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Research article

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Revision of the African cichlid fish genus *Ctenochromis* (Teleostei, Cichliformes), including a description of the new genus *Shuja* from Lake Tanganyika and the new species *Ctenochromis scatebra* from northern Tanzania

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[5] urn:lsid:zoobank.org:author:65B20206-1EA0-4768-965F-F45D36571E4C
[6] urn:lsid:zoobank.org:author:54B30AB7-E628-4A71-BDE8-8353C2E0E07F
[7] urn:lsid:zoobank.org:author:AEDC8D10-A40D-4C37-910D-8F564BA6E43E
[8] urn:lsid:zoobank.org:author:EEE277DC-D33D-4F24-8830-FF07DD514F9E

Abstract. Molecular phylogenetic evidence clearly resolves the African cichlid fish genus *Ctenochromis*, as defined by Greenwood (1979), as paraphyletic. Here, we redefine the genus *Ctenochromis* and assign *Ctenochromis horei*, a member of the Tropheini from Lake Tanganyika, to a new genus *Shuja* gen. nov. We restrict *Ctenochromis* to *Ctenochromis pectoralis* and *Ctenochromis scatebra* sp. nov., both of which are endemic to the Pangani River catchment in northern Tanzania, and are resolved as sister taxa in a phylogenetic analysis using genome-wide data. *Ctenochromis pectoralis* is the type species of the genus and described from specimens collected near Korogwe, Tanzania. The species was declared extinct in a 2016 IUCN Red List Assessment. We confirm the continued presence of a population of

C. pectoralis within the Ruvu tributary linking Lake Jipe to Nyumba ya Mungu Reservoir. The new taxon *Ctenochromis scatebra* sp. nov. is described from Chemka Springs, and recognised on the basis of differences from *C. pectoralis* in tooth and jaw morphology.

Keywords. Cichlidae, East Africa, endemic species, freshwater fish, haplochromine.

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Introduction

The haplochromine cichlid fish genus *Ctenochromis* Pfeffer, 1893 has been the source of some taxonomic confusion and nomenclatural instability since its inception, for example, in a recent review of the cichlid diversity of Lake Tanganyika, it is applied to two endemic species from different tribes (Ronco *et al.* 2020). The original generic description was based on two species, *Ctenochromis pectoralis* Pfeffer, 1893 and *Ctenochromis strigigena* Pfeffer, 1893. The latter was then synonymised with *Astatotilapia bloyeti* (Sauvage, 1883) by Regan (1922a), which remains the situation until today (Turner *et al.* 2021). While regarding *Ctenochromis* as a junior synonym of *Haplochromis* Hilgendorf, 1888, Regan (1922a) also designated the type species as *Ctenochromis pectoralis* Pfeffer, 1893. This species was originally described from specimens collected from the Pangani River near Korogwe in Tanzania in 1888 (Fig. 1),

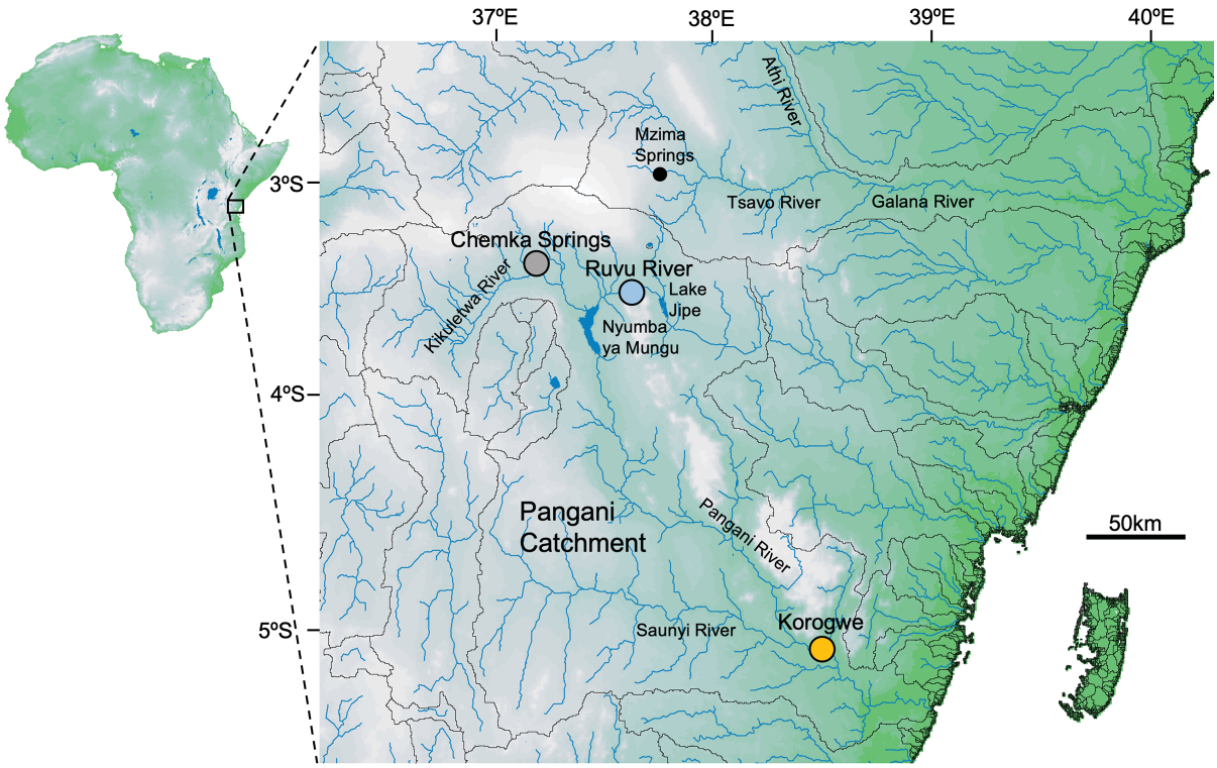

Fig. 1. The type locality of *Ctenochromis pectoralis* Pfeffer, 1893 is Korogwe, in the lower section of the Pangani River system. Collection sites of specimens of *Ctenochromis* for this study were Chemka Springs and the Ruvu River (which flows between Lake Jipe to the east, and Nyumba ya Mungu Reservoir to the west). A further population of *Ctenochromis* has been reported from Mzima Springs, in the Tsavo River system of Kenya.

Greenwood (1979) revised the genus *Ctenochromis* and formally identified key morphological diagnostic features, including the possession of a sharp break in chest scale size between the pectoral and pelvic fins, and one or more naked (scale-free) patches on chest. On the basis of similarities in chest squamation, Greenwood (1979) considered the genus to contain *Ctenochromis pectoralis* and a further four taxa, namely *Ctenochromis luluae* (Fowler, 1930), *Ctenochromis oligacanthus* (Regan, 1922), *Ctenochromis polli* (Thys van den Audenaerde, 1964) and *Ctenochromis horei* (Günther, 1894). Notably none of these four taxa are geographically contiguous with *Ctenochromis* from the Pangani River system, and the placement of these taxa within *Ctenochromis* has never been universally accepted (e.g., Daget *et al.* 1991; Fricke *et al.* 2021).

Since being collected at Korogwe in 1888, *C. pectoralis* does not appear to have been collected in the field until specimens were used in a phylogenetic analysis of African cichlids by Mayer *et al.* (1998), but the precise collecting localities of the analysed specimens were not noted. A decade later, the species was again reported in a phylogenetic analysis of haplochromines, this time by Koblmüller *et al.* (2008), with the collection locality reported as the Nyumba ya Mungu Reservoir, approximately 250 km upstream of Korogwe in the Pangani drainage. We note that specimens collected by Lother Seegers in 1974 from Nyumba ya Mungu Reservoir and identified as *C. pectoralis*, deposited in 1998 at the Natural History Museum in London (accessions BMNH 1998.10.9.1–19), are *Astatotilapia bloyeti* (identifier Martin Genner). De Graaf (2011) also reported *C. pectoralis* populations from the Nyumba ya Mungu Reservoir, as well as the nearby Chemka Springs. Later, van Heusden (2015) reported *C. pectoralis* from Chemka Springs, as well as the nearby Ruvu River tributary that connects Nyumba ya Mungu Reservoir to Lake Jipe. More recently, Kalacska *et al.* (2017) reported the *Ctenochromis* population from Chemka Springs, referring to it as an undescribed species, but not listing any characteristic features. More detail on the coloration and ecology of the Chemka Springs population was described by Schedel (2019). In phylogenetic analyses, *Ctenochromis pectoralis* has consistently been resolved as a phylogenetically unique taxon, the sister group of all other haplochromines (Koblmüller *et al.* 2008; Dunz & Schliewen 2013; Meier *et al.* 2017; Schedel *et al.* 2019).

Ctenochromis luluae, as referred to by Greenwood (1979), is known only from the Lulua River and Kasai River in the Democratic Republic of Congo (Lamboj 2004). It was originally described as *Tilapia luluae* Fowler, 1930. It has variously been referred to subsequently as *Haplochromis luluae* (Daget *et al.* 1991) and "*Haplochromis*" *luluae* (Decru *et al.* 2017) and suggested as plausibly synonymous with *Haplochromis stigmatogenys* (Boulenger, 1913) (Stiassny *et al.* 2011). Currently, this species is considered valid as *Haplochromis luluae* by Fricke *et al.* (2021). As of now, no specimens that have been unambiguously assigned to *Haplochromis luluae* have been included in any molecular phylogenetic studies, to our knowledge. The plausibly synonymous and geographically proximate *Haplochromis stigmatogenys*, however, appears closely related to the southern and central African haplochromine taxa, including the serranochromines, rather than sequenced specimens assigned to East African *Ctenochromis pectoralis* (Schwarzer *et al.* 2012; Meier *et al.* 2017).

Ctenochromis oligacanthus, as referred to by Greenwood (1979), is known from the Ubanghi River at Bangi, Central African Republic. It was originally described as *Haplochromis oligacanthus* Regan, 1922, until placed in *Ctenochromis* by Greenwood (1979). It has subsequently been referred to as *Haplochromis oligacanthus* (van Oijen *et al.* 1991) and "*Haplochromis*" *oligacanthus* (Wamuini Lunkayilakio & Vreven 2010). Currently this species is considered valid as *Haplochromis oligacanthus* by Fricke *et al.* (2021). In molecular phylogenetic analyses, *Haplochromis oligacanthus* forms a sister clade to *Haplochromis polli*, which shares a phylogenetic affinity with a larger clade of southern and central African haplochromines, including the serranochromines, rather than sequenced specimens assigned to *Ctenochromis pectoralis* (Koblmüller *et al.* 2008; Schwarzer *et al.* 2012).

Ctenochromis polli, as referred to by Greenwood (1979), is known from the lower and middle Congo River Basin within the Democratic Republic of the Congo. The species was originally described as *Haplochromis polli* Thys van den Audenaerde, 1964. The type locality is Pool Malebo, historically referred to as "Stanley Pool". The upstream limit has been listed as Luozi-Manianga, and the downstream limit being Kwamouth (Froese & Pauly 2021). The species has been resolved as a sister taxon to *Haplochromis oligacanthus* (Schwarzer *et al.* 2012), and thus also comprises part of a larger clade of southern and central African haplochromines that includes the serranochromines. Therefore, Greenwood's *Ctenochromis polli* is only distantly related to sequenced specimens assigned to *Ctenochromis pectoralis* (Koblmuller *et al.* 2008; Schwarzer *et al.* 2012).

Ctenochromis horei was originally described as *Chromis horei* Günther, 1894. It is an endemic of the catchment of Lake Tanganyika, being present in the shallow marginal waters of the lake and immediate riverine habitats. The species was placed in *Ctenochromis* by Greenwood (1979), and Fricke *et al.* (2021) currently consider the species valid as *Ctenochromis horei*. In addition, the species has since been variously referred to as *Haplochromis horei* (van Oijen *et al.* 1991), "*Haplochromis*" *horei* (Wamuini Lunkayilakio & Vreven 2010) and "*Ctenochromis*" *horei* (Takahashi 2003). In recent literature, the species is still referred to as *Ctenochromis horei* (Kullander & Roberts 2011; Konings 2015; Ronco *et al.* 2021). In phylogenetic analyses, the species has been reliably resolved as a member of the endemic tribe of Tropheini from Lake Tanganyika, alongside members of the genera *Simochromis* Boulenger, 1898, *Tropheus* Boulenger, 1898, *Gnathochromis* Poll, 1981, *Petrochromis* Boulenger, 1898, *Pseudosimochromis* Nelissen 1977, *Lobochilotes* Boulenger, 1915, *Limnotilapia* Regan, 1920, and *Interochromis* Yamaoka, Hori & Kuwamura, 1998 (Ronco *et al.* 2021).

Historically, other species have also been referred to as *Ctenochromis*. These include the Lake Tanganyikan endemic initially described as *Haplochromis benthicola* Matthes, 1962. The species is now widely referred to as both *Ctenochromis benthicola* (Ronco *et al.* 2021) and *Trematochromis benthicola* (Konings 2015) and is considered valid as *Trematochromis benthicola* by Fricke *et al.* (2021), who cite Geerts (2006) and Konings (2015, 2019). In molecular phylogenetic analyses, the species is placed within the endemic Lake Tanganyikan clade Cyphotilapini, alongside *Cyphotilapia frontosa* (Boulenger, 1906) and *Cyphotilapia gibberosa* Takahashi & Nakaya, 2003 (Ronco *et al.* 2021).

Another species historically associated with *Ctenochromis* is the Lake Victorian endemic *Haplochromis sauvagei* (Pfeffer, 1896), which was originally described by Pfeffer as *Ctenochromis sauvagei.* The species was redescribed and transferred to *Ptyochromis* Greenwood, 1980 by Greenwood (1980) with a neotype identified as the holotype was presumed lost. Seegers (2008) later relocated and reviewed the holotype of *H. sauvagei*, and considered this to be a representative of *Paralabidochromis* Greenwood, 1956 (conspecific with *Haplochromis* "rockkribensis"). Nevertheless, Seegers (2008) retained it as *Haplochromis sauvagei*, which is still considered as valid by Fricke *et al.* (2021). Additionally, Seegers (2008) described Greenwood's neotype of *Ptyochromis sauvagei* as a new species *Haplochromis* (*Ptyochromis*) *fischeri* Seegers, 2008. *Haplochromis sauvagei* reliably falls within the Lake Victorian lineage of haplochromine cichlids, for example in Meier *et al.* (2017), where it is referred to as *H.* "rockkribensis".

In August 2015, we sampled the populations of *Ctenochromis* from the Ruvu River near Nyumba ya Mungu Reservoir, and from the Chemka Springs, both within the Pangani River system (Figs 1–2). Using these samples, and considering published phylogenetic evidence, we here redefine *Ctenochromis* to render it a monophyletic genus, including transferring *C. horei* to a new genus, *Shuja* gen. nov. We also explore how two populations within the upper reaches of the Pangani River system (Ruvu River and Chemka Springs) differ morphologically from another, and from the type material of *C. pectoralis* collected from Korogwe in 1888. We quantify the extent of genetic differences between the populations from the Ruvu River and Chemka Springs, using evidence from genomic variants derived from double

digest restriction-site associated DNA (ddRAD) sequencing. Based on clear morphological and genetic differences between the two extant populations in the Pangani River system, we describe the population from Chemka Springs as *Ctenochromis scatebra* sp. nov. We also discuss the conservation status of the valid species of *Ctenochromis*.

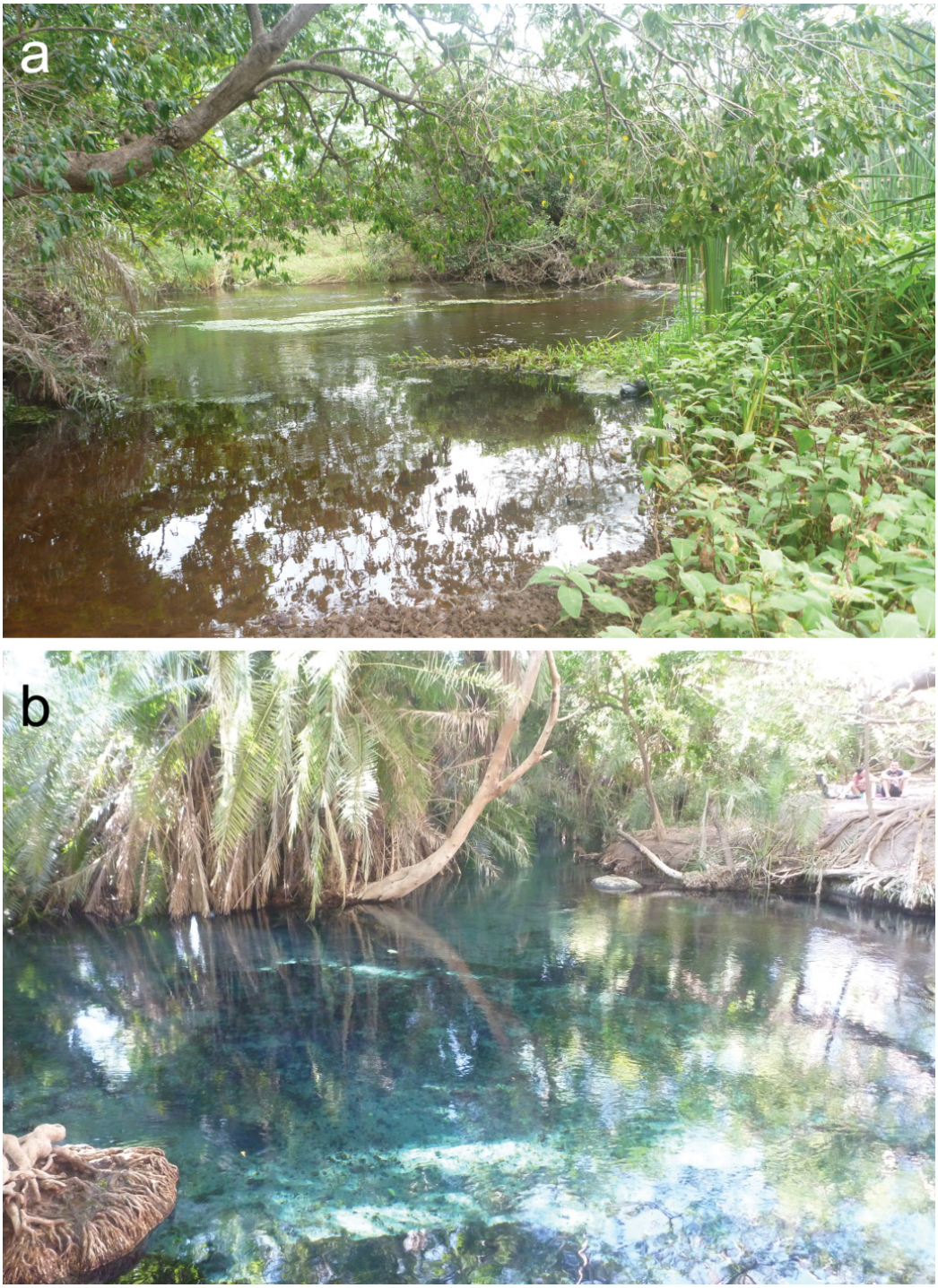

Fig. 2. a. Ruvu River where specimens of *Ctenochromis pectoralis* Pfeffer, 1893 were collected. **b**. Chemka Springs where specimens of *C. scatebra* Genner, Ngatunga & Turner sp. nov. were collected. See Table 1 for collection details.

Material and methods

Sample collection

Collections of *Ctenochromis pectoralis* were made in the Ruvu River between Lake Jipe and Nyumba ya Mungu Reservoir (BMNH 2021.7.15.1–3), while *C. scatebra* sp. nov. was collected at Chemka Springs (Table 1; Fig. 2; BMNH 2021.7.15.4–13). Collections were made using seine nets, and immediately after capture fish were euthanised using clove oil. To maintain body shape during preservation, the fresh specimens were pinned against a polystyrene board with fins erect, and they were then sprinkled with ethanol which was allowed to dry. The pins were then removed and specimens were preserved in absolute ethanol. Later, a genetic sample (fin clip of the right pectoral fin) was removed and placed in ethanol.

Comparative genetic material

For ddRAD analyses, samples of three haplochromine species from Lake Malawi [*Otopharynx speciosus* (Trewavas, 1935), *Maylandia zebra* (Boulenger, 1899) and *Rhamphochromis longiceps* (Günther, 1864)] (n = 1 per species, reference material not retained) and three haplochromine species from Lake Tanganyika (*Gnathochromis pfefferi* (Boulenger, 1898), *Lobochilotes labiata* (Boulenger, 1898) and *Shuja horei* gen. et comb. nov.) (n = 1 per species, BMNH 2021.7.15.14–16) were obtained from commercial fishers (Table 1), and again a genetic sample (fin clip of the right pectoral fin) was removed and placed in ethanol. We analysed these specimens (total n = 6) alongside specimens of *Ctenochromis pectoralis* from Ruvu River (n = 3) and *C. scatebra* sp. nov. from Chemka Springs (n = 3). For an outgroup we used *Orthochromis malagaraziensis* (David, 1937), employing published sequence data (SRR9673860; Ronco *et al.* 2021).

For mtDNA analyses, we generated new NADH2 sequences for *Ctenochromis pectoralis* from Ruvu River (n = 3) and *C. scatebra* sp. nov. from Chemka Springs (n = 3). These sequences were compared to published data for three haplochromine species from Lake Malawi (same species as ddRAD analyses; Genbank accessions AF305323, GU192220, JQ950395; Shaw *et al.* 2000; Mims *et al.* 2010; Wagner *et al.* 2012) and three haplochromine species from Lake Tanganyika (same species as ddRAD analyses listed above, Genbank accessions EF679245, KY366718, EU753935; Wagner *et al.* 2009; Meier *et al.* 2017; Koblmüller *et al.* 2008). They were also compared to two published sequences of *Ctenochromis pectoralis* collected from Nyumba ya Mungu (Genbank accessions EU753938, EU753939; Koblmüller *et al.* 2008). Again, as an outgroup we used available sequence data for *Orthochromis malagaraziensis* (Genbank accession AF398232; Salzburger *et al.* 2002).

Comparative morphological material

We examined *Ctenochromis pectoralis* (lectotype ZMH402, paralectotypes ZMH403 1–3) from Korogwe collected in 1888 (n = 4). The type locality has the name "Rufu" on the original collection label, that we assume to refer to "Ruvu", and 19th century maps indicate the present day Pangani was historically referred to as the "Ruvu" across its entire length (www.oldmapsonline.org/map/cuni/1169611). This material from ZMH was examined using photographs taken by Thilo Weddehage of the ZMH; material was not physically accessible due to COVID-19 restrictions. We also examined *Ctenochromis pectoralis* (paralectotype BMNH 1899.2.27.1) from Korogwe collected in 1888 (n = 1). This specimen was formerly part of the type series from the ZMH. We analysed these Korogwe specimens (total n = 5) alongside specimens of *Ctenochromis pectoralis* from Ruvu River (n = 3) and *C. scatebra* sp. nov. from Chemka Springs (n = 10).

To compare oral jaw morphology of species of Tropheini from Lake Tanganyika, we viewed published x-ray computed tomography scans of a total of 82 specimens of a total of 19 described species (Table 2; Ronco *et al.* 2021). These data were accessed on MorphoSource.org.

Table 1. Samples included in the genetic analyses for this study. Decimal coordinates are presented. * indicates vouchers are accessioned within a group. Abbreviations: MG = Martin Genner; AS = Alan Smith, BN = Benjamin Ngatunga, AHS = Asilatu H. Shechonge.

Species	Location	Latitude	Longitude	Date	Collector(s)	Isolate	Genbank Accession (MtDNA)	SRA Accession (ddRAD)	Voucher Accessions (NHM)
Ctenochromis pectoralis Pfeffer, 1893	Ruvu River	3.529° S	37.573° E	14/08/2015	MG,AS,BN,AHS	A10-01; LL69	OL694092	SRX13301695	BMNH 2021.7.15.1–3 *
Ctenochromis pectoralis Pfeffer, 1893	Ruvu River	3.529° S	37.573° E	14/08/2015	MG,AS,BN,AHS	A10-05; LL70	OL694093	SRX13301696	BMNH 2021.7.15.1–3 *
Ctenochromis pectoralis Pfeffer, 1893	Ruvu River	3.529° S	37.573° E	14/08/2015	MG,AS,BN,AHS	A10-06; LL71	OL694094	SRX13301699	BMNH 2021.7.15.1–3 *
Ctenochromis scatebra sp. nov.	Chemka Springs	3.443° S	37.194° E	17/08/2015	MG,AS,BN,AHS	18.1; LL75	OL694095	SRX13301700	BMNH 2021.7.15.4
Ctenochromis scatebra sp. nov.	Chemka Springs	3.443° S	37.194° E	17/08/2015	MG,AS,BN,AHS	18.2; LL76	OL694096	SRX13301701	BMNH 2021.7.15.5–13 *
Ctenochromis scatebra sp. nov.	Chemka Springs	3.443° S	37.194° E	17/08/2015	MG,AS,BN,AHS	18.3; LL77	OL694097	SRX13301702	BMNH 2021.7.15.5–13 *
Shuja horei gen. et comb. nov.	Malagarasi River, Ilagala	5.211° S	29.842° E	28/07/2016	MG,AS,BN,AHS	MG061; LL35	Not sequenced	SRX13301703	BMNH 2021.7.15.14
Gnathochromis pfefferi (Boulenger, 1898)	Kagera Market, Ujiji	4.907° S	29.665° E	27/07/2016	MG,AS,BN,AHS	LT.01; LL68	Not sequenced	SRX13301704	BMNH 2021.7.15.15
Lobochilotes labiata (Boulenger, 1898)	Kagera Market, Ujiji	4.907° S	29.665° E	29/07/2016	MG,AS,BN,AHS	MG111; LL36	Not sequenced	SRX13301705	BMNH 2021.7.15.16
Rhamphochromis longiceps (Günther, 1864)	Metangula Market	12.689° S	34.810° E	30/08/2014	MG	2014-08#01-10; LL92	Not sequenced	SRX13301706	–
Otopharynx speciosus (Trewavas, 1935)	Chiwanga	12.639° S	34.791° E	01/09/2014	MG	2014-08#02-89; LL93	Not sequenced	SRX13301697	–
Maylandia zebra (Boulenger, 1899)	Minos Reef	12.904° S	34.748° E	31/08/2014	MG	2014-08#03-77; LL94	Not sequenced	SRX13301698	–

Table 2 continued on next two pages). Images of Lake Tanganyika Tropheni species examined (from Ronco *et al*. 2021). Images and data were accessed via MorphoSource.org.

Species	physical_object_ID	Ark identifier	Oral jaw characteristics (after Barel 1977)
Gnathochromis pfefferi (Boulenger, 1898)	unibas:ic:IWG8	ark:/87602/m4/M156463	retrognathus
	unibas:ic:IWG9	ark:/87602/m4/M156464	retrognathus
	unibas:ic:IWH1	ark:/87602/m4/M156465	retrognathus
	unibas:ic:IWC7	ark:/87602/m4/M156462	retrognathus
Interochromis loocki (Poll, 1949)	unibas:ic:IYF7	ark:/87602/m4/M155982	isognathus
	unibas:ic:IYF8	ark:/87602/m4/M155983	isognathus
	unibas:ic:IYG8	ark:/87602/m4/M155984	isognathus
	unibas:ic:IYE5	ark:/87602/m4/M155981	isognathus
Limnotilapia dardennii (Boulenger, 1899)	unibas:ic:GPF5	ark:/87602/m4/M155571	isognathus
	unibas:ic:IMD6	ark:/87602/m4/M155572	isognathus
	unibas:ic:JBF6	ark:/87602/m4/M155573	isognathus
	unibas:ic:GPB6	ark:/87602/m4/M155570	isognathus
Lobochilotes labiata (Boulenger, 1898)	unibas:ic:IYF9	ark:/87602/m4/M155879	retrognathus
	unibas:ic:IYG2	ark:/87602/m4/M155880	retrognathus
	unibas:ic:IYG3	ark:/87602/m4/M155881	retrognathus
	unibas:ic:IYE2	ark:/87602/m4/M155878	retrognathus
Petrochromis ephippium Brichard, 1989	unibas:ic:JCG8	ark:/87602/m4/M155669	retrognathus
	unibas:ic:JCH7	ark:/87602/m4/M155670	retrognathus
	unibas:ic:JCI7	ark:/87602/m4/M155671	retrognathus
	unibas:ic:JCG7	ark:/87602/m4/M155668	retrognathus
Petrochromis famula Matthes & Trewavas, 1960	unibas:ic:JCH2	ark:/87602/m4/M155688	retrognathus
	unibas:ic:JCH4	ark:/87602/m4/M155689	retrognathus
	unibas:ic:JCI8	ark:/87602/m4/M155690	retrognathus
	unibas:ic:JCH1	ark:/87602/m4/M155687	retrognathus
Petrochromis fasciolatus Boulenger, 1914	unibas:ic:GPI3	ark:/87602/m4/M155714	isognathus, but ‘chin’ slightly protuding
	unibas:ic:JBB9	ark:/87602/m4/M155716	isognathus, but ‘chin’ slightly protuding
	unibas:ic:JBD4	ark:/87602/m4/M155717	isognathus, but ‘chin’ slightly protuding
	unibas:ic:GPI2	ark:/87602/m4/M155713	isognathus, but ‘chin’ slightly protuding

Table 2 continued). Images of Lake Tanganyika Tropheni species examined (from Ronco *et al*. 2021). Images and data were accessed via MorphoSource.org.

Species	physical_object_ID	Ark identifier	Oral jaw characteristics (after Barel 1977)
Petrochromis horii Takahashi & Koblmüller, 2014	unibas:ic:IWC2	ark:/87602/m4/M155839	retrognathus
	unibas:ic:JBF8	ark:/87602/m4/M155840	retrognathus
	unibas:ic:JBF9	ark:/87602/m4/M155841	retrognathus
	unibas:ic:IWC1	ark:/87602/m4/M155838	retrognathus
Petrochromis macrognathus Yamaoka, 1983	unibas:ic:LDC5	ark:/87602/m4/M155992	retrognathus
	unibas:ic:LJB2	ark:/87602/m4/M155993	retrognathus
	unibas:ic:LJB3	ark:/87602/m4/M155994	retrognathus
	unibas:ic:LDC4	ark:/87602/m4/M155991	retrognathus
Petrochromis orthognathus Matthes, 1959	unibas:ic:JZD2	ark:/87602/m4/M156376	isognathus
	unibas:ic:KDF6	ark:/87602/m4/M156377	isognathus
	unibas:ic:KDF8	ark:/87602/m4/M156378	isognathus
	unibas:ic:JXH5	ark:/87602/m4/M156370	isognathus
Petrochromis polyodon Boulenger, 1898	unibas:ic:IYE9	ark:/87602/m4/M156502	retrognathus
	unibas:ic:IYG5	ark:/87602/m4/M156503	retrognathus
	unibas:ic:JXD2	ark:/87602/m4/M156504	retrognathus
	unibas:ic:IYD4	ark:/87602/m4/M156500	retrognathus
Petrochromis trewavasae Poll, 1948	unibas:ic:IWH8	ark:/87602/m4/M157188	retrognathus
	unibas:ic:IWH9	ark:/87602/m4/M157189	retrognathus
	unibas:ic:IWI1	ark:/87602/m4/M157190	retrognathus
	unibas:ic:IWH6	ark:/87602/m4/M157186	retrognathus
Pseudosimochromis marginatus (Poll, 1956)	unibas:ic:LEI1	ark:/87602/m4/M156041	retrognathus
	unibas:ic:LEI3	ark:/87602/m4/M156043	retrognathus
	unibas:ic:KHG3	ark:/87602/m4/M156039	retrognathus
	unibas:ic:LEI2	ark:/87602/m4/M156042	retrognathus
Shuja horei gen. et comb. nov.	unibas:ic:JAD9	ark:/87602/m4/M155813	prognathus
	unibas:ic:JDB2	ark:/87602/m4/M155814	prognathus
	unibas:ic:JDB5	ark:/87602/m4/M155815	prognathus
	unibas:ic:JDB6	ark:/87602/m4/M155816	prognathus

Table 2 continued). Images of Lake Tanganyika Tropheni species examined (from Ronco *et al*. 2021). Images and data were accessed via MorphoSource.org.

Species	physical_object_ID	Ark identifier	Oral jaw characteristics (after Barel 1977)
Shuja horei gen. et comb. nov.	unibas:ic:JDC3	ark:/87602/m4/M155817	prognathus
	unibas:ic:JDC8	ark:/87602/m4/M155818	prognathus
	unibas:ic:JDD9	ark:/87602/m4/M155819	prognathus
	unibas:ic:JDE1	ark:/87602/m4/M155820	prognathus
	unibas:ic:KAE4	ark:/87602/m4/M155821	prognathus
	unibas:ic:IQI6	ark:/87602/m4/M155812	prognathus
Simochromis diagramma (Günther, 1894)	unibas:ic:JDB9	ark:/87602/m4/M155614	retrognathus
	unibas:ic:JDC1	ark:/87602/m4/M155615	retrognathus
	unibas:ic:JDC2	ark:/87602/m4/M155616	retrognathus
	unibas:ic:JDB8	ark:/87602/m4/M155613	retrognathus
Tropheus annectens Boulenger, 1900	unibas:ic:LEG8	ark:/87602/m4/M156487	retrognathus
	unibas:ic:LEG9	ark:/87602/m4/M156488	retrognathus
	unibas:ic:LEH1	ark:/87602/m4/M156489	retrognathus
	unibas:ic:LEG7	ark:/87602/m4/M156486	retrognathus
Tropheus brichardi Nelissen & Thys van den Audenaerde, 1975	unibas:ic:JZA9	ark:/87602/m4/M155378	retrognathus
	unibas:ic:JZB1	ark:/87602/m4/M155379	retrognathus
	unibas:ic:JZB2	ark:/87602/m4/M155380	retrognathus
	unibas:ic:JZA6	ark:/87602/m4/M155376	retrognathus
Tropheus duboisi Marlier, 1959	unibas:ic:KHF9	ark:/87602/m4/M155634	retrognathus
	unibas:ic:KHG1	ark:/87602/m4/M155635	retrognathus
	unibas:ic:KHI7	ark:/87602/m4/M155636	retrognathus
	unibas:ic:KHF8	ark:/87602/m4/M155633	retrognathus
Tropheus moorii Boulenger, 1898	unibas:ic:JBH7	ark:/87602/m4/M156197	retrognathus
	unibas:ic:JBH9	ark:/87602/m4/M156198	retrognathus
	unibas:ic:JBI1	ark:/87602/m4/M156199	retrognathus
	unibas:ic:JBH6	ark:/87602/m4/M156196	retrognathus

Abbreviations of museums and institutions

BMNH = British Museum of Natural History, London, UK
ZMH = Zoological Museum Hamburg, Germany

Abbreviations

AFBL	=	anal-fin base length
ASOFT	=	anal-fin soft rays
ASPINES	=	anal-fin spines
BD	=	body depth
CPD	=	caudal-peduncle depth
CPL	=	caudal-peduncle length
ddRAD	=	double digest restriction-site associated DNA
DFBL	=	dorsal-fin base length
DSOFT	=	dorsal-fin soft rays
DSPINES	=	dorsal-fin spines
ED	=	eye diameter
HL	=	head length
LATLOW	=	lower trunk lateral-line scales
LATUP	=	upper trunk lateral-line scales
LONG	=	longitudinal-line scales
PCA	=	Principal Components Analysis
PCR	=	polymerase chain reaction
PRA	=	preanal distance
PRD	=	predorsal distance
PRP	=	prepectoral distance
PRV	=	prepelvic distance
SL	=	standard length
SNL	=	snout length
SNP	=	single nucleotide polymorphism
TL	=	total length

Morphometric measurements and meristic counts

The left side of preserved fish was photographed from a standard orientation with a scale. With ImageJ ver. 2 (Rueden *et al.* 2017) images were calibrated and morphological measurements were taken from all individuals larger than 30 mm standard length (Table 1). All morphological measurements follow Snoeks (2004), and in total 14 measurements were taken from each fish, namely 1) SL, 2) TL, 3) BD, 4) HL, 5) SNL, 6) ED, 7) DFBL, 8) AFBL, 9) PRD, 10) PRA, 11) PRP, 12) PRV, 13) CPL, 14) CPD. Meristic counts were taken following Snoeks (2004), including i) DSPINES, ii) DSOFT, iii) ASPINES, iv) ASOFT, v) LONG, vi) LATUP and vii) LATLOW. Meristic counts were not possible using the available images of the ZMH material.

Continuous morphological measurements were $\log_{10}$ transformed, regressed against standard length, and residuals were generated. Principal Component Analysis was conducted on the residuals using the prcomp function from stats ver. 3.6.2 in R (R Core Team 2019) employing a covariance matrix.

Micro-CT scans of representative specimens

Whole body scans of four specimens of *Ctenochromis* were obtained using a Nikon XTH225ST micro-computed tomography (micro-CT) scanning system. Beam energy was set to 80 kV with an exposure time of 708 ms. Each specimen generated approximately 400 projections at a voxel size of 47 µm. Scan

images were reconstructed in CT Pro 3D ver. 4.4.3 (Nikon Metrology) before export to VG Studio Max ver. 3.4 (Volume Graphics GmbH) for 3D visualisation. Final images were captured in Avizo Lite ver. 9.7 (Thermo Scientific) using the Volume Rendering function.

Genomic data – ddRAD sequences

Genomic DNA was extracted from fin clips using a modified CTAB (cetyl trimethylammonium bromide) method. Fin tissues were cut to approximately 4 mm^2 and placed into 1.5 ml centrifuge tubes. 200 µl of CTAB buffer and 2.5 µl of proteinase K (Qiagen) were added into the tube per sample. After 30 minutes of 60°C incubation, 200 µl of chloroform was added to each sample. Products were then vortexed followed by a 5 min centrifuge at 14 600 rpm. The supernatant was removed and placed into a new tube with 400 µl 100% ethanol, which was then vortexed before being centrifuged for 5 min at 14 600 rpm. The supernatant was then removed and the pellets in the tube were allowed to dry overnight. The pellet was then eluted into 50 µl of ddH_2O. This genomic DNA was then purified using a QIAquick PCR Purification Kit (Qiagen), and the DNA quality was measured using both 260/280 and 230/260 nm absorbance ratios using a Nanodrop (ThermoFisher). The final DNA concentration was determined using a dsDNA High Sensitivity Assay in a Qubit 4 fluorometer (ThermoFisher) and diluted using ddH_2O to a standardised concentration of 200 ng of DNA per 15 µl sample.

Reduced representation sequencing of the genome was completed using the original ddRAD protocol from Peterson *et al.* (2012). First, barcoded adapters were prepared by annealing adapter stocks, which were then diluted ten times with ddH_2O to 0.4 µM (concentration adjusted by the ligation molarity calculator provided in the original protocol). DNA was then subjected to a restriction-ligation, using the barcoded adapters. Each restriction-ligation contained 15 µl DNA template (200 ng), 5 µl 1 × CutSmart buffer (NEB), 0.1 µl EcoRI (NEB), 0.1µl MspI (NEB), 0.5 µl T4 ligase (NEB), 0.5 µl ATP (NEB), 2 µl Adapter1, 2 µl Adapter2 and 24.8 µl H_2O, and was performed for 3 h at 37°C, followed by 15 min at 68°C.

For each sample, we conducted four replicate 10-cycle PCR reactions. Each reaction was in a 20 µl volume, and included 4 µl of ligated DNA, 10 µl of 2 × Phusion Flash PCR Master Mix (ThermoFisher), 1 µl Index Primer1, 1 µl Index Primer2, 0.5 µl Bovine Serum Albumin (NEB), 3.5 µl ddH_2O. Cycling conditions for the PCR were an initial denaturation at 98°C for 60 seconds, followed by 10 cycles of 98°C for 10 seconds, 55°C for 35 seconds, and 72°C for 90 seconds. This was followed by final extension step of 72°C for 7 minutes. The PCR products were then pooled and AMPure XP beads (1.8 ×) (Beckman Coulter) were used to clean the library. Three size selections were then performed using 2% agarose E-Gel SizeSelect II gels (ThermoFisher), targeting the fragments between 650–765 basepairs. The size-selected product was then sequenced using an Illumina MiSeq v3 600 cycle kit (2 × 300 bp) at the University of Bristol Genomics Facility. The sequences for the adaptors and indexed PCR primers used are available in Appendix 1.

Analyses of ddRAD sequence data

The ddRAD sequences were demultiplexed and cleaned using cutadapt 2.1 (Martin 2011). The reference genome of the haplochromine cichlid *Astatotilapia calliptera* (Günther, 1894) (assembly fAstCal1.2; GCA_900246225.3) was referenced using the "index" function in bwa 0.7.17 (Li 2013). Trimmed sequences from each sample, and the whole genome sequence from the outgroup taxon (*Orthochromis malagaraziensis* (David, 1937); SRR9673860; Ronco *et al.* 2021), were then aligned to the reference using the "mem" function in bwa, and the resultant sam files were converted into bam files using the "view" function in SAMtools 1.1 (Li *et al.* 2009). The bam files were then sorted using the "sort" function in SAMtools, before duplicates marked with the "MarkDuplicates" function and read group tags added using the "AddOrReplaceReadGroups" function, both in picard ver. 2.23.3 (http://broadinstitute.github.io/picard/). Variant calling was undertaken using freebayes ver. 1.3.2

(Garrison & Marth 2012) using the default settings. The resultant vcf file was then filtered using vcftools 0.1.16 (Danecek *et al.* 2011) (– remove-indels – minDP 2 – max-missing 1). The overall between-population F_{ST} was calculated using the "weir-fst-pop" function in vcftools. The vcf file was converted to phylip format using the python script vcf2phylip (https://github.com/edgardomortiz/vcf2phylip), enabling a phylogeny of the individuals to be constructed using RAxML-NG (Kozlov *et al.* 2019) using the general time reversible (GTR)+Γ model and 100 bootstrap replicates. The demultiplexed sequences with adaptors removed are available within Sequence Read Archive (SRA) BioProject PRJNA785572.

Mitochondrial DNA data

We amplified the NADH2 gene from six *Ctenochromis* specimens (Table 1, same specimens as for the ddRAD analyses) using the primers ND2–MET (5'–CAT ACC CCA ACA TGT TGG T–3') and ND2–TRP (5'–GAG ATT TTC ACT CCC GCT TA–3') (Kocher *et al.* 1995). All polymerase chain reactions (PCRs) were performed in 25 µl reactions including 12.5 µl GoTaq Green Master Mix 2X (Promega), 1 µl forward primer (10 µm), 1 µl reverse primer (10 µm), 9.5 µl nuclease free water and 1 µl genomic DNA (~50 ng). PCR conditions were as follows: 1 min at 95 °C; then 40 cycles of 95°C for 30 s, 50°C for 1 minute, and 72°C for 1 min, followed by 72°C for 5 min. PCR products were cleaned using Ampure XP beads (Beckman Coulter) and sequenced in both directions by Eurofins Genomics. Chromatograms were checked using 4 Peaks 1.8 (Nucleobytes). The newly generated sequences have Genbank Accessions OL694092–OL694097. Data from these specimens, in additional to published comparative sequences (see section on comparative genetic material**)**, were aligned using Muscle ver. 3.8.31 (Edgar 2004) and a phylogeny was reconstructed using RAxML-NG (Kozlov *et al.* 2019) using the general time reversible (GTR)+Γ model and autoMRE bootstrapping (0.03 cutoff).

Results

Morphometric analyses

Using PCA on external measurements, Axis 1 captured 54.6% of the observed variation, while Axis 2 captured 19.9% of the observed variation (Fig. 3). These axes separate the three groups of *Ctenochromis* individuals by sampling location. Most prominently, *C. scatebra* sp. nov. has a shorter anal-fin base length than the type specimens of *C. pectoralis* from Korogwe and *C. pectoralis* from the Ruvu River (Tables 3–4). Meanwhile, *C. pectoralis* from Korogwe has a shallower body depth, a shorter caudal-peduncle length, a larger eye and a longer snout than specimens of *C. pectoralis* from the Ruvu River (Tables 3–4). We find no discriminatory differences in meristic counts we made of *C. scatebra* sp. nov or *C. pectoralis* sampled from the Ruvu River (Table 3). Diagnostic differences in craniofacial morphology are however present between the species (see diagnosis of *C. scatebra* sp. nov.).

Genetic analyses

In total, after filtering, we identified 11 288 SNPs across all 13 individuals included in the analysis. The resultant phylogeny showed strong support for the *Ctenochromis* populations from the Pangani system collectively representing a monophyletic group (Fig. 4a). Consistent with previous analyses, the phylogeny resolved *C. horei* (herein assigned genus *Shuja* gen. nov.) within a clade with members of the Tropheini from the Lake Tanganyika (*Gnathochromis pfefferi*, *Lobochilotes labiata*), which in turn is more closely related to other 'modern haplochromines' represented here by members of the Lake Malawi haplochromine species flock (*Maylandia zebra*, *Otopharynx speciosus* and *Rhamphochromis longiceps*). The population of *Ctenochromis* from Ruvu River and that of *Ctenochromis* from Chemka Springs were resolved as reciprocally monophyletic, coupled with strong population genetic structure (Unweighted $F_{ST}=0.100$; Weighted $F_{ST}=0.247$).

Table 3 (continued on next page). Morphometric measurements and meristics of the populations of *Ctenochromis* Pfeffer, 1893 used in this study from the Pangani Catchment, the measurements are presented as absolute measurements (in mm), and as percentages of standard length (SL) or head length (HL).

	Ctenochomis pectoralis Pfeffer, 1893 (Korogwe, n = 5)			***C. pectoralis*** (Ruvu River, n = 3)			***C. scatebra*** sp. nov. (Chemka Springs, n = 10)		
Measurement	**Lectotype**	**Average**	**Range**	**Largest**	**Average**	**Range**	**Holotype**	**Average**	**Range**
SL (mm)	51.81	43.04	33.23–51.81	63.24	58.83	53.68–63.24	54.86	41.86	33.79–59.09
TL (mm)	64.05	52.41	41.38–64.05	80.49	72.86	66.15–80.49	66.12	52.23	41.52–71.31
BD (mm)	18.58	14.77	11.88–18.58	23.55	22.33	20.08–23.56	19.82	14.51	11.75–20.66
HL (mm)	18.20	15.23	12.32–18.20	22.59	20.06	18.09–22.59	19.54	15.41	12.13–20.16
DFBL (mm)	27.86	20.77	17.65–27.86	32.5	30.52	27.63–32.50	26.99	21.68	16.60–29.50
AFBL (mm)	10.46	7.08	6.11–10.46	12.67	12.05	11.41–12.67	8.88	8.28	5.33–9.52
CPD (mm)	6.67	5.11	4.64–6.67	8.46	7.95	7.21–8.46	6.67	5.55	3.86–7.08
PRD (mm)	20.71	18.43	14.11–20.71	25.63	23.35	21.19–25.63	23.51	17.66	15.27–24.49
PRA (mm)	35.60	29.11	22.56–35.60	41.94	38.49	34.71–41.94	37.59	28.33	23.83–40.81
PRP (mm)	19.54	15.88	12.54–19.54	24.05	21.87	19.65–24.05	20.24	15.75	13.41–21.60
PRV (mm)	22.78	18.11	13.99–22.78	26.51	24.62	22.10–26.51	23.78	17.94	12.06–25.17
CPL (mm)	7.10	7.31	4.71–7.10	11.08	9.50	8.29–11.08	8.71	5.76	5.85–8.71
ED (mm)	5.72	4.22	4.02–5.97	5.75	5.62	4.98–5.75	4.74	5.03	3.60–5.27
SNL (mm)	5.16	4.21	3.20–5.16	5.66	4.96	4.61–5.66	5.59	4.10	3.10–6.24
Measurement	**Lectotype**	**Average**	**Range %**	**Largest %**	**Average %**	**Range %**	**Holotype %**	**Average %**	**Range %**
BD % of SL	35.85	34.64	33.52–35.85	37.24	37.96	37.24–39.25	33.90	34.17	32.30–36.12
HL % of SL	35.13	36.94	35.13–39.12	35.72	34.05	32.74–35.72	35.68	35.49	34.13–36.41

Table 3 (continued). Morphometric measurements and meristics of the populations of *Ctenochromis* Pfeffer, 1893 used in this study from the Pangani Catchment, the measurements are presented as absolute measurements (in mm), and as percentages of standard length (SL) or head length (HL).

	Ctenochomis pectoralis Pfeffer, 1893 (Korogwe, n=5)			***C. pectoralis*** (Ruvu River, n=3)			***C. scatebra*** sp. nov. (Chemka Springs, n=10)		
Measurement	**Lectotype**	**Average**	**Range %**	**Largest %**	**Average %**	**Range %**	**Holotype %**	**Average %**	**Range %**
DFBL % of SL	53.77	51.80	48.64–53.77	51.39	51.88	51.39–52.79	48.4	48.12	44.22–51.27
AFBL % of SL	20.19	19.73	18.38–20.57	20.03	20.51	20.03–21.25	17.35	16.41	14.65–19.08
CPD % of SL	12.87	13.27	12.19–13.95	13.38	13.52	13.38–13.74	10.90	11.83	10.90–12.74
PRD % of SL	39.97	42.35	39.97–44.15	40.53	39.66	38.98–40.53	42.78	42.96	41.45–45.19
PRA % of SL	68.72	67.62	66.38–68.72	66.32	65.38	64.65–66.32	66.44	67.56	65.40–71.21
PRP % of SL	37.71	37.66	36.40–38.75	38.03	37.14	36.59–38.03	35.48	36.89	35.48–41.24
PRV % of SL	43.97	42.77	41.20–44.18	41.92	41.83	41.17–42.40	41.10	41.97	35.59–43.34
CPL % of SL	13.70	13.76	13.19–14.49	17.52	16.10	15.33–17.52	17.04	17.18	14.36– 18.81
ED % of HL	32.81	32.68	32.24–33.78	29.04	27.93	27.32–29.04	30.59	28.01	24.07–32.07
SNL % of HL	28.33	26.51	25.59–28.33	25.06	24.75	23.62–25.56	28.30	27.32	23.42–30.92
Meristics	–	–	–	**Largest**	**Median**	**Range**	**Holotype**	**Median**	**Range**
DSPINES	–	–	–	15	15	15–16	14	14	14–15
DSOFT	–	–	–	7	8	7–9	9	8	8–9
ASPINES	–	–	–	3	3	3	3	3	3
ASOFT	–	–	–	8	8	8	8	8	7–8
LONG	–	–	–	30	30	30	31	29	27–31
LATUP	–	–	–	19	20	19–21	21	18	15–21
LATLOW	–	–	–	11	11	11–12	10	8	7–11

Table 4. Principal Component Analysis factor loadings. The variables contributing most substantially to the two axes (< -0.4 and > 0.4) are highlighted in bold.

Measurement	PC1	PC2
BD	0.057	-0.185
HL	0.103	0.160
DFBL	0.205	-0.063
AFBL	**0.524**	-0.345
CPD	0.288	-0.235
PRD	-0.039	0.134
PRA	-0.001	0.122
PRP	0.054	-0.015
PRV	0.042	0.013
CPL	**-0.589**	-0.334
ED	**0.482**	0.080
SNL	0.033	**0.782**

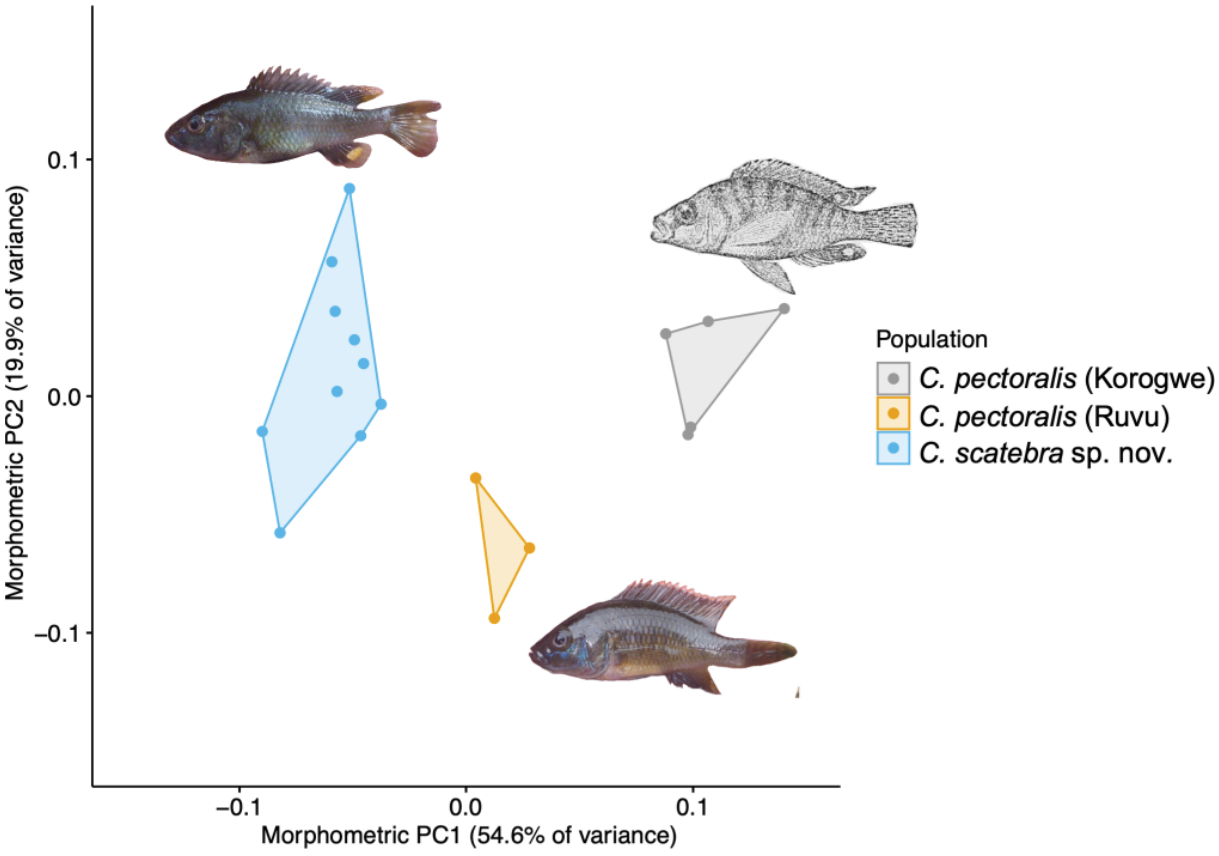

Fig. 3. Principal Component Axes 1 and 2 of morphological measurements of specimens of *Ctenochromis pectoralis* Pfeffer, 1893 of the type series from Korogwe, compared with specimens of *C. pectoralis* from the Ruvu River, and *C. scatebra* Genner, Ngatunga & Turner sp. nov. from Chemka Springs. The image of *C. pectoralis* from Korogwe is from the original description (Pfeffer 1893). Collection localities are in parentheses following the species names. In total, Principal Component Axes 1 and 2 captured 74.5% of the observed morphological variation.

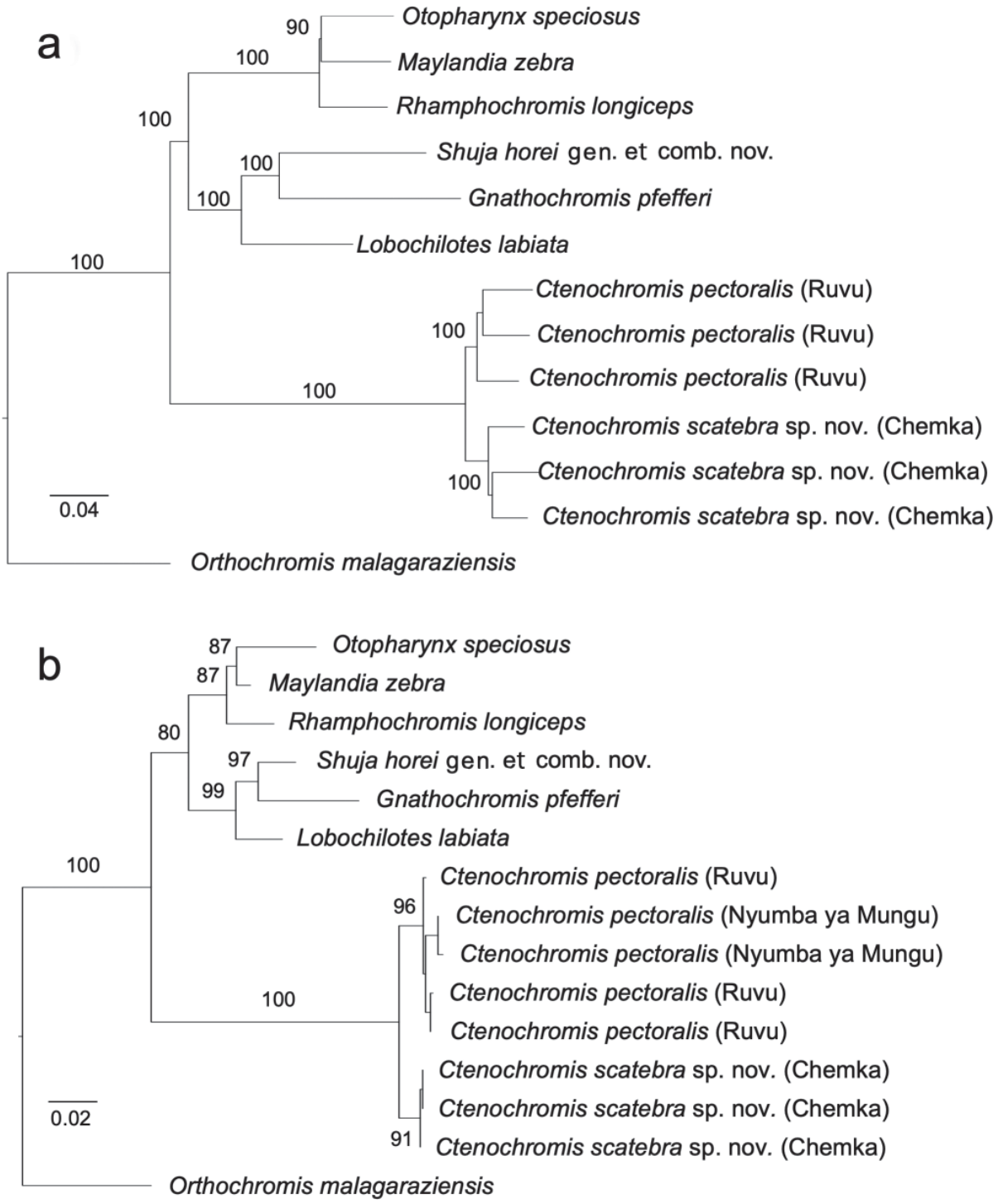

Fig. 4. Phylogenetic reconstructions of representatives of populations of *Ctenochromis* Pfeffer, 1893, as well as representatives of the Lake Malawi haplochromine radiation, and the Lake Tanganyika Tropheini Poll, 1986. **a**. Maximum Likelihood phylogenetic reconstruction based on 11 288 SNPs. **b**. Maximum Likelihood phylogenetic reconstruction based on 1047 basepairs of the entired NADH2 mtDNA gene. In both trees, numbers on branches indicate percentage bootstrap support, and branches with > 70% support are shown. The scale bars represent a measure of genetic distance. See Table 1 for sampling details. Collection localities are in parentheses. Samples from Nyumba ya Mungu have accessions EU753938 and EU753939 and are from Koblmüller *et al.* (2008).

Phylogenetic analyses of the mitochondrial NADH2 gene revealed an identical topology of the species to the ddRAD analyses (Fig 4b). This analysis also included two published NADH2 sequences from *Ctenochromis pectoralis*, collected from Nyumba ya Mungu Reservoir (Koblmüller *et al.* 2008), these were nested in the clade with the specimens of *C. pectoralis* collected from the Ruvu River.

Taxonomy

Phylum Chordata Haeckel, 1874
Class Actinopterygii Klein, 1885
Order Cichliformes Betancur-R *et al.*, 2013
Family Cichlidae Bonaparte, 1840
Subfamily Pseudocrenilabrinae Fowler, 1934
Tribe Tropheini Poll, 1986

Genus ***Shuja*** Genner, Ngatunga & Turner gen. nov.
urn:lsid:zoobank.org:act:7862EC4A-51D5-4535-98D5-650D6CC165A7

Type species

Chromis horei Günther, 1894.

Diagnosis

Shuja gen. nov. can be diagnosed as a genus of haplochromine cichlid within the Tropheini. According to Takahashi (2003: 379), the Tropheini have "extensively granulated cycloid scales at midbody", with the "granulations comprising irregularly arranged, variously shaped protrusions over almost entire exposed surface". *Shuja* gen. nov. is the only representative of the Tropheini with a prognathous lower jaw, versus the retrognathus or isognathus jaw in other genera within the Tropheini (Table 2).

Etymology

The genus name is derived from the Swahili noun 'shujaa', translated into English as 'brave person' or 'warrior', referring to the notable territorial behaviour of the males of this genus.

Description

One species in genus *Shuja horei* gen. et comb. nov. Species description, from original German text (Günther 1894: 630): "Dorsal fin 14 spines 8 rays, L. lat 28, L. trans. 4/9. Teeth bicuspid, cusps subequal, slightly tinged with brown; 28–31 each side of upper jaw outer series. Cheeks naked or few extremely thin scales. In specimen nearly 5 inches (12.70 cm) long eye diameter nearly equal to depth of soft part of cheek, a little less than width of preorbital and interorbital space, which is flat. Preopercular limbs at right angle. Body height less than length of head, and one third of total (without caudal). Last dorsal spine longest, two fifths of head length. Pectoral fin to, or nearly to, origin of anal fin. Caudal scaleless. Scales rough, some with margins ciliated. Body light greenish, with incomplete brownish cross-bands on upper part of body. Largest specimen cheek and snout with irregular deep brown spots. Soft dorsal and caudal fin with scattered ocelli; milky-white spot between last two anal rays." In *Shuja horei* gen. et comb. nov. hypertrophied lips absent.

Remarks

Shuja gen. nov. belongs to Tropheini tribe of African cichlids, originally defined by Poll (1986). The diagnosis of Tropheini is the presence of "extensively granulated cycloid scales at midbody" (Takahashi 2003: 379). Our observations suggest such granularity of the flank/midbody scales in Tropheini is present in *Shuja horei* gen. et comb. nov., *Gnathochromis pfefferi* and *Lobochilotes labiata*, at least, but also small ctenii are present. These ctenii are sparse and largely restricted on the margins of this caudal edge of

the scale. Viertler *et al.* (2021) report multiple species in the Tropheini with ctenoid scales on the central flank area. Further detailed work of all described species is needed to determine if the distribution of the granulation and ctenii on midbody scales is diagnostic of the Tropheini. Nevertheless, irrespective of morphological traits, the tribe is unambiguously monophyletic in genome-scale molecular phylogenetic analyses (Ronco *et al.* 2021), and endemic to Lake Tanganyika and immediate river systems. Within the tribe, *Shuja* gen. nov. is a monotypic genus and can be distinguished from other representatives of the Tropheini by the presence of a prognathous jaw (Fig. 5a–c), when all other described species in the Tropheini have a retrognathus or isognathus jaw (Table 2). These include *Gnathochromis pfefferi*, and representatives of *Interochromis*, *Limnotilapia*, *Lobochilotes*, *Petrochromis*, *Pseudosimochromis*, *Simochromis* and *Tropheus* (Table 2).

Shuja horei Günther, 1894 gen. et comb. nov.
Fig. 5

Chromis horei Günther, 1894: 630, pl. 58a.
Tilapia horii Boulenger, 1899: 122.
Tilapia fasciata tanganaicae Borodin, 1936: 29.

Haplochromis horei – Trewavas 1946: 242. — van Oijen *et al.* 1991: 131. — Wamuini Lunkayilakio & Vreven 2010: 280.
Tilapia fasciata tanganaicae – Trewavas 1946: 242.
Ctenochromis horii – Greenwood 1979: 289–290.
Ctenochromis horei – Poll & Gosse 1995: 229. — De Vos *et al.* 2001: 130, 132. — Kullander & Roberts 2011: 362, 369. — Konings 2015: 223; 2019: 237.

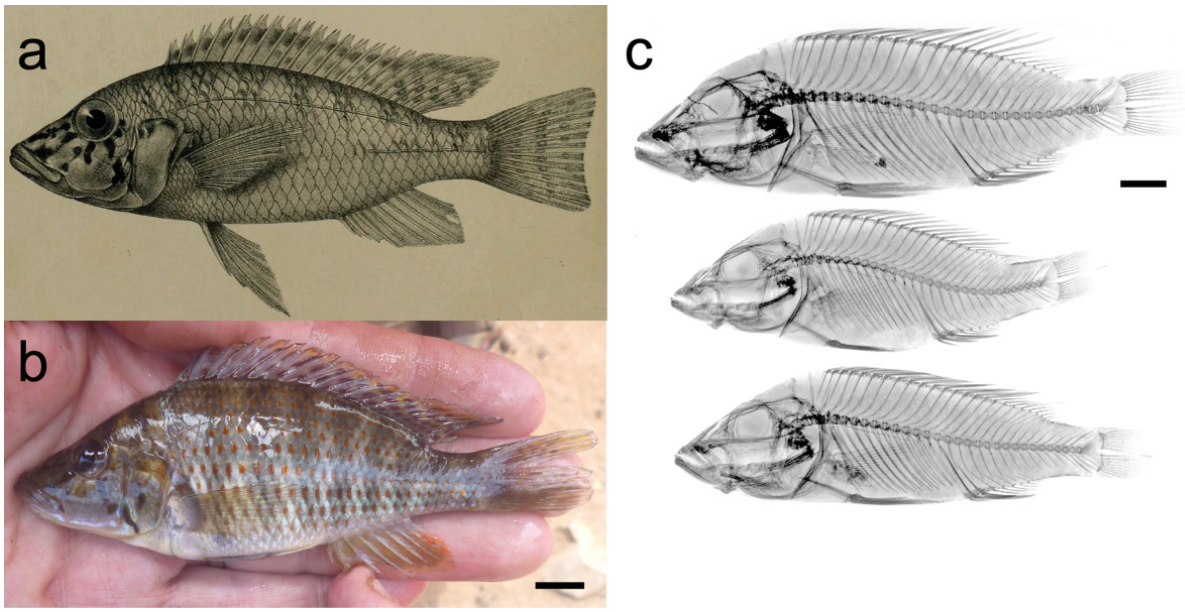

Fig. 5. *Shuja horei* gen. et comb. nov. **a**. Illustration from the original type specimen (Günther 1894). **b**. A freshly caught specimen collected in 2016 from the Malagarasi River, Ilagala (BMNH 2021.7.15.14). **c**. Radiographs of the type series (syntypes) from the Natural History Museum (BMNH 1889.1.30.13–15). Note the prognathous jaw that distinguishes *Shuja* Genner, Ngatunga & Turner gen. nov. from other genera within the Tropheini Poll, 1986. Radiographs from the Natural History Museum, London (Creative Commons Attribution License (CC BY 4.0)). Scale bars: 10 mm.

Material examined

Syntypes

TANZANIA • 3 individuals; near Ujiji (Boulenger 1915), Lake Tanganyika; BMNH 1889.1.30.13–BMNH 1889.1.30.15 (Fig. 5C).

Additional material

TANZANIA • 1 individual; Malagarasi River, Ilagala; 28 Jul. 2016; BMNH 2021.7.15.14 (Fig. 5B).

Distribution

Lake Tanganyika and proximate rivers.

Tribe Haplochromini Poll, 1986

Genus ***Ctenochromis*** Pfeffer, 1893

Type species

Ctenochromis pectoralis Pfeffer, 1893 (by original designation).

Diagnosis

Ctenochromis is a haplochromine cichlid genus restricted to species with the combination of four key characters, following Greenwood (1979): 1) "The abrupt size transition between the very small chest scales and the larger scales on the ventrolateral aspects of the anterior flanks", 2) "a naked area on either side of the chest", 3) "a failure of cheek squamation to reach the ventral margin of the cheek", and 4) "anal fin markings of male fishes are in the form of one or two (rarely three) non-ocellate spots".

Remarks

As noted by Greenwood (1979: 288), non-ocellate spots are "without a dark margin or clear surround". In Greenwood (1979) the significance of the non-ocellate male egg spots in the diagnosis of *Ctenochromis* is unclear, hence a rediagnosis has been provided here. Using the four diagnostic characters for *Ctenochromis* listed above, the genus currently includes only *C. pectoralis* and *Ctenochromis scatebra* sp. nov. described herein.

Of Greenwood's (1979) five species of *Ctenochromis*, namely *C. pectoralis*, *C. horei*, *C. luluae*, *C. oligacanthus* and *C. polli*, Greenwood notes that three species have non-ocellate egg spots but did not specifically mention which species, although it is likely that Greenwood considered his *C. polli* to have this trait, given the mention of a colour photograph of the species in Voss (1977: 74). Based on photographs of field collected specimens, or specimens kept in aquaria, we are aware of only one of Greenwood's five species that unquestionably possesses non-ocellate egg spots, namely the type species *C. pectoralis* (Fig. 6). Aquarium specimens of Greenwood's *C. horei* (herein *Shuja horei* gen. et comb. nov.) are clearly in possession of ocellate egg spots (see Konings 2015). In contrast to Greenwood, we consider Voss (1977: 74) to show a specimen of Greenwood's *C. polli* with an ocellate spot, as does a photograph in Lamboj (2004: 212). There is a photograph of a specimen of *C. luluae* in Lamboj (2004: 211) with ocellate eggspots. We are unaware of any unambiguous evidence of the precise egg spot characteristics of Greenwood's *C. oligacanthus*. Hence, we follow Daget *et al.* (1991) and Fricke *et al.* (2021) in assigning Greenwood's *C. polli*, *C. luluae* and *C. oligacanthus* to the catch-all genus *Haplochromis*, as *Haplochromis polli*, *Haplochromis luluae* and *Haplochromis oligacanthus*, respectively. These three taxa will require a comprehensive taxonomic evaluation. In addition, the specimens we observed of *Shura horei* gen. et comb. nov. do not possess the "naked area on either side of the chest" characteristic of *Ctenochromis*, but instead have a single scaleless area at the anterior of

the chest. This single scaleless area at the anterior of the chest is also shared with the phylogenetically proximate *Gnathochromis pfefferi*.

Ctenochromis pectoralis Pfeffer, 1893
Figs 6a–d, 7a–f, 8a–b

Tilapia pectoralis – Boulenger 1899: 130.
Haplochromis pectoralis – Regan 1922a: 685. — Jordan 1923: 219. — van Oijen *et al.* 1991: 161.

non *Harpagochromis pectoralis* – Kaufman 1996: 1.

Material examined

Lectotype
TANZANIA • Korogwe; 1888; F. Stuhlmann leg.; ZMH, ZMH402.

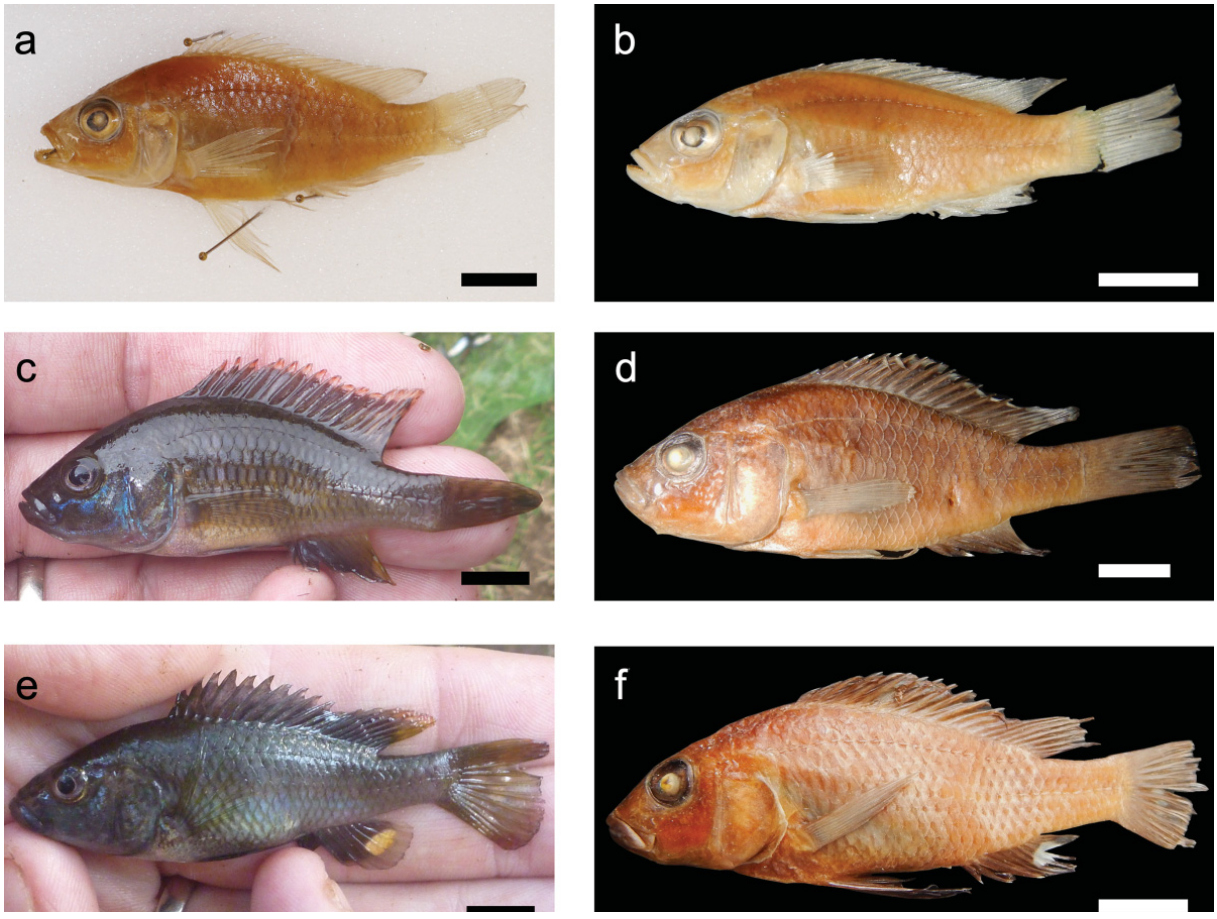

Fig. 6. a. *Ctenochomis pectoralis* Pfeffer, 1893, lectotype ZMH402 from Korogwe (imaged by Thilo Weddehage). **b**. *C. pectoralis*, paralectotype BMNH 1899.2.27.1 from Korogwe. **c**. *C. pectoralis*, ♂ from Ruvu River shortly after capture (part of BMNH 2021.7.15.1-3). **d**. *C. pectoralis*, ♂ from Ruvu River preserved state (part of BMNH 2021.7.15.1-3). **e**. *C. scatebra* Genner, Ngatunga & Turner sp. nov., ♂ from Chemka Springs shortly after capture (part of BMNH 2021.7.15.1-3). **f**. *C. scatebra* sp. nov. holotype BMNH 2021.7.15.4. Scale bars: 10 mm.

Paralectotypes

TANZANIA • 3 individuals; Korogwe; 1888; F. Stuhlmann leg.; ZMH403 1–3 • 1 individual; Korogwe; 1888; F. Stuhlmann leg.; BMNH 1899.2.27.1.

Additional material

TANZANIA – **Ruvu River** (between Lake Jipe and Nyumba ya Mungu Reservoir) • 3 specimens; 14 Aug. 2015; M. Genner, A. Shechonge, A. Smith and B.P. Ngatunga leg.; BMNH 2021.7.15.1–BMNH 2021.7.15.3.

Distribution

Pangani River system, specimens only known from the Korogwe, Nyumba ya Mungu Reservoir, and the Ruvu River (between Lake Jipe and Nyumba ya Mungu Reservoir).

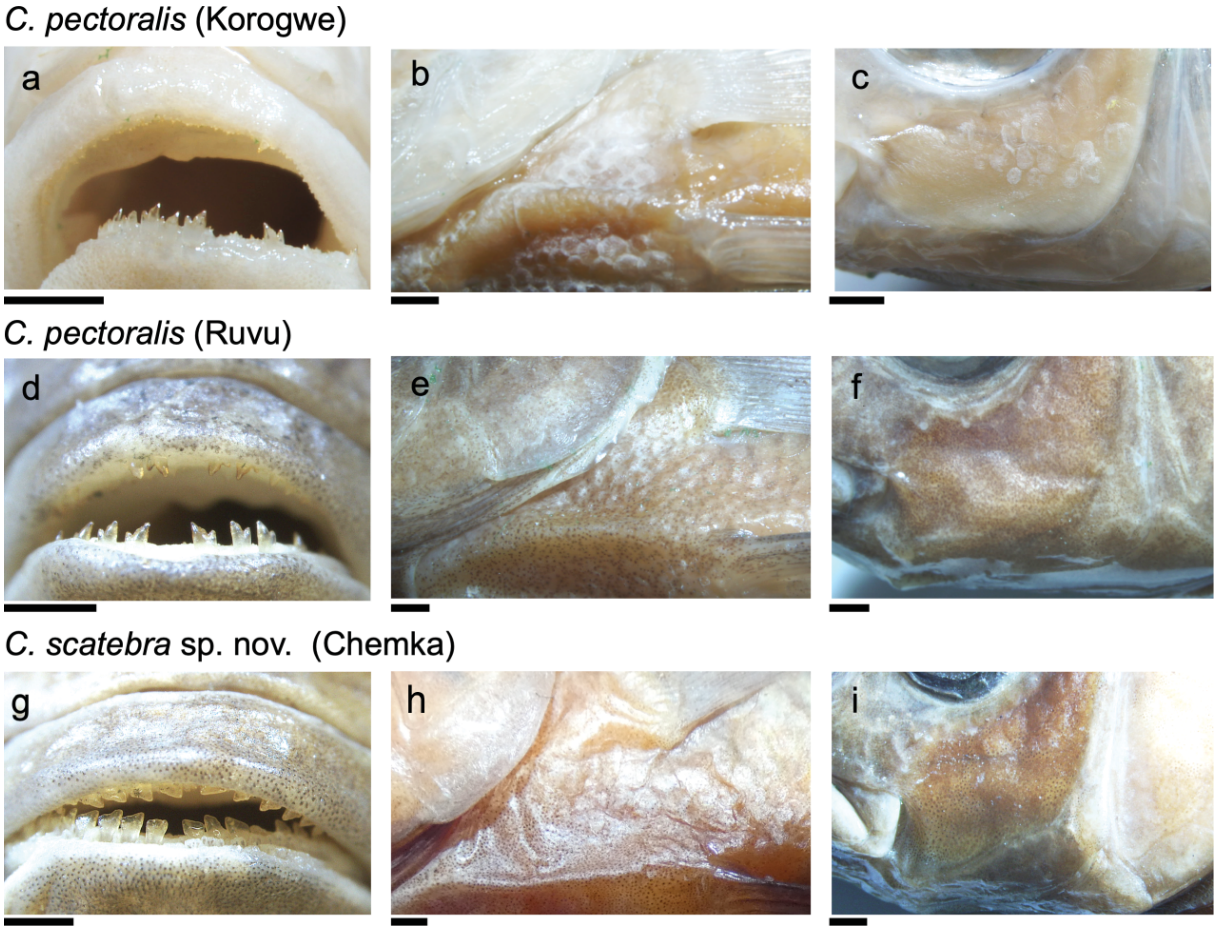

Fig. 7. Morphology of *Ctenochromis* Pfeffer, 1893. **a, d, g**. Oral teeth. **b, e, h**. Chest squamation illustrating scale-free patches. **c, f, i**. Cheek squamation illustrating the reduction in scale number towards the ventral section of the cheek. a–c. *Ctenochomis pectoralis* Pfeffer, 1893 from Korogwe (paralectotype BMNH 1899.2.27.1); d–f. *C. pectoralis* from the Ruvu River (part of BMNH 2021.7.15.1-3); g–i. *C. scatebra* Genner, Ngatunga & Turner sp. nov. from Chemka Springs (holotype BMNH 2021.7.15.4). Scale bars: 1 mm.

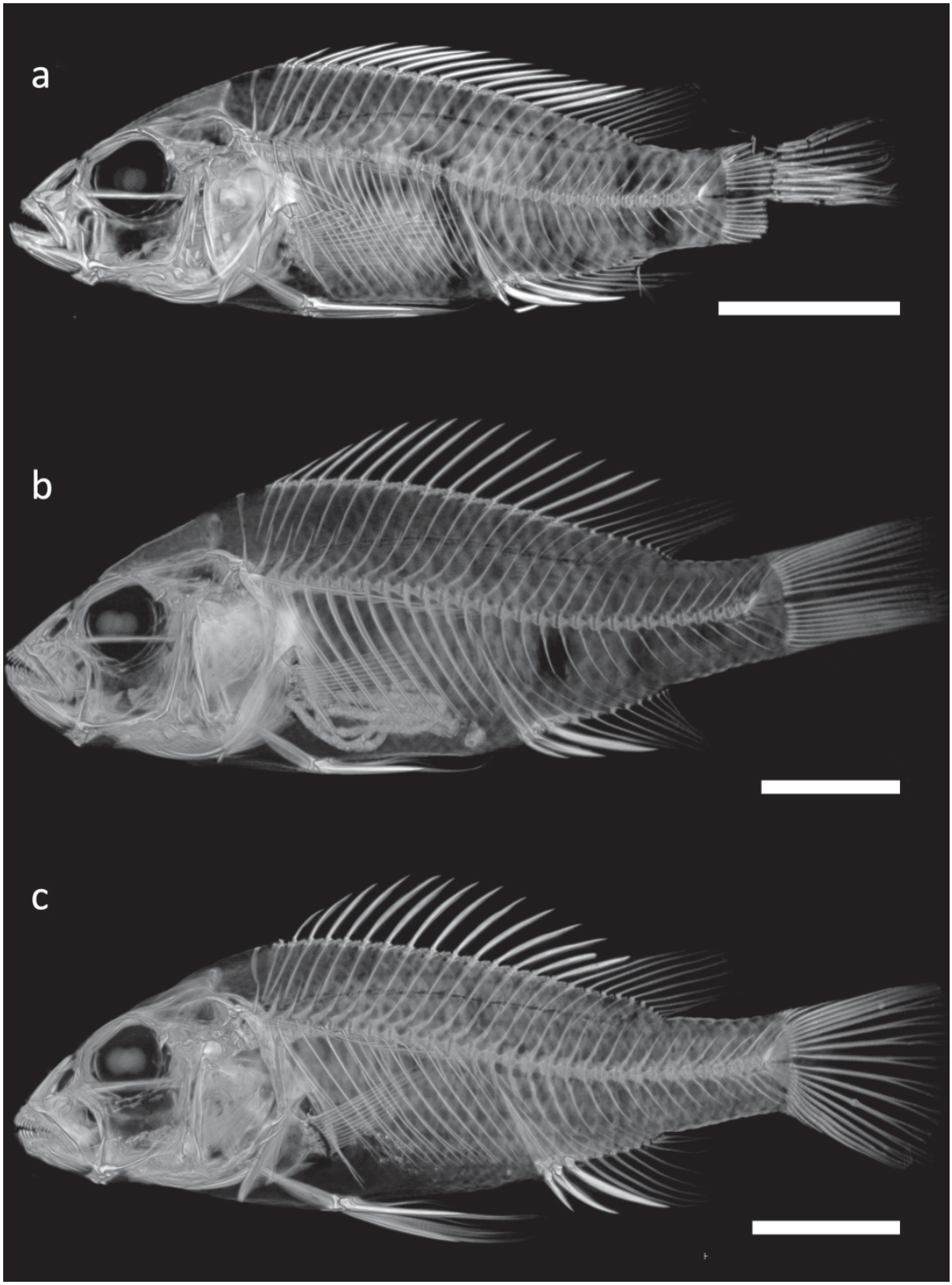

Fig. 8. Morphology of *Ctenochromis* Pfeffer, 1893, imaged using x-ray tomography micro CT. **a**. *Ctenochomis pectoralis* Pfeffer, 1893, paralectotype BMNH 1899.2.27.1 from Korogwe. **b**. *C. pectoralis* from the Ruvu River (part of BMNH 2021.7.15.1-3). **c**. *C. scatebra* Genner, Ngatunga & Turner sp. nov., holotype BMNH 2021.7.15.4. Scale bars: 10 mm.

Ctenochromis scatebra Genner, Ngatunga & Turner sp. nov.
urn:lsid:zoobank.org:act:8B105DD6-6E73-4679-81D1-1A63CF668E5C
Figs 6e–f, 7g–i, 8c

Ctenochromis pectoralis – de Graaf 2011: 38 (specimens from Chemka Springs). — van Heusden 2015: 24–27, 29 (part, specimens from Chemka Springs). — Schedel *et al.* 2019: 27–30. — Carleton *et al.* 2020: 4961, 4964, fig 2.
Ctenochromis sp. – Kalacska *et al.* 2017: 4–6,18, fig. 2g–j.

Diagnosis

Ctenochromis scatebra sp. nov. is recognised as a member of *Ctenochromis*. This is because it possesses the diagnostic feature of a sharp break from small anterior scales to large posterior scales between the pectoral and pelvic fins, and it possesses scaleless areas on either side of the chest (Greenwood 1979). In *C. scatebra* sp. nov. squamation is absent from the ventral part of the cheek, which is characteristic of the genus *Ctenochromis.* Mature adult male *C. scatebra* sp. nov. possess at least one clear non-ocellate egg spot on the anal fin.

Etymology

The species is named from the Latin noun 'scatebra', meaning 'spring' or 'a gush of water from the ground', referring to the type locality which is a spring in northern Tanzania.

Material examined

Holotype

TANZANIA • ♂ (54.9 mm SL); Chemka Springs; 3.443° S, 37.194° E; 17 Aug. 2015; BMNH 2021.7.15.4 (Figs 6f, 7g–i, 8c).

Paratypes

TANZANIA • 9 individuals (between 33.8 and 59.1 mm SL); same collection data as for holotype; BMNH 2021.7.15.5–BMNH 2021.7.15.13.

Description

Holotype and paratype measurements in Table 3. Body laterally compressed, deeper than wide. Head (lateral view) slightly convex between eye and dorsal fin. Snout straight in lateral view, rounded in dorsal view. Mouth retrognathus. Lips slightly thickened, equally developed. Teeth in outer row primarily unicuspid, widened (shovel shaped), often slanted. Side teeth in outer row unequally bicuspid and pointed. Teeth in inner rows small, in fleshy tissue. Pectoral fin origin above dorsal fin origin, pelvic fin origin slightly more anterior. Caudal-peduncle longer than deep (caudal-peduncle depth 62.0–83.4% of caudal-peduncle length). Scales ctenoid on flanks. Scales cycloid on head, between pectoral fin and anal fin, along dorsal-fin base. Scales absent from chest. Lateral-line scales 15–21/7–11, Dorsal fin XIV–XV, 8–9, Anal fin III, 7–8.

Colour

Live colouration from images of live specimens in natural habitat (Schedel 2019). Mature males: dorsal body grey-blue, flanks lighter than dorsal with blueish sheen. Depending on mood, a very faint midline stripe and 4–5 very faint vertical bars present. Head dark grey-blue, blue sheen below and posterior to eye. Blue tinge to lower lip. Dorsal fin grey-blue with orange-red lappets, red posteriorly. Pectoral fins black. Pelvic fins with red base. Anal fin grey/blue, red posteriorly, with one or two (rarely three) non-ocellate egg spots (multiple spots tightly packed). Caudal fin light grey-blue, with red tinges at the dorsal and ventral tips. Euthanised fish: colours darker (Fig. 6e). Females and subadult males: flank

grey-brown base colour, white ventrally. Fins uniformly light grey-brown. Flank with 6–8 irregularly shaped and irregularly spaced vertical bars, alongside partially complete midlateral and dorsolateral stripes. Bar and stripe patterns variable among individuals, faded in some specimens (photo in van Heusden 2015). Preserved coloration: in ethanol brown or beige. Male non-ocellate egg spots on anal fin sometimes visible.

Distribution

The species is restricted to Chemka Springs and the surrounding water bodies immediately adjacent to the Springs. Water from Chemka Springs flows southwards into the Kikuletwa River towards Nyumba ya Mungu Reservoir. Surveys are needed further downstream from the site of the spring, into the river, to determine the full species distribution.

Life history

The species has been observed feeding upon epilithic and epiphytic algae in Chemka Springs, as well as sifting soft sediment (Schedel 2019), and pecking on skin of swimmers. The species is therefore most likely an omnivorous generalist. Only two other fish species are known from Chemka Springs, *Garra* cf. *dembeensis* (Rüppell, 1835) and *Clarias gariepinus* (Burchell, 1822). The water maintains a steady 28.4°C (Røhr *et al.* 2002).

Remarks

Phylogenetic analyses, based on genome-wide genetic markers, place *C. scatebra* sp. nov. as a sister to the type species *C. pectoralis* (Fig. 4). Specimens of *C. scatebra* sp. nov. can be distinguished from *C. pectoralis* based on two aspects of trophic morphology: 1) *C. scatebra* sp. nov. has front teeth in the outer row on both jaws that are primarily unicuspid, widened (shovel shaped) and often slanted (Fig. 7g), while side teeth in the outer row are unequally bicuspid and pointed; by contrast all front and side teeth in the outer row of *C. pectoralis* are all unequally bicuspid and pointed (Fig. 7a, d); 2) *Ctenochromis scatebra* sp. nov. has a retrognathus jaw, while *C. pectoralis* has a marginally prognathous jaw (Figs 6, 8).

Discussion

We transferred *C. horei* to the new genus *Shuja* gen. nov. Assuming the recognition of *Trematochromis benthicola* as valid (e.g., Konings 2015), this ensures that there are no longer any *Ctenochromis* spp. within Lake Tanganyika. Additionally, with *C. luluae*, *C. polli* and *C. oligacanthus* placed in the catch-all genus *Haplochromis* alongside other Congolese haplochromines (including the plausibly related *Haplochromis stigmatogenys*), then *Ctenochromis* is now restricted to coastal rivers of East Africa. The formal status of those species assigned to genus *Haplochromis* now requires investigation and resolution.

A further haplochromine population potentially related to *C. pectoralis* and *C. scatebra* sp. nov. is known from Mzima Springs, in the Tsavo River catchment of Kenya (see Fig. 1); referred to as *C.* aff. *pectoralis* (Okeyo 1998) and *C. pectoralis* (Seegers *et al.* 2003). It has been suggested that this species may be distinct from *C. pectoralis* (Seegers *et al.* 2003), but to date we are unaware of any morphological or molecular systematic work that has included this Mzima Springs population. It would be valuable to resolve the systematics of these relative to *C. pectoralis* and *C. scatebra* sp. nov. Notably, the Tsavo River catchment is directly adjacent to the Pangani River catchment, perhaps enabling mixing of fish assemblages during historic periods of flooding.

The type material of *C. pectoralis* was collected from the Pangani River at Korogwe in 1888. We are unaware of any subsequent collections in the Korogwe area, and no habitat notes are included in

the original description. If the type population is ecologically similar to the population in the Ruvu River, it is most likely that the species will be present in slow flowing shallow and vegetated riverine environments (Fig. 2). Focused surveys would be useful to establish whether the species continues to persist at the type locality. We (MJG, BPN, AS) surveyed multiple waterbodies in the lower Pangani River catchment in 2015, and recovered only two species of haplochromine cichlids, the native *Astatotilapia bloyeti* and non-native *Astatoreochromis alluaudi* Pellegrin, 1904. The latter was historically introduced beyond its native range in Tanzania for snail control (Bailey 1966). Currently the IUCN assessment of *C. pectoralis* conducted in 1996 lists the species as Extinct (Kaufman 1996). It is unclear from the IUCN document precisely what evidence was used to justify the classification, but our analyses of the specimens collected from the Ruvu tributary of the Pangani River in 2015 strongly suggest the species is extant at that location, at least.

The results of our analyses of genetic data, both ddRAD and mtDNA, showed *C. pectoralis* (from the Ruvu River and Nyumba ya Mungu Reservoir) and *C. scatebra* sp. nov. were reciprocally monophyletic, with strong genetic differentiation between the species. It is possible that this differentiation has been driven exclusively by geographic separation of the populations. We are unaware of any present-day physical barriers to movement, such as rapids or waterfalls, but plausibly these may exist, or have existed historically, prior to the hydrological changes linked to the formation of the Nyumba-ya-Mungu Reservoir. The genetic divergence could also have resulted from limited gene flow due to ecological differences. Chemka Springs is a strikingly different environment to both the Ruvu River and Nyumba-ya-Mungu, in terms of the water chemistry, food resources, and co-occurring fish species. It is therefore plausible that these different environments have promoted divergent adaptation to these environmental regimes, which in turn has promoted restricted admixture irrespective of any geographic distance.

Notably, the IUCN assessment states *Ctenochromis pectoralis* to be synonymous with a taxon referred to as "*Harpagochromis pectoralis*". The latter, however, almost certainly refers to the Lake Victoria species currently considered by Fricke *et al.* (2021) to be valid as *Haplochromis squamulatus* Regan, 1922. This species from Lake Victoria was originally described as *Paratilapia pectoralis* Boulenger, 1911 but placed in *Haplochromis* by Regan (1922b) alongside the Pangani species described as *Ctenochromis pectoralis*. Since the specific epithet was already occupied by the species originally described as *Ctenochromis pectoralis*, Regan gave the Victorian taxon the new specific epithet *Haplochromis squamulatus*, although Greenwood (1980) referred to this taxon as *Harpagochromis pectoralis* (Boulenger, 1911). Given the clear distinction between *Ctenochromis pectoralis* and *Haplochromis squamulatus*, these taxa should avoid being conflated in future IUCN assessments.

Despite evident differences in morphometric measurements between the *C. pectoralis* specimens from the type locality in Korogwe and the population within the Ruvu River joining Lake Jipe to Nyumba ya Mungu, we have considered them conspecific. This is because of a lack of clear diagnostic characters that separate the populations, based on the material we studied at least. Consequently, the known extant range of *C. pectoralis* is the Ruvu River and neighbouring Nyumba ya Mungu (Koblmüller *et al.* 2008; De Graaf 2011), therefore making this a narrow endemic species. Seegers *et al.* (2003) also lists the species from Lake Jipe, suggesting a potentially broader distribution, but we did not encounter the species during a survey of littoral habitat on the Tanzania side of Lake Jipe in 2015 (MJG, BPN, AS). In contrast to *C. pectoralis*, *Ctenochromis scatebra* sp. nov. should be considered a point endemic, until surveys of the proximate water bodies are conducted. Collectively, due to these narrow ranges, both species are vulnerable to potential habitat change, including encroaching agriculture such as palm plantations (Kalacska *et al.* 2017). Notably, Chemka Springs is a tourist attraction in the region, and there are consequent risks if the site would be further developed for visitors. We suggest updating the IUCN Red List, to include the new species *C. scatebra* sp. nov., and revise the status of *C. pectoralis* based on updated information provided here.

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Conflicts of interest

The authors declare no conflicts of interest

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Appendix 1

Adapter barcode and PCR index sequences.

Adapter stock barcode	
GCATG_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATG-3’
AACCA_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACCA-3’
CGATC_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATC -3’
TCGAT_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAT -3’
TGCAT_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAT -3’
CAACC_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAACC -3’
GGTTG_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTG -3’
AAGGA_EcoRI_1.1	5’- ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGA -3’
GCATG_EcoRI_1.2	5’-[PHO]AATTCATGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT -3’
AACCA_EcoRI_1.2	5’-[PHO]AATTTGGTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
CGATC_EcoRI_1.2	5’-[PHO]AATTGATCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TCGAT_EcoRI_1.2	5’-[PHO]AATTATCGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TGCAT_EcoRI_1.2	5’-[PHO]AATTATGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
CAACC_EcoRI_1.2	5’-[PHO]AATTGGTTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GGTTG_EcoRI_1.2	5’-[PHO]AATTCAACCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
AAGGA_EcoRI_1.2	5’-[PHO]AATTTCCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
MspI_2.1	5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3’
MspI_2.2	5’-[PHO]CGAGATCGGAAGAGCGAGAACAA-3’
Primer Index	
PCR1	5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG-3’
PCR2_Idx_1	5’-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_2	5’-CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_3	5’-CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_4	5’-CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_5	5’-CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_6	5’-CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_7	5’-CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_8	5’-CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_9	5’-CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_10	5’-CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_11	5’-CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGC-3’
PCR2_Idx_12	5’-CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGC-3’