Molecular Phylogenetics and Evolution 79 (2014) 415–421

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Phylogeny of suckermouth catfishes (Mochokidae: *Chiloglanis*) from Kenya: The utility of Growth Hormone introns in species level phylogenies

Ray C. Schmidt [a,*], Henry L. Bart Jr. [a], Dorothy Wanja Nyingi [b], Nathan Ndegwa Gichuki [c]

[a] Department of Ecology and Evolutionary Biology, Tulane University, 400 Boggs Hall, New Orleans, LA 70118, United States
[b] Ichthyology Section, National Museums of Kenya, PO Box 40658-00100, Nairobi, Kenya
[c] School of Biological Sciences, University of Nairobi, PO Box 30197-00100, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 8 January 2014
Revised 18 July 2014
Accepted 19 July 2014
Available online 29 July 2014

Keywords:
Gap coding
East Africa
Nuclear markers

ABSTRACT

African suckermouth catfishes (Mochokidae: *Chiloglanis*) occur in freshwater throughout tropical Africa. Specimens from all major drainages across Kenya were collected over three field seasons. Here we present a phylogeny inferred from both mitochondrial cytochrome b (cyt *b*) and introns of the nuclear Growth Hormone gene (GH). The phylogeny inferred from introns is largely congruent with the results from an analysis of cyt *b*. The length and variability of GH introns make them ideal species level nuclear markers without the problem of introgression commonly encountered with mitochondrial genes. This analysis confirmed the presence of two previously known undescribed *Chiloglanis* species and also suggests the presence of previously unknown diversity within the Athi River system. The resulting phylogeny also indicates the presence of two separate lineages within *C. brevibarbis*. The historical biogeography of *Chiloglanis* within Kenya is discussed. The utility of GH intron for species level phylogenies of Siluriformes is compared to that in other groups.

1. Introduction

The suckermouth catfishes of the genus *Chiloglanis* are the second most species rich genus within family Mochokidae. There are 49 described species of *Chiloglanis* found throughout sub-Saharan Africa; with more species awaiting description (Ferrais, 2007). These diminutive (<10 cm) fishes inhabit rivers of all sizes and are typically associated with flowing water. Habitats utilized by these fishes vary by species; with many associated with boulders and smaller rocks in rifles, while others are found near aquatic vegetation and woody debris. Members of the genus are distinct from most other Mochokidae genera in possessing an oral disk formed by modified maxillary and mandibular barbels. The disk helps them to maintain position in turbulent flow and aids in feeding. Mandibular teeth crowded around the mandibular symphysis distinguish *Chiloglanis* from the other chiloglanidin genera (*Atopodontus*, *Atopochilus*, and *Euchilichthys*; Vigliotta, 2008). Two oval premaxillary tooth patches along with buccal suction provided from the disk hold the fish steady in fast flowing water, while a row of curved mandibular teeth scrape aufwuchs and other organisms off of rocks, plants, and woody debris (Friel and Vigliotta, 2011). These fish are relatively poor swimmers; they are said to resemble tadpoles once they enter the water column (Daget and Durand, 1981).

Three recognized species and two undescribed species are reported from the freshwaters of Kenya (Seegers et al., 2003) (Fig. 1). *Chiloglanis deckenii* Peters 1868 occurs in the Pangani River of southern Kenya and northern Tanzania. *Chiloglanis brevibarbis* Boulenger 1902 occurs throughout the Tana and Athi River basins within central Kenya. *Chiloglanis somereni* Whitehead 1958 inhabits streams of the Lake Victoria basin in western Kenya. Two other species have previously been discovered but have not been formerly described (Seegers et al., 2003). One species is found in the northern Ewaso Nyiro River just below Chanler's Falls. The range of this species is unclear but it is likely restricted to the area between the falls and the Lorian swamp. Another undescribed species inhabits the headwaters of the Kerio River which flows into Lake Turkana in northern Kenya (Seegers et al., 2003). These Kerio River specimens represent the only known populations within the Lake Turkana drainage basin.

Here we investigate phylogeography of *Chiloglanis* in Kenya to confirm the presence of undescribed species and hypothesize the historical biogeography of these species. Presently, no

* Corresponding author. Fax: +1 504 862 8706.
E-mail address: rschmidt@tulane.edu (R.C. Schmidt).

http://dx.doi.org/10.1016/j.ympev.2014.07.011
1055-7903/

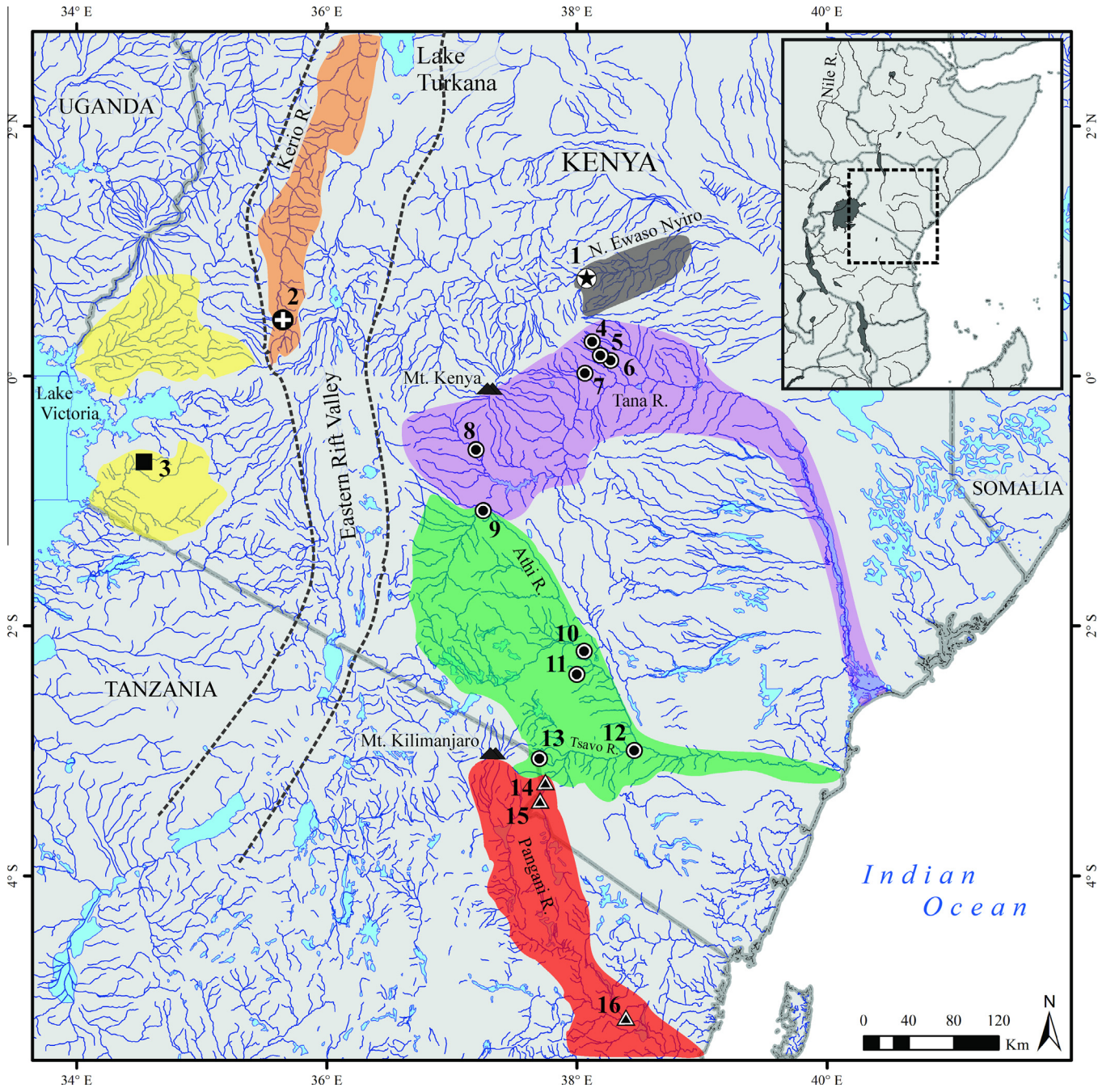

Fig. 1. Localities of the study taxa in this analysis. Symbols representing the species collected (square – *Chiloglanis somereni*, cross – *Chiloglanis* sp. "Kerio River", star – *Chiloglanis* sp. "Chanler's Falls", circle – *C. brevibarbis*, triangle – *C. deckenii*) and extent of drainages and hypothetical distributions denoted by color (yellow – Lake Victoria, orange – Kerio River, dark gray – Northern Ewaso Nyiro, purple – Tana River, green – Athi River, and red – Pangani River). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

biogeographic hypothesis exists for the freshwater fishes of Kenya. We also investigate the utility of GH introns for inferring phylogenetic relationship of these closely related, co-occurring taxa.

2. Material and methods

2.1. Taxon sampling and protocols

Chiloglanis specimens were collected at 15 localities across southern Kenya during field expeditions of the International Research Experience for Students (IRES) project from 2010 to 2012 (Fig. 1). Tissue samples were stored in 95% ethanol and voucher specimens were fixed in 10% formalin. Specimens and tissue samples were identified then deposited in the Ichthyology section of the National Museums of Kenya, the Royal D. Suttkus Fish Collection at the Tulane University Biodiversity Research Institute, and genetics resources collections for long term storage. In addition to the Kenyan specimens, species from West and Central Africa, and Tanzania were also sequenced and included in the analysis with published *Chiloglanis* sequences (see Supplementary Data).

Molecular markers, cytochrome *b* and Growth Hormone (GH) introns 3 and 4, were used to infer the phylogenetic relationships

of the Kenyan *Chiloglanis*. Although not previously used in phylogenies of Siluriformes, the unique and rapid evolution of introns has been reported on in studies of salmonids (Oakley and Phillips, 1999), cyprinids (Moyer et al., 2009), and catostomids (Bart et al., 2010a; Clements et al., 2012). The ambiguous areas caused by insertion and deletion events can be coded and analyzed along with the nucleotide alignment (Simmons and Ochoterena, 2000; Müller, 2006). In many cases the inclusion of these gap data improves the resolution of the analysis (Simmons et al., 2001; Kawakita et al., 2003; Malhotra et al., 2010; Nagy et al., 2012).

Total DNA was extracted from preserved muscle and/or fin tissue using an Invitrogen PureLink Genomic DNA mini prep kit. The cytochrome *b* region was amplified using primers Glu-2 (5′-AACC ACCGTTGTTATTCAACTA-3′) and Pro-R1 (5′-TAGTTTAGTTTAGAA TTCTGGCTTTGG-3′) (Hardman and Page, 2003). The amplification protocol was a touchdown format with a 1 min denaturation at 94 °C; 30 s at 94 °C, 30 s at 58 °C, 90 s at 72 °C (5 cycles). This protocol repeated as follows with the annealing temperature decreasing with each set of cycles (5 cycles at 56 °C, 10 cycles at 54 °C, and 15 cycles at 52 °C). The primers used to amplify the targeted regions of GH and the specific introns are listed in Table 1. A nested PCR protocol was used to obtain the desired products. Primers GHe.cat.10F and GHe5.183R amplified a portion of the GH gene (exon 2 through exon 5). The protocol for this amplification was an initial denaturation at 94 °C for 60 s; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 100 s at 72 °C, followed by a final 5 min extension step. The amplified products were diluted 49:1 and used as the template in the intron specific reactions. The protocol for intron 3 using primers ghe3.min.61F and ghe4.cat.121R was the same as above. The protocol for intron 4, using primers ghe4.cat.14F and ghe5.173R, was 94 °C for 60 s; 15 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, 15 cycles with the annealing temperature at 53 °C, following by an 5 min extension period at 72 °C.

Amplified products were visualized by electrophoresis on Ethidium bromide stained gels. Fragments were purified with ExoSAP-IT (Amersham Biosciences) and sequenced with the same primers using the BigDye Terminator Reaction Mix from Applied Biosystems. Sequencing reactions were visualized on an ABI 3730XL DNA Analyzer. Sequence contigs were formed and visually edited in Sequencher 4.6 (Gene Codes Corporation). GenBank accession numbers for each sequenced product are listed in the Supplementary Data.

2.2. Sequence alignment and phylogenetic analysis

The partial cytochrome *b* sequences were aligned using ClustalW version 1.4 (Thompson et al., 1994) and manually edited in BioEdit version 7.0.9 (Hall, 1999). Four datasets utilizing the intron data were created. Intron 3 and intron 4 sequences were individually aligned with Prankster (Löytynoja and Goldman, 2008). Partitioned datasets including the sequence data and gap codes (binary characters) were created in FastGap version 1.2 (Borchsenius, 2009) from the prank alignments of intron 3, intron 4, and a concatenated alignment of introns 3 and 4 (see Supplementary Data). FastGap employs the simple indel coding method (Simmons and Ochoterena, 2000) which is just as effective as more complicated methods in coding the gap (indel) characters (Simmons et al., 2007). Analyzing the two introns separately and combined allowed us to determine the phylogenetic signal of each intron. A final alignment of the concatenated introns 3 and 4 with all gap areas removed allow us to assess the strength of including the gap characters in the analysis.

The cytochrome *b* and non-gap coded alignments were analyzed in MrModeltest 2.0 to find the optimal model of nucleotide evolution. A maximum likelihood (ML) and Bayesian inferences analysis were performed to infer the phylogeny. GARLI 2.0 (Zwickl, 2006) via the CIPRES Science Gateway (Miller et al., 2010) was used for ML analysis because it allows partitioned analysis. An initial analysis of 5 runs with 500 search reps allowed us to confirm that all runs were converging on the same tree topology. Nodal support was inferred by bootstrap proportions after 1000 bootstrap replicates (3 search reps). Bootstrap values were obtained by importing the resulting trees in Mesquite (Maddison and Maddison, 2011) and generating a majority rule consensus tree. The Bayesian analysis of the cytochrome *b* data was performed in MrBayes version 3.2 (Ronquist et al., 2012). Posterior probabilities were assessed with 5 million generations in which trees and parameters were sampled every 100 generations. The first 25% of trees were discarded as burn-in. Alignments for each analysis and associated configuration files included in the Supplementary Data.

3. Results

3.1. Cytochrome b

A 1053 base-pair (bp) alignment of the partial cytochrome *b* gene was obtained after including all sequences and trimming to the shortest sequence (HF565994.1). Within this alignment there were 317 parsimony informative (PI) characters shared by at least two different sequences. Pairwise uncorrected p-distance ranged from 15% to 20% among outgroup and ingroup taxa, and from 1% (Tana River to Athi River *C. brevibarbis* populations) to 20% (*C.* sp. "Chanler's Falls" to *C. brevibarbis*) among ingroup taxa. The model of evolution for the cytochrome *b* analysis selected by AIC and implemented in both ML and BI analysis was TVM + I + G.

A majority rules consensus tree obtained from the ML analysis with bootstrap support and posterior probabilities is shown in Fig. 2 (Fig. 2A). *Chiloglanis micropogon* from the Democratic Republic of Congo was defined as the outgroup. *Chiloglanis* cf. *occidentalis* from Guinea, West Africa were also included in the analysis. The analysis recovered a well-supported East African clade with a clade containing *Chiloglanis* sp. from Chanler's Falls; sister to *C. niloticus* (Nile R. basin) and *C. somereni* from the Lake Victoria basin (Fig. 2A). This group is sister to the remaining populations of *Chiloglanis* sampled. *Chiloglanis* sp. from the Kerio is sister to an unresolved polytomy comprising the *C. brevibarbis* group, *C. deckenii* group, and *C. asymetricaudalis* from Tanzania. Bootstrap support for this topology is moderate (75%). The BI analysis resolved the

Table 1
Nucleotide sequence for primers used in amplifying and sequencing Growth Hormone introns.

Primer name	Primer sequence (5′–3′)	Region	Source
GHe3.cat.10F	TGTTGCCTGAAGAACGCAAACA	GH exon 3	P. Unmack unpublished data
GH35.183R	CTACAGGGTGCAGTTGGAATC	GH exon 5	Moyer et al. (2009)
GHe3.min.61F	GCAACTCTGACTCCATTGAGGC	GH exon 3	Moyer et al. (2009)
GHe4.cat.121R	GCCCATTTTCAGGTCAGCCAGC	GH exon 4	P. Unmack unpublished data
GHe4.cat.14F	CACACCTCCTACCGTCTGAT	GH exon 4	P. Unmack unpublished data
GHe5.173R	CAGTTGGAATCCAGGGATCTC	GH exon 5	Moyer et al. (2009)

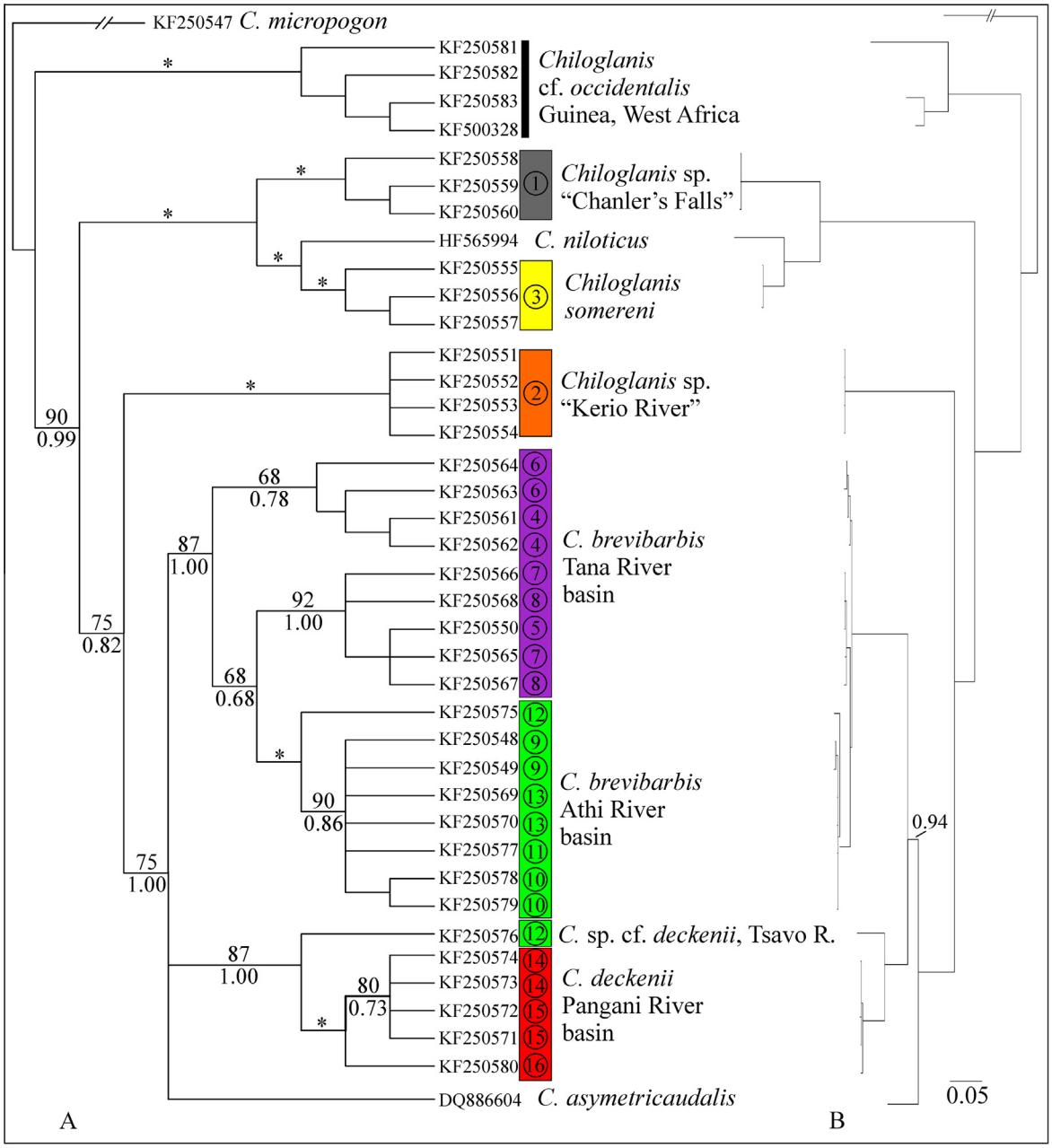

Fig. 2. Phylogenies inferred from cytochrome *b*. Majority rules consensus tree with bootstrap support above and posterior probabilities below (A); asterisks denote support >95%. Phylogram inferred from Bayesian analysis (B). Locality number and drainage colors correlate with those in Fig. 1.

polytomy with high Bayesian posterior probability (1.00): *C. asymetricaudalis* is sister to a group comprising all *C. brevibarbis* and *C. deckenii* (Fig. 2B). *Chiloglanis brevibarbis* forms a monophyletic group; however, populations from the Tana River basin are paraphyletic (Fig. 2A). *Chiloglanis* sp. cf. *deckenii* from the Tsavo River is sister to *C. deckenii* from the Pangani drainage with strong support.

3.2. Growth Hormone

The model of evolution selected by AIC for each GH intron alignment was TPM3 uf + G. Length of introns varied by species with intron 3 ranging from 844 bp in *C. brevibarbis* to 987 bp in *C.* cf. *deckenii*. Intron 4 was shorter ranging from 266 bp in *Chiloglanis* sp. from Chanler's Falls to 388 bp in *C. brevibarbis*. No allelic variation was detected within the individuals sampled in this study. The intervening exon 4 was 132 bp in length and was excluded from analyses. The concatenated dataset of introns 3 and 4 with indels removed had 791 characters (PI = 123), gap coded intron 3 alignment had 1612 nucleotide characters (PI = 149) and 76 gap coded binary characters (PI = 30), gap coded intron 4 alignment had 421 nucleotide characters (PI = 49) and 23 gap characters (PI = 12), and concatenated and gap coded introns 3 and 4 had 2033 nucleotide characters (PI = 198) and 99 gap characters (PI = 43). Intron 3 had an uncorrected p-distance ranging from 2.1% to 12.5% after removing indels. Intron 4 had an uncorrected p-distance ranging from 1.0% to 15.0% after removing indels.

Majority rule consensus trees inferred from each intron alignment are shown in Fig. 3. In all analyses specimens of *Chiloglanis* sp. from Chanler's Falls are sister to *C. somereni*, this group being sister to all other ingroup taxa. Specimens of *Chiloglanis* sp. from the Kerio River are sister to the *C. brevibarbis* and *C. deckenii* groups.

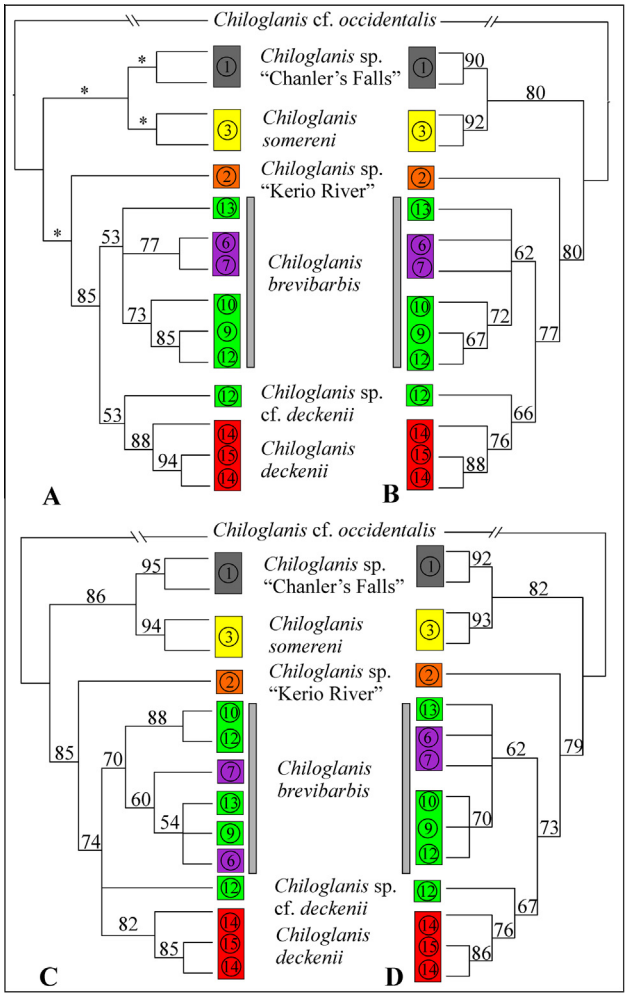

Fig. 3. Phylogenies inferred from Growth Hormone (GH) introns 3 and 4. Introns 3 and 4 concatenated with indels removed (A), intron 3 with coded indels (B), intron 4 with coded indels (C), and concatenated introns 3 and 4 with indels coded (D). Bootstrap support assessed after 1000 replicates.

Chiloglanis brevibarbis and *C. deckenii* are resolved as sister taxa in all analyses except for the gap-coded analysis of intron 4. Although this relationship is resolved in the analysis with indels removed, it is poorly supported (Fig. 3A). Including the binary characters derived from indel events improves the support for these branches (Fig. 3D).

4. Discussion

4.1. Undescribed Chiloglanis species in Kenya

The phylogenies inferred from cytochrome *b* and GH intron data are largely congruent and reveal unknown diversity within *Chiloglanis*. The analyses confirm the presence of the two known undescribed species. These species were earlier hypothesized to be distinct but were not described due to the paucity of specimens (Seegers et al., 2003). It was suggested that both undescribed taxa were closely related to *C. niloticus*; which the present study partially confirms (Seegers et al., 2003). *Chiloglanis* sp. "Chanler's Falls" specimens are sister to *C. niloticus* and *C. somereni*, while *C.* sp. "Kerio River" specimens are sister to *C. brevibarbis*, *C. deckenii*, and *C. asymetricaudalis* groups (Fig. 2). Both taxa are morphologically distinct from other taxa in the area and are presently being formally described by the authors.

The results also provide genetic evidence of additional unrecognized diversity within *Chiloglanis* in Kenya. Specimens related to *Chiloglanis deckenii*, which was thought to be confined to the Pangani system, were collected in the Tsavo River (Athi River basin) along with *C. brevibarbis* specimens. The specimens are morphologically similar to *C. brevibarbis* but display significant genetic divergence within cyt *b* (9.5% uncorrected p-distance). Additional specimens from this and surrounding areas are needed to confirm the presence of this potentially undescribed species. The authors are completing a morphological study of *C. brevibarbis* populations to determine if this and other genetic diversity observed within *C. brevibarbis* warrants species recognition.

4.2. Historical biogeography of the Kenyan Chiloglanis

The rivers of Kenya have undergone major alterations from the late Eocene through the Quaternary (Ojany, 1972). The most drastic alterations have been caused by the formation and continuation of the East African Rift System. This system, bisecting the eastern and western halves of the country, runs from Lake Turkana in the north to Lake Natron in the south. The uplift of the shoulders of the rift system commenced 45–40 MYA and was completely formed by 20 MYA (Roberts et al., 2012). Major updoming in Eastern Kenya from 3.2 to 2.75 MYA formed the main river courses observed today (Ojany, 1972; Veldkamp et al., 2007). Rivers in western Kenya have been altered by the formation of Lake Victoria. The lake was likely formed by down warping and subsequent uplifting along the eastern edge of the Western Rift Valley around 400,000 BP (Johnson et al., 2000). The rivers currently flowing into Lake Victoria would have previously been a part of Congo River basin (Ojany, 1972). Although there has been a general drying of the African climate over the last 8 MYA; water levels within the major lakes, smaller water bodies, and rivers have likely fluctuated throughout Kenya during this time (Hamilton and Taylor, 1991; Ryner et al., 2007). These fluctuations would allow for potential river capture events and localized extinction events as seen in the periods of desiccation in Lake Victoria (Johnson et al., 2000).

In both analyses specimens from Chanler's Falls (Northern Ewaso Nyiro) and *Chiloglanis somereni* from the Lake Victoria basin form the basal sister group to all other Kenyan *Chiloglanis. Chiloglanis niloticus* from Ethiopia is sister to *C. somereni* in the cyt *b* tree (Fig. 2). These results point to a recently shared Nilo-Sudan ancestor that gave rise to *Chiloglanis* sp. "Chanler's Falls" and *C. niloticus*. Subsequent dispersal of the *Chiloglanis* specimens into the Lake Victoria basin, when water levels rose and the outflow into the Nile commenced, likely gave rise to *C. somereni*. A different pattern is observed in *Synodontis afrofischeri* (Mochokidae), a Lake Victoria endemic, which shows closer affinities to species from the Eastern Congo tributaries than Nilo-Sudan species (Pinton et al., 2013; Day et al., 2013). Additional specimens of *Chiloglanis* from the Eastern Congo need to be included in the analyses before final determinations are made.

Chiloglanis sp. "Chanler's Falls" was likely once widely distributed throughout the Northern Ewaso Nyiro with climatic fluctuations occurring in the Pleistocene causing the species to be restricted below the falls. Waterfalls have not been generally viewed as a dispersal barrier for *Chiloglanis* because of their oral disc. Chanler's Falls, composed of two main channels falling roughly 20 m through chutes of white trachyte and dark basaltic rock (Broun, 1906), appears to be an effective barrier for upstream migration for some fishes within the Northern Ewaso Nyiro. In addition to *Chiloglanis* sp. "Chanler's Falls", *Labeo* sp. cf. *mesops* (Cyprinidae) has only been collected below Chanler's Falls.

Chiloglanis specimens collected from the upper reaches of the Kerio River are the only known *Chiloglanis* species within the Lake Turkana drainage. It is interesting that these are sister to the

eastern and southern Kenyan species vs. sister to the other Eastern Rift Valley and Lake Victoria taxa (*C. somereni* and *C. niloticus*). Including additional East and Central African taxa into the analysis may allow for better resolution of these relationships.

Within *C. brevibarbis* there is clear, but slight (0.8% cyt *b*) divergence between Athi River and Tana River (type locality) specimens. However, the trees suggest either that gene flow has continued between the rivers or lineage sorting within the Tana River is incomplete (Figs. 2 and 3). The proximity of the Athi and Tana rivers would allow for river capture events between the basins.

Connections between Lake Jipe and the Pangani River occur seasonally through a network of swamps and could allow for faunal exchanges between basins (Lowe, 1955). The close proximity of the Lumi River, source of Lake Jipe, to the Rombo River (Athi River basin) could allow for exchanges to occur. The presence of *Chiloglanis* sp. cf. *deckenii* in the middle Athi River (Tsavo River) provides evidence for past faunal exchanges between the Athi and Pangani river basins. This pattern of shared species in the Athi and Pangani rivers is also observed in *Amphilius kreftii* (Amphiliidae) and *Garra* sp. cf. *dembeensis* (Cyprinidae) (unpublished data). The authors are examining other co-distributed taxa to determine if they show similar patterns of relationships.

4.3. Utility of GH introns for phylogenetics

This analysis shows that GH introns are effective nuclear markers in phylogenetic studies involving closely related taxa. The independent analyses of GH introns 3 and 4 were largely congruent with the phylogeny inferred from cytochrome *b*. Intron 3 was longer and more informative than intron 4 in all *Chiloglanis* species samples. In some West African *Chiloglanis* taxa, intron 3 is much longer (~1500 bp), which can make sequencing difficult (unpublished data). Few intron sequences from Siluriformes are published; intron 3 from *Ictalurus punctatus* (S69215.1) is 716 bp and intron 4 is 340 bp (Tang et al., 1993). Characters derived from coding the indel regions within introns proved informative in this analysis. Inclusion of the gap characters resolved the polytomy between the *C. brevibarbis* and *C. deckenii* group and improved branch support (Fig. 3). These results provide further evidence for including the gap characters when possible (Simmons et al., 2001; Kawakita et al., 2003; Malhotra et al., 2010; Nagy et al., 2012).

This analysis adds to the existing body of work showing the utility of Growth Hormone introns in species-level phylogenetic analyses (Oakley and Phillips, 1999; Moyer et al., 2009; Bart et al., 2010a; Clements et al., 2012). A phylogeny inferred from GH introns and exons in *Hybognathus* (Cyprinidae) was largely congruent with results from mitochondrial markers and previous morphological studies (i.e., *Hybognathus* recovered as monophyletic); whereas, analysis of S7 introns recovered *Hybognathus* as paraphyletic (Moyer et al., 2009). GH introns in *Chiloglanis* display more intra-generic divergence than observed in the recently studied catostomids. Sampled members of *Ictiobus* (Catostomidae) had an interspecific nucleotide difference of 1.9–5.6% in GH1 (Bart et al., 2010a). A more complete study of the tribe Moxostomatini (Catostomidae) revealed ingroup differences from 1.4% to 4.4% (Clements et al., 2012). The higher level of divergence observed within the *Chiloglanis* likely resulted in a slightly stronger supported GH phylogeny (Fig. 3) than observed in the phylogeny of Moxostomatini (Clements et al., 2012).

The higher level of divergence in cyt *b* and GH introns in *Chiloglanis* compared to that observed in catostomids is likely the result of differences in gene flow among populations and selection within the two groups. Catostomid species generally maintain more contiguous distributions across a wider range of stream sizes and lakes, with more gene flow among populations than is typical of the distribution of *Chiloglanis* species. The higher rates of gene flow among populations slow the accumulation of nucleotide substitutions. Investigating rates of intron evolution across other groups of African Siluriformes will provide a better understanding of the importance of dispersal on rates of intron evolution. Coding regions of genes are generally under strong purifying selection. Results of selection tests have shown this to be the case for duplicate copies of GH in catostomids (Bart et al., 2010b). In such cases, selection operates to slow the divergence of the coding regions of gene copies until the genes can adaptively subfunctionalize (Nielsen et al., 2010). The coding regions of the different paralogs of the catostomids display slightly different rates of divergence; 3.33% and 3.22% for GH1 and GH2 respectively (Bart et al., 2010b). Non-coding introns apparently are not subject to the same selective forces.

The conserved nature of exon regions of GH within the Cypriniformes and Siluriformes allow primers placed in these regions (including the primers published herein) to easily amplify the intron regions of interest. This combined with the ideal lengths of these markers, presence of indels that can be gap coded, and a slower substitution rates than cyt *b*; make GH introns effective species level nuclear marker.

Acknowledgments

Funding provided by NSF OISE: 0968727; 1215395. The following participants of the IRES program whose dedication and hard work allowed the project to succeed. Principal investigators: D. Sigana (University of Nairobi), M. Ogada (Laikipia Wildlife Forum), and G. Talarchek (Tulane University). IRES student participants: R. Anderson, D. Lach, L. Mathews, N. Moses, H. Strobel, and T. Woods (Tulane University); S. Abade, J. Abong'o, L. Losili, A. Lydiah, R. Omwonga, C. Mburu, P. N'gang'a, M. Odhiambo, W. Owako, O. Mlati, P. Tanui (University of Nairobi); A. Ewing and M. Patterson (Xavier University of New Orleans); and J. Gathua, G. Kosgei, T. Ndiwa (Kenya Wetlands Biodiversity Research Group). In addition we thank the Kenyan Wildlife Service (KWS) particularly J. Nyunja (Wetlands program) and the Warden and staff at Meru National Park for allowing us to collect within the park boundaries. We thank the Mpala Research Center and its staff for their hospitality. Peter Nyamenya (Kisumu Museum) and Dr. W. Ojwang (KEMFRI) were of great assistance in Nyanza County. Our drivers A. Mukiri and L. Nzangi Kioko (NMK), A. Mureithi and I. Mogoi (UoN) provided wonderful service. We would also like to thank K. Githui and A. Mwaura (NMK), F. Nyaga and J. Samoei (UoN) for their assistance in their respective laboratories. The staff of the NMK Ichthyology and Herpetology sections provided invaluable support while in the field and in the laboratory. J.P. Friel at Cornel University Museum of Vertebrates (CUMV) provided genetic material. P.J. Unmack provided the unpublished GH primers and initial protocols. Comments and discussions from M.H. Doosey greatly improved the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.07.011.

References

Bart Jr., H.L., Clements, M.D., Blanton, R.E., Piller, K.R., 2010a. Discordant molecular and morphological evolution in buffalofishes (Actinopterygii: Catostomidae). Mol. Phylogenet. Evol. 56, 808–820.

Bart Jr., H.L., Reneau, P.C., Doosey, M.H., Bell, C.D., 2010b. Evolutionary divergence of duplicate copies of the growth hormone gene in suckers (Actinopterygii: Catostomidae). Int. J. Mol. Sci. 11, 1090–1102.

Borchsenius, F., 2009 FastGap 1.2. Distributed by author Aarhus, Denmark: Department of Biological Sciences, University of Aarhus. <http://192.38.46.42/aubot/fb/FastGap_home.htm> (Downloaded May 2012).
Broun, W.H., 1906. A journey to the Lorian swamp, British East Africa. Geogr. J. 27 (1), 36–51.
Clements, M.D., Bart Jr., H.L., Hurley, D.L., 2012. A different perspective on the phylogenetic relationships of the Moxostomatini (Cypriniformes: Catostomidae) based on cytochrome *b* and growth hormone intron sequences. Mol. Phylogenet. Evol. 63, 159–167.
Daget, J., Durand, J.R., 1981. Poissons. In: Durand, J.R., Lévêque, C. (Eds.), Flore et faune aquatiques de l'Afrique Sahelo-Soudanienne. ORSTOM, Paris, vol. 2.
Day, J.J., Peart, C.R., Brown, K.J., Friel, J.P., Bills, R., Moritz, T., 2013. Continental diversification of an African catfish radiation (Mochokidae: *Synodontis*). Syst. Biol. 62 (3), 351–365.
Ferrais, C.J., 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. Zootaxa, 1418.
Friel, F.P., Vigliotta, T.R., 2011. Three new species of African suckermouth catfishes, genus *Chiloglanis* (Siluriformes: Mochokidae), from the lower Malagarasi and Luiche rivers of western Tanzania. Zootaxa 3063, 1–21.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis programs for Windows 95/98/NT. Nucleic Acids Res. 41, 95–98.
Hamilton, A.C., Taylor, D., 1991. History of climate and forests in tropical Africa during the last 8 million years. Climatic Change 19 (1–2), 65–78.
Hardman, M., Page, L.M., 2003. Phylogenetic relationships among bullhead catfishes of the Genus *Ameiurus* (Siluriformes: Ictaluridae). Copiea 2003 (1), 20–33.
Johnson, T.C., Kelts, K., Odada, E., 2000. The Holocene history of Lake Victoria. Ambio 29 (1), 2–11.
Kawakita, A., Sota, T., Ascher, J.S., Ito, M., Tanaka, H., Kato, M., 2003. Evolution and phylogenetic utility of alignment gaps withing intron sequences of three nuclear genes in bumble bees (*Bombus*). Mol. Biol. Evol. 20 (1), 87–92.
Löytynoja, A., Goldman, J., 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. Science 320, 1632–1635.
Lowe, R.H., 1955. New species of Tilipia (Piesces, Cichlidae) from Lake Jipe and the Pangani River, East Africa. Bull. Br. Mus. (Nat. Hist.) Zool. 2 (12), 349–368.
Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 <http://mesquiteproject.org>.
Malhotra, A., Creer, S., Pook, C.E., Thorpe, R.S., 2010. Inclusion of nuclear intron sequence data helps to identify the Asian sister group of New World pitvipers. Mol. Phylogenet. Evol. 54, 172–178.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of The Gateway Computing Environments Workshop (GCE), 14.11.10, New Orleans, LA, pp. 1–8.
Moyer, G.R., Remington, R.K., Turner, T.F., 2009. Incongruent gene trees, complex evolutionary processes, and the phylogeny of a group of North American minnows (*Hybognathus* Agassiz 1855). Mol. Phylogenet. Evol. 50, 514–525.
Müller, K., 2006. Incorporating information from length-mutational events into phylogenetic analysis. Mol. Phylogenet. Evol. 38, 667–676.
Nagy, L.G., Kocsubé, S., Csanádi, Z., Kovács, G.M., Petkovits, T., Vágvölgyi, C., Papp, T., 2012. Re-mind the gap! insertion – deletion data reveal neglected phylogenetic potential of the nuclear ribosomal internal transcribed spacer (ITS) of fungi. PLoS One 7 (11), e49794.
Nielsen, M.G., Gadagkar, S.R., Gutzwiller, L., 2010. Tubulin evolution in insects: gene duplication and subfunctionalization provide specialized isoforms in a functionally constrained gene family. BMC Evol. Biol. 10 (113), 1–21.
Oakley, T.H., Phillips, R.B., 1999. Phylogeny of Salmonine fishes based on growth hormone introns: Atlantic (*Salmo*) and Pacific (*Oncorhynchus*) salmon are not sister taxa. Mol. Phylogenet. Evol. 11 (3), 381–393.
Ojany, F.F., 1972. Drainage evolution in Kenya. In: Ominde, S.H. (Ed.), Studies in East African Geography and Development. University of California Press, Berkley and Los Angeles, pp. 137–145.
Pinton, A., Angèse, J.-F., Paugy, D., Otero, O., 2013. A large-scale phylogeny of *Synodontis* (Mochokidae, Siluriformes) reveals the influence of geological events on continental diversity during the Cenozoic. Mol. Phylogenet. Evol. 66 (3), 1027–1040.
Roberts, E.M., Stevens, N.J., O'Connor, P.M., Dirks, P.H.G.M., Gottfried, M.D., Clyde, W.C., Armstrong, R.A., Kemp, A.I.S., Hemming, S., 2012. Initiation of the western branch of the East African Rift coeval with the eastern branch. Nat. Geosci. 5, 289–294.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61 (3), 539–542.
Ryner, M., Gasse, F., Rumes, B., Verschuren, D., 2007. Climatic and hydrological instability in semi-arid equatorial East Africa during the late Glacial to Holocene transition: a multi-proxy reconstruction of aquatic ecosystem responses in northern Tanzania. Palaeogeogr. Palaeocl. 248, 440–458.
Seegers, L., De Vos, L., Okeyo, D.O., 2003. Annotated checklist of the freshwater fishes of Kenya (excluding the lacustrine haplochromines from Lake Victoria). J. East Afr. Nat. Hist. 92, 11–47.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analysis. Syst. Biol. 49 (2), 369–381.
Simmons, M.P., Ochoterena, H., Carr, T.G., 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Syst. Biol. 50 (3), 454–462.
Simmons, M.P., Müller, K., Norton, A.P., 2007. The relative performance of indel-coding methods in simulations. Mol. Phylogenet. Evol. 44, 724–740.
Tang, Y., Lin, C.M., Chen, T.T., Kawauchi, H., Dunham, R.A., Powers, D.A., 1993. Structure of the channel catfish (*Ictalurus punctatus*) growth hormone and its evolutionary implications. Mol. Mar. Biol. Biotech. 2 (4), 198–206.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Veldkamp, A., Buis, E., Wijbrans, J.R., Olago, D.O., Boshoven, E.H., Marée, R.M., van Saparoea, V.D.B., 2007. Late Cenozoic fluvial dynamics of the river Tana, Kenya, an uplift dominated record. Quaternary Sci. Rev. 26 (22–24), 2897–2912.
Vigliotta, T.R., 2008. A phylogenetic study of the African catfish family Mochokidae (Osteichthyes, Ostariophysi, Siluriformes), with a key to genera. Proc. Acad. Nat. Sci. Phila. 157 (1), 73–136.
Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.