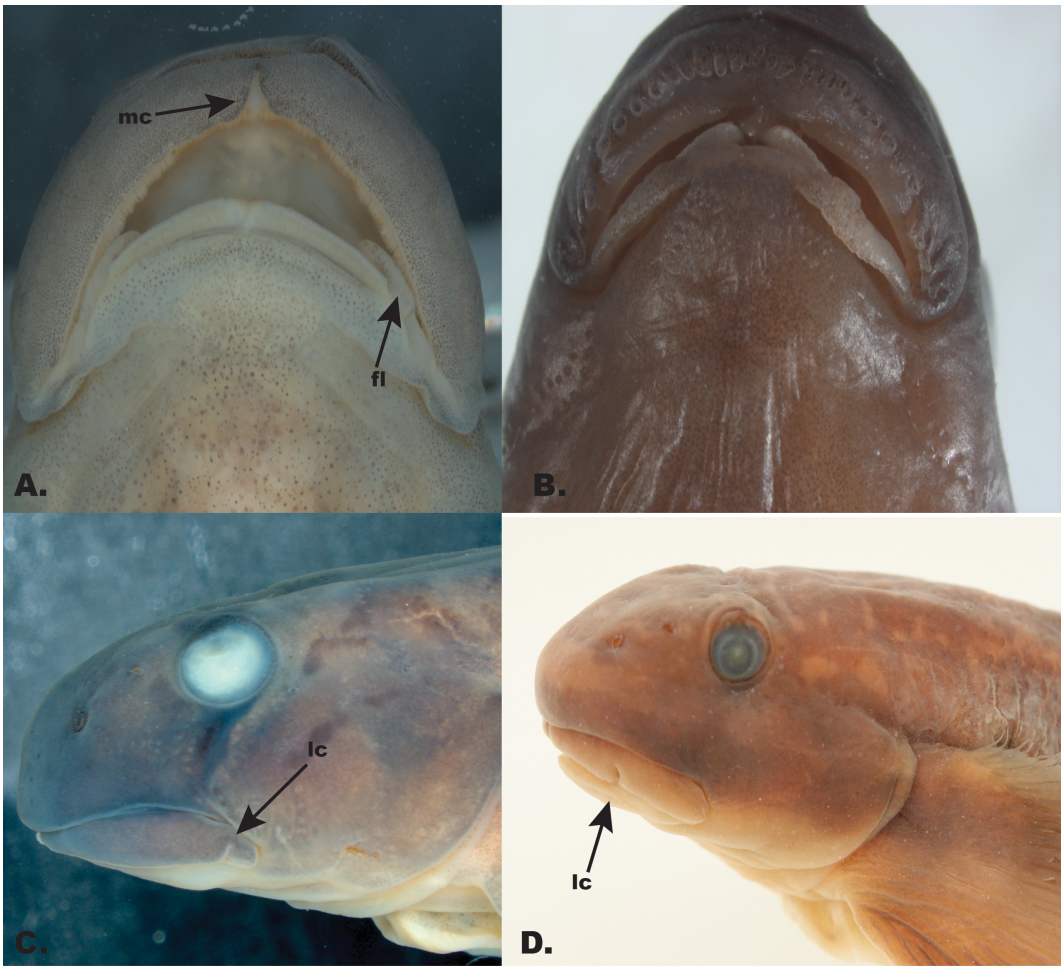

Figure 5 Lip morphology. A) Medial Cleft (mc) and fleshy lobe (fl) of *Sicydium brevifile* B) Ventral view of the mouth of *Sicyopterus lividus* showing the absence of a felshy lobe between the labial and dentary teeth. Lateral clefts (lc) in the upper lip are found in two different locations in C) *Sicydium salvini* it is near the corner of the mouth. D) In *Sicyopterus eudentatus* the lateral cleft is anterior to the corner of the mouth

Character 7. Lateral cleft of the upper lip. 0=absent; 1=present (Fig. 5, C-D). *Sicydium* and *Sicyopterus* are the only two sicydiine genera that have a lateral cleft in the upper lip (Fig. 5, C-D). All other genera including the two outgroup taxa lack a lateral cleft. Therefore the presence

of a lateral cleft in *Sicydium* and *Sicyopterus* was considered to be a derived character by Parenti & Maciolek (1993).

Character 8. Lateral cleft in the corner of the mouth (7). 0=no lateral cleft in the corner of the mouth; 1=present (Fig. 5, C). The presence of a lateral cleft at the corner of the mouth is found only in *Sicydium* (Fig. 5, C).

Character 9. Lateral cleft anterior to the corner of the mouth (8). 0= absent. 1= present (Fig. 5, D). The presence of a lateral cleft anterior to the corner of the mouth is found only in *Sicyopterus.* This character is absent in *Awaous, Stenogobius,* and all other sicydiine gobies examined.

Character 10. Swelling of tissue between the labial and conical teeth of the lower jaw. 0=absent (Fig. 5, B), 1=present (Fig. 5, A). *Sicydium* and *Parasicydium* possess a swelling of tissue between the labial and conical teeth of the lower jaw (Fig 5, A). *Awaous, Stenogobius,* and all other genera of Sicydiinae examined here lack this swelling. It is therefore considered to be the derived state. This character has been used to distinguish between *Sicydium* and *Sicyopterus* by other authors (Akihito and Meguro, 1979; Parenti and Maciolek, 1993). Harrison (1993) noted this character was also present in *Parasicydium* and *Sicydium.*

Character 11. Oculoscapular-canal pores. 0=separate H' & K' pores, 1= fusion of the H' & K' pores. Pezold (1993) defined a distinct oculoscapular-canal pore pattern in *Sicydium* and *Sicyopterus.* The H and K pores of *Sicydium* and *Sicyopterus* are fused into a single pore. Parenti

and Maciolek (1993) noted that *Parasicydium* also shares this same oculoscapular pore pattern of *Sicydium* and *Sicyopterus*. In other sicydiine genera and *Stenogobius* the H' and K' pores are separate. Fusion of the H and K pores is found in a few species of *Awaous* (Pezold 1993). This character represents a homoplasy in some *Awaous* species.

Character 12. Tooth arrangement. 0=single row (Fig. 6, C), 1= alternating rows (Fig. 6, A-B). Most species have a single row arrangement (Fig. 6, C). Alternating tooth arrangement gives the appearance of two tooth rows on the premaxilla (Fig. 6, A-B). The alternating row character state is only in *Sicydium salvini, S. plumieri, S. altum, S. brevifile, S. hildebrandi,* and *S. cocoensis* and is interpreted as the derived character state.

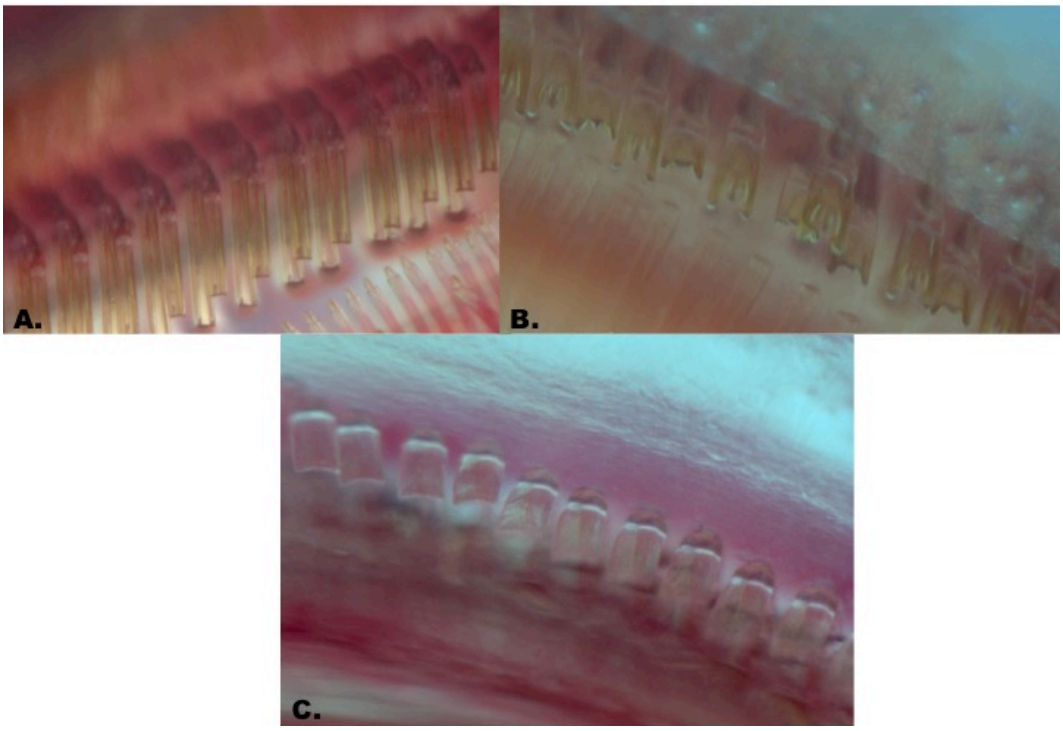

Figure 6 Tooth Arrangement of A) *Sicydium altum,* alternating B) *Sicydium salvini*, Alternating, and C) *Sicydium buscki*, Aligned

Character 13. Tooth orientation in the upper jaw. 0=outward (Fig. 7, B), 1=anterior (Fig. 7, A). In some species, teeth on lateral sides of mouth are oriented anteriorly, almost parallel with the premaxilla. Lateral teeth in mouth appear to be in a line. In others, all teeth face anterior (Fig. 7, A). In *Awaous, Stenogobius* and most sicydiine species teeth face outward from the curvature of the premaxilla (Fig. 7, B). The derived character state can be found in *Sicydium buscki, S. bustamantei, S. crenilabrum* "B", *S. punctatum, S. gymnogaster* and *Sicyopterus eudentatus.*

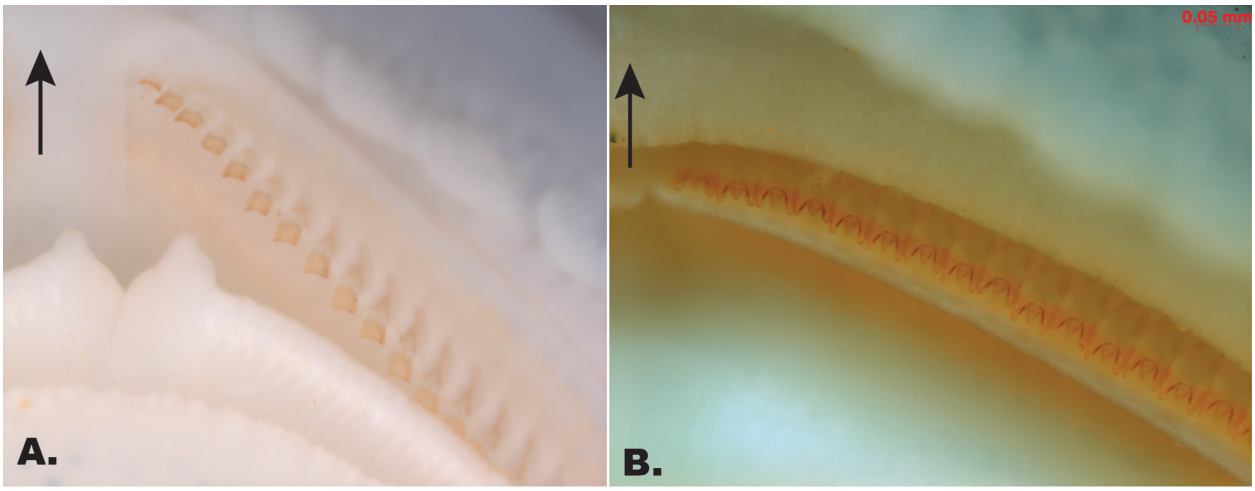

Figure 7 Premaxillary tooth orientation. A) Anterior, *Sicydium crenilabrum* "B" B) Outward, *Sicydium crenilabrum* "A". Arrow indicates the anterior direction.

Character 14. Extent of filamentous first dorsal fin in males. 0= Reaching to the origin of the second dorsal fin when appressed, 1= extending beyond the middle of the second dorsal fin. The filamentous first dorsal fins of males in outgroups *Awaous* and *Stenogobius* do not reach beyond the origin of the second dorsal fin. This long, filamentous dorsal fin is found in *Sicydium plumieri, S. altum, S. salvini, S. cocoensis, S. hildebrandi, S. adelum,* and *S. gilberti.* It was also found in a single *Sicyopterus* species (*S. eudentatus).*

Character 15. Crenulate upper lip. 0=crenulations absent (smooth, Fig. 8, A), 1=crenulate (Fig. 8, B), 2= heavily crenulate (Fig. 8, C). Harrison (1993) distinguished *S. crenilabrum* from other species of *Sicydium* in West Africa by the presence of distinct crenulations in the upper lip. *Sicydium crenilabrum* "A"*, S. brevifile,* and *S. cocoensis* possess crenulate upper lips. Heavily crenulations upper lip is found only in *Sicydium crenilabrum* "B" and *Sicyopterus eudentatus* has a heavily crenulated upper lip similar to the character state that is seen in *S. crenilabrum* "B". Character state 1, crenulate upper lip, appears to be the intermediate form between the smooth and heavily crenulate. *Awaous* and *Stenogobius* have smooth lips, therefore a crenulate upper lip is the derived character state.

Figure 8 Upper lip. A) Smooth lip, *S. brevifile* B) Crenulate, *S. crenilabrum* "A" C) Heavily crenulate, *S. crenilabrumm* "B"

Character 16. Posterior extension of the jaw. 0= to a vertical through the middle of the eye (Fig. 9, A), 1= beyond the posterior margin of the eye (Fig. 9, B). Harrison (1993) and Pezold et al. (2006) used this character to distinguish between *S. brevifile* and other West African *Sicydium* species. In addition to *S. brevifile* the derived state is also found in *S. plumieri, S. altum, S.*

salvini, S. gilberti and *S. adelum.* Upon examination of this character in other sicydiine genera and the outgroups *Awaous* and *Stenogobius*, the jaws of do not extend past the middle of the eye.

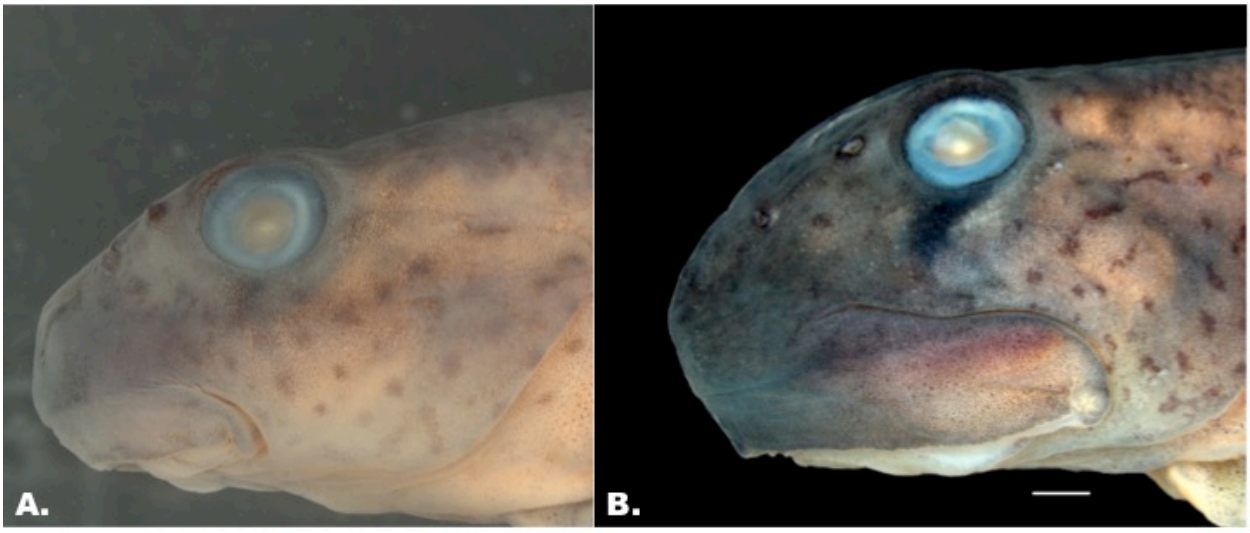

Figure 9 Extent of jaws. A) Jaw not extending beyond the middle of the eye, *S. bustamantei*

B) Jaw extending past the posterior margin of the eye, *S. brevifile*

Character 17. Diagonal stripe towards the dorsal extent of the caudal fin. 0=absent, 1=present (Fig. 10). In Sicydium plumieri, S. altum, S. cocoensis, S. brevifile, S. salvini and the three species of Sicyopterus examined here a black stripe borders the light margin at the dorsal ridge of the caudal fin (Fig. 10). This character was not found in Awaous, Stenogobius, Parasicydium, Lentipes and Stiphodon. Therefore the presence of the stripe is considered to be derived.

Figure 10 Diagonal stripe on the dorsal portion of caudal fin.

Characters 18-22. Morphology of the upper jaw teeth. The morphology of the premaxillary teeth is only known to dramatically vary within *Sicydium* and *Sicyopterus*. Different tooth morphologies in *Sicyopterus* have not been thoroughly examined as systematic or diagnostic tools, however they have been explored for *Sicydium* in this study and in others (Ogilvie-Grant, 1884; Watson, 2000; Pezold et al., 2006). The tooth morphologies found in *Sicydium* are unique among sicydiine gobies and the outgroup taxa. For the tooth morphologies found in *Sicydium,* individual morphologies were coded as individual characters.

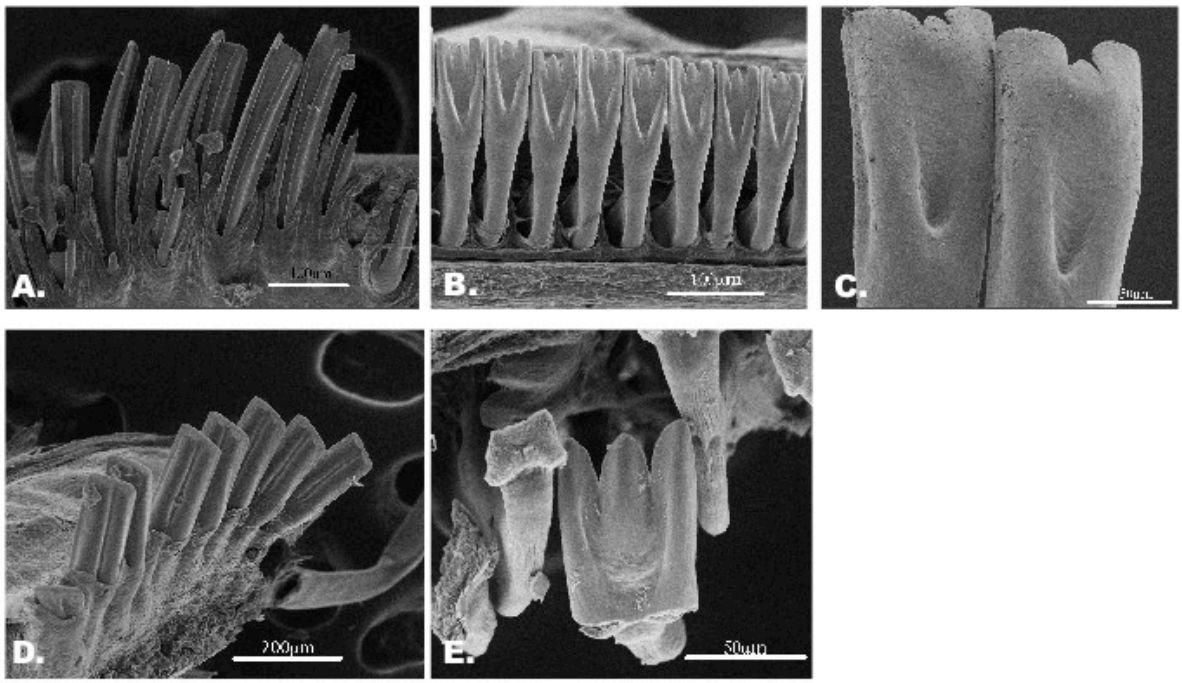

Figure 11 Morphology of the premaxillary teeth of *Sicydium*. SEM images of the premaxillary teeth of A. *Sicydium altum,* B. *S. salvini,* C. *S. punctatum,* D. *S. buscki,* E. *S. crenilabrum* "A"

Character 18. Upper jaw teeth narrow, long, and shallowly tricuspid. 0=absent, 1=present (Fig. 11, A). The tooth is narrow, long, and shallowly tricuspid with two large parallel ridges along the tooth face, the small cusps wear away giving the teeth a unicuspid appearance. The outgroup taxa have conical/canine morphologies in the upper jaw teeth. The derived state can be found in *Sicydium plumieri, S. altum, S. cocoensis, S. hildebrandi,* and *S. brevifile.*

Character 19. Upper jaw teeth trident, all cusps equal in length and pointed. 0=absent, 1=present (Fig. 11, B). The cusps of this trident-like morphology are equal length with the two lateral cusps forming a ridge along the outside of the teeth creating a valley where the medial

cusp is located (Figure 3B). Among *Sicydium,* this derived state is found in *S. salvini, S. gilberti,* and *S. adelum*.

Character 20. *Tricuspid tooth with two large lateral cusps that flank the small medial cusp*. 0= absent, 1= present (Fig. 11,C). This derived tooth morphology is found in two western Atlantic species, *Sicydium punctatum* and *S. gymnogaster*.

Character 21. *Upper jaw teeth unicuspid tooth with a medial furrow down*. 0= absent, 1= present (Fig. 11, D). The furrow in the center of this tooth is superficial, not marking the division between two cusps. Unicuspid teeth are found in Sicydium buscki, S. bustamantei, and S. crenilabrum "B".

Character 22. Tricuspid tooth with tapering cusps, the two lateral cusps taper towards the interior of the tooth, whereby the medial cusp is convex, tapering laterally. 0= absent, 1= present (Fig. 11, E). This morphology is present in Sicydium crenilabrum "A" and S. condotense.

Character 23. Protrusion of soft tissue in the lower jaw at the symphysis of the dentaries. 0=absent, 1=present (Fig. 12). Akihito and Meguro (1979) used this character to distinguish *Sicydium* from *Sicyopterus.* Upon examination for this study, it was found to be present in *Parasicydium* and absent in *Awaous, Stenogobius, Sicyopus, Smilosicyopus, Sicyopterus, Stiphodon* and *Lentipes.* This character is considered to be derived due to its absence in the outgroups.

Figure 12 Protrusion of soft tissue at the symphysis of the dentaries (arrow) of *Sicydium brevifile*

Character 24. Lower jaw teeth. 0=uniform in size, 1=anterior teeth larger (Fig. 13). Akihito and Meguro (1979) used this character to distinguish between *Sicydium* and *Sicyopterus.* In the three *Sicyopterus* species, the anterior conical teeth of the lower jaw are larger than the rest (Fig. 13). *Awaous, Stenogobius,* and all other sicydiine genera have uniform conical teeth in the lower jaw. Large anterior teeth are considered to be derived because the lower jaw teeth in most sicydiines and the outgroups are uniform.

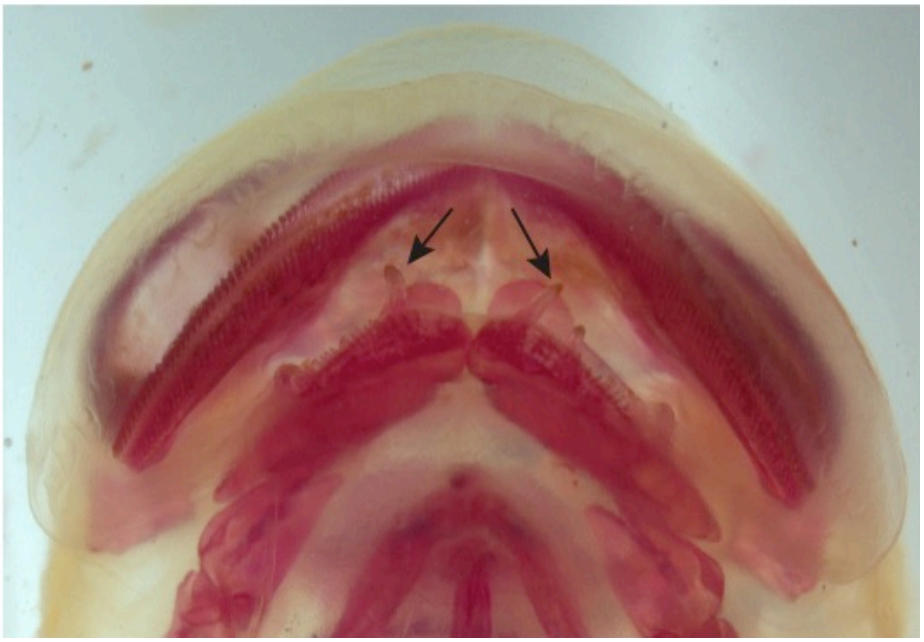

Figure 13 Ventral view of the mouth of *Sicyopterus lividus.* The anteriormost teeth of the lower jaw are indicated by arrows.

Character 25. Maxilla. 0=not expanded (Fig. 14, A-B), 1=greatly expanded (Fig. 14, C-D). Akihito and Meguro (1979) used a greatly expanded maxilla with a protruding anterior portion to distinguish *Sicyopterus* from *Sicydium.* Harrison (1989) came to a similar conclusion. In *Awaous, Stenogobius, Sicyopus, Smilosicyopus,* and *Stiphodon* the maxilla is not greatly expanded (Fig. 14, A-B). In the present study, the derived state of having a greatly expanded maxilla is also present in the *Sicydium* species (Fig. 14, C-D) and the three *Sicyopterus* species.

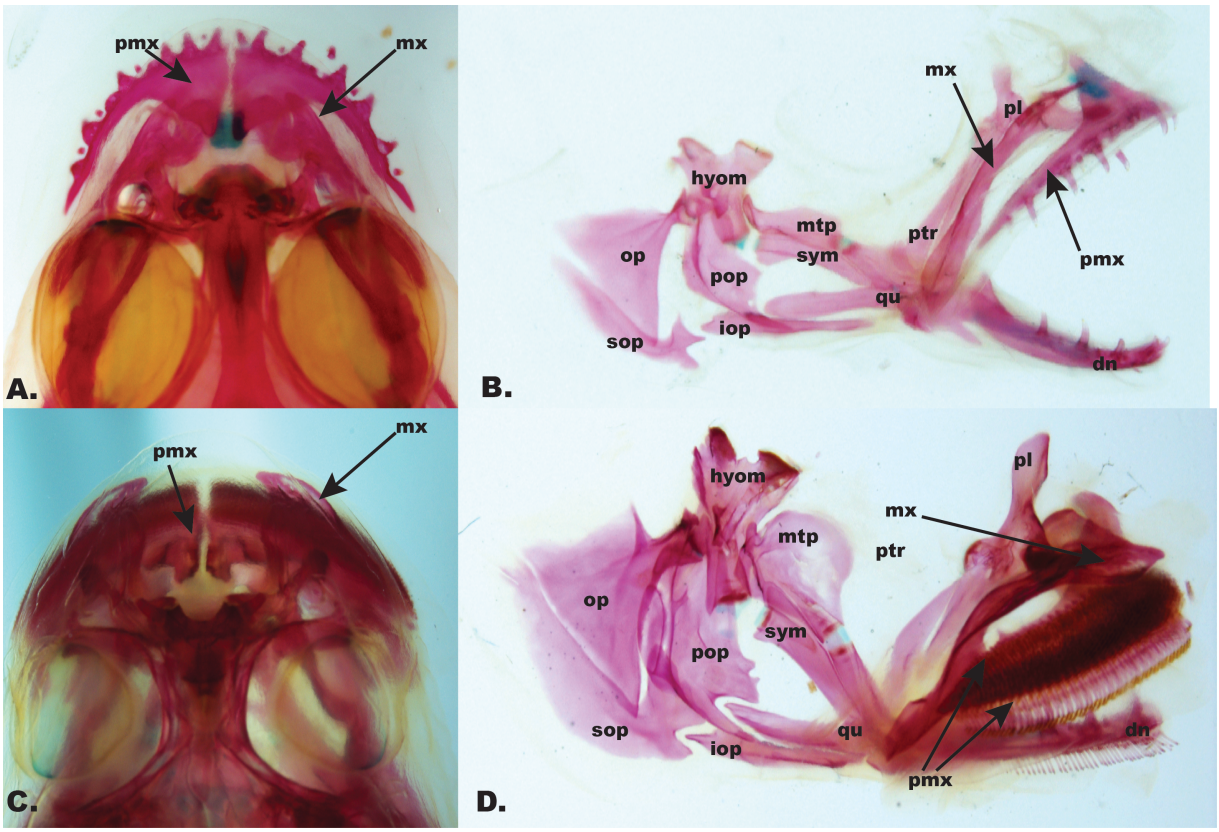

Figure 14 Comparison of the maxilla and premaxilla. Dorsal View of the head of A) *Sicyopus zosterophorum* and C) *Sicydium salvini.* Lateral view of the suspensorium of B) *S. zosterophorum* and D) *S. salvini*. List of abbreviations same as in Fig. 3.

Character 26. Premaxilla. 0=not expanded dorsally (Fig. 15, B; Fig. 3, A), 1= expanded dorsally (Fig. 15, A; Fig. 14, A-D). The premaxilla of sicydiine gobies is expanded dorsally (Harrison 1989) with little to no differentiation between the ascending and articular processes (Fig. 15, A) (Parenti & Maciolek 1993). Harrison (1989) proposed this as a synapomorphy for Sicydiinae. From examination of the specimens for this study, the sicydiine genera all possessed

this character. In *Awaous* and *Stenogobius*, the premaxilla is not expanded dorsally and the ascending and articular processes are separated (Fig. 15, B; Fig. 3, A).

4. Discussion

The phylogenetic analysis recovered a monophyletic *Sicydium* (Fig. 1), supported by the presence of a lateral cleft at the corner of the mouth (Fig. 1). The sister relationship between *Sicydium* and *Sicyopterus* recovered here is concordant with previous morphological (Parenti & Thomas 1998) and molecular studies (Keith et al., 2011; Taillebois et al., 2014) of the subfamily Sicydiinae. In the present analysis, *Sicydium* and *Sicyopterus* are united by the following synapomorphic characters: (1) presence of a short, blunt ascending/articular process of the premaxilla (Fig. 3, B); (2) lateral clefts of the upper lip (Fig. 5, C-D); and (3) an expanded maxilla with a protruding anterior portion (Fig. 14) Harrison (1993) constructed a cladogram based on morphological data of Parenti (1991) and Parenti & Maciolek (1993) that showed different relationships between genera from this study. Specifically a trichotomy was found for *Sicydium, Stiphodon,* and *Sicyopterus* that was sister to *Parasicydium*. The character that was used to unite the trichotomy was the presence of setiform teeth across the entire upper jaw. *Parasicydium* shows sexual dimorphism in the types of upper jaw teeth. In males the anterior premaxillary teeth are setiform (tricuspid) while teeth at the posterior part of the upper jaw are canines (Risch 1980). This tooth arrangement is also found in species of *Lentipes* (Harrison, 1993). Taillebois et al. (2014) obtained *Parasicydium* to be nested within *Sicydium* based upon molecular data. Risch (1980) described *Parasicydium* as a "*Sicydium*-like" genus. However in the phylogenetic hypothesis resulting from the phylogenetic analysis conducted herein *Parasicydium* is not recovered as the closest relative to *Sicydium* but as the closest relative to

Sicydium + *Sicyopterus* clade (Fig. 1). *Parasicydium* can be distinguished from *Sicydium* and *Sicyopterus* by the presence of canines in the upper jaw in males and the absence lateral clefts in the upper lip. This relationship is supported by the presence of an oculoscapular canal pore pattern with fused H & K pores with both Sicydium and Sicyopterus. In addition *Parasicydium* possesses a fleshy lobe between the labial and dentary teeth (Fig. 5, A) and a protrusion of tissue at the symphysis of the dentaries (Fig. 12), characters shared with *Sicydium*. Given its position as a sister group to *Sicydium* and *Sicyopterus,* these fleshy labial features are hypothesized to have been lost in *Sicyopterus.*

This section is reworked according to Kevin's comments.Within *Sicydium*, three well-supported (Fig. 1) monophyletic clades were recovered, including: *Sicydium* clade 1 *(S. plumieri, S. altum, S. brevifile, S. cocoensis, S. hildebrandi, S. salvini, S. adelum* and *S. gilberti*); Sicydium clade 2 (*S. buscki, S. bustamantei, S. punctatum, S. gymnogaster*, and *S. crenilabrum* "B") and *Sicydium* clade 3 (*S. condotense* and *S. crenilabrum* "A"). Unfortunately, the relationships between these three clades were not well-resolved in this analysis. Monophyly of clade 1 is supported by the presence a filamentous first dorsal fin in males extends past the middle of the second dorsal fin and a jaw that extends beyond the middle of the eye (usually beyond the posterior margin). Within clade 1, *Sicydium plumieri, S. altum, S. brevifile, S. cocoensis* and *S. hildebrandi* formed a clade that is united by the presence of narrow, ridged, shallow tricuspid teeth (Fig. 11, A) and fingerlike projections along the gums (Fig. 15) that almost completely cover the entire tooth. Within this clade 1, *S. cocoensis* and *S. hildebrandi* have lost a longer jaw, which unites both as sister taxa. *Sicydium brevifile* is sister to *S. cocoensis* and *S. hildebrandi* based on the homoplasious character of having a crenulate upper lip a state that also appears in the more distantly related *Sicydium crenilabrum* “A”. An alternate tooth replacement pattern on

the premaxillae (Fig. 6, A-B) and the presence of a diagonal stripe at the dorsal surface of the caudal fin supports the sister relationship between *S. salvini* and the *S. plumieri* + *S. altum* + *S. brevifile* + *S. cocoensis* + *S. hildebrandi* clade. The presence of identical tooth morphologies, trident like tricuspid tooth (Fig. 11, B) did not unite *S. salvini, S. adelum,* and *S. gilberti.*

The species of *Sicydium* clade 2 are united by a unique arrangement of upper jaw teeth. On the lateral sides of the jaw, the teeth are angled to become nearly parallel with the premaxilla. Within this clade, a sister group relationship between *S. punctatum* and *S. gymnogaster* was obtained, supported by the presence of a unique tricuspid tooth morphology (Fig. 11,C). These two species generally appear similar and share the same spotted pigment pattern on the lateral sides of the head that extend onto the pectoral fin base, and have scales with large melanophores across the entire body. *Sicydium buscki, S. bustamantei,* and *S. crenilabrum* "B" all have unicuspid teeth (Fig.11,D). *Sicydium buscki* did form a single clade, but the relationship with *S. crenilabrum* "B" is unresolved. This is due in part to the analysis placing the evolution of a unicuspid tooth at the base of the larger clade, then a subsequent loss of unicuspid teeth in the lineage containing *S. gymnogaster* and *S. punctatum* (Fig. 1). Furthermore *S. crenilabrum* "B" has a heavily crenulate upper lip that is unique among other members of this clade and *Sicydium* as a whole. Within Sicydiinae, a heavily crenulate upper lip was also observed in *Stiphodon atratus* and *Sicyopterus eudentatus*. The final clade of *Sicydium* (*Sicydium* clade 3) includes *Sicydium condotense* and *S. crenilabrum* "A" supported by the presence of a tricuspid tooth morphology tapering cusps (Fig 11, E). In this unique tooth morphology, the medial cusp is convex with two lateral cusps that taper inward.

The importance of oral morphology in systematics of sicydiine interrelationships has been stressed by numerous authors (Akihito & Meguro 1979; Sakai & Nakamura 1979; Parenti

& Maciolek 1993; Pezold et al. 2006). In the current study, many aspects of oral morphology are shown to be useful in determining relationships between species of *Sicydium.* In most situations, premaxillary tooth morphology and arrangement appears to be useful in diagnosing species or supporting larger groups of species. Table 4 shows the distribution of characters between the species of *Sicydium* examined for this study. Many of the characters cannot fully distinguish all species of *Sicydium* without taking geographic distribution into consideration.

Some characters in the current analysis were homoplasious and likely the result of parallel evolution. A close relationship between *Sicydium* and *Sicyopterus* has been shown by many authors (Harrison 1993; Parenti & Maciolek 1993; Keith et al. 2011; Taillebois et al. 2014). Parallel evolution is expected in closely related lineages because they share genetic backgrounds and developmental potentials (Eldredge & Cracraft 1980; Mayr & Ashlock 1991). If closely related lineages live in similar environments it is likely they experience similar selective pressures and therefore similar characteristics are more likely to evolve in parallel (Eldredge & Cracraft 1980; Mayr & Ashlock 1991). Parallel evolution between morphological traits has been shown in whitefish (Bernatchez et al. 2010) and in the threespine stickleback (Foster & Baker 2004). *Sicydium* and *Sicyopterus* are both herbivorous and they occur in the same types of habitats. Because of the similarity between their environments species of *Sicydium* and *Sicyopterus* are likely to encounter similar selective pressures. These pressures may have led to the similar characters that appear to have evolved independently within *Sicydium* and *Sicyopterus* (e.g., heavily crenulate upper lip, anterior premaxillary tooth orientation).

The morphological characters used in this study could not resolve the full relationships between the species of *Sicydium.* Future analysis of molecular data could help further illuminate

the species relationships as well as character evolution within *Sicydium.* A thorough taxonomic review of *Sicydium* and *Sicyopterus* is also needed.

Table 4 Distribution of characters among the *Sicydium.* For the tooth morphology column, the letter indicates the corresponding morphology in Fig. 11. List of Abbreviations: OB=Ocean Basin; TM=Tooth Morphology; TA= Tooth Arrangement; TO= Tooth Orientation; DF= Extent of filamentous dorsal fin; UL= Upper lip; LC= Lateral clefts; MC= Medial Cleft; JE= Jaw extent; Y= Yes; N= No

Species	OB	TM	TA	TO	DF	UL	LC	MC	JE
S. salvini	E. Pacific	B	Alternate	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. hildebrandi	E. Pacific	A	Alternate	Outward	At least to middle of 2nd	Crenulate	Y	Y	Middle of eye
S. condotense	E. Pacific	E	Sing	Outward	Up to 2nd Dorsal	Smooth	Y	Y	Middle of eye
S. cocoensis	E. Pacific	A	Alt	Outward	Beyond middle of 2nd dorsal	Crenulate	Y	Y	Middle of eye
S. punctatum	Caribbean	C	Sing	Anterior	Up to 2nd Dorsal	Smooth	Y	Y	First 1/4 of eye
S. buscki	Caribbean	D	Sing	Anterior	Up to 2nd Dorsal	Smooth	Y	Y	First 1/4 of eye
S. plumieri	Caribbean	A	Alternate	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. adelum	Caribbean	B	Sing	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. gilberti	Caribbean	B	Sing	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. altum	Caribbean	A	Alt	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. gymnogaster	Gulf of Mexico	C	Sing	Outward	Up to 2nd Dorsal	Smooth	Y	Y	First 1/4 of eye
S. brevifile	E. Atlantic	A	Alt	Outward	Beyond middle of 2nd dorsal	Smooth	Y	Y	Beyond middle of eye
S. bustamantei	E. Atlantic	D	Sing	Anterior	Up to 2nd Dorsal	Smooth	Y	N	To middle of eye
S. crenilabrum "A"	E. Atlantic	E	Sing	Outward	Up to 2nd Dorsal	Crenulate	Y	Y	Almost to anterior of eye
S. crenilabrum "B"	E. Atlantic	D	Sing	Anterior	Up to 2nd Dorsal	Heavily Crenulate	Y	Y	Does not reach eye

Appendix

Materials Examined

Sicydium adelum. Alcohol Specimens: Costa Rica: REC10-20(2); REC10-25 (2); REC10-19(1); REC10-16 (3) ;UCR 1299-24 Paratype(1). SEM Costa Rica: REC289. Cleared and Stained: Costa Rica: REC479; REC480

Sicydium altum. Alcohol Specimens: Costa Rica:REC10-20(10); REC10-25 (9); REC10-19(7);REC10-26(10). Puerto Rico: SLU1439. SEM: Costa Rica: REC381. Cleared and Stained: Costa Rica: REC384; REC388; REC478; REC481; Rio Bonanito

Sicydium brevifile. Alcohol Specimens: Cameroon:CR4(1); BMNH 1866.6.26.10(1), Holotype; Sao Tome and Principe: CAS214398(10); CAS224095(20); CAS224095(13); CAS224099(10); Cleared and Stained:CAS:214413(10)

Sicydium buscki. Alcohol specimens: Puerto Rico: RECO9-07 (6); REC09-02 (2); 09-27(9). Jamaica: SLU6344(2). St Croix: UF180989(5); UF183289(4); UF181001(5). Dominican Republic: UF101796(4). SEM: Puerto Rico: SLU1160. Cleared and Stained: Puerto Rico: SLU1226; SLU1373; SLU1378; SLU1379; SLU1485

Sicydium bustamantei. Alcohol Specimens: Sao Tome and Principe: CAS214115(20); CAS214550 (19); CAS214407(6). Cleared and Stained: Sao Tome and Principe: CAS214389(5)

Sicydium cocoensis. Alcohol Specimens: Cocos Islands: UCR736.006 (17); UCR8.002 (1); UCR6.001(1). Cleared and Stained: Cocos Islands: UCR736.006 (2)

Sicydium condotense. Alcohol Specimens: ROM93685 (2); BMNH 1914.5.18.109 (holotype)

Sicydium crenilabrum "A". Alcohol Specimens: Cameroon: CAS61408(9); MCZ48156(13); Pettrade(3). Liberia: Lib14-17(1); Lib14-23(1); Lib14-24(2). SEM:CR7; Cleared and Stained:CR7

Sicydium crenilabrum "B". Alcohol Specimens: Liberia: Lib14-24(6); Lib14-17(5)

Sicydium gilberti. Alcohol Specimens: Puerto Rico: REC09-02(1); REC09-07(6); REC09-09(1); REC09-10(2); REC09-16(1). St Croix: UF180989(2); UF181001(1). Cleared and Stained: Puerto Rico: SLU1295

Sicydium gymnogaster. Alcohol specimens: Mexico: Wisc 11690(13); UMMZ18392(3)

Sicydium hildebrandi. Alcohol Specimens: Colombia:USNM313635 (14); CAS46151 (1)-Paratype. Cleared and Stained: USNM 410085(2)

Sicydium plumieri: Alcohol specimens: Puerto Rico: 09-17(5); 09-09 (13). Dominican Republic: UF90952 (1). Jamaica: SLU6348(1). St Croix: UF180989(1); UF183291(1).

SEM: Puerto Rico: SLU1449. Cleared and Stained: SLU1276; SLU1228; SLU1229; SLU1287; SLU1364; SLU148; SLU1482.

Sicydium punctatum. Alcohol Specimens: Puerto Rico: 09-29(4); 09-21(11); REC09-32(2). Jamaica: SLU6344(2). St Croix: UF180989(4); UF183289(2); UF181001(1). Martinique: ULM48(3). SEM: SLU1210; Cleared and Stained: SLU1218; SLU1498; SLU1504; SLU1532.

Sicydium salvini. Alcohol Specimens: Costa Rica: REC10-32(20); REC10-05(04); REC10-06(8); REC10-34(10); Panama: BMNH 1864.1.26.413 (Holotype); UF101789(2)-Panama. Colombia: USNM12022(2). Guatemala: UMMZ194163. SEM:REC006. Cleared and Stained: REC066; REC092; REC134; SLU1831; SLU1854

Parasicydium bandama. Alcohol Specimens: Liberia: Lib14-24(1); Lib14-17(3). SEM: LIB1477; Cleared and Stained: Cameroon: Pettrade.

Sicyopterus lividus. Alcohol Specimens: Pohnpei: Senipehn river (12); WP007 (10) 1 CS – Pohnpei. SEM: WP007. Cleared and Stained: WP007

Sicyopterus lagocephalus. Alcohol Specimens: Pohnpei: Mahnd River(1). Moorea: LT11-08(5). SEM: LT11-08(2); Cleared and Stained: Solomon Islands: DB09

Sicyopterus eudentatus. Alcohol Specimens: Pohnpei: Wp15(5) SEM: WP15

Smilosicyopus nigroradiatus: Pohnpei: No Data (5); Wp007(3). Cleared and Stained: WP12

Smilosicyopus chloe. Alcohol Specimens: Solomon islandsDB09 (8); Cleared and Stained: DB09(2).

Stiphodon attratus. Alcohol specimens: Solomon islands(4) SEM: Solomon Islands

Stiphodon elegans. Cleared and Stained: 1(No Data).

Cotylopus acutipinnis. Cleared and Stained: Reunion Islands: MNHN 2012-0019(1)

Lentipes concolor. Alcohol Specimens: Hawaii: LSU8481(4) Cleared and Stained: LSU8481(2).

Stenogobius sp. Palau (1).

Awaous banana. Alcohol Specimens: Costa Rica: 10-17(2); 10-20(3). Cleared and Stained: 10-20(1).

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CHAPTER II: Molecular Phylogeny of Gobies in the Genus *Sicydium* (Gobiidae: Sicydiinae)

Abstract

Amphidromous gobies in the genus *Sicydium* (Teleostei: Gobiidae: Sicydiinae) are common inhabitants of tropical river systems of the western hemisphere and eastern Atlantic. Plagued by inconsistent character diagnosis, geographically limited studies and high morphological variation, the inference of the number of species and their relationships has been problematic. Here a molecular phylogenetic study is presented based upon two mitochondrial genes and two nuclear genes. *Sicydium* was found to be monophyletic and sister to *Parasicydium* in the concatenated analysis. Furthermore seven lineages corresponding to previously described species were recovered in the analysis of the concatenated and mtDNA data sets. The relationships between these species are discordant between the mtDNA gene trees and the concatenated data set. Incomplete lineage sorting resulted in the paraphyly of certain morphologically distinct species.

1. Introduction

The gobies in the genus *Sicydium* are abundant inhabitants of tropical coastal and insular rivers and streams in the Atlantic and eastern Pacific basins (Keith 2003; Lyons 2005). *Sicydium* species are known to be amphidromous like the other genera and species in the subfamily Sicydiinae (Keith 2003; Tabouret et al. 2011; Lejeune et al. 2014). Amphidromy is a life history in which adults live and breed in freshwater, but the larvae develop in the ocean. During spawning, eggs are attached to the undersides of rocks and guarded by males (Keith, 2003). Once hatched, the larvae are passively swept out to sea where they live pelagically. Planktonic larval duration varies among species and seasons (Bell et al. 1995; Bell 2009; Taillebois et al. 2012). Upon entering the river estuaries sicydiine gobies undergo a metamorphosis accompanied by both physiological and morphological changes (Valade et al. 2009; Taillebois et al. 2011). Once metamorphosis is complete they migrate back upstream to colonize adult habitats. This larval phase has allowed sicydiine gobies to maintain adult populations in isolated streams sharing a common current and perhaps to invade new and distant habitats (McDowall 2004; Chabarria & Pezold, 2013). The duration of the pelagic larval phase, the strength and direction of currents both past and present, and the extent of coastal plain traversed by a river can have a strong influence on the distribution of amphidromous species (Iguchi & Mizuno 1999; Lyons 2005; Crandall et al. 2010).

The number and distributions of species in the genus *Sicydium* have long been confused due to parochial studies ignoring the pelagic marine larval phase, inadequate descriptions, and poorly understood diagnostic character suites. There are 23 nominal species of *Sicydium* (Table 1), of which only18 are accepted valid (Eschmeyer 2015). Of the valid species, seven live in the

eastern Pacific. *Sicydium multipunctatum* Regan 1906 is considered to occur from Mexico to El Salvador, whereas *S. salvini* Ogilvie-Grant 1884 is typically thought to range from Nicaragua to Panama (Lyons 2005). Chabarria and Pezold (2013) confirmed the hypothesis that a single species, *S. salvini,* occurs from Mexico to western Panama. Three species, *S. condotense* Regan, 1914*, S. rosenbergii* Boulenger 1899*,* and *S. hildebrandi* Eigenmann 1918 are thought to occur from southeastern Panama to Ecuador. There are two endemic insular species, *S. fayae* Brock 1942 from the Tres Marias islands off the west coast of Mexico and *S. cocoensis* Heller & Snoodgrass 1903 from the Cocos Islands of Costa Rica. The remaining 11 species live within the Atlantic basin. *Sicydium brevifile* Ogilvie-Grant 1884*, S. bustamantei* Greef 1884*,* and *S.crenilabrum* Harrison 1993 are found in the coastal and insular streams of tropical West Africa. In the Caribbean, there are currently seven recognized species: *S. buscki* Evermann & Clark 1906*, S. gilberti* Watson 2000*, S. montanum* Hubbs 1920*, S. punctatum* Perugia 1896*, S. plumieri* (Bloch 1786)*, S. altum* Meek 1907*,* and *S. adelum* Bussing 1995. It should be noted that *S. altum* and *S. plumieri* co-occur in the Caribbean and have identical morphologies. Therefore the identities of these species is uncertain at this time, see discussion for further elaboration. A single species, *S. gymnogaster* Ogilvie-Grant 1884*,* can be found in SE Mexico in rivers draining into the southern Gulf of Mexico. Most published works on the genus have been descriptions of new species with little or limited reference, and often understanding, of the described diversity within the genus. Several recent studies have examined species diversity within a part of the range of the genus (e.g.Watson 2000; Pezold et al. 2006; Chabarria & Pezold 2013).

Even the limits of the genus have been slow in definition. *Sicydium* and *Sicyopterus* were not clearly diagnosed for more than a century after their recognition as genera. Akihito and Meguro (1979) distinguished *Sicydium* and *Sicyopterus* primarily using differences in oral

morphology. *Sicydium* is separated from *Sicyopterus* by the presence of a fleshy swelling behind the symphysis of the dentaries in the lower jaw, a lateral cleft of the upper lip at the corner of the mouth (vs. anterior to the corner of the mouth), labial teeth that begin at the anterior tip of the dentaries (vs. beginning behind the anterior tip of the dentaries), and by lacking a fleshy swelling on the inner side of the posterior part of the upper jaw (Akihito & Meguro 1979). A recent molecular analysis of the subfamily Sicydiinae using two mitochondrial genes and one nuclear gene supports the monophyly of *Sicydium* and its sister relationship with *Sicyopterus* (Keith et al., 2011). A sister relationship between *Sicydium* and *Sicyopterus* was also indicated by morphological studies of the subfamily Sicydiinae (Parenti & Maciolek 1993; Parenti & Thomas 1998). This sister relationship was supported by the shared presence of lateral clefts in the upper lip (though in different locations), a short, blunt ascending process of the premaxilla, and fused H and K oculoscapular pores (Parenti & Maciolek 1993; Pezold 1993). *Parasicydium,* a monotypic sicydiine genus, has been placed in *Sicydium* by some authors based on a shared synapomorphy (Parenti and Maciolek, 1993). While *Sicydium* and *Parasicydium* do share some similarities, including the hypothesized synapomorphy of a dorsolateral fleshy lobe on the lower lip, they are distinguished by three features of lip morphology, squamation and tooth morphology (Harrison, 1993). Specifically, the genera are distinguished by the presence of lateral clefts in the upper lip in *Sicydium*, and the occurrence of sexually dimorphic premaxillary teeth and the absence of dorsal squamation anterior to the origin of the second dorsal fin in *Parasicydium.* However, a recent molecular phylogeny of the subfamily Sicydiinae found *Parasicydium* nested within *Sicydium* (Taillebois et al. 2014). In a morphological phylogenetic analysis of *Sicydium*, Chabarria and Pezold (unpubl.) all three genera were recovered as monophyletic with *Parasicydium* basal to *Sicydium* and *Sicyopterus.*

The purpose of this study was to examine the species diversity of *Sicydium* and to test the monophyly of *Sicydium* relative to *Parasicydium* and *Sicyopterus* in a phylogenetic analysis using multiple mitochondrial and nuclear genes.

2. Materials and Methods

2.1 Sampling

Tissues were collected for this study by several means. Extensive collecting of *Sicydium* was conducted in Puerto Rico and along the Atlantic and Pacific versants of Costa Rica. Specimens were collected using a Smith-Root Electrofisher. Upon collection, specimens were euthanized with MS-222. A tissue sample was taken from the right pectoral fin and stored in 95% ethanol (ETOH). Voucher specimens were then fixed in a 10% formalin solution and transferred to 75% ETOH for long-term storage. Additional tissues from Liberia, Honduras, Jamaica, Mexico, and Panama were acquired from museum collections. Specimens of *S. brevifile* and *S. crenilabrum* were obtained from the pet trade with a proclaimed origin of Cameroon. Tissues of *Parasicydium bandama* (Cameroon (pet trade) and Liberia) and *Sicyopterus lagocephalus* (Micronesia) were also included in the analysis. Previous morphological analysis placed *Stiphodon* closely related to *Sicydium* and *Sicyopterus* (Harrison, 1993; Parenti, 1991; Parenti and Thomas, 1998). Phylogenetic studies based on molecular data however have placed *Stiphodon* as sister to *Sicyopus* (Keith et al. 2011) or as the basal sicydiine genus (Taillebois et al. 2014). *Stiphodon* was chosen as the outgroup because it has always been found as distinct from *Sicydium, Sicyopterus* and *Parasicydium.* A list of all tissues and vouchers can be seen in Table 5. Species were identified based on species descriptions and comparisons to the type specimens where available (See Appendix).

2.2 DNA Amplification Methods

DNA was extracted using the DNeasy Tissue kit (Qiagen) following the manufacturer's protocol. Samples will run out on a 1.0% agarose gel and stained with SYBR Green to assess the quality and amount of extracted DNA. For each sample the mitochondrial gene *cytochrome b* (*cyt b*) was amplified using the primers AJG15A and H5 (Akihito et al., 2000). The internal *cyt b* primers SiCBH and SiCBL from Chabarria and Pezold (2013) were used for direct sequencing of cyt b. The primers GOBY H7696 and GOBY L6468 (Thacker 2003) were used in polymerase chain reaction (PCR) amplification and sequencing of *cytochrome oxidase I* (COI). Two nuclear fragments were amplified and sequenced for this study, recombination activating gene 2 (Rag 2) and Ptr. The nuclear marker Ptr was amplified and sequenced using primers from Li et al. (2007). Recombination activating gene 2 (Rag2) was also amplified and sequenced using primers from Thacker et al. (2011). PCR consisted of 25µl reactions using GoTaq (Promega) Master Mix that comprised 13µl GoTaq master mix, 0.1µl of each primer (100µM), 2-4µl DNA, and ddH_2O for the rest of the mix. Thermocycler protocols used to amplify fragments went as follows: 95°C for 10 Min, 30 cycles of 1 Min at 95°C, 45sec at 54-58°C, 1min at 72°C, with a seven minute extension period at 72°C. PCR products were assessed on a 1% agarose gel stained with SYBR green. Successful amplification products were sent to MCLAB for direct sequencing.

Table 5 List of specimens, localities and genes sequenced for this study

Specimen #	Species	Locality	Cyt b	COI	Rag 2	Ptr
CR3	*Parasicydium bandama*	Pet trade/Cameroon	X	X	X	X
REC395	*S. adelum*	Costa Rica	X	-	X	X
REC450	*S. adelum*	Costa Rica	X	X	X	X
REC460	*S. adelum*	Costa Rica	X	X	X	X
SLU1778	*S. adelum*	Costa Rica	X	X	X	X
SLU1779	*S. adelum*	Costa Rica	X	X	X	X
REC293	*S. altum*	Costa Rica	X	X	X	X
REC294	*S. altum*	Costa Rica	X	X	X	X
REC312	*S. altum*	Costa Rica	X	X	X	X
REC389	*S. altum*	Costa Rica	X	-	X	X
REC391	*S. altum*	Costa Rica	X	-	X	X
REC400	*S. altum*	Costa Rica	X	X	X	X
REC404	*S. altum*	Costa Rica	X	X	X	X
REC406	*S. altum*	Costa Rica	X	X	X	X
REC424	*S. altum*	Costa Rica	X	X	X	X
REC426	*S. altum*	Costa Rica	X	X	X	X
SLU1760	*S. altum*	Costa Rica	X	X	X	X
SLU1765	*S. altum*	Costa Rica	X	X	X	X
SLU1439	*S. altum*	Puerto Rico	X	X	X	X
SS-06	*S. altum*	Honduras	X	X	-	X
CR4	*S. brevifile*	Pet trade/Cameroon	X	X	X	X
SLU1143	*S. buscki*	Puerto Rico	X	X	X	X
SLU1153	*S. buscki*	Puerto Rico	X	X	X	X
SLU1226	*S. buscki*	Puerto Rico	X	X	X	X
SLU1235	*S. buscki*	Puerto Rico	X	X	X	X
SLU1358	*S. buscki*	Puerto Rico	X	X	X	x
SLU1378	*S. buscki*	Puerto Rico	X	X	X	X
SLU-TC 1874	*S. buscki*	Jamaica	X	X	X	X
T13582	*S. condotense*	Ecuador	X	X	X	X
T13583	*S. condotense*	Ecuador	X	X	X	X
CR1	*S. crenilabrum A*	Pet trade/Cameroon	X	X	X	X
CR2	*S. crenilabrum A*	Pet trade/Cameroon	X	X	X	X
CR5	*S. crenilabrum A*	Pet trade/Cameroon	x	X	X	X
Lib200	*S. crenilabrum B*	Liberia	X	X	X	X
Lib203	*S. crenilabrum B*	Liberia	X	X	X	X
Lib254	*S. crenilabrum B*	Liberia	X	X	X	X
SLU1158	*S. gilberti*	Puerto Rico	X	X	X	X
SLU1214	*S. gilberti*	Puerto Rico	-	X	X	X
SLU1233	*S. gilberti*	Puerto Rico	X	X	X	X

Table 5 cont'd.

SLU1236	*S. gilberti*	Puerto Rico	X	X	X	X
SLU1255	*S. gilberti*	Puerto Rico	X	X	X	X
SS-23	*S. gymnogaster*	Mexico	X	X	X	X
SS-13	*S. multipunctatum*	Mexico	X	X	X	-
SLU1156	*S. plumieri*	Puerto Rico	X	X	X	-
SLU1289	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1303	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1364	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1455	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1472	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1473	*S. plumieri*	Puerto Rico	X	X	-	X
SLU1492	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1517	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1519	*S. plumieri*	Puerto Rico	X	X	X	X
SLU1211	*S. punctatum*	Puerto Rico	X	X	X	X
SLU1218	*S. punctatum*	Puerto Rico	X	X	X	X
SLU1423	*S. punctatum*	Puerto Rico	X	X	X	X
SLU1424	*S. punctatum*	Puerto Rico	X	X	X	X
SLU1454	*S. punctatum*	Puerto Rico	X	X	-	X
SLU1481	*S. punctatum*	Puerto Rico	X	X	-	X
SLU1521	*S. punctatum*	Puerto Rico	X	X	X	-
SS-02	*S. punctatum*	Honduras	X	X	X	-
REC019	*S. salvini*	Costa Rica	X	X	X	-
REC021	*S. salvini*	Costa Rica	X	X	X	-
REC022	*S. salvini*	Costa Rica	X	X	X	-
REC129	*S. salvini*	Costa Rica	X	X	X	X
REC130	*S. salvini*	Costa Rica	X	X	X	X
REC131	*S. salvini*	Costa Rica	X	-	X	X
SLU1803	*S. salvini*	Costa Rica	X	X	X	X
SLU1804	*S. salvini*	Costa Rica	X	X	X	X
SLU1805	*S. salvini*	Costa Rica	X	X	X	X
SicyopDDB	*Sicyopterus sp.*	Solomon Islands	X	X	X	X
StiphDDB	*Stiphodon sp.*	Solomon Islands	X	X	X	X

2.3 DNA Analysis and Phylogenetic Inference

Bayesian inference of phylogeny was conducted using the program MrBayes 3.2.5 (Ronquist et al., 2012). A model selection analysis was conducted in the program jModelTest

(Posada, 2008). Sequence sizes and models selected for the analysis can be seen in Table 6. The Bayesian analysis was run for 10 million generations, with tree sampling every 1000 generations. Burn-in was assessed using the program Tracer (Rambaut and Drummond, 2007); all trees before stationarity were discarded. The analyses were run multiple times to ensure convergence. If posterior probabilities in independent runs were within 3% then convergence was assumed (Huelsenbeck et al., 2002).

Table 6 Number of aligned base pairs and model used in the analysis by data set.

Locus	Aligned Bases	Model
Cyt b	1097	TIM2+I+G
COI	1066	TrN+I+G
Rag2	686	TIM2+I+G
Ptr	612	TIM1+I+G
Concatenated Data	3461	GTR+I+G

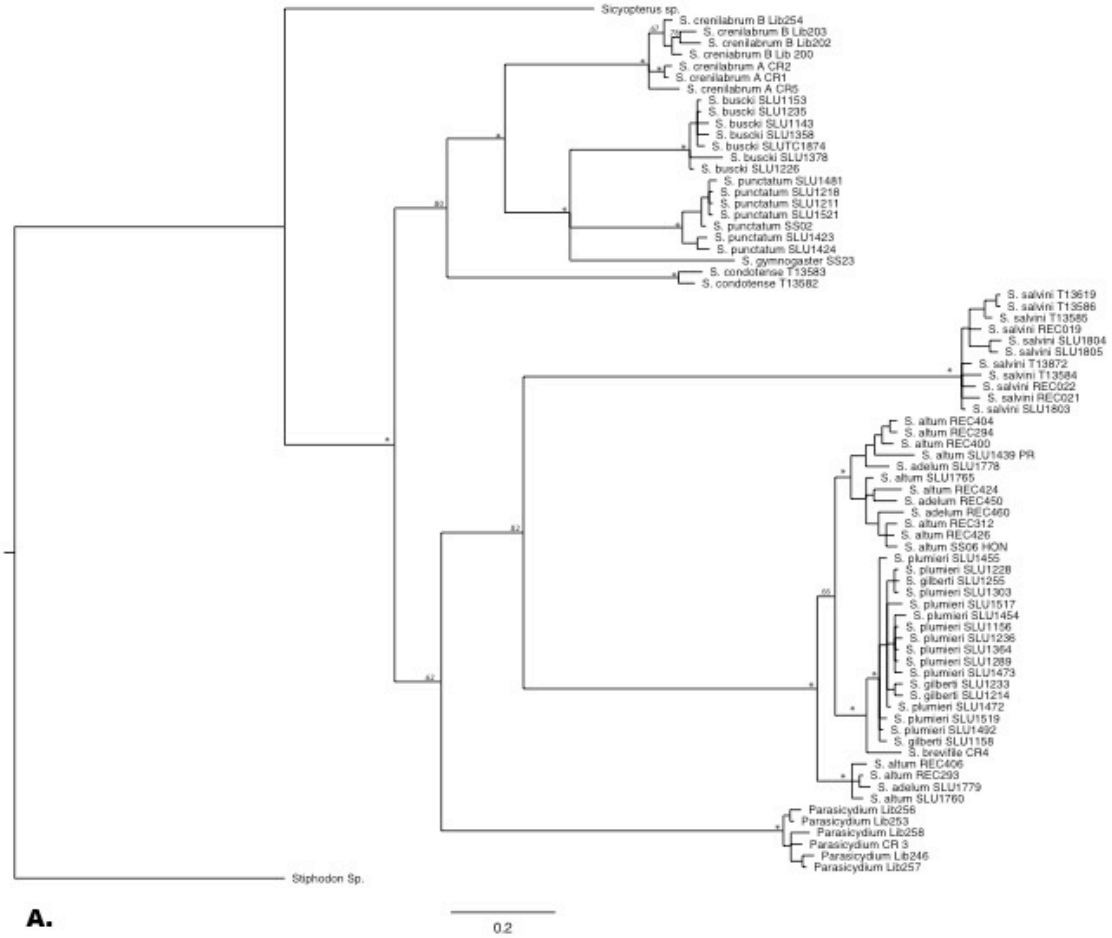
3. Results

3.1 Conflicting Gene Trees

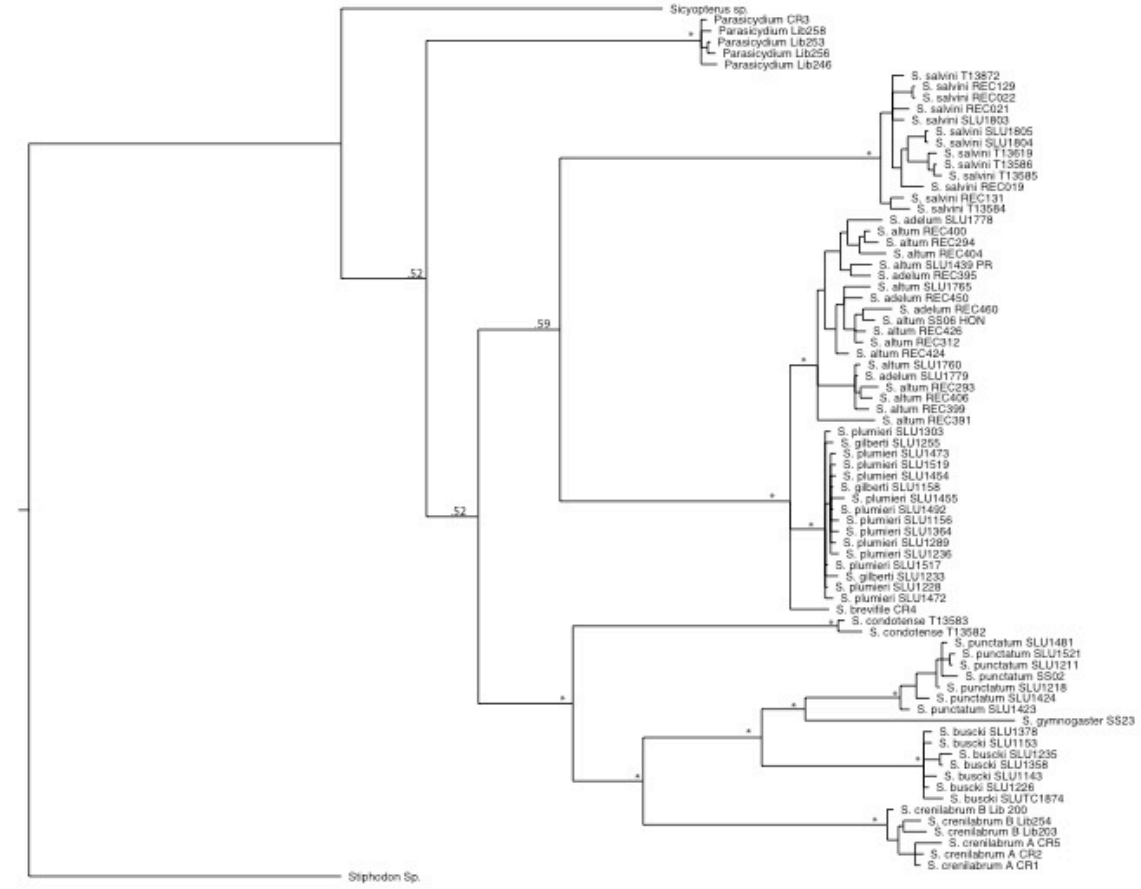
The individual gene trees for Bayesian analyses on Cytb, COI, and Rag2 can be seen in Figure 15. The sequencing results for the individual DNA markers are outlined in Table 2. *Sicydium punctatum, S. buscki, S. salvini, S. brevifile, S. gymnogaster,* and *S. condotense* were all recovered as distinct lineages in the analyses of the individual mtDNA and concatenated data sets. The two mtDNA trees (Fig. 15 A, B) were mostly consistent, returning similar monophyletic lineages with the exception of *S. cf. altum/S. adelum* which were recovered as paraphyletic to *S. brevifile,* and *S. cf. plumieri/S. gilberti* for COI. The COI phylogeny also found

Parasicydium to be nested within *Sicydium* whereas the Cyt b phylogeny found *Parasicydium* to be sister to *Sicydium.* Neither mtDNA tree recovered distinct lineages for *S. adelum* and *S. gilberti.* *Sicydium gilberti* was nested within *S. cf. plumieri,* in both mtDNA analyses. In both mtDNA trees, individuals of *S. adelum* were mixed with representatives of *S. cf. altum*, but as above the *Sicydium cf. altum/S. adelum* grouping was monophyletic in the cyt B tree and paraphyletic in the CO1 phylogeny. Both mtDNA trees showed a close relationship between *S. crenilabrum, S. punctatum, S. buscki,* and *S. gymnogaster,* with *S. crenilabrum* sister to a clade of the western Atlantic species. In the COI gene tree, the relationship between *S. punctatum*, *S. buscki,* and *S. gymnogaster* was collapsed with a posterior probability of <50, while in the Cyt b phylogeny, *S. buscki* was sister to *S. punctatum* and *S. gymnogaster*. In the Cyt b phylogeny *S. salvini* was found to be sister to the clade containing *S. crenilabrum, S. buscki, S. punctatum* and *S. gymnogaster*, but the COI phylogeny placed *S. salvini* sister to the clade containing *S. adelum, S. cf. altum, S. brevifile, S. gilberti* and *S. cf. plumieri.*

The gene tree for Ptr was completely unresolved due to a low number of variable nucleotide positions and therefore the tree was not shown. The Rag2 phylogeny (Fig 15 C) was mostly unresolved for species and relationships. Like the Cyt b phylogeny, Rag 2 recovered *Parasicydium* distinct from *Sicydium,* but it was sister to *Sicyopterus* and *Sicydium.* The Rag 2 phylogeny (Fig. 15, C) did recover *S. crenilabrum* and *S. buscki* as monophyletic species. *Sicydium punctatum* and *S. gymnogaster* formed a monophyletic clade in this tree, however *S. punctatum* was paraphyletic.


Sicyopterus sp.
S. crenilabrum B Lib254
S. crenilabrum B Lib203
S. crenilabrum B Lib202
S. crenilabrum B Lib 200
S. crenilabrum A CR2
S. crenilabrum A CR1
S. crenilabrum A CR5
S. buscki SLU1153
S. buscki SLU1235
S. buscki SLU1143
S. buscki SLU1358
S. buscki SLUTC1874
S. buscki SLU1378
S. buscki SLU1226
S. punctatum SLU1481
S. punctatum SLU1218
S. punctatum SLU1211
S. punctatum SLU1521
S. punctatum SS02
S. punctatum SLU1423
S. punctatum SLU1424
S. gymnogaster SS23
S. condotense T13583
S. condotense T13582
S. salvini T13619
S. salvini T13586
S. salvini T13585
S. salvini REC019
S. salvini SLU1804
S. salvini SLU1805
S. salvini T13872
S. salvini T13584
S. salvini REC022
S. salvini REC021
S. salvini SLU1803
S. altum REC404
S. altum REC294
S. altum REC400
S. altum SLU1439 PR
S. adelum SLU1778
S. altum SLU1765
S. altum REC424
S. adelum REC450
S. adelum REC460
S. altum REC312
S. altum REC426
S. altum SS06 HON
S. plumieri SLU1455
S. plumieri SLU1228
S. gilberti SLU1255
S. plumieri SLU1303
S. plumieri SLU1517
S. plumieri SLU1454
S. plumieri SLU1156
S. plumieri SLU1236
S. plumieri SLU1364
S. plumieri SLU1289
S. plumieri SLU1473
S. gilberti SLU1233
S. gilberti SLU1214
S. plumieri SLU1472
S. plumieri SLU1519
S. plumieri SLU1492
S. gilberti SLU1158
S. brevifile CR4
S. altum REC406
S. altum REC293
S. adelum SLU1779
S. altum SLU1760
Parasicydium Lib256
Parasicydium Lib253
Parasicydium Lib258
Parasicydium CR 3
Parasicydium Lib246
Parasicydium Lib257
Stiphodon Sp.
0.2

A.


Sicyopterus sp.
Parasicydium CR3
Parasicydium Lib258
Parasicydium Lib253
Parasicydium Lib256
Parasicydium Lib246
S. salvini T13872
S. salvini REC129
S. salvini REC022
S. salvini REC021
S. salvini SLU1803
S. salvini SLU1805
S. salvini SLU1804
S. salvini T13619
S. salvini T13586
S. salvini T13585
S. salvini REC019
S. salvini REC131
S. salvini T13584
S. adelum SLU1778
S. altum REC400
S. altum REC294
S. altum REC404
S. altum SLU1439 PR
S. adelum REC395
S. altum SLU1765
S. adelum REC450
S. adelum REC460
S. altum SS06 HON
S. altum REC426
S. altum REC312
S. altum REC424
S. altum SLU1760
S. adelum SLU1779
S. altum REC283
S. altum REC406
S. altum REC399
S. altum REC391
S. plumieri SLU1303
S. gilberti SLU1255
S. plumieri SLU1473
S. plumieri SLU1519
S. plumieri SLU1454
S. gilberti SLU1158
S. plumieri SLU1455
S. plumieri SLU1492
S. plumieri SLU1156
S. plumieri SLU1364
S. plumieri SLU1289
S. plumieri SLU1236
S. plumieri SLU1517
S. gilberti SLU1233
S. plumieri SLU1228
S. plumieri SLU1472
S. brevifile CR4
S. condotense T13583
S. condotense T13582
S. punctatum SLU1481
S. punctatum SLU1521
S. punctatum SLU1211
S. punctatum SS02
S. punctatum SLU1218
S. punctatum SLU1424
S. punctatum SLU1423
S. gymnogaster SS23
S. buscki SLU1378
S. buscki SLU1153
S. buscki SLU1235
S. buscki SLU1358
S. buscki SLU1143
S. buscki SLU1226
S. buscki SLUTC1874
S. crenilabrum B Lib 200
S. crenilabrum B Lib254
S. crenilabrum B Lib203
S. crenilabrum A CR5
S. crenilabrum A CR2
S. crenilabrum A CR1
Stiphodon Sp.
.52
.59
.52
0.06

B.

Figure 15 Bayesian phylogeny of A) COI dataset of 1066 aligned bases, B) Cyt B dataset of 1181 aligned bases, and C) Rag2 dataset of 804 aligned bases. Values on branches are posterior probabilities. Clades with posterior probabilities of less than 0.50 were collapsed. * =posterior probabilities of 0.95 or greater.

3.2 The Concatenated Data Set

The concatenated data set of both the mtDNA and nDNA markers resulted in 3662 aligned bases. In the concatenated phylogeny (Fig. 16), *Sicydium* was found to be monophyletic with respect to *Parasicydium* and *Sicyopterus* with support >0.95. Nine lineages of *Sicydium* were strongly

supported with posterior probabilities of >0.95. The species lineages *Sicydium brevifile, S. buscki, S. crenilabrum* A & B*, S. condotense, S. gymnogaster, S. punctatum,* and *S. salvini* were all found to be monophyletic. *Sicydium cf. plumieri* and *S. gilberti* were paraphyletic within a well-supported single clade and a monophyletic clade containing paraphyletic *S. cf. altum* and *S. adelum* was recovered and well-supported. Both of the mtDNA gene trees strongly influenced the recovery of species and their relationships observed in the concatenated phylogeny. The Rag2 and Cyt b data sets influenced the position of *Parasicydium bandama*. While COI had *P. bandama* nested within *Sicydium* with low posterior probability support*,* both Rag 2 and Cyt b had *P. bandama* as distinct from *Sicydium.* Whereas the Cyt b data set found *Sicyopterus* sister to a clade with reciprocally monophyletic *Parasicydium* and *Sicydium,* but with low posterior probabilities, analysis of the concatenated data set found *Parasicydium bandama* to be sister to a well-supported clade containing *Sicyopterus* and *Sicydium*. The position of *S. salvini* in the concatenated tree is congruent with that of the COI data. The monophyly of the mixed *S. c.f. altum/S. adelum* clade appears to be driven by the Cyt b data set compared to the COI data that did not recover the S. *c.f. altum*/*S. adelum* clade as monophyletic.

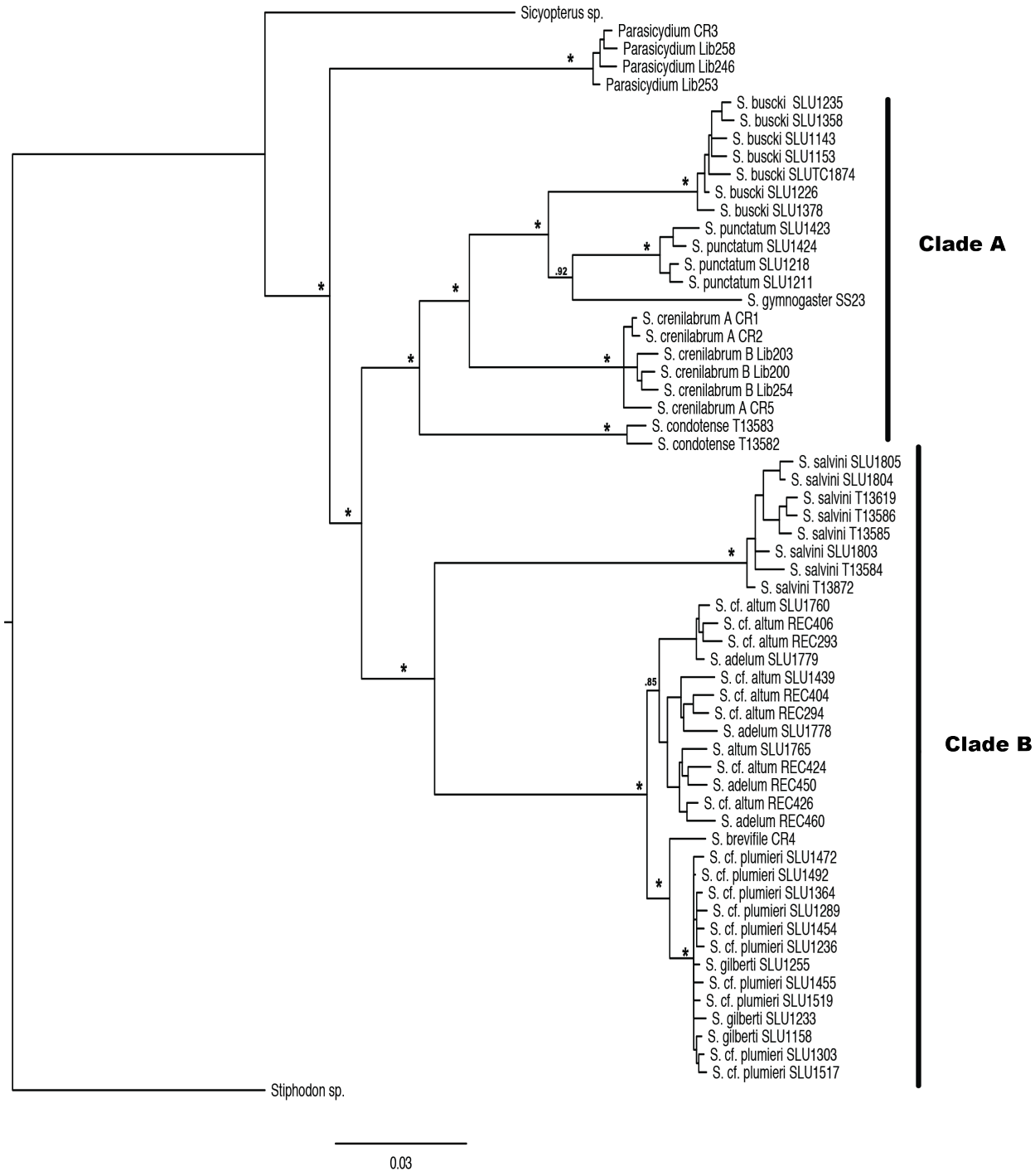


Figure 16 Bayesian phylogeny of concatenated dataset of 3662 aligned bases. Values on branches are posterior probabilities. Clades with posterior probabilities of less than 0.50 were collapsed. * =posterior probabilities of 0.95 or greater.

4. Discussion

4.1 Sicydium, Parasicydium and Sicyopterus

Despite incongruence between gene trees, there are multiple lineages that were found to be monophyletic using the mtDNA and concatenated data sets. All mtDNA gene trees and the concatenated data sets recovered *Sicydium* as a lineage distinct from *Sicyopterus.* In the only phylogenetic analysis of *Sicydium* to date, Chabarria and Pezold (unpubl.) found *Sicyopterus* sister to *Sicydium* based on morphology. *Parasicydium* was sister to *Sicydium* and *Sicyopterus.* Parenti and Maciolek (1993) recognized *Sicydium* and *Sicyopterus* as sister groups based on the synapomorphies of "a short, blunt ascending/articular process of the premaxilla" and a fusion of oculoscapular pores H and K to form a single pore "HK" (Pezold 1993). They also hypothesized monophyly of *Sicydium* based on "a large, fleshy, swelling between the posterior extent of the labial (horizontal)" and unicuspid dentary teeth (Parenti & Maciolek 1993). Yet this swelling is also shared with *Parasicydium* (Harrison 1993) and Parenti and Maciolek (1993) considered *Parasicydium* to be a synonym of *Sicydium.* A sister relationship between *Sicydium* and *Sicyopterus* was also found in two different molecular studies of the subfamily Sicydiinae (Keith et al. 2011; Taillebois et al. 2014).

Parasicydium is a monotypic genus described by Risch (1980) as a "*Sicydium*-like" genus from West Africa, and in fact was considered by Parenti and Maciolek (1993) to be a synonym of *Sicydium. Parasicydium bandama* has a large fleshy swelling between the unicuspid dentary teeth and horizontal teeth at the posterior extent of the jaw. Parenti and Maciolek (1993) considered this sweeling to be a synapomorphy of *Sicydium* which led them to place *Parasicydium* in its synonymy. Morphologically, *Parasicydium* differs from *Sicydium* in having canine teeth in the upper jaw in males, lacking a lateral cleft of the upper lip, having more

conical teeth on the dentaries, and in lacking scales on the anterior part of the dorsum (Risch, 1980). Harrison (1993) also noted that *Parasicydium* lacks a medial tubercle on the upper lip that is found in *Sicydium.* Both the Cyt b (Fig 15, B) and Rag 1 (Fig 15, C) analyses found *Parasicydium* to be separate from *Sicydium*. In the Rag 1 data set, *Parasicydium* was sister to *Sicydium* and *Sicyopterus.* The analysis of Cyt b showed *Parasicydium* to be sister to *Sicydium* forming a monophyletic lineage sister to *Sicyopterus.* The COI phylogeny was the only gene tree to recover *Parasicydium* nested within *Sicydium*. Incongruence between gene trees can be a result of differences in the history of the genes, or the result of ancestral polymorphisms leading to incomplete lineage sorting (Degnan & Rosenberg 2009). Taillebois et al. (2014) found *Parasicydium* to be nested within *Sicydium* in a subfamily-level phylogeny based upon two nuclear genes and smaller fragments of the same mtDNA genes (COI and Cyt b) used in our analysis. However the sample size in that study included only four species of *Sicydium,* which could have lead to an inaccurate phylogenetic estimation. Sparsely sampled trees can include long branches which can lead to homoplasy being treated as homology. Increasing taxon sampling can reduce the effects of long branches by distributing homoplasy across the tree leading to more accurate phylogenetic estimation (Heath et al. 2008).

4.2 Taxonomy of Sicydium cf. plumieri and S. cf. altum

Geographically limited reviews (e.g. Erdman, 1961; Watson, 2000) and poor species descriptions (e.g. Jordan and Evermann, 1898; Meek, 1907) have led to taxonomic problems in interpreting phylogenetic results of this study. *Sicydium cf. plumieri* and *S. cf. altum* both have long slender tricuspid teeth (Fig. 17, A), and fingerlike projections of the gums that cover almost the entire tooth (Fig. 18)(Watson, 2000, Bussing, 1996). With the current descriptions of these

species, they cannot be distinguished morphologically. In the analysis of the mtDNA and concatenated datasets two different lineages from the Caribbean have this morphology. One lineage contains specimens from Puerto Rico and the other lineage, primarily continental in distribution, contains specimens from Costa Rica, Honduras, and a single specimen from Puerto Rico. The original description of *S. cf. plumieri* was based on a painting based on a specimen from Martinique. Watson (2000) redescribed this species designating a neotype from the Dominican Republic. *Sicydium cf. altum* was described by Meek (1907) from Costa Rica. The only other species besides *S. cf. altum* reported from the Atlantic coast of Costa Rica is *S. adelum,* which does not have the same tooth morphology. Taxonomically the identity of the *S. cf. altum* lineage specimens could be *S. antillarum, S. siragus, S. vincente,* or *S. caguitae,* all of which were described before *S. cf. altum* and have priority. Watson (2000) synonymized those four species with *S. cf. plumieri* based upon tooth morphology and meristic data. He examined species from the Dominican Republic, Martinique, Puerto Rico, and Panama. However he neither examined the type specimen of *S. altum* nor acknowledged its existence (Watson, 2000). Therefore for this study we label the clade with all specimens from Puerto Rico to be *S. cf. plumieri* and the clade with most specimens from Central America as *S. cf. altum*. This designation is solely based on traditional views of the distribution of these two species. Without closer examination of the morphology of the type specimens of all species in the Caribbean with long slender teeth, alternating replacement pattern, and finger-like projections from the gums (Fig. 18), the correct identity of these specimens from the Caribbean cannot be determined.

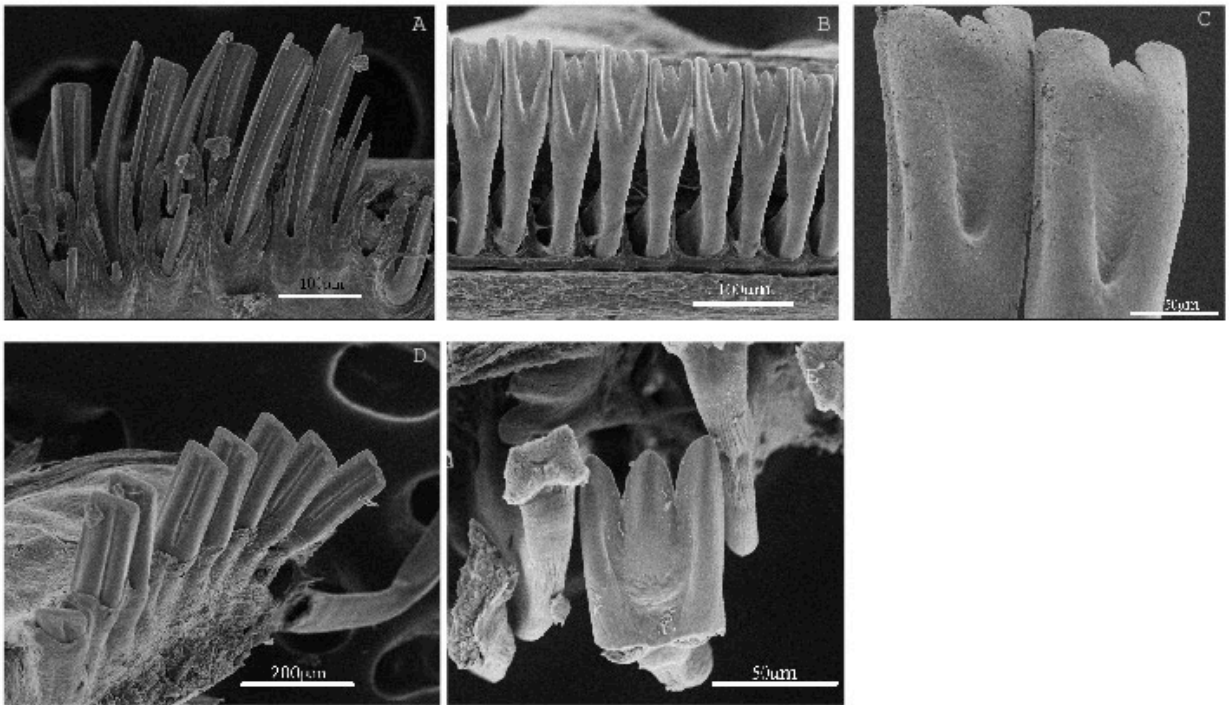


Figure 17 Scanning electron microscopy images of the premaxillary teeth of A) *Sicydium plumieri*, B) *S. salvini,* C) *S. punctatum,* D) *S. buscki,* and E) *S. crenilabrum "A"*

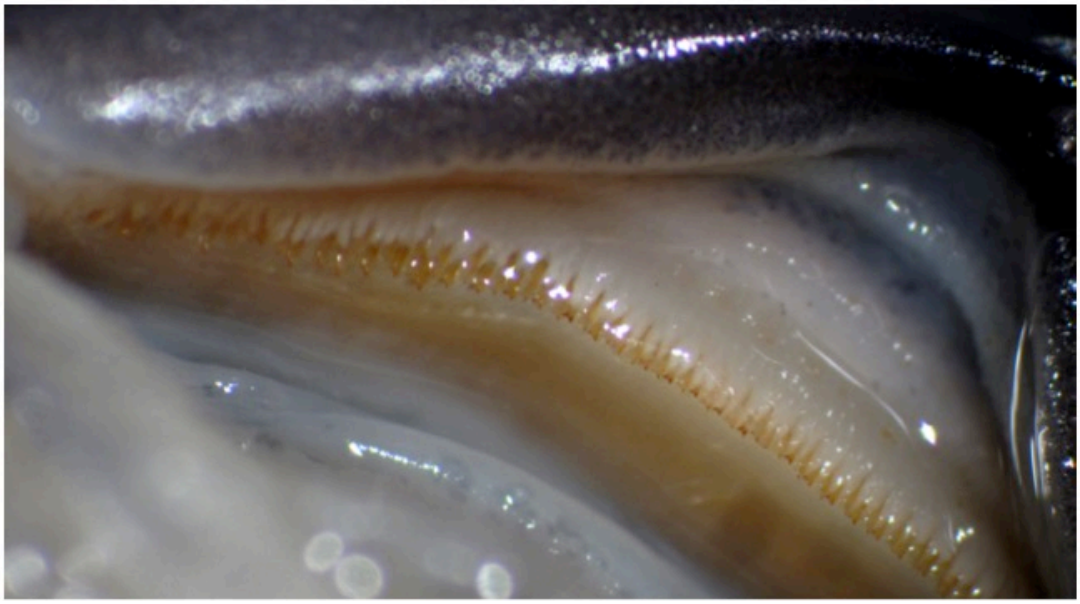


Figure 18 Photograph of the premaxillary teeth and gums of *Sicydium plumieri.* Teeth are alternately arranged and gums have finger like projections that cover most of the tooth face

4.3 Taxonomy and tooth morphology in Sicydium

Tooth morphology has been used as an important character to distinguish species within *Sicydium* (Ogilvie-Grant 1884; Watson 2000; Pezold et al. 2006). Previous authors have also suggested close relationships between species based upon shared tooth morphology. In the concatenated tree (Fig. 16) and both mtDNA trees (Fig 15, A & B) the eight monophyletic lineages cannot be distinguished from one another based on tooth morphology. However most species of *Sicydium* with identical tooth morphologies were found to be closely related (Figure 5, Table 3), for example *Sicydium punctatum* (Caribbean) and *S. gymnogaster* (Gulf of Mexico) both have tricuspid teeth with large lateral cusps and short medial cusps (Fig 17, C) and were recovered as sister taxa. *Sicydium brevifile* (E. Atlantic)*, S. cf. plumieri* (Caribbean) and *S. cf. altum* (Caribbean) all have long slender shallowly tricuspid teeth (Fig 17, A) with fingerlike projections of the gums (Fig. 18) (Bussing, 1996; Watson, 2000). All three of these species were found to be closely related and sister to *S. salvini* (Fig. 16). However of those three species, *S. brevifile* is the only one that was found to be monophyletic. A monophyletic *Sicydium crenilabrum* (forms A&B) clade was recovered. Chabarria and Pezold (unpubl.) reported two different forms (A&B) of *Sicydium crenilabrum.* *Sicydium crenilabrum* form A, has a tricuspid tooth similar to that found in *S. condotense* from the eastern Pacific (Fig. 17, E). *Sicydium crenilabrum* form B possesses unicuspid teeth similar to what is seen in *S. buscki* (Fig. 17,D). In the present study, specimens of *S. crenilabrum* "A" are from Cameroon (pet trade) and those of *S. crenilabrum* "B" are from Liberia. However both forms are syntopic. Specimens of both forms were collected from the same rivers in Liberia. Despite distinct morphological differences, these two forms did not separate in the molecular analysis; most likely due to incomplete lineage sorting.

Sicydium cf. plumieri and *S.cf. altum* were found to be paraphyletic with *S. gilberti* and *S. adelum,* respectively. *Sicydium gilberti* and *S. adelum* both have trident-like tricuspid teeth like *S. salvini* (Fig. 17, B). As seen in the concatenated tree (Fig. 16), specimens of *S. adelum* are paraphyletic and nested within *S. cf. altum* forming a well-supported clade, and specimens of *S. gilberti* are nested in a well-supported clade with *S. cf. plumieri*. Based upon tooth and oral morphology *S. gilberti* and *S. adelum* are distinct from *S. cf. plumieri* and *S. cf. altum*. When Watson (2000) described *S. gilberti,* he noted it appeared identical to *S. cf. plumieri* except in tooth morphology. But he argued it was most closely related to *S. salvini* presumably because they share the same tooth morphology (Fig. 17, B).

There are several possible explanations for the lack of resolution of the morphological species in these two clades. The first is that *S. cf. plumieri* and *S. cf. altum* both have two tooth morphotypes. Two co-occurring morphotypes differing in pharyngeal tooth structure have been found in the cichlid *Herichthys minckleyi* (Hulsey et al. 2005; Hulsey & Garcia de Leon 2005; Hulsey et al. 2006). The morphotypes are linked to trophic differences – individuals with molariform teeth tend to crush and eat snails, while those with papilliform teeth consume more plant matter (Hulsey et al. 2006). It is possible that *S. adelum* and *S. gilberti* represent trophic morphotypes of *S. cf. altum* and *S. cf. plumieri,* respectively. The primary dietary component of species of *Sicydium* is algae (Erdman, 1961; Watson, 2000), which they scrape from rocks using the teeth in the upper jaw. It is possible that trident teeth allow individuals to take advantage of a different type of algae (encrusted vs. filamentous). Trophic species have been seen in the cichlids of Lake Tanganyika, however the authors noted that there have been no known examples of trophic morphotypes differing in oral morphology (Rüber et al. 1999). As there have been no

examples of differing adaptive foraging tooth morphotypes in gobies, it is an unlikely scenario for *Sicydium.*

It is also possible that the lack of resolution reflects the recency of species divergence. From the individual gene phylogenies in figure 1, the mtDNA gene trees (Fig. 15, A & B) show the most resolution compared to the poorly resolved Rag 2 data set (Fig. 15, C). The fast mutation rate of mtDNA genes makes it favorable for and widely used in species phylogenies (Avise, 2000). Despite those favorable properties for phylogenetics, mtDNA markers can sometimes show processes other than the phylogenetic history (Doyle, 1997; Nichols, 2001). One such process that can confound phylogeny is incomplete lineage sorting (Leache and McGuire, 2006; McGuire et al., 2007). In this case, divergence of *Sicydium adelum* from *S. c.f. altum* and *S. gilberti* from *S. c.f. plumieri* would have to have happened recently and gene lineages have not sorted into individual lineages. Considering the conflict between the gene trees this is a more likely scenario.

Hybrid introgression is another process that can confound phylogeny. In this scenario past hybridization between *S. c.f. plumieri* and *S. c.f. altum* has led to a 'hybrid morphology'. Although introgression has been shown in the goby genus *Tridentiger* (Mukai et al. 1997), hybridization does not appear to explain the data here. Typically hybrid introgression is seen in contact zones between allopatric species (Seehausen 2004) and the ranges of these *Sicydium* species overlap extensively. Furthermore there is no evidence of the trident tooth morphology being a hybrid morphology.

In the eastern Pacific, two lineages exist both morphologically and genetically in our data, *Sicydium salvini* and *S. condotense.* We obtained specimens for the analysis of both *S. multipunctatum* from Mexico and *S. salvini* from Costa Rica, lack four other putative species

from the eastern Pacific based upon possible distribution differences: *S. hildebrandi* (Ecuador), *S. fayae* (Tres Marias Islands, Mexico), *S. cocoensis* (Cocos Islands), and *S. rosenbergii* (Colombia). *Sicydium hildebrandi* and *S. cocoensis* have morphologies distinct from the sampled specimens included in this analysis. They were included in the morphological analysis of the genus and found to be closely related to *S. cf. plumieri, S. cf. altum, S. brevifile* and *S. salvini* (Chabarria and Pezold, Unpublished). *Sicydium fayae* and *S. rosenbergii* were poorly described and no specimens in the current analysis could be associated with these species. Without examination of the type specimens the validity of these species is unknown. *Sicydium multipunctatum* was believed to occur from Mexico to Honduras and *S. salvini* from Costa Rica to Panama (Reis et al. 2003). The concatenated phylogeny shows our samples representing a single species occurring from Mexico to Panama. This is in agreement with the population level study of *S. salvini* by Chabarria & Pezold (2013). Based on a large Cyt b data set of specimens from Mexico to Panama they found no genetic or phylogeographic structure between populations from Mexico and Central America. Furthermore, the type specimens of *S. multipunctatum* and *S. salvini* could not be morphologically distinguished. Given this information and the priority of *S. salvini,* it is likely there is a single species occurring in continental rivers from Mexico to Panama.

4.4 The Evolution of Clade A

This clade contains *Sicydium buscki, S. crenilabrum "A", S. crenilabrum "B", S. gymnogaster, S. condotense* and *S. punctatum*. The close relationship between these five species lineages was recovered in both mtDNA phylogenies (Fig. 15, A & B) and the concatenated data set (Fig. 16). *Sicydium crenilabrum* is unique among other West African species in having a

crenate upper lip (Harrison 1993; Pezold et al. 2006) and trident-like tricuspid teeth. Crenulations in the upper lip are also present in two species found in the eastern Pacific (*S. hildebrandi & S. cocoensis*). However there are two distinct forms of *S. crenilabrum* which differ in tooth and lip morphologies. Despite the morphological distinctions, the two forms were not recovered as separate lineages. In the concatenated phylogeny the specimens that correspond to form B are in a well-supported (>.95) clade (Fig. 16). This lineage is part of a larger polytomy with specimens of form A.

A close relationship between *S. crenilabrum* and the other three species in this clade has never been suggested. However, previous authors have suggested a close relationship between *S. crenilabrum* and other species not obtained here. Watson (2000) noted that *S. gilberti* had teeth similar to *S. crenilabrum.* Although similarities do exist between the teeth of *S. crenilabrum* (Fig. 17, E) and *S. gilberti* (Fig. 17, B), they are not identical. The teeth of *Sicydium gilberti* (Fig. 17, B) are indented below the medial cusp of the tooth, which appears to be absent in *S. crenilabrum* (Fig. 17, E). Harrison (1993) stated that squamation of *S. bustamantei* was similar in meristics to that of *S. crenilabrum*. *Sicydium crenilabrum* and *S. bustamantei* also have indistinct neuromasts and share a similar pattern in the anterior part of oculoscapular canal. *Sicydium bustamantei* is an unsampled African species that appears to be similar to *S. buscki.* Both species have unicuspid teeth with a medial groove (Fig. 17, D; Pezold et al. 2006, Fig. 10B). The close relationship between *S. bustamantei* and *S. buscki* has never been suggested before, but having the same derived tooth morphology, suggests that they are closely related.

A close relationship between *S. punctatum* and *S. buscki* was hypothesized by Evermann and Clark (1906) based on coloration and number of scales. Watson (2000) seemed to agree with this relationship, but added that the two species differed in upper jaw teeth and that *S.*

punctatum has more distinct markings on the fins and body. *Sicydium punctatum* has tricuspid upper jaw teeth (Fig. 17, C), whereas in *S. buscki* the teeth are unicuspid (Fig. 17, D). The tooth morphologies of these two species are unique within the Caribbean. *Sicydium gymnogaster* and *S. punctatum,* recovered as sister species, possess identical tricuspid teeth (Fig. 17, C). *Sicydium gymnogaster* is restricted to the southwestern Gulf of Mexico and *S. punctatum* to the Caribbean from Venezuela to Honduras.

Similarities in tooth morphology can indicate a close relationship between species. However in some clades it appears that differences in tooth morphology could influence species divergence. Watson (2000) hypothesized that different tooth morphologies, such as those observed here for *S. buscki* and *S. punctatum/S. gymnogaster*, could be due to dietary differences. Selective pressures can lead to the divergence of species with the appearance of novel tooth morphologies especially in areas where numerous species occur (Schluter & McPhail 1993; Orr & Smith 1998; Rüber et al. 1999). Evolution of different tooth morphologies has been shown to repeatedly have evolved in cichlids from Lake Tanganyika (Rüber et al. 1999). Dentition patterns and jaw structures have been shown to have evolved to that allow for specialized feeding patterns in butterflyfishes (Motta 1988; Motta 1989). Motta (1988) found that two Chaetodontidae sister species, *Forcipiger longirostris* and *F. flavissimus,* had diverged based on feeding morphology and behavior. Lujan et al. (2011) showed how syntopic loricariid fishes utilize different wood-associated food resources with differing oral morphologies including tooth shape and number. Roberts (1974) describe different tooth polymorphisms within and between species of algae eating *Saccodon.* The different tooth morphologies of *Saccodon* was hypothesized to correlate with eating different types of algae (Roberts, 1974). *Sicydium* species are thought to be primarily herbivorous scraping algae from rocks (Erdman 1961;

Watson 2000). It is possible that the evolution of different tooth morphologies in *Sicydium* allowed for utilization of unique food types. Yet without detailed information about the diet of the individual species this cannot be determined.

Despite large dispersal capabilities of amphidromous taxa, biogeographic factors often influence species relationships. The divergence between *S. gymnogaster* and *S. punctatum* lineages can be explained by vicariance and isolation of *S. gymnogaster* in the southwestern Gulf of Mexico. McMahan et al. (2013) found a similar distinct lineage of the diadromous mountain mullet *Agonostomus monticola* in the southwestern Gulf of Mexico. In fact, specimens of *A. monticola* collected from the panhandle of Florida in the northeastern Gulf of Mexico were more closely related to populations in the Caribbean. This further suggests that there may be limited or no gene flow between diadromous fishes in the southwestern Gulf of Mexico and the Caribbean Sea. Isolation of *Agonostomus monticola* in the Southwestern Gulf of Mexico was attributed to the lack of suitable habitat on the Yucatan Peninsula. This would mean the Yucatan is a barrier to gene flow with the Caribbean lineage (McMahan et al., 2013). Like *Sicydium, Agonostomus* prefers fast moving high gradient rivers which do not occur on the Yucatan Peninsula (Lyons, 2005; Miller et al., 2006). Two species of the genus *Elacatinus* were described by Taylor and Akins (2007) that are endemic to the western Gulf of Mexico. They were described from Veracruz, Mexico, the same area where *S. gymnogaster* is found. Futhermore, *Ctenogobius claytonii* is also endemic to the western Gulf of Mexico (Meek 1902). It appears that this area harbors isolation like that found in the lineages of *Sicyidum* and *Agonostomus.* This could be related to available habitat or the overall direction and circulation of currents keeping *Sicydium gymnogaster* from entering the Caribbean Sea. This isolation would have led to the divergence of *S. gymnogaster* and *S. punctatum.*

The basal member of clade A (*S. crenilabrum*) is native to the Gulf of Guinea in the Eastern Atlantic. A close relationship between eastern Atlantic and western Atlantic species has been seen in many other fishes including *Bathygobius* (Tornabene & Pezold 2011), *Diplodus* (Summerer et al. 2001), *Nicholsina* and *Sparisoma* (Robertson et al. 2006), *Hippocampus* (Casey et al. 2004), and *Gnatholepis* (Rocha et al. 2005). The other West African *Sicydium* species in this study, *S. brevifile,* was nested within Clade B. Additionally *S. bustamantei* was found to be closely related to the species recovered in clade A based on morphology (Chabarria and Pezold, Unpublished). This suggests that multiple species lineages were present in West Africa before the separation of American lineages from West African lineages.

4.5 Evolution of Clade B

In the concatenated phylogeny, clade 'B' contains *Sicydium adelum, S. cf. altum, S. brevifile, S. gilberti, S. cf. plumieri,* and *S. salvini*. This same clade was recovered by the morphological analysis of the genus (Chabarria and Pezold, unpublished). Based on tooth morphology (Fig. 17, A) and gum morphologies (Fig. 18) alone, *S. brevifile, S. cf. altum,* and *S. cf. plumieri* would all be closely related. A close relationship between tooth morphologies is congruent with the molecular (Fig. 15 A&B, Fig 16) and morphological analyses (Chabarria and Pezold, unpublished). However *S. adelum* and *S. gilberti* nested within *S. cf. altum* and *S. cf. plumieri,* respectively, confounds the relationship. The relationship between *S. adelum* and *S. gilberti* with the other species in this clade was unresolved in the phylogenetic analysis based on morphology. Bussing (1996) suggested that *S. adelum* is closely related to *S. salvini* because of the presence of trident-shaped tricuspid teeth (Fig. 17, B), a tooth morphology also shared by *S. gilberti.* Watson (2000) suggested that *S. gilberti* was most closely related to *S. salvini* despite

noting similarities in tooth morphology with *S. crenilabrum.* The shared tooth morphology does suggest a close relationship between *S. adelum, S. gilberti* and *S. salvini.* Based on morphology, *S. salvini* was found to be sister to a clade that included *S. cf. plumieri, S. brevifile,* and *S. cf. altum* (Chabarria and Pezold, unpublished). These species all have alternating tooth replacement pattern and this relationship is congruent with the molecular analysis.

The amphidromous life history of *Sicydium* allows for dispersal through the marine larval phase. There are many factors that can affect the range of an amphidromous species including pelagic larval duration, biogeography and ecology (Keith et al., 2011). The strength and direction of oceanic currents is another factor that can restrict or aid in the dispersal abilities of species with pelagic larvae (Planes 1993; Muss et al. 2001; Crandall et al. 2010). Biogeographic patterns in this clade suggest that both vicariance and dispersal has influenced this lineage of *Sicydium.* Rosen (1975) proposed a vicariant hypothesis in which the Eastern and Western Atlantic biota diverged because of spreading of the Atlantic during the Cenozoic. Subsequently lineages of the eastern Pacific diverged due to the closing of the Isthmus of Panama (Rosen 1975). Alternatively, divergence between the eastern and western Atlantic could be the result of dispersal events. Taillebois et al. (2014) suggested an origin of the *Sicydium* + *Parasicydium* clade to be Polynesia. This suggests colonization of the Atlantic from the Pacific via the Isthmus of Panama prior to its closing. Expansion of *Sicydium* + *Parasicydium* to the eastern Atlantic would have happen from the western Atlantic. Considering there are multiple species of *Sicydium* from both sides of the Atlantic suggests multiple invasions to West Africa. Muss et al. (2001) reasoned this dispersal happened via the easterly flowing equatorial undercurrent in *Ophioblennius.* This west to east dispersal was also found in *Hippocampus* (Casey et al., 2004).

Divergence within the Caribbean of the *Sicydium cf. altum/S. adelum* clade from the *S. cf. plumieri/S. brevifile* clade may be attributed to the Caribbean Current (Fig. 19). *Sicydium cf. altum* and *S. cf. plumieri* have identical tooth morphologies and both can be found in Puerto Rico. This suggests that they could be the same species. Only two species (*S. c.f. altum* and *S. adelum)* of the six Caribbean species of *Sicydium* can be found in Costa Rica and northeast Panama. This would indicate that the Caribbean current acts as a barrier for *S. buscki, S. gilberti, S. cf. plumieri,* and *S. punctatum*. The Caribbean current appears to be a semipermeable barrier for the species in the western Caribbean. Eddies off of the Panama-Colombia Gyre (Fig. 19) seem to allow larvae to escape the western Caribbean and co-occur with the primarily insular Caribbean species. This is evident by the presence of *S. cf. altum* in Honduras and Puerto Rico but the Caribbean current itself may keep other species out of the southwestern Caribbean. Although Watson (2000) did report *S. punctatum* in Panama, those specimens were not available for this study. A single specimen of *S. punctatum* from Honduras was included in the present study. However this specimen was nested within a larger clade of *S. punctatum* from Puerto Rico. No other specimens have been reported from Costa Rica. Alternatively, *S. cf. altum* could represent an old species that is distributed on both sides of the now presumed Caribbean current barrier.

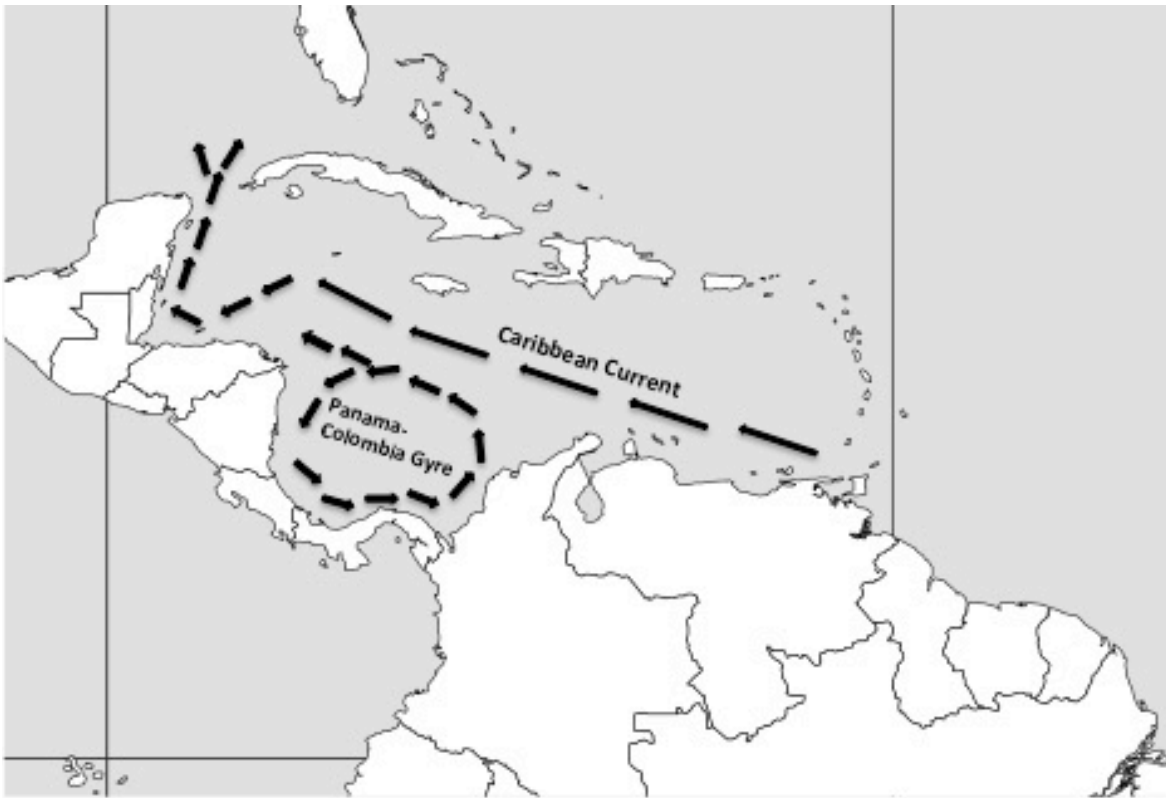


Figure 19 Map of the Caribbean. Arrows indicate direction and location of the Caribbean Current and the Panama-Colombia gyre.

The marine larval phase of *Sicydium* species considered important for the distribution of species as currents have been shown to affect reef fish larvae (Cowen et al. 2006). Based on biophysical models in the Caribbean, Cowen et al. (2006) predicted that reefs near the Panama-Colombia gyre would be isolated from other reefs in the Caribbean due to high self recruitment. Salas et al. (2010) tested this isolation hypothesis on *Stegastes partitus,* and found structure between reefs near Costa Rica-Panama and the Mesoamerican Barrier Reef System of the coast of Belize. This suggests that there was higher self-recruitment in the Costa Rica-Panama area. In *Elacatinus illecebrosus,* there are two color forms. The yellow color form is endemic to Nicaragua, Costa Rica, and Panama (Colin 2010). Taylor and Hellberg (2005) found genetic

differences between these the two color morphs. *Elacatinus horsti,* has a yellow form that occurs in Costa Rica and Panama that is genetically distinct from yellow populations in the Bahamas (Taylor & Hellberg 2005; Colin 2010). This "Yellow-South" form of *E. horsti* also occurs in Venezuela (Colin 2010), indicating that the barrier of the Panama-Colombia gyre is semi-permeable for this species like it is for *Sicydium cf. altum/S. adelum.* But the Caribbean current is apparently a non-permeable barrier for the other *Sicydium* species in the Caribbean. Heavier population level sampling is needed especially along northern South America to look for genetic breaks and faunal changes.

4.6 Missing taxa and gene tree conflicts

One issue that could be confounding the phylogeny presented here is missing taxa. Of the currently recognized species of *Sicydium* (Eschmeyer, 2014), we are missing five. In the Eastern Pacific we are missing molecular samples of *S. cocoensis, S. hildebrandi, S. rosenbergi* and *S. fayae. Sicydium bustamantei,* from West Africa, is the only Atlantic basin species not represented. Missing taxa have been shown to confound phylogenies because of long branches (Huelsenbeck 1995; Heath et al. 2008). Increased taxon sampling has been shown to increase phylogenetic accuracy (Huelsenbeck 1995). Another issue could be the limited resolution and number of the nDNA markers used. More sensitive nDNA markers are needed to improve the quality of the phylogenetic estimate. Inclusion of independent multilocus markers could clear up the paraphyly of *Sicydium cf. plumieri* and *S. cf. altum,* and allow a phylogeny that is more representative of the species phylogeny. Most of the relationships between species were driven by the mtDNA. This could be problematic because of incomplete lineage sorting (Leaché & McGuire 2006) and homoplasy associated with these highly variable markers.

5. Conclusion

This study presented a well-supported concatenated phylogeny for the genus *Sicydium* based on two mitochondrial and two nuclear markers. This represents the first and most complete phylogenetic study of *Sicydium.* The two clades recovered from the molecular analysis were mostly congruent with morphological data. Divergence within *Sicydium* seems to be strongly associated biogeographic factors and possibly tooth morphology. Paraphyly of *Sicyidum plumieri, S. cf. altum, S. adelum* and *S. gilberti* is likely the result of incomplete lineage sorting. Many of the relationships were driven by the mtDNA data which is known to show processes other than descent with modification (Nichols 2001; McGuire et al. 2007; Degnan & Rosenberg 2009). Increasing the number of independent nuclear markers could help sort out the relationships between these four species. Conflicting gene trees appear to be the result of incomplete lineage sorting and incomplete taxon sampling.

Appendix

Type specimens examined: *Sicydium hildebrandi* (CAS46151, Paratype); *S. salvini* (BMNH 1866.6.26.10, Holotype); *S. multipunctatum* (BMNH 1906.6.1.421, Holotype); *S. condotense* (BMNH 1914.5.18.109, Holotype)

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CHAPTER III: Phylogeography and historical demography of *Sicydium* salvini in the eastern Pacific

Abstract

Amphidromy, characterized by freshwater adult and marine larval stages, has been shown to be an important influence on the genetic structure of aquatic populations. *Sicydium salvini* is a widespread goby species (Teleostei: Gobiidae: Sicydiinae) in the eastern Pacific with a continuous distribution from Mexico to Panama. Here, we use mitochondrial data to infer population genetic and historical demography of this species. Sequences were collected for the mitochondrial gene cytochrome b for 162 specimens sampled across the range of *S. salvini* with a concentration on rivers in Costa Rica. No genetic structure was detected between regions or rivers in the AMOVA analysis; the phylogeny for this species showed no geographic affinities and very little resolution. Historical demographic analyses indicated a population expansion during the late Pleistocene. These results are consistent with a panmictic population with expansion influenced strongly by Pleistocene glacial cycles and geologic uplift.

1. Introduction

Sicydiine gobies (Teleostei: Gobiidae) have a circumtropical distribution, being commonly found in freshwater streams on oceanic islands and continental streams with high gradients, good flow and short coastal plains (Keith et al. 2011). Fishes in the genus *Sicydium* are the only representatives of the subfamily in the New World. *Sicydium salvini* is distributed in rivers of the eastern Pacific with a nearly continuous distribution from Mexico to Panama (Reis et al. 2003; Lyons 2005). Because of the close proximity of mountains to the coast, the rivers along the Pacific slope of Mexico and Central America tend to have short coastal plains (<65 km) and ample rocky habitat available for this species (Lyons 2005).

Like other members of the subfamily Sicydiinae, *S. salvini* is thought to be amphidromous (McDowall 2008). This life history is a diadromy type in which adults live and breed in freshwater environments. Upon spawning, eggs are deposited on the undersides of rocks and are guarded by males until hatching (Keith 2003). Larvae are passively carried out to sea where they have a planktonic lifestyle for a particular time period. Once the larval life cycle is complete, they move into estuaries where they undergo a transformation into post-larvae and re-enter streams. This life history was confirmed by Tabouret et al. (2011) for *Sicydium punctatum* based on otolith microchemistry.

Marine larval dispersal is important in shaping the distribution and population genetic patterns of species (Parenti 1991; McDowall 2008; Cook et al. 2009). Unlike typical freshwater taxa, most amphidromous species tend to show little to no genetic structure between river populations (Chubb et al. 1998; Cook et al. 2009; Lord et al. 2012). Natal stream retention has not been reported for any sicydiines, but Sorensen & Hobson (2005) found that larval

amphidromous gobies may prefer inshore habitats near the mouths of rivers to wait for proper recruitment conditions. Recently, Lord et al. (2010) showed that in species of *Sicyopterus* larval duration influenced the ability of species to disperse. They found that species with larger distributions had a longer larval duration than species that were endemic to particular islands or island systems, although this might not be the case in all amphidromous genera (Taillebois et al. 2012). The specific larval duration of *S. salvini* is unknown and Bell et al. (1995) has shown that larval duration is species dependent. Larval duration periods for two *Sicydium* species in the Caribbean range from 54 to 139 days (Bell et al. 1995; Bell 2009).

Population structure and demography of amphidromous species can show the effects of both marine and freshwater climatic and geologic history. While amphidromy may stimulate the evolution of large species ranges, allowing species to exploit new habitats (McDowall 2004), it is countered by marine and geologic barriers to produce sometimes cryptic zoogeographic patterns even for far- ranging species (e.g., Hoareau et al. 2012). The closing of the Isthmus of Panama 3 to 3.5 mya separated the eastern Pacific from the Caribbean. Since that time, Central America has been a geologically active area (Mann et al. 2007). The collision of the Cocos, Nazca and Caribbean plates has caused rapid uplift in various parts of Costa Rica. Climatically, it is known that Pleistocene glacial cycles have influenced the growth and abundance of corals (Cortes 1997) and that high peaks in Central America were glaciated during the glacial periods as recent as 10.5 kya (Lachniet 2007). Although there have been no studies on the population genetics and history of amphidromous fishes from the eastern Pacific, the varied climatic and geological history of the region makes it a suitable choice for studying the effects of climatic and geologic events on the population genetics and demography of amphidromous species. The goal of this study was to examine the phylogeographic, demographic and genetic structure of *Sicydium*

salvini populations to better understand the interplay of life history, and climatic and geologic events on this amphidromous species' population structure and history.

2. Materials and Methods

2.1 Sampling

Sicydium salvini is typically thought to occur from Nicaragua to Panama (Lyons 2005). Specimens found from Mexico to El Salvador have been traditionally assigned to *Sicydium multipunctatum* (see Miller et al. 2006). Unfortunately, *S. multipunctatum* was inadequately described to distinguish it from *S. salvini*. Furthermore, examination of the type specimens of both *S. salvini* and *S. multipunctatum* and specimens used in this study failed to separate these two species. Because the name *S. salvini* has priority over *S. multipunctatum*, we consider *S. salvini* to range from Mexico to Panama. Extensive collecting of *S. salvini* was conducted along the Pacific slope of Costa Rica (Fig. 1) in the summer of 2010. Sampling along the Pacific slope of Costa Rica focused on four different areas of the coast delimited by potential barriers to larval movement that could result in genetic differentiation among populations of *S. salvini* (Fig. 1, Table 7). Samples were collected from rivers draining into two different embayments (Golfo de Nicoya, B in Fig. 1; Golfo Dulce, D in Fig. 1) and two open coast areas (A and C in Fig. 1) within Costa Rica. Samples from Mexico/El Salvador (E in Fig. 1) and Panama (F in Fig. 1) were added to increase sampling from across the range of *S. salvini*. Sampling from the extent of the known distribution of *S. salvini* could show the influence of distance on the population structure of this species.

Figure 20 Map showing sampling localities for Sicydium salvini. Letters correspond to localities in Table 1. Arrows indicate the direction of the Costa Rican Counter Current (CRCC) drawn based on Glynn and Ault (2000)

Table 7 List of localities with the number of samples (N) and number of haplotypes (H) at each of those localities. Grouping indicates the regional grouping used for the AMOVA

Locality	N	H	Grouping
Río Nosara	23	23	A
Río Guacimal	28	26	B1
Río Seco	27	26	B2
Río Portolon	18	18	C1
Río Hatillo Viejo	7	7	C2
Río Piedras Blancas	24	24	D1
Río Esquinas	22	19	D2
Mexico/ El Salvador	6	6	E
Panama	7	7	F

Specimens were collected using a Smith-Root Electrofisher. Upon collection, specimens were killed with MS- 222. A tissue was taken from the right pectoral fin and stored in 95 % ethanol (EtOH). Voucher specimens were then fixed in a 10 % formalin solution and transferred to 75 % EtOH for long-term storage. In addition to the specimens collected in Costa Rica, additional specimens from museum tissue holdings were used from Mexico, Panama and El Salvador (Fig. 1).

2.2 DNA extraction and sequencing

DNA was extracted using the DNeasy Tissue kit (Qiagen) following the manufacturer's protocol. Samples were run out on a 1.0 % agarose gel and stained with SYBR Green to assess the quality and amount of extracted DNA. For each sample, the mitochondrial gene cytochrome

b (cyt b) was amplified using the primers AJG15A and H5 (Akihito et al. 2000). Cytochrome b shows considerable variation and has been used in previous studies of gobies at the population level (Chubb et al. 1998; Larmuseau et al. 2010; Hoareau et al. 2012; Lord et al. 2012). Polymerase chain reactions (PCR) consisted of 25 µl reactions using GoTaq (Promega) Master Mix that comprised 13 µl GoTaq master mix, 0.1 µl of each primer (100 lM), 2–4 µl DNA and ddH_2O for the rest of the mix. Thermocycler programs used to amplify fragments were as follows: 95 °C for 10 min, 30 cycles of 1min at 95°C, 45s at 54–58°C, 1 min at 72°C, with a seven minute extension period at 72 °C. The PCR products were assessed on a 1 % agarose gel stained with SYBR Green. Successful amplification products were sent to MCLAB for direct sequencing. Because of initial difficulties in sequencing the internal primers, SiCBL and SiCBH were designed. Sequences were edited and aligned by eye in Sequencher 4.8 (Gene Codes). Genbank accession. All sequences were deposited in Genbank under the accession numbers KF276809– KF276971.

2.3 Phylogenetic analysis

Bayesian inference of phylogeny was conducted using the program BEAST version 1.4.8 (Drummond et al. 2005; Drummond & Rambaut 2007). A model selection analysis was conducted in the program jModelTest (Posada 2008). The best fitting model chosen was GTR + I + G according to the Akaike information criterion in jModelTest. The Bayesian analysis was run for 10 million generations, with tree sampling every 1,000 generations. Burn-in was assessed using the program Tracer (Rambaut & Drummond 2007); all trees before stationarity were discarded. The analyses were run multiple times to ensure convergence. If posterior probabilities in independent runs were within 3 %, then convergence was assumed (Huelsenbeck et al. 2002).

2.4 Population genetic analysis

The calculation of haplotype diversity (h) and nucleotide diversity (p) was conducted in DnaSP version 5.10 (Librado & Rozas 2009). To test for population genetic structure, an analysis of molecular variance (AMOVA) was performed between regions, between rivers and within rivers (Excoffier et al. 1992). Regional groupings (Table 7, Fig. 20) were made according to geographic proximity of rivers and the relationship of their mouths to coastal structure (e.g., within Golfo Dulce vs. open coast). Specimens from Panama were grouped together and specimens from Mexico and El Salvador were grouped together as regions and treated as population groups because of a lack of adequate sampling of rivers in those countries. The AMOVA was run in Arlequin version 3.5 (Excoffier & Lischer 2010).

2.5 Historical demography

To test for population neutrality, Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) were calculated in DnaSP version 5.10 by constructing 1,000 coalescent simulations. A population in neutrality is one that is constant in size and panmictic and the effects of selection are negligible. If the population fulfills the expectations of neutrality, Tajima's D and Fu's F_s should be at or near zero. If the values are significantly negative, this could indicate that there has been a recent population expansion or selection because of an excess of rare alleles, while a significantly positive value indicates that a population has recently undergone a bottleneck (Tajima 1989). A mismatch distribution was calculated using DnaSP version 5.10 to test for population size changes. If the distribution is unimodal, more rare alleles exist than expected, indicating a recent population expansion (Rogers & Harpending 1992). A bimodal distribution indicates that the population has undergone a recent bottleneck resulting in fewer rare alleles than expected, or two populations are coming back together (Rogers & Harpending 1992). The genealogical method of

testing for changes in the effective population size (N_e), Bayesian Skyline Plots (BSP), was also performed using BEAST version 1.4.8 (Drummond et al. 2005; Drummond & Rambaut 2007). Tajima's D, Fu's F_s and mismatch distribution can only test if there was a population size change. Bayesian skyline analysis allows inference of the shape of population growth (constant vs. sudden) through time (Fontanella et al. 2008). For optimal inference of timing of changes in N_e, fossil calibrations are preferred (Ho 2007). Unfortunately, there are no known fossils of *S. salvini*; in fact, there are no known sicydiine goby fossils available to calibrate the molecular clock. To calculate mutation rate for *S. salv*ini, the method of Rocha et al. (2005)was used. In their study, the mutation rate (k) was calculated by solving the formula, k = d/2T, where d is the genetic distance and T the divergence time. Genetic distance was calculated between *S. salvini* and its Atlantic geminate species group. The divergence between them was 10.1% with a mean T of 3.0 mya corresponding to the closing of the Isthmus of Panama. The mutation rate for *S. salvini* was calculated to be 1.67 % per million years. This rate is similar to the rate found by Rocha et al. (2005) for a geminate species pair of *Evorthodus*, a genus thought to be closely related to Sicydiinae (Thacker & Roje 2011). The BSP analysis was run for 50 million generations with sampling every 1,000 generations. The plots were visualized in Tracer (Rambaut & Drummond 2007).

2.6 Relative sea level

Evidence from previous studies indicates that historical sea levels can influence the demographic history of amphidromous species. The rela- tive sea level has been estimated based on oxygen isotope levels used to determine the volume of ice for the past 450 kyr (Waelbroeck et al. 2002). These data were used to graph the relative sea level for the past 185 kyr. The sea level

graphs were transposed onto the BSP plots to look for a relationship between sea level and demographic changes.

3. Results

Sequencing resulted in an alignment of 968 bp of cyt b for 162 individuals across the range of *Sicydium salvini* with 142 total haplotypes. There were very few shared haplotypes among rivers with a majority of the haplotypes from each river being unique (Table 1). Haplotype diversity was 0.996 with no redundancy within six streams sampled and very little in the other three. Nucleotide diversity (π) was 0.01296. The Bayesian phylogeny (Figs. 21–24) showed very little resolution across the entire tree. Where clades were well supported, there were no concordant geographic associations. Furthermore, genetic structure was not detected between regions or between rivers within regions in the AMOVA with nearly 100 % of the variation found within rivers (Table 8). Most of the pairwise ϕ_{st} values between rivers were at or near zero, but there was significant population variation between the rivers of the Panama and Rio Guacimal (Table 9). Similar ϕ_{st} values, although not significant, were found between the Panama rivers and rivers in Mexico and El Salvador (Table 9). \

Table 8 Hierarchical AMOVA showing that nearly 100 % of the variation exists within river populations. There is no apparent structuring between regions or among rivers within regions

Source of variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among groups (regions)	5	32.123	0.06344 Va	0.99
Among Populations (rivers) within groups (regions)	3	13.338	−0.09886 Vb	−1.54
Within populations (rivers)	153	985.41	6.44059 Vc	100.55
Total	161	1030.87	6.40517	

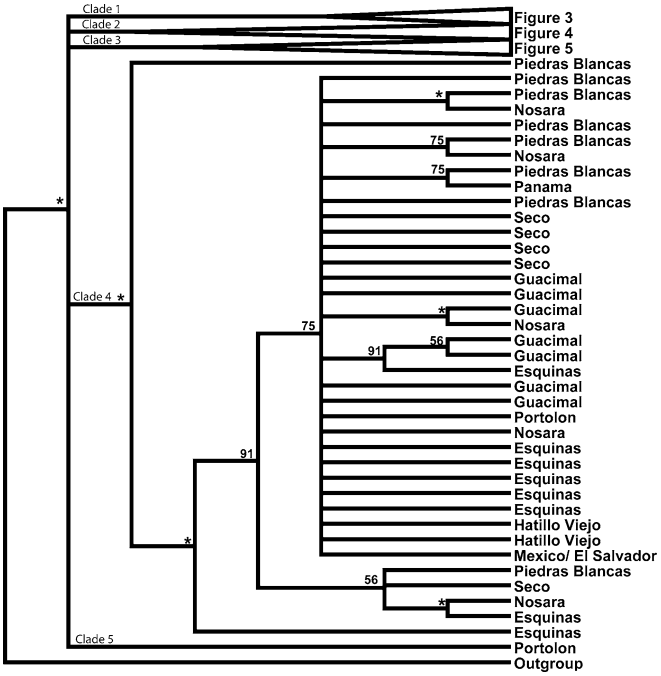

Figure 21 Bayesian phylogeny of Sicydium salvini. Names indicate sampling localities. Numbers above branches correspond to posterior probabilities (pp). Nodes with pp > 0.95 are labeled with an asterisk

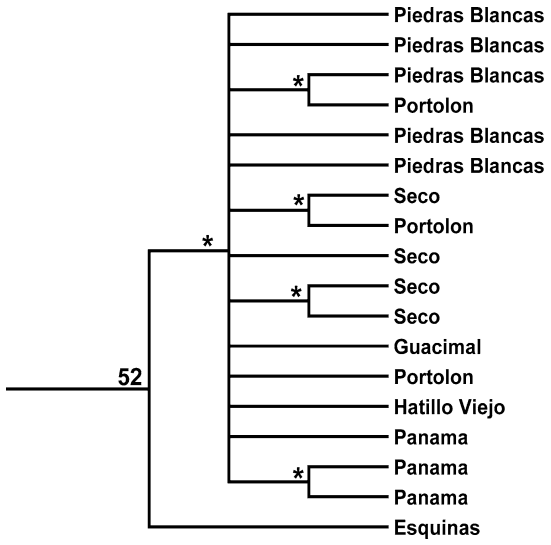

Figure 22 Bayesian phylogeny of clade 1 in Sicydium salvini. Names indicate sampling localities. Numbers above branches correspond to posterior probabilities (pp). Nodes with pp>0.95 are labeled with an asterisk

Table 9 Pairwise ϕ values between river populations; most values are at or near zero indicating no structure. There was one significant (p = 0.046) difference between Panama and the Rio Guacimal populations.

	Nosara	Guacimal	Seco	Portolon	Hatillo Viejo	Piedras Blancas	Esquinas	Mexico/El Salvador
Nosara	0							
Guacimal	−0.0042	0						
Seco	−0.0075	−0.0104	0					
Portolon	−0.0092	0.0105	−0.0184	0				
Hatillo Viejo	−0.0378	−0.0388	−0.0483	−0.0508	0			
Piedras Blancas	0.0031	0.011	−0.0079	−0.0059	−0.0459	0		
Esquinas	−0.0120	−0.0165	−0.0146	0.0012	−0.0413	−0.0080	0	
Mexico/El Salvador	−0.0448	−0.0414	−0.0447	−0.0236	−0.0524	−0.0081	−0.0406	0
Panama	0.0449	0.0988	0.0330	−0.0113	−0.0340	−0.0061	0.0679	0.0934

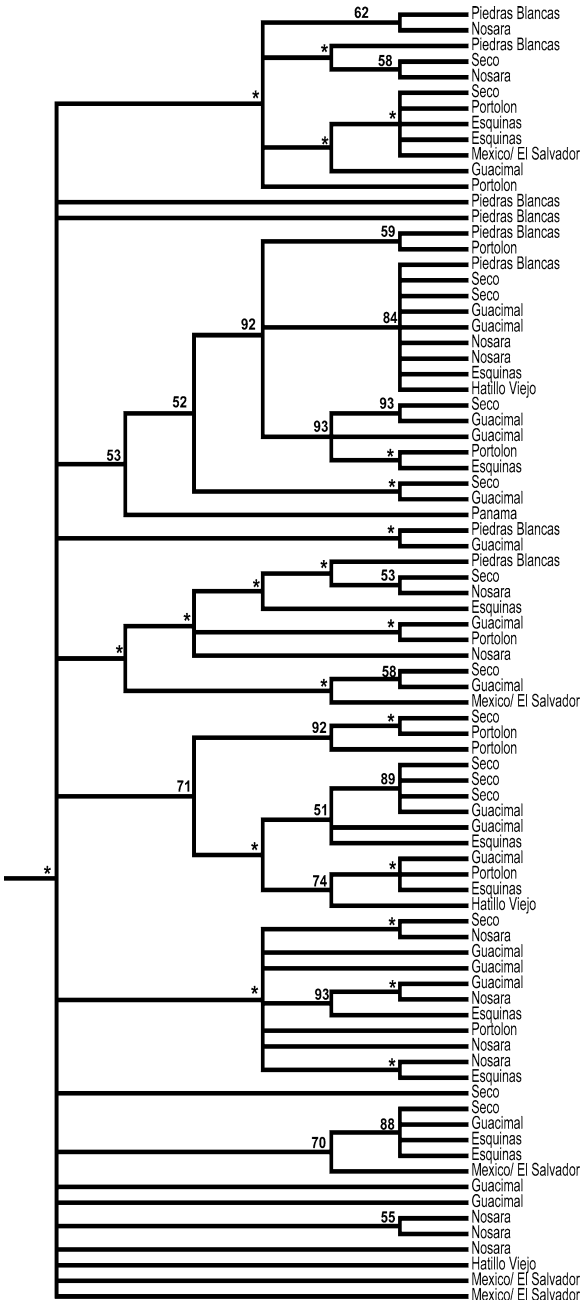

Figure 23 Bayesian phylogeny of clade 2 in Sicydium salvini. Names indicate sampling localities. Numbers above branches correspond to posterior probabilities (pp). Nodes with pp > 0.95 are labeled with an asterisk

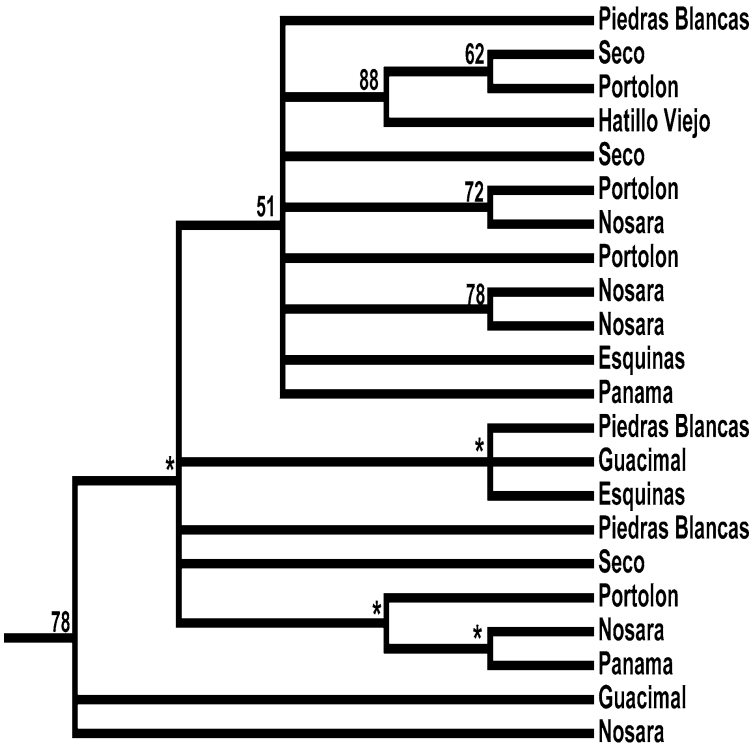

Figure 24 Bayesian phylogeny of clade 3 in Sicydium salvini. Names indicate sampling localities. Numbers above branches correspond to posterior probabilities (pp). Nodes with pp > 0.95 are labeled with an asterisk

Due to the overall lack of geographic structure in the AMOVA analysis, *S. salvini* was treated as a single population in the historical demographic analysis. Tajima's D was -0.1374 and significant ($p< 0.0001$); Fu's F_s was also significantly negative (-0.8912, $p<0.0010$). This indicated that either the population had undergone an expansion or selection (Tajima 1989; Fu 1997). The result of the mismatch analysis was a unimodal distribution (Fig. 25), which indicated that the population might have undergone an expansion, but it could also indicate a selective sweep where a favored allele was pushed to fixation (Rogers & Harpending 1992). The population expansion appears to have been rapid according to the shape in the BSP plots (Fig.

26). The BSP plot indicated an increasing N_e with a sudden expansion between 125–175 kyr ago (Fig. 26). The relative sea levels superimposed onto the BSP plots showed that an expansion occurred throughout the warming and cooling periods.

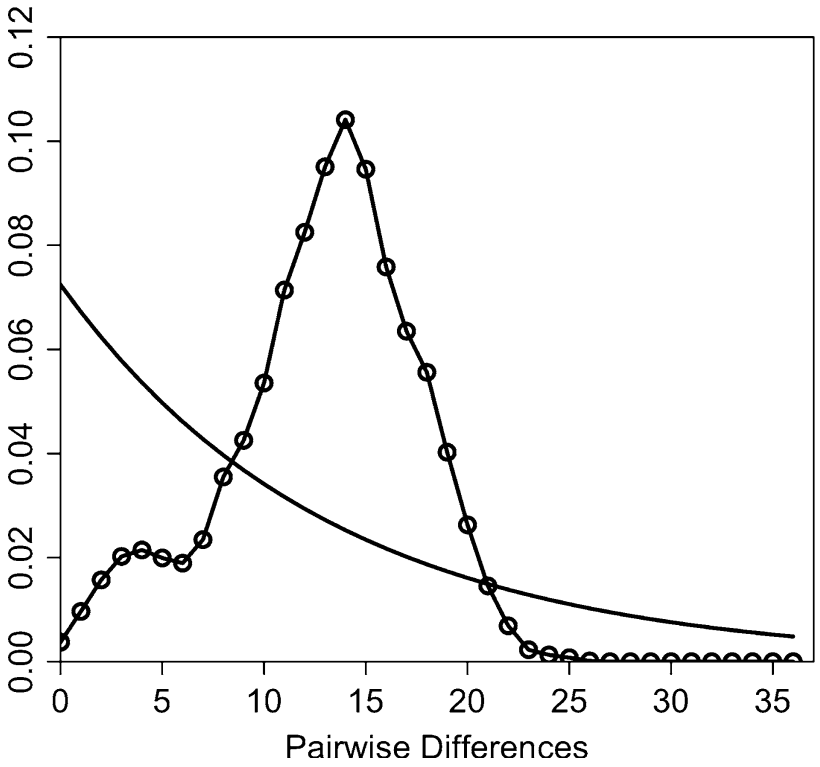

Figure 25 Results of the mismatch analysis for Sicydium salvini of individuals from all river populations sampled. The solid line indicates the expected mismatch distribution under a sudden expansion model. The observed pairwise differences (solid line, open circle) show a unimodal curve

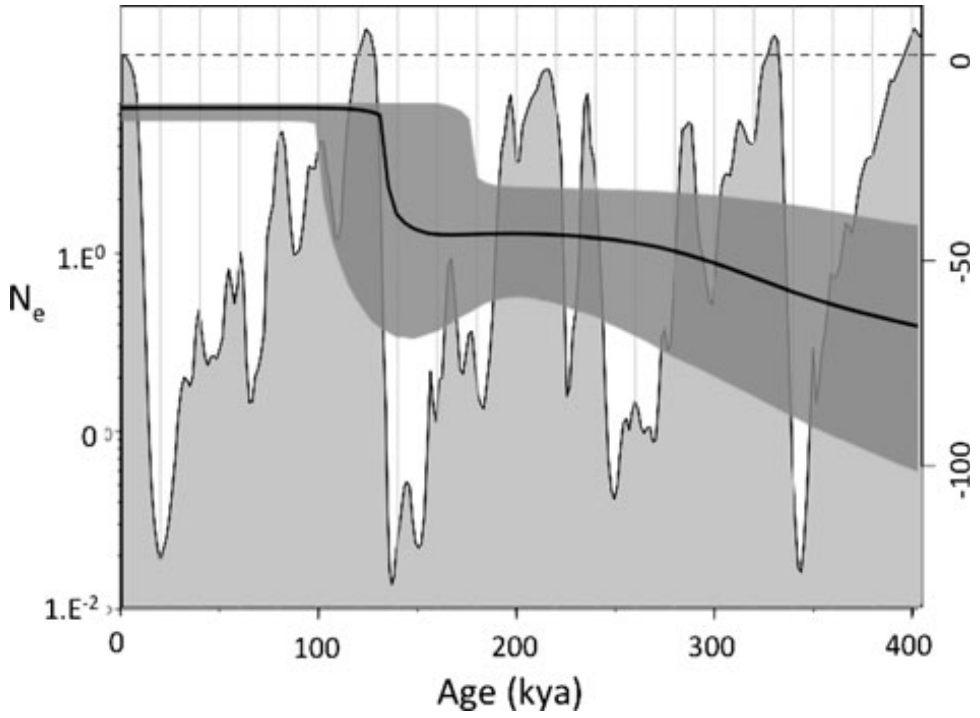

Figure 26 Results of the Bayesian skyline plot using a strict molecular clock with a substitution rate of 1.6 % myr. Superimposed onto plot is the relative sea level. The solid line is the median of the log of the population size. The shaded region around the median line represents the upper and lower bounds of the 95 % credible intervals

4. Discussion

The results of the study showed no phylogeographic structure among river populations across the range of *Sicydium salvini*. The phylogenetic reconstruction (Figs. 21–24) of the *S. salvini* samples showed no clustering of river systems or geographic areas, suggesting this species was panmictic. This adds evidence that there is a single species ranging from Mexico to Panama in the eastern Pacific. Furthermore, no genetic structuring at any hierarchical levels of

the AMOVA analysis was found. This indicated that there could be a high level of gene flow among populations. In addition, the genetic diversity of *S. salvini* is high (haplotype diversity = 0.996) with few shared haplotypes (only 20 out of 162 individuals). The haplotype diversities of three Caribbean *Sicydium* species from Puerto Rico were similarly found to be high (0.85 to 0.97) by Cook et al. (2009). Lord et al. (2012) found overall high haplotype diversities with *Sicyopterus lago- cephalus, Sicyopterus sarasini* and *Sicyopterus aiensis*. For *Stenogobius hawaiiensis, Lentipes concolor* and *Awaous guamensis*, Chubb et al. (1998) found similarly high haplotype diversities, but for *Sicyopterus stimpsoni* the haplotype diversity was only 0.47. There was a slightly significant pairwise ϕ_{st} value between two of our smallest groupings (Panama and Rio Guacimal, 9). This significance could have resulted from differences in sample size or a founder's effect (see additional discussion below).

The importance of the pelagic larval stage in shaping the population structure and history of amphidromous species is apparent from a growing number of studies (Watanabe et al. 2006; Cook et al. 2009; Lord et al. 2012). Larval duration seems to be important in shaping the ranges of sicydiine gobies. Lord et al. (2010) found that the most wide-ranging species in their study, *Sicyopterus lagocephalus*, had the longest pelagic larval duration (PLD) that varied between regional populations with means of 131– 200 days. Species of *Sicyopterus* with more narrow geographic ranges than *S. lagocephalus* had considerably shorter larval durations with means of 76 and 79 days. However, in the wide- ranging species *Sicyopus zosterophorum*, the PLD was not significantly different from species with narrow ranges in the genera *Smilosicyopus* and *Akihito* (see Taillebois et al. 2012). This strongly suggests that PLD is not the only factor determining the range of a sicydiine species. In the Caribbean, (Bell et al. 1995)found that the larval duration of two Sicydium species [*S. punctatum* and *Sicydium antillarum* (synonym of *S.*

plumieri)] ranged from 63–139 days. The larval duration for *S. salvini* is unknown; therefore, we do not know if there is sufficient time during the larval period to have larval movement across the entire range of the species. However, there is no evidence of a stepwise progression of relatedness between rivers or river basins along the coast. In an ongoing study of the genus, *S. salvini* appears to be closely related to *S. plumieri* (Chabarria and Pezold, unpublished data). If *S. salvini* and *S. antillarum* share a similar larval period (63–139 days, Bell et al. 1995), there would be sufficient time for migration of larvae across the range of *S. salvini*. Two haplotypes from the Mexican populations are shared with individuals in the R´ıo Esquinas in the Golfo Dulce in southern Costa Rica. Shared haplotypes between streams that are about 1900 km apart also suggest that there is gene flow across the range of *S. salvini*. This could be a genetic relict, but that is not likely because of the overall lack of structuring in the phylogeny and AMOVA indicating panmixia.

The regional groupings with their hypothesized geomorphic barriers to gene flow for *S. salvini* do not correlate with the genetic data. The apparent panmixia indicates that the potential barriers defining our regions do not impede larval movement. In addition, it seems that larvae can tolerate time away from nearshore habitat when crossing an area with little to no continental shelf (Oso Peninsula). One driver for this apparent panmixia could be the Costa Rican Coastal Current (Fig. 20). This northward current can reach the Baja Peninsula during its strongest times of the year (Glynn & Ault 2000) and may aid in the transport of larvae from the southern portion of the species' distribution northward.

Comparisons with other studies at the regional level can be difficult because regions may be recognized at different scales. Phylogenetic structuring between mtDNA haplotype groups associated with three large biogeographic regions (southwestern Indian Ocean, Melanesia and

Polynesia) has been observed in *Sicyopterus lagocephalus* (Hoareau et al. 2012; Lord et al. 2012). Hoareau et al. (2012) attributed this structuring to low sea levels during the Pleistocene fragmenting larval dispersion. A broader study (Lord et al. 2012) confirmed the existence of three distinct genetic lineages within this species, but offered a more complex explanation for their origins. They agreed that Pleistocene sea level changes likely produced the divergence between Indian Ocean and western Pacific populations, but the divergence of populations in Polynesia was attributed to isolation by ocean current patterns. No population structuring was observed at a smaller regional scale for multiple amphidromous species in Puerto Rico by Cook et al. (2009), where the two regions were defined as the Atlantic and Caribbean versants of the island. In fact, studies of amphidromous gobies from streams within island archipelagos (Chubb et al. 1998) or of island populations (Berrebi et al. 2005) generally have shown no structure. Chubb et al. (1998) found no structure across the Hawaiian Islands for four amphidromous goby species (*Stenogobius hawaiiensis*, *Lentipes concolor, Sicyopterus stimpsoni* and *Awaous guamensis*). Watanabe et al. (2006) found no structure among populations of *Sicyopterus japonicus* in the Japanese Archipelago. No structure was found among populations of *Sicyopterus lagocephalus* within large biogeographic haplogroups by Hoareau et al. (2012). This finding was also mirrored in a larger analysis of *S. lagocephalus* by Lord et al. (2012). The overall trend seen with amphidromous species is a lack of genetic structuring between individual river systems.

Although there was structure found in our study between *S. salvini* populations in Panama and the Rio Guacimal in Costa Rica, we believe this could be an artifact of low sample size. Similar structure between two populations of *Sicydium buscki* in Puerto Rico was attributed to non-equilibrium dynamics (Cook et al. 2009). Waples (1998) pointed out that having few

samples or nonrandom sampling efforts may mislead population structure calculations and may not reflect the genetic variation over the entire population. In our study, the number of specimens from Panama (n = 7) covered four different river systems; therefore it is likely that the structure is an artifact of sampling.

The unimodal curve of the mismatch distribution (Fig. 25) could indicate that the population has undergone a population expansion or that a selective sweep has taken place, but the Bayesian demographic analyses indicate that *S. salvini* underwent a rapid population expansion in the Pleistocene between 125–175 kyr (Fig. 26). Given the results of the Bayesian skyline analysis, it is likely that negative Tajima's D, Fu's Fs and the unimodal mismatch distribution indicate population expansion and not selection. The Pleistocene was characterized by glacial cycles that had an impact on both terrestrial and aquatic (marine and freshwater) species' distributions and demographies (Hewitt 2004). Previous studies of inshore fishes (Larmuseau et al. 2009; Ravago-Gotanco & Juinio-Meñez 2010), reef fishes (Gaither et al. 2010; Gaither et al. 2011) and sicydiine gobies (Hoareau et al. 2012; Lord et al. 2012) have all shown impacts of Pleistocene glacial cycles on genetic lineages and historical demography. The data here, however, suggest little influence of the Pleistocene climate on the historical demography of *S. salvini*. The BSP plot shows a gradual increase in population with a sharp increase occurring 125–175 kyr (Fig. 26). This lack of influence on the demography of *S. salvini* by Pleistocene climate changes indicates that other factors have led to the increase in population size.

Dramatic geologic events have shaped the river systems of Central America. The Quaternary was marked by extensive geologic activity (Gardner et al. 1987; Marshall 2007). The Chorotega block in southern Central America contains all of Costa Rica and western Panama (Marshall 2007). This area is composed primarily of Neogene– Quaternary volcanic belt. The

subduction of the Cocos plate under the Costa Rican margin has created a pronounced uplift. This uplift is variable across Costa Rica, but can be dramatic (up to 6.1 m/kyr on the Oso Peninsula) (Marshall 2007). This uplift would have transformed stream habitats from low relief to high relief, creating greater suitable habitat for *S. salvini* expansion. Specific information on geological history is not available for many of the streams sampled, but there is a general trend of increased tectonic activity from north to south in Costa Rica (Menges 1987; De Boer et al. 1995; von Huene & Ranero 2009). This increase in tectonic activity would have drastically influenced the physical characteristics of streams. For example, the tributaries of the upper Rio Esquinas (D2 in Fig. 20) are adjacent to areas of high mountain tectonic activity (Menges 1987). This uplift increases the gradient of rivers that flow through them. Furthermore, within stream systems such as the Rio Naranjo, local uplift can influence gradients at multiple points along a river (Menges 1987). High tectonic activity can change a meandering river into a steep gradient river possessing a short coastal segment and abundant suitable habitat for S. salvini. Changes to stream characteristics are not the only factor relevant to the species population expansion. During the middle to late Quaternary, volcanic activity led to changes in river drainage (Marshall et al. 2001; Marshall et al. 2003). During this period, the stream capture events and reversals led to a change from Caribbean direction to Pacific direction drainages in the Valle Central (Marshall et al. 2001). Not only would this have changed the landscape, but it would have also increased the number of streams along the Pacific slope. By the late Pleistocene, continued uplift of the Cordillera Central would have solidified the reversals in flow (Marshall et al. 2001). These newly formed habitats associated with the uplift would mirror the formation of oceanic island river systems. Given the ability of amphidromous species to invade new habitats, this would have been the perfect opportunity to expand into new habitats unavailable to primary freshwater

fishes. Like other amphidromous taxa, the marine larval stage of *S. salvini* has been an important driver for the patterns of population expansion and connectivity. This ability to exploit new habitats combined with the increase in available habitat allowed populations of *S. salvini* to expand.

We have shown that *S. salvini* is a genetically diverse species with a wide distribution. Demographic analyses show that *S. salvini* underwent a historical population expansion. The increase in population size may have been influenced by the coincidental occurrence of Pleistocene global glacial fluctuations and geologic uplift in Central America. These two factors could have led to an increase in habitat availability allowing *S. salvini* to expand its population. This expansion was facilitated by the amphidromous life history of this species, which has been shown to be an important influence on the population structure of *S. salvini*. There appears to be high connectivity and gene flow between stream populations across the species' known range. Determining the level and direction of gene flow will require using finer-scale molecular markers (e.g., microsatellites). In addition, this is a very abundant species (Chabarria and Pezold, unpublished data) without competition from congeners in the river systems. In Venezuela, population densities of *S. plumieri* dominated all fish species (Penczak & Lasso 1991). (Erdman 1961) estimated a migration of 50 million fish over a 50 hour period from a single river in Puerto Rico. If S. salvini post-larval migrations happen at this magnitude in the eastern Pacific, the diversity could be much higher, and to understand the dynamics between river systems sampling would have to increase.

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SUMMARY AND FUTURE RESEARCH

This dissertation has provided new insight into the evolution of *Sicydium*. In the first chapter the evolutionary relationships between the species of *Sicydium* were hypothesized based upon morphological characters. The results of the study concluded that there is a monophyletic *Sicydium* that is sister to *Sicyopterus*. *Parasicydium,* a monotypic genus that was previously proposed to be nested within *Sicydium* (Taillebois et al. 2014), was hypothesized to be basal to *Sicydium* and *Sicyopterus*. The species of *Sicydium* were grouped into three clades that were primarily distinguished by oral morphology, fin morphology and pigmentation. The relationships between species within the clades are largely unresolved. In the second chapter a phylogeny of *Sicydium* was constructed based upon mitochondrial and nuclear DNA. The phylogeny of the combined data set recovered a well-resolved phylogeny of *Sicydium*. The species formed two large clades that largely reflected the morphological phylogeny. Seven independent lineages were recovered that correspond to previously described morphological species. Most likely because of incomplete lineage sorting, the morphologically distinct forms *Sicydium plumieri/S. gilberti, S. altum/S. adelum,* and *S. crenilabrum* "A" and "B" did not separate in the analyses. Also *Parasicydium* was found to be sister to *Sicydium,* a relationship that was not indicated by the morphological analysis. In the third chapter I show that *Sicydium salvini* is a genetically diverse, panmictic population from Mexico to Panama. From data gathered in chapter two, increased sampling, and examination of type specimens it is shown that *S. multipunctatum* is likely synonym of *S. salvini*. Furthermore it is concluded that geologic uplift and ocean currents have played a role in the population dynamics and size of *Sicydium salvini*. The marine larval phase allows for high connectivity between stream populations. This larval phase was also

important in a historic population expansion of *S. salvini.* Amphidromous fishes have an amazing capability to invade new habitats because of the marine larval phase. It is suspected that new habitats resulting from geologic uplift and glacial cycles allowed *S. salvini* populations to expand.

The phylogenetic hypotheses of the first two chapter shed light on relationships between species. The relationships and species lineages of the molecular analysis showed that species diversification was the result of biogeographic and possibly morphological evolution. Our understanding of the diversity within *Sicydium salvini* has shown further evidence of concordance between genetic and morphological species hypotheses. Additionally new characters for *Sicydium* presented here will aid future taxonomic reviews. Under a phylogenetic framework, new questions may be asked in regards to the evolution of morphology and species in *Sicydium*. The chapters of dissertation show that the story is not complete for *Sicydium.*

Discordance between molecular and morphological data shows that more information is needed. The inclusion of many independent loci from next gen sequencing could help tease out the problems associated with incomplete lineage sorting. Further testing at the subfamily level would be useful to understand the relationship between the genera, especially *Sicydium, Sicyopterus,* and *Parasicydium.* Recent molecular analyses of the subfamily (Keith et al. 2011; Taillebois et al. 2014) have shown different relationships between different studies and from previous and current morphological studies.

Finally, a thorough taxonomic revision is needed for *Sicydium.* This dissertation has brought new information about the relationships between species. However as evident from some of the data presented here, our understanding of species diversity in *Sicydium* is poor. This study provides a stepping stone by introducing new characters to investigate taxonomic

boundaries within *Sicydium*. A strong taxonomic hypothesis is needed for species of *Sicydium* for several reasons. As conservation decisions are based upon taxonomic units, it is critical to have a clear understanding of the diversity represented by the genus (Bortolus 2008). *Sicydium* species often constitute one of the most abundant components of the fish communities in which they occur. Being largely herbivorous, they are major primary consumers in their host streams and also serve as a potential food source for larger fishes in those systems (i.e. *Gobiomorus* sp., *Eleotris* sp.). Although adult *Sicydium* are not harvested, fisheries exist for the fry (Bell 1999). Large numbers of post-larvae are taken during migrations upstream in Puerto Rico (Bell 1999) and Jamaica (Aiken 2006). This harvest is not sustainable (Keith 2003) and, therefore, could be detrimental to local populations. Human activities such as production of municipal and agricultural waste seem to negatively affect the abundance and health of individuals living in these areas (Pers. Observ.). Their abundance may also be negatively influenced by the introduction of invasive fish species such as *Tilapia* (Pers. Observ.). The larvae of *Sicydium* are important coastal inhabitants contributing to another trophic level in marine systems. Understanding the evolution and diversity of *Sicydium* species is critical to their management and conservation.

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BIOGRAPHICAL SKETCH

Ryan Chabarria received his Bachelor of Science degree in Zoology from Louisiana State University in Baton Rouge in 2002. His interest in phylogenetics and evolution was sparked when he worked with Dr. Jim McGuire. During his time working in the McGuire lab, Ryan worked with flying lizards and hummingbirds learning molecular phylogenetic. It was also during his time at LSU where his interest in fishes really sparked. He had the pleasure of taking Ichthyology from Dr. Mike Fitzsimons.

After a short time teaching high school Biology, Ryan returned to academia enrolling in a Master's program Southeastern Louisiana University in 2005. His Master's thesis was a phylogeographic study of *Necturus beyeri.* In addition to research on salamanders, Ryan was also a co-author with Dustin Siegel on the glue glands of the frog *Gastrophryne carolinensis.* Also he was able to work on two fish projects in the lab of Dr. Kyle Piller.

Upon completion of his Master's degree in 2008, he enrolled in the Marine Biology PhD program at Texas A&M University-Corpus Christi in the lab of Dr. Frank Pezold. During his PhD research, Ryan's research focus included amphidromous fishes, systematics, morphological evolution and biogeography. After a couple of field seasons, a marriage and a few moves Ryan completed his dissertation in 2015.

Ryan's research interests in fishes continue to grow in addition to evolution, systematics and biogeography. He has a strong interest in fieldwork, having spent time in the field in the Continental United States collecting amphibians, reptiles and fishes and expeditions to Puerto Rico and Costa Rica to collect *Sicydium.* To date Ryan has authored or co-authored five publications including a book chapter.