Body shape evolution of African/Asian minnows of the genus *Labeo* Cuvier 1817 (Cyprinidae, Labeonini) and variations in *Labeo parvus*.

by

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Abstract

Morphological variation or similarities among organisms are not only a result of common evolutionary history but can also emerge because of convergent adaptations to similar habitats. Therefore, an organism's morphology is strongly correlated with environmental gradients and plays an important role in growth, survival, and reproduction. In aquatic habitats for instance, body shape plays a significant role in foraging, locomotion, defense, and habitat exploitation. Understanding shape variation and evolution within a group of organisms can provide insights about strategies for habitat colonization, food resource use, and even species diversity within a group. Herein, I combined geometric morphometrics, molecular phylogeny, and phylogenetic comparative methods to assess body shape variation and evolution among species of the African/Asian minnows of the genus *Labeo*. Additionally, I assessed species diversity and distribution patterns within *Labeo parvus*. I found that *Labeo* body shape varies significantly across species and clades. The greatest variation in body shape among *Labeo* species and clades occurs in body depth and width. I also found that the similarities in body shape observed between some species are not always due to common evolutionary history. Indeed, both the visual examination of the phylomorphospaces and SURFACE analyses revealed multiple instances of convergent evolution across *Labeo* phylogeny. Furthermore, I found that *Labeo parvus* is a species complex of several species and that the true *Labeo parvus* is endemic to the Congo basin.

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List of Abbreviations

AMCC	Ambrose Monell Cryo Collection
AMNH	American Museum of Natural History
AUFT	Auburn University Museum Fish Tissues
AUMNH	Auburn University Museum of Natural History
BI	Bayesian Inference
BOLD	Barcode of Life Data System
CI	Confidential Interval
GMYC	General Mixed Yule Coalescent
Ma	Millions of years ago
MAP	Maximum *a posteriori*
ML	Maximum Likelihood
PTP	Poisson Tree Processes

General Introduction

Two centuries ago George Cuvier erected the subgenus *Labeo* to designate a subgroup of *Cyprinus* species characterized by a long dorsal fin, thick lips, and lack of spines (Cuvier et al. 1817). The genus *Labeo* belongs to the subfamily Labeoninae of the family Cyprinidae (Nelson et al., 2016; Zheng et al., 2012). With an estimated number of 3,006 species, Cyprinidae represents the largest family within the order Cypriniformes and is the largest freshwater fish family in the world (Chen and Mayden, 2009; Nelson et al., 2016). Cyprinids are found in Africa, Europe, Asia, and North America (Winfield and Nelson, 1991; Mayden et al., 2009). The fishes of this family exhibit high levels of both genetic and morphological diversity, with the highest diversification on the Asian continent, where nearly 1,200 species have been reported (Wang et al., 2007). Cyprinids are likely very diverse in Africa, where about 526 species occur, representing almost 17% of the continental ichthyofauna (Lévêque and Paugy, 2006). Cyprinids constitute the second-largest fish family on the African continent and are predominant in almost all African river systems (Lévêque and Paugy, 2006). In number of described species, they are only exceeded by Cichlidae (Paugy 2010). Members of Labeoninae, one of the 12 Cyprinidae subfamilies, are characterized by a high diversity of mouth morphologies. Modifications of the mouth have been described from structures such as: lips, rostral cap, barbels, and mental adhesive disc (Stiassny and Getahun, 2007; Yang et al., 2012; Zheng et al.,2016). Labeonines are widely distributed in Asian and African river systems, where they are represented by almost 40 genera and over 450 species (Yang et al., 2012; Eschmeyer et al., 2017).

After *Garra*, the Afro-Asian genus *Labeo* is the second-most diverse genus of Labeoninae. Over 106 species of *Labeo* have been described. Of the 73 known from Africa, 35 inhabit the Congo basin (Froese & Pauly, 2016; Moritz and Neumann, 2017) and 28 live in

South and Southeast Asia (Jayaram 2010; Lal et al. 2015). While the monophyly of *Labeo* has been strongly supported by morphological studies (Stiassny and Getahun, 2007), this is not the case with genetic work to date, which has suggested that *Labeo* is paraphyletic (Yang et al. 2012; Zheng, Yang, and Chen 2012). *Labeo* species are widely distributed throughout Africa, South and South East Asia, where they occur in a wide variety of habitats (Weyl and Booth 1999; Moritz and Neumann 2017; Lysell 2009; Stiassny, Teugels, and Hopkins 2007). They are mainly herbivores and feed on algae and detritus from benthic substrates (Reid 1985; Skelton 2001; Pwema et al. 2015). Most *Labeo* are adapted to swift currents with rocky substrates and are strong swimmers (Skelton 2001; Pwema et al. 2011). Based on the fossil record, Van Couvering and Greenwood (Winfield and Nelson 1991), think that Danionidae (Stout et al., 2016) and Cyprinidae (Labeonine and Barbine) migrated from Southeast Asia to Africa, via the Arabian Peninsula, as early as 18 million years ago (Ma). Ren and Mayden (2016), using fossils of different cyprinid groups, found that the African and Asian *Labeo* clades diverged about 18.1 Ma.

The African species of *Labeo* have been reviewed by Reid (1985) and Tshibwabwa (1997). Prior to these two major studies on African *Labeo*, Jegu and Lévêque (1984) reviewed the West African species of *Labeo* closely related to *Labeo parvus*, one of the widespread species of the genus. Reid (1985), based on morphometric and anatomical data, subdivided African *Labeo* species into six species-groups with distinct geographical ranges: *L. gregorii* group (East Coast), *L. macrostoma* group (West Coast and Congo basin), *L. umbratus* group (South West Cape), *L. niloticus* group, *L. coubie* group, and *L. forskalii* group (pan-African excluding the North African and South West Cape for the three last groups). The Asian *Labeo* species have been reviewed by Jayaram and Dhas (2000), who sorted them into eight groups

based on morphometric, meristic, and anatomical data: *L. gonius* group, *L. pangusia* group, *L. dero* group, *L. porcellus* group, *L. fimbriatus* group, *L. ariza* group, *L. boga* group, and *L. potail* group. Subsequently, Lal et al. (2015) reviewed the *L. gonius* subgroup species. Despite these efforts, the taxonomy of *Labeo* remains problematic, especially in Africa, and species identification is difficult (Lowenstein et al., 2011; Van Steenberge et al., 2014; Van Steenberge et al. 2016).

Lowenstein et al. (2011) were the first to investigate the phylogenetic relationships of *Labeo* species from the Congo and Lower Guinea ichthyoprovinces (Fig. 1) using molecular data. Their study corroborated the hypothesis of two main clades of *Labeo* species within these provinces that had been suggested earlier in a morphology-based study by Tshibwabwa (1997). Yang et al. (2012) brought more insight about the phylogenetic relationship of *Labeo* species. More recently, Adeoba et al. (2018) published a phylogenetic tree based on a COI dataset that for the first time incorporated *Labeo* species from the Southern African ichthyoprovince (Fig. 1). However, these phylogenies did not include all *Labeo* species, leaving the relationships among several species unknown. Thus, a more inclusive phylogenetic study is necessary to resolve and understand the phylogenetic relationship within the genus.

Considering adaptive radiation as a result of natural selection driving the divergence of an ancestral species into descendants that are better able to take advantage of the environmental conditions that maintain that lineage and generate divergence (Glor 2010; Wellborn and Langerhans 2015) and given the paraphyly of *Labeo* species (shared common ancestor) and their level of diversification, *Labeo* constitutes a good group for understanding speciation, adaptive radiation and evolution. Yet, studies on the evolution of the genus are almost inexistent.

In the body of this thesis, I assess the overall body shape evolution among *Labeo* species. In chapter 1, I build a large-scale phylogeny of *Labeo* species, assess their overall body shape variation by using geometric morphometrics, and establish the presence of and quantify evolutionary convergence by testing for the phylogenetic signal and estimating a macroevolutionary landscape that measures the extent of convergence. In chapter 2, I combine traditional morphometric, geometric morphometrics, and molecular phylogeny to provide insights about the diversity and distribution of an African *Labeo* species complex (*Labeo parvus*).

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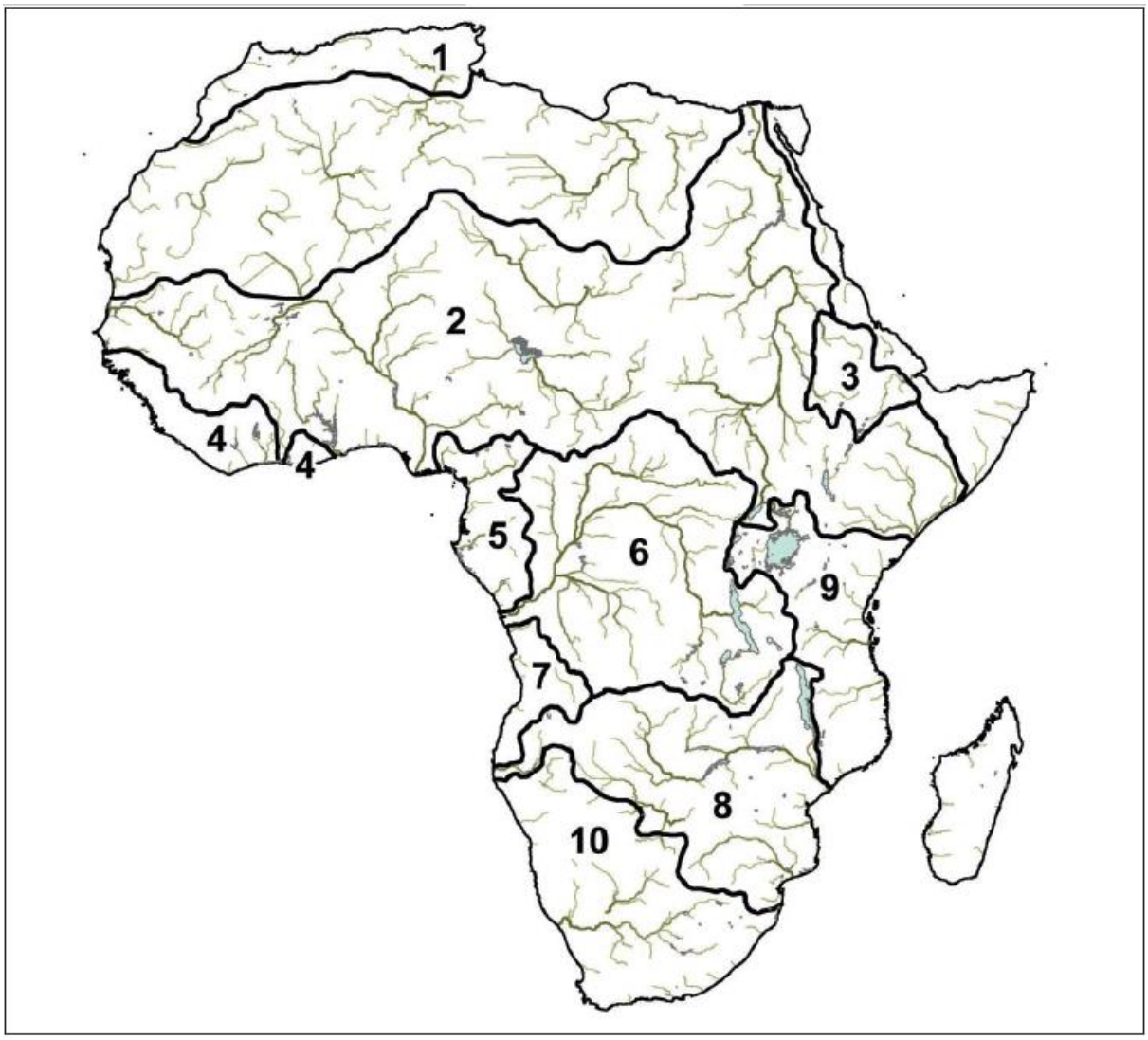

Figure 1: The major ichthyofaunal provinces of continental Africa, from Snoek *et al.* (2011). (1) Maghreb, (2) Nilo-Sudan, (3) Abyssinian Highlands, (4) Upper Guinea, (5) Lower Guinea, (6) Congo (Zaire), (7) Quanza (Kwanza), (8) Zambezi, (9) East Coast, (10) Southern (including Cape of Good Hope).

Chapter I. Phylogeny, variation, and convergent evolution in overall body shape within the genus *Labeo*

ABSTRACT

The Afro-Asian genus *Labeo* is the second-most diverse genus of the subfamily Labeoninae with over 106 species described. The members of *Labeo* are widely distributed throughout Africa and South and Southeast Asia, where they occur in a wide variety of habitats. Due to their ability to adapt to different habitats, *Labeo* species are morphologically diverse. Though they are economically important and highly diverse, studies on *Labeo* species evolution, adaptive radiation, and body shape variation are very rare. In this study, we used a molecular phylogeny and geometric morphometrics to investigate shape variation within the genus *Labeo* and test for convergent evolution in body shape across these species. Genomic DNA was extracted from 98 specimens of *Labeo* from different localities and two genes (COI and RAG1) were amplified by PCR and Sanger sequenced. Bayesian Inference and Maximum Likelihood analyses were used for phylogenetic reconstruction. A total of 530 *Labeo* individuals belonging to at least 41 valid species were photographed in ventral and lateral views. The obtained digital images were landmarked and analyzed in MorphoJ using Principal Components and Canonical Variates Analyses. The phylogenies were mapped onto the resulting morphospaces to test for phylogenetic signal, adaptive radiation, and convergent evolution using MorphoJ, SURFACE, and Geomorph. We found that *Labeo* body shape is considerably diverse and associated with specific habitats. The phylogenetic signal test suggested body shape conservation within subclades. However, we identified multiple instances of convergence in body morphology of *Labeo* species.

INTRODUCTION

Throughout the history of life, evolutionary processes have yielded a spectacular morphological diversity of living organisms. Within vertebrates, fishes are not only the most speciose group but also the one that presents the greatest body shape range (Nelson et al., 2016) due to their ability to adapt to a variety of habitats. Indeed, the invasion of a new habitats by fish species often results in diet and morphological changes. In aquatic habitat, body shape plays an important role in foraging, locomotion, reproduction, defense, and habitat exploitation (Schluter, 1993; Langerhans, 2008;Webster et al., 2011; Foster et al., 2015). Thus, the understanding of body shape divergence can provide insights about species strategies for habitat colonization and food resource use.

In freshwaters, fishes often show large amounts of body shape variation across divergent habitats (Foster et al., 2015) and niches. The ability of organisms to proliferate both in number of taxa and diversification of shape in relationship with habitat exploitation is known as adaptive radiation (Schluter, 1993; Foote, 1997). A spectacular case of adaptive radiation in freshwater fishes is the radiation of cichlid species flocks in the East African Great Lakes (Ruber and Adams, 2001; Seehausen, 2006; Brawand et al., 2015), but cichlid fishes are not the only group to undergo adaptive radiation in freshwater.

Cyprinidae is the largest freshwater fish family in the world (Chen and Mayden, 2009; Nelson et al., 2016), and members of this family exhibit high levels of both genetic and morphological diversity, with the highest diversity on the Asian and African continents (1,200 and 526 species respectively) (Lévêque and Paugy, 2006; Wang et al., 2007). Within Cyprinidae, the Afro-Asian genus *Labeo* is the second-most diverse genus of the subfamily Labeoninae, with over 106 species described. Of the 73 known from Africa, 35 inhabit the Congo basin (Froese &

Pauly, 2016; Moritz and Neumann, 2017) and 28 live in South and Southeast Asia (Jayaram, 2010; Lal et al., 2015). While the monophyly of *Labeo* has been strongly supported by morphological studies (Stiassny and Getahun, 2007), this is not the case with genetic work to date, which has suggested that *Labeo* is paraphyletic (Yang et al. 2012; Zheng et al., 2012).

Labeo species are widely distributed throughout Africa and South and Southeast Asia, where they occur in a wide variety of habitats (Weyl and Booth, 1999; Moritz and Neumann, 2017; Lysell, 2009; Stiassny et al., 2007). They are mainly herbivores and feed on algae and detritus from benthic substrates (Reid, 1985; Skelton, 2001; Pwema et al., 2015). Most *Labeo* are adapted to swift currents and a rocky substratum (Skelton, 2001; Pwema et al., 2011). Their adaptation to different habitats during a long and complex evolution yielded diverse morphology and a high level of endemism. The fossil records and molecular evidence reveal that *Labeo* migrated from Southeast Asia to Africa as early as 18 million years ago (Ma) when Africa contacted the Arabian Plate (Couvering, 1977; Ren and Mayden, 2016).

Despite their high morphological and genetic diversity, studies on the evolution of body shape variation and ecomorphology within *Labeo* radiation are almost nonexistent. To date, the phylogenetic relationships of most *Labeo* species remain unknown and there is no large-scale phylogenetic study that has addressed their Phylogeography. Furthermore, there is no study on evolutionary mechanisms responsible for their morphological diversity.

In this study, we used molecular phylogenetics to assess the relationships of the species of *Labeo* and geometric morphometrics to assess body shape variation and its evolution among *Labeo* clades and species. We hypothesize that body shape similarities among some species of *Labeo* are due to environmental selective pressures leading to evolutionary convergence. We tested this hypothesis and measured the extent of morphological convergence.

MATERIALS AND METHODS

Morphological data collection and analyses

Fish taxa used in this study include forty-one valid and undescribed *Labeo* species from the collections at the Auburn University Museum (AUM) and American Museum of Natural History (AMNH). These species are mainly form sub-Saharan Africa and Eastern Asia. Representative individuals of each species were photographed in ventral and lateral (left side) views using a mounted Canon EOS 600D digital camera. Analyses included juveniles and adults of both sexes. Photographs from 516 individuals were used to create digital images of geometric morphometric (GM) landmarks, following Armbruster (2012), using TpsDIG2 (Rohlf 2016a) to describe species' body shape. The x-y coordinates of landmarks generated by TpsDig2 were saved in a tps file with TpsUtil 1.70 (Rohlf 2016b). A Generalized Procrustes Analysis (GPA) was performed in MorphoJ 1.06d (Klingenberg 2011) to scale landmarks of each specimen to a common body size, to rotate each individual to a common alignment, and to generate a consensus shape by calculating the average shape of all specimens in the analysis. After checking for outliers, a covariate matrix was constructed to prepare the dataset for Principal Components Analyses (PCA) and Canonical Variates Analyses (CVA), which were conducted in MorphoJ. The PCA was performed to assess body shape variation among individuals, whereas the CVA and permutation test for pairwise distance with 10,000 iterations assessed species differences. In addition to CVA, a Procrustes ANOVA was also performed in MorphoJ. The broken stick model (Borcard et al. 2011) was used to determine which of the principal components or canonical variate axes were significant.

DNA Extraction

Cataloged tissues from the AUM Fish Tissue collections and the AMNH Department of Ichthyology Tissue collections were used for genomic DNA extractions. These tissues were collected during different collecting trips, preserved in 95% ethanol and frozen at -80 °C. Total genomic DNA was extracted from 98 individuals using either Omega BioTek E.Z.N.A. or Qiagen Dneasy Tissue kits according to the methods provided by the manufacturers.

Gene Amplification and Sequencing

DNA amplification was conducted by polymerase chain reaction (Mullis et al. 1986; Saiki et al. 1988) for part (about 652 bp) of the mitochondrial cytochrome oxidase subunit 1 (COI) and part (about 800 bp) of the nuclear Recombination-Activation gene 1 (RAG1). The COI was amplified following Ivanova et al. (2007) while the part b of the RAG1 was amplified following López, Chen, and Ortí (2004) and Lowenstein et al. (2011) using the following primers: RAG1_R1 (5'-CTGAGTCCTTGTGAGCTTCCATRAAYTT-3') and RAG1_JHL_Fi (5'-ATGCACGCTCTGCGACTCAA-3'). DNA extractions and amplifications were performed in the Bond/Armbruster molecular laboratory at Auburn University. The obtained amplicons were sent for Sanger sequencing at Genewiz (https://www.genewiz.com). Additional COI and RAG1 sequences were imported from Genbank (www.ncbi.nlm.nih.gov/genbank) and the Barcode of Life Data System (BOLD; http://boldsystems.org/index.php). Some sequences from the Barcode of Life Data System were renamed following the AMNH and Lowenstein et al. (2011) identifications because the identifications provided by the BOLD system were incorrect for those sequences.

Phylogeny Construction

We used 398 *Labeo* COI sequence reads, from which 91 came from our dataset and 207 were imported form Genbank and BOLD. These were aligned and edited by hand using

Geneious (v.11.0.2). Three additional COI sequences (two *Opsariichthys* and one *Danio*) were used as outgroups. One hundred and seventy-nine *Labeo* sequences for COI (596 bp) and RAG1 (624 bp) were concatenated (1220 bp) using Geneious and exported as PHYLIP and NEXUS files for further analyses. Optimal models and partitioning schemes were determined using Partionfinder2 (v. 2.1.1.) (Lanfear et al., 2016) using the PHYLIP formatted files exported from Geneious. The GTR+I+G model was used for the COI dataset and GTR+ I+G or GTR+I models were used for the five subsets of the concatenated dataset. Bayesian inference and Maximum likelihood analyses were conducted for COI on the concatenated datasets with MrBayes 3.2.2 and RAxML v8.2.X (Stamatakis, 2014), respectively, using the CIPRES Science Gateway V.3.3 (http://www.phylo.org). Markov Chain Monte Carlo (MCMC) analyses were run for $6 * 10^7$ generations, with trees sampled every 3,000 generations for Bayesian analyses. One thousand (1,000) bootstrap replicates were used to evaluate branch support in RAxML. The obtained phylogenetic trees were visualized and annotated with FigTree v1.4.3 (Rambaut, 2016).

Detecting evolutionary convergence

Testing for phylogenetic signal

To test for phylogenetic signal, the Bayesian inference and maximum likelihood phylogenetic trees were pruned, using the phytools (Revell, 2012) package in R 3.4.1. (R Core Team 2013), to match the taxa on the phylogeny with those of the morphospace datasets. The pruned trees were imported in MorphoJ and mapped onto the morphospaces (PCA and CVA) to generate phylomorphospaces. The permutation test for phylogenetic signal, with 10,000 iterations and weighted by branch length, was applied to the resulting phylomorphospaces to assess the direction of body shape change along the evolutionary axes. We conducted an evolutionary principal component analysis (EPCA) on the changes along the branches produced

by the phylogenetic signal test (Schlick-Steiner et al., 2006) to assess the shape changes that account for most of the evolutionary differentiation along tree branches.

Quantifying convergent evolution

To detect and quantify evolutionary convergence, meaning to measure the frequency of convergence, we used SURFACE analysis (Ingram and Mahler, 2013), which uses regime fitting with Akaike Information Criterion (AIC) to model convergent evolution. Two stepwise procedures were carried out to locate the number of regime shifts (k) on the phylogeny and then identify whether any of these shifts (change in the parameters of the model) had evolved independently in multiple lineages. During this process the regime shifts are iteratively added to a Hansen model, shifts are then iteratively removed to identify convergent regimes (k'). The reduction in complexity (k-k') corresponds to the number of regimes that can be collapsed into an existing regime (i.e. convergence). To visualize distinct body shape evolutionary regimes convergent and non-convergent regimes were overlaid onto the phylogeny (Ingram and Mahler, 2013).

RESULTS

Geometric morphometrics

Both PCA and CVA indicate an important dispersion among individuals, species, and species groups across morphospaces. The broken stick model analysis reveals that the first four principal components are significant in both lateral and ventral views, whereas only the two first canonical variate axes were found to be significant in both lateral and ventral views (Fig. 1).

Body shape variation among individuals (PC axes)

In lateral view, the first four principal components describe 71.2% of the total body shape variation among *Labeo* individuals, with 29.2% of the variation described by the first principal

component (PC1). This principal component mainly described the depth of the body. Extreme positive PC1 values were associated with individuals that had large orbital length (eye diameter), large head, deep but short caudal peduncle, long dorsal-fin base, and deep body (e.g., *L. longipinnis*) whereas extreme negative PC1 values were associated with individuals that had small orbital length, shallow but long caudal peduncle, small head, short dorsal-fin base, and shallow body (e.g., *L. alluaudi*) (Fig. 2). The second and third principal components explained 17.2% and 14.7% of total shape variation, respectively. Specimens associated with high positive values on PC2 were mainly characterized by a relatively long distance between the tip of snout and the mouth opening, a relatively long snout, and nearly dorsolateral eyes; specimens associated with negative PC2 values were characterized by a relatively short distance between snout tip and the mouth opening, a relatively short snout, and lateral eyes (Fig. 3). The third PC mainly described the size of the head, the snout length, eye positions, and the mouth opening position relative to the tip of snout. Specimens with high positive PC3 values had a very small head, a mouth opening very close to the snout tip, a very short snout, and lateral eyes (e.g., *L. mesops*) whereas specimens with high negative PC3 values had their mouth opening far from their snout tip, a very large head, a very elongate snout, and almost dorsal eyes (e.g, *L. longipinnis* and *L. fulakariensis*) (Fig. 4).

In ventral view, 76.4% of the total shape variation among individuals were described by the first four principal components, with 31.2% of the variation explained by the first principal component. PC1 mainly described the thickness of the body. Extreme positive values of PC1 were associated with individuals that had a slim body (very short inter-pectoral and inter-pelvic widths), a blunt snout, a small mouth (short gape width), and a short vent-anal distance (distance between the anal fin and the anus) (e.g., *L. bata* and *L. weeksii*); negative PC1 values were

associated with individuals that had a thick body, pointed snout, large mouth, and large vent-anal distance (e.g., *L. sorex* and *L. nasus*) (Fig. 5). The second principal component described 21.5% of the total variation and captured variation in the head-base size. The positive values on this axis were associated with individuals that had relatively small head-base, whereas the negative values were associated with the ones with a relatively large head-base. Figure 6 illustrates variation associated with PC2.

Body shape variation among species (CV axes)

The two interpretable canonical variate axes (CV1 and CV2) captured 62.2% of the total variation in lateral view between species, with the first CV describing 38.8% of the variation and the second CV 24.7%. Positive values of CV1 were associated with species that had a pointed snout and dorsoventrally compressed body (e.g., *L. sorex* and *L. parvus*), while negative CV1 values were associated with the species with a blunt snout and deep body (e.g., *L. longipinnis* and *L. chrysophekadion*). In addition to capturing the body depth, CV2 was negatively correlated with the caudal peduncle depth of species (Fig. 7).

In ventral view, the two interpretable CV axes (CV1 and CV2) explained 68.1% of shape variation among species with the first axis describing 48.4% of the total variation observed between *Labeo* species. CV1 captured the same variation as PC1. Negative CV1 values were associated with species with slim body (lateral compression), blunt snout, small mouth, and short vent-anal distance (e.g., *L. bata*), while positive values were associated with species with a large mouth, thick body (lateral expansion), pointed snout, and long vent-anal distance (e.g *L. sorex* and *L. nasus*). The second CV axis captured 19.7% of the total variation of the shape. Its extreme negative values were associated with the species that had a thick body, large mouth, and short vent-anal distance (e.g., *L. fulakariensis, L. cyclorhynchus,* and *L. macrostama*) in contrast, the

extreme positive values were associated to species with relative small mouth, and a large vent-anal distance (e.g., *L. cylindricus*).

The permutation tests for Procrustes and Mahalanobis distances among species revealed that there were significant overall body shape differences among the majority of *Labeo* species but not all of them.

The simultaneous examination of the lateral and the ventral views showed that individuals or species with deep body tended to have a short inter-pectoral distance whereas those with shallower bodies trended toward longer inter-pectoral distance. PCA and CVA of *Labeo* overall body shape revealed a tendency of species toward a dorsoventral body compression or a lateral body compression.

Phylogeny

Cytochrome oxidase subunit 1

Model-based phylogenetic analyses under both maximum likelihood (ML) and Bayesian inference (BI) supported the hypotheses of a monophyletic African *Labeo* as suggested by Lowenstein et al. (2011). In contrast, the Asian *Labeo* species were found to be paraphyletic. The BI analyses suggested the existence of four main clades within the African *Labeo* sampled in this study (Fig. 8) but the relationships among the four clades was an unresolved polytomy. Maximum likelihood suggested a similar topology with slight differences regarding the relationship between these clades (Fig. 9). For most clades, ML returned lower bootstrap support values than posterior probabilities given by BI. Therefore, the results presented in this section are based on BI analyses. The clade A was made up of two main subclades: clade Aa and clade Ab (Fig. 8). The subclade Aa was comprised exclusively of the *L. forskallii* group species from the Congo and Kwanza Rivers (*L. parvus, L. sorex, L. nasus, L. lukulae, L. simpsoni, L. kirki, L.*

annectens, *L. chariensis*, *L. quadribarbis*, etc.). Its sister clade, Ab, included *L. forskallii* group species from the Niger, St-Paul, Senegal, Little Scarcies, Konkouré and Cross Rivers in West Africa; Sanaga River in Central Africa; Nile River in East Africa; and Zambezi and Incomati Rivers in Southeast Africa.

Clade B consisted of species from four different Reid's groups (Reid, 1985): *L. coubie* group (*L. longipinnis, L. barbatus, L. curriei,* etc.), *L. niloticus* group (*L. lineatus, L. senegalensis, L. weeskii,* etc.), *L. forskallii* group (*L. alluaudi*), and *L. macrostoma* group (*L. greenii, L. batesii*, etc.). These species belong to both the plicate-lipped and the papillary-lipped *Labeo* groups of Tshibwabwa (1997). Although this clade was not well-supported in ML analyses, several subclades within this clade were well-supported by both BI and ML. However, relationships between these subclades were not resolved. The first subclade, Ba, was made up of *L. weeskii*, *L. altivelis, L. rosae*, *L. lineatus, L. senegalensis* and *L. horie*. Within Ba, *L. horie* was supported as sister to *L. senegalensis* and *L. rosae, L. weeskii*, and *L. altivelis* closely related. The second subclade (Bb) was made up of *L. longipinnis, L. coubie, L. cyclorhynchus*, and *L. macrostoma*. Within this subclade *L. longipinnis* from the Congo River was supported as being sister to *L. coubie* from the Niger and Cross Rivers and *L. cyclorhynchus* sister to *L. macrostoma*. The third well supported subclade (Bc) was composed of *L. lividus*, *L. barbatus*, *L. reidi, L. clylopinnis,* and *L. fulakariensis.* Within it, *L. barbatus* was supported as sister to closely-related *L. lividus* and *L. cyclopinnis*. Whereas *L. fulakariensis* were closely related to *L. reidi*. However, BI analyses grouped *L. greenii*, *L. alluaudi*, and *L. curriei* in a fourth subclade, with *L. curriei* sister to *L. alluaudi.* The existence of this clade was supported by neither BI nor ML. The phylogenetic relationships of *L. batesii* from the Sanaga River within the remaining species of clade B was not resolved.

Clade C consisted of *L. camerunensi*, a *Labeo* species identified as *L. batesii*, *L. capensis* and *L. umbratus*, with *L. camerunensi* supported as sister to *L. batesii* and *L. capensis* sister to *L. umbratus*.

Clade D was made of *L. ruddi* from Kunene River, *L. vulgaris* and *L. niloticus* from the Nile River and Lake Omo, with *L. niloticus* as sister to *L. vulgaris*.

The Asian *Labeo* species included in our analyses nested in seven distinct clades. Clade E included *L. angra* and an undescribed species; clade F included *L. fimbriatus*, *L. caeruleus*, and *L. rohita*; clade G included *L. calbasu* and *L. chrysophekadion*; clade H included *L. rajasthanicus*, *L. dussumieri*, and *L. gonius*; clade I included *L. dyocheilus*, *L. pangusia*, *L. pierrei* and *L. yunnanensis*, clade J included *L. boggut*, *L. boga*, *L. bata* and other species from different genera; and a last clade composed of specimens identified as *L. bata* that we refer to here as *L.* cf. *bata*. Both BI and ML suggested clade E to be sister to the African *Labeo* species. Phylogenetic relationships between clades F, G, H and J were not revolved. These clades resulted into a polytomy with the African clades and the Asian clade E.

Concatenated dataset (COI and RAG1)

The analyses of the concatenated dataset resulted in similar topologies as the ones obtained with the COI dataset. However, slight differences were observed regarding the phylogenetic relationship between clades and some species (Fig. 10). These analyses confirmed the existence of most of the clades suggested by the COI analyses with higher posterior and bootstrap values.

Body shape evolution

Body shape variation among clades

As the COI dataset included more species than the concatenated one and as the tree topologies obtained with the two datasets were similar, we used the BI COI topology to define clades and subclades (Fig. 8). The visual inspection of morphospaces using both CV and PCA revealed clusters that corresponded to clades and subclades (Fig. 11). The subclade Aa occupied the extreme values of PC1(negative values) and CV1 (positive values) associated with the shallow body and overlapped with some species from subclade Ab, which displayed variation between different groups. The African clade B and the Asian clades generally occupied the opposite extremes values along PC1 and CV1, which were mainly associated with a medium and deep body (Fig. 11). The permutation test for the Procrustes distance revealed significant differences between clades (Tables 1 and 2). That observation is corroborated by the Procrustes ANOVA, which also indicated significant differences in both lateral and dorsal view of body shape among genetic lineages ($F = 25.26$, $p < 0.0001$ in lateral view and $F = 19.6$, $p < 0.001$ in ventral view). However, some species (*L. greenii*, *L. alluaudi*, *L. camerunensis*) displayed strong similarities in body shape with other species from distantly related clades.

Phylogenetic signal

The tests for phylogenetic signal, both in lateral and ventral views, were highly significant (p-value < 0.0001 both for PCA and CVA in MorphoJ; $p = 0.001$, $K = 0.7569$ for CVA and $p = 0.001$, $K = 0.7086$ for PCA in lateral view; $p = 0.001$, $K = 0.8079$ for CVA and $p = 0.001$, $K = 0.817$ for PCA in geomorph) even by using only the interpretable axes in geomorph ($p = 0.001$, $K = 0.9505$ and $p = 0.001$, $K = 1.7938$ respectively in PCA and CVA in ventral view; $p = 0.001$, $K = 0.9421$ and $p = 0.001$, $K = 2.1099$ respectively for PCA and CVA in lateral view). Hence, these tests results support the alternative hypothesis that phylogeny accounts for overall body shape similarities observed among closely related *Labeo* species (clades) in general. That

is, the observed similarities in overall body shape between closely-related *Labeo* taxa suggest a shared evolutionary history. However, the visual inspection of phylomorphospace plots has revealed several instances of multiple branches independently arriving in the same regions of the plots (Fig. 12). That clustering illustrates instances of evolutionary convergence between distantly-related species in overall body shape. For example, *L. camerunensis*, *L. alluaudi*, and *L. greenii* morphologically converge with several species of the subclades Aa (Congolese *L. forskalii* group species). They resemble in overall body shape something between *L. chrysophicadion* from Asia and *L. longipinnis*, which is likely another interesting case of convergent evolution.

Evolutionary regimes, adaptive peaks, and convergence

SURFACE was used to further evaluate and quantify the observed convergence. This resulted in an estimation of a landscape with nine adaptive peaks, including four convergent peaks that attracted 2.75 lineages on average, and five nonconvergent peaks (Fig.13 and Table 3). The number of shifts towards convergent regimes occupied by multiple lineages and the proportion of shifts toward convergent regimes were respectively estimated to be eight and 0.615. The first adaptive convergent peak was occupied by species forming the subclade Aa (Congo and Kwanza provinces), some species in the clade Ab (Nilo-Sudan, Upper and Lower Guinea provinces), *L. alluaudi* in clade B (Upper Guinea province), and *L. camerumensis* in clade C (Lower Guinea), corroborating the phylomorphospace results. That convergent peak was namely characterized by a shallow and relatively thick body. The second convergent peak was occupied by species in clade Ab from the Zambezi, Nilo-Sudan, and East Coast provinces. That optimum was characterized by a relative shallow and thick body. The third convergent peak was occupied by some species in clade B (from the Congo) and *L. niloticus* in clade D (from the

Nilo-Sudan). The last convergent adaptive peak was occupied by *L. umbratus* in clade C from the Southern provinces, *L. bata,* and *L*. cf. *bata* from Asia.

DISCUSSION

Body shape variation and habitat

Morphological variation or similarities among organisms are not only a result of common evolutionary history but can also emerge as a result of convergent adaptations to similar habitats (Foster et al., 2015; Armbruster et al., 2016). Therefore, an organism's morphology is strongly correlated with environmental gradients and plays an important role in growth, survival, and reproduction (Schluter, 1993; Hausch et al., 2013). *Labeo* species are known to live in a wide variety of habitats and some species exhibit a characteristic morphology associated with their habitat (Stewart and Roberts, 1976; Reid, 1985; Pwema et al., 2011). One of the objectives of this study was to investigate body shape variation among *Labeo* species and clades. The results have revealed a variety of patterns of body shape variation among *Labeo* species and clades. The major features associated with the overall body shape variation of *Labeo* species and clades as captured by PC1 and CV1 were body depth, orbital length, head size, snout length, caudal-peduncle depth, in lateral view; gape width, body width (inter-pectoral and inter-pelvic width), and vent-anal distance (Figs. 2, 4, and 5). Interestingly, some of these features have been found in *Labeo* and other species to relate to habitat adaptation (Stewart and Roberts, 1976; Reid, 1985). For instance, eye reduction or microphthalmia, dorsoventral flattening of the body (variations in body depth, inter-pectoral and inter-pelvic widths), a downturned mouth (mouth opening position relative to the tip of snout), and a sucker-like mouth (variations in gape width) are morphological adaptations associated with benthic rheophilic species (Lujan and Conway,

2015; Zuluaga-Gómez et al., 2016). Whereas, normal or large eyes, deeper and narrower body (lateral compression), and robust caudal peduncle are morphological adaptations associate with lentic habitats (Webster et al., 2011; Foster et al., 2015).

The variation in body depth is not only an adaptation to water characteristics, but also associated with defensive strategies adapted by species accordingly to the habitat where these species are living. While a shallower body might not seem to procure protection against gape-limited predators, that morphology is very efficient in lotic habitats because it allows species to seek refuges from predators and water current in narrow crevices. Conversely, a deeper body can augment water drag and limit the fishes' mobility, but in lentic habitats these disadvantages are compensated by the protection that deeper body offers against gape-limited predators and more propulsion power offered by a robust caudal peduncle both in fast-start or burst swimming to escape from predators, as well in response to the important hydrodynamic drag due to deeper body (Webster et al., 2011).

Although correlations between morphological variation and habitat exploitation or trophic differences were not addressed here, previous studies clearly suggest that *Labeo* body shape diversification has been strongly driven by habitat and ecological adaptation (Stewart and Roberts, 1976; Reid, 1985; Pwema et al., 2011; Armbruster et al., 2016).

Phylogenetic relationships and biogeography of African *Labeo* species

Previous studies that addressed the phylogenetic relationships among *Labeo* species did not include several species from different African ichthyofaunal provinces, nor did any include specimens from the upper Guinea province, indicating that the phylogenetic relationship within *Labeo* had not been resolved, especially with respect to Africa (Yang and Mayden, 2010; Lowenstein et al., 2011; Nwani et al., 2011; Adeoba et al., 2018). An Asian origin of *Labeo* had

been supported by both molecular and paleontological studies (Zheng et al., 2012; Stewart and Murray, 2017). However, what remains poorly documented are the migration routes, the dispersal mechanisms, and the biogeography of these species within the African continent, which is where they reached their highest diversity. A secondary objective was to reconstruct a large-scale phylogeny of African *Labeo* species by including species sampled from across a wide geographical distribution.

The present study differs from the results of previous studies by suggesting the existence of more than two main *Labeo* clades in Africa, and by providing information regarding subdivisions within these clades. We elucidated the phylogenetic positions for species not previously analyzed. Examples include: *L. alluaudi*, which has been resolved as most closely related to *L. curriei*; species identified as *L. parvus* from west Africa have been resolved as most closely related to *L. nunensis* and *L. sanagaensis* and are distantly related to true *L. parvus*; *L. ansorgii* previously hypothesized to be sister to *L. lunatus* (Adeoba et al., 2018) has been resolved as sister to *L. molybdinus*; and *L. reidi* was found to be sister to *L. fulakariensis* (Figs. 8, 9 and 10). *Labeo alluaudi* is the only species placed by Reid (1985) in the *L. forskalii*-group that was not nested in clade A. Van Steenberge et al. (2016) reviewed the Congolese papillate *Labeo* species, synonymized *L. weeksii* with *L. altivelis* based on COI and morphometric analyses. COI-based analyses presented here, which included *L. rosae*, revealed that the genetic difference between *L. weeksii*, *L. altivelis* and *L. rosae* are very small, suggesting a recent divergence of these species but that the species may be valid. Therefore, the synonymy proposed by Van Steenberge et al. (2016) may not be correct. For a robust delimitation of species, it will be necessary to review the systematics of these species by including more genes, *L. mesops* specimens, and more *L. altivelis* specimens for the Zambezi River.

Though the estimation of *Labeo* species biogeography within the African continent is beyond the scope of this study, our phylogenetic analyses did provide preliminary molecular support for geographical range delimitation of several species. Several *Labeo* species are considered to be widespread throughout African content. Among these species, *L. parvus*, *L. cylindricus*, and *L. coubie* have the largest geographical ranges. Our results show that the sisters to these species are generally found in the same ichthyofauna province, suggesting that these species are probably endemic to the provinces in which they were originally described. Tshibwabwa (1997) reported that the Congo province shares three *Labeo* species with the Lower-Guinea province: *L. lukulae*, *L. annectens*, *L. chariensis*. But none of the *L. forskalii*-group species from the Lower-Guinea province, included in the presented study, nested with the Congolese *L. forskalii*-group species which are closely related to species from the Quanza province. Therefore, it may be possible that the geographic ranges of *L. lukalae*, *L. annectens*, and *L. chariensis* are restricted to the Lower Guinea province, and that species identified in the Congo basin as *L. lukulae, L. annectens and L. chariensis* may represent different species. This hypothesis seems plausible given the fact that each of these species represents a species complex in the Congo basin (Figs. 8 and 9).

Within the framework of the proposed Asian origin of *Labeo* species (Zheng et al., 2012; Stewart and Murray 2017) and their historical biogeographic range and radiation throughout the African continent likely occurring by way of the Awash River (Van Couvering 1977; Stewart and Murray 2017), it can now be hypothesized that multiple independent colonizations of *Labeo* species occurred into the Congo, Zambezi, Nile and Niger river systems. The colonization into the Congo would have been made possible via a past connectivity between the Congo and the Nile basins (Otero et al. 2009). Pinton et al. (2013) proposed that the isolation

between the Nile and the Congo basins may be 16.9 Ma old. After the isolation of the two regions, *Labeo* species may have dispersed from the Congo to the surrounding river systems (Kwanza, Sanaga, and Zambezi) and from the Nile to other river systems (Niger, St-Paul in West Africa and Zambezi, Kunene in southeastern Africa) without any direct reconnection between the Nile and the Congo. The hypothesis of a subsequent *Labeo* species migration from the Congo back to the Nile River, via the Niger River, is the most plausible to explain the current distribution of *L. horie, L. coubie*, and *L. senegalensis*, of which their common ancestral population seems to have originated from the Congo. This hypothesis is given support by the results of Goodier et al. (2011). Indeed, Goodier et al. (2011) suggest that the genus *Hydrocynus* (African tigerfishes) originated from the Congo basin and migrated to the Nile via the Sanaga and Niger Rivers. Goodier et al. (2011) dated the divergence event that separated the Congo lineage and the Nilo-Sudan lineage at the late Miocene (6.8 Ma, CI:10.8-3.2). An estimation of *Labeo* species divergence time is therefore necessary to corroborate these hypotheses.

Phylogenetic signal, adaptive radiation, and convergent evolution

One of the primary objectives in this chapter was to provide insights on the body shape evolution of *Labeo* species. The application of *K* statistic (Blomberg et al., 2003; Adams 2014) to measure the phylogenetic signal has revealed moderate to strong phylogenetic signal (Ackerly, 2009; Kamilar and Cooper, 2013), suggesting that closely related species are more similar in body shape than expected under Brownian motion model of evolution (Adams 2014). From that observation (presence of high phylogenetic signal), we concluded that *Labeo* species body shape states are in large part determined by common ancestry. According to Ackerly (2009) the presence of high phylogenetic signal indicates that subclades within a clade (*Labeo* herein) are highly conserved. The visual examination of Figures 11 and 12 shows the high body shape

conservation within *Labeo* subclades, suggesting that the major variation in body shape happened early in the evolutionary history of the genus. Although Revell et al., (2008) argued against the interpretation of evolutionary process based on the estimations of the phylogenetic signal and Losos (2008) concluded that the phylogenetic signal is not sufficient evidence for the existence of phylogenetic niche conservationism, we hypothesize that the high phylogenetic signal observed in *Labeo* body shape may indicate the occurrence of phylogenetic niche conservationism given the fact that their body shape is an adaptation to the habitat. Future studies incorporating diet and habitat characteristics are necessary to verify that hypothesis. Although high and significant phylogenetic signal have been recovered in body shape among *Labeo* species, which is mainly the case when working in large scale (Cavender-Bares et al., 2006), our results suggest several instances of body shape convergence within *Labeo* species (Fig. 12).

Convergent evolution has not previously been neither quantified nor identified within the genus *Labeo*. Our SURFACE analysis has identified four convergent adaptive peaks attracting at least thirty-four species (Fig. 13). The largest converging community has been identified within the Congo-Quanza subclade. We hypothesize that the extreme environmental conditions in the Congo are the main factors driving that extreme convergence in that river basin. That hypothesis is complemented by the fact that several convergent evolution cases have been detected in different fish groups living in extreme Congo river environments (Alter et al., 2015). Apart from the four convergent peaks, our results supported five other adaptive peaks along the *Labeo* phylogeny. Fewer species, seventeen in total, have been attracted by those peaks. In addition to the convergence within subclades, our SURFACE analysis has also detected convergence between subclades (Fig. 13).

Converging species live in flowing water and most of them have morphology characteristic of lotic inhabitants. However, convergent evolution has also been identified between species adapted to the lentic habitat such as *L. lineatus*, *L. altivelis*, *L. senegalensi* and *L. niloticus* (Fig. 13). Hence, the convergent evolution among *Labeo* species is mainly happening among species that occupy the same habitats. The findings support our hypothesisthat *Labeo* body shape diversification has been strongly driven by habitat and ecological adaptation. On the other hand, the same environmental factors have exerted a constraining pressure that has limited, in a certain level, body shape diversification throughout the evolutionary history of *Labeo*. This finding supports our hypothesis of broad-scale phylogenetic niche conservationism.

CONCLUSION

As suggested by Zheng et al. (2012) and Zheng et al. (2016), *Labeo* is a paraphyletic genus especially with its Asian clades. African species of this genus are monophyletic and can be subdivided into at least three well supported main clades. Several African *Labeo* species have been resolved as sister to a congener within the same river system or ichthyofaunal province, suggesting that *Labeo* is a good study model to understand local adaptive diversification. The phylogeny elucidated in this study revealed that the number of *Labeo* species is underestimated and several species need to be described. However, support for several internal nodes were weak; leaving phylogenetic relationships of subclades unresolved. Therefore, a larger scale phylogenetic study including more samples and more markers is necessary to reevaluate the number of *Labeo* species and their phylogenetic position.

We found significant variation in body shape among *Labeo* species, suggesting that the shape within that genus is considerably diverse. The greatest variation in body shape among

Labeo species and clades occurs in body depth and width. Although we did not assess the correlation between the body shape variation and habitat, our results in light of other studies suggest that the body shape within *Labeo* is associated with their habitat.

The test of phylogenetic signal revealed that the similarity in body shape observed among closely related species is mainly due to common evolutionary history. Nevertheless, further analyses revealed multiple instances of convergent evolution within and between subclades. This study not only is the first to use geometric morphometrics to assess body shape variation across the entire *Labeo* genus but is also the first to identify and quantify convergent evolution in body shape among *Labeo* species.

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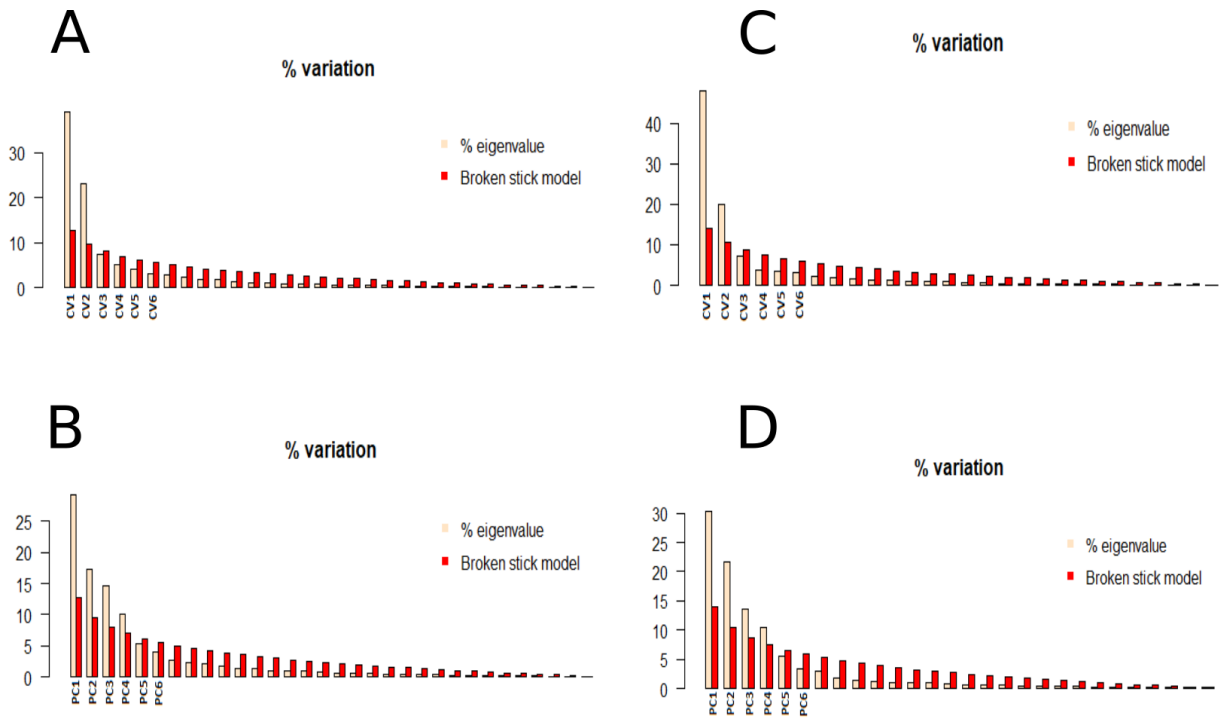

Figure 1: Comparison of broken stick model results to the eigenvalues. Interpretable axes are the ones with larger eigenvalue (orange bars) than the corresponding piece of stick (red bars) generated by the broken stick model. A: CVAs in lateral view, B: PCs in lateral view, C: CVAs in ventral view, D: PCs in ventral view.

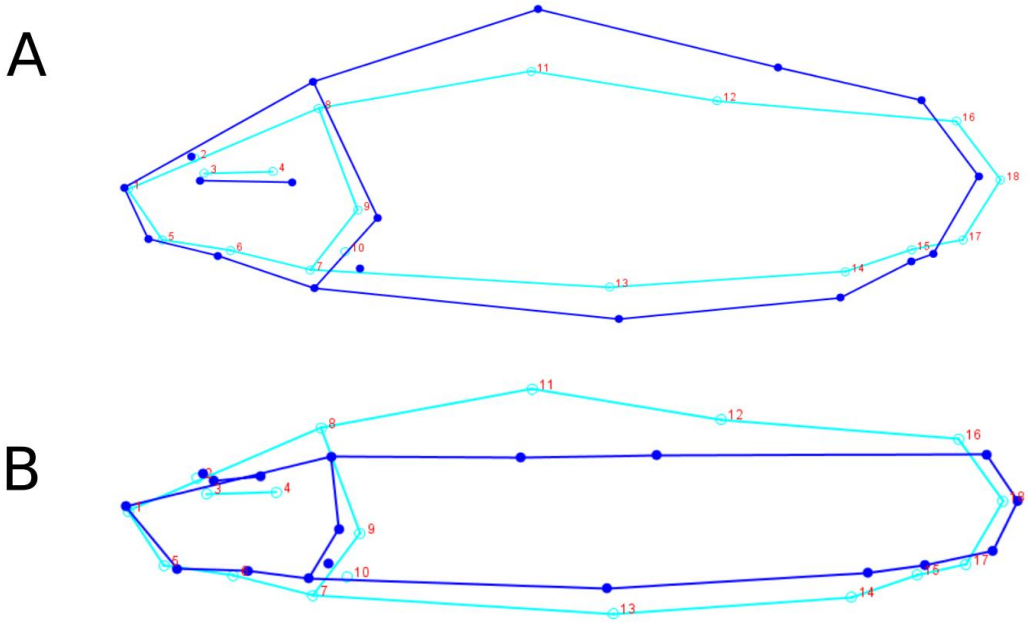

Figure 2: Wireframe visualization of lateral view shape variation along principal component one. In panel A dark blue landmarks represent the shape change associated with extreme positive PC1 values whereas in panel B they represent the shape change associated with the extreme negative PC1 values. Light blue landmarks represent the average shape of average specimens.

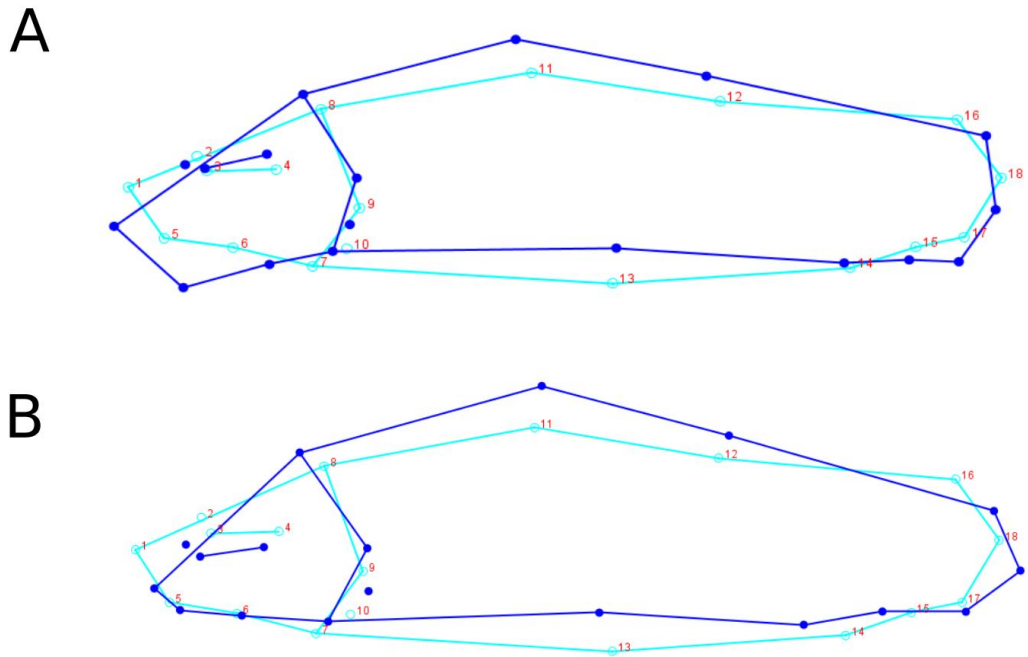

Figure 3: Wireframe visualization shape variation in lateral view along principal component two. Panel A showing variation associate with extreme positive values and panel B variation associate with extreme negative values.

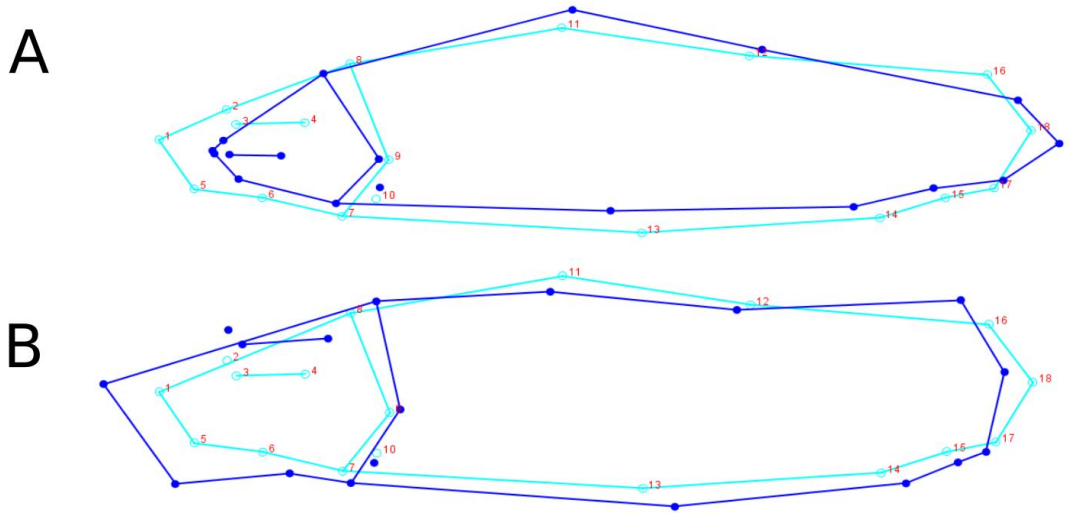

Figure 4**:** Wireframe visualization shape variation in lateral view along principal component three. Panel A showing variation associate with extreme positive values and panel B variation associate with extreme negative values.

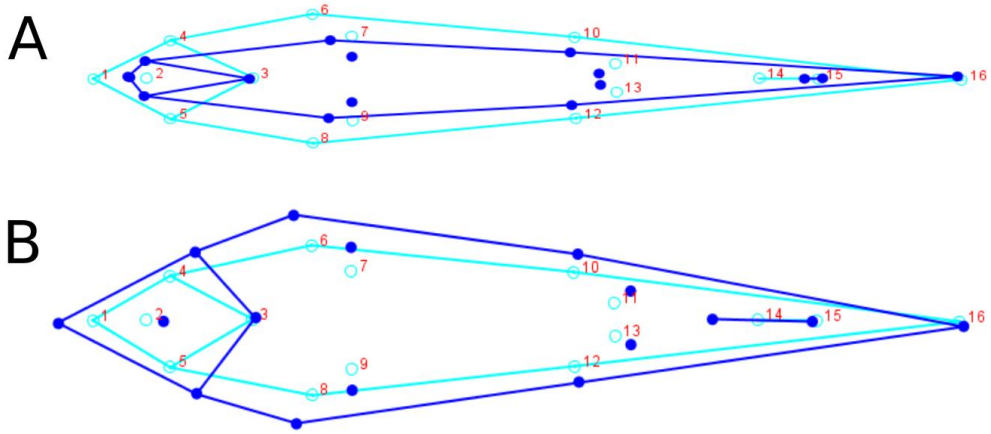

Figure 5: Wireframe visualization shape variation in ventral view along principal component one. Panel A showing variation associate with extreme positive values and panel B variation associate with extreme negative values.

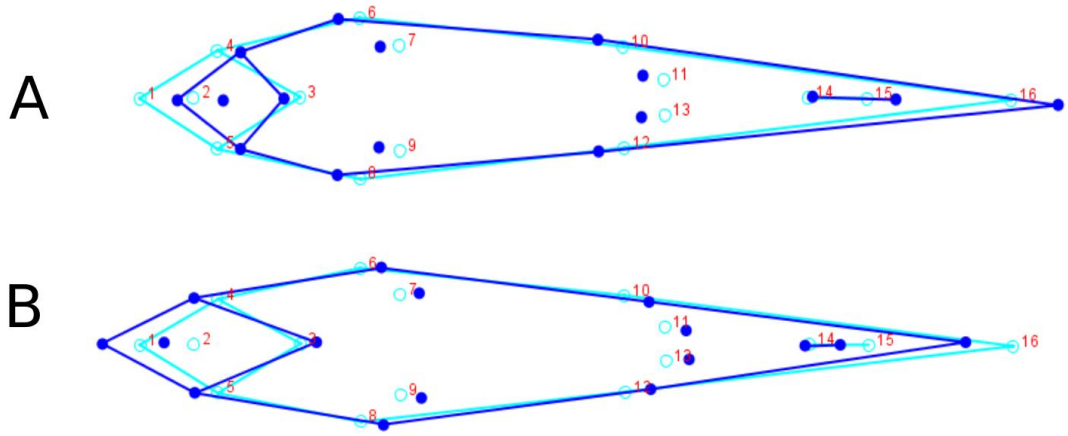

Figure 6: Wireframe visualization shape variation in ventral view along principal component two. Panel A showing variation associate with extreme positive values and panel B variation associate with extreme negative values.

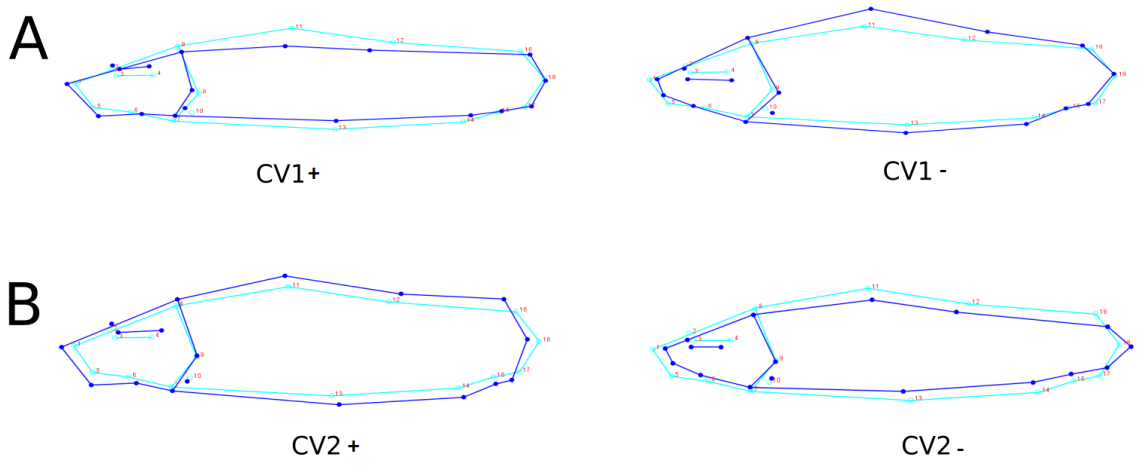

Figure 7: Wireframe visualization shape variation in ventral view along canonical variate axes (A) one and (B) two. Images on the left and right show variation associated with extreme positive and negative values, respectively.

Figure 8: The 50% majority rule consensus tree with mean branch lengths summarizing the posterior sample collected by our Bayesian phylogenetic analysis of the COI dataset of *Labeo* taxa. (**) represents branch posterior probability support above 99% and (*) branch posterior probability support above support above 90%.

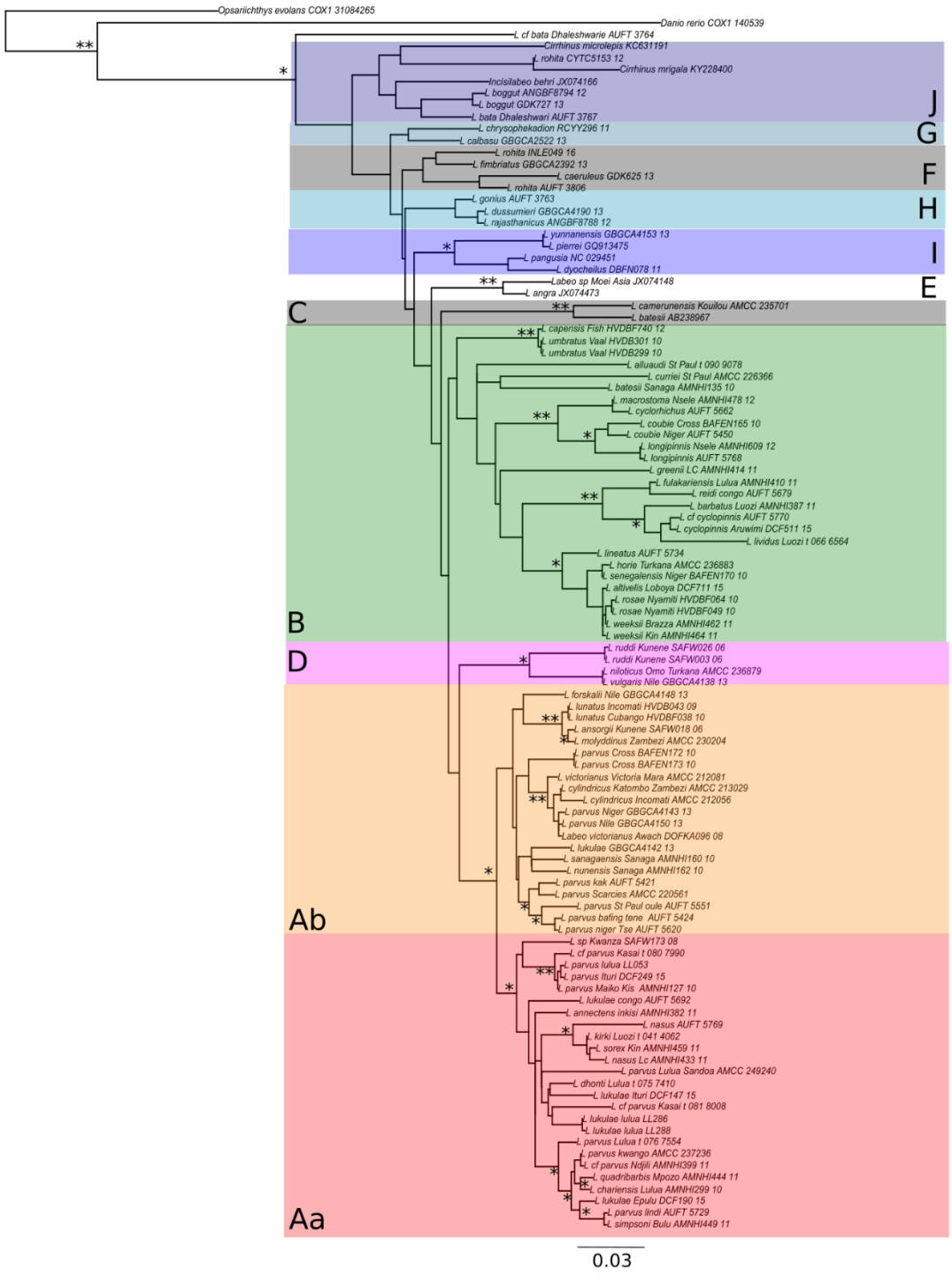

Figure 9: Maximum likelihood tree inferred from the analysis of COI dataset of *Labeo* taxa. (**) represents branch bootstrap support above 98% and (*) branch bootstrap support above 70%.

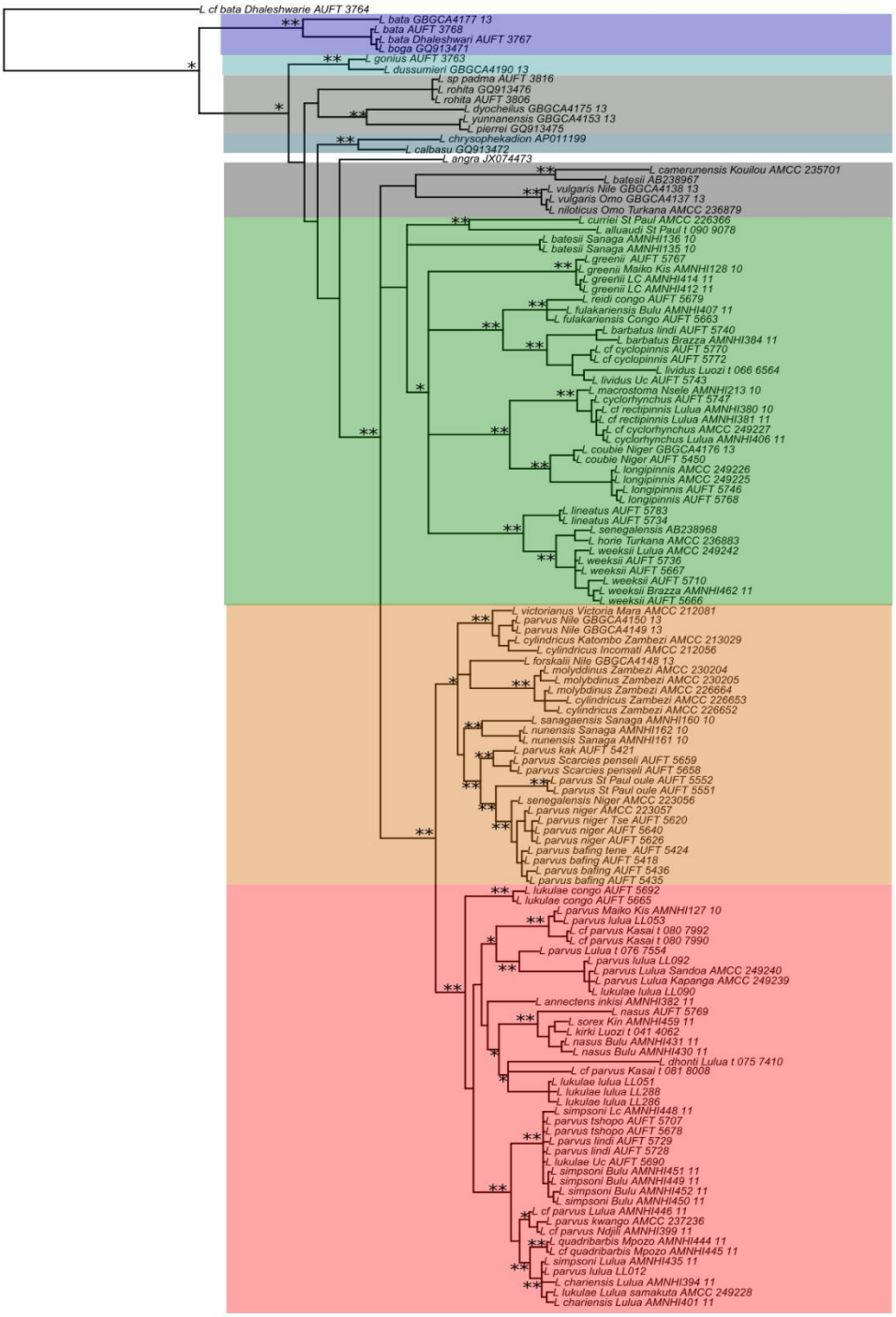

Figure 10: Bayesian phylogenetic tree inferred from the analysis of the concatenated sequence dataset (COI and RAG1) of *Labeo* taxa. (**) represents branch posterior probability support above 99% and (*) branch posterior probability support above support above 90%.

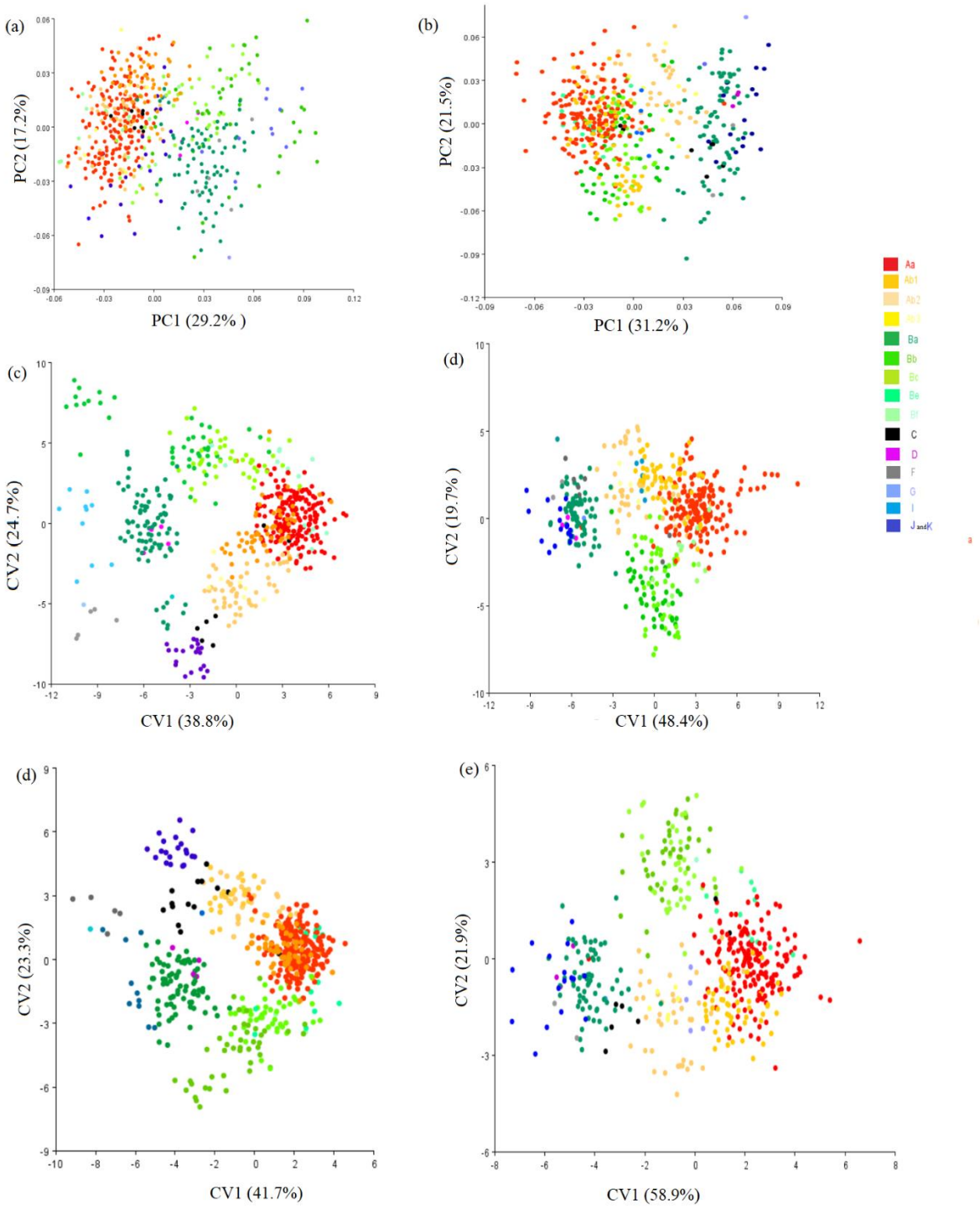

Figure 11: Morphospace projections of *Labeo* species body shape. **a** and **b**: first two principal components in respectively lateral and ventral views; **c** and **d**: first two canonical axes in respectively lateral and ventral views (predefined groups=species); **e** and **f**: first two canonical axes in respectively lateral and ventral views (predefined groups=clades and subclades). Colors are indicative of clade or subclade of each species.

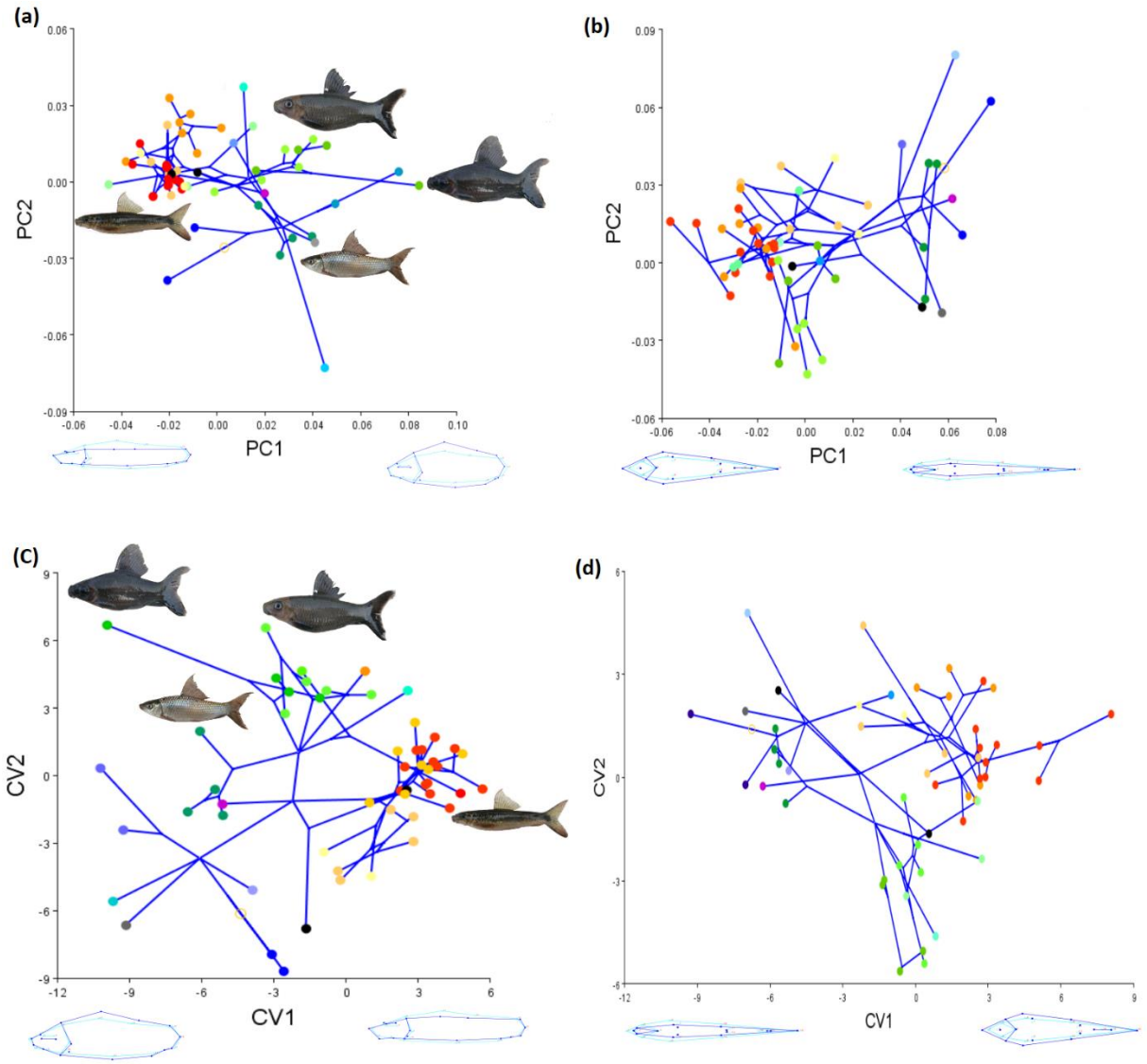

Figure 12: Phylomorphospace projections of *Labeo* species overall body shape. **a)** two first principal components in lateral view, **b)** two first principal components in ventral view, **c)** two first canonical variates axes in lateral view and **d)** the two first canonical variates axes in ventral view. Colors at each tip are indicative of clade or subclade of each species.

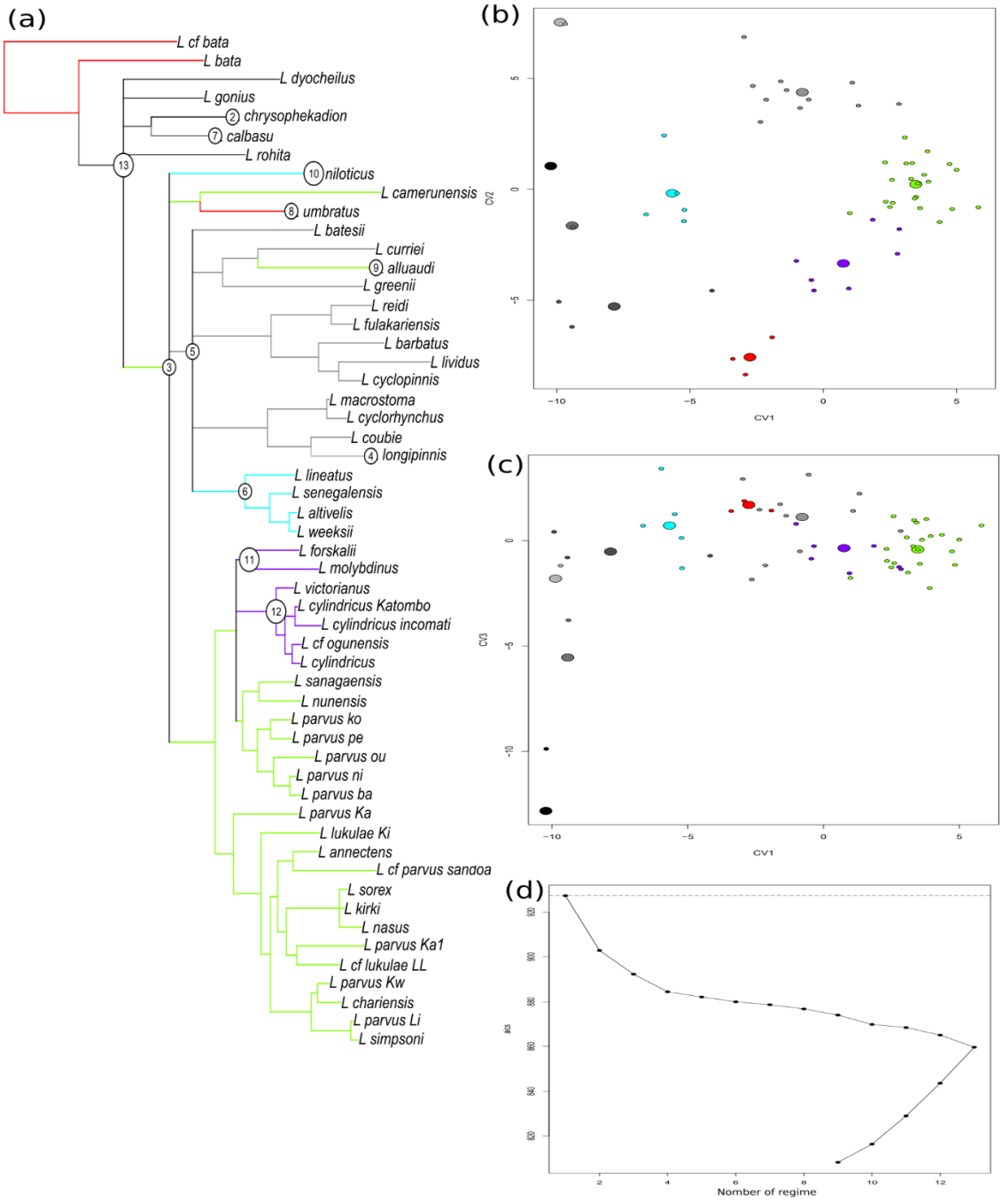

Figure 13: Results from SURFACE Analysis of body shape in lateral view among *Labeo* species. (a) phylogenetic tree, with convergent (coloured) and nonconvergent (greyscales) regimes estimated from the best-fit model. (b and c) Trait values (phylogenetic canonical variates) for each species (small circles) and estimated optima (large circles), with regime colours matching the ones in the tree. (d) Changes in AICc during the forward and backward phases of the analysis.

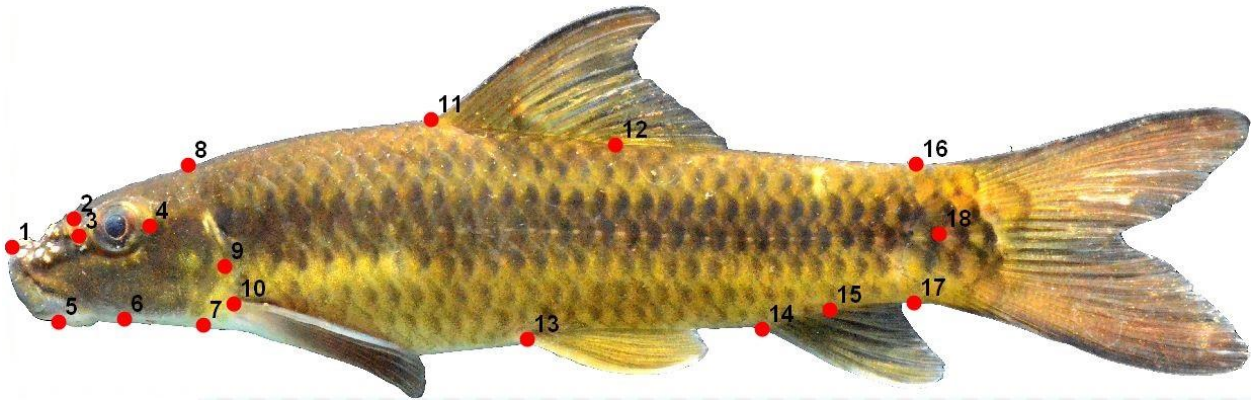

Figure 14: Homologous landmarks used in lateral view following Armbruster 2012.

Table 1: *P*-values from permutation tests (10, 000 permutation rounds) for Procrustes distances among clades and subclades in ventral view

	Aa	Ab1	Ab2	Ab3	Ba	Bb	Bc	Be	C	D	F	G	I	J
Ab1	<.0001													
Ab2	<.0001	<.0001												
Ab3	<.0001	<.0001	0.0785											
Ba	<.0001	<.0001	<.0001	0.0016										
Bb	<.0001	<.0001	<.0001	<.0001	<.0001									
Bc	<.0001	<.0001	<.0001	<.0001	<.0001	0.3777								
Be	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001							
C	<.0001	<.0001	<.0001	0.0039	0.0295	<.0001	<.0001	<.0001						
D	<.0001	<.0001	<.0001	0.0072	0.222	<.0001	<.0001	0.0002	0.0156					
F	0.0001	0.0003	0.0011	0.0032	0.3431	0.0065	0.0004	0.003	0.0969	0.1025				
G	0.0002	0.0293	0.0065	0.0205	0.0003	0.0001	0.0002	<.0001	0.0133	0.0121	0.0178			
I	0.0071	0.0127	0.2042	0.246	0.1819	0.0068	0.0034	0.0005	0.0659	0.1663	0.3299	0.1603		
J	<.0001	<.0001	<.0001	0.0001	0.0153	<.0001	<.0001	<.0001	0.0008	0.3222	0.1884	0.0001	0.4964	
K	0.0023	0.0082	0.0068	0.1233	0.0923	0.0082	0.0049	0.0011	0.0773	0.1639	0.3395	0.1375	1	0.4097

Table 2: *P*-values from permutation tests (10, 000 permutation rounds) for Procrustes distances among clades and subclades in lateral view

	Aa	Ab1	Ab2	Ab3	Ba	Bb	Bc	Be	C	D	F	G	H	I	J
Ab1	<.0001														
Ab2	<.0001	<.0001													
Ab3	<.0001	<.0001	0.2739												
Ba	<.0001	<.0001	<.0001	<.0001											
Bb	<.0001	<.0001	<.0001	<.0001	<.0001										
Bc	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001									
Be	0.0057	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001								
C	<.0001	<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	0.0002							
D	<.0001	<.0001	<.0001	0.0004	0.0034	<.0001	<.0001	<.0001	0.0019						
F	<.0001	<.0001	<.0001	0.0001	0.0005	<.0001	<.0001	0.0002	0.0007	0.0054					
G	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0015				
H	0.0011	0.0136	0.0073	0.0204	0.0044	0.0433	0.0216	0.059	0.1009	0.069	0.0848	0.0331			
I	0.0007	0.0054	0.0409	0.0531	0.0033	0.0294	0.0086	0.0586	0.1105	0.1349	0.1656	0.0716	1		
J	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0001	<.0001	0.0335	0.0823	
K	<.0001	0.0006	0.0178	0.0104	<.0001	0.0005	0.0009	0.0061	0.0068	0.0405	0.0399	0.0109	0.3356	0.3294	0.1543

Table 3: Parameters representing evolutionary processes and features of the adaptive landscape as obtained from SURFACE

Adaptive peak shifts (***k***)	13
Convergent adaptive peak shifts (***k'***)	9
Adaptive peaks	9
reduction in complexity of the adaptive landscape when accounting for convergence (Δ***k***)	4
Number of shifts that are towards convergent regimes occupied by multiple lineages (**c**)	8
Number of convergent regimes reached by multiple shifts (***k'*conv**)	4
Number of nonconvergent regimes reached by multiple shifts (***k'noncov***)	5
Relative reduction in complexity of the adaptive landscape when accounting for convergence (Δ***k /k***)	30.7%
Convergence fraction (convergent peak shifts/total peak shifts)	30.7%
Average number of lineages converging to each shared adaptive peak	2.75
Proportion of shifts that are towards convergent regimes (**c**/***k***)	61.5%

Chapter 2: Geometric morphometrics and molecular phylogeny provide new insights about convergent evolution, distribution (biogeography) and diversity within the African carp: *Labeo parvus*.

ABSTRACT

Labeo is the third most diverse African cyprinoid genus and is widely distributed across the continent. *Labeo parvus*, a small species originally described from the Congo basin, seems to be the only species of the *forskalii*-group to be distributed in four different African ichthyo-provinces (Nilo-Sudan, Congo, and Upper and Lower-Guinea). Several nominal species have been synonymized with *L. parvus* despite the proposition to restrict the occurrence of that species to the Congo basin. The distinction between *L. parvus* and close relatives remains problematic. We used geometric morphometrics and molecular phylogenetics to assess the biological diversity within *L. parvus* and reevaluate its distribution. Our phylogenetic analysis strongly supports a non-sister relationship between the West African (Nilo-Sudan and Upper Guinea ichthyoprovinces) and the Central African (Congo ichthyoprovince) *Labeo parvus* lineages. Permutation tests for Procrustes and Mahalanobis distances, applied on the overall body shape dataset, reveal a significant difference (P-value < 0.0001, PD= 0.0267 and MD= 4.1437) between the West Africa and Congolese *L. parvus* lineages. Because geometric morphometric analysis corroborates phylogenetic reconstruction, we conclude that *L. parvus* is an endemic species of the Congo basin and does not occur in West Africa where it is replaced by *L. ogunensis*. Additionally, our analyses suggested that both *L. parvus* and *L. ogunensis* are species complexes. We discuss our findings in terms of convergent and divergent evolution by mapping the phylogeny into the morphospace and testing for phylogenetic signal.

INTRODUCTION

The African carp, *Labeo parvus*, was originally described from Mobayi-Mbongo (formerly Banziville) in the northern part of the Democratic Republic of the Congo by Boulenger (1902; Jegu and Lévêque 1984; Reid 1985; Eschmeyer et al., 2017). Fifty-two years after its description, Daget (1954) mentioned, for the first time, the occurrence of *Labeo parvus* in the Niger basin. Subsequently, the species would be found in several coastal basins in West Africa including the Senegal, the Ouémé and Volta Rivers (Jegu and Lévêque 1984; Lévêque et al., 1990). Presently, *L. parvus* is a widespread species distributed from western Africa (Senegal basin) to eastern Africa (Malagarasi River basin) via central Africa (Congo basin) (Hanssens et al., 2010; Lévêque et al., 1990; Montchowui et al., 2009). The taxon has been reported to also be present in the Lake Chad system (Jegu and Lévêque 1984) and the Nile River (Yang et al., 2012) making *L. parvus* one of the most widespread *Labeo* species in Africa.

Considering the geological history of the Africa drainages (Goudie 2005; Stankiewicz and de Wit 2006) and several physical boundaries that exist between the Congo, the Niger and Nile drainages, the present distribution of *L. parvus* is very intriguing. The species is found in four different ichthyoprovinces (Stiassny et al., 2007; Snoeks et al., 2011), which have their own distinct set of endemic taxa. Reid (1985) was the first to notice this problem. He proposed restricting the distribution of *L. parvus* because this species is replaced in western African by *Labeo ogunensis* and the two species occur allopatrically. Reid also considered several west African *Labeo* nominal species as synonyms of *L. ogunensis* while considering *Labeo obscurus* and others valid (Reid 1985; Lévêque et al., 1990). These proposals were considered controversial and not taken into account in several publications (Lévêque et al., 1990; Montchowui et al., 2009; Montchowui et al., 2011; Montchowui et al., 2012). Presently, *L.*

obscurus, considered by Reid as a distinct species, and *L. ogunensis* are considered synonyms of *L. parvus (Eschmeyer et al., 2017)*.

Lowenstein et al. (2011), were the first, after the revision of *Labeo* species of Congo and the Lower Guinea by Tshibwabwa (1997), to show using molecular data that there were problems in the delimitation of *Labeo* species in the Congo basin, including *L. parvus,* which was not a monophyletic taxon. The same observation was made by Decru et al. (2016) using material from different localities in the same river system. To date, no study has addressed the *Labeo parvus* distribution problem using modern taxonomic tools such as DNA barcoding or geometric morphometrics.

Several hypotheses can explain the confusion that exists in the delimitation of *L. parvus*. The first is that the morphological resemblance that exists among these species is due to their overall convergent shape evolution. The second hypothesis is that morphological characteristics used to identify *Labeo* species may not be very discriminative (Steenberge et al., 2016). Lévêque et al. (1990) recognized to have grouped under *L. parvus*, in west Africa, several species that are apparently identical based on the morphological criteria used. The third hypothesis suggests that in his original description Boulenger (1909) may have included several species under the description of *L. parvus*. Indeed, apart from the two specimens from the type locality of Mobayi Mbongo (Ubangi Rivers), Boulenger included in the type series specimens from Lindi River (Tshopo basin, Kisangani, DRC), Aruwimi River (Uturi, DRC) and Bange Ngola (Angola) (Boulenger 1909). Pictures of two syntypes and one specimen obtained from the Natural History Museum in London, include three specimens that are morphologically very different (Figure 1).

In this study we used geometric morphometrics and phylogenetic reconstruction to compare, morphologically and genetically, the Congo Basin *Labeo parvus* populations to the

West African (Niger, Senegal, Little Scarcies, Konkouré and St-Paul Basins) populations. Our Chapter 1 analysis shows that *L. parvus* is a polyphyletic taxon and that the West African *L. parvus* nests in a different clade than the Congolese ones. Therefore, we hypothesize that similarities between them are due to convergent evolution. Thus, we expect to demonstrate that *L. parvus* is an endemic species of the Congo basin and that the specimens identified as *L. parvus* from West Africa belong to different species (*L. ogunensis* and/or *L. obscuris*) as proposed by Reid (1985).

MATERIAL AND METHODS

Morphological Data Collection and Analyses

Materials examined in this study include 103 specimens identified as *Labeo parvus* or *Labeo cf. parvus* from the Auburn University Museum (AUM) and American Museum of Natural History (AMNH) collections. Fifty-three of these specimens are from Central Africa (Congo River) whereas fifty are from West Africa (Niger, St. Paul, Little Scarcies, Konkouré, Senegal Rivers). In addition, we included several individuals of closely related species that are frequently misidentified as *L. parvus*. Among these are nine individuals of *Labeo lukulae* from Kisangani (Congo River), 11 individuals of *Labeo chariensis* and 11 *Labeo* cf. *lukulae* from Lulua River (Congo), 12 individuals of *Labeo simpsoni* from the Lower Congo, and 10 individuals of *Labeo ogunensis* from lake Guelta d'Archei in Chad. *Labeo* specimens from the lake Guelta d'Archei were originally described as *Labeo tibestii* by Pellegrin in 1919 (Burgis and Symoens, 1987) and synonymized to *L. ogunensis* by Reid (1985), thus we refer to these specimens as *L. ogunensis*.

Each specimen was photographed in ventral and lateral (left side) views using a mounted Canon EOS 600D digital camera. Both juvenile and adult individuals, of both sexes, in good condition were included in the analyses. Additional photographs of two syntypes and one specimen of *Labeo parvus* were obtained from the Natural History Museum data portal (http://data.nhm.ac.uk/dataset/collection-specimens). The obtained photographs were used to create digital images of Geometric Morphometric (GM) landmarks, following Armbruster 2012, using TpsDIG2 (Rohlf 2016a) to describe individual body shape. The x-y coordinates of landmarks generated by TpsDig2 were saved in a tps file with TpsUtil 1.70 (Rohlf 2016b). A Generalized Procrustes Analysis (GPA) was performed, using MorphoJ 1.06d (Klingenberg 2011), to scale landmarks of each specimen to a common body size, to rotate each individual to a common alignment, and to generate a consensus shape by calculating the average shape of all specimens included in the analysis. After checking for outliers, a covariate matrix was constructed to prepare data for Principal Components Analysis (PCA) which was conducted in MorphoJ. To assess variations across different groups of *Labeo parvus*, a Canonical Variates Analysis (CVA) incorporating a permutation test for pairwise differences with 10000 iterations was conducted in MorphoJ.

Additionally, traditional meristic counts, following Tshibwabwa et al. (2006) and Reid (1985), were conducted on a subset of specimens. We used X-ray images to count the number of total (abdominal and caudal) vertebrae, the number of pleural ribs, the number of procurrent and simple dorsal-fin rays, the number of procurrent and simple anal-fin rays, and the number of principal and procurrent caudal-fin rays. Different from Tshibwabwa et al. (2006), we counted all vertebrae possessing a haemal spine as caudal vertebrae whereas those with ribs and those

with haemal arches but lacking a haemal spine were counted as abdominal vertebrae (Aguirre et al., 2014). Weberian apparatus vertebrae were not included in the counts.

Molecular data collection and analyses

DNA Extraction

Genomic DNA was extracted from 52 individuals representing *Labeo* species of the forskalii-group and one individual of *L. camerunensis* used as an outgroup. Several individuals of *Labeo parvus* from different localities were included in the analysis. Extractions were conducted using the Omega BioTek E.Z.N.A. or Qiagen Dneasy Tissue kit following the methods provided by the manufacturers.

Gene Amplification and Sequencing

DNA amplification was conducted by polymerase chain reaction (Mullis et al., 1986; Saiki et al., 1988) for part (about 652 bp) of the mitochondrial cytochrome oxidase subunit 1 (COI) and part (about 800 bp) of the nuclear Recombination-Activation Gene 1 (RAG1). The COI was amplified following Ivanova et al. (2007) while the part b of the RAG1 was amplified following López et al. (2004) and Lowenstein et al. (2011) using the following primers: RAG1_R1 (5'-CTGAGTCCTTGTGAGCTTCCATRAAYTT-3') and RAG1_JHL_Fi (5'-ATGCACGCTCTGCGACTCAA-3'). The obtained amplicons were submitted to Genewiz (https://www.genewiz.com) for Sanger sequencing. Additional COI and RAG1 sequences were imported from Genbank (www.ncbi.nlm.nih.gov/genbank) and the Barcode of Life Data System (http://boldsystems.org/index.php).

Phylogeny Reconstruction

A total of 103 *Labeo* sequence reads for CO1 (596 bp) and RAG1 (624 bp) were concatenated (1220 bp) using Geneious and exported as PHYLIP and NEXUS formatted files for

downstream analyses. Optimal models and partitioning schemes were determined using Partionfider2 (v. 2.1.1.) (Lanfear et al., 2016) using the PHYLIP formatted files exported from Geneious. The GTR+I+G or GTR+I models were used for the five subsets of the concatenated dataset. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted on the concatenated dataset using MrBayes 3.2.2 and RAxML v8.2.X (Stamatakis 2014) implemented on the CIPRES Science Gateway V.3.3 (http://www.phylo.org). Using MrBayes, Markov Chain Monte Carlo (MCMC) analyses were run for 60 million generations, with trees sampled every 3000 generations. One thousand (1000) bootstrap replicates were used to evaluate branch support in RAxML. The obtained phylogenetic trees were visualized and annotated with FigTree v1.4.3 (Rambaut 2016).

Phylogenetic Signal Test

BI and ML phylogenetic trees were pruned, using the phytools (Revell 2012) package in R3.4.1. (R Core Team 2013), to match the taxa on the phylogeny with those on the morphospace datasets. The pruned trees were imported in MorphoJ and mapped onto the morphospaces (PCA and CVA) to generate phylomorphospaces (Sidlauskas 2008). The permutation test for phylogenetic signal, with 10,000 iterations and weighted by branch length, was applied to the resulting phylomorphospaces to assess the direction of body shape change along the evolutionary axes. Our data were also tested for the phylogenetic signal using the physignal function of the R package geomorph (Adams and Otárola-Castillo 2013). For that, we used the K_{mult} method (Adams 2014) with 1000 random permutations.

Species Delimitation

To estimate the number of species within *Labeo parvus* complex, we used General Mixed Yule Coalescent (GMYC) (Pons et al., 2006) and the Poisson Tree Processes (PTP) models

(Zhang et al., 2013). The GMYC model was applied on a single-locus dataset of 114 COI sequences from individuals identified as *L. parvus* and closely related species. As the GMYC requires an ultrametric tree, we used the software package Beast v2.4.8 (Bouckaert et al., 2014) to estimate such a tree under a coalescent prior that assumes a constant population size. The obtained ultrametric tree was later used to estimate the number of species with GMYC using the function gmyc of the R package Splits v1.0-19 (Ezard et al., 2009). The PTP was applied on the BI tree obtained from the analyses of the concatenated dataset. The calculation was implemented in the bPTP web server (http://species.h-its.org/ptp/).

RESULTS

Labeo parvus phylogenetic relationship

The results of our ML and BI analyses of the concatenated dataset were similar (Figs. 2 and 3). In this section, we focus on the results of the BI analysis**.** Our phylogenetic analysis strongly supports a non-sister relationship between the West African and the Central African *Labeo parvus*. The two groups are not closely related. The West African *Labeo parvus* nested with *L. victorianus* from the Mara River in the Lake Victoria system, *L. cylindricus* and *L. molybdinus* from the Zambezi system, and *L. nunensis* and *L. sanaganensis* from the Sanaga system whereas the Congolese *Labeo parvus* forms a monophyletic group with other plicate-lipped *Labeo* species from the Congo such as *L. chariensis*, *L. nasus*, *L. quadribarbis*, *L. simpsoni*, *L. sorex*, etc. (Fig.2).

Within the Western Africa group, *L. parvus* from Bafing River (Senegal system) is nested within *L. parvus* from the Niger River. The two populations are separated with a very short branch length and seem to share an identical haplotype. In addition, the node separating the two

groups is weakly supported (68% for posterior probability). This group is estimated as sister to *L. parvus* from Oulé River (St-Paul system). On the other hand, the Konkouré specimens are sister to the Little-Scarcies specimens and form a well-supported group (99%) that is sister to the Niger, Senegal, and St-Paul group. Based on these groupings, the West African *L. parvus* might be a species complex composed of at least four species.

As in the West African clade, specimens identified as *Labeo parvus* from the Congo are represented in multiple clades that can be grouped to two principle clades. Six individuals from Kisangani (Tshopo and Congo River) are closely related to 12 Lower Congo (LC) individuals identified as *L. simpsoni* that seem to share the shame haplotype. Four individuals identified as *L. chariensis* from Lulua River shared the same haplotype with seven individuals identified as *L.* cf. *parvus*, as well as one individual identified as *L. parvus*, one as *L. lukulae*, and two as *L. simpsoni* from the Lulua River. These results suggest that these individuals represent the same species, therefore we will refer to them as *L. chariensis*. That group has been resolved as sister to two specimens of *L. quadribarbis* from Mpozo River, a tributary of the Lower Congo. Three other individuals, one from the Kwango River, one from Ndjili River and the last one from the Lulua River form a sister group to the *L. chariensis* and *L. quadribarbis* group. All these specimens form the first *L. parvus* clade within the Congo. The second clade is made of species that are closely related to *L. nasus*. Within this clade are 11 specimens among which four are identified as *L. lukulae*, four as *L.* cf. *parvus*, two as *L. parvus*, and one as *L. dhonti*, from the Lulua River, clustered together. We consider them a single species and refer to them as *L.* cf. *lukulae*_LL (LL emphasizes the fact that they are endemic to the Lulua River) because of their morphological similarity to *L. lukulae* from Kisangani. This group forms a polytomy with two specimens from the Lower Congo River (LC) and Kasai River, one specimen from Kisangani

Region (Mayiko River) and another specimen identified as *L. dhonti* from the Lulua. They have been resolved as sister to *L. nasus*. Six individuals identified as *L. parvus* from the upper Lulua (Katanga province) clustered together with high posterior probabilities. We referred to this group as *L.* cf. *parvus*_Sandoa in the present study to emphasize the fact that these specimens may represent an undescribed species. This species has been resolved as sister to a group made up of four individuals from Kasai River (*L. parvus*_Ka), Lulua and Kisangani. Finally, two specimens identified as *L. lukulae* from Kisangani in the Upper Congo River (UC), that we referred as *L. lukulae*_Ki, has been resolved as sister to all the Congolese *L. parvus*.

Geometric morphometric analysis

Individuals of *Labeo parvus, L. lukulae,* and other *Labeo* species from Central Africa overlapped with those of *L. parvus* from West Africa in the PCA (in both lateral and ventral views) of all *Labeo parvus*-like species included in the present study (Fig 4). The observed overlap suggests a certain level of body shape similarity between the West and Central African *Labeo parvus*. However, PCA of the averaged Procrustes coordinates of each group reveals a different scenario that separates the West Africa group from the Central Africa (Fig. 5). That trend was already perceptible in the PCA of all individuals. The split between the Central and West Africa *L. parvus*-like species becomes more noticeable when we used the Canonical Variate Analysis (CVA). In fact, the permutation tests (CVA) for Procrustes distances (PD) and Mahalanobis distances (MD) reveal a significant difference (P-values < 0.0001, PD= 0.0267 and MD= 4.1437 in lateral view and PD= 0.0307 and MD= 3.0572 in ventral view) between the West Africa and Congolese *L. parvus* groups. Differences between the Congolese *L. parvus* species and the West Africa *L. parvus* were mainly depicted by PC1(32.1% and 28.02%, respectively) and CV1(36.3% and 41.13, respectively) in both lateral and ventral views. The Congolese clade

presents a shallower and thicker body whereas the West Africa species possess a relatively deeper and narrower body. In addition, the West Africa species possess a shorter vent-anal distance than the Congolese species (Figs. 6 and 7).

As our phylogenetic analysis suggests, the existence of several species within *L. parvus* both in West Africa and in the Congo basin, we used CVA to assess the differences among these groups or species. It stands from these analyses that most of these groups are significantly different both in ventral and lateral view (Figs. 6 and 7, Table 1 and 2). However, we also found non-significant differences between distantly related species or groups suggesting similarities in overall body shape between non-related species. For instance, there are no significant differences between specimens of *L. parvus* from the Niger River and *L. chariensis* from the Lulua River (Congo River). In West Africa, specimens from the Niger River are significantly different to the specimens from the Bafing River (*L. parvus*-Ba) and these from the St-Paul River (*L. parvus*-Ou) but not to these from the Scarcies River (*L. parvus*-Pe). Though *L.* cf. *lukulae*-LL tends to occupy the same morphospace as *L. lukulae*-Ki, the permutation tests for the Procrustes distance show that there is a significant difference in overall body shape between these two species (Table 1 and 2). The considerable similarity observed among these groups corroborates the PCA results and suggests convergent evolution of these species.

Phylogenetic signal test

The permutation tests for phylogenetic signal were significant in MorphoJ for both PCA ($P< 0.0001$ in lateral view and $p= 0.0068$ in ventral view) and CVA ($p<0.0001$ in lateral view and $p=0.005$ in ventral view). As the broken stick model analysis (Borcard et al. 2011) has revealed that only the first four principal components were significant, in both lateral and ventral views, only these axes were used to test for the phylogenetic signal in geomorph. The test was

significant in the lateral view (p=0.049 and *K*= 0.524) and non-significant in ventral view (p=0.326 and *K*= 0.3778). For CVA, the tests were significant both in lateral (p=0.003 and *K*=0.6583) and ventral (p=0.001 and *K*=0.7725) views. In general, these results support the hypothesis that phylogenetic relatedness among these species is responsible for their overall body shape similarity. However, the low values of *K* (low phylogenetic signal) could be explained by shape similarities among distantly species (i.e. convergence). In addition, the phylogenetic signal test was non-significant in ventral view, supporting the idea of convergent evolution. Figure 8 presents the phylomorphospaces on which the test was conducted.

Species diversity with *Labeo parvus*

Both the MGYC and the PTP support the occurrence of multiple *Labeo* species recognized as *L. parvus* in both West and Central Africa (Figs. 9 and 10). In West Africa, three species have been delimited within the Upper Guinea province (St. Paul, Little Scarcies, and Konkouré basins), one within the Nilo-Sudan province (Niger and Senegal basins), and one within the Lower Guinea province (Cross River). In central Africa (Congo basin), more than 12 *Labeo* species, identified as *L. parvus*, *L.* cf. *parvus*, *L. chariensis*, and *L. lukulae* have been delineated (Likelihood Ratio test highly significant: LR=49.247 and *p*=2.023615e-11) for MGYC. We recovered the same species delimitation from PTP (Fig.10). Some of these species are limited to one ecoregion whereas others are widespread within the Congo basin.

DISCUSSION

Distribution of *Labeo parvus*

One of the present study objectives was to assess whether the actual distribution of the African carp *L. parvus* was relevant or not. Indeed, Reid (1985) supported the hypothesis that

the geographical distribution of *L. parvus* is limited within the Congo basin and probably in some Angolan coastal rivers. He suggested that the species did not occur in West Africa and is instead replaced by *L. ogunensis* which, morphologically, is closely related to *L. parvus*.

Our results support Reid's hypothesis of *Labeo parvus* being an endemic species of the Congo basin. In fact, our phylogenetic analysis strongly supported that none of the Congolese groups identified or susceptible to be misidentified as *L. parvus* are closely related to West African groups identified as *L. parvus*. The two groups are members of two genetically distinct sister clades with high posterior probability and bootstrap support. Moreover, our results support the hypothesis of the Congolese species identified as *L. parvus* forming a monophyletic group with several other endemic species of the Congo basin such *L. nasus, L. sorex, L. simpsoni, L. kirki*, etc. (Tshibwabwa 1997). On the other hand, the fact that the West African *L. parvus* clade is closely related to *Labeo* species from some coastal Lower Guinea ichthyoprovince rivers and these from the east Africa (Nile and Zambezi) place them evolutionary very distant from the Congolese *L. parvus*. Therefore, the occurrence of *L. parvus* in West Africa or in any other African ichthyoprovinces, as reported by several authors (Jegu and Lévêque 1984; Guégan, Lambert, and Euzet 1988; Christian Lévêque, Paugy, and Teugels 1990; Lalèyè et al. 2004; Nwani et al. 2011) is incorrect. The erroneous reports of *L. parvus* in West Africa were not based on misidentifications but on the inability to discriminate these species using the traditional morphological traits used to delimitate *Labeo* species (Lévêque et al. 1992). That problem is enhanced by the morphological similarities observed between the *L. parvus* originally described from the Congo and the Niger species recognized as *L. parvus*.

Diversity within *Labeo parvus* in West Africa

Several *Labeo* species in West Africa have been placed in the synonym of *L. parvus (Jegu and Lévêque 1984; Reid 1985)*. These species include *L. ogunensis* described from Ogun river by Boulenger in 1910, *L. obscurus* described from Badi river a tributary of Konkouré river by Pellegrin in 1908, *L. toboensis* described from Gambia river by Svensson in 1933, *L. tibestii* described from the Tibesti mountains region by Pellegrin 1919, etc. (Eschmeyer et al. 2017). Two specimens from the Kakrima river, a tributary of the Konkouré river, included in our analyses present similar characteristics as *L. obscurus* Pellegrin 1908 (Boulenger 1909; Jegu and Lévêque 1984; Reid 1985): 10 branched dorsal rays, 32+3 (35) scales in lateral series, 4.5 between the lateral line and the dorsal fin, 3 scales between the lateral line and the ventral fin, and 12 scales around the caudal peduncle (Table 3), and a blackish brown body that makes the lateral band barely visible. Reid (1985), reports that *L. obscurus* has 30 vertebrae. The species analyzed in the present studies has 29 vertebrae (Table 3). *Labeo rouaneti* Daget (1962) has been described from the Kakrima River, but that species has higher scale and vertebral counts: 36+3 (39) in lateral line, 4.5 between the lateral line and ventral fin,16 around caudal peduncle, and 31 vertebrae (Reid 1985). Therefore, we are resurrecting *Labeo obscurus* Pellegrin, 1908 as a valid species of *Labeo* in the Upper Guinea. Our results support *Labeo obscurus* as sister to individuals from Penselli river, a tributary of Little Scarcies river, with whom it shares the same color pattern. These individuals differ from *L. obscurus* by having 14 to 15 scale rows around the caudal peduncle, 34+4 to 34+3 (37 to 38) scales on lateral line series, and 30 to 31 vertebrae (Table 3). These characteristics are close to the one of *L. rouaneti*, but the latter has 11 to 12 branched dorsal fin rays, and 4.5 scale rows between the lateral line and ventral fin (Reid 1985). Further investigations, incorporating samples of *L. rouaneti*, are required to determine if these individuals represent an undescribed species.

Individuals from the upper Niger river and Bafing river, a tributary of the Senegal river, included in this study, present the same characteristics (Table 3) and these characteristics match the diagnosis of *L. parvus (Boulenger 1909; Jegu and Lévêque 1984; Reid 1985)*: 31+3 scale on the lateral line series, 12 scales around caudal peduncle, 4.5 scale rows between lateral line and the dorsal fin, 3.5 scale rows between lateral line and ventral fin, and 28 vertebrae. These characteristics slightly differ from the ones provided by Tshibwabwa (1997) especially for the number of vertebrae which, according to him, varies from 29 to 31. That species may be the one identified as *L. parvus* by Daget (1964; Jegu and Lévêque 1984). We refer to that species as *L. ogunensis* based on Reid's idea that it replaces *L. parvus* in West Africa. However, its characteristics do not match those of *L. ogunensis* which, according to Reid (1985), has a higher scale and vertebral counts: 33+3 (36) in lateral line, 5.5 to 6.5 between the lateral line and the dorsal fin, 4.5 between the lateral line and the ventral fin, 12 to 16 (14 frequently) scale rows around the caudal peduncle, and 30 vertebrae. The original description of Boulenger (1910) for *L. ogunensis* reports 12 scale rows around the caudal peduncle (Boulenger 1910; Jegu and Lévêque 1984). It is almost certain that the individuals we are referring to as *L. ogunensis* belong to a different species. Additional research is ongoing to determine the differences between the two species and provide new descriptions for both. The CVA of the body shape revealed a significant difference between the Senegal river (Bafing river) and Niger river populations ($P>0.0001$). But a closer look revealed that the observed significant difference is due to the allometric growth observed in the Bafing population. Besides the allometric growth, that species is morphologically closely related to specimens from Oulé river and has been, genetically, resolved as sister to them.

Specimens from Oulé river, a tributary of Saint-Paul river, cannot be distinguished using traditional meristics from specimens that we are calling *L. ogunensis* in this study (Table 3). The two species have a similar body shape. Nevertheless, the Oulé adult individuals are much larger than the *L. ogunensis* ones. That species is a morphologically cryptic species of *L. ogunensis,* and it is referred in this study as *Labeo* sp. A description of that species as a new species is in preparation.

Specimens from lake Guelta d'Archei, in Chad, are characterized by a high number of scale and vertebral counts (table 3). In addition, their body color and shape, and the size of their barbels are different to any other species examined in this study. The comparison of these individuals to the descriptions of *L. meroensis (Moritz 2007),* a *L. parvus*-like species from the Nile river, and *L. latebra (Moritz and Neumann 2017)* shows clear difference between them. These individuals may represent a lake ecomorph of one of the Nile *Labeo* species or a different species. We are referring to that species as *L. tibestii*. That species was not included in the molecular analysis because all the specimens were preserved in formalin.

Diversity within *Labeo parvus* in the Congo basin

Although *L. parvus* was originally described from the Congo basin, individuals recognized as *L. parvus* from that ichthyological province belong to multiple species (Lowenstein et al., 2011; Decru et al., 2016). The taxonomic revision conducted by Tshibwabwa and Teugels (1995) on *Labeo* species from the Congo ichthyoprovince revealed that none of the synonymy of *L. parvus* from the Congo was correct. However, the description they provided for *L. parvus* corresponds to the diagnoses of different species. Morphological characters used by the authors (such as the shape of the dorsal fin) to discriminate these species is one of the reasons that leads to that confusion (Steenberge et al., 2016).

Specimens from Tshopo river (main channel and its tributary Lindi river) and some from the main channel of the Upper Congo River (UC) in Kisangani region (AUM 51572, 51582), referred to as *L. parvus*_Li, examined in this study share the same haplotype with specimens of *L. simpsoni* from the lower Congo (AMNH 247071, 243589, 243591). We think that these specimens are representatives of the species *L. simpsoni*; however, the distinction of the two species is not easy. According to Tshibwabwa (1997) the major difference between the two species is the shape of their dorsal fin with *L. parvus* possessing a concave dorsal fin while *L. simpsoni* has a falciform one. A significant difference in body shape ($p= 0.0306$ for the CVA) has been observed between the UCR and the LCR populations of *L. simpsoni* in this study. That difference can be due to the polymorphism of that species (some specimens from Epulu have been identified as *L. chariensis*), population variations, or the allometric growth given the fact that most of the UCR specimens in the present were juveniles. Five out of six individuals examined had 28 vertebrae (Table 4), while only one had 29. Tshibwabwa (1997) reports that individuals of *L. simpsoni* have 29 to 30 vertebrae.

Several species from the Kasai river are misidentified as *L. parvus* because of their morphological similarity. Specimens from the Kasai river and its tributaries included in this study have been clustered, based on molecular data, into more than five distinct species. One of them, referred in this study as *L. lukulae*_LL because of its similarities with *L. lukulae* from Kisangani (Table 4 and Figure 2), seems to be endemic to the Lulua river (a tributary of the Kasai). The meristic characteristics of that species are close to *L. luluae* (Fowler 1930) except for the circumpeduncle scale rows, which is reported to vary from 13 to 14 by Tshibwabwa (1997): 29 vertebrae, 34 scales on the lateral line, 10 branched dorsal rays, 3 simple dorsal rays. However, Fowler's description of *L. luluae* does not match with the present species. Another

species, from the upper portion of Lulua river, referred to here as *L. cf. parvus* Sandoa shares *L. parvus* characters but is genetically different to any other *Labeo* species included in this study. Further investigations are required for the description of that species.

Some specimens from the main channel of the Kasai river, referred as *L. parvus*_Ka share the same haplotype (CO1) with several *L. parvus* individuals from Lomami, Aruwimi and Ituri rivers (Decru et al., 2016).This group may represent the true *L. parvus*; however, the vertebral count of these individuals is lower (28) than the one presented by Tshibwabwa's results (29 to 31).

Body shape convergence and divergence within *L. parvus* groups

The results presented in this study reveal that *L. parvus* is a species complex and that species from West Africa, identified as *L. parvus*, belong to a different clade than *L. parvus* from the Congo basin. Yet, the morphological similarities between these species are remarkable. Those similarities are observable both in ventral and lateral views, as presented in this study, by the overlapping of individuals of the two groups in the PCA of the overall body shape morphospace. Even by using the CVA, which maximizes the difference between groups (Webster and Sheets 2010), several species in both groups were not significantly different in shape. It is known that body morphology of aquatic animals, fishes in this case, is a response to environmental pressure (Bryant 1977; Knouft 2003) and that sympatric species or allopatric species that occupy a similar niche tend to produce similar body shape as adaptation to their habitat conditions (Knouft 2003; Armbruster et al., 2016). Our results of phylogenetic signal, in general, show that there is phylogenetic signal in overall body shape of these taxa. This suggests that the West African species resemble each other more than they resemble the Congo species and vice versa. In other words, the two groups are diverging, and the similarities among closely

related species are due to shared evolutionary history. However, these results do not explain why the West Africa species are more similar to the Congo species than they are to the lower Guinea and the East Africa (Nile and Zambezi) species with whom they share a more recent common ancestor (see Chapter 1). The observed body shape similarities between those distantly related species is probably a result of convergent evolution. That convergence might be the result of an adaptation to rapid and rocky substrate habitats where most of these species are found. A recent study (Alter et al. 2015) has demonstrated that extreme rapids in the lower Congo river, has yielded similar phenotypic convergence in distantly related eel species. That phenotypic convergence in rapids is not limited to eel species but is also observed in several other groups of fishes (Mormyrids, Cichlids, Catfish and Cyprinids) in that region. The similarity between the West African and the Congo basin *L. parvus*-like species is not limited to shape but include also the coloration which is characterized by the presence of dark-brown and brown lateral bands.

Conservation implications

Labeo parvus is currently listed as least concern in the IUCN Red List of Threatened Species based on the idea that the species is widely distributed throughout the African continent and seems to have no major widespread threat (Hanssens et al. 2010). Our results have demonstrated that is not the case. Species that were recognized as *L. parvus* seem to be endemic to specific river basins. Therefore, their conservation status is likely of higher concern than is currently recognized. This is supported by the fact that fishing pressure keeps increasing in Africa due to demographic growth. In East Africa for instance, populations of '*L. parvus*' have been assessed as endangered (Hanssens et al. 2010). In West Africa, studies report that, in certain countries, '*L. parvus*' is one of the most popular foods and one of the most harvested fish species for commercial interests (Montchowui et al. 2009; Montchowui et al. 2011). Though assessed as

least concerned in central Africa, the status of '*L. parvus'* in that region remains uncertain. Hence, a regional re-evaluation of the conservation status of each species, previously included in the *L. parvus* complex, is necessary to determine what measures or conservation actions need to be taken for the preservation of these species.

CONCLUSION

In conclusion, *L. parvus* is endemic to the Congo basin where it was originally described. Due to morphological similarities, several other species of *Labeo* in west and east Africa have been erroneously synonymized to *L. parvus*. Our results confirm the endemicity of *L. parvus* in the Congo basin and demonstrate that *L. 'ogunensis'* and *L. obscurus*, respectively from the Nilo-Sudan and the Upper Guinea ichthyoprovinces, are valid and distinct species. The two groups are members of different clades. Several cryptic species have been delimited in the Congo basin and in West Africa. The overall body shape similarities observed between West African and Central African species are results of convergent evolution. The two groups were also converging in meristic characters such as vertebral counts, scale number in lateral line and circumpeduncle scale rows. Hence, these characters alone are not sufficient for the distinction of the *L. parvus*-like species between different ichthyoprovinces, thus explaining why distinct species were mistaken as *L. parvus*. Further analyses are needed to distinguish *L. ogunensis* from its cryptic congeners and provide adequate description of these species.

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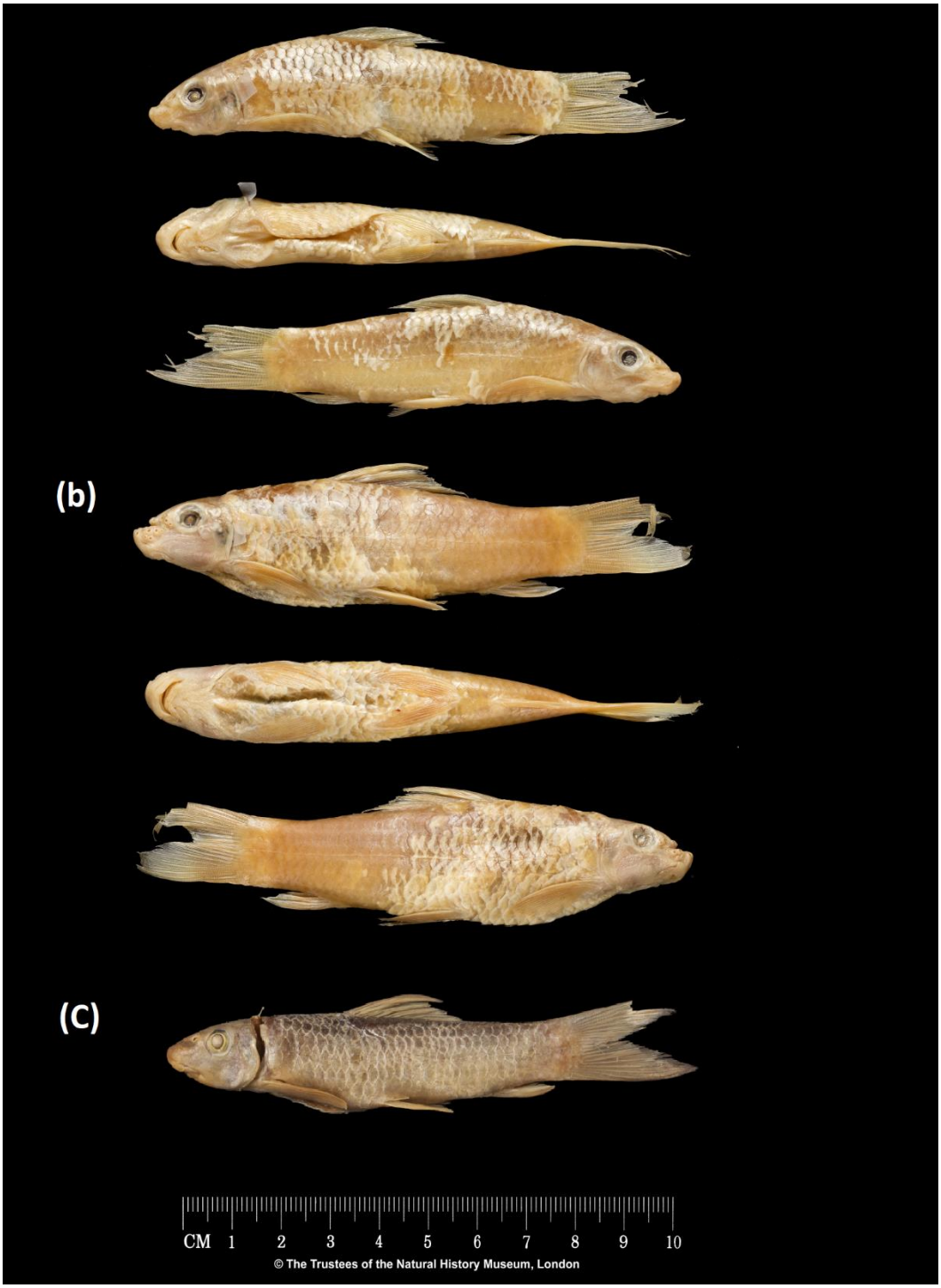

Figure 1: Type series of *Labeo parvus* in the Natural History Museum (London). (**a** and **b**): BMNH 1901.12.26.24-25, Syntypes from Ubaghi river (Banziville, DRC); (**c**): BMNH 1907.4.20.38, Specimen from Aruwini River (DRC).

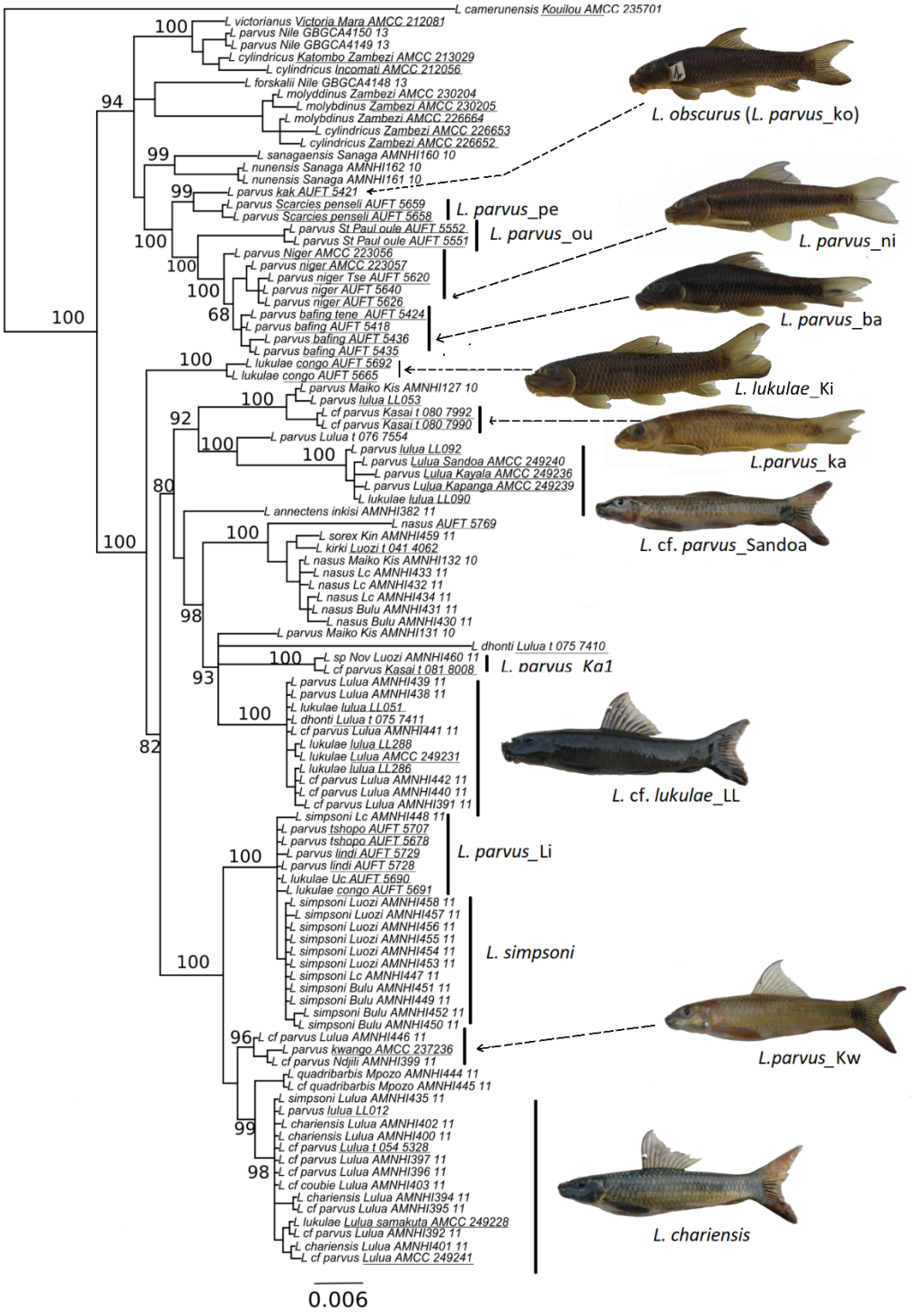

Figure 2: The MAP tree with mean branch lengths summarized from posterior sample from our Bayesian phylogenetic analysis of the concatenated sequence dataset (COI and RAG1) of the African *Labeo* species of the *L. forskalii*-group. Posterior probabilities are reported on branches with > 67 posterior probability.

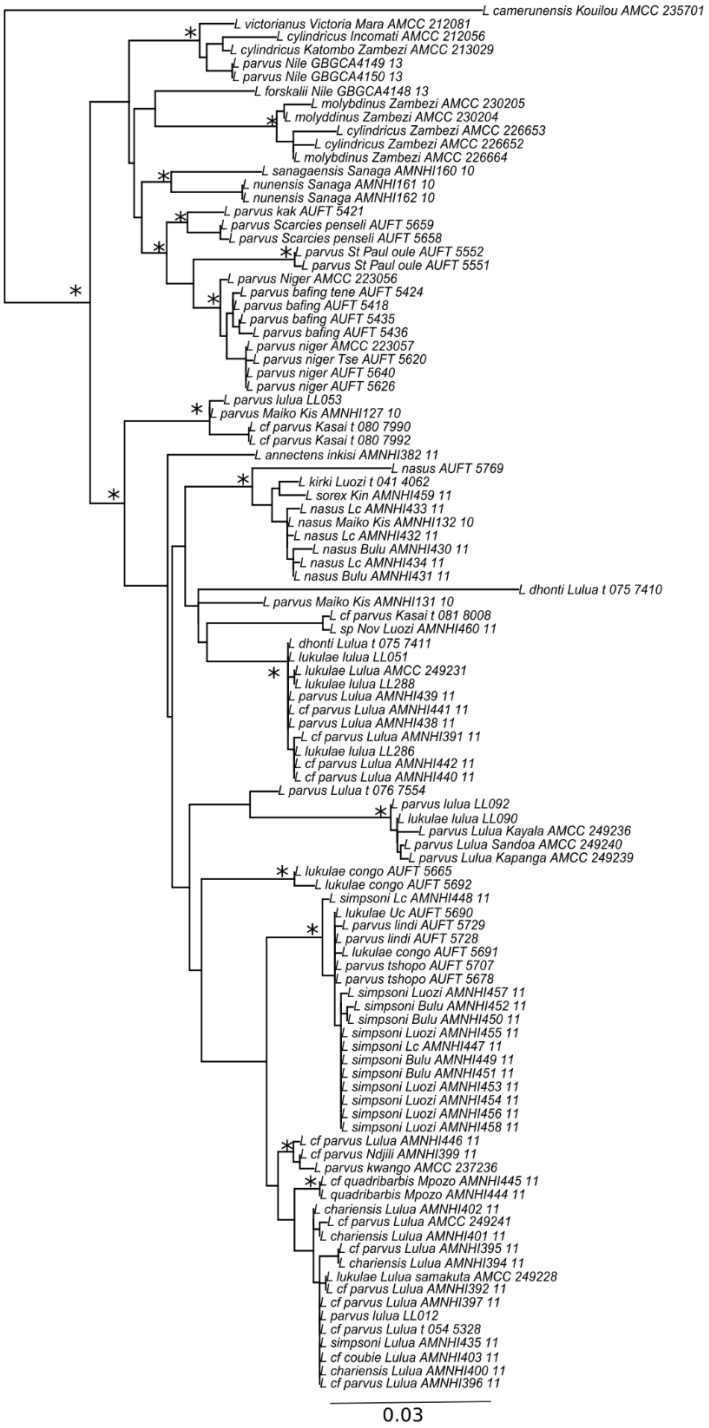

Figure 3: Phylogram inferred from Maximum-Likelihood analysis of the concatenated sequence dataset (COI and RAG1) of the African *Labeo* species of the *L. forskalii*-group. (*) indicates branch bootstrap support above 70%.

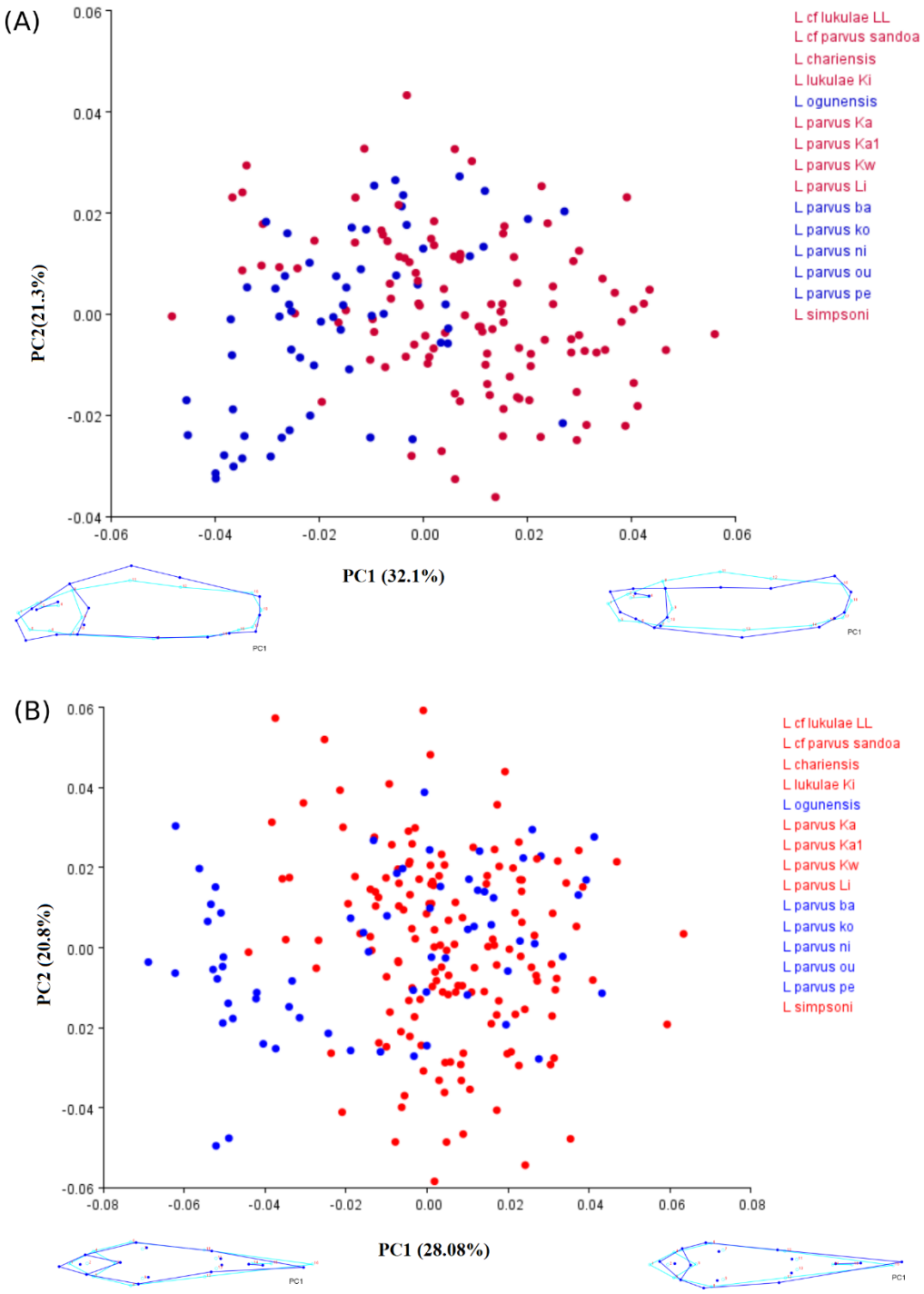

Figure 4: Principal component analysis on different individuals of *Labeo parvus*-like species in Congo (red dots) and in west Africa (blue dots). **A**) Lateral view; and **B**) Ventral view.

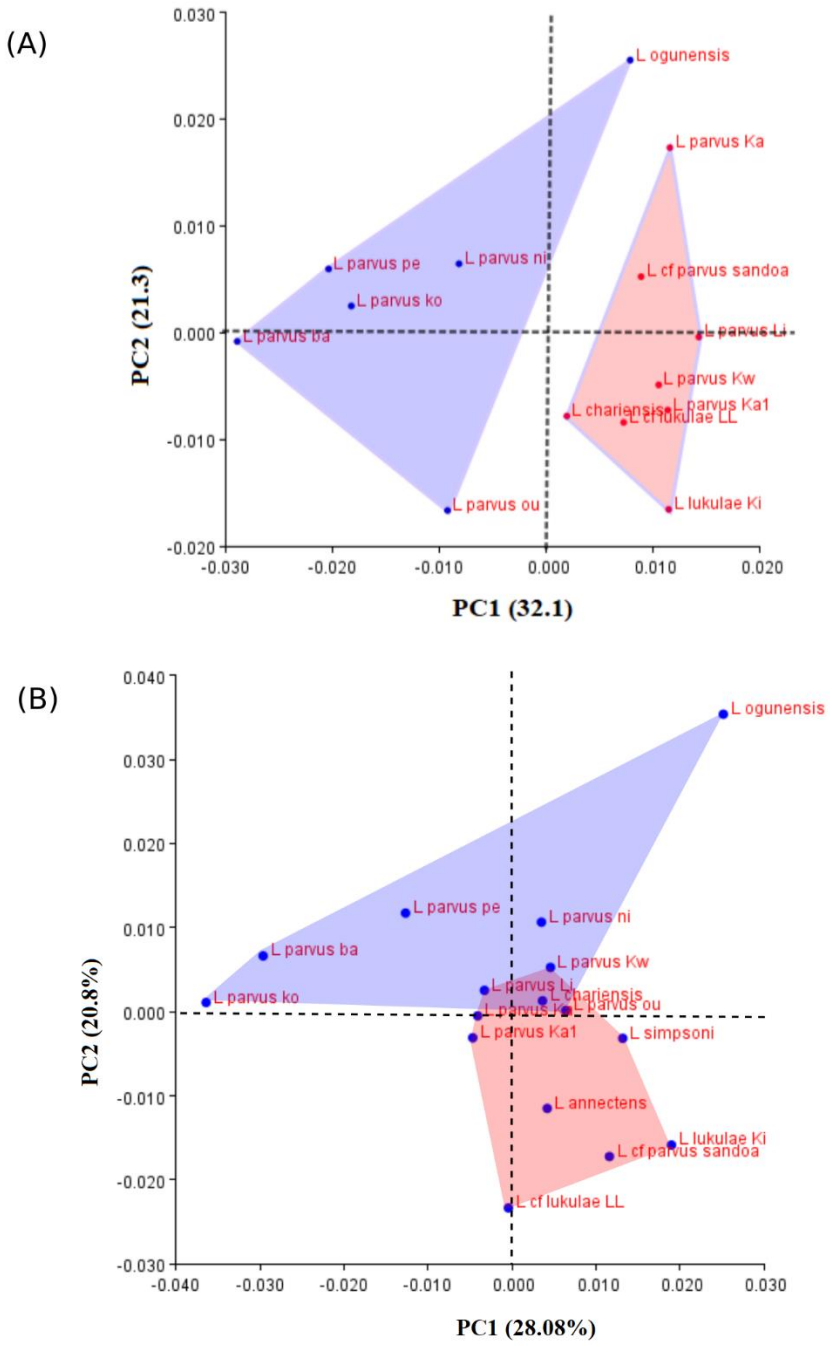

Figure 5: Principal component analysis on average Procrustes coordinates of individuals of each *Labeo parvus*-like species from Congo (red polygon) and West Africa (blue polygon). **A**) Lateral view; and **B**) Ventral view.

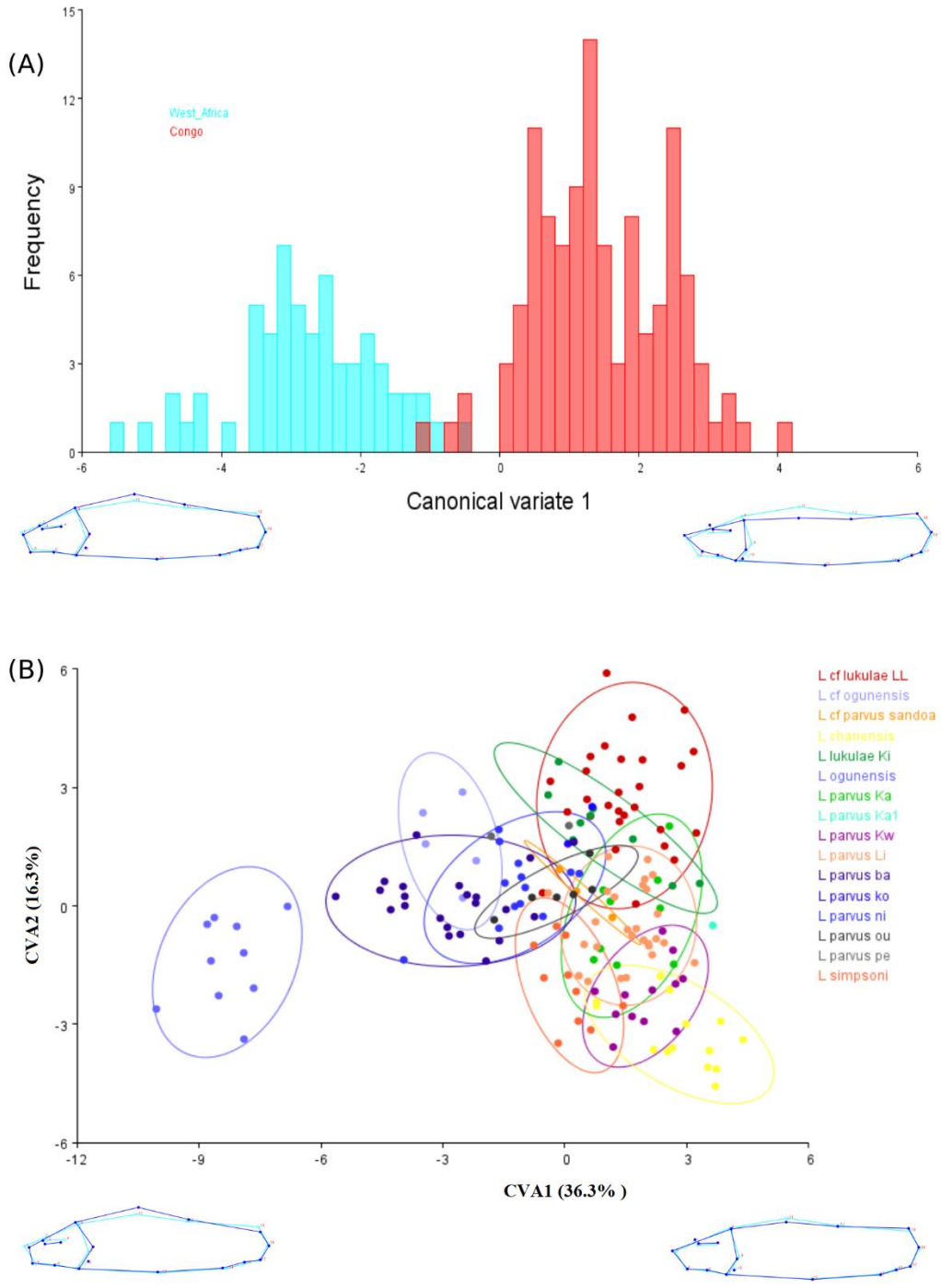

Figure 6: Morphospace plot visualizing body shape variation (in lateral view): (**A**) between the West Africa and the Congo clades; and B) between West Africa (blue and greyscales) and Congo species (remaining colors) with 90% confidence ellipses.

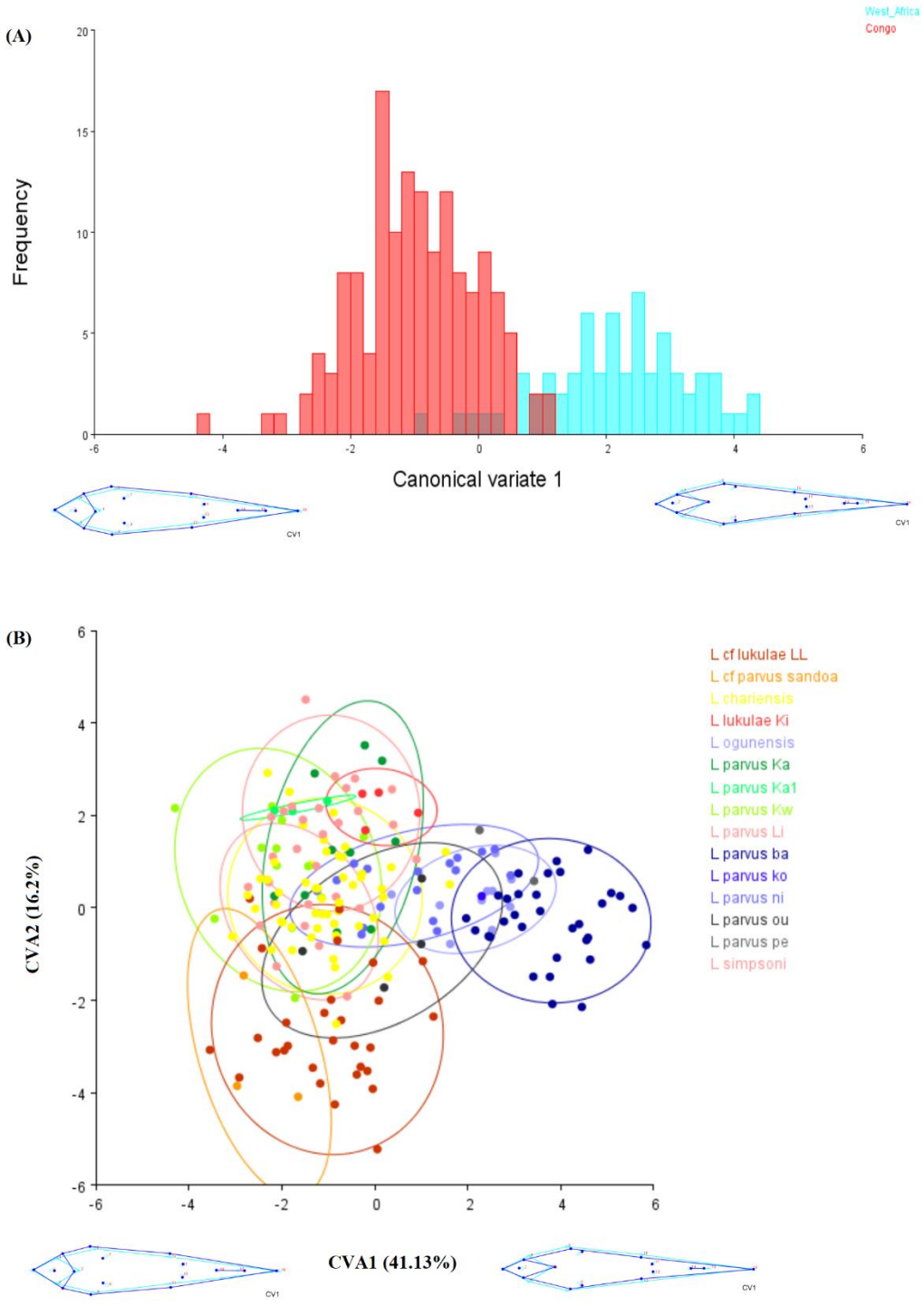

Figure 7: Morphospace plot visualizing body shape variation (in ventral view): (**A**) between the West Africa and the Congo clades; and B) between West Africa (blue and greyscales) and Congo species (remaining colors) with 90% confidence ellipses.

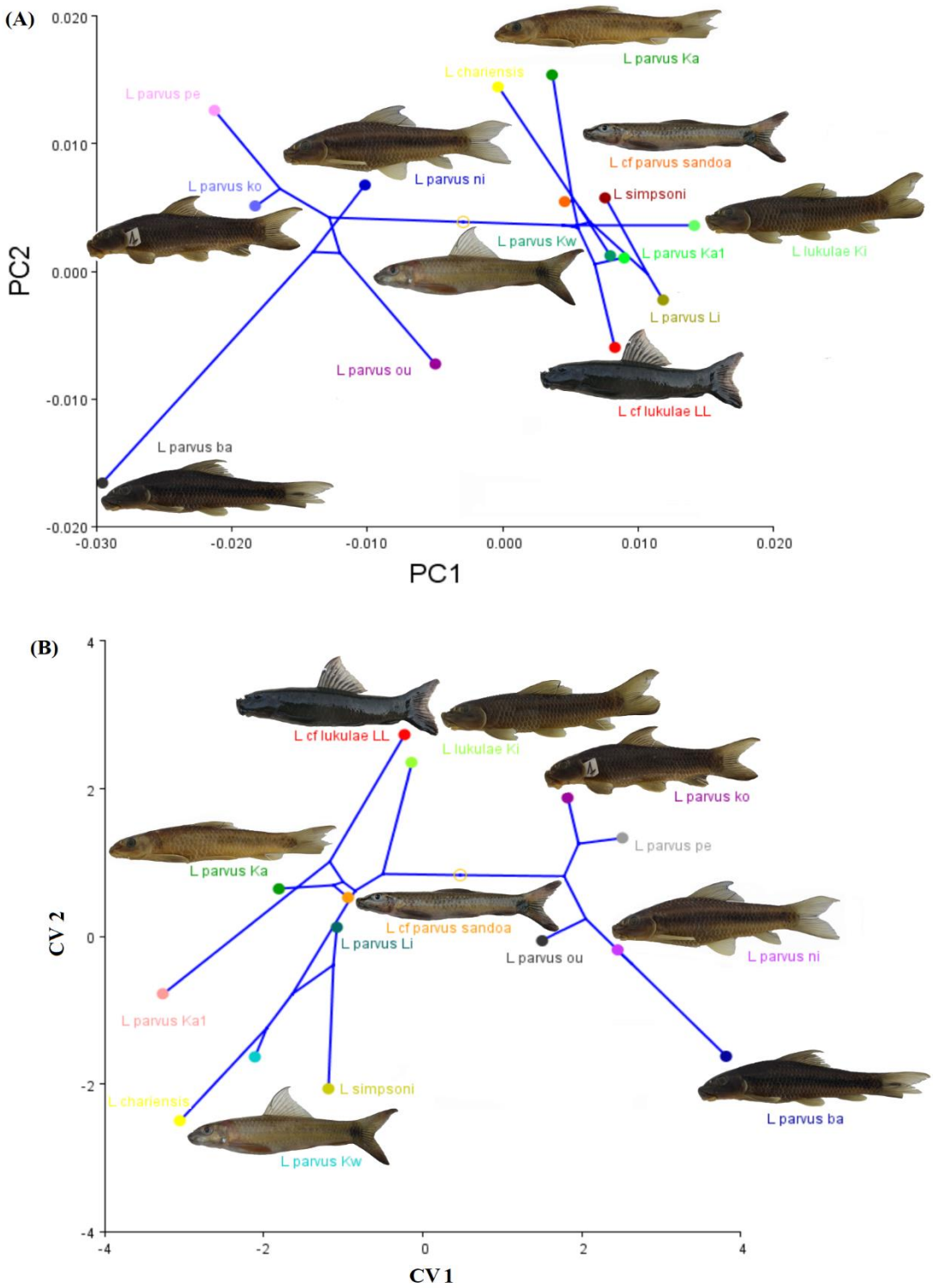

Figure 8: Phylomorphospace plot of body shape of *Labeo parvus*-like species from Congo and West Africa. **A**) PC1 vs PC2 and **B**) CV1 vs CV2.

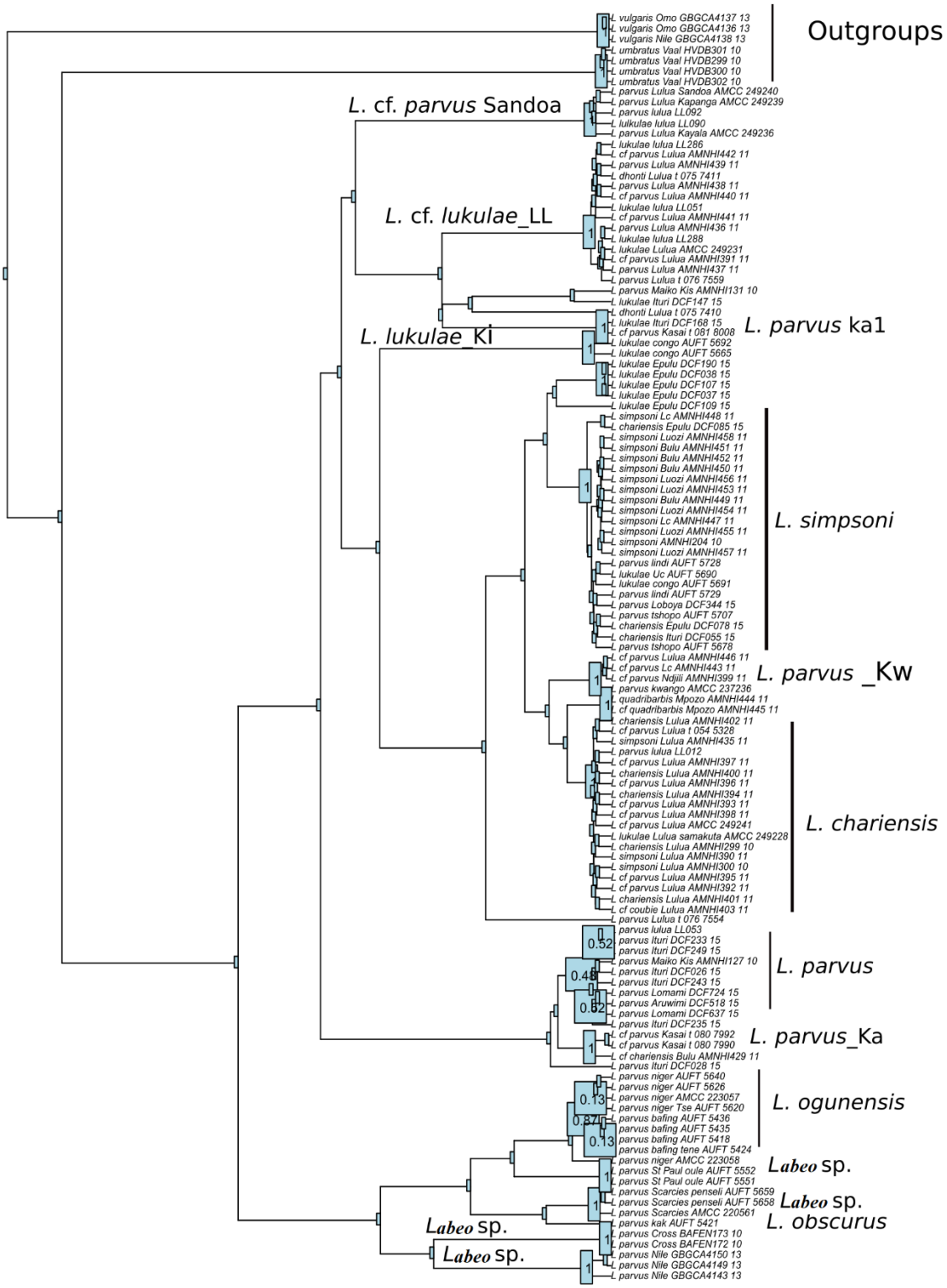

Figure 9: GMYC species delimitation solution

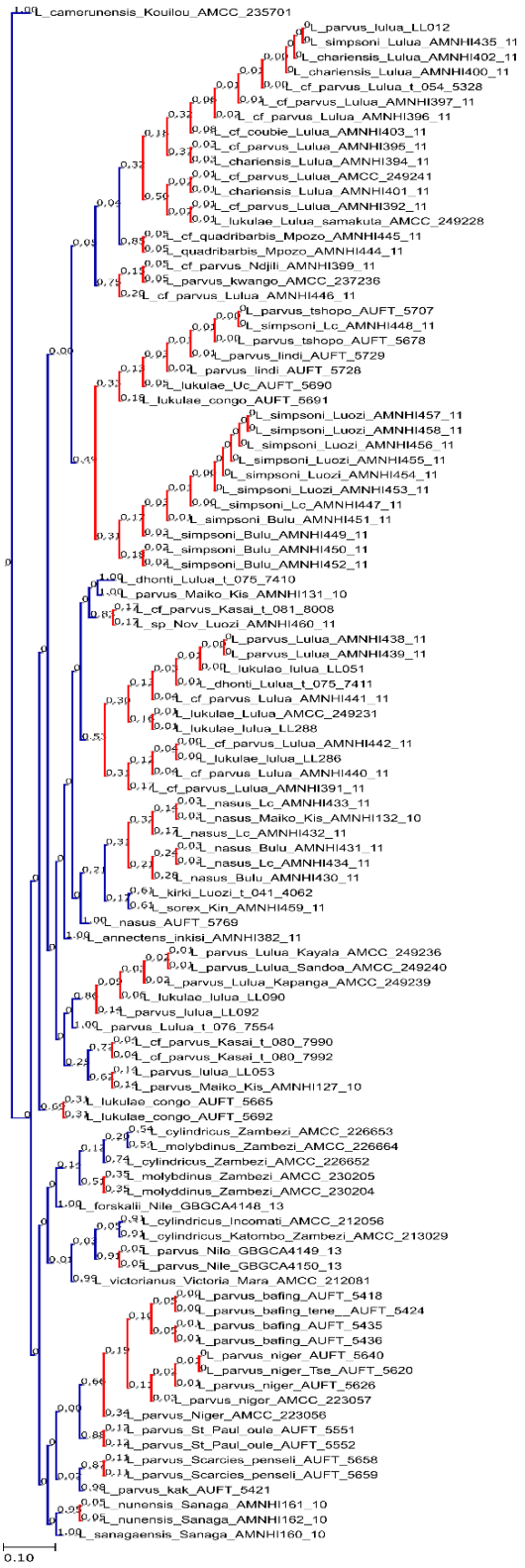

Figure 10: PTP species delimitation Maximum Likelihood solution

Table 1: *P*-values from permutation tests (10000 permutation rounds) for Procrustes distances among species in lateral view

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *L* cf *lukulae* LL														
2. *L* cf. *parvus*_Sandoa	0.4418													
3. *L chariensis*	<.0001	0.1212												
4. *L lukulae* Ki	0.0028	0.0963	0.0035											
5. *L ogunensis*	<.0001	0.003	<.0001	<.0001										
6. *L parvus* Ka	0.0002	0.223	<.0001	0.0001	<.0001									
7. *L parvus* Ka1	0.7545	0.831	0.3809	0.2144	0.092	0.9478								
8. *L parvus* Kw	0.0022	0.6373	0.0827	0.0079	<.0001	0.0034	0.6244							
9. *L parvus* Li	<.0001	0.292	<.0001	0.0001	<.0001	0.0013	0.5601	0.0368						
10. *L parvus* Ba	<.0001	0.0012	<.0001	<.0001	<.0001	<.0001	0.0905	<.0001	<.0001					
11. *L parvus* Ko	0.1609	0.2942	0.0476	0.0399	0.0162	0.0259	0.3317	0.1183	0.0321	0.0309				
12. *L parvus* Ni	<.0001	0.0109	<.0001	0.0002	<.0001	<.0001	0.2035	0.0004	<.0001	<.0001	0.0947			
13. *L parvus* Ou	0.004	0.0516	0.0227	0.0329	<.0001	0.0007	0.3986	0.0442	0.0001	0.0001	0.0934	0.0023		
14. *L parvus* Pe	0.0454	0.1026	0.063	0.0563	0.0157	0.0905	0.3257	0.0597	0.0304	0.0037	0.331	0.4556	0.1356	
15. *L simpsoni*	0.0014	0.6927	0.2428	0.0193	<.0001	0.0189	0.4579	0.3507	0.0304	<.0001	0.1823	0.0012	0.0346	0.1532

Table 2: *P*-values from permutation tests (10000 permutation rounds) for Procrustes distances among species in ventral view

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *L* cf. *lukulae* LL														
2. *L* cf. *parvus* Sandoa	0.2554													
3. *L chariensis*	<.0001	0.1072												
4. *L lukulae* Ki	0.0194	0.3955	0.0154											
5. *L ogunensis*	<.0001	0.0036	<.0001	0.0019										
6. *L parvus* Ka	0.0001	0.3465	0.03	0.0336	<.0001									
7. *L parvus* Ka1	0.0355	0.1933	0.4895	0.2671	0.0015	0.4965								
8. *L parvus* Kw	<.0001	0.0329	0.0539	0.0107	<.0001	0.2397	0.3512							
9. *L parvus* Li	0.0001	0.2385	0.0052	0.0355	<.0001	0.7458	0.7201	0.6425						
10. *L parvus* Ba	<.0001	0.0002	<.0001	0.0001	<.0001	<.0001	0.0003	<.0001	<.0001					
11. *L parvus* Ko	0.2422	0.0775	0.3025	0.5704	0.0621	0.615	0.2491	0.0733	0.3359	0.0244				
12. *L parvus* Ni	<.0001	0.0038	0.0822	0.0314	<.0001	0.0133	0.0835	0.0009	0.0025	<.0001	0.3245			
13. *L parvus* Ou	0.0083	0.0102	0.3809	0.4514	0.0002	0.0689	0.1517	0.0024	0.0611	0.0001	0.1829	0.5269		
14. *L parvus* Pe	0.0218	0.0315	0.2845	0.416	0.004	0.3692	0.1953	0.0108	0.2613	0.0018	1	0.4392	0.1751	
15. *L simpsoni*	<.0001	0.3827	0.4671	0.1649	<.0001	0.0963	0.3639	0.0919	0.058	<.0001	0.3066	0.0366	0.3389	0.1763

Table 3: Meristic characters of *L. parvus*-Like species in West African. Numbers in parenthesis indicate number of specimens sharing this character.

	Labeo ogunensis							***Labeo* sp.**		
	*L. parvus*_Ba				*L. parvus*_Ni			*L. parvus*_Ou		
	consensus	Max	Min		consensus	Max	Min	consensus	Max	Min
Procurrent dorsal fin rays	**2**	2 (6)	2(6)		**2**	2 (5)	2(5)	**2**	2(3)	1(1)
Simple dorsal fin rays	**2**	2(6)	2(6)		**2**	2(5)	2(5)	**2**	2(4)	2(4)
Branched dorsal fin rays	**10**	10 (10)	10 (10)		**10**	10 (5)	10 (5)	**10**	10 (4)	10 (4)
Scales in lateral line	**31+3**	31+3 (9)	30+3(1)		**31+3**	31+3 (4)	30+3 (1)	**31+3**	32+3 (1)	31+3(6)
Scale rows between lateral line and dorsal fin	**4.5**	4.5 (10)	4.5 (10)		**4.5**	4.5 (4)	4 (1)	**4**	4.5 (2)	4 (5)
Scale rows between lateral line and pelvic fin	**3.5**	3.5 (10)	3.5 (10)		**3.5**	3.5 (5)	3.5 (5)	**3**	3.5 (2)	3(5)
Scales around caudal peduncle	**12**	12 (10)	12 (10)		**12**	13 (2)	12 (3)	**12**	12 (7)	
Predorsal scales	**9**	9 (10)	9 (10)		**9**	10 (1)	9(4)	**9**	9(7)	
Principal caudal-fin rays	**19**	19 (10)	19 (10)		**19**	19 (5)	19 (5)	**19**	19(7)	
Upper procurrent caudal-fin rays	**10**	10 (5)	9 (1)		**9**	10 (2)	9(3)	**9**	9(4)	
Lower procurrent caudal-fin rays	**8**	8 (3)	6 (1)	7 (2)	**7**	8 (1)	7(4)	**7**	7(3)	6(1)
Simple pelvic fin rays	**1**	1 (10)	1(10)		**1**	1(5)	1(5)	**1**	1(4)	
branched pelvic-fin rays	**8**	8(10)	8(10)		**8**	8 (5)	8 (5)	**8**	8(4)	
Procurrent anal fin rays	**1**	1(6)	1(6)		**1**	1 (5)	1(5)	**1**	1 (4)	
Simple Anal-fin rays	**2**	2(10)	2(10)		**2**	2 (5)	2 (5)	**2**	2 (4)	
Branched anal-fin rays	**5**	5 (10)	5 (10)		**5**	5(5)	5(5)	**5**	5 (7)	
Total vertebrae	**28**	28 (5)	27 (1)		**28**	28(5)	28(5)	**28**	28 (4)	
Abdominal vertebra	**16**	16 (6)	16(6)		**16**	16 (5)	16 (5)	**15**	16 (1)	15 (13)
Caudal vertebra	**12**	12 (6)	12(6)		**12**	12 (5)	12 (5)	**13**	13 (3)	12(1)
Pleural ribs	**12**	12 (5)	11 (1)		**12**	12 (5)	12 (5)	**11**	12(1)	11(3)

Table 3 (Continued)

	Labeo obscurus			***Labeo* sp.**			***Labeo tibestii***			
	*L. parvus*_Ko			*L. parvus*_Pe			*L. ogunensis*			
	consensus	Max	Min	consensus	Max	Min	consensus	Max	Min	Others
Procurrent dorsal fin rays	**2**	2(2)	2(2)	**2**	2(2)	2(2)	**2**	2 (5)		
Simple dorsal fin rays	**2**	2(2)	2(2)	**2**	2(2)	2(2)	**2**	2(9)		
Branched dorsal fin rays	**10**	10 (2)		**10**	10(2)		**10**	10(9)		
Scales in lateral line	**32+3**	32+3 (2)		**34+3**	34+4 (1)	34+3 (1)	**34+3**	35+3(4)	34+3(5)	
Scale rows between lateral line and dorsal fin	**4.5**	4.5(2)		**4.5**	4.5(2)		**5**	5.5(3)	5(6)	
Scale rows between lateral line and pelvic fin	**3**	3(2)		**3.5**	3.5(2)		**4**	4.5(2)	3.5(1)	4(6)
Scales around caudal peduncle	**12**	12 (2)		**14~15**	15 (1)	14(1)	**15**	16 (3)	14(1)	15(6)
Predorsal scales	**9**	9(2)		**10**	10(2)		**10**	11(3)	10(6)	
Principal caudal-fin rays	**19**	19(2)		**19**	19(2)		**19**	19(9)		
Upper procurrent caudal-fin rays	**9**	9(2)		**10**	10(1)	9(1)	**9**	9(4)	8(1)	
Lower procurrent caudal-fin rays	**7**	7(2)		**8**	8(1)	7(1)	**8**	8(3)	7(2)	
Simple pelvic fin rays	**1**	1(2)		**1**	1(2)		**1**	1(9)		
branched pelvic-fin rays	**8**	8(2)		**8**	8(2)		**8**	8(9)		
Procurrent anal fin rays	**1**	1(2)		**1**	1(2)		**1**	1(5)		
Simple Anal-fin rays	**2**	2(2)		**2**	2(2)		**2**	2(9)		
Branched anal-fin rays	**5**	5(2)		**5**	5(2)		**5**	5(9)		
Total vertebrae	**29**	29 (2)		**31**	31(1)	30(1)	**31**	32(1)	30(1)	31(3)
Abdominal vertebra	**16**	16 (2)		**18**	18(1)	17(1)	**16**	17(2)	16(3)	
Caudal vertebra	**13**	13 (2)		**13**	13(2)		**15**	15(3)	14(2)	
Pleural ribs	**12**	12(2)		**12**	12(2)		**14**	14(5)		

Table 4: Meristic characters of *L. parvus*-Like species in the Congo basin. Numbers in parenthesis indicate number of specimens sharing this character.

	***L. parvus*_Li**				***L. parvus*_Ka**			***L. parvus*_Ka1**		***L.* cf. *parvus*_Sandoa**	
	Consensus	Max	Min	Others	Consensus	Max	Min	Consensus	Max	Consensus	Max
Procurrent dorsal fin rays	**2**	2 (7)	2(7)		**2**	2(4)		**2**	2 (1)	**2**	2(1)
Simple dorsal fin rays	**2**	2(13)	2(13)		**2**	2(6)		**2**	2(1)	**2**	2(1)
Branched dorsal fin rays	**10**	10 (13)	10 (13)		**10**	10(6)		**10**	10(1)	**10**	10(1)
Scales in lateral line	**31+3**	32+3 (3)	30+3(4)	31+3(6)	**31+3**	31+3(5)	30+3(1)	**31+3**	31+3(1)	**31+3**	31+3(1)
Scale rows between lateral line and dorsal fin	**4.5**	4.5 (12)	4 (4)		**4.5**	4.5(4)	4(2)	**4.5**	4.5(1)	**4**	4(1)
Scale rows between lateral line and pelvic fin	**3**	3.5 (4)	3 (9)		**3**	3(6)		**3**	3(1)	**3**	3(1)
Scales around caudal peduncle	**12**	12 (13)			**12**	12(6)		**12**	12(1)	**12**	12(1)
Predorsal scales	**9**	10 (3)	9 (10)		**9~10**	10(3)	9(3)	**10**	10(1)	**10**	10(1)
Principal caudal-fin rays	**19**	19 (13)			**19**	19(6)		**19**	19(1)	**19**	19(1)
Upper procurrent caudal-fin rays	**8~9**	9 (3)	8(3)		**8**	8(4)		**8**	8(1)	**8**	8(1)
Lower procurrent caudal-fin rays	**7**	8 (2)	7 (4)		**7**	7(3)		**7**	7(1)	**7**	7(1)
Simple pelvic fin rays	**1**	1 (13)			**1**	1(6)		**1**	1(1)	**1**	1(1)
branched pelvic-fin rays	**8**	8(13)			**8**	8(6)		**8**	8(1)	**8**	8(1)
Procurrent anal fin rays	**1**	1(6)	1(6)		**1**	1(4)		**1**	1(1)	**1**	1(1)
Simple Anal-fin rays	**2**	2(13)			**2**	2(4)		**2**	2(1)	**2**	2(1)
Branched anal-fin rays	**5**	5 (13)			**5**	5(6)		**5**	5(1)	**5**	5(1)
Total vertebrae	**28**	29(1)	28 (5)		**28**	28(4)		**28**	28(1)	**28**	28(1)
Abdominal vertebra	**15**	15 (5)	16(1)		**15**	15(4)		**14**	14(1)	**16**	16(1)
Caudal vertebra	**13**	13 (6)			**13**	13(4)		**14**	13(1)	**12**	12(1)
Pleural ribs	**11**	12 (1)	11 (5)		**12**	12(4)		**11**	11(1)	**12**	12(1)

Table 4 (continued)

	***L. lukulae*_Ki**				***L. lukulae*_LL**				***L. chariensis***			
	consensus	Max	Min	Others	consensus	Max	Min	Others	consensus	Max	Min	Others
Procurrent dorsal fin rays	**1~2**	2(1)	1(1)		**1**	2(1)	1(15)		**2**	2(3)		
Simple dorsal fin rays	**2**	2(7)			**2**	2(25)			**2**	2(23)		
Branched dorsal fin rays	**10**	10(7)			**10**	10(23)	9(2)		**10**	10(23)		
Scales in lateral line	**32+3**	32+3(5)	31+3(2)		**31+3**	32+3(2)	30+3(2)	31+3(21)	**31+3**	32+3(5)	31+3(18)	
Scale rows between lateral line and dorsal fin	**4.5**	4.5(6)	4(1)		**4**	4.5(4)	4(20)		**4**	4.5(6)	4(17)	
Scale rows between lateral line and pelvic fin	**3.5**	3.5(5)	3(2)		**3**	3.5(7)	3(17)		**3**	3(22)	3.5(1)	
Scales around caudal peduncle	**12**	13(3)	12(4)		**12**	12(24)			**12**	13(1)	12(22)	
Predorsal scales	**10**	12(1)	9(1)	11(2);10(3)	**9**	10(6)	9(18)		**9**	10(4)	8(5)	9(14)
Principal caudal-fin rays	**19**	19(7)			**19**	19(25)			**19**	19(23)		
Upper procurrent caudal-fin rays	**9**	9(2)			**9**	9(12)	8(4)		**8**	9(1)	8(2)	
Lower procurrent caudal-fin rays	**7**	7(2)			**7**	7(10)	6(5)		**6**	7(1)	6(2)	
Simple pelvic fin rays	**1**	1(7)			**1**	1(24)			**1**	1(23)		
branched pelvic-fin rays	**8**	1(7)			**8**	8(24)			**8**	8(23)		
Procurrent anal fin rays	**1**	1(2)			**0**	1(4)	0(12)		**1**	1(3)		
Simple Anal-fin rays	**2**	2(7)			**2**	2(24)			**2**	2(23)		
Branched anal-fin rays	**5**	5(7)			**5**	5(25)			**5**	5(23)		
Total vertebrae	**29**	29(2)			**29**	29(12)	28(4)		**28**	28(3)		
Abdominal vertebra	**16**	16(2)			**16**	16(15)	15(1)		**15**	16(1)	15(2)	
Caudal vertebra	**13**	13(2)			**13**	13(13)	12(3)		**13**	13(2)	1(2)	
Pleural ribs	**12~13**	13(1)	12(1)		**12**	13(3)	12(13)		**12**	12(2)	11(1)	