Title
A novel phylogeny for the Neotropical triplefin blennies (Teleostei: Tripterygiidae): Contrasting molecules and morphology

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A novel phylogeny for the Neotropical triplefin blennies (Teleostei: Tripterygiidae):
Contrasting molecules and morphology.

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science
in
Biology
by
Elizabeth Christina Miller

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2014
The thesis of Elizabeth Christina Miller is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-Chair

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University of California, San Diego

2014
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This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
ABSTRACT OF THE THESIS

A novel phylogeny for the Neotropical triplefin blennies (Teleostei: Tripterygiidae):
Contrasting molecules and morphology

by

Elizabeth Christina Miller

Master of Science in Biology
University of California, San Diego, 2014
Professor Phil Hastings, Chair
Professor Jonathan Shurin, Co-Chair

The triplefin blennies (Teleostei: Tripterygiidae) are a diverse group of cryoptobenthic fishes found globally, though usually associated with rocky or coral reefs. The Neotropics contain four genera and 25 species, many of which have only been recently described. A recent molecular phylogeny (Lin and Hastings 2013) contrasts with previous phylogenies based on morphology in recovering the four Neotropical genera as a single clade with respect to the Indo-Pacific genera; however, relationships within and
among genera were poorly resolved. This study reports a novel topology based on an expanded seven-gene molecular dataset. The monophyly of *Axoclinus* is well supported, but *Enneanectes* is paraphyletic with the inclusion of *Axoclinus* and *Crocodilichthys*. A morphological dataset was constructed, but did not provide resolution. Apparent sister species appear have disjunct ranges, and allopatric speciation due to oceanographic breaks is a likely driver of speciation in the Tropical Eastern Pacific. Improved taxa sampling within the Neotropical species, in addition to sampling additional genera outside of the Neotropics, is necessary to further improve resolution and confirm the monophyly of the Neotropical triplefins.
INTRODUCTION

The Tripterygiidae are a diverse blennioid family, composed of 29 genera and 171 species (Fishbase). Triplefins are found worldwide, in both temperate and tropical oceans, where they are often associated with rocky or coral reef habitats. Their peak diversity is in New Zealand, where 20 of the 26 endemic species have resulted from an adaptive radiation (Wellenreuther et al 2007, Hickey et al 2009). In addition, two Indo-Pacific genera, *Enneapterygius* and *Helcogramma*, are highly diverse with over 53 and 39 species, respectively (Fricke 2009). Due to their cryptobenthic lifestyle, tripterygiids have been the focal species for studies on marine radiations (Carreras Carbonell et al 2005, Wellenreuther 2007), cryptic speciation (Victor 2013), life history (Longnecker and Langston 2005, Riginos and Victor 2001) and isolation by distance (Riginos and Nachman 2001, Hickey et al 2009).

The Neotropics contain four genera and 25 species of triplefins (Figure 1), many of which have only been described in the past 25 years (Allen and Robertson 1991, Allen and Robertson 1992, Rosenblatt et al 2013, Victor 2013). Of the 25, seven are island endemics. Three genera are endemic to the Tropical Eastern Pacific (TEP): *Axoclinus* (six species), *Lepidonectes* (three species), and *Crocodilichthys* (one species). The fourth and most diverse, *Enneanectes*, contains 15 species distributed in both the TEP and Western Atlantic. Although their ecological contribution is unclear, tripterygiids comprise a significant portion of the ichthyofauna of rocky reef communities in the TEP, especially in the Gulf of California (Aburto-Oropeza and Balart 2001, Thomson and Giligan 2002, Galland 2013).
Previous phylogenetic hypotheses of the generic relationships within the Tripterygiidae have been based solely on morphology. Rosenblatt (1959) placed the four Neotropical genera in a clade containing Indopacific genera in his unpublished dissertation (Figure 2). Fricke (1994) later revised the family, recognizing eight tribes, and proposed different placements for the Neotropical genera. *Lepidonectes* was placed in the tribe Norfolkini with the South African genus *Cremnochorites*, and the other three genera were placed in the tribe Tripterygiini with the Mediterranean genus *Tripterygion*. Both studies hypothesized each genus to be monophyletic. However, the four Neotropical genera do not form a monophyletic clade in either study, implying multiple colonizations of the Neotropics.

In contrast, Lin (2009) and Lin and Hastings (2013) constructed a 5-gene molecular phylogeny that recovered the four Neotropical genera as monophyletic, with implications of a single colonization event from the Indo-Pacific. That analysis only included seven of 29 genera (including the four Neotropical genera), and so it is difficult to make robust conclusions on their relationships to triplefins from other regions. An interesting, but unanswered question concerns the biogeographic origins of the Neotropical triplefins (Hastings 2009) given their apparently poor dispersal ability, a consequence of their benthic, territorial adult behavior, demersal eggs, short pelagic larval duration, and near-shore development (Lin 2009). Indeed, other blennioid families with more resolved phylogenetic relationships show strong geographic restriction (Lin and Hastings 2013).

The species-level relationships of triplefins included in Lin and Hastings (2013) are unresolved. Although the four Neotropical genera were recovered as monophyletic
with strong support, most nodes within genera had very low support. Curiously, both
*Enneanectes* and *Axoclinus* were paraphyletic, with *E. reticulatus* and *A. nigricaudus*
falling out as sister species. They attributed the poor resolution within the Tripterygiidae
to poor taxon sampling, missing sequence data, and several indels in sampled nuclear
genes (Lin and Hastings 2013). Additionally, there are thought to be several rapid
speciation events within the family (Carreras-Carbonell *et al* 2005; Wellenreuther 2007),
causing many short branches, which can complicate phylogenetic reconstruction.

This study expands the Lin and Hastings (2013) molecular dataset to more fully
resolve the species-level relationships within the four Neotropical genera. The expanded
molecular dataset contains seven markers, including new sequence data for three
mitochondrial genes. In addition, a morphological dataset was constructed in an attempt
to reconcile the disparity between previously published morphology and molecular
phylogenies.

*Submission for Publication*

This thesis will be submitted for publication. The thesis author was the primary
investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-
author for her contribution of sequences for analysis.
MATERIALS AND METHODS

Taxon Sampling

For molecular analysis, tissues from 12 species from four genera were taken from voucher specimens stored in the Scripps Institution of Oceanography Marine Vertebrate Collection (SIO), with the exception of three specimens from the University of Kansas Natural History Museum (KU). Lepidonectes corallicola was selected as an outgroup based on Lin and Hastings (2013). Species not included are predominantly island endemics for which tissues were not available. For morphological analysis, Lepidonectes clarkhubbsi was substituted for L. corallicola, and the additional outgroups Helcogrammoides cunninghami and Helcogramma elliotti were examined. See Tables 1, 2, and 3 for a description of included taxa.

Molecular Data

A total of four mitochondrial markers (12S, 16S, Cytochrome C Oxidase 1 (CO1) and Cytochrome b) and three nuclear markers (Rag-1, Rhodopsin, and TMO-4C4) were included in this study, compared to five total markers in Lin and Hastings 2013 (CO1, Rag1, Rhodopsin, TMO-4C4, and Histone H3). New sequences from CO1, Rhodopsin, and TMO-4C4 were obtained and compared with sequences from Lin and Hastings (2013) to confirm identity. The following sequence data from Lin and Hastings (2013) were included in this dataset: Rag1 (HQ168761-68) and TMO-4C4 (HQ169008, 9, and 12). Histone H3 sequences were not included because the marker seems to evolve more slowly than the others and were not informative (Lin and Hastings 2011, 2013).
Total genomic DNA was extracted from muscle tissue using a Qiagen (Chatsworth, CA) DNeasy Blood and Tissue kit by following the manufacturer’s instructions. When available, multiple individuals per species were used. Primers used to obtain all 7 markers are described in Table 4. PCR was performed under the following conditions: 94°C for one minute for initial denaturing, 34-35 cycles of 94°C for 30 s, 50–56°C for 45 s, and 72°C for 45 s, followed by 72°C for five minutes as the final extension. PCR products were purified using a Sephadex gel matrix (Sigma-Aldrich), and sequenced in both directions using the amplifying primers via Retrogen, Inc (San Diego, CA).

*Morphological Data*

Whole specimens were examined and observations were made on meristics, osteology, sensory pores, and squamation (Table 5). Most characters were chosen *a priori* based on previous studies on triplefins (Fricke 1994, Rosenblatt 1959, Wellenreuther 2010, Jawad 2005, 2008). To assist examination of osteology, specimens were cleared in aqueous 1% KOH and trypsin, and stained using alcian blue and alizarin red following methods of Dingerkus and Uhler (1997) and Taylor and Van Dyke (1985). Sensory pores were examined by drying specimens with a small air pump.

*Phylogenetic Analysis*

Sequences were assembled and edited in Sequencher 5.2 (Gene Codes, Ann Arbor, MI), and imported into Mesquite 2.75 (Maddison and Maddison 2011), where they were aligned using MUSCLE (Edgar 2004). Alignments were checked by eye. For
the protein-coding markers (all but 12S and 16S), codon position was assigned by minimizing stop codons, and translating sequences to ensure that no stop codons were present.

Due to the documented high rates of mtDNA evolution in blennies (Eytan 2010), each gene was tested for saturation, with protein-coding genes separated by 1+2 and 3rd codon positions in DAMBE 5 (Xia 2013). The number of transitions and transversions versus genetic distance was plotted to look for plateaus in accumulated changes. The Xia test for saturation (Xia 2003) was implemented as well, providing two measures to determine saturation.

To compare the impact of partitioning scheme on topology and support (Dornburg 2008), the seven-gene concatenated dataset was analyzed according to two partitioning schemes: by gene (seven partitions) and according to the best-fit scheme selected by PartitionFinder (Lanfear 2012). The best-fit model of evolution for individual markers was determined in JModelTest 2 using the AIC criterion (Posada 2008). The best-fit partitioning scheme and appropriate substitution models for the entire dataset and individual codon positions were determined in PartitionFinder using the AIC criterion and the “greedy” heuristic algorithm (Table 6). A second PartitionFinder analysis was run with the number of models restrained to only those recognized by MrBayes (Ronquist and Huelsenbeck 2003).

Individual gene trees were analyzed separately, as well as seven concatenated datasets: mtDNA (four markers), mtDNA with saturated sited removed, nuDNA (three markers), and all seven markers partitioned either by gene or via PartitionFinder, with or without saturated sites. Bayesian Metropolis coupled Markov chain Monte Carlo
(MCMC) analyses were conducted in Mr. Bayes v. 3.2.2 (Ronquist and Huelsenbeck 2003). Bayesian inference was performed with four independent MCMC runs with three heated and one cold chains, for 10 million generations, sampling every 1000 generations, and conservatively discarding the first 30% of samples as burn-in. Convergence of runs was confirmed based on plots of lnL scores versus generation time, as well as ESS values, visualized in Tracer (Drummond and Rambaut 2007). Maximum likelihood analyses (Felsenstein 1981) were conducted in the program RAxML v7.4.2 (Stamatakis, 2006) via raxmlGUI v1.3 (Silvestro and Michalak, 2012), using the thorough bootstrapping algorithm and 100 replicates. The model GTR+GAMMA was assigned to all partitions. Maximum parsimony analyses were conducted in PAUP 4.0b10 (Swofford 2003) using 1000 heuristic search replicates with random addition, and jackknife node support with 10 additional replicates.

Morphological characters were scored assigned binomial or numerical values. The character matrix was constructed in Mesquite. Phylogenetic inference was conducted in PAUP and Mr. Bayes under the same conditions, with the exception of sampling every 100 generations in the Bayesian analysis.

Submission for Publication

This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
RESULTS

Molecular Data

Sequence data from seven markers totaling 4198 bp were obtained and analyzed (Table 7). The number of parsimony-informative sites was 776 (19%) for the total dataset with all codons, and 376 (10%) when saturated sites were removed. Nodes with bootstrap values above 70 and posterior probabilities above 95% were considered well-supported.

The 3rd codon positions of both CO1 and Cytochrome b were saturated as demonstrated both graphically and statistically (Table 8, Figure 3). The plot of transitions vs. transversions for Rag1 also plateaued; however, saturation was rejected according to Xia’s test. Therefore, the 3rd codon positions of CO1 and Cytochrome B were removed in three of the datasets, but all codon positions of Rag1 were included. After removing saturated sites, both CO1 and Cytochrome b had a very low number of parsimony-informative sites (11 and 26 respectively).

Each individual gene tree had poor resolution overall, and few nodes were supported until genes were concatenated (Figures 4, 5, and 6). The ideal partitioning scheme determined by PartitionFinder combined 12S and 16S into one partition, and partitioned the remaining markers by codon position for a total of 16 partitions (Table 6). Models assigned to individual codon positions ranged from simple (F81+G) to complex (GTR+I+G), revealing great heterogeneity in substitution rates within the dataset.

Mitochondrial and Nuclear Trees

Overall, any conflicts between the mitochondrial and nuclear trees, and the mtDNA and nuDNA trees with the total seven-gene trees had low node support. Node
support was low overall for both mtDNA datasets (Figures 7 and 8). As expected, removing saturation resulted in much shorter branch lengths in the ML tree. The mtDNA trees recovered the three Atlantic species as a clade including *E. carminalis*, a result not seen in the other datasets. Instead of *E. carminalis*, *A. nigricaudus* and *E. reticulatus* were the outgroups to the remaining species. The same (*E. macrops (E. glendae-C. gracilis)*) clade as the total seven-gene dataset was recovered with fair support, with *A. lucillae* and *A. storeyae* as a sister clade.

There was overall strong congruence between ML, parsimony, and Bayesian nuclear trees (Figure 9). The ML and Bayesian nuclear trees recovered the same placement of *E. carminalis* and the Atlantic species as the total seven-gene dataset. *Axoclinus* is recovered as monophyletic with high support. The only deviation from the seven-gene topology is in the placement of *E. macrops* and *E. reticulatus*, with low support.

**Effects of Saturation and Partitioning**

The mtDNA dataset with saturated sites included (saturated sites comprised about 20% of this dataset) recovered a similar topology as the dataset without saturation, with the exception of *E. pectoralis* and *E. boehlkei* changing positions (Figures 7 and 8). Removing saturation resulted in greater congruence between ML, parsimony, and Bayesian trees. Bootstrap support was comparable in both mtDNA ML trees, but removing saturation improved bootstrap support in the Bayesian trees (not shown).

Both removing saturation and increasing partitioning of the total seven-gene dataset had minor effects on node support and topology (Figures 10-14). Partitioning by
codon did improve support for basal nodes in the ML trees, but the impact on shallower nodes was ambiguous. The only changes in topology between these four datasets concerned placements with low support in all trees. For the total dataset partitioned by gene, removing saturation resulted in comparable node support in both ML and Bayesian trees as the dataset with saturated sites. Within each dataset, ML and Bayes trees recovered the same topology except with *E. reticulatus* in a polytomy at the base of the remaining Pacific species. The dataset with saturation recovered *E. boehlkei* and *E. pectoralis* as sister species, while removing saturation produced no sister species pairs among the three Atlantic species, all with low support. Removing saturation had similar effects in the total dataset partitioned by codon. In both, *E. boehlkei* and *E. pectoralis* were sister species. Node support was slightly higher for shallower nodes when saturation was left in and slightly lower when it was removed. The reverse was true for deeper nodes.

The only difference in topology between the total dataset partitioned by gene and partitioned by codon was that in the ML trees, *E. reticulatus* was the sister species to *Axoclinus* in the dataset partitioned by codon. In all Bayesian trees, it was placed in a polytomy with *Axoclinus* and the clade containing *E. macrops, E. glendae*, and *C. gracilis*.

*Morphological Data*

The morphological dataset included 41 parsimony-informative characters and two autapomorphies (Tables 5 and 10). The following characters were constant for all taxa and were excluded from the analysis: two elements supported on last anal fin
pterygiophore (Fishbase), autogenous bony stay present on last anal fin pterygiophore (Fishbase), scapula and cleithrum in close proximity (Jawad 2008), pelvic girdle without sutured halves (Jawad 2008), and two anal-fin spines (Rosenblatt 1959).

According to the maximum parsimony reconstruction, *Axoclinus* and *Helcogramma* were distinct from the remaining genera (Figure 16). Characters supporting this are related to the lateral line position and squamation (13, 19, 21, 24, 31), lack of scales on the anal and first dorsal fin base (17, 18) and lack of branched rays in the 3rd dorsal fin (32; see Table 5). Unfortunately, the dataset was unable to resolve relationships any further than this. The Bayesian analysis did not recover any resolution at all, and is not shown.

**Phylogenetic relationships**

According to the seven-gene concatenated datasets, *Axoclinus* is a monophyletic genus, with *A. storeyae* and *A. lucillae* as sister species with high support from almost every gene (Figures 6, 10-14). *Enneanectes* is paraphyletic with the inclusion of *Axoclinus* and *Crocodilichthys*. After *Lepidonectes*, *E. carminalis* is the outgroup to the remaining species. The three Atlantic species are not monophyletic; *E. boehlkei* and *E. pectoralis* are sister species, but with low support. The remaining seven Pacific species form a clade with high support. Within this clade, *E. glendae* and *C. gracilis* are sister species, with *E. macrops* as a sister group to them. Finally, the placement of *E. reticulatus* within this clade is unresolved.
Submission for Publication

This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
DISCUSSION

Phylogenetic relationships

This study proposes a novel topology for the Neotropical tripterygiids. Lin and Hastings (2013) reported *A. nigricaudus* and *E. reticulatus* as sister species, making both *Axoclinus* and *Enneanectes* paraphyletic (Figure 2). The present study is consistent with previous morphological phylogenies (Rosenblatt 1959, Fricke 1994) in recovering a monophyletic *Axoclinus*, but like Lin and Hastings (2013), did not support the monophyly of *Enneanectes*. While *Axoclinus* is well defined morphologically, sharing the synapomorphies of a continuous lateral line and blunt head shape, members of *Enneanectes* are more varied. *Crocodilichthys gracilis* and *E. glendae* were recovered as sister species with high support across several markers (Figure 6). This is consistent with Lin and Hastings (2013), as well as morphological similarities including a long, slender body and high meristic counts (Rosenblatt 2013).

In both this study and previous phylogenetic reconstructions (Rosenblatt 1959, Lin and Hastings 2013), the Atlantic species are not monophyletic but they are not closely related to any eastern Pacific species. These Caribbean species are found primarily on coral reefs (Rosenblatt 1960), while TEP triplefins are associated with rocky reefs (Hastings 2009). This pattern is similar to other groups of blennioids that include coral specialists. For example, the chaenopsid genus *Emblemariopsis* is a coral specialist and restricted to the Caribbean basin. However, relationships among the Atlantic species of *Enneanectes* are unresolved. This is most likely due to poor sampling, with only 3 of 10 Atlantic *Enneanectes* sampled (Victor 2013).
The placement of *E. reticulatus* is unresolved in this study. In the Bayesian analyses, it is placed either as the outgroup to *Axoclinus* or as the sister species of *E. macrops* (Figure 13). The latter result is consistent with its placement by Rosenblatt (1959) based on morphological similarity. Interestingly, the mtDNA data set recovers *E. reticulatus* and *A. nigricaudus* as outgroups to all remaining *Enneanectes* species (Figures 7 and 8). This is possibly due to the rapid evolution of mitochondrial markers, resulting in long branch attraction. A likely solution is to improve taxon sampling in this group to break up these long branches.

**Clade support**

It is interesting that although each individual gene tree had overall low support, support for most nodes was high when genes were concatenated (Figures 4, 5, and 6). This is especially true for basal nodes, many of which were collapsed into polytomies in individual gene trees. Low basal node support is indicative of rapid radiation early in this group’s history. Rapid radiation events have been proposed for other triplefin clades (Carreras-Carbonell et al. 2005, Wellenreuther 2007), as well as other blennioids (Lin and Hastings 2011, Eytan 2010). In addition, this result may be an example of “hidden support,” where individual genes actually contain consistent support for nodes, but due to a low number of parsimony-informative sites, support is not apparent until all genes are concatenated (Townsend 2011). In this case, concatenation is an appropriate and effective method for phylogenetic reconstruction, especially when attempting to resolve relationships by increasing the number of loci.
Removing saturated sites had a stronger impact on the mtDNA dataset, for which these sites comprise about 20% of base pairs (Figures 5 and 6). However, removing saturation had minor effects on the total concatenated set, where saturated sites are only 10% of base pairs. Interestingly, including 3rd codon positions may have improved support for some nodes (Figures 7-14). This may be because these codon positions may indeed include some sites with phylogenetic signal, and the inclusion of nuclear genes may overwhelm conflicting signal from the saturated sites.

The effects of partitioning a dataset in phylogenetic analyses is controversial; some argue that over-parameterizing can increase error (Burnham and Anderson 2003), while empirically the impacts may actually be slight (Dornburg 2008). The PartitionFinder analysis revealed a large degree of heterogeneity in codon substitution sites within the molecular data (Table 6) that is not taken into account when models are applied to whole genes. In practice, partitioning by codon position improved the support for basal nodes in the ML trees. However, there did not seem to be an effect on shallower nodes due to partition scheme. The fact that neither removing substitution nor changing partitioning scheme had strong effects on topology (except for poorly supported relationships) indicates that portions of the topology recovered in this study are robust.

Morphology

Traditionally, as with other groups, phylogenetic relationships in tripterygiids were inferred exclusively from morphology, and without the use of algorithms that are widespread in modern phylogenetic analyses (Figure 2). Despite including several morphological characters that Rosenblatt (1959) used to distinguish species-level
relationships within the Neotropical species, the morphological dataset in this study provided almost no resolution. This is most likely due to the small number of parsimony-informative characters, but may also reflect limited taxon sampling and a high degree of homoplasy. Rosenblatt’s species-level relationships within *Enneanectes* and *Axoclinus* are exclusively based on external characteristics such as squamation and spination patterns (1959). He hypothesized that there was a great degree of convergence in these characters, which he thought had complicated phylogenetic inference in his study (1959).

The Neotropical triplefins appear to have less variation in osteological characters as some New Zealand tripterygiid species (Jawad 2008), providing relatively few osteological characters for analysis.

Despite the inability of morphological characters to provide resolution in this study, some morphological characters reported here provide support for the clades recovered by molecular data (Table 5). In congruence with previous studies (Rosenblatt 1959, Fricke 1994), the monophyly of *Axoclinus* is supported by the presence of 1 upper unbranched pectoral-fin ray unattached to the scapula (3, 33), broad shape of the 4th pterygiophore (6), and a continuous lateral line (13) with position and squamation distinct from that of *Enneanectes* (19, 21, 24, 31). Some of these characters are shared with *Helcogramma ellioti*, which resulted in their placement in a clade in the parsimony trees (Figure 16) as well as in Rosenblatt’s phylogeny (Figure 2). The monophyletic Pacific clade containing *Axoclinus*, *Crocodilichthys*, and some members of *Enneanectes* is supported by the thin shape of the coracoid (4).

Sister-species relationships reported by molecules have ambiguous support from the morphological dataset; for example, morphology supports the sister species pair *A.*
storeyae and *A. nigricaudus* instead of *A. storeyae* and *A. lucillae*, despite high support from gene trees (Figures 6 and 16). This may be due to homoplasy of characters associated with squamation and supination (Rosenblatt 1959).

**Biogeography**

According to the topology recovered with the seven-gene dataset, the Pacific species do not form a monophyletic group. Instead, *E. carminalis* is the sister group of all remaining species. The so-called ”Delicate Triplefin” has the broadest distribution of any TEP triplefin, occurring from the Gulf of California to Panama. It and the similar island endemic *E. exsul* are distinctive among the region's triplefins in having short, robust bodies (Rosenblatt et al. 2013). The Atlantic species of *Enneanectes* included in this study also do not form a monophyletic group. Several additional species, including cryptic species (Victor 2013), are known from the western Atlantic but were unavailable for this study. The remaining Pacific species included in this study, currently allocated among three genera (*Axoclinus*, *Enneanectes* and *Crocodilichthys*), form a monophyletic group, but increased taxon sampling is necessary to verify their monophyly.

Given that most blennies, including triplefins, are poor dispersers with short larval phases (Lin and Hastings 2011, Riggins and Victor 2001), allopatric speciation caused by oceanographic or vicariant barriers are often a driving force in their diversification (Hastings 2000, 2009). The ranges of the Pacific species in this study appear to fall within the three biogeographic provinces discussed by Hastings (2000): the Cortez Province including the Gulf of California and southern Baja California peninsula, the Mexican Province corresponding to Mazatlán, Sinaloa to the Isthmus of Tehuantepec in
southern Mexico, and the Panamic Province extending from the Gulf of Fonseca in Nicaragua to the Gulf of Guayaquil, Peru. Interestingly no transisthmian geminate species pairs were recovered in this study, despite the occurrence of several other examples of transisthmian geminates among Neotropical blennioids (Hastings 2009).

Within the Pacific species, the sister species pair of *C. gracilis* and *E. glendae*, are allopatric, separated by the Sinaloan Gap, a region of coastline largely devoid of rocky reefs (Hastings, 2000). A second species pair, *A. storeyae* and *A. lucillae*, occur allopatrically in the Cortez and Panamic provinces, respectively, but their ranges overlap in the intervening Mexican province. Tissue samples of several TEP triplefins endemic to various oceanic islands were unavailable. Their inclusion in a phylogenetic analysis would help to further clarify the biogeography of eastern Pacific triplefins.

**Concluding Remarks**

This study supports the monophyly of the genus *Axoclinus*, but not of *Enneanectes*. Given the uncertainty of relationships within the species currently allocated to *Enneanectes*, we do not recommend nomenclatural changes at this time. In addition, the monophyly of this entire group of Neotropical triplefins cannot be adequately tested without more thorough taxon sampling, despite the fact that triplefins are limited in their dispersal. Key taxa include the genera *Tripterygion* (6 species), *Ceratobregma* (2), *Springerichthys* (2), and *Enneapterygius* (53) that Fricke (1994) included in the Tripterygiini along with *Axoclinus, Crocodilichthys* and *Enneanectes* (Table 2). Given the morphological variation among the species currently included in *Enneanectes*, as well as the paraphyly revealed in this study, it is possible that the
included species have affinities with different lineages of triplefins from other regions and that these Neotropical triplefins represent multiple invasions from other parts of the world.

Submission for Publication

This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
Table 1: Summary of species sampled

<table>
<thead>
<tr>
<th>Species</th>
<th>Catalogue # of Exemplar Individual</th>
<th>Locality of Exemplar Individual</th>
<th>Range (FishBase and STRI Shorefishes Database)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidonectes corallicola</td>
<td>SIO 02-82</td>
<td>Isla Pinzon, Ecuador</td>
<td>Pacific: Galapagos Islands</td>
</tr>
<tr>
<td>Enneanectes carinalis</td>
<td>SIO 01-170</td>
<td>Golfo de Fonseca, El Salvador</td>
<td>Pacific: Central Baja to Gulf of California to Panama</td>
</tr>
<tr>
<td>Enneanectes boehlkei</td>
<td>KU 166</td>
<td>Carrie Bow Cay, Belize</td>
<td>Atlantic: Florida, Bahamas, Yucatan Peninsula, to Venezuela</td>
</tr>
<tr>
<td>Enneanectes pectoralis</td>
<td>KU 167</td>
<td>Carrie Bow Cay, Belize</td>
<td>Atlantic: Florida, Bahamas, Yucatan Peninsula to Venezuela</td>
</tr>
<tr>
<td>Enneanectes altivelis</td>
<td>KU 225</td>
<td>Carrie Bow Cay, Belize</td>
<td>Atlantic: Florida, Bahamas to Brazil</td>
</tr>
<tr>
<td>Enneanectes reticulatus</td>
<td>SIO 11-355</td>
<td>Isla Espiritu Santo, Mexico</td>
<td>Pacific: Lower Gulf of California</td>
</tr>
<tr>
<td>Enneanectes glendae</td>
<td>SIO 01-182</td>
<td>Puerto Vallarta, Mexico</td>
<td>Pacific: Central Mexico and surrounding islands</td>
</tr>
<tr>
<td>Enneanectes macrops</td>
<td>SIO 01-182</td>
<td>Puerto Vallarta, Mexico</td>
<td>Pacific: Gulf of California, Central and southern Mexico</td>
</tr>
<tr>
<td>Axoclinus nigricaudus</td>
<td>SIO 07-50</td>
<td>Punta Chivato, Mexico</td>
<td>Pacific: Western and northern Gulf of California</td>
</tr>
<tr>
<td>Axoclinus storeyae</td>
<td>SIO 09-267</td>
<td>Isla Tortuga, Mexico</td>
<td>Pacific: Southern Baja California and central Gulf of California to central Mexico</td>
</tr>
<tr>
<td>Axoclinus lucillae</td>
<td>SIO 01-164</td>
<td>Isla Taboquilla, Panama</td>
<td>Central Mexico to Colombia</td>
</tr>
<tr>
<td>Crocodilichthys gracilis</td>
<td>SIO 06-55</td>
<td>Bahia de Los Angeles, Mexico</td>
<td>Gulf of California</td>
</tr>
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</table>
Table 2: Distribution of species sampling effort.

<table>
<thead>
<tr>
<th>Genus</th>
<th># Sampled/Total</th>
<th>Pacific: # Sampled/Total</th>
<th>Atlantic: # Sampled/Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Described (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidonectes</td>
<td>1/3 (33%)</td>
<td>1/1</td>
<td>0</td>
</tr>
<tr>
<td>Enneanectes</td>
<td>7/15 (47%)</td>
<td>4/5</td>
<td>3/10</td>
</tr>
<tr>
<td>Axoclinus</td>
<td>3/6 (50%)</td>
<td>3/6</td>
<td>0</td>
</tr>
<tr>
<td>Crocodilichthys</td>
<td>1/1 (100%)</td>
<td>1/1</td>
<td>0</td>
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</tbody>
</table>
Table 3: Additional material examined for morphological analysis

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Number</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crocodilichthys gracilis</em></td>
<td>SIO 09-264</td>
<td>Isla Espiritu Santo, Southern Gulf of California, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 65-323</td>
<td>Isla Carmen, Central Gulf of California, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 08-120</td>
<td>Bahia de los Angeles, Central Gulf of California, Mexico</td>
</tr>
<tr>
<td><em>Axoclinus nigricaudus</em></td>
<td>SIO 61-265</td>
<td>Isla Espiritu Santo, Southern Gulf of California, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 65-330</td>
<td>Isla Ildefonso, Central Gulf of California, Mexico</td>
</tr>
<tr>
<td><em>Axoclinus storeyae</em></td>
<td>SIO 04-145</td>
<td>Isla Dazante, Central Gulf of California, Mexico</td>
</tr>
<tr>
<td><em>Axoclinus lucillae</em></td>
<td>SIO 67-36</td>
<td>Pearl Islands, Panama</td>
</tr>
<tr>
<td></td>
<td>SIO 67-38-61</td>
<td></td>
</tr>
<tr>
<td><em>Enneanectes reticulatus</em></td>
<td>SIO 65-331</td>
<td>Isla Ildefonso, Central Gulf of California, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 65-261-61</td>
<td>Isla San Jose, Southern Gulf of California, Mexico</td>
</tr>
<tr>
<td><em>Enneanectes carinalis</em></td>
<td>SIO 62-56</td>
<td>Tres Marias Islands, Mexico</td>
</tr>
<tr>
<td><em>Enneanectes glendae</em></td>
<td>SIO 70-155</td>
<td>Zihuatanejo, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 70-163</td>
<td>Bahia Navidad, Mexico</td>
</tr>
<tr>
<td><em>Enneanectes macrops</em></td>
<td>SIO 70-155</td>
<td>Zihuatanejo, Mexico</td>
</tr>
<tr>
<td></td>
<td>SIO 11-399</td>
<td>Isla Venado, Mexico</td>
</tr>
<tr>
<td><em>Enneanectes altivelis</em></td>
<td>LACM 6727-9</td>
<td>Lagoen, Curacao, Netherland Antilles</td>
</tr>
<tr>
<td></td>
<td>LACM 8938-17</td>
<td>Montego Bay, Jamaica</td>
</tr>
<tr>
<td><em>Enneanectes boehlkei</em></td>
<td>SIO 06-64</td>
<td>Navassa Island, Caribbean, U.S.A</td>
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<td>LACM 54087</td>
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<td><em>Enneanectes pectoralis</em></td>
<td>LACM 6162</td>
<td>Lime Cay, Jamaica</td>
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<td></td>
<td>LACM 6160</td>
<td>Pedro Cays, Jamaica</td>
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<td></td>
<td>LACM 5943</td>
<td>Runaway Bay, Jamaica</td>
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<tr>
<td><em>Lepidonectes clarkhubbsi</em></td>
<td>SIO 67-39</td>
<td>Pearl Islands, Panama</td>
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<tr>
<td><em>Helcogrammoides cunninghami</em></td>
<td>SIO 65-678</td>
<td>La Ventana, Valparaiso, Chile</td>
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<tr>
<td><em>Helcogramma elliotti</em></td>
<td>CAS 217490</td>
<td>Tiapea Point, American Samoa</td>
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Table 4: Summary of genes and primers.

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<th>Primer Sequences</th>
<th>Primer Reference</th>
<th>Model by JModelTest</th>
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<td>12SFor</td>
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<td>Almada et al 2005</td>
<td>GTR+I+G</td>
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<td>12S Rev</td>
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<td>16S</td>
<td>16SAR</td>
<td>CGGCTGGTTATCAAAACAT</td>
<td>Palumbi et al., 1991</td>
<td>GTR+I</td>
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<td>16SBR</td>
<td>CCGTCTGAACTCAGATCAGT</td>
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<td>CO1</td>
<td>Fish-F1</td>
<td>TCAACACACCAAAGACATTGGCAC</td>
<td>Ward et al., 2005</td>
<td>GTR+I+G (1+2 codons only: TrN+I)</td>
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<td>Fish-R2</td>
<td>ACTTCAGGGTGACCGAAGAATCAGA</td>
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<td>ExoCBFwd</td>
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<td>Sevilla et al., 2007</td>
<td>GTR+I+G (1+2 codons only: TrN+I)</td>
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<td></td>
<td>AACTTCAGGGTGACCGAAGAATCAGA</td>
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<td>Rhodopsin</td>
<td>RhodF</td>
<td>CCGTCATGGGGCGCTAYATGTTYT</td>
<td>Taylor &amp; Hellberg, 2005; Lin and Hastings 2013</td>
<td>TrN+I</td>
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<td>RhodR</td>
<td>CAGCACAGGGTGATCATRCARTG</td>
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<td>Rhod-BleF</td>
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<td></td>
<td>Rhod-BleR</td>
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<tr>
<td>Rag-1</td>
<td>Rag-F1</td>
<td>AGCAGGCTACCTGTCCTCAT</td>
<td>Lin and Hastings 2011</td>
<td>GTR+G</td>
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<tr>
<td></td>
<td>Rag-R2</td>
<td>GGGTGATGGAGTGCAAGCCACCATGTT</td>
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<tr>
<td>TMO-4C4</td>
<td>TMO-F2</td>
<td>GAKTGGTTGAAAATGACTCGCTA</td>
<td>Near et al. 2004, Lin and Hastings 2011, Hastings 2013</td>
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<td>TMO-F3</td>
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<tr>
<td>Number</td>
<td>Description</td>
<td>Character States</td>
<td>PI?</td>
<td>Reference</td>
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<td>--------</td>
<td>-----------------------------------------------</td>
<td>----------------------------</td>
<td>-----</td>
<td>--------------------</td>
</tr>
<tr>
<td>1</td>
<td># Epurals</td>
<td>2 or 3</td>
<td>No</td>
<td>Fricke 1994</td>
</tr>
<tr>
<td>2</td>
<td>Palatine teeth</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Fishbase</td>
</tr>
<tr>
<td>3</td>
<td>Dorsal-most pectoral rays free from scapula</td>
<td>0, 1, or 2</td>
<td>Yes</td>
<td>Jawad 2008</td>
</tr>
<tr>
<td>4</td>
<td>Coracoid shape</td>
<td>Thin (1) or squat (0)</td>
<td>Yes</td>
<td>Jawad 2008</td>
</tr>
<tr>
<td>5</td>
<td>Pterygiophore formula</td>
<td>Variations 1, 2, and 3*</td>
<td>No</td>
<td>Jawad 2008</td>
</tr>
<tr>
<td>6</td>
<td>Shape of 4th dorsal pterygiophore</td>
<td>Broad (1), moderate (2), thin (3)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>7</td>
<td>Overall size of head pores</td>
<td>Small (1), moderate (2), large (3)**</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>8</td>
<td>Overall number of head pores</td>
<td>Many (1), Moderate (2), Few (3)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>9</td>
<td>Paired on mandibular</td>
<td>2+ pairs (1), one pair (2), none (3)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>10</td>
<td>Paired on preopercle</td>
<td>Yes (1) or no (0)</td>
<td>Yes</td>
<td>This study</td>
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<tr>
<td>11</td>
<td>Paired on subocular</td>
<td>Yes (1) or no (0)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>12</td>
<td>Mandibular pit</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>13</td>
<td>Lateral line</td>
<td>Continuous (1) or discontinuous (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>14</td>
<td>Belly scalation</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959, Fricke 1994</td>
</tr>
<tr>
<td>15</td>
<td>Operculum scalation</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959, Jawad 2005</td>
</tr>
<tr>
<td>16</td>
<td>Pectoral fin base scalation</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959, Jawad 2005</td>
</tr>
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<td>17</td>
<td>First dorsal base scalation</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959, Jawad 2005</td>
</tr>
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<td>18</td>
<td>Anal fin base scalation</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959, Jawad 2005</td>
</tr>
<tr>
<td>19</td>
<td># Scale rows above anterior lateral line</td>
<td>3, 4, 5 or more</td>
<td>Yes</td>
<td>Jawad 2005</td>
</tr>
<tr>
<td>20</td>
<td>Scale cteni</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Jawad 2005</td>
</tr>
<tr>
<td>21</td>
<td>Shape of pored lateral line scales</td>
<td>Rounded (1) or angular (0)</td>
<td>Yes</td>
<td>Jawad 2005</td>
</tr>
<tr>
<td>22</td>
<td>Shape of lateral line canals</td>
<td>Straight (1) or constricted (0)</td>
<td>Yes</td>
<td>Jawad 2005</td>
</tr>
<tr>
<td>23</td>
<td>Length of lateral line canals</td>
<td>Long (1) or short (0)</td>
<td>Yes</td>
<td>Jawad 2005</td>
</tr>
<tr>
<td>24</td>
<td>Location of lateral line canal</td>
<td>Center (1) or angled (0) on scale</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>25</td>
<td>Scales above lateral line larger than lateral line scales</td>
<td>Yes (1) or no (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
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</tbody>
</table>
Table 5, continued.

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Character States</th>
<th>PI?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Scales on cheek</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>27</td>
<td>Spinules on nasal bone</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>28</td>
<td>Spinules on anterior orbital margin</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>29</td>
<td>Spinules on top of head</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>30</td>
<td>Spinules on supraorbital margin</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>31</td>
<td>Lateral line location</td>
<td>Middle (1) or upper half (0) of body</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>32</td>
<td>Branched rays in 3rd dorsal</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>33</td>
<td>Upper unbranched pectoral rays</td>
<td>1, 2, or 3+ (3)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>34</td>
<td>Membrane connecting spinous dorsals</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>35</td>
<td>Pelvic rays united by membrane</td>
<td>Yes (1), very small (2), no (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
<td>36</td>
<td>Supraorbital cirrus</td>
<td>None (0), Simple (1), Flaplike (2), Frilled (3)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
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<tr>
<td>37</td>
<td>Head shape</td>
<td>Rounded (0), Pointed (1), Strongly pointed (2)</td>
<td>Yes</td>
<td>Fricke 1994</td>
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<td>38</td>
<td>Edge of cheek with spinules</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
</tr>
<tr>
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<td>First spine of first dorsal with spinules</td>
<td>Present (1) or absent (0)</td>
<td>Yes</td>
<td>Rosenblatt 1959</td>
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<tr>
<td>40</td>
<td>Second dorsal spines***</td>
<td>Low (0), moderate (1), high (2)</td>
<td>Yes</td>
<td>This study</td>
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<tr>
<td>41</td>
<td>Pectoral fin rays***</td>
<td>Low (0), moderate (1), high (2)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>42</td>
<td>Anal fin rays***</td>
<td>Low (0), moderate (1), high (2)</td>
<td>Yes</td>
<td>This study</td>
</tr>
<tr>
<td>43</td>
<td>Third dorsal rays***</td>
<td>Low (0), moderate (1), high (2)</td>
<td>Yes</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Pterygiophore variations: 3-N-0-0-1-1-1 (1); 3-N-0-1-1-1-1 (2); 3-N-0-1-0-1-1 (3)
** If variable in size from moderate to large, scored as moderate
*** Meristics scored as follows:

<table>
<thead>
<tr>
<th></th>
<th>Anal fin rays</th>
<th>Second Dorsal Spines</th>
<th>Third Dorsal Rays</th>
<th>Pectoral Fin Rays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>14-16</td>
<td>11</td>
<td>7 to 8</td>
<td>14</td>
</tr>
<tr>
<td>Moderate</td>
<td>17-18</td>
<td>12 to 13</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>High</td>
<td>19+</td>
<td>14+</td>
<td>10+</td>
<td>16+</td>
</tr>
</tbody>
</table>

Note: If variation was recorded, scored as moderate if range values included those in the “moderate” score.
Table 6: Best partition scheme and models determined by PartitionFinder.
(All Models: Scheme AIC= 23172.66; lnL= -15944.33;
MrBayes Models: Scheme AIC= 32192.08; lnL= -15941.04)

<table>
<thead>
<tr>
<th>Partition #</th>
<th>Partitions</th>
<th>Best Model</th>
<th>Best Model (MrBayes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12S, 16S</td>
<td>GTR+I+G</td>
<td>GTR+I+G</td>
</tr>
<tr>
<td>2</td>
<td>CO1_1</td>
<td>GTR+G</td>
<td>GTR+G</td>
</tr>
<tr>
<td>3</td>
<td>CO1_2</td>
<td>F81+I</td>
<td>F81+I</td>
</tr>
<tr>
<td>4</td>
<td>CO1_3</td>
<td>TrN+I+G</td>
<td>GTR+I+G</td>
</tr>
<tr>
<td>5</td>
<td>CytB_1</td>
<td>TrNef+I+G</td>
<td>SYM+I+G</td>
</tr>
<tr>
<td>6</td>
<td>CytB_2</td>
<td>TVM</td>
<td>GTR</td>
</tr>
<tr>
<td>7</td>
<td>CytB_3</td>
<td>TIM+I+G</td>
<td>GTR+I+G</td>
</tr>
<tr>
<td>8</td>
<td>Rag1_1</td>
<td>TrN+G</td>
<td>F81+G</td>
</tr>
<tr>
<td>9</td>
<td>Rag1_2</td>
<td>TVMef+G</td>
<td>K80+G</td>
</tr>
<tr>
<td>10</td>
<td>Rag1_3</td>
<td>HKY</td>
<td>HKY</td>
</tr>
<tr>
<td>11</td>
<td>Rhod_1</td>
<td>TrN+I+G</td>
<td>GTR+I+G</td>
</tr>
<tr>
<td>12</td>
<td>Rhod_2</td>
<td>K81uf+I</td>
<td>HKY+I</td>
</tr>
<tr>
<td>13</td>
<td>Rhod_3</td>
<td>TVM+G</td>
<td>GTR+G</td>
</tr>
<tr>
<td>14</td>
<td>TMO_1</td>
<td>GTR+G</td>
<td>GTR+G</td>
</tr>
<tr>
<td>15</td>
<td>TMO_2</td>
<td>TrN+G</td>
<td>GTR+G</td>
</tr>
<tr>
<td>16</td>
<td>TMO_3</td>
<td>K81uf+G</td>
<td>GTR+G</td>
</tr>
</tbody>
</table>
Table 7: Summary of sites sampled.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Codon Position</th>
<th>Size (bp)</th>
<th>Constant Sites</th>
<th>P-I Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>12S</td>
<td>Non-coding</td>
<td>368</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>16S</td>
<td>Non-coding</td>
<td>549</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>CO1</td>
<td>1</td>
<td>190</td>
<td>162</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>190</td>
<td>190</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1+2</td>
<td>380</td>
<td>352</td>
<td>11</td>
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<tr>
<td></td>
<td>3</td>
<td>189</td>
<td>11</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>569</td>
<td>363</td>
<td>162</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>1</td>
<td>283</td>
<td>229</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>283</td>
<td>272</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1+2</td>
<td>566</td>
<td>501</td>
<td>26</td>
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<td></td>
<td>3</td>
<td>284</td>
<td>6</td>
<td>249</td>
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<tr>
<td></td>
<td>All</td>
<td>850</td>
<td>507</td>
<td>275</td>
</tr>
<tr>
<td>Rag 1</td>
<td>1</td>
<td>256</td>
<td>207</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>257</td>
<td>257</td>
<td>12</td>
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<tr>
<td></td>
<td>3</td>
<td>256</td>
<td>104</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>769</td>
<td>515</td>
<td>159</td>
</tr>
<tr>
<td>Rhodopsin</td>
<td>1</td>
<td>243</td>
<td>230</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>243</td>
<td>239</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>243</td>
<td>173</td>
<td>30</td>
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<tr>
<td></td>
<td>All</td>
<td>729</td>
<td>642</td>
<td>39</td>
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<tr>
<td>TMO-4C4</td>
<td>1</td>
<td>122</td>
<td>110</td>
<td>4</td>
</tr>
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<td></td>
<td>2</td>
<td>121</td>
<td>112</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>121</td>
<td>46</td>
<td>30</td>
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<tr>
<td></td>
<td>All</td>
<td>364</td>
<td>268</td>
<td>36</td>
</tr>
</tbody>
</table>
Table 8: Summary of Xia’s test of saturation in DAMBE. Only those positions that had evidence of saturation are shown.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Iss (symmetric tree)</th>
<th>Iss.c (symm. Tree)</th>
<th>P-value (symm. Tree)</th>
<th>Iss.c (asymmetric tree)</th>
<th>P-value (asymm. Tree)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO1 3rd codon</td>
<td>0.6112</td>
<td>0.6919</td>
<td>.0097</td>
<td>.5540</td>
<td>.0653</td>
<td>“Useless sequences” if asymm. tree</td>
</tr>
<tr>
<td>CytB 3rd codon</td>
<td>0.6904</td>
<td>0.6906</td>
<td>.99</td>
<td>0.5274</td>
<td>0.00</td>
<td>“Useless sequences” if asymm. tree</td>
</tr>
<tr>
<td>Rag1 3rd codon</td>
<td>0.5037</td>
<td>0.7361</td>
<td>0.000</td>
<td>0.6539</td>
<td>0.00</td>
<td>“Little saturation”</td>
</tr>
</tbody>
</table>
Table 9: Summary of datasets.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Number of Genes/Number of Partitions</th>
<th>Size (bp)</th>
<th>Parsimony-Informative Sites</th>
<th>Score of Best Tree in PAUP</th>
<th>lnL Score of ML Tree</th>
<th>lnL Score range of MrBayes runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA,</td>
<td>4/7</td>
<td>2336</td>
<td>542</td>
<td>2191</td>
<td>-10521.03</td>
<td>-11380 to -11340</td>
</tr>
<tr>
<td>mtDNA, saturation removed</td>
<td>4/5</td>
<td>1863</td>
<td>142</td>
<td>575</td>
<td>-5150.58</td>
<td>-5250 to -5205</td>
</tr>
<tr>
<td>nuDNA</td>
<td>3/9</td>
<td>1862</td>
<td>234</td>
<td>653</td>
<td>-5288.43</td>
<td>-5340 to -5290</td>
</tr>
<tr>
<td>Total, partition by gene</td>
<td>7/7</td>
<td>4198</td>
<td>776</td>
<td>2867</td>
<td>-17217.87</td>
<td>-17190 to -17135</td>
</tr>
<tr>
<td>Total, partition by gene, sat. removed</td>
<td>7/7</td>
<td>3725</td>
<td>376</td>
<td>1239</td>
<td>-11073.46</td>
<td>-11090 to -11030</td>
</tr>
<tr>
<td>Total, partition by codon</td>
<td>7/16</td>
<td>4198</td>
<td>776</td>
<td>2867</td>
<td>-15961.21</td>
<td>-16030 to -15960</td>
</tr>
<tr>
<td>Total, partition by codon, sat. removed</td>
<td>7/14</td>
<td>3725</td>
<td>376</td>
<td>1239</td>
<td>-10565.48</td>
<td>-10620 to -10565</td>
</tr>
<tr>
<td>Morphology</td>
<td>-</td>
<td>43 (characters)</td>
<td>41</td>
<td>144</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 10: Morphological matrix analyzed in this study. (-) represents missing or ambiguous data.

| Species                   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| A. lucilla                 | 2  | 0  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1  | 4  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 1  |
| A. nigricaudus             | 2  | 0  | 1  | 1  | 1  | 2  | 1  | 2  | 3  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 5  | 1  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  |
| A. storeyae               | 2  | 0  | 1  | 1  | 2  | 1  | 2  | 3  | 2  | 1  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 5  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  |
| C. gracilis               | 2  | 0  | 2  | 1  | 2  | 2  | 2  | 3  | 3  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 1  | 5  | 1  | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 1  |
| E. altivelis              | 2  | 0  | 0  | 0  | 0  | 2  | 2  | 3  | 3  | 3  | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 1  | 3  | 1  | 0  | 1  | 0  | 1  | 1  | 0  | 0  | 0  |
| E. boehlkei               | 2  | 0  | 2  | 0  | 2  | 3  | 1  | 3  | 3  | 3  | 3  | 3  | 3  | -  | -  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 3  | 1  | 0  | 0  | 0  | 0  | 0  |
| E. carminalis             | 2  | 0  | 2  | 0  | 2  | 3  | 2  | 2  | 3  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 1  | 1  | 2  | 1  | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  |
| E. glendae                | 2  | 0  | 2  | 1  | 2  | 1  | 3  | 3  | 0  | 1  | 1  | 0  | 0  | 1  | 0  | 0  | 1  | 1  | 3  | 1  | 0  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  |
| E. macrops                | 2  | 0  | 2  | 1  | 2  | 3  | 1  | 3  | 2  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 1  | 1  | 3  | 1  | 0  | 1  | 1  | 1  | 0  | 0  | 1  | 1  |
| E. pectoralis             | 2  | 0  | 1  | 0  | 2  | 3  | 1  | 2  | 2  | 0  | 1  | 0  | 0  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 3  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  |
| E. reticulatus            | 2  | 0  | 2  | 1  | 2  | 3  | 1  | 3  | 2  | 1  | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 3  | 1  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 0  |
| Helcogramma ellioti       | -  | 1  | 0  | 0  | 2  | -  | 2  | 2  | 2  | -  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | -  | 0  | 1  | 1  | 1  | 1  | 0  | 0  | 0  |
| L. clarkhubbsi            | 3  | 1  | 1  | 1  | 2  | 3  | 2  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 1  | 4  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 1  | 1  |
| Helcogrammoidec cunningham | 2  | -  | 2  | 1  | 2  | 1  | 2  | 2  | 3  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 1  | 1  | 4  | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 1  | 1  |

Table continued...
Submission for Publication

This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
Figure 1: Photographs of genera included in this study. Top left: *Enneanectes reticulatus*. Top right: *Lepidonectes corallicola*. Bottom Left: *Axoclinus lucillae*. Bottom Right: *Crocodilichthys gracilis*. Photographs by Ross Robertson and Gerald Allen, STRI Shorefishes of the Tropical Eastern Pacific Database.
Figure 2: Previous hypotheses of relationships among the Tripterygiidae. a) Rosenblatt 1959 family-level phylogeny based on morphology. Neotropical genera in bold; b) Fricke 1994 family-level phylogeny based on morphology. Neotropical genera in bold; c) Rosenblatt 1959 phylogeny of *Enneanectes* species based on morphology; d) Rosenblatt 1959 phylogeny of *Axocinus* species based on morphology; e) Lin and Hastings 2013 family-level phylogeny based on 4 nuclear and 1 mitochondrial markers. Support values indicate: ML bootstrap, parsimony bootstrap, and Bayesian posterior probabilities.
Figure 3: Plots of transitions vs. transversions for each marker.
Figure 4: Bayesian gene trees for mitochondrial markers. a) 12S; b) 16S; c) CO1; d) Cytochrome b. Stars represent well-supported nodes (>0.95 posterior probabilities).
Figure 5: Bayesian gene trees for nuclear markers. a) Histone H-3, b) Rag-1, c) Rhodopsin, d) TMO-4C4. Stars represent well-supported nodes (>0.95 posterior probabilities).
Figure 6: Topology of 7-gene dataset, showing support from individual sources. Black shading indicates the node is supported; grey indicates node not supported due to polytomies or missing data, but not in conflict; white indicates conflicting node was supported. “Historical” means the node was recovered by Lin and Hastings 2013; none of the nodes recovered by this study were shared with previously published morphological phylogenies. * = Node was well supported (>70) in ML analysis of 7-gene dataset partitioned by PartitionFinder. *Lepidonectes* outgroup is excluded for clarity.
Figure 7: Maximum likelihood tree for mtDNA dataset with all codons. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 8: Maximum likelihood tree for mtDNA dataset with saturation removed. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 9: Maximum likelihood tree for nuclear dataset. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 10: Maximum likelihood tree for total dataset, partitioned by gene, with all codons. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 11: Maximum likelihood tree for total dataset, partitioned by gene, with saturation removed. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 12: Maximum likelihood tree for total dataset, partitioned by codon position, with all codons. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 13: Maximum likelihood tree for total dataset, partitioned by codon position, with saturation removed. Support values are: ML bootstrap, Parsimony jackknife, and Bayesian posterior probabilities.
Figure 14: Bayesian trees for total dataset: a) partitioned by gene, all codons; b) partitioned by gene, saturation removed; c) partitioned by codon position, all codons; d) partitioned by codon position, saturation removed. Support values are Bayesian posterior probabilities. *Lepidonectes* outgroup excluded for clarity.
Figure 15: Range mapped on topology from total dataset, with table of distribution among TEP provinces (Hastings 2000)
Figure 16: Maximum parsimony trees for morphological dataset. Left: All nodes are shown (“KEEPALL” command); Right: Nodes with jackknife support values <50 are collapsed as polytomies.
Submission for Publication

This thesis will be submitted for publication. The thesis author was the primary investigator of this material. In addition to P.A. Hastings, H.C. Lin will be listed as a co-author for her contribution of sequences for analysis.
REFERENCES


(Subfamily Chaenopsinae, Suborder Blennioidei) based on phylogenetic analysis of combined molecular and morphological data. *Molecular Phylogenetics and Evolution, 60*(2), 236-248.


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Robertson, D. R., & Allen, G. R. 2008. Shorefishes of the Tropical Eastern Pacific online information system. *Smithsonian Tropical Research Institute, Balboa* (available at: [www.stri.org/sftep](http://www.stri.org/sftep)).


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