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Letter from the Editors:

This past year we have witnessed a wave of change and enthusiasm sweep across the biology community at UCSD, and it has affected Saltman Quarterly in ways that have left us excited for the future. It is difficult to speak of change in the context of biology without invoking Charles Darwin, a man who drew biology together into a single unified science, and forever changed our vision of life and the natural world. As we move forward in the twenty-first century, it is important to keep in mind the timeless principles discovered by Darwin, which are continually affirmed and deepened by the research of scientists today – particularly at UCSD. It was a great honor for SQ to celebrate Darwin's 200th birthday this year, and to have had the privilege of sponsoring the division's first Undergraduate Dean's Symposium, "Why Darwin Matters." The campus-wide excitement for this event and the creative contribution of undergraduates (which can be seen on the following page), show that biology at UCSD is more alive than ever. SQ is proud to be a part of this scientific community, and we anticipate a sustained interest for years to come.

This year we are pleased to publish SQ earlier than ever, as a result of the abundant volume of submissions we received. We are equally impressed with the level of excellence which is exhibited in the published manuscripts. In the Research Section, we begin by looking at tumors of the vestibular nerve, and then move to four studies focusing on different regions of the ocean, and the creatures inhabiting it. These studies focus first on the shallow sea, with sand dollars and pipefish, and then plunge deeper into the ocean with the secondary fluorescence maximum and the Oxygen Minimum Zone. It is fitting that our topics cluster around marine life, because the diversity and unique adaptations in this environment are a living record of the power of evolution.

In the Features section we take a foray into phage therapy, gene therapy, and lung cancer vaccines; three promising new approaches to combating infection and disease. We then enter the borderlands of science fiction with brain-computer interface technology, and wrap up with a discussion of alternative biofuel. We even have two Research Reviews which explore the regenerative power of neurons, both endogenously and via stem cell therapy, and the implications this has for neurodegenerative diseases and vision restoration.

In the final pages you will find abstracts of our biology student's honors theses, and a salute to the man who inspired this journal, Dr. Paul Saltman. In his spirit we hope this journal provides you with an opportunity to express your love for science communication; and as fellow students, we encourage you to remember the grand tradition in which you are participating and the many giants who forged the way ahead of you. The science of life is truly inexhaustible, and there is no better place to experience it than here at UCSD. We eagerly anticipate future revolutions of "Darwinian" proportions in the years ahead, and have thus made it our mission to involve all biology majors in the exciting research of our time. At this unique place where discovery comes to life, we hope you enjoy Volume 6.

Tyler Green and Christine Cho
Editors-in-Chief

On the Cover:
This photo of a Golden Orb Weaver was taken by Alice Ho in the countryside of southern Taiwan. Alice is a freshman majoring in Bioengineering at Warren College.

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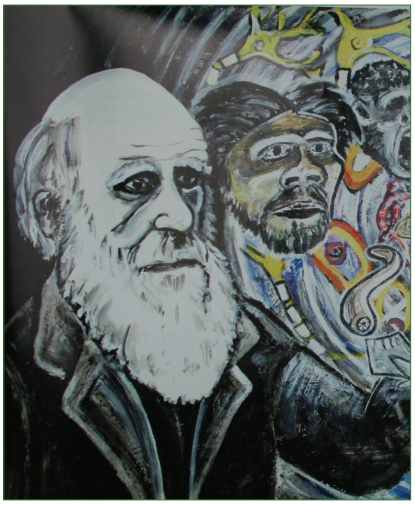

FEATURES

RESEARCH

REVIEW

"Why Darwin Matters" contest entries, clockwise from top left:

"Why Darwin Matters"
Eran Agmon, Marshall College
"Brilliant!"
Sarah Choi, Sixth College
"Natural Selection"
Kacie Paik, Muir College
"Darwin Brings Us Together"
Kim Cyprian, Marshall College

correlations between the world's OMZs will give us insight into where the oceans are headed, as well as the impact that expanding OMZs will have on the planet.

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Interspecific hybridization as a source of genetic variation in eastern Pacific *Syngnathus* species

Eric Garcia,[1*] Stuart Sandin,[2] Tony Wilson[3]

While hybridization is thought to be an important source of genetic variation, it has not yet been reported in any *syngnathid* species (pipefish and seahorses). However, hybridization may contribute to genetic variation in exceptionally dense and diverse communities of *Syngnathus* pipefish along the coast of California and Baja California. We used both genetic and morphological analyses to investigate hybridization in Californian pipefish. A *Syngnathus*-specific non-coding nuclear sequence and mitochondrial DNA were obtained from distinctly preserved specimens collected from the area under investigation. Genetic results of each species were compared and used to construct a phylogeny. In addition to successful DNA extraction and amplification from samples fixed in formalin, results documented novel *Syngnathus* sequences as well as a new *S. exilis* haplotype. Although *S. exilis* indicates some degree of hybridization, we were not able to attribute this variation directly to hybridization. Nonetheless, this study presents a useful framework for further research detailing the great, but unresolved, genetic variation of the eastern Pacific *Syngnathus* species.

Introduction

Scientists have expressed hybridization as an important source of novel genetic variation and have illustrated that interspecific hybridization may improve the ability of wild populations to adapt to new environments (*1*). The scientific interest in hybridization has increased over the last three decades, especially after recent improvements in molecular genetic techniques that have contributed to the discovery of many more hybrid species. These findings suggest that hybrid taxa may be more frequent than was previously thought (*2*, *3*). Most cases of natural hybridization occur between closely related species. However, it was found that incidents which bring ecologically divergent parent species together could also produce a speciation event (*3*). Although hybridization is very common in plants, and generally very rare in animals, hybrid species are not unusual in fish (*4*, *5*, *3*). For example, the *Gila seminuda* population of the Moapa River, Nevada, is proposed to have a hybrid origin. Similarly, stickleback fish have been shown to have the ability to generate fertile hybrids between divergent populations from different parts of the globe (*6*, *7*).

The *Syngnathus* pipefish species of the eastern Pacific Ocean could possibly be another case of interspecific hybridization. A total of eight *Syngnathus* species inhabit almost the entire Pacific shoreline of North, Central and South America (*8*). The coast of California and Baja California exclusively shelters five of these species: *Syngnathus exilis*, *S. euchrous*, *S. carinatus*, *S. insulae*, and *S. californiensis* (*8*). The particularity of the distributions

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Sample	Species	Sampling Location/ SIO collection #	Preservation	Donator / Source
EG1	*Syngnathus exilis*	SIO# **02**-71	Ethanol	HJ Walker
exT2	*Syngnathus exilis*	SIO# **02**-71	Ethanol	HJ Walker
exi 1-15	*Syngnathus exilis*	SIO# **47**-106	Formalin	HJ Walker
cari 1-15	*Syngnathus carinatus*	SIO# **47**-53	Formalin	HJ Walker
auli 1-15	*Syngnathus auliscus*	SIO# **50**-69	Formalin	HJ Walker
insu 1-15, EG3	*Syngnathus insulae*	SIO# **63**-169	Formalin	HJ Walker
euch 1-15, EG 2	*Syngnathus euchrous*	SIO# **47**-68	Formalin	HJ Walker
lep 1-125, EG 5-6	*Syngnathus leptorhynchus*	Bred in lab **04**	Ethanol	Dr. Marchetti
EG 7	*Syngnathus leptorhynchus*	San Diego Bay **06**	Frozen -80°C	Dr. Wilson
EG4	*Syngnathus californiensis*	Mission beach **04**	Dry	Dr. Marchetti
cali2	*Syngnathus californiensis*	Mission beach **04**	Dry	Dr. Marchetti

Table 1. Specimen information chart. Bold numbers specify the year of the specimen's collection. For example, **50** = 1950, **02** = 2002, and so on.

and high density of Californian pipefish has generated scientific interest to investigate the cause of their unique high diversity. Although hybridization has never been recorded in any syngnathid species, there is evidence of interspecific mating between two other eastern Pacific pipefish, *Syngnathus leptorhynchus* (occurring from Alaska to Baja California) and *Syngnathus auliscus* (occurring from northern Peru to California), whose wide distributions overlap in Californian waters as well (*1*). This interspecific mating and the high diversity of *Syngnathus* species co-occurring in high densities in California and Baja California suggest that hybridization may be contributing variation to these *Syngnathus* species. By employing genetic analyses of DNA extracted from specimens preserved in an extensive variety of methods (including formalin preservation), and morphological analyses involving meristic traits quantification, this project investigated the role of hybridization as a source of genetic variation in these highly dense and diverse communities of Californian pipefish.

Materials and methods

Specimen compilation

Pipefish specimens were provided by former UCSD investigator, Dr. Marchetti, and by H.J. Walker, manager of the marine vertebrate collection of Scripps Institution of Oceanography (SIO). Most of the specimens obtained from Dr. Marchetti were bred in lab, but some were captured from Mission Beach, CA. Those coming from the marine vertebrate collection were acquired from several points along the coast of California and Baja California, including the SIO pier, the bay of San Diego, and the previously mentioned Mission beach. The exact sampling coordinates of the SIO's specimens can be found in the institution's database using the given specimens' collection number (Table 1).

Morphological analysis

The morphological examination consisted of contrasting and identifying all pipefish according to the *Syngnathus* species morphological key provided by Fritzsche, which uses a meristic approach involving trunk ring, tail ring, and dorsal fin ray quantifications (*8*). All measurements required were performed as described by the *Syngnathus* species morphological key.

Genetic Analysis

1. Standard ethanol precipitation DNA extractions

For ethanol-preserved, dried and frozen specimens, DNA was extracted from lateral caudal muscle tissue and isolated as indicated by the DNeasy 96 Tissue Kits for purification, using the animal DNA purification protocol. The DNA was then diluted to a concentration of 2 ng/μL as preparation for mtDNA and nuclear sequence analysis (*1*). While there were no preliminary procedures performed for frozen and ethanol-preserved samples, dry specimens were submerged in 70% ethanol for 17 days prior to the extraction. For practicality and to account for equipment limitations, few modifications were made to the DNeasy protocol. However, of the changes that were made, DNA was incubated at 65ºC rather than 70ºC.

Primer	Sequence	Analysis
16Sar	CGCCTGTTTAT-CAAAAACAT	16S gene mtDNA
16Sbr	CCGGTCTGAACTCA-GATCACGT	16S gene mtDNA
SlepA1f	ATCTGAGCCAGC-GGGCCGAGCAG	Nuclear Sequence
SlepA1r	TGGAGCGCGGCTTG-CAGTCGTG	Nuclear Sequence

Table 2. Primer information. All primers were obtained from Tony Wilson.

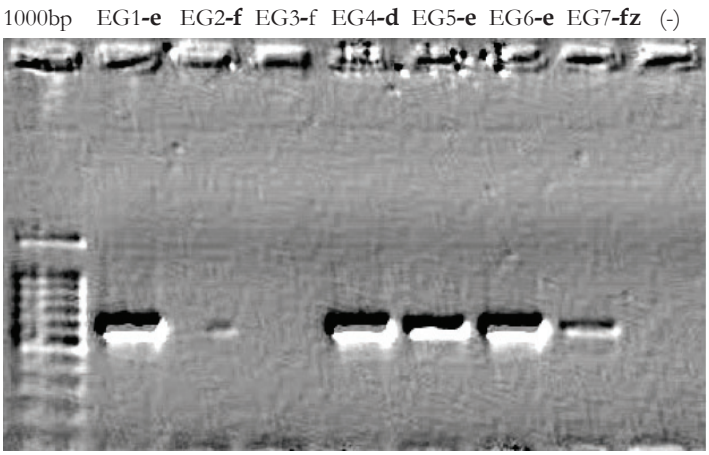

Figure 1. 16S gene mtDNA gel electrophores showing 550-600 bp bands. EG# represents the sample's name and the final highlighted letter denotes the preservation method of the sample. **-e** : ethanol-preserved, **-f** : formalin-preserved, **-d** : dry sample, and **–fz** :frozen sample. 1000 bp symbolizes a 1000 base pair bench ladder.
EG1: *S. exilis*, EG2: *S. euchrous*, EG3: *S. insulae*, EG4: *S. californiensis*, EG5-EG7: *S. leptorhynchus*, (-) : negative control.

Secondly, DNA centrifugation for the removal of residual ethanol was done at the speed of 13,000 rpm instead of 14,000 rpm. Thirdly, final elution was not repeated as it was irrelevant for the purpose of this research.

2. DNA extraction from formalin-preserved specimens

A formalin-specific protocol outlined by Schedlock et al. was followed for extracting and isolating DNA from specimens preserved in formalin (*9*). Muscle tissue was extracted using the previous technique for all formalin-preserved specimens, except for *S. auliscus* samples, which were too small to extract enough muscle tissue. Instead, the complete right half of the trunk was removed, and chopped and used as a DNA source. Also, a small number of ethanol-stored specimens were included as positive controls. As with the ethanol precipitation extractions, few changes were made to the protocol for practical and time management reasons. Prior to washing, extracted tissues were submerged in 70% ethanol overnight. Then they were poured into petri dishes to be collected and placed in new tubes, where they were air-dried in an incubator at 37˚C for 55 minutes. Secondly, purified DNA was precipitated by adding approximately 2 volumes instead of 2.5 volumes of cold ethanol stored at -21˚C, rather than -80˚C. The purified DNA was then placed at -21˚C, which completed precipitation after 14 hours instead of 24 hours.

3. PCR protocol

The genetic study included a *Syngnathus*-specific non-coding nuclear sequence and the mitochondrial 16S gene to determine maternal inherence of the examined pipefish species. Both genes were amplified using a 25μL polymerase chain reaction (PCR) protocol containing 1 U *Taq* (Promega), 2.5 μL 10X reaction buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 1.0 μm dNTPs (Promega), 200 nM primers, and 20-50 ng DNA (1). Amplification and sequencing of the 16S mitochondrial gene was done with 16Sar and 16Sbr primers, and with a PCR process starting at 94°C (3 min), followed by 39 cycles of 94°C (30 sec), 50°C (30 sec), 72°C (1 min), 72°C (5 min) and finishing with 14°C. Amplification and sequencing of the non-coding nuclear sequence was done with SlepA1f and SlepA1r primers and with a PCR process starting at 92°C (10 sec), followed by 39 cycles of 92°C (10 sec), 59°C (30 sec), 68°C (2 min) and finishing with 14°C.

Nuclear and mitochondrial DNA sequences were edited, compared, and analyzed with BioEdit© sequence alignment editor software (*10*). Following this, Forcon© software was utilized to convert the format of the sequencing data into Mega format (*11*). Finally, the Molecular Evolutionary Genetics Analysis© (MEGA4) program was used to construct a phylogeny that provides insight into the origin of genetic variation among these Californian *Syngnathus* species (*12*).

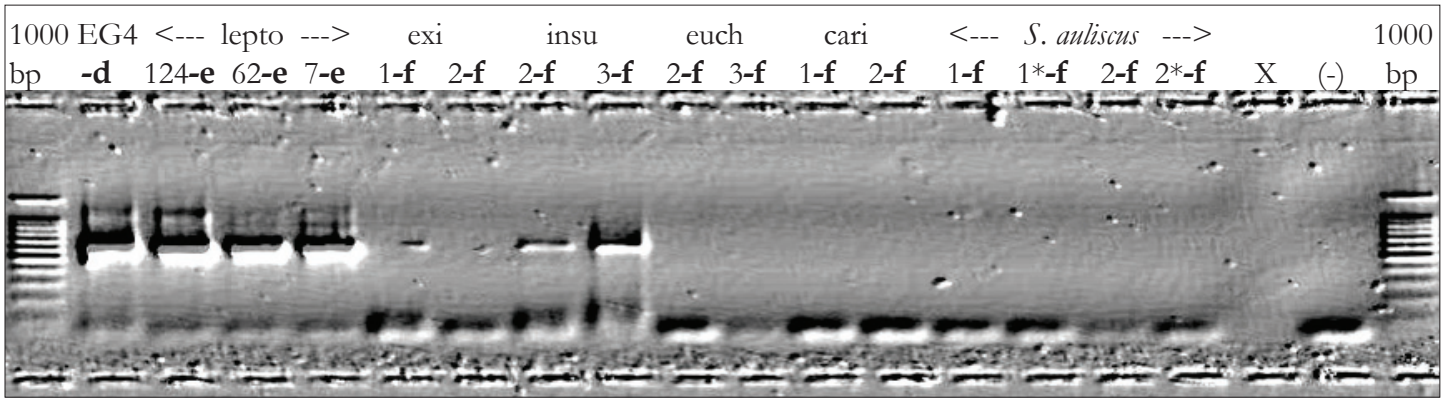

Figure 2. 16S gene mtDNA gel electrophoresis showing 550-600 bp bands. 1000 bp represents 1000 base pair bench ladder, and numbers represent specimen names. EG4: *S. californiensis* (positive control), lepto: *S. leptorhynchus* (positive controls), exi: *S. exilis*, insu: *S. insulae*, euch: *S. euchrous*, cari: *S. carinatus*; *S. auliscus* 1*and 2* are replicates of *S. auliscus* 1 and 2. Final highlighted letter denotes the preservation method of the sample, **-d**: dry sample, **-e**: ethanol preserved, **-f**: formalin-preserved; (-) : negative control, X: extra.

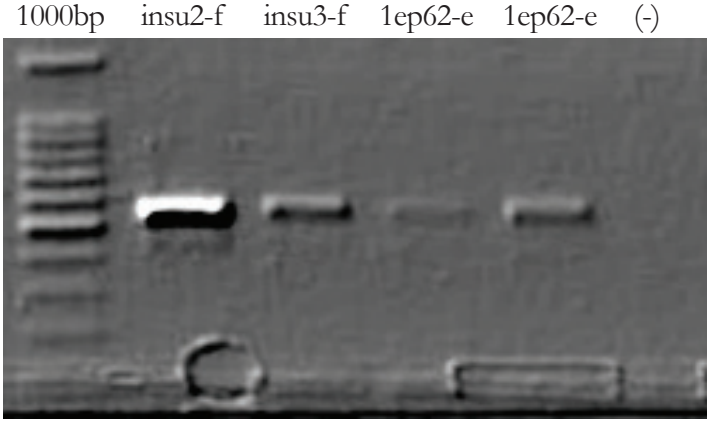

Figure 3. Nuclear sequence gel electrophoresis showing 550-600 bp bands. insu: *S. insulae*, lep: *S. leptorhynchus*, **-e**: ethanol preserved, **-f** : formalin-preserved, (-) : negative control.

Results

The first round of DNA extractions followed standard ethanol precipitation methods and successfully yielded genetic material from all specimens, except those stored in formalin. Extracted DNA was then used to amplify the mtDNA 16S gene. As expected, gel electrophoresis showed a band of approximately 550-600 base pairs for all samples except for formalin-fixed ones, indicating the presence of the 16S gene fragment (Fig. 1). Following this, the 16S gene was effectively sequenced for specimens: EG1, EG4, EG5, EG6, and EG7.

The second set of DNA extractions followed the protocol for formalin-preserved specimens, and yielded genetic material from the ethanol-preserved positive controls, *S. californiensis* and *S. leptorhynchus*, but also from two individuals of the formalin-stored *S. insulae* (Fig. 2).

Finally, a total of six 16S gene sequences were obtained from *S. exilis*, *S. californiensis*, *S. leptorhynchus*, as well as two nuclear sequences from *Syngnathus exilis*

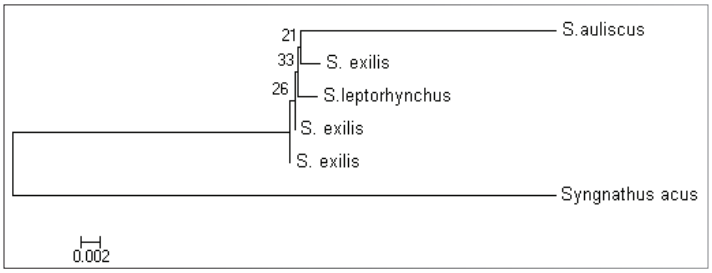

Figure 5. Nuclear sequence neighbor-joining phylogeny for the Californian *S. exilis*, *S. leptorhynchus*, *S. auliscus* and the European *S. acus* (outgroup). Not scaled in time but in species genetic difference. Presented bootstrap values (30, 38, 21) indicate node and branch confidence. The maximum value of 100 represents a well-supported node or branch.

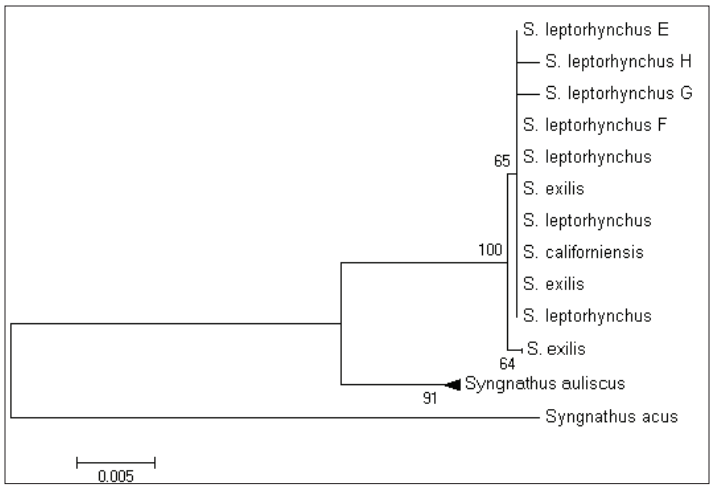

Figure 4. 16S gene mtDNA neighbor-joining phylogeny for the Californian *S. exilis*, *S. californiensis*, *S. leptorhynchus*, *S. auliscus* and the European *S. acus* (outgroup). Not scaled in time but in species genetic difference. Presented bootstrap values (91, 100, 65, 64) indicate node and branch confidence. The maximum value of 100 represents a well-supported node or branch. GenBank accession numbers for *S. leptorhynchus* haplotypes are as follows: E, DQ309797; H, DQ309799; G, DQ309800; and F, DQ309798.

and *californiensis*. These eight sequences were edited and compared with already published *Syngnathus* sequences to create phylogenies for both mitochondrial and nuclear sequences (Figs. 4 and 5).

Discussion

Standard DNA extractions yielded six novel 16S gene mtDNA sequences from specimens in preservations other than formalin. Remarkably, DNA extractions from formalin-fixed samples also contributed two new nuclear sequences. In addition, genetic analysis revealed a previously unknown *S. exilis* haplotype.

As expected, phylogenies identified the European outgroup, *S. acus*, as the most divergent of all species examined in this study. In agreement with Wilson, the illustrated mitochondrial phylogeny (Fig. 4) classified *S. auliscus*, the most widely dispersed of the analyzed pipefish (along with *S. leptorhynchus*), as the most genetically distinct species of Californian pipefish (*1*). Because *S. auliscus* is one of the most dispersed species, and is also the most genetically distinct, it can expected that pipefish such as *S. exilis*, *S. euchrous*, *S. insulae* and *S. californiensis*, which have restricted and overlapping ranges, are therefore more closely related. In addition, since most cases of natural hybridization occur between closely related species (*3*), these pipefish are more likely to be hybrid species.

Interestingly, both phylogenies illustrated *S. exilis* in multiple braches, suggesting that *S. exilis* is a hybrid species. In contrast, the 16S gene phylogeny exhibits *S. leptorhynchus*, *exilis* and *californiensis* in a single branch, implying that all three pipefish are actually one single species with different possible morphs. However,

Species	16S gene mtDNA		Nuclear Sequence	
	Amplified	sequenced	Amplified	sequenced
S. exilis	Y	Y	Y	Y
S. californiensis	Y	Y	Y	
S.leptorhynchus	Y	Y	Y	Y
S. auliscus	Y	Y	Y	Y
S. insulae	Y		Y	
S. carinatus	Y			
S. euchrous	Y			
S. macrobrachium				

Table 3. Final data summarizing whether or not 16S gene mtDNA and analyzed nuclear DNA sequence were amplified and sequenced from each species; Y = Yes.

discrepancies in results may be a product of misidentifying specimens and/or mixing samples while analyzing genetic data. Although genetic data presented in phylogenies can be a powerful indicator of species status, this study did not consider other important factors such as the age of the divergence of the Californian pipefish radiation. Furthermore, only two sequences of approximately 550 bp each were analyzed per sample; hence, a larger data set and additional DNA markers are needed to make a statement about the true identity, the absolute species richness, and the role of hybridization in the eastern Pacific *Syngnathus* species.

An unanticipated and extremely valuable outcome was the success of the DNA extraction from pipefish stored in formalin. This DNA extraction protocol will permit researchers to obtain DNA sequences from samples found at the many aquariums, museums, universities and other institutions that possess a formalin-preserved fish collection. By successfully amplifying DNA from two 46 year-old *S. insulae* individuals, the extractions were proven to work efficiently with specimens collected more than four decades ago. However, DNA yields were lower in quantity and quality when using older specimens, suggesting that higher quality sequences should be obtained from younger specimens, which are extensively abundant in natural collections.

In summary, while the formalin protocol will be greatly valuable to a broad array of investigations, this study mainly serves as a framework for further research in the status of the eastern Pacific *Syngnathus* species. Due to time restrictions, the genetic data obtained was limited, producing results that offered suggestions rather than definite answers. Nevertheless, our presented framework sets the basis for supplementary genetic analysis that can demonstrate definitively whether these pipefish are phenotypically plastic or species abundant. Additional research, therefore, has the potential to lower the species number of what is currently regarded as the highly diverse Californian *Syngnathus* pipefish.

Future studies and limitations

In order to better understand the origin of diversity and phylogenetic relationships between these organisms, further investigation is encouraged in at least two directions: the completion of this project by sampling missing species and including supplementary sequences, or the development of a new study enclosing additional nuclear and/or mtDNA markers. There are strong suggestions that further analysis has the potential to report for the first time hybridization in any *Syngnathidae* species, and unmask the true identity of these pipefish species. Additional analyses will most likely provide significant insights into the origin of the high diversity among these *Syngnathus* species.

Potential sources of error could have been the resolving power of the markers used, and the possibility of specimen misidentification and/or mixing during genetic analysis. Nonetheless, time was the most prevalent limiting factor of this study. This included issues such as gathering pipefish specimens in the allotted time, delays in the arrival of some specimens, the long duration of protocols, and the time consuming final genetic analysis.

Acknowledgements

I would like to express my gratitude to Kai Stoelting for helping supervise my project, to my great and always smiling lab partners for the extra tutoring, and to Dr. Wilson, Dr. Michael and Dr. Denise Hengartner for their support and hospitality at the University of Zurich. Equally sincere is my gratitude to donors of specimens and to all the people who assisted me at my home institution, especially Dr. Jacquie Azize for always being enthusiastic and supportive about my project. Finally, I am especially grateful to those who financially nourished this research endeavor.

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