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RESEARCH ARTICLE

The Genetic and Morphological Evolution of Fish through the Protein Cytochrome C Oxidase Subunit One

Sammer Marzouk,

University of Chicago Laboratory Schools, 1362 E 59th St, Chicago, IL 60637,
Email: marzouk.sammer@gmail.com

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ABSTRACT

Background: Cytochrome C Oxidase Subunit 1 (COX1) is a protein that helps to catalyze the reduction of water into oxygen in Eukaryotes. Through the analyzation of COX1 from online public genetic databases in 16 species of fish, an evolutionary phylogeny of fish was derived from the data. This paper considered three hypotheses: Axolotl (*Ambystoma mexicanum)* and Tiktaalik (*Tiktaalik roseae)* share a common ancestor was determined; that *A.gueldenstaedtii* and *P.spathula* both share a common ancestor; and that *P.marinus* and *S.acanthias* are the outliers of the phylogeny. The evolutionary phylogeny used the percent ID between the two species of fish. From these differences, analysis is done to the data and the data is used to make phylogenies based on the morphological and genetic evolution of these fish.

Results: From the data derived from the phylogenies, the results demonstrates the claims that Axolotl (*Ambystoma mexicanum)* and Tiktaalik (*Tiktaalik roseae)* share a common ancestor was determined, *A.gueldenstaedtii* and *P.spathula* both share a common ancestor, and *P.marinus* and *S.acanthias* are the outliers of the phylogeny.

Conclusion: The data gathered can be used to connect tetrapods to fish, and contribute to the theory that tetrapods came from fish. The information presented in this paper can be used to make a complete phylogeny of all organisms in the biosphere.

Keywords: Cladogram, Evolutionary Biology, Ichthyology, Phylogeny, and Evolution.

INTRODUCTION

Of the 30,000 species of fish, the phylogenetic trees of fish have not been heavily studied on built upon for the reason that their has not been a reliable connection between fish and tetrapods (Fiz-Palacios *et al*., 2013). Charles Darwin first proposed that animals could be categorized by the morphological traits they possessed, and supported this by categorizing finches based on their beaks. This type of phylogeny made by data from morphological traits is the morphological phylogeny. As DNA sequencing technology because in greater use, a new type of phylogeny from which protein-sequences were used was also used to categorize animals. These two types of phylogenies would show a hypothesis of the evolution of certain beings. But sometimes there would be differences in these two types of phylogenies. This demonstrates the need of how can a cladogram be inferred using genetic data be reconciled with a cladogram using only morphological data? From a sample size of 16 species of fish, a morphological phylogeny was previously derived (Fig.1). The phylogeny has 21 traits and shows which traits evolved in which species (Fig.1). From the morphological phylogeny, it is shown that gills, spiracles, and a cartilage skeleton are the basic traits that make this clade of species (Fig.1). Overtime, more diverse and complex traits divided up the fish into separate taxa (Fig.1). In the morphological phylogeny Tiktaalik (*Tiktaalik roseae),* and Axolotl (*Ambystoma mexicanum)* were both in the same clade, which lead to the hypothesis that if the morphological and protein phylogenies were compared, then it would determine that they shared a common ancestor (Fig.1). Also, in the morphological phylogeny, it is shown that *A.gueldenstaedtii* and *P.spathula* both share a common ancestor, and *P.marinus* and *S.acanthias* are the outliers of the phylogeny (Fig. 1). In order to get the most accurate results, this morphological data was combined with the protein-sequence of Cytochrome C Oxidase Subunit 1 (COX1) (Table 2). Cytochrome C Oxidase Subunit 1 (COX1) is one out of the three mitochondrial DNA subunits, the others being MT-CO2, MT-CO3 that are part of respiratory complex IV (Tsukihara *et al*., 1996). In respiratory complex IV, this enzyme completes the electron transport system, and its function of catalyzing the reduction of water into oxygen (Tsukihara *et al*., 1996). Most Eukaryotes, and all vertebrates have this protein (Tsukihara *et al*., 1996). The COX1 from the Spiny Dogfish (*Squalus acanthias),* Sea Lamprey (*Petromyzon marinus),* Saddled Bichir (*Polypterus endlicheri),* Paddlefish (*Polyodon spathula),* Ghost Shark (*Callorhynchus milii),* Coelacanth (*Latimeria chalumnae),* Bowfin (*Amia calva),* Pollen`s Cichlid (*Paratilapia pollen),* Tiktaalik (*Tiktaalik roseae),* Ropefish (*Erpetoichthys calabaricus),* Great White Shark (*Carcharodon carcharias),* Axolotl (*Ambystoma mexicanum),* Cuvier's Bichir (*Polypterus senegalus),* Russian Sturgeon (*Acipenser gueldenstaedtii),* Australian Lungfish (*Neoceratodus forsteri),* and Goldfish (*Carassius auratus)* were sequenced and compared in order to find the percent ID (Table 1). This percent ID was used in order to make a phylogeny. The protein-sequenced phylogeny was compared with the morphological phylogeny to make a final phylogeny. This paper describes the morphological and protein similarity between the 16 species of fish, and describes the use of building a more extensive phylogenetic tree.

Table 1: Sample group of 16 species of fishes

Common Name:	***Genus Species:***
Spiny Dogfish	*Squalus acanthias*
Sea Lamprey	*Petromyzon marinus*
Saddled Bichir	*Polypterus endlicheri*
Paddlefish	*Polyodon spathula*
Ghost Shark	*Callorhynchus milii*
Coelacanth	*Latimeria chalumnae*
Bowfin	*Amia calva*
Pollen`s Cichlid	*Paratilapia pollen*
Tiktaalik	*Tiktaalik roseae*
Ropefish	*Erpetoichthys calabaricus*
Great White Shark	*Carcharodon carcharias*
Axolotl	*Ambystoma mexicanum*
Cuvier's Bichir	*Polypterus senegalus*
Russian Sturgeon	*Acipenser gueldenstaedtii*
Australian Lungfish	*Neoceratodus forsteri*
Goldfish	*Carassius auratus*

MATERIALS AND METHODS

Morphological Phylogeny

The morphological tree phylogeny was constructed based off of 21 synapomorphies of a sample group of 16 species of fish (Fig. 1). A species would be compared with the other species of fish. If the species had or did not have a specific trait, they would be placed in a different clade than species that did not did not have another trait (Fig. 1). The continuation of this pattern would give a complete morphological phylogeny (Fig. 1). The morphological phylogeny was based off of a data matrix which was derived from the characteristics that the species of fish might or might not have (Table 3). For every characteristic, if it was present in that species of fish it had a “Y” otherwise it had a “N” in it (Table 3).

Protein Sequences and Construction of Phylogeny

The protein sequence of the fish was derived from an online public genomic database (Table 4). The data was then compared and analyzed by a series of specialized computer algorithms. These computer algorithms gave the specific percent identity (specific as in rounded to the nearest tenth) and the specific percent similar. These percentages were then used, and compiled on a genetic chart. From the genetic chart, the phylogenetic tree was derived (Table 4). In order to make the phylogeny, the data on the first species, *S.acanthias* was compared to the rest of the fish (Fig. 2). The one with the largest percent identity was put into a clade with *S.acanthias* (Fig. 2). After this, the next species that was closest to *S.acanthias* was put in the same clade the two previous fish.

Combination of Morphological and Protein Phylogeny

In order to combine the types of phylogenies, further examination on the similarities and differences of the phylogenies would be conducted (Fig. 3). Similarities would be kept and considered a constant of both phylogenies (Fig. 3). Differences in species placement were decided by comparing the two phylogenies and deriving a spot for the species based on a specialized computer algorithm. Any contradictions would be resolved by analysing the percent similar and determining which species had the greater percent similar (Fig. 3). This species would then be placed accordingly (Fig. 3).

2.4 Data Analysis

All sequenced protein data has been verified by the proper scientific body. Data analysis was by a specialized computer algorithm developed by William Pearson and run on the LALIGN server. All data was tested for specific outliers and the percent similar induced function of amino acids. percent identity included exact amino acid comparisons. Identical and similar percentages were rounded to the nearest tenth.

RESULTS

From the data, three specific conclusions were drawn. The first conclusion drawn from the data was that Axolotl (*Ambystoma mexicanum)* and Tiktaalik (*Tiktaalik roseae)* share a common ancestor (Fig.1, Fig.2, Fig.3).

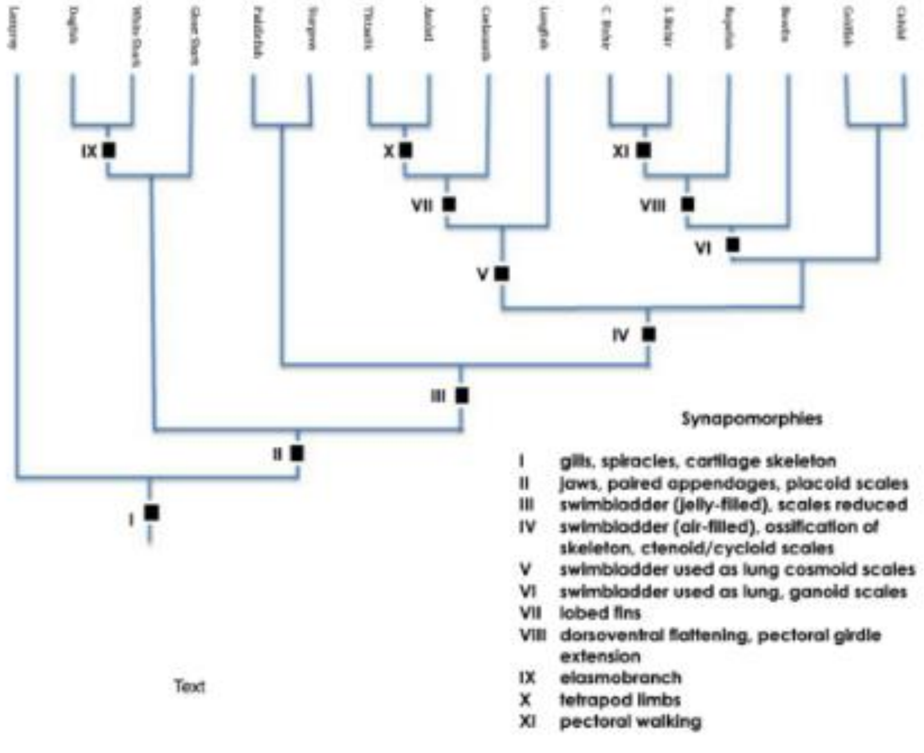

Fig. 1 Morphological Phylogeny. The morphological phylogeny is showing the 16-different species of fish, and their synapomorphic distinctions. From the table, it is shown the morphologically, the Sea Lamprey is most distinct to pollens Cichlid. Not all possible traits are shown by the phylogeny.

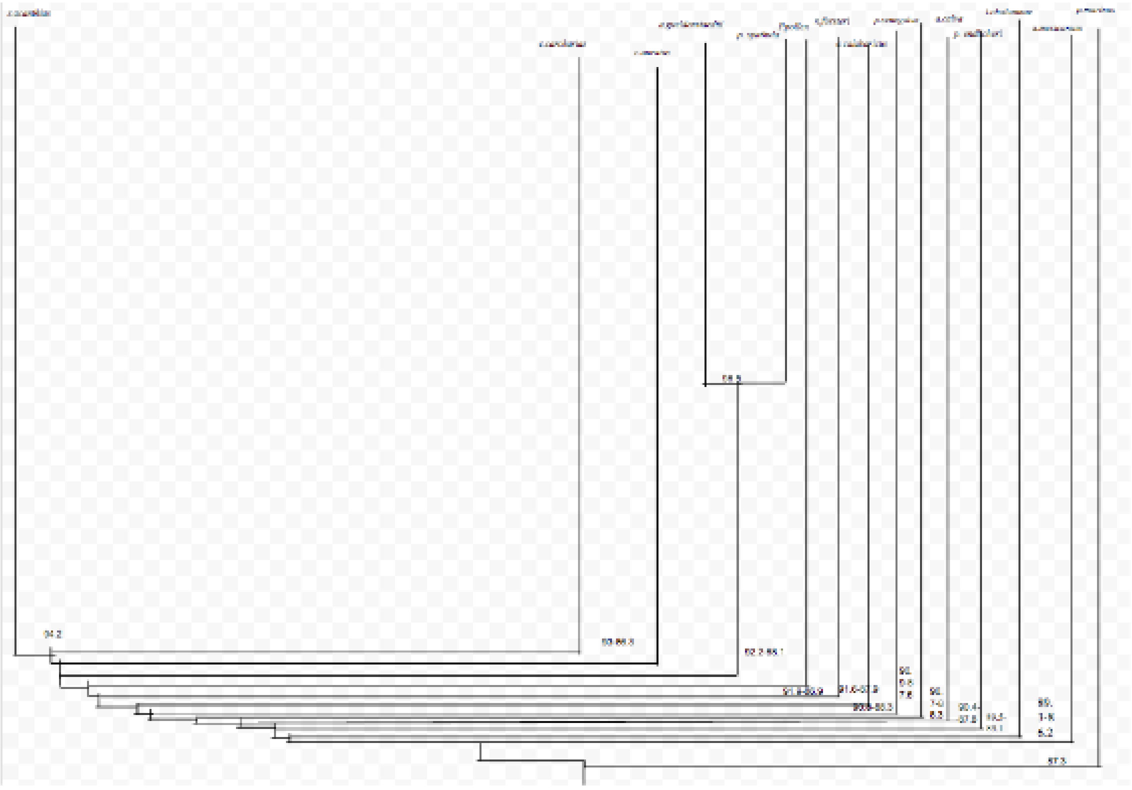

Fig. 2 Protein-Derived Phylogeny. This is the phylogeny that was derived using the protein comparisons of COX 1 from the 16 species of fish. The data demonstrates that the first species, the Sea Lamprey is an outlier from the rest of the fish.

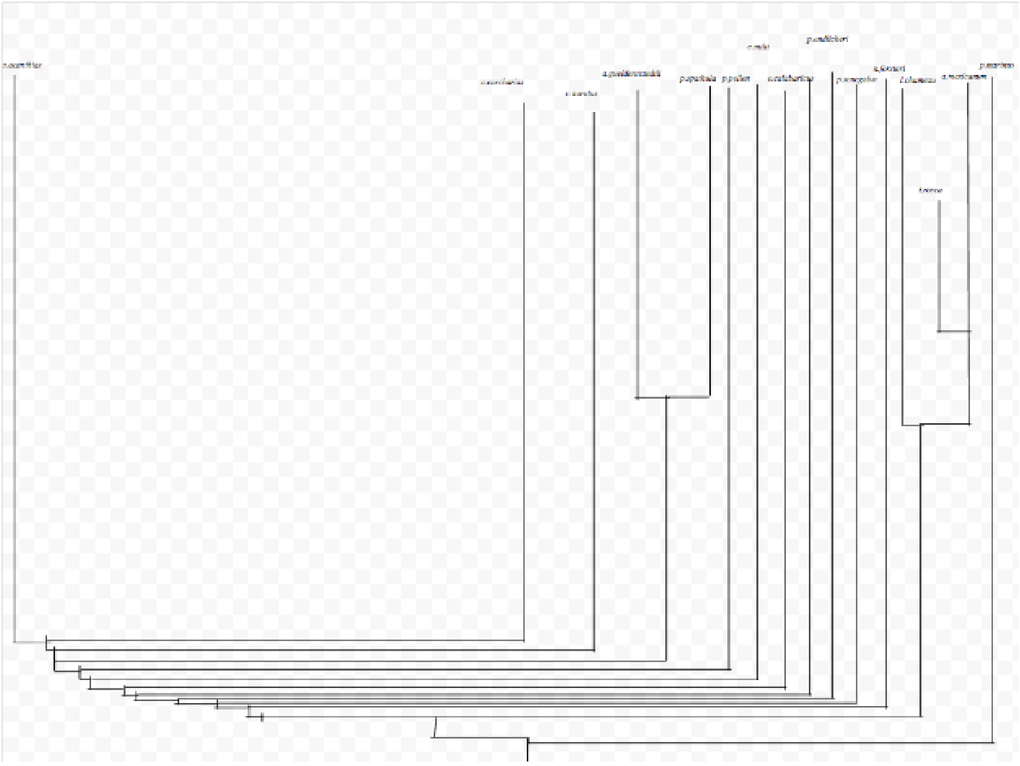

Fig. 3 Final Phylogeny. This is the final phylogeny that was made by combining the morphological phylogeny and the protein derived phylogeny. The final phylogeny demonstrates the impformation that Tiktaalik is closely related to pollens Cichlid.

Table 2 : Morphological data was combined with the protein-sequence of Cytochrome C Oxidase Subunit 1 (COX1) from the 16 species of fish

	Sea Dogfish	Sea Lamprey	Saddled Bichir	Paddlefish	Ghost Shark	Coelacanth	Bowfin	Pollen`s Cichlid	Tiktaalik	Ropefish	Great White Shark	Axolotl	Cuvier's Bichir	Russian Sturgeon	Australian Lungfish	Goldfish
Sea Dogfish	100%	87.3/97.9	90.4/98	92.4/98.4	90.9/97.7	89.5/97.7	90.7/98.3	92.2/98.6		91.6/98.4	94.2/98.5	89.3/97.9	90.8/98.6	92.4/98.6	91.9/98.6	93/98.6
Sea Lamprey	87.3/97.9	100%	87.9/96.5	88.9/97.5	87.6/96.3	89.1/97.3	88.3/97.3	88.1/97.1		87.9/96.9	86.3/97.	86.2/96.5	88.3/96.9	89.1/97.9	86.9/97.7	88.9/97.5
Saddled Bichir	90.4/98	87.9/96.5	100%	92.4/98.6	90.6/98	91.5/98.3	91.7/98.6	92.6/98.3		98.5/99.6	90.4/98.2	90.3/97.7	98.6/99.4	92.6/99.2	91.6/99	92.2/98.8
Paddlefish	92.4/98.4	88.9/97.5	92.4/98.6	100%	90.5/98.6	93.8/99	95.1/99.4	95.3/99.6		93.4/99	91.2/98.1	89.1/97.5	90.8/98.2	98.5/99.8	93.2/99.6	95.9/99.4
Ghost Shark	90.9/97.7	87.6/96.3	90.6/98	90.5/98.6	100%	91/98.2	90/98.4	91.1/98.4		90.6/98.2	91.2/98.1	89.1/97.5	90.8/98.2	90.9/98.8	91.1/98.8	90.1/98.3
Coelacanth	89.5/97.7	89.1/97.3	91.5/98.3	93.8/99	91/98.2	100%	92.2/98.8	93.4/99		92/98.2	89.5/97.9	90.8/98.6	92/98.4	93.8/99.2	93.8/98.8	92.8/98.8
Bowfin	90.7/98.3	88.3/97.3	91.7/98.6	95.1/99.4	90/98.4	92.2/98.8	100%	93.6/99.2		93/99.2	89.9/98.3	91.6/98.4	92.4/98.8	95.1/99.6	92.4/99.2	93.4/99
Pollen`s Cichlid	92.2/98.6	88.1/97.1	92.6/98.3	95.3/99.6	91.1/98.4	93.4/99	93.6/99.2	100%		93.9/99	92.2/99	93.6/98.6	93.8/98.8	95.9/99.8	94.7/99.4	94.9/994
Tiktaalik									100%							
Ropefish	91.6/98.4	87.9/96.9	98.5/99.6	93.4/99	90.6/98.2	92/98.2	93/99.2	93.9/99		100%	91/98.6	90.8/98.2	98.6/99.8	95.9/99.6	91.5/99	92/98.8
Great White Shark	94.2/98.5	86.3/97.1	90.4/98.2	91.2/99	91.2/98.1	89.5/97.9	89.9/98.3	92.2/99		91/98.6	100%	89.3/97.9	90.6/98.8	91.9/98.6	92.2/99	91.8/99
Axolotl	89.3/97.9	86.2/96.5	90.3/97.7	92.4/98.9	89.1/97.5	90.8/98.6	91.6/98.4	93.6/98.6		90.8/98.2	89.3/97.9	100%	91.4/98.4	92.8/99	91.2/98.6	91.8/99
Cuvier's Bichir	90.8/98.6	88.3/96.9	98.6/99.4	93.2/99.2	90.8/98.2	92/98.4	92.4/98.8	93.8/98.8		98.6/99.8	90.6/98.8	91.4/98.4	100%	92.8/99	93/99.6	93/99.4
Russian Sturgeon	92.4/98.6	89.1/97.9	92.6/99.2	98.5/99.8	90.9/98.8	93.8/99.2	95.1/99.6	95.9/99.8		93.6/99.6	91.9/98.6	92.8/99	95.9/99.6	100%	94.2/99.8	95.9/99.6
Australian Lungfish	91.9/98.6	86.9/97.7	91.6/99	93.2/99.6	91.1/98.8	93.8/98.8	92.4/99.2	94.7/99.4		91.5/99	92.2/99	91.2/98.6	93/99.6	94.2/99.8	100%	93/99.6
Goldfish	93/98.6	88.9/97.5	92.2/98.8	95.9/99.4	90.1/98.3	92.8/98.8	93.4/99	94.9/994		93.2/99.2	92/98.8	91.8/99	93/99.4	95.9/99.6	93/99.6	100%

Table 3 : The morphological characteristics that the species of fish might or might not have

Feature	Spiny Dogfish	Sea Lamprey	Saddled Bichir	Paddlefish	Ghost Shark	Coelacanth	Bowfin	Pollen's Cichlid	Tiktaalik	Ropefish
gills	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
spiracles	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Cartilage skeleton	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
jaws	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
Paired appendages	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
Placoid scales	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
Jelly filled swim bladder	N	N	Y	N	N	Y	Y	Y	Y	Y
Scales reduced	N	N	Y	N	N	Y	Y	Y	Y	Y
Air filled swim bladder	N	N	Y	N	N	Y	Y	Y	Y	Y
Ossification of skeleton	N	N	Y	N	N	Y	Y	Y	Y	Y
Cycloid skeleton	N	N	Y	N	N	Y	Y	Y	Y	Y
Swimbladder used as lung	N	N	N	N	N	Y	N	N	Y	N
Cosmoid scales	N	N	N	N	N	Y	N	N	Y	N
Ganoid scales	N	N	Y	N	N	N	N	N	N	Y
Lobed fins	N	N	Y	N	N	Y	Y	Y	N	Y
Dorsoventral flattening	N	N	Y	N	N	N	N	Y	N	Y
Pectoral girdle extension	N	N	Y	N	N	N	N	Y	N	Y
elasmobranch	Y	N	N	N	N	N	N	N	N	N
Tetrapod limbs	N	N	N	N	N	N	N	N	Y	N
Pectoral walking	N	N	Y	N	N	N	N	N	N	N

Morphologically, *A.mexicanum,* and *T.roseae* share the synapomorphies of tetrapod limbs, lobed fins, cosmoid scales, an air-filled swim bladder, an ossified skeleton, cycloid scales, a reduced amount of scales, jaws, paired appendages, and having a cartilage skeleton (Fig. 1). Since *T.roseae* is an extinct species, no protein sequence of COX1 from *T.roseae* was available (Fig. 2). Only the morphological phylogeny could be used to infer where *T.roseae* goes on the final phylogeny (Fig. 1). In the final phylogeny, when the data from the previous phylogenies were combined, it was shown that *A.mexicanum* and *T.roseae,* both from protein comparisons and morphological traits, shared a common ancestor (Fig.1, Fig.2, Fig.3).

In the morphological phylogeny, it is shown that *A.gueldenstaedtii* and *P.spathula* are in the same clade as they both have jelly-filled swim bladders, jaws, paired appendages, placoid scales, gills, spiracles, and cartilage skeletons (Fig. 1). And in the protein-derived phylogeny, it is shown that *A.gueldenstaedtii* and *P.spathula* have a 98.5 percent identity with each other.(Fig. 2). In the final phylogeny, it was shown that *A.gueldenstaedtii* and *P.spathula* with the 98.5 percent identity and the similar synapomorphies are related and have once shared a common ancestor (Table 2).

The third conclusion derived from the data is that *P.marinus* and *S.acanthias* are the outliers of the phylogeny (Fig.1). In the morphological phylogeny, *P.marinus* and *S.acanthias* were shown to share many similar synapomorphies (Fig. 1). These include gills, spiracles, and cartilage skeletons (Fig. 1). But in the protein-derived phylogeny, it was demonstrated that the COX1 comparison between the two species of fish yield an 87.3 percent identity (Table 2). This is a high average, but compared to the other relationships, such as *S.acanthias* and *A.gueldenstaedtii* sharing a 90.4 percent identity, and *P.marinus* and *P.pollen*, who morphologically are different, only sharing three synapomorphies by having a 90.8 percent identity (Fig.1, Fig.5). In the final phylogeny, it was decided that *S.acanthias* and *A.gueldenstaedtii* would be the outliers of the group, because the protein-derived data was more accurately studied, and the synapomorphies could be modified in a way to change the position of any of the two fish (Fig. 3). These three conclusions were the three main results. Another finding revealed that all of the 16 fish species are all related (Table 2). Of the 15 COX1 protein sequences that were available, all of them had an percent identity greater than 88% (Table 2). Morphologically, the species of fish are shown to all have similar traits, except *P.marinus*, which is shown to be the outlier of the group (Table 2). Tis information correlates to the protein-derived phylogeny in which *P.marinus* is also the outlier of the clade, and also in the final phylogeny in which *P.marinus* is the outlier of the clade (Fig.1, Fig.2, Fig.3). *S.acanthias* is also shown to be constant in its position in all three phylogenies. In the morphological, protein-derived, and final phylogenies, *S.acanthias* was shown to always be in the same clade as *C.carcharias* (Fig.1, Fig.2, Fig.3). Every species on the morphological and protein-derived phylogeny were in the same position in the final phylogeny except *P.senegalus, P. Endlicheri* and *A.calva* (Fig.1, Fig.2, Fig.3). In protein-derived phylogeny, it was demonstrated that *P. Endlicheri* was similar to *P.senegalus* while in the morphological phylogeny, it was demonstrated that *C.milii* and *A.calva were* more related *P.senegalus* then *P. Endlicheri* was (Fig.1, Fig.2, Fig.3).

DISCUSSION

In an effort to build a phylogeny that demonstrates a link between fish and tetrapods, COX1 from 16 different species was sequenced and made into a protein-derived phylogeny (Fig. 3). This phylogeny was combined with a morphological phylogeny with 21 synapomorphies of the 16 species of fish to make a final phylogeny (Fig. 1). From this final phylogeny, three main conclusion were derived: *A.mexicanum* and *T.roseae* share a common ancestor; *A.gueldenstaedtii* and *P.spathula* both share a common ancestor; and *P.marinus* and *S.acanthias* are the outliers of the phylogeny. The first conclusion which was Axolotl (*Ambystoma mexicanum)* and Tiktaalik (*Tiktaalik roseae)* share a common ancestor was derived from the morphological phylogeny (Fig. 1). In the morphological phylogeny, both *A.mexicanum* and *T.roseae* formed a clade, and shared 15 synapomorphies (Fig. 1). In the protein-derived phylogeny, there was no evidence of a relationship between *A.mexicanum* and *T.roseae* as *T.roseae* is extinct (Fig. 2). *A.mexicanum* and *T.roseae* have been shown to share many synapomorphies (Ericsson *et al*., 2012). *A.mexicanum* and *T.roseae* both share a specific organization of neck muscles, which goes around their gills and allows them to move the head (Ericsson *et al*., 2012). *A.mexicanum* and *T.roseae* also both share appendicular skeleton which connect to their neck muscles (Ericsson *et al*., 2012). *A.mexicanum* and *T.roseae* have both been shown to be closely related as they have been put on the same clade on morphological phylogenies (Shubin *et al*., 2014). *A.mexicanum* and *T.roseae* share a unique pelvic girdle and fin that allows for movement, and it is demonstrated that *T.rosales* pelvic girdle was a transitional phase *for* A.mexicanums pelvic girdle (Shubin *et al*., 2014). The second conclusion was that *A.gueldenstaedtii* and *P.spathula* both share a common ancestor, which was derived from the morphological and protein-based phylogeny (Fig.1, Fig. 2). In the morphological phylogeny, *A.gueldenstaedtii* and *P.spathula* were in the same clade and shared 8 synapomorphies (Fig. 1). In the protein-derived phylogeny, *A.gueldenstaedtii* and *P.spathula* shared a 98.5 percent identity (Table 2). Morphologically, it is supported that *A.gueldenstaedtii* and *P.spathula* are related as they both have eggs with funnels and clear groves (Ludwig, 2008). Also, *A.gueldenstaedtii* and *P.spathula* share the same sized micropylar opening (Ludwig, A, 2008). Genetically the data is supported as *A.gueldenstaedtii* and *P.spathula* have similar heteroplasmic movements in the mtDNA control region (Wang *et al*., 2010). This allows both species to have lengths of central units 78-83 bp (Wang *et al*., 2010). The conclusion that *A.gueldenstaedtii* and *P.spathula* are related shown through the fact that *A.gueldenstaedtii* and *P.spathula* are in the same Kingdom, Phylum, Superclass, Class, and order (Carroll R, 1998). This demonstrates a support for the claim that *A.gueldenstaedtii* and *P.spathula* are closely related (Carroll, 1998). The third conclusion was that *P.marinus* and *S.acanthias* are the outliers of the phylogeny, which was derived from the morphological and protein-derived phylogeny (Fig. 2, Fig. 3). In the morphological phylogeny, *P.marinus* and *S.acanthias* share three synapomorphies, and were in different clades (Fig. 1). In the protein-derived phylogeny, *P.marinus* and *S.acanthias* share an 87.3 percent identity (Fig. 2). This is the lowest percent identity of the 15 species of fish that had a COX1 protein sequence available (Table 2). It is supported that *P.marinus* and *S.acanthias* are morphologically different, as *P.marinus* and *S.acanthias* have different secretory granule compositions (Wilson *et al.*, 2002). *P.marinus* and *S.acanthias* also have a different type of microfilament network (Wilson *et al.*, 2002). Genetically, the claim that *P.marinus* and *S.acanthias* are outliers of the fish phylogeny is supported. It has been shown that *P.marinus* and *S.acanthias* have different gas transfer rates due to the difference in respiratory system IV (Randall, 1995). And it has been demonstrated that *P.marinus* and *S.acanthias* are both in genetically separate groups, which share a 0.400 similarly plasma level rate (Steve *et al.*, 1998). *P.marinus* and *S.acanthias* are also shown to be put into separate clades (Grim *et al.*, 2011). *P.marinus* and *S.acanthias* are demonstrated to be not very related, as they are in different classes, subclasses, orders, families and genuses (Grim *et al.*, 2011). This finding that *P.marinus* and *S.acanthias* shown to be a support for the idea that *P.marinus* and *S.acanthias* are not very related (Grimm *et al.*, 2011).

Table 4: The protein sequence of the fish was derived from an online public genomic database

Spiny Dogfish: >sp|Q9ZZ52|COX1_SQUAC Cytochrome c oxidase subunit 1 OS=Squalus acanthias GN=MT-CO1 PE=3 SV=1 MAINRWFFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELSQPGTLLGDDQIYNVIVTA HAFVMIFFMVMPVMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLLLLLASAGVEA GAGTGWTVYPPLAGNMAHAGASVDLAIFSLHLAGISSILASINFITTIINMKPPAISQYQ TPLFVWSILVTTILLLLSLPVLAAAITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH PEVYILILPGFGMISHVVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD TRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWETPLLWALGFIFLFTVGGLTGIVLAN SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAGFIHWFPLFSGYTLHSTWTKTQFLVMFIG VNLTFFPQHFLGLAGMPRRYSDYPDAYALWNTVSSIGSLISLVAVIMFLFIIWEAFAPSG KVLSVELPHTNVEWLHGCPPPYHTYEEPAFVQVQRTYF
Sea Lamprey >sp|Q35536|COX1_PETMA Cytochrome c oxidase subunit 1 OS=Petromyzon marinus GN=MT-CO1 PE=3 SV=1 MTHIRWLFSTNHKDIGTLYLIFGAWAGMVGTALSILIRAELSQPGTLLGDDQIFNVIVTA HAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLLLLLASAGVEA GAGTGWTVYPPLAGNLAHTGASVDLTIFSLHLAGVSSILGAVNFITTIFNMKPPTMTQYQ TPLFVWSVLITAVLLLLSLPVLAAAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGH PEVYILILPGFGIISHVVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD TRAYFTSATMIIAIPTGVKVFSWLATLHGGKIVWHTPMLWALGFIFLFTVGGLTGIVLSN SSLDIILHDTYYVVAHFHYVLSMGAVFAIMAGFVHWFPLFTGYTLNETWAKAHFIIMFAG VNLTFFPQHFLGLAGMPRRYSDYPDAYTTWNIISSIGSTVSLIAVMLFMFILWEAFSAKR KAIATDLLNTNLEWLHGCPPPYHTYEEPAFVQTNFKK
Saddled Bichir: >tr|F2W4F6|F2W4F6_9ACTI Cytochrome c oxidase subunit 1 OS=Polypterus endlicherii congicus GN=COX1 PE=3 SV=1 MTITRWLFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELGQPGALMGDDQIYNVIVTA HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLLLLLTSSAVEA GVGTGWTVYPPLAGNLAHAGASVDLAIFSLHLAGVSSILGAINFITTIINMKSPSTSQYQ TPLFVWSVLVTAILLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH PEVYILILPGFGMISHIVAYYSGKNEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD TRAYFTSATMIIAIPTGVKVFSWLATLHGGAIKWETPMLWALGFIFLFTVGGLTGIILAN SSLDIMLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLHPTWTKIHFGIMFIG

VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNSLSSIGSMISLTAVIMFLFILWEAFAAKR
EVQMVNLTYTNVEWLHGCPPPYHTYEEPAFVQSPQARE

Paddlefish:
>tr|Q8HMQ8|Q8HMQ8_POLSP Cytochrome c oxidase subunit 1 OS=Polyodon spathula GN=COI PE=3 SV=1
MAITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAVFQYQ
TPLFVWSVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWDTPLLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGAFVHWFPLFTGYTLHSTWSKIHFAVMFVG
VNLTFFPQHFLGLAGMPRRYSDYPDAYALWNTVSSVGSLISLIAVIMFLFILWEAFAAKR
EVLSVELTATNVEWLHGCPPPYHTYEEPAFVQVQSTN

Ghost Shark:
>tr|D7RWS9|D7RWS9_CALMI Cytochrome c oxidase subunit 1 OS=Callorhinchus milii GN=COX1 PE=3 SV=1
MTINRWLFSTNHKDIGTLYLLFGAWAGMVGTALSLLIRAELSQPGALMGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLIPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASAGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGISSILASINFITTIINMKPPSITQYQ
TPLFVWSILITTILLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHVVTYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGNIKWDTPMLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAGLVHWFPLFTGYTLHETWTKIHFGLMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNSVSSIGSLMSLLAVILFLFILWEAFASKR
TLSHVMMSSTNNEWLHGCPPPHHTFEEPAFVQIQVNKKG

Ghost Shark:
>tr|D7RWS9|D7RWS9_CALMI Cytochrome c oxidase subunit 1 OS=Callorhinchus milii GN=COX1 PE=3 SV=1
MTINRWLFSTNHKDIGTLYLLFGAWAGMVGTALSLLIRAELSQPGALMGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLIPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASAGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGISSILASINFITTIINMKPPSITQYQ
TPLFVWSILITTILLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHVVTYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGNIKWDTPMLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAGLVHWFPLFTGYTLHETWTKIHFGLMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNSVSSIGSLMSLLAVILFLFILWEAFASKR
TLSHVMMSSTNNEWLHGCPPPHHTFEEPAFVQIQVNKKG

Coelacanth:
>sp|O03167|COX1_LATCH Cytochrome c oxidase subunit 1 OS=Latimeria chalumnae GN=MT-CO1 PE=3 SV=1
MMITRWLFSTNHKDIGTLYMIFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVVVTA
HAFVMIFFMVMPIMIGGFGNWLIPLMIGAPDMAFPRMNNMSFWLLPPSLLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKPPTMTQYQ
TPLFIWSVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGVTKWDTPLLWALGFIFLFTVGGLTGIVLAN
SSLDIILHDTYYVVAHFHYVLSMGAVFAIMGGLVHWFPLMTGYTLHNTWTKIHFGVMFTG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTVSSIGSLISLIAVIMFMFILWEAFSAKR
EVLIVEMTTTNVEWLHGCPPPHHTYEEPAFVQAPR

Bowfin:
>tr|Q8HQL9|Q8HQL9_AMICA Cytochrome c oxidase subunit 1 OS=Amia calva GN=COI PE=3 SV=1
MTITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVIVTA
HAFVMIFFMVMPVMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLASNLAHAGASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAASQYQ
TPLFVWSVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMVSHIVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMVIAIPTGVKVFSWLATLHGGAIKWETPLLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLHPTWSKIHFGVMFVG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTVSSIGSLVSLIAVVMFLFMLWEAFAAKR
EVLAVEYAATNVEWLHGCPPPYHTYEEPAYVQAHRA

Pollen`s Cichlid:
>tr|B5DCF6|B5DCF6_9CICH Cytochrome c oxidase subunit 1 OS=Paratilapia polleni GN=CO1 PE=3 SV=1
MAITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLIPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGPSVDLTIFSLHLAGVSSILGAINFITTIINMKPPAISQYQ
TPLFVWSVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGAIKWDTPLLWALGFIFLFTVGGLTGIVLAN
SSLDIMLHDTYYVVAHFHYVLSMGAVFAIVGGFVHWFPLFSGYTLHSTWTKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTVSSIGSMISLVAVIMFLFIIWEAFAAKR
EVLSAELTTTNAEWLHGCPPPYHTFEEPAFVQVQQA

Ropefish:
>tr|Q8HMU1|Q8HMU1_ERPCA Cytochrome c oxidase subunit 1 OS=Erpetoichthys calabaricus GN=COI PE=3 SV=1
MTITRWLFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELGQPGALMGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLTSSAVEA
GVGTGWTVYPPLAGNLAHAGASVDLAIFSLHLAGVSSILGAINFITTIINMKPPATSQYQ
TPLFVWSVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYSGKNEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGAIKWETPMLWALGFIFLFTVGGLTGIILAN
SSLDIMLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLHPTWTKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNSLSSIGSMISLTAVIMFLFILWEAFAAKR
EVQTVNLTYTNVEWLHGCPPPYHTYEEPAFVQSPNSRE

Great White Shark:
>tr|I1U3D7|I1U3D7_CARCH Cytochrome c oxidase subunit 1 OS=Carcharodon carcharias PE=3 SV=1
MAINRWLFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELGQPGSLLGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASAGVEA
GAGTGWTVYPPLASNLAHAGPSVDLAIFSLHLAGISSILASINFITTIINMKPPAISQYQ
TPLFVWSILVTTILLLPALPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHVVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGTIKWDTPLLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAGFIHWFPLMSGFTLHQTWTKIQFTVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNAISSIGSLISLVAVIMLLFIIWEAFASKR
EVLSIELPHTNVEWLHGCPPPYHTYEEPAFVQIQRPSF

Axolotl:

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>tr|Q70EE6|Q70EE6_AMBME Cytochrome c oxidase subunit 1 OS=Ambystoma mexicanum GN=COI PE=3 SV=1
MMITRWLFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVIVTA
HAFVMIFFMVMPVMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTSINMKPASMSQYQ
TPLFVWSVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPVLYQHLFWFFGH
PEVYILILPGFGMISHIVTYYSAKKEPFGYMGMVWAMMSIGLLGFIVWAHHMFTVDLNVD
TRAYFTSATMIIAIPTGVKVFSWLATMHGGAIKWDAAMLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLHSTWSKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTVSSIGSLISLVAVIMMMFIIWEAFASKR
EVLSTELTSTNIEWLHNCPPPYHTFEEPSFVQSRI
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Cuvier's Bichir:

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>tr|Q8HMS1|Q8HMS1_POLSE Cytochrome c oxidase subunit 1 OS=Polypterus senegalus senegalus GN=COI PE=3
SV=1
MTITRWLFSTNHKDIGTLYLIFGAWAGMVGTALSLLIRAELGQPGALMGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLLLLLTSSAVEA
GVGTGWTVYPPLAGNLAHAGASVDLAIFSLHLAGVSSILGAINFITTIINMKPPSTSQYQ
TPLFVWSVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYSGKNEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGAIKWETPMLWALGFIFLFTVGGLTGIILAN
SSLDIMLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLHSTWTKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNSLSSIGSMISLTAVIMFLFILWEAFAAKR
EVQTVELTYTNVEWLHGCPPPYHTYEEPAFVQSPQARE
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Russian Sturgeon:

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>tr|C1J916|C1J916_ACIGU Cytochrome c oxidase subunit 1 OS=Acipenser gueldenstaedtii GN=cox1 PE=3 SV=1
MAITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVIVTA
HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAVSQYQ
TPLFVWSVLITAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGMISHIVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWDTPLLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGAFVHWFPLFTGYTLHGTWSKIHFAVMFVG
VNLTFFPQHFLGLAGMPRRYSDYPDAYALWNTVSSIGSLISLVAVIMFLFILWEAFAAKR
EVMSVELTTTNVEWLHGCPPPYHTYEEPAFVQVQSTS
```

Australian Lungfish:

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>tr|Q94XJ5|Q94XJ5_NEOFS Cytochrome c oxidase subunit 1 OS=Neoceratodus forsteri GN=COX1 PE=3 SV=1
MTITRWFFSTNHKDIGTLYMIFGAWAGMVGTALSLLIRAELSQPGALLGDDQIYNVLVTA
HAFVMIFFMVMPIMIGGFGNWLIPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGSINFITTIINMKPPAISQYQ
TPLFIWSVMITTILLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGYGMISHIVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWETPLLWALGFIFLFTVGGLTGIVLAN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLMTGYTLHNTWTKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYTLWNTVSSIGSLISLVAVIMLLFIIWEAFAAKR
EVMSIELSPTNVEWLHGCPPPHHTFEEPAFVQVQTSQR
```

Goldfish:

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>sp|O78681|COX1_CARAU Cytochrome c oxidase subunit 1 OS=Carassius auratus GN=mt-co1 PE=3 SV=1
MAITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTA
HAFVMIFFMVMPILIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA
GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQ
TPLFVWSVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGH
PEVYILILPGFGIISHVVAYYSGKKEPFGYMGMVWAMMAIGLLGFIVWAHHMFTVGMDVD
TRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWETPMLWALGFIFLFTVGGLTGIVLSN
SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAAFVHWFPLLTGYTLHSAWTKIHFGVMFIG
VNLTFFPQHFLGLAGMPRRYSDYPDAYALWNTVSSIGSLISLVAVIMFLFILWEAFAAKR
EVLSVELTMTNVEWLHGCPPPYHTYEEPAFVQIQSN
```

From the phylogenies, it was shown that species that were in the same genus and families were more closely related morphologically and genetically (Fig. 3). It was also shown that species that were morphologically similar were also similar in their protein structures (Fig. 3). A point of controversy on the topic of combining the protein-derived phylogeny and the morphological phylogeny is that other versions have put *P.marinus* and *S.acanthias* closer on the final phylogeny as they are morphologically similar (Fig. 3). This idea of putting *P.marinus* and *S.acanthias* close to each other because they have similar synapomorphies disrupts the phylogeny as *A.gueldenstaedtii* and *P.spathula* are more morphologically similar and have similar COX1 sequences (Fig.5, Fig. 1). Putting *P.marinus* and *S.acanthias* in place of *A.gueldenstaedtii* and *P.spathula* would push *A.gueldenstaedtii* and *P.spathula* apart on the phylogeny, even though they are more closely related (Fig. 3). In the phylogeny, there are only 16 limited taxa for the 16 species of fish to be put on. If one fish's position in the phylogeny changes, it will impact the rest of the phylogeny. These results build upon the idea that tetrapods and fish may share a common ancestor, and tetrapods may once been fish. This idea has implications throughout biological sciences (Nancy *et al*., 2002). A phylogeny connecting tetrapods to fish could build a more detailed connection of the evolution of genes (Nancy *et al*., 2002).

CONCLUSION

This information could be more helpful as it could be used in the medical field to find specific new nutrients and new antibiotics (Nancy B *et al*., 2002). A phylogeny between tetrapods to fish could also allow for new connection to be made between primates and other animals (Nancy *et al*., 2002). This connection could allow for medicine to be tested on fish before the medicines use of primates (Nancy *et al*., 2002). A limitation of the protein-derived phylogeny was that *T.roseae* was extinct and did not have any sequences of COX1 available. Because of this, *T.roseae* only had one source for its placement on the final phylogeny, and that was the morphological phylogeny. The morphological phylogeny was only based off of certain traits. If these traits were chosen in specific ways, the morphological phylogeny could be theoretically controlled and be made to look any way the synapomorphies allow it to be. This could have affected the final phylogeny, as it allows for variations of the phylogeny. The process of combining morphological and protein-derived phylogenies is a flawed process in itself (Gontcharova. A *et al*., 2003). Even though computers and mathematical formulas make the combination more precise, phylogenies made from the combination of morphological and protein-derived phylogenies suffer from inadequate, noncongruent taxon sampling, long-branch attraction, or conflicting evolutionary models of the genes analyzed (Gontcharova *et al*., 2003). In summary, the present data suggests that as time progressed, fish started to gain more tetrapod-like synapomorphies, and supports the theory that tetrapods came from fish. Additional studies concerning the morphological and genetic similarity between fish and terrestrial organisms might help better understand the link between tetrapods and fish.

Conflicts of interest: The authors stated that no conflicts of interest.

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