

# Phylogenomics and Morphology of the African Fish Genus *Brycinus* with Revalidation of *Brachyalestes* and Description of a New Species from the Congo Basin (Teleostei: Alestidae)

Melanie L. J. Stiassny<sup>1</sup>, Cooper Keane<sup>1</sup>, José J. M. M. Mbimbi<sup>2</sup>, and Bruno F. Melo<sup>1</sup>

A time-calibrated phylogeny, based on nuclear ultraconserved elements and including representatives of all major alestid lineages, strongly supports two distantly related clades within the currently accepted concept of *Brycinus*. The first, which includes the type species of the genus, *B. macrolepidotus* (herein *Brycinus*), and a second, composed of taxa previously referred to as the *B. nurse* group (herein *Brachyalestes*), are both resolved as monophyletic. These results provide strong evidence for the restriction of the genus *Brycinus* to nine species, and for the revalidation of the genus *Brachyalestes* to accommodate 20 valid species. Within *Brachyalestes*, a new species from the Lulua River basin, initially misidentified as *Brycinus kingsleyae*, is described and resolved as sister to the widespread, central Congolese lowland species, *Brachyalestes bimaculatus*. Within *Brachyalestes*, a subclade mostly restricted to the Central Congo basin is estimated to have undergone diversification within the last 10 million years, suggesting that Late Neogene riverine reorganization likely influenced their allopatric speciation. The split of the new species, endemic to high elevation tributaries of the Lulua River, from its lowland sister species, *Brachyalestes bimaculatus*, suggests a Late Miocene/Early Pliocene colonization into the upland river ecosystems of the Katanga plateau in the southwestern Democratic Republic of Congo.

**RESHWATER** fishes of the family Alestidae constitute by far the greatest diversity of African characiforms, with current estimates of 118 species distributed among 19 genera with species found throughout Africa's freshwater ecosystems (Froese and Pauly, 2019; Fricke et al., 2022). Despite significant progress toward the resolution of interfamilial, suprageneric, and generic groupings, much uncertainty remains (Murray and Stewart, 2002; Calcagnotto et al., 2005; Hubert et al., 2005; Arroyave and Stiassny, 2011; Melo and Stiassny, 2022). This is particularly true for the largest alestid genus, *Brycinus*, and a lack of consensus regarding the systematics of this species-rich and morphologically heterogeneous assemblage is in many respects emblematic of ongoing uncertainties surrounding the systematics of Alestidae as a whole.

In the most recent revisional study of the genus, Paugy (1986) divided *Brycinus* into three informally named species groups: the *macrolepidotus* group, the *longipinnis* group, and the *nurse* group. Subsequent morphological (Murray and Stewart, 2002; Zanata and Vari, 2005) and molecular (Calcagnotto et al., 2005; Hubert et al., 2005; Arroyave and Stiassny, 2011) studies, while differing markedly in taxon coverage and details of resolved relationships, consistently refuted the monophyly of *Brycinus*. In agreement with both morphological and molecular studies, Zanata and Vari (2005) reassigned Paugy's *longipinnis* group to the genus *Bryconalestes* (type species *Bryconalestes longipinnis*), and now including *B. bartoni*, *B. derhami*, *B. intermedius*, *B. tessmanni*, and *B. tholloni*, thereby restricting membership of *Brycinus* to the *macrolepidotus* and *nurse* groups. The monophyly of

Brycinus as so restricted, was supported by Zanata and Vari (2005) based on four non-exclusive morphological synapomorphies: four teeth in the outer row of each premaxilla (reversed to five or six in some members of the *macrolepidotus* group, or reduced to three in some members of the nurse group); circuli oriented posteriorly over the scales and relatively straight or slightly inclined towards the horizontal midline of each scale (widespread among alestids); deep-lying midlateral stripe extending from midbody to the caudal peduncle (a heterogeneous feature, reversed in numerous species); development of a median anal-fin lobe in males formed by the relative elongation of the fifth to eighth anal-fin rays (reversed in macrolepidotus group, and present in numerous alestid genera). However, subsequent molecular studies (Calcagnotto et al., 2005; Arroyave and Stiassny, 2011; present study) resolve Brycinus (exclusive of Bryconalestes) into two, distantly related clades, suggesting the likely homoplastic nature of these and other characters thought to support the monophyly of Brycinus (Murray and Stewart, 2002; Hubert et al., 2005). The first clade corresponds to the *macrolepidotus* group and includes the type species of the genus, *B. macrolepi*dotus, and B. brevis, B. carmesinus, B. grandisquamis, B. poptae, B. rhodopleura, and B. schoutedeni, and is restricted here as Brycinus. The second, corresponding to Paugy's nurse group, is herein assigned to Brachyalestes with B. nurse as the type species, and additionally including B. abeli, B. affinis, B. bimaculatus, B. carolinae, B. comptus, B. epuluensis, B. ferox, B. fwaensis, B. humilis, B. imberi, B. jacksonii, B. kingsleyae, B. lateralis, B. minutus, B. nigricauda, B. opisthotaenia, B. peringueyi, B. sadleri, and B. taeniurus.

<sup>&</sup>lt;sup>1</sup> Department of Ichthyology, American Museum of Natural History, 200 Central Park West, New York, New York 10024; ORCID: (MLJS) 0000-0001-8220-4768; and (BFM) 0000-0002-0499-567X; Email: (MLJS) mljs@amnh.org; (CK) coop.keane@gmail.com; and (BFM) bmelo@amnh.org. Send correspondence to BFM.

<sup>&</sup>lt;sup>2</sup> Département de Biologie, Faculté des Sciences, Université de Kinshasa, B.P. 190 Kin XI, Kinshasa, République Démocratique de Congo; ORCID: 0009-0001-7666-1820; Email: jjmbimbishambuyi@gmail.com.

Submitted: 17 May 2023. Accepted: 8 September 2023. Associate Editor: R. E. Reis.

<sup>© 2023</sup> by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/i2023033 Published online: 22 November 2023

Species	Catalog number	Tissue code	Locality
Alestes inferus	AMNH 242137	AMCC 252818	Mpozo River, Bas Congo, Kongo Central, DRC
Alestopetersius leopoldianus	AMNH 269850	AMCC 253460	Fimi River, Bandundu Province, DRC
Bathyaethiops caudomaculatus	AMNH 258018	AMCC 220996	Mai Ndombe, Bandundu Province, DRC
Brachyalestes bimaculatus	AMNH 242467	AMCC 269183	Lac Ikenge, Bandundu Province, DRC
Brachyalestes bimaculatus	AMNH 242469	AMCC 269265	Lac Ilungu, Bandundu Province, DRC
Brachyalestes comptus	AMNH 250111	AMCC 257155	Luozi, Congo River, Bas Congo, Kongo Central, DRC
Brachyalestes imberi	AMNH 258959	AMCC 212044	Komati River, Mpumalanga, South Africa
Brachyalestes jackiae	AMNH 251302	AMCC 254786	Lulua River, Kasai Central, DRC
Brachyalestes kingsleyae	AMNH 262921	AMCC 205344	Ogooue-Ivindo, Gabon
Brachyalestes lateralis	AMNH 259432	AMCC 213056	Mulungushi River, Zambia
Brachyalestes nurse	AMNH 254138	AMCC 226340	Conakry, Litoral Province, Guinea
Brachyalestes opisthotaenia	AMNH 271745	AMCC 257973	Rio Mbia, Litoral, Equatorial Guinea
Brycinus grandisquamis	AMNH 263305	AMCC 227411	Congo River, Boma, Bas Congo, Kongo Central, DRC
Brycinus macrolepidotus	AMNH 250128	AMCC 257153	Congo River, Luozi Region, Kongo Central, DRC
Bryconalestes longipinnis	AMNH 261781	AMCC 221615	Kindia, Guinea
Hepsetus odoe	AMNH 249532	AMCC 257613	Lokoundje River, Kribi, Cameroon
Hydrocynus goliath	AMNH 239463	AMCC 221086	Congo River, Brazzavile, Rep. Congo
Micralestes acutidens	AMNH 241049	AMCC 313059	Congo River, Bulu, Bas Congo, DRC
Nannopetersius ansorgii	AMNH 258412	AMCC 211560	Lake Youbi, Kouilou, Rep. Congo
Phenacogrammus interruptus	AMNH 254757	AMCC 235942	N'Sele River, Kinshasa, DRC
Rhabdalestes rhodesiensis	AMNH 215603	DNA-BM262	Kataba, Western Province, Zambia

**Table 1.** List of species, voucher numbers, and locality information of specimens used for the molecular analysis.AMCC = Ambrose Monell CryoCollection.DRC = Democratic Republic of Congo.

In addition to resolution of generic assignments and intergeneric relationships of Brycinus s.l., motivation for the current study is the discovery of a putatively undescribed species of Brachyalestes from high elevation tributaries of the middle Lulua River (Kasai ecoregion, Congo basin, Democratic Republic of Congo). The Lulua River, a large right bank tributary of the Kasai River, is recognized as one of the most species-rich communities within the entire Congo basin (Mbimbi Mayi Munene et al., 2021; Liyandja and Stiassny, 2023), and notably more than 14% of species recorded from the system are found exclusively in tributaries and have yet to be reported from the main channel. Among these are numerous specimens initially identified as Brycinus kingsleyae (Mbimbi Mayi Munene et al., 2021); however, closer examination suggests that similarity with *B. kingsleyae* is superficial, and that the Lulua specimens likely represent an undescribed species. As no investigation of the composition and relationships among Brycinus s.l. of westcentral Africa has been undertaken, we investigate the status and phylogenetic relationships of the Lulua specimens within Brachyalestes with a focus on Central Congo basin taxa, using phylogenomic methods and morphological comparisons to formally describe and place the new species. Additionally, we provide museum-based evidence clarifying the geographical distribution of *B. bimaculatus* and *B. kingsleyae* in west-central Africa and discuss a time-calibrated phylogeny and biogeographical implications of the new species of Brachyalestes endemic to upland tributaries in the Kasai basin.

## MATERIALS AND METHODS

**Morphological approach.**—Counts and measurements follow Stiassny et al. (2021) and Melo and Stiassny (2022). Point-to-point linear distances were measured using digital calipers with a precision of 0.1 mm. In the description, an asterisk designates the value for the holotype, and parentheses indicate the number of examined specimens for a given count. Radiographs

were obtained for the holotype, paratypes, and representative congeners and related genera. Fused PU1+U1 centra are counted as a single element, and Weberian vertebrae are counted as four elements. Two adult specimens (AMNH 251301: 70 mm SL, 80 mm SL) and numerous comparative materials (see Data Accessibility) were µCT scanned at the Microscopy and Imaging Facility at AMNH using a GE Phoenix v|tome|x with a 240 kV Nano Tube (General Electric, Fairfield, CT) with resolution ranging from 15.7 to 25.2 µm, with beam energy set at 110 kV and 166 mA. Scans were reconstructed using Phoe-nix datos|x (General Electric, Wunstorf, Germany) and rendered using VGStudio Max 3.5.1 (Volume Graphics, Heidelberg, Germany). Stomach and intestinal contents of three specimens of the new species (AMNH 251301, 1 ex., AMNH 252702, 2 ex.) were identified by entomologist David Grimaldi (AMNH). Abbreviations follow Sabaj (2020) and are: Ambrose Monell Cryo Collection, New York (AMCC/ AMNH); American Museum of Natural History, New York (AMNH), Cornell University Museum of Vertebrates, Ithaca (CUMV), Field Museum of Natural History, Chicago (FMNH), and Royal Museum for Central Africa (RMCA). Standard length (SL), head length (HL), radiographed specimen(s) (RD), µCT scanned specimen(s) (CT).

*Molecular phylogenetics and time-calibration analysis.*—Prior knowledge of alestid relationships based on molecular data (Arroyave and Stiassny, 2011; Melo and Stiassny, unpubl. data) and the most recent revisional study of *Brycinus* (Paugy, 1986) guided the selection of taxa for a molecular analysis aimed at resolving the status and relationships of the putatively new *Brachyalestes* from the Lulua drainage (Table 1). Sequences were generated as part of an ongoing phylogenomic analysis of African characiforms (Melo and Stiassny, unpubl. data), and ultraconserved elements (UCEs; Faircloth et al., 2012) of two species of *Brycinus* s.s. and eight

*Brachyalestes* (with a focus on Congo basin taxa) were sequenced. Representatives of major alestid clades were included in the analysis in order to interrogate the monophyly of *Brycinus* and estimate the time of divergence of the Lulua species (Table 1). DNA was extracted using DNeasy tissue kit (Qiagen Inc., Germantown, MD) with concentration ranging from 5 to 100 ng/µl. The sample of the Lulua species of *Brachyalestes* (AMNH 251302, tissue AMCC-254786) had 37.1 ng/µl. Detailed protocols for UCE-phylogenomics are as in recent publications for characiform fishes (Mateussi et al., 2020; Melo et al., 2022). Using the ostariophysan probe set including 2,708 ultraconserved element loci (Faircloth et al., 2020), genomic libraries were quantified and enriched using the MYbaits Target Enrichment system (Arbor Biosciences, Ann Arbor, MI).

The UCE package PHYLUCE (Faircloth, 2015) with Illumiprocessor was used for adapter cleaning, filtering, and locating UCE loci for alignment. The contigs were aligned using MUS-CLE (Edgar, 2004) in two distinct matrices accounting for different amounts of missing data: a 50% complete matrix using loci present in at least 50% of taxa, and a 70% complete matrix using loci present in at least 70% of taxa. Maximum likelihood was utilized to determine the identity and phylogenetic position of the new species. RAxML v8.2.11 (Stamatakis, 2014) ran ten maximum likelihood inferences using the GTRGAMMA model and ten distinct randomized maximum parsimony trees on the initial alignment; it also ran 1000 non-parametric bootstrap inferences via the autoMRE function.

We estimated the best-fit models of evolution for each UCE partition using ModelFinder (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017) within IQ-TREE v2.2.2.6 (Minh et al., 2020). The 50% complete matrix used 1030 partitions and the 70% complete matrix used 480 partitions. Then, IQ-TREE v2.2.2.6 applied distinct models for each partition, estimated a maximum likelihood tree, and generated 1000 samples for ultrafast bootstrap (log-likelihood of consensus tree: -606812.138). Potential problems involving cross-contamination of specimens, tissues, or DNA were evaluated by the position of terminals in the larger phylogenomic dataset of Alestidae (Melo and Stiassny, unpubl. data) and compared with our current understanding of the systematics of the group. Terminals with relatively long branches resulted primarily from the low coverage of sequencing or degraded samples in ML analyses and were removed from downstream analyses.

Estimates of divergence times were conducted to investigate potentially impactful biogeographic events associated with the establishment of species of Brachyalestes in upland habitats of the Kasai basin. Two fossil-based priors and one root constraint were used to calibrate the UCE tree. The first primary calibration is represented by the earliest reported alestid teeth from the Ager basin (Eocene 54-49 Ma) of Spain (de la Peña Zarzuelo, 1996). Similarities between the fossil dentition and that of modern Alestes, Brycinus s.s., Brachyalestes, and Bryconaethiops (Melo and Stiassny, 2022) necessitated a conservative placement at the most recent common ancestor (MRCA) at the node including all sampled alestids (mean = 51.9; sigma = 2.0; 95-5% quantiles: 55.2-48.6). The second primary calibration corresponds to the highly distinctive, earliest fossil teeth of Hydrocynus, from the Dur At-Talah deposit (Eocene 48.6-33.9 Ma) of Libya (Otero et al., 2015). In accordance with previous morphological and molecular phylogenies (Murray and Stewart, 2002; Arroyave and Stiassny, 2011) and our ongoing research, Hydrocynus is resolved as sister to Alestes; we

therefore assign this calibration to the node separating *Hydrocynus* and *Alestes* (mean = 44.5; sigma = 2.0; 95–5% quantiles: 47.8–41.2). Finally, a secondary calibration is applied as a root constraint reflecting the estimated divergence period between Alestidae and Hepsetidae in the Late Cretaceous at around 82 Ma (104–65 Ma, 95% highest posterior density, HPD; mean = 82.2; sigma = 10.0; 95–5% quantiles: 98.6–65.8; Melo et al., 2022). Calibration priors are available in the .xml input file (see Data Accessibility).

In Beauti v2.6.3 (Bouckaert et al., 2019), we used the 70% complete matrix with 21 terminals (119,993 bp) and applied the GTR+G+I site model, the birth-death tree prior, a fixed 70% complete ML tree, and a relaxed lognormal clock model (Drummond et al., 2006). We performed two independent BEAST v2.4.8 (Bouckaert et al., 2014) runs of 100 million generations each (total of 200 million generations), saving one tree every 10,000 generations. Tracer v1.6 (Bouckaert et al., 2014) was used to examine log scores, effective sample size (ESS > 200), and checked for stationary convergence. LogCombiner v2.6.3 (Bouckaert et al., 2014) combined the 20,002 trees, and TreeAnnotator v1.8.2 used the last 18,002 trees (burn-in: 10%) to generate the maximum clade credibility tree. Topologies are available in Supplementary Material (see Data Accessibility).

## RESULTS

Maximum likelihood analyses of ultraconserved elements provide strong support for two distantly related clades within *Brycinus* s.l., the first formed of representatives of *Brycinus* s.s., and the second composed of all sampled representatives of *Brachyalestes* (Fig. 1). Although with limited representation of taxa, this division is also supported by the molecular phylogenies of Calcagnotto et al. (2005) and Arroyave and Stiassny (2011). Phylogenetic relationships within *Brachyalestes* are described in detail below (see Phylogenetic relationships).

Morphological comparisons across Alestidae reveal a suite of derived character states shared among species of *Brycinus*. Unfortunately, such is not the case for *Brachyalestes*; however, in addition to strong molecular support for that clade (100%), we provide a simple, unique combination of two morphological traits that readily distinguish *Brachyalestes* from all other alestid (and characiform) genera. While it is anticipated that an ongoing, more densely sampled study of the entire Alestidae and allied families (Melo and Stiassny, unpubl. data) will provide additional morphological character data supporting the monophyly of *Brachyalestes*, based on the combined evidence of genomic and morphological data and shared modifications among species of these clades, we restrict the genus *Brycinus* to *B. macrolepidotus* and related species, and formally revalidate *Brachyalestes*. The new composition of *Brachyalestes* is presented in Table 2.

### Brycinus Valenciennes, 1850

*Type species.*—*Brycinus macrolepidotus* Valenciennes, 1850. Type by monotypy.

*Included species.*—(9) *Brycinus brevis, Brycinus carmesinus, Brycinus grandisquamis, Brycinus leuciscus, Brycinus luteus, Brycinus macrolepidotus, Brycinus poptae, Brycinus rhodopleura,* and *Brycinus schoutedeni.* 



Fig. 1. Maximum likelihood tree of *Brachyalestes* and related alestid genera based on a 70% complete dataset of 481 ultraconserved element loci (119,993 bp). *Brachyalestes jackiae* highlighted in red. Photographs: J. Cutler, J. Mbimbi, M. Stiassny, R. Palmer, and South African Institute for Aquatic Biodiversity (used with permission).

**Remarks.**—Zanata and Vari (2005: fig. 43) resolved two representatives of *Brycinus* (*B. macrolepidotus* and *B. brevis*) as a subclade nested within *Brycinus* s.l. and proposed two synapomorphies for that subclade. The first is the absence of a

supraneural anterior to the fourth Weberian vertebral neural spine (e.g., Fig. 2A vs. Fig. 3A). However, our own examination across Alestidae reveals a far more widespread occurrence of the loss of an anteriorly located supraneural than

Table 2.	List of species	previously	assigned to Br	<i>ycinus</i> and n	ew arrangement into	<b>Brachyalestes</b>	based on the	present study.
----------	-----------------	------------	----------------	---------------------	---------------------	----------------------	--------------	----------------

Species under the prior concept of Brycinus	New arrangement
Brycinus abeli (Fowler 1936)	Brachyalestes abeli (Fowler 1936)
Brycinus affinis (Günther 1894)	Brachyalestes affinis (Günther 1894)
Brycinus bimaculatus (Boulenger 1899)	Brachyalestes bimaculatus (Boulenger 1899)
Brycinus carolinae (Paugy and Lévêque 1981)	Brachyalestes carolinae (Paugy and Lévêque 1981)
Brycinus comptus (Roberts and Stewart 1976)	Brachyalestes comptus (Roberts and Stewart 1976)
Brycinus epuluensis Decru, Vreven, Sadio, and Snoeks 2016	Brachyalestes epuluensis (Decru, Vreven, Sadio, and Snoeks 2016)
Brycinus ferox (Hopson and Hopson 1982)	Brachyalestes ferox (Hopson and Hopson 1982)
Brycinus fwaensis Géry 1995	Brachyalestes fwaensis (Géry 1995)
Brycinus humilis (Boulenger 1905)	Brachyalestes humilis (Boulenger 1905)
Brycinus imberi (Peters 1852)	Brachyalestes imberi (Peters 1852)
Brycinus jacksonii (Boulenger 1912)	Brachyalestes jacksonii (Boulenger 1912)
Brycinus kingsleyae (Günther 1896)	Brachyalestes kingsleyae (Günther 1896)
Brycinus lateralis (Boulenger 1900)	Brachyalestes lateralis (Boulenger 1900)
Brycinus minutus (Hopson and Hopson 1982)	Brachyalestes minutus (Hopson and Hopson 1982)
Brycinus nigricauda (Thys van den Audenaerde 1974)	Brachyalestes nigricauda (Thys van den Audenaerde 1974)
Brycinus nurse (Rüppell 1832)	Brachyalestes nurse (Rüppell 1832)
Brycinus opisthotaenia (Boulenger 1903)	Brachyalestes opisthotaenia (Boulenger 1903)
Brycinus perinqueyi (Boulenger 1923)	Brachyalestes perinqueyi (Boulenger 1923)
Brycinus sadleri (Boulenger 1906)	Brachyalestes sadleri (Boulenger 1906)
Brycinus taeniurus (Günther 1867)	Brachyalestes taeniurus (Günther 1867)



**Fig. 2.** Brycinus macrolepidotus, AMNH 270775. (A) Posterior neurocranium, Weberian apparatus, predorsal vertebrae, and associated structures (lateral view). (B) Neurocranium, premaxillae, and circumorbitals (dorsal view). (C) Isolated maxilla in lateral (left) and dorsal (right) views.

suggested by Zanata and Vari (2005). As a result, the utility of this feature as diagnostic for Brycinus s.s. requires further investigation in a phylogenetic context, across all alestid lineages (Melo and Stiassny, unpubl. data). The second derived feature recognized by Zanata and Vari (2005), and corroborated here, is the loss of a sexually dimorphic anal fin. Some degree of sexual dimorphism in finnage, particularly of the anal fin, is pervasive among Alestidae, and the complete loss of such dimorphism appears to be found exclusively in Brycinus. Another apomorphic condition, identified here for the first time, is the presence of a characteristic expansion of the dorsal surface of the maxillary head (Fig. 2C). While considerable variation in the morphology of the maxilla and maxillary head is found within Alestidae (Melo and Stiassny, unpubl. data), the presence of such a broad, spoon-shaped dorsal expansion is not present in any other alestid or, as far as we can assess, any other characiform. An additional derived feature recognized here is a broad, laterally expanded nasal bone present in all Brycinus; a similar expansion is not present in any other taxon examined (see Fig. 2B vs. Fig. 3B-E).

#### Brachyalestes Günther, 1864

*Type species.—Brachyalestes nurse* (Günther, 1864). Type by subsequent designation.

Included species.—(21) Brachyalestes abeli, Brachyalestes affinis, Brachyalestes bimaculatus, Brachyalestes carolinae, Brachyalestes comptus, Brachyalestes epuluensis, Brachyalestes ferox, Brachyalestes fwaensis, Brachyalestes humilis, Brachyalestes imberi, Brachyalestes jackiae, new species, Brachyalestes jacksonii, Brachyalestes kingsleyae, Brachyalestes lateralis, Brachyalestes minutus, Brachyalestes nigricauda, Brachyalestes nurse, Brachyalestes opisthotaenia, Brachyalestes peringueyi, Brachyalestes sadleri, and Brachyalestes taeniurus.

**Remarks.**—Although we have yet to find uniquely derived features diagnostic for *Brachyalestes*, in the absence of the

synapomorphies of Brycinus, a simple combination of two morphological features serves to differentiate members of the genus from all other alestid and characiform genera. The first is the presence of 6–10 molariform inner row premaxillary teeth (3-5 teeth on each side); among Alestidae multiple molariform premaxillary teeth are also present in Alestes, Bryconaethiops, and Brycinus, and are present but somewhat reduced in *Bryconalestes*. The second is the cranial fontanel, which, when present, is usually restricted to the parietal-supraoccipital region; it never extends anterior to the epiphyseal bar, even in juveniles, and thus an anterior frontal fontanel is never present in Brachyalestes (Fig. 3B-D vs. Fig. 3E). The absence of Brycinus synapomorphies and the combination of these two features (presence of multiple molariform premaxillary teeth, and the absence of an anterior frontal fontanel) readily serve to distinguish Brachyalestes from all other alestid genera.

#### Brachyalestes jackiae Stiassny and Melo, new species

urn:lsid:zoobank.org:act:6D67A7C4-5749-4256-8454-F02942DFC5E2 Figures 4–7, Table 3

*Brycinus kingsleyae.*—Mbimbi Mayi Munene et al., 2021: 16 (list of species; Lulua basin).

*Holotype.*—AMNH 253243, 105.8 mm SL, RD, male, Democratic Republic of Congo, Kasai Central, Mutefu, Tshimayi River, Lulua river, Kasai system, Congo basin, -6°11'37.5714", 22°44'23.712", J. J. Mbimbi Mayi Munene, 29 January 2010.

Paratypes.--All from tributaries of the Lulua River, Kasai Central Province, Democratic Republic of Congo: AMNH 251302, 2, RD, 91.6-97.1 mm SL, Bampanya, Lunyenga River, -6°7'28.272", 22°31'34.896", J. J. Mbimbi Mayi Munene, 2009; AMNH 252702, 8, 68.8-76.4 mm SL, Dijiba, Moyo River, -6°11'14.0994", 22°29'4.3794", J. J. Mbimbi Mayi Munene, 15 July 2010; AMNH 252787, 2, RD, 102.6-113.8 mm SL, Kalumba, Lubi River, -5°59'43.5006", 22°33'0.255", J. J. Mbimbi Mayi Munene, 28 January 2010; AMNH 277628, 15, 2 CT, 59.4-96.1 mm SL, Bampanya, Lunyenga River, -6°7'28.272", 22°31'34.896", J. J. Mbimbi Mayi Munene, 2009; (RMCA) BE\_RMCA\_Vert.2023.015.P.0001-0002, 2, 83.4–96.4 mm SL, Bampanya, Lunyenga River, -6°7'28.272", 22°31′34.896″, J. J. Mbimbi Mayi Munene, 2009; CUMV 10028, 2, 91.8-94.6 mm SL, Dijiba, Moyo River, -6°11'14.0994", 22°29'4.3794", J. J. Mbimbi Mayi Munene, 15 July 2010; FMNH 150030, 2, 83.0-86.2 mm SL, Dijiba, Moyo River, -6°11'14.0994", 22°29'4.3794", J. J. Mbimbi Mayi Munene, 15 July 2010; OS 26722, 2, 89.9–91.9 mm SL, Dijiba, Moyo River, -6°11'14.0994", 22°29'4.3794", J. J. Mbimbi Mayi Munene, 15 July 2010.

Differential diagnosis.—Brachyalestes jackiae is readily distinguished from congeners other than *B. carolinae*, *B. epuluen*sis, *B. jacksonii*, *B. kingsleyae*, *B. lateralis*, *B. opisthotaenia*, *B.* peringueyi, and *B. taeniurus* by the presence of a conspicuous midlateral stripe (vs. pigmentation restricted to a caudalpeduncle spot or blotches). It differs from *B. carolinae*, *B.* epuluensis, *B. jacksonii*, *B. lateralis*, and *B. taeniurus* in the form of the midlateral stripe, which is a diffuse narrow (*B. jacksonii*, *B. lateralis*, and *B. taeniurus*) or broad (*B. carolinae* and *B. epuluensis*), straight band of dark pigmentation



Fig. 3. (A) Alestes liebrechtsii, AMNH 254612, posterior neurocranium, Weberian apparatus, predorsal vertebrae, and associated structures (lateral view); (B) Alestes liebrechtsii, AMNH 254612, neurocranium, premaxillae, and circumorbitals (dorsal view); (C) Bryconaethiops microstoma, AMNH 253823; (D) Bryconalestes longipinnis, AMNH 59626; (E) Brachyalestes nurse, AMNH 215629.

passing from the caudal blotch to midbody or extending to the opercle (Fig. 8A). In contrast, the midlateral stripe of *B. jackiae*, *B. kingsleyae*, *B. opisthotaenia*, and *B. peringueyi* is strongly demarcated and distinctively club-shaped anteriorly (Figs. 4A, B, 8B, C). *Brachyalestes jackiae* differs from *B. kingsleyae* in the presence of an anterior extension of the club-shaped midlateral stripe in males (vs. terminal club restricted to below the level of the dorsal fin in both males and females), lack of humeral spot (vs. presence), 21–23 lateral line scales (vs. 24–30), and caudal peduncle depth 9.0– 10.6% of SL (vs. 11.4–15.1% of SL). In addition to the absence of an anterior extension of a club-shaped midlateral stripe in males and the absence of a humeral spot, *B. jackiae* differs further from *B. opisthotaenia* and *B. peringueyi* in possessing far fewer pored lateral line scales (21–23 vs. 27– 32 and 29–30, respectively). *Brachyalestes jackiae* is readily distinguished from its sister species, *B. bimaculatus* (Fig. 8D, E), by the presence of a dark club-shaped midlateral stripe (vs. absence) and absence of dark blotches (vs. presence of first blotch midlaterally located on the flank and the second on the caudal peduncle).

**Description.**—Morphometric data summarized in Table 3. Medium-sized species (maximum observed size 113.8 mm SL) with general appearance as in Figure 4. Body elongate, moderately robust, particularly so in adult females. Dorsal head profile straight from snout to supraoccipital, convex to dorsal-fin origin, slightly concave along dorsal-fin base,



Fig. 4. Brachyalestes jackiae. (A) AMNH 253243, holotype, male, 105.8 mm SL, Mutefu, Tshimayi River, Lulua-Kasai system, Kasai Central, Democratic Republic of Congo. (B) AMNH 252787, paratype, female, 114.1 mm SL, Kalumba, Lubi River, Lulua, Kasai Central, Democratic Republic of Congo. Scale bars = 1 cm.

smoothly convex to origin of caudal fin. Ventral body profile smoothly convex to anal-fin origin, anal-fin base strongly convex, expanded and proximally scaled. Caudal peduncle longer than deep.

Adipose eyelid restricted to translucent band immediately anterior to orbit. Snout rounded, length equal to, or slightly shorter than, orbital diameter. Mouth terminal and horizontally aligned with center of orbit. Two rows of multicuspid premaxillary teeth (Fig. 5A). Outer row with 3 (1), 4\* (16), or 5 (3) stout, tricuspid teeth; inner row with 4\* (20) molariform teeth with 4-5 lingually oriented, cutting-edge cusps, and 2-3 molariform cusps on moderately developed buccal shelves. Fourth tooth elongate, laterally aligned with single molariform cusp on reduced buccal shelf. Contralateral premaxillae interdigitating medially with two strongly overlapping sutures. Maxilla edentulous, robust, with deeply bifurcated head (Fig. 5B). Dentary with 4\* (20) outer row teeth bearing five to seven buccally oriented cusps (Fig. 5C). Fourth tooth markedly smaller than others. One inner row pair of large, robust, strongly shouldered conical teeth at dental symphysis. Contralateral dentaries strongly interdigitating lingually.

Distal margin of dorsal fin rounded or slightly emarginate, iii,8\* (20) fin rays, first unbranched ray very short. Pectoral fin pointed or slightly rounded, adpressed fin tip reaching two or three scales short of vertical through pelvicfin origin, i,13 (5) or i,14\* (15) rays. Pelvic-fin rays i,8\* (17) or i,9 (3), adpressed fin tip reaching 2–3 scales short of analfin origin. Caudal fin weakly forked. Small adipose fin present. Anal-fin rays iii,11 (2), iii,12 (12), or iii,13\* (6); first ray very short. Anal fin straight or slightly emarginate in females, distal margin broadly rounded in males with middle branched rays elongated forming anal-fin lobe. Anal-fin base covered proximally by sheath of scales.

Body covered with large, regularly imbricated cycloid scales, circuli on exposed portions with horizontal orientation. Lateral line scales from supracleithrum to hypural joint 21 (1), 22 (5), or 23\* (13), with 2\* (16) or 3 (4) scales extending over caudal-fin base. Anteriormost 3–4 scales of lateral line descending steeply to below midlateral body plane. Scales in transverse series from lateral line to dorsal-fin origin 4.5\* (20). Scales in transverse series from lateral line to pelvic-fin insertion 2 (1) or 2.5\* (19). Middorsal series from tip of supraoccipital to dorsal-fin origin 7 (1), 8 (4), 9\* (13), or 10 (2). Circumpeduncular scales 9 (1) or  $10^*$  (19).

Supraneurals  $7^*$  (4) or 8 (2), first supraneural associated with fourth Weberian vertebral spine (Fig. 6A); predorsal vertebrae 7 (6); precaudal vertebrae 17 (5) or 18 (1); total vertebrae 36 (2), 37 (2), or 38\* (2).



Fig. 5. Brachyalestes jackiae, AMNH 277628. (A) Premaxillae and dentition (ventral view); (B) isolated maxilla in lateral (left) and dorsal (right) views; (C) lower jaw (dorsal view); (D) circumorbital series.

**Osteological features.**—Circumorbital series complete, forming uninterrupted ring around orbit (Fig. 5D). Supraorbital elongate, strongly sigmoid anteriorly, ventral process present, articulating with dorsal face of lateral ethmoid. Antorbital in contact with supraorbital posteriorly, overlapping first infraorbital anteriorly. Three pairs of posttemporal fossae present (Fig. 6B). Median posttemporal fossae located entirely within epioccipitals. Dorsal posttemporal fossae well developed, bounded by supraoccipital, parietals, and epioccipitals; dorsal fossae slightly smaller than ventral posttemporal fossae, bounded by epioccipitals and pterotics. Exoccipital foramen slightly smaller than foramen magnum. Presence of small parietal–supraoccipital fontanel correlated with size (Fig. 6C; 70 mm SL), completely absent in larger individuals (Fig. 6D; 80 mm SL). Well-developed, prong-like pterotic processes.

**Color in alcohol.**—Ground coloration yellowish brown. Snout, dorsal portion of head, opercle, and body above and including lateral line markedly darker than yellowish pale ventrum. Deep-lying, club-shaped black stripe along midlateral surface from vertical through rear of dorsal-fin base to median caudal-fin rays in females and juveniles (Fig. 4B). Broad dark stripe from cheek connecting to midbody stripe in males, attenuating at level of anal fin and over caudal peduncle, terminating at median caudal-fin rays (Fig. 4A). Dorsal, caudal, and anal fins outlined by small, dark chromatophores. First rays of pectoral and pelvic fins with scattered dark



Fig. 6. Brachyalestes jackiae, AMNH 277628. (A) Posterior neurocranium, Weberian apparatus, predorsal vertebrae, and associated structures (lateral view); (B) neurocranium (posterior view); (C) neurocranium (dorsal view), 70.9 mm SL; (D) posterior neurocranium (dorsal view), 79.8 mm SL.

chromatophores. Adipose fin with small chromatophores concentrated along distal margins.

**Color in life.**—Overall silvery iridescence. Fins generally orange/yellow. Green iridescent pigments overlying midbody scales, partially obscuring anterior extension of deep lying club-shaped midlateral stripe of male specimens. Darker pigmentation over basal portions of anal and caudal fins in males (Fig. 7A, B).

*Sexual dimorphism.*—Males of *Brachyalestes jackiae* differ from females in the possession of a conspicuous (in preservation), broad, dark band that extends from club-shaped midlateral stripe to the snout. Males also have an elongation of branched anal-fin rays forming a distinct anal-fin lobe, bordered proximally by 2–3 scale rows over the anal-fin base (Figs. 4A, 7A), while females have a dark club-shaped stripe confined to the posterior midbody region and straight analfin margin, with a single scale row along the anal-fin base (Figs. 4B, 7B).

*Distribution.*—*Brachyalestes jackiae* is abundant in forested tributaries of the Lulua River (Lubi, Luna, Lunyenga, Mbuyi, Moyo, and Tshimayi) of the Congo basin, Democratic Republic of Congo (Fig. 9A). However, due to inaccessibility much



Fig. 7. Brachyalestes jackiae, live coloration: (A) male, (B) female, (C) digestive tract (slightly unraveled for clearer depiction of morphology), after removal of liver, pancreas, gallbladder, spleen, and adherent tissues.

Table 3.	Morphometric data	for Brachyalestes jackiae.	Range includes holotype and	19 paratypes. SD = standard	deviation.
----------	-------------------	----------------------------	-----------------------------	-----------------------------	------------

	Holotype	п	Mean	Range	SD
Standard length (mm)	105.8	20		62.6-113.8	_
% Standard length					
Greatest body depth	31.6	20	31.9	28.7-36.8	2.2
Snout to dorsal-fin origin	51.3	20	53.6	51.1-55.7	1.4
Snout to pectoral-fin origin	26.9	20	28.1	26.6-30.9	1.1
Snout to pelvic-fin origin	50.9	20	54.5	50.9-58.0	2.1
Snout to anal-fin origin	74.7	20	78.9	74.7-85.0	2.7
Eye to dorsal-fin origin	39.7	20	41.0	38.6-43.1	1.4
Dorsal-fin origin to caudal-fin origin	52.8	20	49.9	47.4-52.8	1.6
Peduncle depth	9.8	20	10.0	9.0-10.6	0.4
Peduncle length	10.8	20	11.3	9.3-14.3	1.3
Pectoral-fin length	19.7	20	21.1	17.5-23.4	1.4
Pelvic-fin length	16.4	20	17.5	14.9-19.3	0.9
Dorsal-fin length	21.5	20	23.1	19.6-25.7	1.3
Dorsal-fin base	13.0	20	11.7	10.6-13.0	0.7
Anal-fin length	13.5	20	13.1	10.4-15.5	1.4
Anal-fin base	17.9	20	14.4	12.0-18.4	2.0
Head length	27.2	20	28.9	27.2-30.9	1.1
% Head length					
Head depth	66.3	20	63.1	59.3-67.5	2.7
Snout length	28.0	20	27.1	24.8-29.9	1.4
Orbital diameter	29.6	20	33.4	29.6-36.1	1.9
Upper jaw length	31.5	20	33.1	31.2-35.9	1.2
Interorbital width	41.2	20	38.1	34.3-42.1	1.9



**Fig. 8.** (A) *Brachyalestes epuluensis*, AMNH 5995, male, 79.7 mm SL, Avakubi, Ituri River, Democratic Republic of Congo (DRC); (B) *Brachyalestes kingsleyae*, AMNH 262921, male, 104.6 mm SL, Ivindo River, near Makokou, Ogooué-Ivindo, Gabon; (C) *Brachyalestes kingsleyae*, AMNH 262921, female, 104.0 mm SL, Ivindo River, Gabon; (D) *Brachyalestes bimaculatus*, AMNH 240760, male, 115.1 mm SL, confluence of Lofongo and Luilaka Rivers, Salonga National Park, DRC; (E) *Brachyalestes bimaculatus*, AMNH 242470, female, 102.7 mm SL, Lac Nkolentulu, Bandundu, DRC. Scale bars = 1 cm.



**Fig. 9.** (A) Map of the central Congo basin and Lower Guinea showing analyzed museum records of *Brachyalestes bimaculatus* (green diamonds), *B. jackiae* (red triangles), and *B. kingsleyae* (yellow circles); collection habitats at (B) Kamuandu, Lubi River, (C) Lunyenga River, (D) Tshimayi River, (E) Luna River. Photographs by José J. M. M. Mbimbi.

of the middle and upper Lulua River has been poorly sampled (Mbimbi Mayi Munene et al., 2021) and it is likely that additional collecting efforts will expand the range of this species.

Ecology.—Brachyalestes jackiae has been collected in tributaries of the Lulua River upstream of the city of Kananga (former Luluabourg) at elevations between 562 and 774 m asl. Each collection locality was bordered by gallery forest/vegetation with swift-flowing, relatively shallow (1-2 m) water flowing over rocks, gravel, and sandy substrates (Fig. 9B–E). Water temperatures ranged from 27°C to 30.5°C, and pH between 5.4 and 6.9 (Mbimbi Mayi Munene et al., 2021). The species appears to be primarily insectivorous, and digestive tract contents of three dissected specimens (AMNH 251301, 1 ex. and AMNH 252702, 2 ex.) contained insect remains of strictly allochthonous origin; no remains of aquatic insect taxa were present. Identified contents included remnants of three coleopteran species (including Chrysomelidae and Scarabaeidae), representatives of three or four hymenopteran genera (including Diapriidae and Camponotus), two lepidopteran, one isopteran worker, one dipteran (probably Dolichopodidae), one hemipteran, one heteropteran, one Auchenorrhyncha,

and one apterygote. The digestive tract (unraveled) is slightly shorter than SL, and consists of a short esophagus, a bulbous stomach encased by six stout pyloric caeca, and a simple, double-looped intestine (Fig. 7C).

*Etymology.—Brachyalestes jackiae* is named for Jackie Black, in recognition of her support and encouragement to MLJS and her Congolese colleagues over many years of work in the Congo basin.

Phylogenetic relationships and timing of diversification.—Our 50% complete matrix consisted of 1031 ultraconserved element loci with 226,210 bp, and analysis resulted in a maximum likelihood (ML) phylogeny with final score -618437.543946 and 50 bootstrap replicates (Fig. S1; see Data Accessibility). The 70% complete matrix consisted of 481 UCE loci with 119,993 bp, and analysis resulted in an ML phylogeny with final score -327188.008263 and 50 bootstrap replicates (Fig. 1). There was a single difference in the relationships using the 50% and 70% complete datasets: the sister clade of Alestopetersius + Nannopetersius was Phenacogrammus in the RAxML analysis (92% and 98%) or Bathyaethiops in the IQ-TREE analysis (99% and 96%). Likelihood analyses with each matrix and both RAxML and IQ-TREE returned identical relationships within Brachyalestes, with minor bootstrap differences supporting relationships between B. imberi and B. nurse (86% and 94%), and a "central Congo" node encompassing B. comptus, B. lateralis, B. jackiae, and B. bimaculatus (80% and 82%; Figs. S2, S3; see Data Accessibility). Downstream analyses utilized the 70% complete matrix. Table 4 contains information about reads for each taxon.

Among central Congo taxa sampled, *Brachyalestes lateralis* appears as sister to *B. comptus* (100%), and *B. bimaculatus* is strongly supported as sister to *B. jackiae* (100%; Fig. 1). This arrangement accords with an earlier multilocus phylogeny in placing *B. comptus* close to *B. lateralis* and *B. fwaensis* (not examined here), with *B. bimaculatus* resolved as sister to that clade (Arroyave and Stiassny, 2011).

The resulting timetree indicates a divergence of Hepsetidae and Alestidae during the Late Cretaceous (83 Ma; 101-67 Ma, 95% HPD), which corresponds to the prior distribution of the analysis based on previous estimates (Melo et al., 2022). Results indicate that the most recent common ancestor (MRCA) of Alestidae began to diversify during the Early Eocene (54 Ma; 57–50 Ma, 95% HPD; Fig. 10). During the Middle Eocene, Brachyalestes diverged from the MRCA of Bryconalestes, Bathyaethiops, Phenacogrammus, Alestopetersius, and Nannopetersius (44 Ma; 49-40 Ma, 95% HPD). The earliest divergence of the Brachyalestes occurred during the Oligocene-Miocene transition (22 Ma; 32-15 Ma, 95% HPD), separating B. nurse and B. imberi from the other species. The Lower Guinean B. opisthotaenia appears to have diverged approximately 14 Ma (20-9 Ma, 95% HPD), whereas B. kingsleyae appears around 11 Ma (16-7 Ma, 95% HPD) during the Late Miocene. The group containing the central Congolese species underwent diversification during the last 10 Ma. Brachyalestes comptus from the lower and middle Congo diverged from B. lateralis from the upper, middle Congo, and Zambezi around 6 Ma (10-3 Ma, 95% HPD), whereas B. jackiae from upland tributaries of the Lulua River diverged from B. bimaculatus from the lowland Cuvette Centrale around 7 Ma (11-4 Ma, 95% HPD).

Table 4.	Summary of ultraconserved	element (UCE)	statistics for each	analyzed specimen.
----------	---------------------------	---------------	---------------------	--------------------

Species/tissue number	UCE contigs	Total bp	Mean length	95 CI length	Min length	Max length	Median length	Contigs >1kb
Alestes inferus 33-328	1716	1102736	642.620047	6.85958874	101	1551	635	207
Alestopetersius leopoldianus 253460	1169	368086	314.872541	6.66457184	39	1269	231	6
Bathiaethiops caudomaculatus 220996	1206	348331	288.831675	4.8729202	45	923	253.5	0
Brachyalestes bimaculatus 269183	1072	300076	279.921642	6.52125741	35	1542	183.5	4
Brachyalestes bimaculatus 269265	1065	285925	268.474178	5.90411365	36	1001	192	1
Brachyalestes comptus 257155	1110	298127	268.582883	5.84750565	42	1085	201.5	6
Brachyalestes imberi 212044	1177	398998	338.995752	7.13988858	43	1270	263	15
Brachyalestes jackiae 254786	1139	361728	317.583845	6.89918383	39	1331	230	9
Brachyalestes kingsleyae 205344	1191	340038	285.506297	6.38745782	43	1723	189	8
Brachyalestes lateralis 213056	1206	426420	353.58209	7.11016032	39	1229	278	15
Brachyalestes nurse 226340	1129	319519	283.010629	6.16744658	35	1282	192	4
Brachyalestes opisthotaenia 257973	1146	348281	303.910122	6.9157757	41	1221	204.5	11
Brycinus grandisquamis 227411	1151	375820	326.516073	7.02288877	40	1341	246	13
Brycinus macrolepidotus 257153	1254	416806	332.38118	6.55360887	43	1770	261	9
Bryconalestes longipinnis 221615	1150	382728	332.806957	6.99946466	36	1295	254.5	6
Hepsetus odoe 257613	1100	402513	365.920909	7.98432506	36	1770	288.5	24
Hydrocynus goliath 221086	1193	387312	324.653814	7.00854609	36	1408	228	16
Micralestes acutidens 313059	1173	327299	279.02728	4.45134814	46	896	237	0
Nannopetersius ansorgii 211560	1240	410480	331.032258	6.37407889	38	1191	264	11
Phenacogrammus interruptus 235942	1143	318963	279.057743	5.93160816	43	1146	200	4
Rhabdalestes rhodesiensis 215603	1040	191694	184.321154	1.71883743	58	461	177	0

## DISCUSSION

Our study has begun resolution of relationships within Bra*chyalestes* by identifying, with strong support, the central Congolese species B. bimaculatus as sister to the newly described species, B. jackiae (Fig. 1). Two morphological synapomorphies support this sister group relationship: both species lack all trace of the humeral spot present in congeners, and adult males possess a broad, scaly sheath covering the proximal portion of the expanded anal-fin lobe (Figs. 4, 8). Within Brachyalestes, B. bimaculatus exhibits a unique pigmentation patterning consisting of two large blotches of dark pigmentation along the flank and caudal peduncle, one located midlaterally immediately below the dorsal-fin base and another over the caudal peduncle (Fig. 8D, E). Additionally, both species have a broad band of chromatophores extending throughout the midlateral portion of the body above lateral line, which is particularly evident in adult males (Figs. 4A, 8D). However, such a dark band over the midbody of males appears in several other species of Brachyalestes (Toham and Teugels, 1997; Paugy and Schaefer, 2007; Decru et al., 2016) and merits further examination in a phylogenetic context.

*Brachyalestes bimaculatus* and *B. jackiae* are allopatrically distributed (Fig. 9). *Brachyalestes bimaculatus*, originally described from Kutu and Lake Leopold II (Lake Mai-Ndombe; Boulenger, 1899), is distributed throughout the Cuvette Centrale of the middle Congo basin, including the tributaries Fimi (Mfimi), Likouala, Lulonga, Ruki, and Sangha (Fig. 9). This distribution suggests a predominance in lowland ecosystems of sediment-rich, large rivers, swamps, and lakes of the Cuvette Centrale of the middle Congo. In contrast, *B. jackiae* is endemic to tributaries (Lubi, Luna, Lunyenga, Mbuyi, Moyo, and Tshimayi Rivers) of the Lulua River (Fig. 9), suggesting an exclusively upland distribution

(above 560 m asl) in small stream habitats currently isolated from the Cuvette Centrale by a series of rapids and waterfalls along the Lulua River as it flows over the Katanga plateau (Mbimbi Mayi Munene et al., 2021).

Brachvalestes kingslevae, the species with which B. jackiae was originally confused, was described from two syntypes collected in the Ogooue River, Gabon (Günther, 1896). In his taxonomic revision of Brycinus s.l., Paugy (1986) reported Brachyalestes kingsleyae from Lower Guinea rivers (Sanaga, Nyong, and Ntem of Cameroon, Nyanga and Ogooue-Ivindo of Gabon, Loeme and Kouilou of the Republic of the Congo, and the Chiloango of the Cabinda province of Angola), but also considered the species to be present in upper sections of the Kasai, Oubangui-Uele, Lualaba, and Luapula-Mweru of the Congo basin. However, Paugy's list of examined material contained only specimens from the Loeme and Ogooue in Gabon and the Republic of the Congo (Paugy, 1986). Toham and Teugels (1997) redescribed B. kingsleyae and B. opisthotaenia based on material from Lower Guinea but, presumably following Paugy (1986), also reported B. kingsleyae as present in the Congo basin. However, based on our own examination of specimens from throughout the region (Supplementary Material; see Data Accessibility), we consider these Congo basin records likely to be the result of misidentifications and we consider Brachyalestes kingsleyae to be restricted to coastal and inland rivers of Lower Guinea from Cameroon to Cabinda (Fig. 9).

Widespread transcontinental uplifting and severe climatic shifts reorganized river networks across the African continent during the Miocene, and this epoch is broadly considered to be a key period for the diversification of a wide array of African fish families (Day et al., 2017, 2023; Bragança and Costa, 2019) including Alestidae (present data; Fig. 10). Following the climatic optimum warm/ humid phase of the Middle Miocene, the second half of the



**Fig. 10.** Time-calibrated phylogeny based on BEAST analyses of ultraconserved elements (70% complete matrix; 481 loci; 119,993 bp). Ma = million years ago; Pal = Paleocene; Olig = Oligocene; Pli = Pliocene; Q = Quaternary. See Data Accessibility for tree file.

Miocene experienced a global cooling trend accompanied by the expansion of arid biomes, including the formation of the Sahara Desert, followed by an extended period of climatic instability (Zachos et al., 2001; Brennan and Keogh, 2018; Bragança et al., 2021). While the geo-hydrological history of the central Congo basin is poorly understood, dynamic climatic and hydrological landscapes during the Late Miocene to Early Pliocene likely facilitated widespread allopatric speciation throughout the region. The current configuration of the Congo River is considered to have arisen around the time of the Miocene–Pliocene transition with the final capture of a large paleo-Congo Lake which is thought to have occupied much of the present-day Cuvette Centrale (Beadle, 1981; Stankiewicz and de Wit, 2006; Stiassny and Alter, 2021). Interestingly, Stiassny et al. (2021) have suggested that the Mfimi-Lukenie River represents the southern boundary of that paleo-Congo Lake, and this corresponds precisely with the present southern limit of the distribution of Brachyalestes bimaculatus. The dating analysis suggest a Late Miocene allopatric speciation between B. bimaculatus and B. jackiae, with one species remaining in lowland central Congo, and the other colonizing upland rivers of the Kasai system on the Katanga plateau; such a hypothesis can be tested with additional evidence from time-calibrated phylogenies for other Lulua River basin endemics.

The present study provides strong support for the restriction of *Brycinus* and provides a foundational phylogeny for *Brachyalestes*; however, many issues and questions remain. A phylogenetic analysis must include the remaining seven species of *Brycinus* and 13 species of *Brachyalestes*, as well as species of the remaining genera. A comprehensive study of the systematics, morphological evolution, and biogeographic reconstruction of the entire family Alestidae are the subject of ongoing research by two authors (MJLS and BFM) in an effort to provide a solid basis for our understanding of the diversification of characiform fishes across the entire African continent.

#### MATERIAL EXAMINED

Material Examined is available as supplemental material (see Data Accessibility).

## DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyology andherpetology.org/i2023033. Raw sequence data are available at NCBI BioProject PRJNA1033584. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License. ZooBank publication urn:lsid:zoo-bank.org:pub:AD53CF8D-5172-42AA-92C0-B6489AEC6B06.

## ACKNOWLEDGMENTS

Our thanks to the collections staff Radford Arrindell, Tom Vigliotta, and Chloe Lewis (AMNH), Caleb McMahan (FMNH), Casey Dillman (CUMV), Mariangeles Arce and Mark Sabaj (ANSP), Emmanuel Vreven (RMCA), and Brian Sidlauskas (OS) for specimen loans, tissues, and curatorial assistance. Claudio Oliveira (LBP) kindly provided additional computational resources and support. We also thank curator David A. Grimaldi (AMNH) for meticulously identifying insects from the digestive tracts of the new species. Financial support for this study was provided by the US National Science Foundation award #1655227 (MLJS), the AMNH Axelrod Research Curatorship (MLJS, JJMMM, BFM), and FAPESP #20/13433-6 (BFM), CNPq #200159/2020-8 (BFM), and the Science Research Mentoring Program (SRMP, AMNH) provided support for the participation of high school intern (CK).

# LITERATURE CITED

- **Arroyave, J., and M. L. J. Stiassny**. 2011. Phylogenetic relationships and the temporal context for the diversification of African characins of the family Alestidae (Ostariophysi: Characiformes): evidence from DNA sequence data. Molecular Phylogenetics and Evolution 60:385–397.
- **Beadle, L. C.** 1981. The Inland Waters of Tropical Africa: An Introduction to Tropical Limnology. Second edition. Longman, London.
- Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C. H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10:e1003537.
- Bouckaert, R., T. G. Vaughan, J. Barido-Sottani, S. Duchêne, M. Fourment, A. Gavryushkina, J. Heled, G. Jones, D. Kühnert, N. De Maio, and M. Matschiner. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology 15:e1006650.
- Boulenger, G. A. 1899. Matériaux pour la faune du Congo. Zoologie.–Série I. Poissons nouveaux du Congo. Quatrième Partie. Polyptères, Clupes, Mormyres, Characins. Annales du Musee du Congo (Ser. Zoology) v. 1 (fasc. 4):59–96, Pls. 30–39.
- **Bragança, P. H. N., and W. J. E. M. Costa.** 2019. Multigene fossil-calibrated analysis of the African lampeyes (Cyprinodontoidei: Procatopodidae) reveals an early Oligocene origin and Neogene diversification driven by palaeogeographic and palaeoclimatic events. Organisms Diversity and Evolution 19:303–320.
- Bragança, P. H. N., J. Van der Zee, A. Chakona, R. C. Schmidt, and M. L. J. Stiassny. 2021. Following the Mangroves: diversification in the banded lampeye *Aplocheilichthys spilauchen* (Dumeril, 1861) (Cyprinodontiformes: Procatopodidae) along the Atlantic coast of Africa. Hydrobiologia 848:1433–1453.

- Brennan, I. G., and J. S. Keogh. 2018. Miocene biome turnover drove conservative body size evolution across Australian vertebrates. Proceedings of the Royal Society B 285:20181474.
- **Calcagnotto**, **D.**, **S. A. Schaefer**, **and R. DeSalle**. 2005. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Molecular Phylogenetics and Evolution 36:135–153.
- Chernomor, O., A. Von Haeseler, and B. Q. Minh. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65:997–1008.
- Day, J. J., A. Fages, K. J. Brown, E. J. Vreven, M. L. J. Stiassny, R. Bills, J. P. Friel, and L. Rüber. 2017. Multiple independent colonizations into the Congo Basin during the continental radiation of African *Mastacembelus* spiny eels. Journal of Biogeography 44:2308–2318.
- Day, J. J., E. M. Steell, T. R. Vigliotta, L. A. Withey, R. Bills, J. P. Friel, M. J. Genner, and M. L. J. Stiassny. 2023. Exceptional levels of species discovery ameliorate inferences of the biogeography and diversification of an Afrotropical catfish family. Molecular Phylogenetics and Evolution 182:107754.
- de la Peña Zarzuelo, A. 1996. Characid teeth from the Lower Eocene of the Ager Basin (Lérida, Spain): paleobiogeographical comments. Copeia 1996:746–750.
- **Decru**, E., E. Vreven, O. Sadio, and J. Snoeks. 2016. *Brycinus epuluensis*, a new species from the Epulu River (Congo basin), Africa (Teleostei: Alestidae). Ichthyological Exploration of Freshwaters 27:49–60.
- Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4:e88.
- Edgar, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113.
- Faircloth, B. C. 2015. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32:786–788.
- Faircloth, B. C., F. Alda, K. Hoekzema, M. D. Burns, C. Oliveira, J. S. Albert, B. F. Melo, L. E. Ochoa, F. F. Roxo, P. Chakrabarty, B. L. Sidlauskas, and M. E. Alfaro. 2020. A target enrichment bait set for studying relationships among ostariophysan fishes. Copeia 108:47–60.
- Faircloth, B. C., J. E. McCormack, N. G. Crawford, M. G. Harvey, R. T. Brumfield, and T. C. Glenn. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Systematic Biology 61:717–726.
- Fricke, R., W. N. Eschmeyer, and J. D. Fong. 2022. Species by family/subfamily. http://research.calacademy.org/ research/ichthyology/catalog/SpeciesByFamily.asp
- Froese, R., and D. Pauly. 2019. Fish Base. World Wide Web Electronic Publication. https://www.fishbase.org
- **Günther**, A. 1896. XXXVII.—Report on a collection of reptiles and fishes made by Miss MH Kingsley during her travels on the Ogowe river and in old Calabar. Journal of Natural History 17:261–285.
- Hubert, N., C. Bonillo, and D. Paugy. 2005. Early divergence among the Alestidae (Teleostei, Ostariophyses, Characiformes): mitochondrial evidences and congruence with morphological data. Comptes Rendus Biologies 328:477–491.
- Kalyaanamoorthy, S., B. Q. Minh, T. K. Wong, A. Von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast

model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

- Liyandja, T. L. D., and M. L. J. Stiassny. 2023. Description of two new *Labeo* (Labeoninae; Cyprinidae) endemic to the Lulua River in the Democratic Republic of Congo (Kasai ecoregion); a hotspot of fish diversity in the Congo basin. American Museum Novitates 2023(3999):1–22.
- Mateussi, N. T., B. F. Melo, R. P. Ota, F. F. Roxo, L. E. Ochoa, F. Foresti, and C. Oliveira. 2020. Phylogenomics of the Neotropical fish family Serrasalmidae with a novel intrafamilial classification (Teleostei: Characiformes). Molecular Phylogenetics and Evolution 153:106945.
- Mbimbi Mayi Munene, J. J., M. L. J. Stiassny, R. J. Monsembula Iyaba, and T. L. Liyandja. 2021. Fishes of the lower Lulua River (Kasai Basin, Central Africa): a continental hotspot of ichthyofaunal diversity under threat. Diversity 13:341.
- Melo, B. F., B. L. Sidlauskas, T. J. Near, F. F. Roxo, A. Ghezelayagh, L. E. Ochoa, M. L. J. Stiassny, J. Arroyave, J. Chang, B. C. Faircloth, D. J. MacGuigan, R. C. Harrington, R. C. Benine, M. D. Burns ... C. Oliveira. 2022. Accelerated diversification explains the exceptional species richness of tropical characoid fishes. Systematic Biology 71:78–92.
- Melo, B. F., and M. L. J. Stiassny. 2022. Systematic review and cranial osteology of *Petersius* with redescription of *P. conserialis* (Teleostei: Alestidae) from the Rufiji and Ruvu Rivers of Tanzania. American Museum Novitates 2022(3992):1–16.
- Minh, B. Q., H. A.Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. Von Haeseler, and R. Lanfear. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37:1530–1534.
- Murray, A. M., and K. M. Stewart. 2002. Phylogenetic relationships of the African genera *Alestes* and *Brycinus* (Teleostei, Characiformes, Alestidae). Canadian Journal of Zoology 80:1887–1899.
- Otero, O., A. Pinton, H. Cappetta, S. Adnet, X. Valentin, M. Salem, and J.-J. Jaeger. 2015. A fish assemblage from the Middle Eocene from Libya (Dur At-Talah) and the earliest record of modern African fish genera. PLoS ONE 10: e0144358.

- Paugy, D. 1986. Révision systématique des Alestes et Brycinus africains (Pisces, Characidae). Editions de l'ORSTOM, Collections Etudes et Thèses, Paris.
- **Paugy, D., and S. A. Schaefer**. 2007. Alestidae, p. 347–411. *In*: The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa. M. L. J. Stiassny, G. G. Teugels, and C. D. Hopkins (eds.). IRD Editions, Paris.
- Sabaj, M. H. 2020. Codes for natural history collections in ichthyology and herpetology. Copeia 108:593–669.
- **Stamatakis**, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.
- Stankiewicz, J., and M. J. de Wit. 2006. A proposed drainage evolution model for Central Africa—did the Congo flow east? Journal of African Earth Sciences 44:75–84.
- Stiassny, M. L. J., and S. E. Alter. 2021. Evolution in the fast Lane: diversity, ecology, and speciation of cichlids in the lower Congo River, p. 107–133. *In*: The Behavior, Ecology and Evolution of Cichlid Fishes. Fish & Fisheries Series, Vol. 40. M. E. Abate and D. L. Noakes (eds.). Springer, Dordrecht.
- Stiassny, M. L. J., S. E. Alter, R. J. M. Iyaba, and T. L. Liyandja. 2021. Two new *Phenacogrammus* (Characoidei; Alestidae) from the Ndzaa River (Mfimi-Lukenie Basin) of Central Africa, Democratic Republic of Congo. American Museum Novitates 2021(3980):1–22.
- Stiassny, M. L. J., S. E. Alter, T. L. Liyandja, M. Y. Modimo, and R. J. M. Iyaba. 2021. Fishes of the Mfimi River in the central Congo basin of the Democratic Republic of Congo. Kasai ecoregion or part of the Cuvette Centrale? Check List 17:1681–1714.
- **Toham, A. K., and G. G. Teugels.** 1997. Redescription of two morphologically similar *Brycinus* species (Ostariophysi: Characidae) from Central Africa. Copeia 1997:363– 372.
- Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.
- Zanata, A. M., and R. P. Vari. 2005. The family Alestidae (Ostariophysi, Characiformes): a phylogenetic analysis of a trans-Atlantic clade. Zoological Journal of the Linnean Society 145:1–144.