This research paper was produced from data collected by myself and with the combined effort of the Spring 2015 and 2016 BIOL 301 Lab students. I was born and raised in Lanikai, Hawaii and this past May, I completed my Bachelors in Science in Marine Biology with two minors; Business and French. My highest academic goal is to receive a MBA in International and Public Relations and a Doctorate in Genomics to enhance my qualifications in science management. My passion for the field of science is all thanks to the incredible Biology, Chemistry, Mathematics and Physics professors I was taught under throughout my years of enrollment at UH Manoa; Dr. David Haymer, Dr. Cynthia Hunter, Dr. Spencer Malecha, Dr. Julie Brock, Garrett Lynch PhD candidate, Dr. Steven Robinow, Dr. Floyd Reed, Dr. Peter Marko, Dr. Stuart Donachie, Dr. Philip Williams, Dr. Michael Nassir, and many more. Each professor brought the best out of me, and instilled in me a passion for personal growth and contribution to the furthering of scientific knowledge. While producing this paper, the toughest obstacle I faced was writing an unbiased scientific research paper, and making sure to address all sides of the controversial topic which my paper was based off of.

Comparison of Species Identification Methods

DNA Barcoding versus Morphological Taxonomy

Sophie Friedheim

Biology 301 (Marine Ecology and Evolution)
Mentor: Dr. Anuschka Faucci

In the field of biological sciences, there are several methods of species identification that are commonly used today. The purpose of this paper is to analyze two of the most popular methods, morphological taxonomy and molecular systematics, or DNA species barcoding, and to determine which technique is the most effective. DNA barcoding uses molecular information to classify species, whereas morphological taxonomy uses morphology and phenotypic characteristics to distinguish between one species and another. It is of great concern to traditional morphological taxonomists that DNA species identification is taking the place of morphological taxonomy, and that this genomic method is not adequate enough to properly do so. To help the spread information regarding species identification, and to reduce the discrepancies within the evolutionary biology community, it is essential that there is a globally accepted method of species identification. Throughout this paper, we address to positives and negatives of both methods, and find that where one method lacks, the other makes up for it. In the conclusion, we suggest that both methods should be used in unison to provide the most efficient, effective, and accurate species identification.

Introduction

With every new species discovery, the 'tree of life' increases in complexity. We gain a better understanding of ourselves as a species as well as how every living thing is connected. This paper will look into the efficacy of both methods of species identification. But first, we must do our best to define what a species is before we can discuss ways of distinguishing one species from another. There are several definitions and species concepts that can be found in biology textbooks and research literature (Wiley, 1978). The definition of *species* has been redefined over time in light of new information. However, a species, at its simplest, is defined as a group of similar living organisms capable of successfully interbreeding, exchanging genes, and producing viable offspring (Cattell, 1898). There are exceptions to this definition: asexual organisms and fertile hybrids of two species. Many of the conflicts that arise in defining species stems from differences in assumptions about the evolutionary process (Hillis, 1974). This in turn produces conflicts in distinguishing species. Reproductive isolation of species is a major component of species definitions, and in some situations cannot be determined through morphological analysis, and can only be determined through molecular systematics when the organisms in comparison look morphologically indistinguishable (Duellmand and Venegas, 2005). The question then becomes, which of the two techniques, morphological or molecular, should be the universally accepted method for species identification and comparison? Several of the components that should be taken into consideration when deciding which technique should be used include speed, accessibility of information, accuracy of

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the information, and overall cost (Herbert and Gregory, 2005). There has become somewhat of a divide within the biological scientific community regarding opinions about which of the methods of specifies identification is better. There have been several publications that declare one method as superior over the other (Frelin and Vuilleumier, 1979; Maxson and Wilson, 1974; Wills and Nei, 1976). It is important to have a universally-accepted system to species identification and species comparison, to allow for a better exchange of knowledge and information around the globe.

For the past 250 years, taxonomy has been defined based on morphological structures; the phenotypic characteristics of individual organisms (Herbert and Gregory, 2005). Morphological systematics originated from comparative anatomy (Hillis 1987). Visually, it is possible to determine the differences amongst species based on their macro-morphological features. For example, an elephant has a trunk and a giraffe has an extended neck. They do not share the same physical characteristics, and therefore they must be different species. There are many advantages to using morphological systematics to identify and compare species. Morphological taxonomy is the basis of all phylogenetic relationship hypotheses of extinct organisms based off of fossil records. It is difficult and time-consuming to extract DNA from fossilized organisms. Morphological systematics has a great advantage in its suitability to the large-scale museum collections of preserved specimen (Hillis, 1987). A large percentage of species have gone extant and can only be studied through the preserved collections (Myers, 1991). Today, many species are protected through rare species conservation acts that prohibit DNA sample collections to take place, or the DNA samples are too difficult or costly to collect.

This study of comparative anatomy is the foundation for all species identifications up until today. Over time, these comparative anatomy differences produced a wide range of terminology used to describe the differences in features amongst species (Bijl and Henk, 2015; Harvey, 2015; Howe, 1960; Matalin, 2015; Park, 2008; Winfield *et al.,* 2009; Young, 1989). One of the issues with morphological systematics is that there are few physical characteristics that are common among major groups of organisms, (e.g., eubacteria and eukaryotes) (Hillis, 1987). Taxonomists tend to have a difficult time agreeing on the convention of how to define one morphological feature from another or one species from another. In light of new information, scientists are constantly defining and redefining species (Malyutina, 2014). One of the issues with morphological taxonomy is that over time, it has become heavily opinionated. Scientists sometimes differ in their assumptions and beliefs about the evolutionary process, which leads to debates within the scientific community (Hillis, 1974). It seems that there lacks a conventional agreement on morphological definitions and species classification (Nazari *et al.,* 2007) and this creates a barrier to the flow of knowledge. It becomes hard to pass on these techniques to the upcoming generations without opinions coming into play. This paper seeks to contribute to a better understanding of species identification methods and encourage the proposal of a universally-agreed method.

Although there are many benefits to morphological descriptions, there are also a lot of downfalls. The majority of the species already described, including microbial species, are difficult, or nearly impossible to see with the naked eye (Savolainen *et al.*, 2005). Cryptic species identification issues have been overcome by molecular techniques with the use of allozymes to distinguish one morphologically identical species from another (Buth, 1984). Another major issue with basing species barriers off of morphological features is that many species go through different life stages, for example caterpillars to butterflies (Park, 2008). Also, larvae look very similar to one another and very different compared to what the species looks like at its final stage of life (Park, 2008). Many species also have sexual dimorphism, meaning that the female looks very different than the male (e.g., birds of paradise) (Savolainen *et al.*, 2005). There are many species that use camouflage, or even mimicry, as a defense mechanism (Park, 2008). This also makes it difficult and often confusing when using morphological features to identify one species from another.

Recently, with the growing understanding of genetics, evolutionary scientists have begun using molecular systematics to identify one species from another (Coyne, 1992; Duenas *et al.,* 2014; Herbert and Gregory 2005; Mallet 1995). Molecular

systematics stemmed largely from population genetics (Hillis, 1974). There are many benefits and downfalls to this method as well. Genetically, each species, and each individual, is unique in the fact that no one genome is identical to the next. Some are very similar (e.g., identical twins) yet they still have their differences in amino acid sequences (Herbert and Gregory, 2005). Using DNA removes the opinion-based disputes amongst scientists on how to define one feature from the next (Herbert and Gregory, 2005). Another benefit to molecular systematics is that sequencing the genetic information from an organism requires very little sample to be taken. From this small sample, a large amount of useable data can be attained; morphological (i.e., physical) characters make up only a small fraction of the species genome (Hillis, 1987). It is relatively quick and easy to perform a DNA extraction (Savolainen *et al.* 2005). And for evolutionary studies, DNA encodes all of the heritable information about the organism and helps provide phylogenetic record from the present dating all the way back to the origin of life (Brown, 1983; Hillis, 1987; Pace *et al.,* 1986).

Geneticists and molecular systematic enthusiasts have suggested a method of 'DNA barcoding' to use as a means of identifying and comparing species. The idea is to produce a database of genomic sequences of specific genes that are commonly found in every living organism. If this database is a success, the ideal goal is to have the entire genome of all sampled specimen stored in this database, not just individual genes. Today, there are multiple genomic databases that are open to everyone to contribute to and learn from. The intention of this molecular systematic research is to help validate the existing morphological species identifications and encourage the acceleration of species discoveries through creation of these barcode databases (Herbert and Gregory, 2005). By using the genome, and studying specific genes, it makes it relatively easy to compare one individual to another, and to determine how closely related one species is to the next.

One gene that is suggested to be used is the CO1 gene, cytosine oxidase 1. This gene has been relatively constrained throughout evolution and so far has been found in every living thing. However, there are a lot of issues with this technique as well, and a lot of assumptions made by geneticists that could turn out to be false. For starters, the idea that one gene can be found in all living things is highly debatable. (Moritz and Cicero, 2004; Salvolainen *et al.*, 2005; Waugh, 2007). There has been evidence that DNA barcoding is very limiting to genetic information at phylogenetic levels the further back in the past (Moritz and Cicero, 2004).

For this method to be useful there must be adequate number of DNA samples, and a wide variation of them, to properly compare one individual from another. Another major issue in phylogenetic studies is that many of the key species in the 'tree of life' have already become extinct, and we cannot produce a viable DNA sample from their fossils to add to the database. The majority of molecular systematic methods require a fresh DNA sample to produce an applicable yield (Dessauer and Hafner, 1984).

Mitochondrial DNA is some of the most rapidly evolving DNA, and it has been useful in phylogenetic population studies (Avise *et al.,* 1979a; Lansman *et al.,* 1981). This rapidly evolving DNA indicates the potential for the creation of a new species; the more mutations occur, the quicker a new species will arise. However, the downfalls to mtDNA is that it is only passed on from one parent to the offspring, and therefore tends to be male biased (Moritz and Cicero, 2004). This creates conflicts when studying the heredity of the species. Another issue that arises with DNA barcoding is retro-viruses and certain parasites that alter to DNA or inject their own through horizontal gene transfer, and therefore potentially provide false data results (Herbert *et al.,* 2003). An assumption that geneticists are making with this DNA barcoding is that all species have fixed characteristics, which goes against everything evolution stands for (Moritz and Cicero, 2004). In the study done by Johnson and Cicero (2004), they find that within a population of avian species, species divergence happens relatively quickly, which would make it difficult to use DNA barcoding to identify or compare species. With new technology decreasing the cost and increasing the efficiency of species comparison and identification, we are getting closer to our goal of globally accessible online "Encyclopedia of Life" (Herbert and Gregory, 2005; Savolainen *et al*).

Traditional morphological taxonomy has been studied for over a century, and is a very well developed method of species identification. Even with a century of continual improvement, there still seems to be some discrepancies within the method, and disagreements about its efficacy throughout the community of those who study it. Genomic techniques to species identification is relatively new, yet quickly progressing. With the rapid developments in technology, genomic studies are evolving exponentially. However, since DNA barcoding is a recently created project, there are many gaps and disparities within the technique. One of the major concerns within the morphological community is that DNA species identification will take the place of traditional taxonomy in means of funding and research efforts (Wheeler, 2004). Traditional taxonomists argue that the genomic species identification method is not sufficient enough to replace morphological phenotypic characterization (Will and Rubinoff, 2004). On the other hand, molecular scientists tend to suggest that morphological taxonomy is dated and time-consuming. Their opinions infer that species identification needs to keep up with the changing times and advances in technology. There have been many research papers over the past two decades of scientists arguing for and against both methods of species identification (Hilis, 1987; Herbert *et al.,* 2003; Herbert and Gregory, 2005; Mortiz and Cicero, 2004; Will and Rubinoff, 2004). It has become clear that some sort of resolution is needed.

The purpose of this report is to use two common species identification methods, morphological analysis and genomic sequencing, to identify fish larvae. Both methods of species identification provide a means to comparing one species from another, however neither one is perfect. In this report, we perform and analyze both methods to put them to the test. Through both experimental analysis and literature review, our goal is to make a conclusion about which method is most effective. Here we suggest that for species identification and comparison to be thorough enough, the combination of both genetic barcoding and morphological taxonomic analysis should be used together to define one species from another.

Methods and Materials

Species Collection:
The fish larvae that were sequenced in this report were collected during a plankton tow that took place on January 27, 2016 on board the *M/V Searcher*. The plankton tow net was 500μM mesh and a diameter of 1m. The collection locations, by Global Positioning System (GPS) coordinates, can be found in Figure 1. The plankton tows collected zooplankton, including the fish larvae, at depths of 1m, 25m and 50m. The fish larvae were collected by students in the 2016 Spring Biology 301 Lab then preserved in 90% ethanol. In this report results, the fish larvae that were sequenced came from the 2015 Spring Biology 301 Lab collection.

Figure 1. GPS coordinates of the 8 plots at which the collections took place on the Plankton cruise, January 27th 2016 (Google, 2016).

Morphological Identification:
Each of the four fish larvae that were preserved in the ethanol were placed under a compound microscope and sketched. Attention to detail was placed on the size and position of the eye and fin, length of the gut, pigments and spots, as well as stage of development. Morphological identification was performed by fish biologist Bruce Mundy. When using the method of morphological species identification, there are several different characteristics used to compare one species to another including body size ratio, eye size and shape, gut morphology, pigmentation, position of fins

throughout life stages and muscle segments. One of the most easily identifiable characteristic is the stage of development (i.e., pre- and post-flexion stages). The morphological characteristic used to identify the fish larvae in Figure 3b and 3c (fish larvae ID C_{46} and C_{47}) was their pre-flexion stage.

DNA Extraction:
The same extraction process was performed on all four of the fish larvae. The DNA extraction process followed the instructions of the commercial Qiagen DNeasy DNA extraction kit. Each of the four fish larvae were placed in four 1.5 mL microtubes with 180µL QIagen ATL Buffer and 20µL of proteinase K. The fish larvae tissue was broken up by vortex, and placed in a 56℃ water bath for approximately two hours. The rest of the DNA extraction process with the spin column was carried out according to the Qiagen DNeasy DNA extraction kit.

The polymerase chain reaction was used to amplify the desired gene of sequencing, cytochrome c oxidase subunit 1or CO1 gene. For this, the required forward and reverse primers were CO1 Fish2. Each of the four fish larvae DNA extractions were combined with the commercial PCR mix, which contains distilled H_2O, PCR Buffer, $MgCl_2$, Forward and Reverse CO1 Fish2 Primers, and Taq polymerase. To save time, two partners produced enough master mix for all eight of the fish larvae that were being sequenced. With the eight fish larvae plus two control PCR reactions and one extra for pipetting error, enough PCR master mix for eleven reactions was produced.

PCR Protocol:
1. 95℃ for 10 minutes
2. *Denaturation* - 95℃ for 30 seconds
3. *Annealing* - 50℃ for 30 seconds
4. *Extension* - 72℃ for 60 seconds
5. *Repeat* - 35 cycles
6. 72℃ for 10 minutes
7. Cool at 4℃ until removed

A 1.5% agarose gel was prepared and set to view the results of the PCR reactions. 6µL of each of the PCR reactions, and 1µL of the dye was combined, loaded in the gel and ran for approximately 30 minutes. After the gel was finished running, it was visualized under the photo system.

The polymerase chain reactions that were successful, and visualized on the agarose gel, were prepared for sequencing. In a new tube, we put 9µL of each of the successful PCR products and 1.125µL of the enzyme. The enzyme is a combination of Exonuclease I from E. coli and FastAP, a thermosensitive Alkaline Phosphatase. Protocols for incubation and denaturation were 37℃ for 60min and 85℃ for 15 min, respectively.

Table 1. Calculations for the PCR master mix of 11 reactions.

Ingredient	Concentration	Volume (in µL)	Number of Reactions +1	Total Volume of MM
d H_2O	-	9.5µL	x 11	104.5µL
MyTaq Red Mix	2x	12.5µL	x 11	137.5µL
Forward CO1 Fish2 Primer	10µM	1µL	x 11	11µL
Reverse CO1 Fish2 Primer	10µM	1µL	x 11	11µL
DNA Template	-	1µL	8 individual	-

5 μL of the product from the previous step, for each of the successful fish larvae PCR reactions, were sent to the ASGPB (Advanced Studies in Genomics, Proteomics and Bioinformatics) facility on the University of Hawai'i, Manoa campus. Through the use of the sequencing machine, each of the nucleotides in the co1 gene sequence were identified through a process with a laser detector that reads the color of the fluorescent tag. The blue, red, yellow tags correspond to the base pairs T, A, G, respectively. Once the PCR products were sequenced, they were returned as chromatogram files, also called trace files.

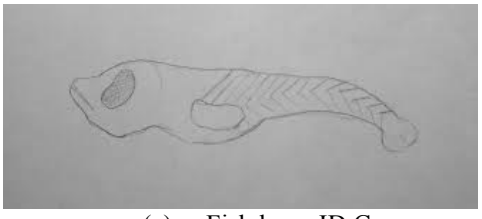
The sequence editing program Sequence Scanner v1.0 was used to view the trace files and clean up the sequences to be analyzed through online databases. Based on the criteria, if there was no more than 20 'N's or ambiguous base calls, the sequence was of good enough quality to be analyzed. We removed the first and last part of the sequence that had too much noise. Then we ran those sequences through two different online databases. The first was a Basic Local Alignment Search Tool (BLAST) search on the database GenBank, administered by the National Center for Biotechnology Information, NCBI. The second database was the Barcode of Life Data systems, BOLD.

Results

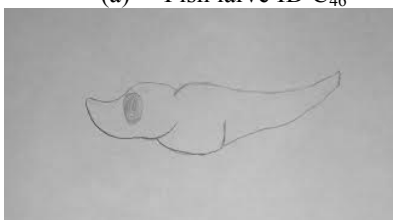
The two sketches of larvae fish can be found in Figure 2. For the four fish larvae, the morphological species identification confidence level was as far down the taxonomic order as genus and species. The results from the morphological analysis performed by Bruce Mundy can be found in Table 2.
The first fish larvae found in Figure 3a - fish larvae ID C_{45}, is *Lestidiops indopacifica* of the genus *Lestitium sp.* of the Barracudinas, in the family Paralepididae. Figure 3b, corresponding to second sketch in Figure 2a - fish larvae ID C_{46}, as a species of lanternfish, the genus *Diapus sp.* of the family Myctophidae. For the third fish larvae, Figure 3c - fish larvae ID C_{47}, it was identified as being a species in the family Gobiidae. The fourth fish larvae, Figure 3d and the sketch of Figure 2b - ID C_{48}, was unknown.

Table 2. Results of the morphological characteristic analysis for species identification.

Fish Larvae ID Number	Corre-spond-ing Picture	Family	Genus	Species
C_{45}	Figure 3 a	Parale-pididae	*Lestidiops*	*indopaci fica*
C_{46}	Figure 3 b	Mycto-phidae	*Diapus*	-
C_{47}	Figure 3 c	Gobii-dae	-	-
C_{48}	Figure 3 d	Un-known	-	-

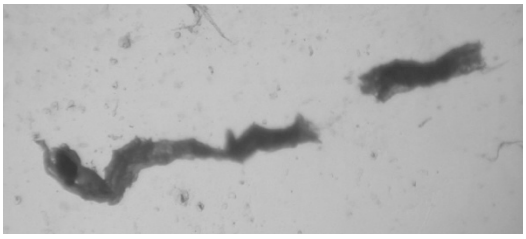


(a) - Fish larve ID C_{46}

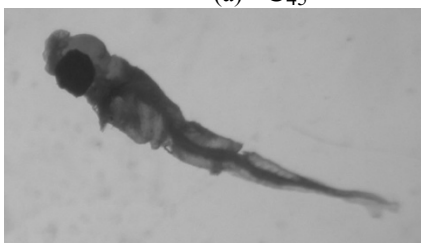


(b) - Fish larvae ID C_{48}

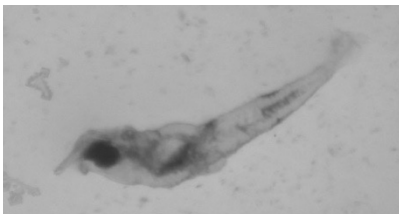
Figure 2. Sketches of fish larvae.



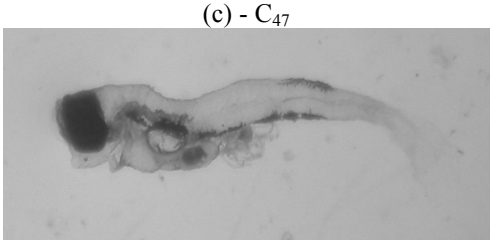
(a) - C_{45}



(b) - C_{46}



(c) - C_{47}



(d) - C_{48}

Figure 3. Photographs of fish larvae ID

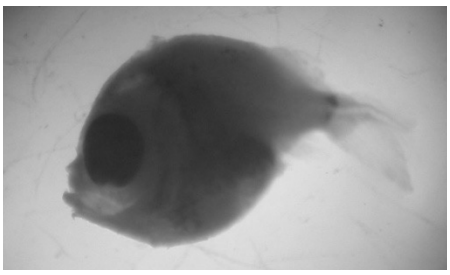
As seen in Figure 4, only two to three of the PCR reactions were successful. Lane 6 and lane 7 have the strongest amplification, corresponding to fish larvae ID numbers C_{49}, and C_{50}. In lane 8, there is a faint result corresponding to fish larvae ID number C_{51}. From these results, we took the PCR amplifications that were successful, and prepared them to be sequenced.

Figure 4. Image of the PCR results ran through the 1.5% agarose gel.

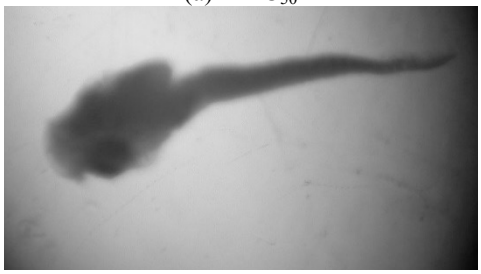
Table 3. Table indicating which lane of the 1.5% agarose gel each of the eight PCR reactions of fish larvae ID's C_{45}, C_{46}, C_{47}, C_{48}, C_{49}, C_{50}, C_{51}, C_{52}. In correspondence to Figure 4.

Lane #	Sample ID
1	Ladder
2	C_{45}
3	C_{46}
4	C_{47}
5	C_{48}
6	C_{49}
7	C_{50}
8	C_{51}
9	C_{52}
10	Positive Attempted
11	Negative

Unfortunately, none of the four fish larvae which our team originally worked on were successful, so for good measure, the two pictures of the fish larvae that were successfully sequenced and analyzed in the DNA barcoding databases were C_{50} and C_{52}, can be found in Figure 5. According to Bruce Mundy's morphological identification, C_{50} is *Ranania laevis*, of the family Molidae. The second larvae C_{52} was unknown.



(a) - – C_{50}



(b) - – C_{52}

Figure 5. Photographs of fish larvae ID

Of the four fish larvae PCR amplification results that were successful, only two of them had sequences clean enough to be run through the two online DNA barcoding databases, Genbank and BOLD. The first sequences corresponded to fish larvae ID C_{50} and was 629bp base pairs long, and the second sequence corresponds to fish larvae ID C_{52} and is 621bp in length. Both of the sequences were identifiable on both of the online databases down to the taxonomic level of species. However, the second sequence had discrepancies and a lower confidence level.

When run through the DNA barcode database Genbank, the first fish larvae sequence ID C_{50} was identified with 99% confidence that it is *Ranzania laevis*, of the family Molidae, found in the Pacific. A similar result came from running fish larvae sequence ID C_{50} through BOLD. The results were 99.84% confidence that the sequence matched the CO1 fish gene of *Ranzania laevis,* and found in Hawai‘i. The results are summarized below in Table 4.

For the second sequence, fish larvae ID C_{52}, the results from the Genbank database came up with a 99% match confidence to the species *Abudufduf vaigiensis,* of the family Pomacentridae, found in the Indo-Pacific. However, when the same sequence was run through the BOLD database, a different species of the same genus was the matching result. For BOLD, the sequence matched the species *Abudufduf abdominalis,* also of the same family, Pomacentridae. This species is found in Hawai‘i.

When looking at the overall data that the 2016 Spring Biology 301 Lab class produced, there are a few trends that are visible. There were approximately 99 sequences that were run through BLAST and BOLD and produced a result at least on the taxonomic level of family. For those that were identified to the family and genus level, the result was the same from both BLAST and BOLD searches. However, on the species level, 13 of the 99 were different when comparing the results from the BOLD and the BLAST searches. Another trend that was visible throughout the class sequence database results was the both databases produced different results as far as location of the species collection. 30 of the 99 results had different locations.

Table 4. Results from fish larvae sequence ID C_{50}, that was successfully run through the online DNA barcode databases Genbank and BOLD.

Database	**ID #**	**Base Pair**	**Match ID**	**Confidence**	**Family**	**Genus**	**Species**	**Location**
Genbank	C_{50}	629	AP006047.1	99%	Molidae	*Ranzania*	*laevis*	Pacific
BOLD	C_{50}	629		99.84%	Molidae	*Ranzania*	*laevis*	Hawai‘i

Table 5. Results from fish larvae sequence ID C_{52}, that was successfully run through the online DNA barcode databases Genbank and BOLD.

Database	**ID #**	**bp**	**Match ID**	**Confidence**	**Family**	**Genus**	**Species**	**Location**
Genbank	C_{52}	621	AP006016.1	99%	Pomacentridae	*Abudufduf*	*vaigiensis*	Indo-Pacific
BOLD	C_{52}	621		94%	Pomacentridae	*Abudufduf*	*abdominalis*	Hawai‘i

Discussion

The purpose of this paper is to analyze two different species identification methods to determine which is relatively better, and should therefore be universally accepted. The two different methods of species identification of interest are morphological characteristics analysis and DNA barcoding. Hillis (1987) makes the argument that it is difficult to compare two techniques that originated for different purposes; morphological systematics for comparative anatomy and molecular systematics for population genetics. This paper suggests that both methods are inadequate on their own in defining, identifying and comparing one species from another. Where one method succeeds, the other has a downfall. To make this process as accurate as possible, both should be required and used in combination to identify and compare species. Due to the complimentary nature of these two methods, the combination of the two techniques has proven to be successful on several occasions (Best *et al.,* 1986; Duellman and Venegas, 2005; Gould *et al.,* 1974; Mickevich and Johnson, 1976; Miyamoto, 1981; Nixon and Taylor, 1977; Shaklee and Tamaru, 1981).

Theoretically, if both methods were truly successful in identifying a species (i.e., determining phylogenetic relations to other species) then the results from both techniques on the same specimen to match. To explain this better, we must address the evolutionary assumption that each species has a single-life history. Therefore, the identification of a species based on its physical characteristics should be congruent with the results produced from the genomic sequence. Discrepancies between the results of the two tests is a good indicator of issues with the techniques and a need for more information to resolve the phylogenetic relationships (Hillis, 1987).

These days, with advancing technology, species identification has begun to shift towards genetic identification. DNA barcoding is the coined term for this species identification and comparison method. Through the use of sequencing specific genes and sharing them universally online, it has become so much easier for the scientific community to share information and pass on knowledge. There are several benefits and downfalls to DNA barcoding. For starters, although this database requires a lot of time, money and effort to be created, it is relatively quick and easy (Savolainen *et al.,* 2005). Also, it is inexpensive to produce a gene sequence and add it to this database, or compare it to sequences already available in the system (Savolainen *et al.,* 2005). Anyone, from student to scientist, can easily perform a PCR (polymerase chain reaction), and produce a sequence to add to the database.

This universal online database is also available to anyone with internet. Although there are many countries that currently do not have access to the internet, and therefore this information, it is only a matter of time before they too have access to this database of knowledge (Doug, 2016). This information can be used for societal benefits such as conservation and management practices. One of the benefit as well as downfalls to having this database available to everyone is that anyone can add any sequence (Herbert and Gregory, 2005). But also, anyone can make mistakes when adding information and claim it is a sequence belonging to something other than what it actually is. This would decrease the accuracy of the database, and therefore provide false results to scientists who use the system. To resolve this potential issue, we suggest that a monitoring system should be put in place by a group of scientists, similar to the way Wikipedia has editors.

To increase the accuracy of the database, a large number of DNA gene sequences need to be added to it. With lowering costs due to technological advancements, it should not be difficult to accumulate an expansive database of genomic sequences (Herbert and Gregory, 2005). Another improvement that could be made to the databases is that each submission of a sequence should require several photographs of the individual organism and descriptions of major morphological characteristics mentioned in the methods. Also, it should be required for sequence submission that the contributor mention how many and which ambiguous base calls were modified based on personal interpretation of the peaks.

It is much easier and less time-consuming to compare species sequences through online databases rather than defining and describing morphological characteristics. However, a major issue with these online databases is that the more databases, the more disagreements arise. With one database, we have nothing to compare our results to. As seen in our

results, Table 4 and Table 5, there were different results that were produced from running the same sequence through two different sequence databases. Also, when looking throughout the entire class data, it was clear that 13 of the 99 species level identifications did not match between both BLAST and BOLD. For location of species collection, there were 30 of the 99 results from BOLD and BLAST that did not have the same location. Recently with the increase of technology, there has been a large surge in the numbers of new species being discovered, and old species being reclassified (Herbert and Gregory, 2005). With new discoveries and new information, it is becoming more difficult to define what a species is exactly without there being many exceptions (Mallet, 1995). Due to this rapid discovery of species and new information, there is also a large number of new species being genetically sequenced, but not enough taxonomic experts to put the time in to naming these new or reclassified species. And so these new individuals go into the database as identification numbers (Landry *et al.,* 2003).

Another benefit of DNA barcoding is that you do not need the entire species to identify it, only a small amount of DNA (Savolainen *et al.,* 2005). Many of the species that exist on this planet are too small to see with the naked eye, and therefore too difficult to describe morphologically (Herbert and Gregory, 2005; Savolainen *et al.,* 2005). Also, by only requiring a small amount of DNA, the species can be sequenced and identified without removing the entire species from its natural habitat. This is important when species are rare, or endangered (Myers, 1991). This allows scientist to get around species management regulations that do not allow the species to be removed from their natural habitat. By using the DNA sequence, it removes the controversial debates amongst taxonomists who disagree on morphological definitions and descriptions (Herbert and Gregory, 2005; Narazi *et al.,* 2007). DNA barcoding boils down to 4 different nucleotide base pair combinations, A, T, G, C, and therefore leaves less room for personal interpretation. With DNA sequencing, it allows scientists to determine the relationships between dimorphic species and those that have different life stages. It also helps to distinguish between different species that look similar, such as with those that use camouflage or mimicry (Park, 2008).

Although there are benefits to using genomics for species identification, it is clear that there are some discrepancies with the molecular technique. Morphological systematics has been used for over 250 years and is the foundation of all current species hypothesis. Morphological taxonomists have stressed concern that molecular systematics is inadequate to replace the morphological identification process. One of the major conflicts that morphological taxonomists have with this new molecular technique is that it will compete for funding and research effort (Wheeler, 2004). Herbert and Gregory (2005) addressed this concern by stating that DNA barcoding will not take the place of morphological systematics because future DNA barcoding sequences depend on the foundation of species definitions produced from morphological analyses; one cannot exist without the other as a reference. The authors also suggested that DNA barcoding will encourage private funding by allowing access of this information to the public. Also, the societal benefits of this DNA barcoding database (e.g., conversation of endangered species, identification of invasive species, etc.) will soon become clear and attract new enthusiasts and non-traditional research funding (Herbert and Gregory, 2005).

Due to the lack of successful DNA extraction and PCR results, the morphological identifications found in Table 2 cannot be compared to the online database results. For fish larvae ID C_{50} found in Figure 5b, we were successfully able to identify the larvae morphologically and genetically. The morphological identification results found in Table 4 match the genomic sequence results from both BOLD and BLAST database searches. These results suggest that both methods are adequate and effective enough in identifying an organism down to its species taxonomic level.

Throughout this analysis of species identification methods, there were several opportunities for sources of error. For starters, with the methods and materials. With the morphological identification, one of the fish larvae was unidentifiable by morphological characteristics. This is an example of how morphological species identification can be inaccurate. When the specimen

has degraded to the point where they become unidentifiable, the method itself become ineffective, and another method of species identification must be used. Because we are working with such a small amount of DNA, there is a higher chance of contamination. If the extraction process is contaminated, then the DNA product will not be good enough to continue on through the PCR reaction. This happened specifically for my four fish larvae. Unfortunately, there was not enough product or some sort of contamination that occurred. This hindered me from working further on the DNA product. There can also be contamination from the DNA to the PCR product stage. This would cause the PCR product to have blank base pairs when the final product is sequenced. When this happens, the sequence is not accurate enough to get a good species identification, and is not high enough quality to be used as a sequence input for the databases. This is an example of another conflict that is commonly faced when using DNA for species identification. Another source of error that was possible after the final PCR products was involved with the accuracy of species identification match from the databases that we used. In several cases with our classes data, there were mismatched identification of the species level between both databases. Also, both databases provide a geographic region of the habitat where the species is commonly found, and in some instances, the databases did not match. This addresses another issue with DNA species identification and emphasizes the need for stronger rubric required for species sequence inputs. However, with more sequences put into the database, the more accurate the databases should become with species matching.

If this database is to be successful, future research is required to advance it and ensure its accuracy. There has been evidence that this database is successful for some groups of organisms in the Eukarya kingdom. However, if this database is going to be the online "Encyclopedia of Life," the next step is creating a protocol for all other kingdoms (Herbert and Gregory, 2005). This database can then be effectively used to validate all current morphological hypotheses that have been produced over the past 250 years of morphological taxonomic studies.

With the constant changing and advancing of scientific information, the best technique for species identification should be the least assumption-based and revisable. Through a literature review of the benefits and disadvantages of both methods, it becomes clear that where one method lacks, the other makes up for it. Through the experimental investigation of both methods of species identification, molecular and morphological systematics, we conclude that both techniques should be used to compliment the other and produce the most accurate means of species identification and comparison. We suggest that for scientific purposes, both methods of species identification should be universally accepted and used in unison.

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