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Molecular systematics of the enigmatic Middle American genus *Vieja* (Teleostei: Cichlidae)

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ABSTRACT

The genus *Vieja* represents a group of heroine cichlids (Teleostei: Cichlidae) distributed on the Atlantic and Pacific slopes of North and Central America from southern Mexico to Panama. Sixteen species of *Vieja* are presently recognized; however, based on long-standing taxonomic problems, the genus itself appears to be weakly defined. A number of different generic designations have been proposed for members of *Vieja*, and recent systematic studies of heroine cichlids have not specifically addressed the validity of the grouping and have not included all species in the genus. Therefore, the purpose of this study was to assess the monophyly of the genus *Vieja* by including all nominal species in the genus using the mitochondrial encoded cytochrome *b* gene and nuclear S7-1 intron. Results of Maximum Parsimony, Bayesian inference, and topology tests (constraint tree searches and post-burn-in Bayesian filtering) indicate that the genus is not monophyletic as it is currently recognized. The genus *Herichthys* was recovered as sister to a clade consisting of a number of *Vieja* species (*V. fenestrata, V. guttulata, V. zonata, V. hartwegi, V. bifasciata, V. breidohri, V. argentea, V. regani, V. melanura*, *V. synspila*, and *V. maculicauda*, as well as *Paraneetroplus bulleri*). A clade consisting of *V. intermedia, V. godmanni*, and *V. microphthalma* was recovered sister to *Theraps*. Additionally, *V. heterospila* and *V. tuyrensis* were recovered outside of *Vieja* and *Herichthys* clades. Based on the results of this comprehensive study, we suggest a revised classification of *Vieja* species.

1. Introduction

In recent years, significant advances have been made into better understanding the systematics and taxonomy of New World cichlids (Teleostei: Cichlidae) (Chakrabarty, 2006a; Concheiro Pérez et al., 2007; Hulsey et al., 2004; López-Fernández et al., 2010; Smith et al., 2008); however, a thorough understanding of the generic status and systematic placement for some groups remains uncertain (Nelson, 2006). In fact, Miller et al. (2005) stated that generic treatment of Middle American cichlids is “both chaotic and frustrating,” due to the high degree of variation in body morphology between and among groups. One such group is the Middle American cichlid genus *Vieja*.

Vieja represents a group of heroine cichlids that occur on both the Atlantic and Pacific slopes of North America from southern Mexico to Panama (Kullander, 2003). *Vieja*, like many other groups of cichlids, is a taxonomically and systematically difficult group, and a number of different taxonomic designations have been proposed for these fishes (Table 1), with many species belonging to the catch-all genus *Cichlasoma* at some time or another. The genus *Vieja* was proposed in 1969 by Fernández-Yépez and originally included four species. *Vieja panamensis* is the type species but was later recognized as a synonym of *V. maculicauda* (Kullander, 2003). Of the remaining three original species assigned to the genus, *Vieja temporale* is a synonym of *Hypselecara temporalis* (Kullander, 1986), *V. coryphaenoides* is a synonym of *Hypselecara coryphaenoides* (Kullander, 1986), and *V. biocellata* is a synonym of *Rocio octofasciata* (Schmitter-Soto, 2007).

Fernández-Yépez (1969) provided a number of characters to define the genus including the length of the pelvic fin, scale counts, and bifid (bicuspid), as opposed to conical teeth; however, there is considerable overlap between the characters used to define *Vieja*, and those used to define the remaining genera described in the same study: *Chuco*, *Copora*, and *Curraichthys* (Stawikowski and Werner, 1998). *Chuco* is the only other genus of Fernández-Yépez (1969) that includes currently recognized species of *Vieja*. The type species for *Chuco* is *Cichlasoma milleri*, now a synonym of *V. microphthalma* (Kullander, 2003). Fernández-Yépez (1969) assigned three additional species to the genus *Chuco*, including *Cichlasoma globossum*, *Cichlasoma manana*, and *Cichlasoma acutum*. *Cichlasoma globossum* and *C. manana* are synonyms of *Vieja maculicauda*

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Table 1
Summary of alternative generic level groupings for species of *Vieja*. Included studies gave alternative groupings for all recognized species.

Species	Original description	Werner and Stawikowski (1989)	Stawikowski and Werner (1998)		Kullander (2003)
		Paratheraps	*Chuco*	*Vieja*	*Vieja*
V. argentea	*Cichlasoma argentea*			X	X
V. bifasciata	*Heros bifasciatus*			X	X
V. breidohri	*Paratheraps breidohri*	X		X	X
V. fenestrata	*Chromis fenestrata*			X	X
V. godmanni	*Heros godmanni*		X		X
V. guttulata	*Heros guttulatus*			X	X
V. hartwegi	*Cichlasoma hartwegi*	X		X	X
V. heterospila	*Cichlasoma heterospilum*			X	X
V. intermedia	*Heros intermedius*		X		X
V. maculicauda	*Cichlasoma maculicauda*			X	X
V. melanura	*Heros melanurus*			X	X
V. microphthalma	*Heros microphthalmus*		X		X
V. regani	*Cichlasoma regani*			X	X
V. synspila	*Cichlasoma synspilum*			X	X
V. tuyrensis	*Cichlasoma tuyrense*			X	X
V. zonata	*Cichlasoma zonatum*			X	X

(Kullander, 2003), and *Cichlasoma acutum* is a synonym of *Amphilophus robertsoni* (Conkel, 1997).

In 1987, Werner and Stawikowski proposed a new genus, *Paratheraps*, which included *P. breidohri*, the type species, and the reassigned *Cichlasoma hartwegi* to *Paratheraps*. Werner and Stawikowski (1987) failed to designate a holotype for this new species and genus, and subsequently published an article correcting the error (Werner and Stawikowski, 1989). *Paratheraps* was later considered to be in synonymy with *Vieja* (Allgayer, 1991; Stawikowski and Werner, 1998), although Stawikowski and Werner (1998) continued to recognize the genus *Chuco* as a valid group. They placed three *Vieja* species into the genus *Chuco*: *V. microphthalma*, *V. intermedia*, and *V. godmanni* and noted that all three of these species have especially large front, middle teeth with rear-facing points, and a small number of lateral scales (32–35).

Kullander (2003) published the most recent checklist of Neotropical fishes and, without a re-diagnosis or formal taxonomic treatment, placed *Chuco* as a synonym of *Vieja*. *Paratheraps* continued to be considered in synonymy with *Vieja*, thereby recognizing 16 species in the genus *Vieja*. Two additional species, *Herichthys pearsei* and '*Cichlasoma*' *ufermanni*, have been recognized as members of *Vieja* (Allgayer, 2002; Miller et al., 2005) although they are not currently (Eschmeyer, 2010; Kullander, 2003).

Recent molecular phylogenetic studies of heroine cichlids (Concheiro Pérez et al., 2007; Hulsey et al., 2004; López-Fernández et al., 2010) have found the genus *Vieja* to be paraphyletic and therefore used alternative taxonomic designations for species within *Vieja.* While we acknowledge that relationships among and within *Vieja* were not the focus of these studies, it is clear that the taxonomic sampling of *Vieja* used for those studies did not allow for a thorough assessment of the genus and subsequent altered generic designations. This is critical, as previous studies have noted the importance of increased taxon sampling on reducing phylogenetic error (Hillis et al., 2003; Rican et al., 2008; Zwickl and Hillis, 2002), which in turn, could result in inaccurate taxonomic changes that may occur as a result of an incomplete phylogeny. Smith et al. (2008) and López-Fernández et al. (2010) specifically note the importance of complete taxon sampling within genera such as *Vieja*.

Two main issues have confounded taxonomy of the genus *Vieja*. First and foremost, the genus was never properly diagnosed such that a set of characters could clearly differentiate *Vieja* from other New World cichlid genera. The original description of the genus is marginal at best, with only a short paragraph to serve as the generic diagnosis. Also, as previously mentioned, many of the characters used by Fernández-Yépez (1969) to diagnose *Vieja* are not exclusive to these fishes. To that extent, the entire genus has never been studied as a whole and with this comes the second issue. Many authors have used a variety of generic names for *Vieja* species without sufficient examination of the entire group (i.e. utilization of all species). While it has been acknowledged for some time that the genus is paraphyletic as currently recognized, arbitrary use of invalid names is counterproductive. For example, many researchers and aquarists have considered a number of *Vieja* species to be in the genus *Paratheraps* (Artigas Azas, 2008; Concheiro Pérez et al., 2007; Hulsey et al., 2004). We find only two *Vieja* species, *V. breidohri* and *V. hartwegi*, to have ever formally been assigned to this genus (Werner and Stawikowski, 1989). Species were assigned to the genus without determining if those species possessed the traits proposed to diagnose *Paratheraps*. Therefore, at present one should not use *Paratheraps* as the generic designation for any additional species. The most recently proposed generic revision of *Vieja* was that of López-Fernández et al. (2010). This study found species formerly assigned to *Chuco* to be within a *Theraps* clade, and *Paraneetroplus bulleri* to be nested well within a clade consisting of the remaining *Vieja* species used in the study. The close relationship of *Vieja* spp. with *P. bulleri* has been shown (Chakrabarty, 2006b; Hulsey et al., 2006, 2004); however, there was little resolution among *Vieja* spp. (Chakrabarty, 2006b).

After nearly 40 years of study, it is clear that the taxonomic status of *Vieja* and its inclusive species remains uncertain. Previous molecular systematic studies (Chakrabarty, 2006a; Concheiro Pérez et al., 2007; Hulsey et al., 2004; López-Fernández et al., 2010; Smith et al., 2008) included a subset of the species that historically have been included in *Vieja*, but were not specifically addressing phylogenetic relationships among species in the genus. Therefore our objective was to utilize available mitochondrial encoded cytochrome *b* and nuclear S7-1 intron sequence data and augment it with additional populations and species to conduct a comprehensive systematic study inclusive of all sixteen species of *Vieja*, to specifically assess the monophyly of the genus and relationships among its species. Complete taxon sampling of the genus renders our study novel and we feel this is a necessity for revision of genera and their respective species. We utilized Kullander (2003) as the most recent taxonomic summary of all recognized species of *Vieja* and *a priori* accept this as the currently valid taxonomy for this genus. Generic names for other herichthyines follow López-Fernández et al. (2010), the most completely sampled Neotropical cichlid phylogeny to date.

2. Materials and methods

2.1. Taxon sampling

All currently valid species of *Vieja* (*sensu* Kullander, 2003) were included, as well as other cichlid species that have been included in the genus at one time or another. For the cytochrome *b* (cyt *b*) dataset, a total of 72 individuals based on previously published studies (Concheiro Pérez et al., 2007; Hulsey et al., 2004; López-Fernández et al., 2010) were used, as well as an additional 18 individuals that included both wild caught and aquarium-hobbyist derived specimens. In total, 90 operational taxonomic units (OTUs) were included in the cyt *b* analyses. The S7-1 dataset included a subset of species, with at least one of each species of *Vieja* as well as additional ingroup and outgroup taxa, for a total of 36 OTUs. Specimen localities and corresponding GenBank accession numbers are summarized in Appendix 1 Leviton et al., 1985. Voucher specimens for all new individuals sequenced were deposited in the Southeastern Louisiana University Vertebrate Museum.

In addition to cichlids from the genus *Vieja*, a number of other representative heroine cichlids were also included in the analysis to further investigate the monophyly and phylogenetic position of *Vieja*. Concheiro Pérez et al. (2007) showed *Herichthys* to be sister to a number of *Vieja* species, and we therefore included all currently recognized species of *Herichthys* in the cyt *b* dataset. Four species of *Herichthys* were used in the S7-1 dataset. Four South American cichlid species, used as outgroups in Concheiro Pérez et al. (2007), were also used as outgroups in the present cyt *b* analyses. We also included *Aequidens coeruleopunctatus*, as an additional non-heroine cichlid. *Aequidens coeruleopunctatus* was used as the outgroup in the S7-1 dataset.

2.2. Molecular methods

Whole genomic DNA was extracted from fin clips preserved in 95% ethanol using the DNeasy Tissue Kit (Qiagen, Inc.). The cyt *b* gene was amplified using the primers GluDG.L and H16460 (Perdices et al., 2002) via polymerase chain reaction in 25 μL samples. Reactions were cycled according to Perdices et al. (2002). The first intron of the S7 ribosomal protein was amplified using primers RP1 and Rpex (Chow and Hazama, 1998) and cycled according to Chow and Hazama (1998). Amplification products were electrophoresed on a 0.8% agarose gel and compared to a standard to assess the presence, size, and intensity of amplified fragments. Products were then purified with ExoSAP (USB Corp.) and subsequently used in sequencing reactions according to the manufacturer's recommendations (Applied Biosystems). Prior to sequencing, gel filtration (Edge Biosystems) was used to remove excess dye terminators, primers, and nucleotides. Chromatographs were checked by eye for ambiguities and sequences were aligned using Sequencher version 4.8.

Given an encountered lack of confidence in the beginning and end of sequence reads, all cyt *b* sequences were truncated from 1137 base pairs to 1080 base pairs after alignment. The resulting 1080 base pair sequences were used in subsequent phylogenetic analyses. The S7-1 dataset was 497 base pairs in length.

2.3. Phylogenetic analyses

Phylogenetic hypotheses were generated using Maximum Parsimony (MP) and Bayesian Inference (BI) methods. For cyt *b* and S7-1 datasets, MP analyses were performed using PAUP$*$ version 4.0 (Swofford, 2002) with all characters equally weighted. A heuristic search was performed with unordered and unweighted data, and Tree Bisection Reconnection (TBR) branch swapping for 1000 random step-wise additions. Bootstrap analysis was then performed with 100 pseudoreplicates. For BI, cyt *b* data were partitioned by codon and a model of evolution selected for each partition in ModelTest 3.06 (Posada and Crandall, 1998) using the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). The S7-1 dataset was not partitioned. Posterior probabilities were estimated using a Metropolis-coupled Markov chain Monte Carlo in Mr. Bayes (Hulsenbeck et al., 2001). Each BI was run for 7,000,000 generations with trees sampled every 100 generations. Stationarity was assessed by visualization of ln likelihood scores vs. generation plots to determine the point at which likelihood values stabilized (100,000 generations but first 500,000 discarded to better ensure stationarity). Post-burn-in trees were used to calculate posterior probabilities (BPP) and results from four independent runs were compared to provide additional confirmation of convergence among likelihood values, tree topologies, and posterior probabilities.

2.4. Hypothesis testing

The support for previously proposed generic level groupings of the genus *Vieja* was assessed by examining alternative tree topologies. Constraint trees were built for each of the previous generic designations in MacClade, thereby forcing each genus into monophyly. The monophyly of the following genera were tested: *Chuco* (*sensu* Stawikowski and Werner, 1998), *Vieja* (*sensu* Stawikowski and Werner, 1998), and *Vieja* (*sensu* Kullander, 2003). Given the small number of available specimens of species previously assigned to the genus *Paratheraps*, this genus was not used for hypothesis testing. Under MP, the length of constrained and unconstrained trees were compared and longer tree lengths in constraint searches allowed for rejection of generic hypotheses. Tests of alternative topologies were also performed using BI filtering of the previously identified constraint trees. If less than 5% of post burn-in trees contained the monophyletic grouping, that topology was statistically rejected as monophyletic based on these data (Weisrock et al., 2006).

3. Results

3.1. Phylogenetic analyses – cytochrome b

Phylogenetic hypotheses generated by both MP (not shown) and BI (Fig. 1) of partial sequences of the cytochrome *b* gene resulted in congruent topologies with four deeply divergent clades of *Vieja* cichlids: (A) a clade containing *V. microphthalma*, *V. godmanni*, and *V. intermedia*; (B) a clade containing *V. heterospila*; (C) a clade containing *V. fenestrata*, *V. guttulata*, *V. zonata*, *V. hartwegi*, *V. breidohri*, *V. bifasciata*, *V. argentea*, *V. regani*, *Paraneetroplus bulleri*, *V. synspila*, *V. melanura*, and *V. maculicauda*; and (D) a *V. tuyrensis* clade. These clades are herein referred to as clades A, B, C, and D. Both analytical methods failed to recover the genus *Vieja* as a monophyletic group. MP analysis of cyt *b* sequences retained 32 trees with shortest tree length of 2315. Consensus tree had a tree length of 2456 (CI = 0.33, HI = 0.67, RI = 0.68). Both MP and BI resulted in similar relationships and results from BI are shown.

Clade A was consistently recovered (BPP = 100) and consists of the species previously assigned to *Chuco* (*sensu* Stawikowski and Werner, 1998): *V. microphthalma*, *V. godmanni*, and *V. intermedia*. There is an average 1.8% cyt *b* sequence divergence among species recovered in clade A. Within this clade, *V. godmanni* and *V. intermedia* were sister to *V. microphthalma*. There is an average 1.9% sequence divergence between *V. godmanni* and *V. microphthalma* and 1.7% between *V. intermedia* and *V. microphthalma*. Between *V. godmanni* and *V. intermedia* there was a 1.3% sequence divergence;

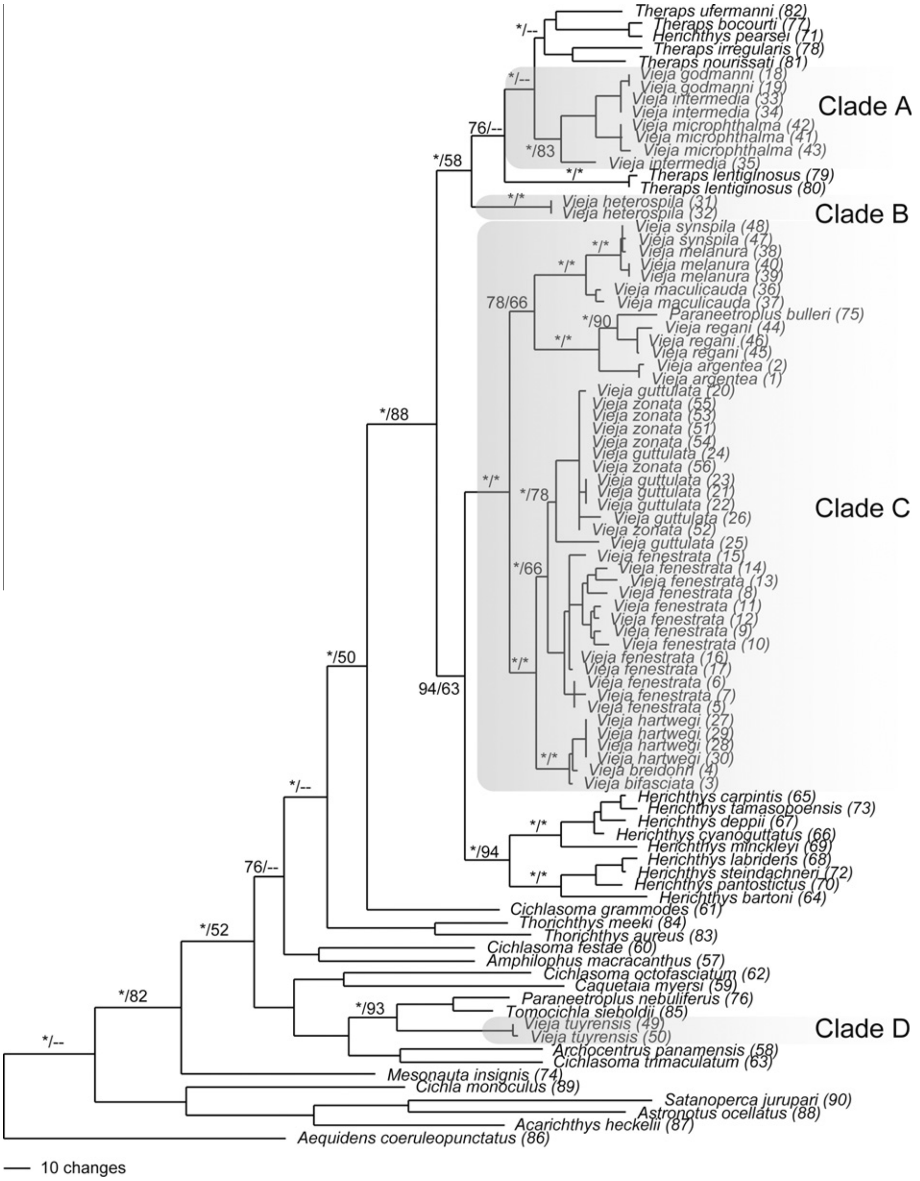

Fig. 1. Phylogram of fifty-percent majority rule consensus tree from a partitioned mixed model Bayesian analysis of partial cytochrome *b* sequences (1080 bp). Majority of *Vieja* spp. recovered in two main clades (A and B). Asterisks indicate posterior probability ⩾95/Bootstrap value ⩾95. Values primarily given for relationships above the species-level to simplify visualization. (- - indicates posterior probability or Bootstrap value below 50). Numbers beside taxa correspond to numbers in Appendix 1.

however, this is driven by a single individual of *V. intermedia* (A.1, number 35) from Río San Pedro (Río Sarstun Drainage) in Mexico, which is 3.2% divergent from the other two individuals of *V. intermedia* (from Guatemala). These species, all formally assigned to *Chuco*, are sister to the *Theraps* clade of López-Fernández et al. (2010), and this clade is 5.7% divergent from the *Theraps* clade based on cyt *b* sequences. *Herichthys pearsei* was recovered within the *Theraps* clade.

Theraps lentiginosus was recovered as sister to a clade consisting of *Theraps* + Clade A. Sister to this was *V. heterospila* (Clade B) with a 6.8% sequence divergence from the *Theraps* clade and 5.7% sequence divergence from Clade A. *Vieja heterospila* is 8.5% divergent from *T. lentiginosus*.

The monophyly of Clade C, encompassing most species of *Vieja*, was consistently recovered (BPP = 100; Bootstrap 99), and includes all currently recognized species of *Vieja* with the exception of those species formerly assigned to *Chuco* (*sensu* Stawikowski and Werner, 1998), *V. heterospila*, and *V. tuyrensis*. As previously reported (Chakrabarty, 2006b; Hulsey et al., 2004; López-Fernández et al., 2010; Rican et al., 2008), *Paraneetroplus bulleri* was recovered as closely related to *Vieja* spp., and in the present study found nested within this clade C. On average there is a relatively low degree of cyt *b* sequence divergence (4.1%) among species within this clade. Clade C was recovered as sister to a monophyletic *Herichthys*.

Two main sub-clades were recovered within clade C. (I) *Vieja fenestrata* was recovered sister to a clade comprised of *V. guttulata* and *V. zonata*. There was an average 2.7% cyt *b* sequence divergence between the two clades (*V. fenestrata* and *V. guttulata* + *V. zonata*), and only a 0.6% sequence divergence between *V. guttulata* and *V. zonata*. These species are sister to a clade containing *V. hartwegi*, *V. breidohri*, and *V. bifasciata*. On average there was only a 0.4% cyt *b* sequence divergence among these three species. (II) *Vieja regani*, *Paraneetroplus bulleri*, and *V. argentea* were recovered sister to a clade comprised of *V. maculicauda*, *V. melanura*, and *V. synspila*. *Vieja regani* and *P. bulleri* were fairly divergent species, with 3.6% sequence divergence between the two. *Vieja argentea* is 3.1%

divergent from the *V. regani* + *P. bulleri* clade. This entire clade (*V. regani* + *V. argentea* + *P. bulleri*) was recovered as sister to *V. melanura*, *V. synspila*, and *V. maculicauda*. On average there was a 0.9% sequence divergence between *V. maculicauda* and *V. melanura* and 0.8% between *V. maculicauda* and *V. synspila*. *Vieja synspila* and *V. melanura* were only 0.3% divergent from each other.

Clade D includes *Vieja tuyrensis* and was consistently recovered (BPP 100; Bootstrap 93). Based on the species included in this dataset, *V. tuyrensis* was recovered well outside of other *Vieja* species and sister to a clade consisting of *Tomocichla sieboldii* and *Paraneetroplus nebuliferus*. *Vieja turyensis* has on average a 14.2% sequence divergence from clade C, which encompasses most species of *Vieja*. Given the sparse sampling of non-*Vieja* species in the dataset, it should not be concluded that the clade comprising *Tomocichla sieboldii* and *Paraneetroplus nebuliferus* is the sister clade to *V. tuyrensis*, only that *V. tuyrensis* is not closely related to other members of *Vieja*.

3.2. Phylogenetic analyses – S7-1

MP analysis of S7-1 sequences retained 4624 trees with a tree length of 137 for the best tree (consensus tree had tree length of 140; CI = 0.88, HI = 0.11, RI = 0.87). Analyses (MP and BI) of the S7-1 dataset showed little resolution among *Vieja* species (Fig. 2). The sequence variation is minute and consistency of clade recovery is low. With the exception of *Thorichthys meeki* and *Cichlasoma festae*, all herichthyines are recovered as a polytomy. Within this clade, the average sequence divergence is only 0.8%. Additionally, average S7-1 sequence divergence between and within the groups recovered in the mitochondrial topology are less than or equal to 0.8%. The divergent relationship of *V. tuyrensis* from all other species of *Vieja* was consistently recovered with BI. The S7-1 sequence for *V. tuyrensis* is 3.6% divergent from other herichthyines (excluding *Thorichthys meeki* and *Cichlasoma festae*).

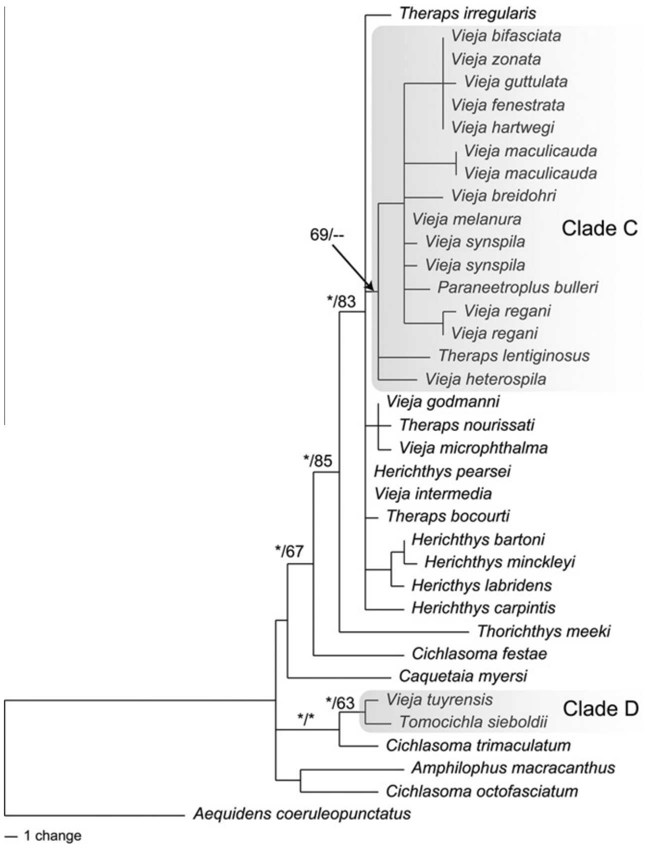

Fig. 2. Phylogram of fifty-percent majority rule consensus tree from a partitioned mixed model Bayesian analysis of ribosomal S7-1 intron sequences (497 bp). *Vieja* spp. recovered in four main clades (A-D). Asterisks indicate posterior probability ⩾95/Bootstrap value ⩾95. Values primarily given for relationships above the species-level to simplify visualization. (- - indicates posterior probability or Bootstrap value below 50).

3.3. Hypothesis testing

For the cyt *b* dataset, none of the constraint tree searches supported the monophyly of the genus *Vieja* (*sensu* Kullander, 2003) (Table 2). The limited amount of sequence variation for the S7-1 sequences for *Vieja* spp. did not allow for complete hypothesis testing and constraint tree searches to be conducted with this data. MP constraint tree searches resulted in trees that were longer than the most parsimonious tree. Bayesian filtering of the post burn-in trees for cyt *b* also failed to recover any trees that contained a monophyletic *Vieja* (*sensu* Kullander, 2003). Similar results were found for cyt *b* constraint searches for *Vieja* (*sensu* Stawikowski and Werner, 1998), as both MP and Bayesian filtering also failed to support a monophyletic *Vieja*. These results allow for the rejection of a monophyletic *Vieja* (*sensu* Kullander, 2003; *sensu* Stawikowski and Werner, 1998).

Dissimilar results were found for the genus *Chuco* (*sensu* Stawikowski and Werner, 1998). For the cyt *b* dataset, MP constraint search result in a shorter tree length than that of the consensus tree (from the unconstrained search), and all post burn-in trees in the BI analysis recovered *Chuco* as monophyletic. Therefore the validity of *Chuco* cannot be rejected.

4. Discussion

4.1. Phylogenetic relationships

The objective of this study was to assess the monophyly of the cichlid genus *Vieja* and the relationships among its inclusive species, using previously proposed taxonomic classifications as a framework (Kullander, 2003; Stawikowski and Werner, 1998). Inclusion of all species in the genus fails to refute the paraphyly of the genus revealed by previous studies which utilized fewer species (Concheiro Pérez et al., 2007; Hulsey et al., 2004; López-Fernández et al., 2010). While the paraphyly of this genus has previously been elucidated, complete taxonomic sampling recovers some different relationships than previously shown and was necessary before comprehensive comments could be made on the taxonomy of these fishes. The results from this study indicate that there is a strong need for a taxonomic revision of this group. Our results are strongly concordant with those of López-Fernández et al. (2010); however, they allow more thorough investigations of species-level relationships in that portion of their phylogeny. Given the complete lack of resolution with the nuclear marker used in the present study, this section will mainly focus on the mitochondrial phylogeny, although the nuclear phylogeny will also be discussed.

Species of *Vieja* were recovered in four main clades. The majority of species were within a single clade (clade C), which includes *V. maculicauda*, the type species for the genus. While the inclusion of *Paraneetroplus bulleri* in a *Vieja* clade is not a new finding, it's sister relationships have not been well understood, and could not be without complete taxon sampling. We find this species to be sister to *V. regani*, with this clade being sister to *V. argentea*. However, Chakrabarty (2006b) found this species to be sister to a clade of *V. regani* and *V. argentea*. Given the seniority of the name *Paraneetroplus* over *Vieja*, and that *P. bulleri* is the type species for *Paraneetroplus*, our results support the nomenclatural hypothesis

Table 2
Alternative topologies tested using constraint parsimony and post burn-in searches for cyt *b* data.

Generic assignment	Maximum Parsimony Tree length (Consensus: 2456)	Bayesian Inference Number of post burn-in trees (Out of 65,000 trees)
Vieja (*sensu* Kullander, 2003)	2552	0
Vieja (*sensu* Stawikowski and Werner, 1998)	2547	0
Chuco (*sensu* Stawikowski and Werner, 1998)	2316	65,000

of López-Fernández et al. (2010) in that *Vieja* spp. in clade C warrant reassignment to *Paraneetroplus*. The results and taxonomic recommendations from the present study can serve as a guide to search for potential characters in future morphological studies that unite species in clade C, which would separate them from *Herichthys* and species of *Vieja* recovered outside of clade C.

The genus *Paratheraps* (including *V. hartwegi* and *V. breidohri* as recognized by Werner and Stawikowski (1989) was recovered as monophyletic; however, given the sparse sampling for these two species in this dataset, and the apparent close relationship with *V. bifasciata*, we did not specifically assess the monophyly of this grouping.

While *Chuco* was recovered as monophyletic and there are morphological characters proposed to diagnose the genus (Fernández-Yépez, 1969; Stawikowski and Werner, 1998), it seems premature to resurrect this genus at present given its poor diagnosis. Diagnostic characters proposed by Fernández-Yépez (1969) overlap with other genera, and no comparative data are provided for the characters given for *Chuco* by Stawikowski and Werner (1998). *Vieja* spp. formerly assigned to the genus *Chuco* (clade A) were recovered sister to *Theraps* (*sensu* López-Fernández et al., 2010). Concheiro Pérez et al. (2007) placed *V. heterospila*, *V. microphthalma*, *V. godmanni*, and *V. intermedia* into the genus *Theraps*, given their close relationship with *T. lentiginosus*. López-Fernández et al. (2010) did the same, but did not include *V. heterospila* in their analyses. We recover a similar *Theraps* clade but find *Herichthys pearsei* to also be nested within this group of fishes and sister to *T. bocourti*. Inclusion of *V. heterospila* also appears to pull *T. lentiginosus* out of the *Theraps* clade, and these species were recovered in a larger clade encompassing clade C, with *V. heterospila* as the basal member.

The inclusion of *Herichthys pearsei* and '*Cichlasoma*' *ufermanni* in *Vieja* has been questioned. Miller et al. (2005) lists *Herichthys pearsei* as *Vieja pearsei*; however, this generic reassignment was based on unpublished data by Miller and Norris (cited in Miller et al., 2005). Results from our study support its removal from *Herichthys* but not its reassignment to *Vieja*. Allgayer (2002) described a new species, *Vieja ufermanni*, but Kullander (2003) does not recognize it as a species of *Vieja*, noting that its generic assignment is still uncertain. However, Miller et al. (2005) continue to recognize this species as *Vieja ufermanni*. While we were unable to include '*Cichlasoma*' *ufermanni* in the S7-1 analyses, it was included in the cyt *b* dataset and found to be nested within the *Theraps* clade, as recovered by López-Fernández et al. (2010). Our results support the inclusion of this species in the genus *Theraps*.

The generic status of *Vieja tuyrensis* has also been a source of debate among cichlid researchers, and this species is recovered well outside of clades containing other *Vieja* species. The phylogenetic position of *V. tuyrensis* elucidated from the present analyses (both mitochondrial and nuclear) support the idea that this species is not actually a *Vieja*, nor does it appear related to *Herichthys* or *Theraps* species.

4.2. Species complexes

Three sets of closely related species were found to either lack reciprocal monophyly or genetic distinctiveness based on cyt *b* sequences: *V. melanura*–*V. synspila*, *V. guttulata*–*V. zonata*, and *V. intermedia*–*V. godmanni*. This may indicate there are fewer species than currently recognized; however, an additional possibility is the occurrence of hybridization. These three complexes represent cases in which genetically similar species occur in the same river system or general area. Inclusion of additional nuclear markers and increased sample sizes and collection localities, as well as revisiting morphological differences between species, will be important for assessing species numbers and limits within these complexes.

4.3. Mitochondrial vs. nuclear phylogenies

Use of nuclear genes for phylogeny reconstruction in Middle American cichlids has certainly been informative and provided useful insight into relationships among tribes and genera (Chakrabarty, 2006b; López-Fernández et al., 2010; Rican et al., 2008; Smith et al., 2008). While the days of solely mitochondrial based phylogenies appear to be numbered, to investigate species-level questions and relationships, at least within this group of cichlids, one may have to rely on mitochondrial genes to provide enough variability to resolve species relationships. Given the potentially fast rate of lineage formation in cichlids, nuclear and even some mitochondrial genes, may be too slowly evolving to contain phylogenetic information at present (Salzburger et al., 2005 and references within). We find that for the 36 herichthyine species utilized in this study there is only a 0.8% average sequence divergence based on S7-1 sequences. López-Fernández et al. (2010) report that for RAG2 sequences, certain species of *Paraneetroplus* (including several *Vieja* spp.), *Herichthys*, as well as five other genera, had identical intra-generic sequences. Everyone is well aware of the caveats of solely mitochondrial based phylogenies; however, for some studies aimed at species-level investigations there may not be enough variation in commonly used nuclear markers to allow for any meaningful conclusions. This certainly appears to be the case for the present study.

4.4. Taxonomic recommendations

Based on the results of the present phylogenetic study, which corroborates the works of others (Chakrabarty, 2006b; López-Fernández et al., 2010), we recommend the following nomenclatural changes (summarized in Table 3). Morphological diagnoses for these clades will be important for further corroborating these changes and are currently being studied (McMahan et al., in prep).

4.4.1. Paraneetroplus (Regan, 1905); Clade C – this study

Included species: P. bulleri (type species), *P. argentea*, *P. bifasciata*, *P. breidohri*, *P. fenestrata*, *P. gibbiceps*, *P. guttulata*, *P. hartwegi*, *P. maculicauda*, *P. melanura*, *P. regani*, *P. synspila*, *P. zonata*.

Vieja (Fernández-Yépez, 1969) is a junior subjective synonym of *Paraneetroplus*. The type species of both genera (*P. bulleri* and *V. maculicauda*) are present in Clade C of this study (Fig. 1). *Paraneetroplus*, being an older name, has priority over *Vieja*. All members of *Vieja* should therefore be reassigned to new genera. Species of *Vieja* recovered in Clade C should henceforth be recognized as members of *Paraneetroplus*. *Paraneetroplus nebuliferus* was not recovered in Clade C and should no longer be recognized as a member of this genus. That species was recovered in a phylogenetic position that does not clarify its taxonomic position so it should be placed under the placeholder genus '*Cichlasoma*' [*incertae sedis*] until additional data become available. *Paraneetroplus gibbiceps* was not sampled

Table 3
Taxonomic recommendations for species of *Vieja*, *Paraneetroplus*, and *Theraps* based on the present study. ∗ indicates type species.

Clades A and B	Clade C	Clade D
Theraps	*Paraneetroplus*	'*Cichlasoma*' [*incertae sedis*]
bocourti	*argentea*	*tuyrensis*
coeruleus	*bifasciata*	
godmanni	*breidohri*	Additional species
heterospila	*bulleri**	'*Cichlasoma*' [*incertae sedis*]
intermedia	*fenestrata*	*nebuliferus*
*irregularis**	*gibbiceps*	
lentiginosus	*guttulata*	
microphthalma	*hartwegi*	
nourissati	*maculicauda*	
pearsei	*melanura*	
ufermanni	*regani*	
	synspila	
	zonata	

in this study and should remain in *Paraneetroplus*. *Vieja tuyrensis* (Clade D) was also recovered in a clade outside of Clade C and sister to taxa with uncertain placement. Until further data are generated to clarify its position we recommend placing this species under the placeholder genus '*Cichlasoma*' [*incertae sedis*] following convention (Kullander, 1983).

4.4.2. Theraps (Günther, 1862); Clades A and B – this study

Included species: T. irregularis (type species), *T. bocourti*, *T. coeruleus*, *T. godmanni*, *T. heterospila*, *T. intermedia*, *T. lentiginosus*, *T. microphthalma*, *T. nourissati*, *T. pearsei*, *T. ufermanni*.

Species in Clades A and B include four members of *Vieja*. Clade A is recovered within a larger clade of species of *Theraps* that includes the type species of the genus, *T. irregularis*. Clade A also contains *V. microphthalma*, the type species for the genus *Chuco*; however, this genus is itself a junior synonym of *Vieja*. Placing members of Clade A in a genus other than *Theraps* would render *Theraps* paraphyletic. *Herichthys pearsei* and '*Cichlasoma*' *ufermanni* are also recovered within this clade. No phylogenetic information is available for *Theraps coeruleus*, so that species should continue to be recognized as a valid member of the genus *Theraps*.

5. Conclusion

This phylogenetic analysis of the cichlid genus *Vieja* recovered the genus as polyphyletic as currently recognized (16 species; *sensu* Kullander, 2003). While previous studies have shed doubt on the monophyly of *Vieja*, no study has encompassed all recognized species in a single analysis. This is a necessary first step before efforts can be made to improve the taxonomy of these fishes, indeed as we have recovered new relationships not before elucidated. This analysis recovered the genus *Herichthys* as sister to most *Vieja* species, including *Paraneetroplus bulleri*, and we now recognize this clade as *Paraneetroplus*. Our analysis supports the removal of *Herichthys pearsei* from the genus *Herichthys*; however, it does not appear this species should be a *Vieja* as proposed by Miller et al. (2005). This species appears nested well within the *Theraps* clade of López-Fernández et al. (2010). This analysis also shows the position of *V. tuyrensis*, well outside of the *Vieja* and *Herichthys* clades. Based on our results, which strongly corroborate the findings of previous studies, taxonomic revisions of cichlids currently assigned to the genus *Vieja* have been recommended.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.09.005.

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