

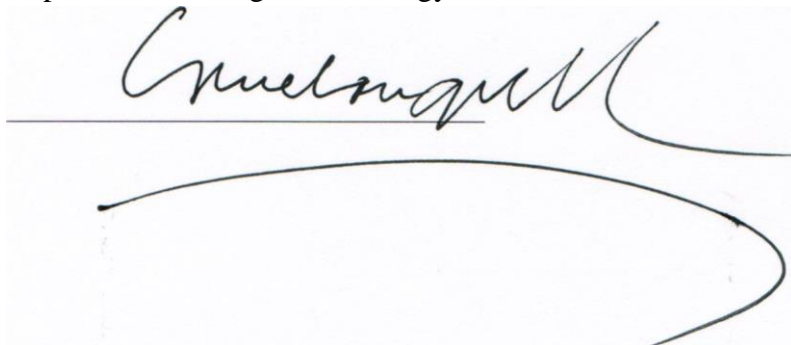
Phylogeny of the Tiger Catfish Group, *Pseudoplatystoma*, Conflicts and Resolutions

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A handwritten signature in black ink, appearing to read 'Uriel Buitrago', written over a horizontal line. Below the signature is a large, stylized, curved flourish that extends from the end of the signature line.

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A handwritten signature in black ink, appearing to read 'Jason Belden', written over a horizontal line. The signature is highly stylized and slanted to the right.

Acknowledgments

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Abstract

The South American catfish family Pimelodidae (long whiskered catfish) includes the tiger catfish genus of *Pseudoplatystoma* (Buitrago-Suárez and Burr 2007). These tiger catfish are an economically, culturally, and ecologically valuable genus, with use in commercial fishing, aquiculture, and as top predators (Carvalho-Costa et al. 2011; Saulo-Machado et al. 2010; Nirchio et al. 2013). There are eight species currently described within the genus, including *P. corruscans*, *P. fasciatum*, *P. tigrinum*, *P. punctifer*, *P. reticulatum*, *P. metaense*, *P. orinocoense*, and *P. magdaleniatum* (Buitrago-Suárez and Burr 2007). The genus has recently received increased attention regarding the species' possible phylogenetic relationships. The present study used GenBank to create parsimonious phylogenetic trees, and then compared these phylogenetic relationships with previous molecular studies. Five trees were created using genes cytochrome oxidase subunit I (COI), cytochrome-b (cyt-b), recombination activating protein 1 (*rag1*) exons 1,2,3 from the 3' end and from the 5' end, and recombination activating protein 2 (*rag2*). The resulting trees formed had congruencies with previous research, finding evidence for *P. metaense* and *P. tigrinum* to be a strongly supported clade (Nirchio et al. 2013; Carvalho-Costa et al. 2011). While more research is needed to determine the exact relationship of these eight species, the present study has found strong evidence to further support the clade of *P. metaense* and *P. tigrinum* with the *Pseudoplatystoma* genus.

Introduction

The genus *Pseudoplatystoma* includes several species of South American catfish within the Family Pimelodidae (Buitrago-Suárez and Burr 2007). *Pseudoplatystoma* is an economically important genus for commercial fishing due to its wide distribution throughout the major river basins of South America (Carvalho-Costa et al. 2011). These boldly striped or spotted catfishes are also important ecologically, playing a significant role as top piscivorous predators and widely distributed migratory fish (Nirchio et al. 2013). They have become a valued resource in aquaculture due to their high growth rate (Saulo-Machado et al. 2010), and are now one of the most common farmed genus of fish in Brazil (Jeronimo et al. 2016) and most expensive catfish species in the fish markets of South America (Nunez et al. 2008). The three original species described within this genus included *P. corruscans*, *P. fasciatum*, and *P. tigrinum*; however, a morphological review of the genus by Buitrago-Suárez and Burr included additional species, bringing the total up to eight species now recognized within the genus. These species include *P. corruscans*, *P. fasciatum*, *P. tigrinum*, *P. metaense*, *P. magdaleniatum*, *P. reticulatum*, *P. orinocoense*, and *P. punctifer* (Buitrago-Suárez and Burr 2007). Monophyly of the genus *Pseudoplatystoma* has now been well supported by both morphological synapomorphies as well as molecular data (Lundbert et al 2011). Better research on the relatedness of this genus will not only further the overall understanding of these fish, but also allow for better farming practices and improved wellness for the *Pseudoplatystoma* fish that are used in aquaculture.



FIGURE 1: *Pseudoplatystoma reticulatum* (above) and *P. corruscans* (below) from Paraná River, near Esquina, Argentina. April 2005. Photo by Mark. H. Sabaj (Buitrago-Suárez and Burr 2007).

The phylogenetic relationships between these species were analyzed in a morphological study that separated the genus into eight species (Buitrago-Suárez and Burr 2007). The exact phylogenetic relationships between these eight species has since been examined and debated. A study by Torrico and others later completed a full phylogenetic review of this group using molecular analysis with the cytochrome-b (*cyt-b*) and mitochondrial control region (CR) genes. Their molecular data showed that *P. tigrinum*, *P. corruscans*, *P. reticulatum*, and *P. magdaleniatum* are highly supported clades, but failed to find differences in *P. metaense* and *P. orinocoense*, as well as *P. punctifer* and *P. fasciatum* at the molecular level (Torrico et al. 2009). A cytogenetic and molecular analyses by Nirchio and others focused on *P. metaense* and *P.*

orinocoense, and their analysis of cytochrome-b (*cyt-b*) and cytochrome oxidase subunit I (COI) did place the specimens into two distinct molecular clades. Regarding *P. metaense* and *P. tigrinum*, however, results from using all the available sequences of *Pseudoplatystoma* *cyt-b* and COI genes failed to distinguish the two as separated clades within their study (Nirchio et al 2013). In another study, the phylogenetics of the family Pimelodidae was examined using nuclear and mitochondrial gene sequences, including *rag1* and *rag2* nuclear genes, as well as 12S, 16S, and cytochrome-b mitochondrial genes. For this study, researchers used four species of *Pseudoplatystoma*, and of their four found an arrangement of *P. magdaleniatum*, *P. corruscans*, *P. fasciatum*, and *P. tigrinum* (Lundbert et al 2011).

For the present study, GenBank was used to establish phylogenetically related species. The two main genes examined were the cytochrome oxidase subunit 1 (COI) and the cytochrome-b (*cyt-b*) gene. Other genes were available in GenBank, but only for a few of the species. Since the goal of this study was to provide a full phylogenetic analysis including all potential members of the genus, the two genes with complete representation by all eight species were used for in depth analysis, with use of two non-encompassing genes mainly for additional reference. The genes that are not available for all species, but are still used for comparison, include *rag1* and *rag2*, which for example, was not available for the *P. fasciatum*, *P. orinocoense*, *P. metaense*, or *P. punctifer* for *rag2*. We examined the phylogenetic trees completed by COI and *cyt-b* for congruencies, and referenced the trees formed from *rag1* and *rag2* for additional support. These trees were then compared to previous molecular analysis.

Methods

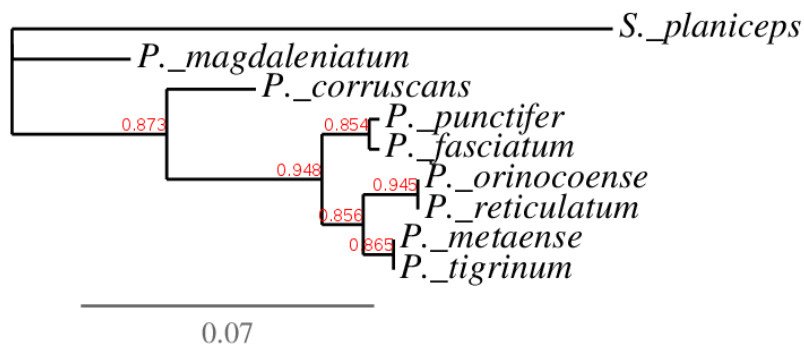
An exhaustive search in GenBank to find genes representative of all eight described species of *Pseudoplatystoma* was performed. The genes cytochrome oxidase subunit I (COI) and cytochrome-b (cyt-b) were found to be sequenced and available for all species. In addition, the recombination activating protein 1 (*rag1*) exons 1,2,3 from the 3' end, recombination activating protein 1 (*rag1*) exons 1,2,3 from the 5' end, and recombination activating protein 2 (*rag2*) were also recovered for this study, but failed to be inclusive of all eight species. Recovered genetic markers from the GenBank search were then used to establish degrees of resolutions and conflicts between potential phylogenetic hypotheses of the group. Sequences were analyzed to recover compelling phylogenetic trees using MABL (Methodes et Algorithmes pour la Bio-informatique LIRMM). Trees were rooted by attaching closed related taxa, *Sorubimichthys planiceps* (*S. planiceps*) in the analyses.

The best phylogenetic trees were recovered using parsimony analysis implemented by MABL. The compelling phylogenetic trees recovered from the genetic markers were compared to determine their resolutions and conflicts. The trees were also compared with previous molecular data, i.e., Nirchio et al. (2013) and Carvalho-Costa et al. (2011). Both, conflicting and resolving nodes for the different phylogenies were analyzed and discussed.

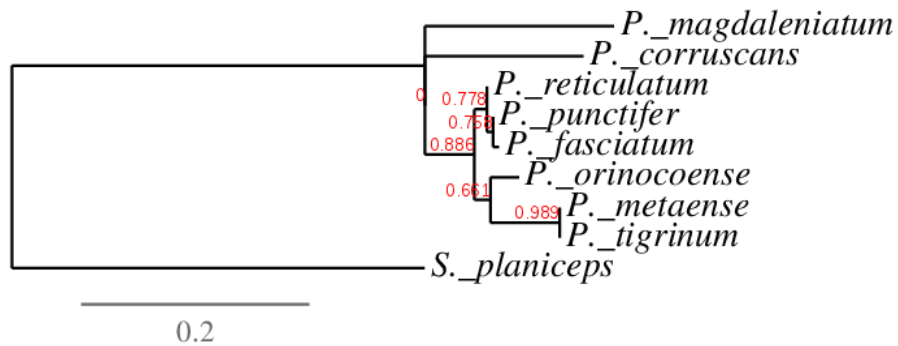
Results

The two markers COI and cyt-b were recovered from the exhaustive search in GenBank, as well as two not complete genes *rag1* (3' and 5' ends) and *rag2*. These four genes were then created into phylogenetic trees to establish their evolutionary relationships. *Sorubimichthys planiceps* (*S. planiceps*) was used as the outgroup that the trees were rooted to. Once phylogenetic trees were created from the MABL program, the best trees were determined using parsimony.

The first tree created was with the cytochrome oxidase subunit 1 (COI) gene, and determined *P. magdaleniatum* to be most primitive and closely related to *S. planiceps*, with *P. corruscans* in the next node. *P. punctifer* and *P. fasciatum* were in their own next node, *P. orinocoense* and *P. reticulatum* in a node together, and *P. metaense* and *P. tigrinum* in the most derive node (Tree 1). The resulting tree from the cytochrome-b (cyt-b) gene also found *P. magdaleniatum* to be most ancestral, and *P. corruscans* to be in the next most primitive node. After those two, *P. reticulatum*, *P. punctifer*, and *P. fasciatum* were in the next node, and *P. orinocoense*, *P. metaense*, and *P. tigrinum* were in the most derived node (Tree 2).

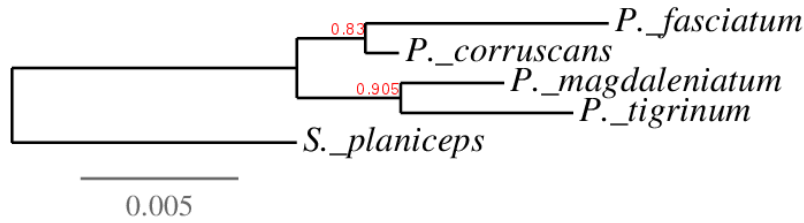


Tree 1: cytochrome oxidase subunit 1 (COI)

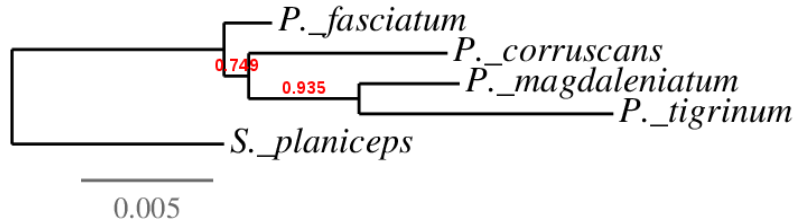


Tree 2: cytochrome-b (cyt-b)

The subsequent three trees were constructed with some, but not all, of the *Pseudoplatystoma* species. Two trees were created with the recombination activating protein 1 (*rag1*) exons 1, 2, and 3 and included the species *P. fasciatum*, *P. corruscans*, *P. magdaleniatum*, and *P. tigrinum*. The first tree was taken from the 3' end (Tree 3a) and the second was formed using the 5' end (Tree 3b). Both trees consistently found *P. fasciatum* to be most ancestral, and *P. magdaleniatum* and *P. tigrinum* to be in their own, most derived, node. The 3' end of *rag1* determined *P. fasciatum* and *P. corruscans* to be in a group together, and *P. magdaleniatum* and *P. tigrinum* to be in a second node together (Tree 3a). The tree formed from the 5' end of *rag1*, however, found *P. fasciatum* to be in a separate more ancestral node than *P. corruscans*, with the two of them being slightly separated (Tree 3b).

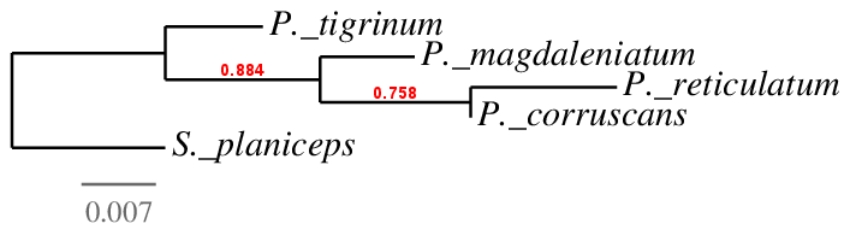


Tree 3a: *rag1*, exons 1, 2, 3; 3' end



Tree 3b: *rag1*, exons 1, 2, 3; 5' end

The last tree for this project was formed from the recombination activating protein 2 (*rag2*) gene. This tree included four species of *Pseudoplatystoma*: *P. tigrinum*, *P. magdaleniatum*, *P. reticulatum*, and *P. corruscans*. It determined *P. corruscans* and *P. reticulatum* to be in a node together, *P. magdaleniatum* to be in its own node, and *P. tigrinum* to be alone and separated most from the group (Tree 4).

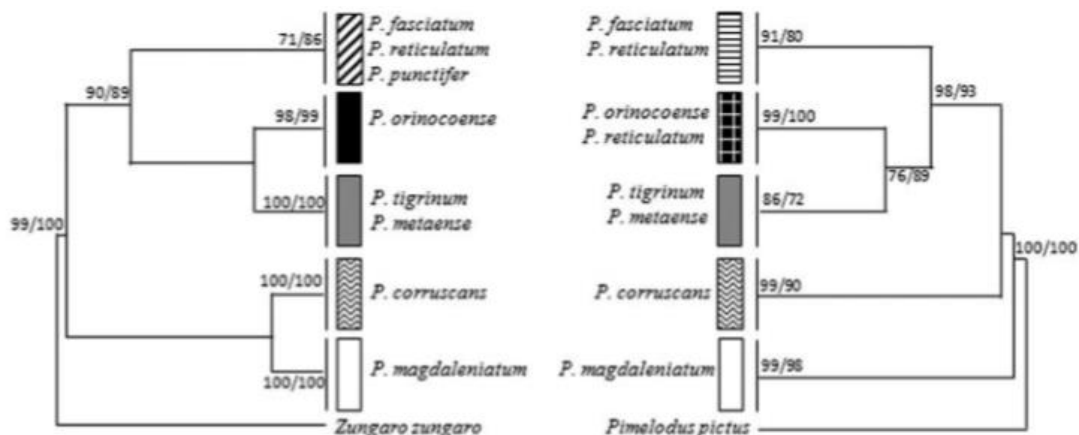


Tree 4: recombination activating protein 2 (*rag2*)

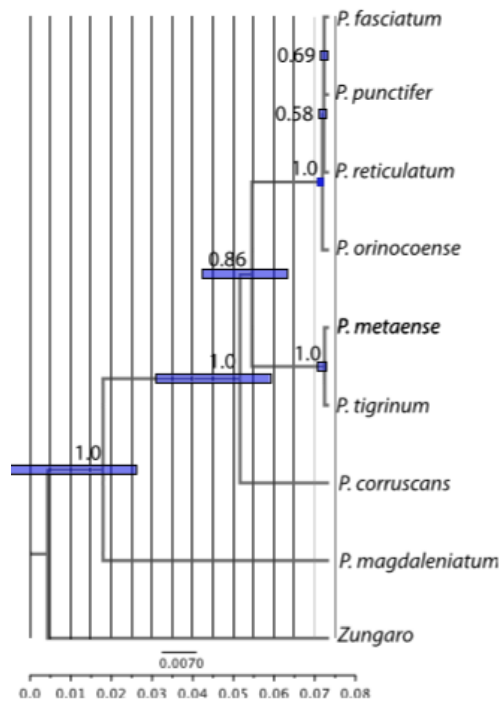
Discussion

The resulting phylogenetic trees created from the four genes examined for this project (COI, cyt-b, *rag1*, and *rag2*) have some consistencies, as well as some conflicts, between them. Both COI and cyt-b placed *P. metaense* and *P. tigrinum* to be in their own node together (Tree 1, Tree 2). These genes also both determined *P. magdaleniatum* to be most ancestral and *P. corruscans* to be next, each in their own nodes (Tree 1, Tree 2). There is some disagreement as to where *P. reticulatum* lies within the genus, with COI placing it with *P. orinocoense*, cyt-b determining it to be in a node with *P. punctifer* and *P. fasciatum*, and *rag2* putting *P. reticulatum* branched with *P. corruscans* (Tree 1, Tree 2, and Tree 4).

When comparing to phylogenetic relationships created by previous scholars' research, some interesting similarities arise. Results from this study agree with work done by Nirchio and others which found that *P. tigrinum* and *P. metaense* are likely in a node together (Tree 5, Nirchio et al. 2013). Further supporting this, another study conducted by Carvalho-Costa and others also found *P. tigrinum* and *P. metaense* to be in a node together (Tree 6, Carvalho-Costa et al. 2011).

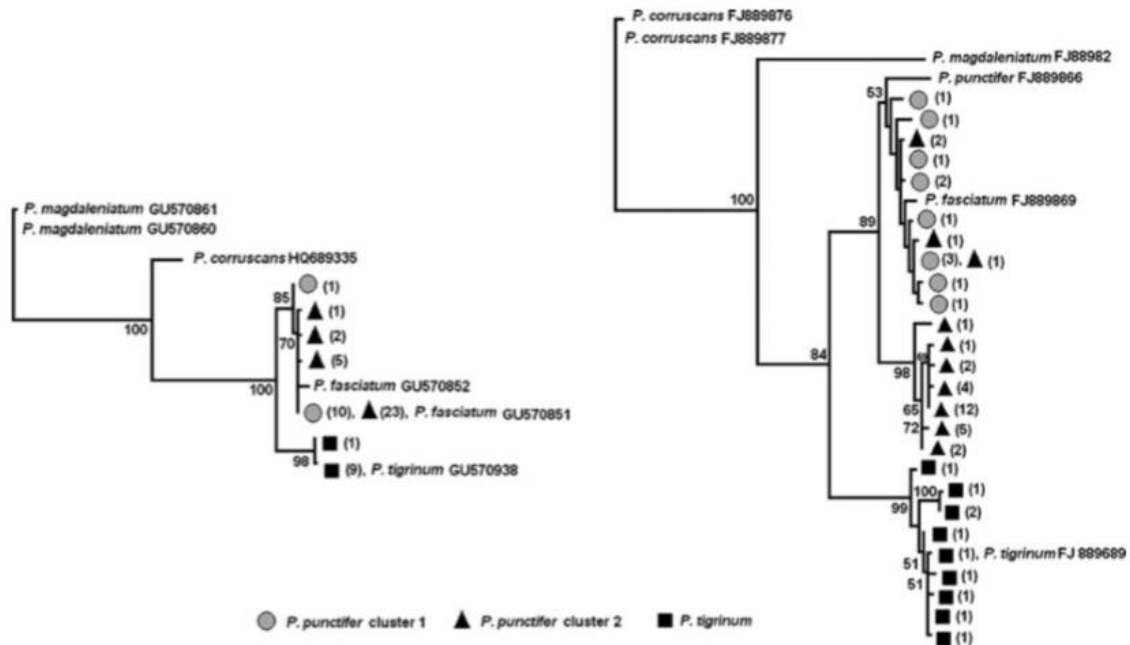


Tree 5: Phylogenetics from Nirchio et al. based on cyt-b and COI genes (Nirchio et al. 2013)



Tree 6: Carvalho-Costa and other maximum clade credibility tree (Carvalho-Costa et al. 2011)

In addition to *P. metaense* and *P. tigrinum* being a strongly supported clade, *P. corruscans* and *P. magdaleniatum* have evidence indicating their places as more ancestral species within the group. The trees created from COI and cyt-b in this study place *P. magdaleniatum* as most primitive in its own node, and *P. corruscans* as second most ancestral in its own node (Tree 1, Tree 2). *Rag1* shows *P. magdaleniatum* and *P. corruscans* as being close to *S. planiceps* along with *P. fasciatum* (Tree 3a, Tree 3b). The work by Nirchio and others found *P. corruscans* to be in a clade with *P. magdaleniatum* (Tree 5; Nirchio et al. 2013). A study by Garcia-Davila and others also found *P. magdaleniatum* and *P. corruscans* to be most primitive (Tree 7; Garcia-Davila et al. 2013). From this study's phylogenetic trees created, and previous research conducted, there appears to be evidence indicating that *P. magdaleniatum* and *P. corruscans* are the most ancestral of the eight *Pseudoplatystoma* species.



Tree 7: Maximum likelihood tree formed by Garcia-Davila et al. (Garcia-Davila et al. 2013)

Overall, the goal of this study was to examine the genus *Pseudoplatystoma* for congruencies and conflicts in the many phylogenetic trees predicted for it. *Pseudoplatystoma* is a unique and appreciated genus of catfish that will likely see more research in the coming years. The strongest congruencies of this study occurred for the placement of *P. tigrinum* and *P. metaense* into their own clade. This clade is supported by congruencies within the trees created for this study, as well as other molecular studies conducted by other researchers (Nirchio et al. 2013; Carvalho-Costa et al. 2011). However, there was conflict between the trees regarding the placement of *P. reticulatum*, with no congruencies being found among any of the phylogenetic trees created. Two other supported placements are that of *P. magdaleniatum* and *P. corruscans*, which both appear to be ancestrally located in the phylogenetic trees. Trees formed from the genes COI, cyt-b, and others appear to agree that these two species should be set on nodes more

primitively placed, close to their outgroup within the phylogenetic tree (Tree 1, Tree 2, Tree 5; Nirchio et al. 2013, and Tree 7; Garcia-Davila et al. 2013). Further research should compare phylogenetic trees created by this and other molecular studies with relationships determined by morphological review. Another area of additional research that will better the understanding of these fish is to sequence specimens of all eight species for genes such as *rag1* and *rag2*, and then compare the trees created from genes for all species within *Pseudoplatystoma*. While the exact phylogenetic relationships of these species has not been fully determined, more and more evidence is being uncovered that will hopefully lead to a better understand of the tiger catfish.

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