



## A new species of cyprinoid fish from the Tana River, Kenya (Actinopterygii: Danionidae)

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### Abstract

Sampling of streams in the middle reaches of the Tana River Basin in Meru National Park, Kenya, from 2010 to 2012 for an NSF-funded International Research Experiences for Students (IRES) project, resulted in the capture of a number of specimens of what were first thought to be *Neobola fluviatilis*. On closer examination the specimens were determined to represent a distinct species, endemic to the Tana River basin, which is herein formally described. The new species is readily diagnosed from *N. fluviatilis* by higher counts of lateral line, pre-dorsal, and caudal peduncle circumferential scales, higher numbers of pectoral rays, lower numbers of anal fin rays, and a shorter anal-fin base length.

**Key words:** *Neobola fluviatilis* (Whitehead 1962), *Neobola kinondo* sp. nov., Cyprinoidei, East Africa

### Introduction

The genus *Neobola* Vinciguerra, as currently recognized comprises five valid species endemic to East-central Africa: *N. bottegoi* Vinciguerra, from the Omo and Webei Shebeli rivers of Ethiopia; *N. fluviatilis* (Whitehead 1962), from the Athi and Tana rivers of Kenya; *N. moeruensis* (Boulenger 1915), from Lake Mweru in the Democratic Republic of Congo; *Neobola nilotica* Werner (1919), from the White Nile River in Sudan; and *Neobola stellae* (Worthington 1932), endemic to Lake Turkana in Ethiopia and Kenya. *Neobola fluviatilis* was originally described as *Engraulicypris fluviatilis* based on six adults (the Holotype, BMNH 1961.5.3.1, 73 MM SL; five Paratypes, BMNH 1961.4.3.2-6, 61-73 mm SL) collected from the Athi River near Kithimani, Kenya (Whitehead 1962). The description references two specimens caught in a floodwater pool in the Tana River at Garissa but provides no information on the whereabouts of these specimens. Howes (1980) restricted *Engraulicypris* Günther (1894) to the type species, *E. sardella*, and re-assigned *Engraulicypris fluviatilis*, and six other species to genus *Neobola* Vinciguerra (1895).

A project funded by the U.S. National Science Foundation's International Research Experiences for Students program in 2010, supported field work by the authors and teams of Kenyan and U.S. students in rivers of central and western Kenya from 2010-2012. Samples taken from streams of the Tana River Basin in Meru National Park yielded a number of specimens that were thought to represent the Tana River population of *N. fluviatilis* (Whitehead 1962). On closer inspection, however, the specimens were found to show marked differences from the description of *N. fluviatilis*. In this paper, we describe the *Neobola* form in Meru National Park as a new species. We diagnose the new species from *N. fluviatilis* and other recognized species of *Neobola*. We also discuss the conservation status of the two *Neobola* species from central Kenya.

### Methods

Study specimens were obtained from the following institutions (symbolic codes in parentheses): Natural History

Museum, London (formerly British Museum of Natural History, BMNH); National Museums of Kenya (NMK); and Tulane University (TU).

Body measurements were made to the nearest millimeter using digital calipers. The measurements were expressed as proportions of either standard length or head length and the resulting proportions were tested for significant differences using non-parametric, Mann-Whitney U tests, a rank-sum test that makes no assumptions about underlying data distributions. Bonferroni corrections were made to the p values based on the number of tests performed. Principal Components Analysis was performed on log-transformed measurements, using the covariance matrix, in MYSTAT (SYSTAT Software Inc.). Allometric correction via reduced-major axis (RMA) regression was implemented in the SMATR package of R (Warton *et al.* 2006). This method examines the relationship between principal components strongly correlated to size (*e.g.*, PC1) and standard length. It determines if allometric scaling (*i.e.* slopes) of the regression lines are similar for each group, and if so, tests whether the elevations (*i.e.*, y-intercepts) are different among groups. Different y-intercepts suggest that other shape variables, in addition to size, are contributing to the variation in PC1, in which case this component should be corrected and retained. Retaining PC1 was shown to be informative for distinguishing recently diverged, cryptic species in two recent studies (Sidlauskas *et al.* 2011; Schmidt *et al.* 2019). Differences in PC2 scores for each group were tested with ANOVA in MYSTAT.

Meristic methods follow Skelton *et al.* (1980) except as noted below. Data were gathered for the following scale counts: lateral line scales, pored lateral line scales, scales above lateral line (to dorsal fin), scales below lateral line (to anal fin), transverse scales (combination of the counts above and below the lateral line plus the lateral line scale), and scales around the caudal peduncle. Fin ray counts are reported three ways: as fin ray formulae (in description), including both simple and branched rays; principal fin rays (in tables), including only the last simple ray (the first full-length ray of the count); and all branched rays with the last two branched rays of the dorsal and anal fins counted as a single ray if the rays converge at their bases. Data were also gathered for the following fin ray counts: dorsal fin rays, anal fin rays, pectoral fin rays, pelvis fin rays, and caudal fin rays.

## Results

### *Morphometrics*

Statistical comparisons of 16 body proportions adjusted for either standard length or head length (Mann-Whitney U tests using a Bonferroni corrected  $\alpha = 0.007143$ ) revealed that the new species has significantly longer means for pre-anal length, pectoral fin length, caudal peduncle depth, and body depth at dorsal fin proportions than *N. fluviatilis*, and a significantly shorter anal fin base length proportion than *N. fluviatilis* (Table 1).

The plot of PC1 vs. PC2 from Principal components analysis of 19 log-transformed body measurements from 43 specimens shows clear separation between the new species and *N. fluviatilis* (Fig. 1A). Principal component 1 is highly correlated with body size (Pearson's correlation = 0.994), but the RMA regression plot of PC1 to Log SL revealed that there is no difference in the allometric trajectories for each of the species groups (Fig. 1B; p-value = 0.1106) but there is a significant difference in the y-intercepts (Fig. 1B; p-value = 0.0219). This suggests that PC1 contains significant variation in body shape factors other than size in the two species and therefore should be retained in the analysis. Principal component 2 is not correlated with size (Pearson's correlation = 0.009) and an ANOVA of PC2 revealed significant differences between the two species (p-value = 0.028). Anal-fin base length, anal fin length, dorsal-fin base length, snout length and caudal peduncle length contribute most to variation in PC2.

### *Neobola kinondo* sp. nov. (Figs. 2A, 4, 7)

*Engraulicypris fluviatilis* (in part), Whitehead 1962:100 (Distribution, Tana River).

*Engraulicypris fluviatilis* (in part), Howes, 1984:156 (Distribution, Tana River).

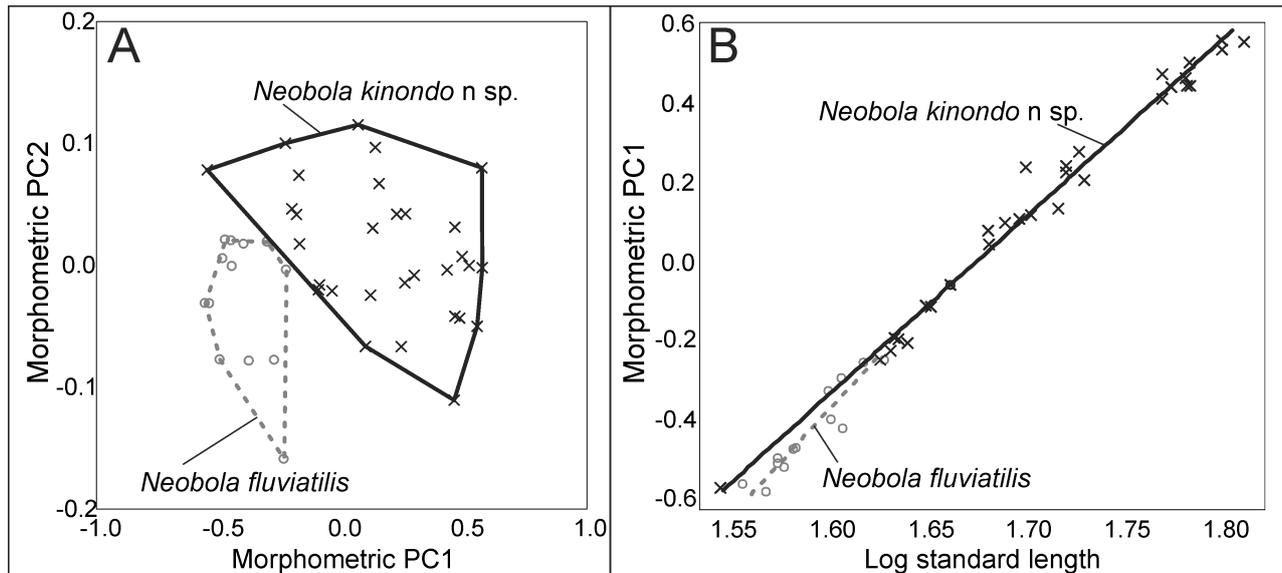
*Engraulicypris fluviatilis* (in part), Lévêque & Daget 1984:326 (Distribution, Tana River)

*Engraulicypris fluviatilis* (in part), Seegers *et al.* 2003:34 (Distribution, Tana River)

Holotype: NMK FW/4810, 44.6 mm SL, Nuptial male, (Fig. 2A), Rojewero River at Kenmare Campsite, Meru National Park, Tana River Basin, Kenya, 14 June 2012.

Paratypes: NMK FW/2786/2-24, Allotype 64.8 mm SL (Fig. 2B) and 22 other paratypes collected with Ho-

lotype. NMK FW/3407/1-24, TU 201866 (8 ex NMK FW/3407/1-32), Mutundu River at low-water bridge, Meru National Park, Kenya, (0.16488333, 38.1880833), 22 July 2010.



**FIGURE 1.** Plot of PC1 vs. PC2 from principal component analysis of 19 log-transformed measurements from 43 specimens of *Neobola* representing the Athi and Tana rivers (A). Reduced-major axis regression of PC1 from morphometrics on log standard length for the two *Neobola* species (B). Trend lines are shown for each group; slopes of groups are equal ( $p$ -value = 0.1106) and  $y$ -intercepts between the two species are significantly different ( $p$ -value = 0.0219).

**TABLE 1.** Means, minimum and maximum values of body measurements of *Neobola kinondo* and *N. fluviatilis* expressed as proportions of standard or head length, showing Mann-Whitney U test  $z$ -scores,  $p$  values and significance test results using a Bonferroni corrected  $\alpha$  of 0.007143 with significantly different means highlighted in bold type.

|                             | <i>Neobola kinondo</i> |       |       | <i>N. fluviatilis</i> |       |       | Mann-Whitney U test |                    |
|-----------------------------|------------------------|-------|-------|-----------------------|-------|-------|---------------------|--------------------|
|                             | $\bar{x}$              | min   | max   | $\bar{x}$             | min   | max   | $z$ -score          | $p$                |
| Predorsal length/SL         | 0.639                  | 0.591 | 0.669 | 0.632                 | 0.588 | 0.658 | 2.3745              | 0.00889            |
| Preanal length/SL           | <b>0.624</b>           | 0.593 | 0.713 | 0.591                 | 0.569 | 0.713 | 4.6674              | <b>&lt; .00001</b> |
| Pectoral fin length/SL      | <b>0.234</b>           | 0.214 | 0.247 | 0.223                 | 0.205 | 0.247 | 3.0615              | <b>0.00111</b>     |
| Caudal peduncle depth/SL    | <b>0.089</b>           | 0.079 | 0.100 | 0.082                 | 0.074 | 0.100 | 3.6915              | <b>0.00011</b>     |
| Body depth at dorsal fin/SL | <b>0.209</b>           | 0.191 | 0.234 | 0.191                 | 0.182 | 0.234 | 3.8652              | <b>0.00005</b>     |
| Dorsal fin base length/SL   | 0.100                  | 0.079 | 0.126 | 0.109                 | 0.097 | 0.126 | -2.2804             | 0.0113             |
| Anal fin base length/SL     | 0.236                  | 0.215 | 0.271 | <b>0.288</b>          | 0.219 | 0.271 | -3.1329             | <b>0.00087</b>     |
| Head length/SL              | 0.219                  | 0.194 | 0.243 | 0.220                 | 0.211 | 0.243 | 0.1890              | 0.42465            |
| Snout length/HL             | 0.257                  | 0.207 | 0.311 | 0.251                 | 0.193 | 0.311 | 0.3654              | 0.35569            |
| Orbit diameter/HL           | 0.282                  | 0.248 | 0.323 | 0.288                 | 0.244 | 0.323 | -1.2725             | 0.10204            |
| Inter-orbital width/HL      | 0.308                  | 0.250 | 0.355 | 0.307                 | 0.272 | 0.355 | -0.2520             | 0.40129            |
| Caudal peduncle length/SL   | 0.142                  | 0.109 | 0.174 | 0.146                 | 0.093 | 0.174 | -1.2977             | 0.0968             |
| Anal fin height/SL          | 0.154                  | 0.133 | 0.178 | 0.150                 | 0.119 | 0.178 | 0.7034              | 0.24196            |

### Diagnosis

*Neobola kinondo* is readily diagnosed from its presumed closest relative, *N. fluviatilis*, by higher counts of lateral line scales (mode 41, range 38–47,  $\bar{x}$  = 41.83 vs. mode 40, range 37–41,  $\bar{x}$  = 38.22 in *N. fluviatilis*, Table 2), predorsal scales (mode 24, range 20–27,  $\bar{x}$  of 23.90 vs. mode 19–20, range 17–22,  $\bar{x}$  = 19.38 in *N. fluviatilis*, Table 3), and caudal peduncle circumferential scales (mode 14, range 12–16,  $\bar{x}$  = 13.90 vs. mode 13, range 10–13,  $\bar{x}$  = 12.57 in *N. fluviatilis*, Table 4), and lower counts of transverse scales (mode 9, range 7–11,  $\bar{x}$  = 9 vs. mode 10, range 8–10,  $\bar{x}$  = 9.64 in *N. fluviatilis*, Table 5), principal dorsal-fin rays (mode 8, range 7–9,  $\bar{x}$  = 7.93 vs. mode 9, range 8–9,  $\bar{x}$  = 8.63 in *N. fluviatilis*, Table 6) and principal anal-fin rays (mode 18, range 18–23,  $\bar{x}$  = 19.23 vs. mode 22, range

20–24,  $\bar{x} = 22.17$  in *N. fluviatilis*, Table 7). Combining lateral line scales and pre-dorsal scales completely separates *N. kinondo* from *N. fluviatilis*. *Neobola kinondo* has a combined count of 61 or more scales; *N. fluviatilis* has fewer than 61 lateral line and predorsal scales (Fig. 3).

**TABLE 2.** Counts of lateral line scales of *Neobola fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 35-36 | 37-38 | 39-40 | 41-42 | 43-44 | 45-47 | N  | $\bar{x}$ |
|-----------------------|-------|-------|-------|-------|-------|-------|----|-----------|
| <i>N. fluviatilis</i> | 3     | 3     | 9     | 3     |       |       | 18 | 38.22     |
| <i>N. kinondo</i>     |       | 2     | 6     | 12    | 6     | 4     | 30 | 41.83     |

**TABLE 3.** Counts of predorsal scales of *Neobola fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 17-18 | 19-20 | 21-22 | 23-24 | 25-26 | 27 | N  | $\bar{x}$ |
|-----------------------|-------|-------|-------|-------|-------|----|----|-----------|
| <i>N. fluviatilis</i> | 3     | 8     | 2     |       |       |    | 13 | 19.38     |
| <i>N. kinondo</i>     |       | 1     | 7     | 10    | 9     | 2  | 29 | 23.90     |

**TABLE 4.** Counts of caudal peduncle circumferential scales of *Neobola fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 10 | 11 | 12 | 13 | 14 | 15 | 16 | N  | $\bar{x}$ |
|-----------------------|----|----|----|----|----|----|----|----|-----------|
| <i>N. fluviatilis</i> | 1  |    | 3  | 10 |    |    |    | 14 | 12.57     |
| <i>N. kinondo</i>     |    |    | 1  | 9  | 12 | 6  | 1  | 29 | 13.90     |

**TABLE 5.** Counts of transverse scale rows of *Neobola fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 6 | 7 | 8 | 9  | 10 | 11 | N  | $\bar{x}$ |
|-----------------------|---|---|---|----|----|----|----|-----------|
| <i>N. fluviatilis</i> |   |   | 1 | 3  | 10 |    | 14 | 9.64      |
| <i>N. kinondo</i>     |   | 2 | 8 | 10 | 8  | 2  | 30 | 9         |

**TABLE 6.** Counts of principal dorsal fin rays of *Neobola fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 7 | 8  | 9  | N  | $\bar{x}$ |
|-----------------------|---|----|----|----|-----------|
| <i>N. fluviatilis</i> |   | 7  | 12 | 19 | 8.63      |
| <i>N. kinondo</i>     | 3 | 26 | 1  | 30 | 7.93      |

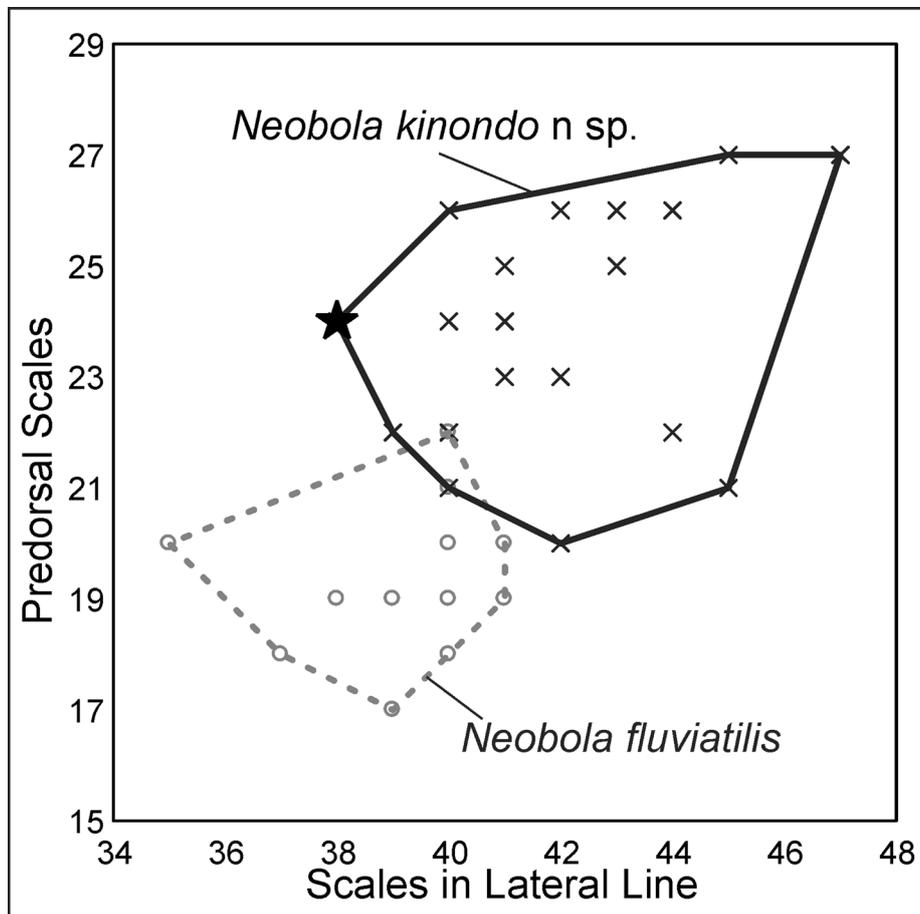
**TABLE 7.** Counts of principal anal fin rays of *N. fluviatilis* and *N. kinondo* with means and numbers of specimens examined.

|                       | 18 | 19 | 20 | 21 | 22 | 23 | 24 | N  | $\bar{x}$ |
|-----------------------|----|----|----|----|----|----|----|----|-----------|
| <i>N. fluviatilis</i> |    |    | 1  | 4  | 6  | 5  | 2  | 18 | 22.17     |
| <i>N. kinondo</i>     | 12 | 8  | 6  | 1  | 1  | 2  |    | 30 | 19.23     |

*Neobola kinondo* differs from *N. bottegi* by its higher numbers of lateral line scales (38–45 vs. 37–40 in *N. bottegi*) and principal anal fin rays (18–23 in *N. kinondo* vs. 14–18 in *N. bottegi*), and a more triangular pectoral axial scale (vs. more lanceolate in *N. bottegi*). *Neobola kinondo* differs from *N. moeruensis* by its higher numbers of principal anal fin rays (18–23 vs. 14 in *N. moeruensis*) and higher caudal peduncle circumferential scales (mode 14 in *N. kinondo* vs. 12 in *N. moeruensis*). *Neobola kinondo* differs from *N. nilotica* by its lower modal numbers of lateral line scales and principal anal fin rays (41 and 18, respectively, vs. 44 and 22, respectively in *N. nilotica*). *Neobola kinondo* is readily distinguished from *N. stellae* by its lower count of gill rakers on the first ceratobranchial (7 vs. 10 in *N. stellae*).



**FIGURE 2** A. Holotype of *Neobola kinondo*, a nuptial male, 44.6 mm SL. B. Paratype (allotype) of *N. kinondo*, a nuptial female, 64.8 mm SL.



**FIGURE 3.** Plot of lateral line scales vs. predorsal scales of *Neobola kinondo* and *N. fluviatilis*, showing the lack of overlap between the species when the two characters are combined.

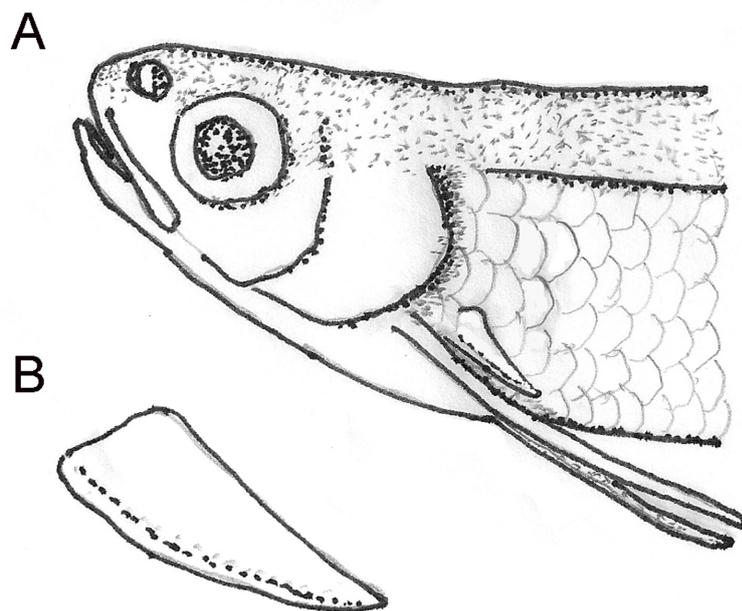
**Description**

*Neobola kinondo* is a species in the Subfamily Chedrinae (Bleeker 1863) of Family Danionidae (Tan & Armbruster 2018). This description of the species is based on 31 adult specimens (35.1–64.8 mm SL) collected at two sites in Meru National Park.

Body compressed, depth at dorsal fin averaging 20.9% of standard length, twice as deep as body width at dorsal fin, which averages 10.3% of SL. Head long, averaging 22% of standard length. Orbit diameter is large averag-

ing 28% of head length, slightly smaller than interorbital width, which averages 31% of head length. Snout short, smaller on average than eye diameter and averaging 26% of head length. Mouth large, upturned and terminal (lips equal), extending roughly to anterior third of orbit. Pre-dorsal and pre-anal distances long and roughly equal, averaging 63.9% and 62.4% of standard length, respectively. Suborbitals broad, covering most of cheek. Scales thin and highly flexible. Gill rakers short, seven on the ceratohyal of the anterior most gill arch. Pharyngeal teeth in two rows, 4.3, dagger like with hooked tips (vs. 4.2 in *N. fluviatilis*, Howes 1984).

Dorsal fin short, averaging 10% of standard length, its origin slightly behind anal fin origin, modally with 8 principal rays, fin ray formula ii, 7. Anal fin long, averaging 23.6% of SL, modally with 18 principal rays, fin ray formula iii, 17. Pectoral fin long, averaging 23.4% of SL, slightly longer than head length and pointed, reaching origin of pelvic fin, modally with 12 principal rays. Pelvic fin length 12.3% of SL, roughly half as long as pectoral fin, modally with 8 principal rays. Caudal peduncle short and narrow, its length averaging 14.2% of SL and depth averaging 8.9% of SL. Caudal fin modally with 19 principal rays, 9 in the upper lobe and 10 in the lower lobe. Pectoral axial scale small averages 31% of pectoral fin length (range 29-35%, N=6), with a scalene triangular shape and a fleshy ventral border (Fig. 4). The pectoral axial scale of *N. fluviatilis* has a similar is triangular shape and insertion behind the pectoral fin.



**FIGURE 4.** A. Drawing of the head and pectoral region of the Holotype of *Neobola kinondo*, showing the location of the pectoral axial scale, medial to the pectoral fin. B. Drawing of a removed pectoral axial scale of *N. kinondo*, showing its scalene triangular shape and fleshy ventral border.

### **Coloration**

In life, dorsum light brown, with intense silver to white on cheeks, operculum and sides from second scale row on dorsum to ventral body margin. Dorsal and pectoral fins unpigmented; pelvic, anal and lower lobe of caudal fin yellow-orange (Fig. 5). In preservative, the dorsum ground color is brown lightening to taupe on the upper sides, with a dusting of brown melanophores and myomeres clearly visible through the skin. A distinct lateral stripe, narrow anteriorly and broadening posteriorly, separates the dorsum from the portion of the sides that is intensely silver in life. The silver color on the cheeks, operculum and sides fades to white in preservative. The fins of preserved specimens are colorless and translucent. The colorless fins and intense silver to white color on the sides are characters shared by *N. fluviatilis* (Whitehead 1962).

### **Distribution**

*Neobola kinondo* is confined to the Tana River of Kenya (Fig. 6). It is known primarily from tributaries of the Tana in Meru National Park and likely also occurs in portions of the Tana River proper bordering the park. The only other record of the species is based on a single juvenile specimen collected from a locality on the lower Tana River near

the Hola Concentration Camps, Tana River County (BMNH 1966.8.25.6), suggesting that the species also inhabits lower portions of the Tana River Basin.



**FIGURE 5.** Photograph of *Neobola kinondo* showing body coloration in life. Note the yellow pigment in the pelvic fin, anal fins and lower lobe of the caudal fin.

### **Ecology**

We collected *N. kinondo* in a variety of habitats in three small rivers in Meru National Park: the Mutundu River, Rojewero River and Ura River. All three sites had swiftly flowing water with large rock outcrops that formed small waterfalls. The Mutundu River specimens were captured over sand and mud bottom in a sluggish pool formed by a road built atop a rock outcrop. Specimens from the other two sites were captured in swift flowing water over rock or sand bottoms. Stomachs dissected from a few specimens were found to contain chironomid larvae, ants, mayflies and various body parts of winged-adult stages of unidentified dipterans.

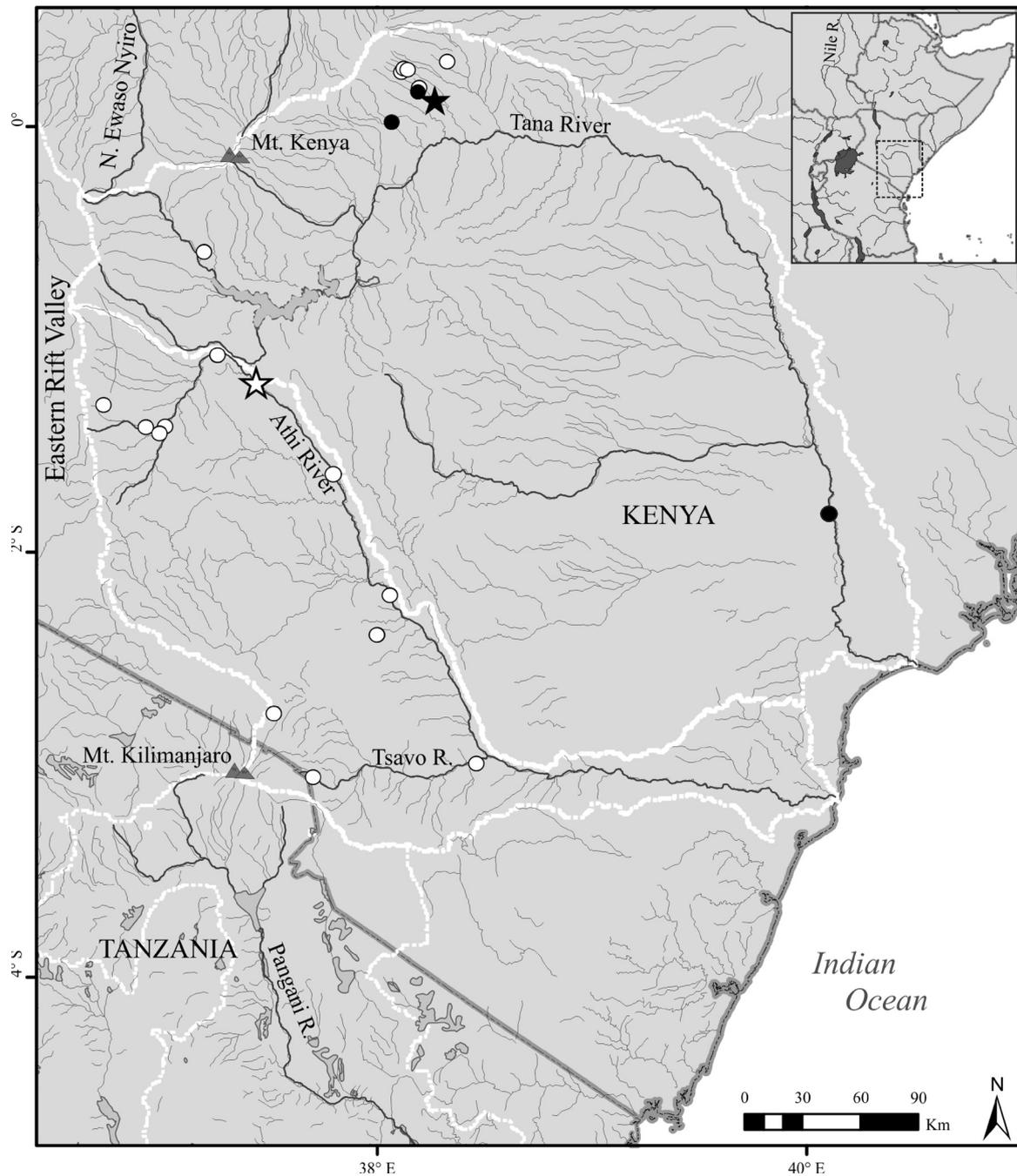
Specimens collected in June are in nuptial condition. Males have breeding tubercles on the top of head, underside of jaws, cheeks, and operculum (Fig. 7), with fine tubercles on the pectoral fin and the ventral sides of the body. Nuptial males also have an orange patch of pigment in the middle of the lower lobe of the caudal fin. Females collected in June have enlarged abdomens and fine tubercles on the underside of the jaws, cheeks and top of the head. A 53 mm SL female had mature, yolked (yellow) ova in its ovaries, suggesting that spawning was imminent if not already occurring. This activity corresponds to the second rainy season in East Africa, which occurs from May through early July. Nuptial females are larger than males; of 21 specimens that were sexed (12 females, 9 males), females averaged 53.7 mm SL (range 44.9–64.8 mm SL); whereas males averaged 42.8 mm SL (range 35.1–48 mm SL).

### **Etymology**

The specific epithet of the new species “kinondo” is the Ameru language word for “silver” and is in reference to the bright silver color of the sides of *N. kinondo*. Species of *Neobola* are commonly referred to as sardines because of their sardine-like appearance. Thus, we suggest the common name, Tana Sardine.

### **Discussion**

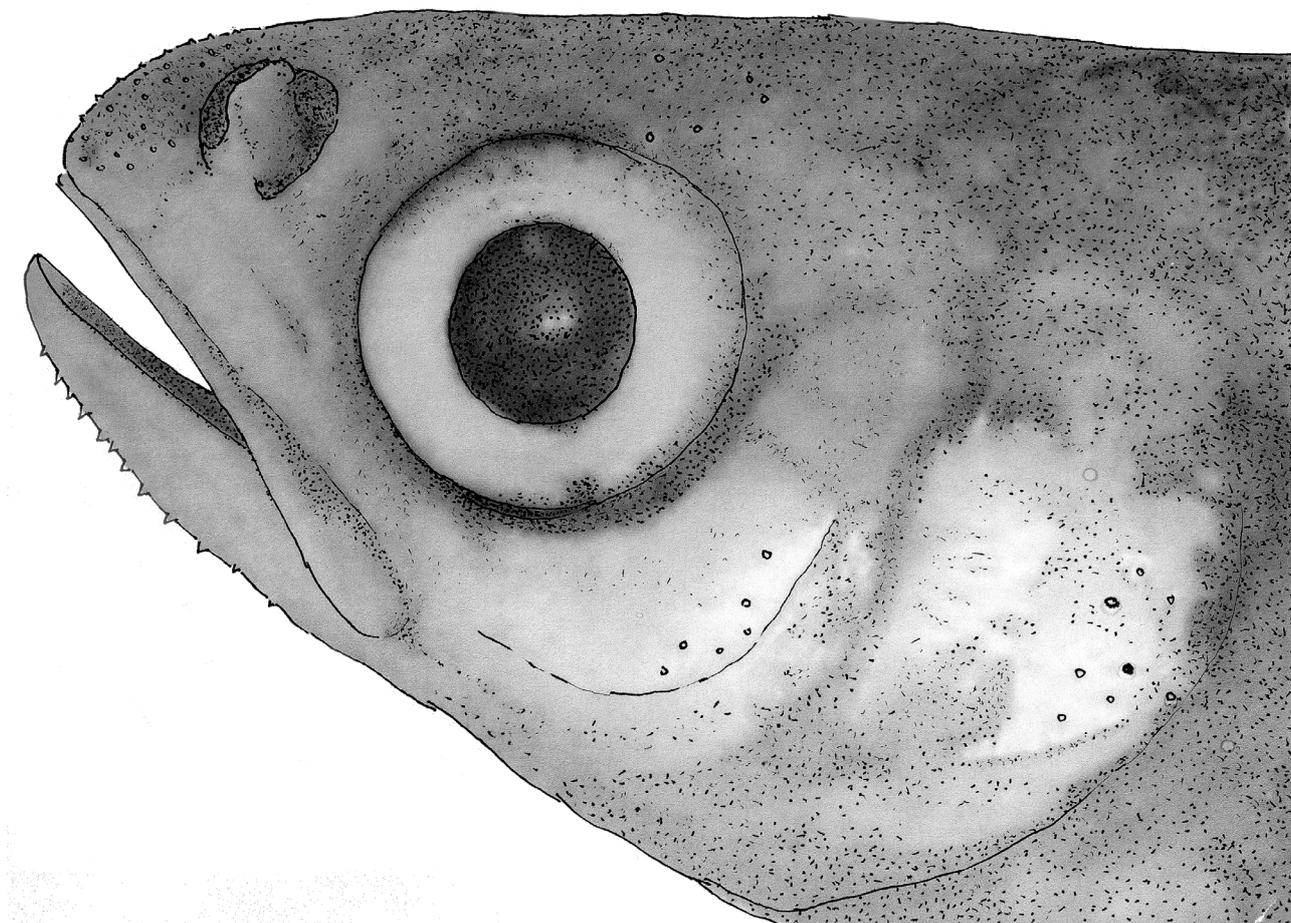
Howes (1983) used a variety of genus group names that sound like subfamily names, but are not recognized as such. One such group is his Neoboline Group, which contains *Neobola Vinciguerra*, *Engraulicypris* Gunther (1894), *Chelaethiops* Boulenger 1899 and *Rastineobola* Fowler (1936). Howes (1984) characterized Neobolines as having a lower jaw articulation extending posterior to the center of the orbit; a broad and dorsally channeled supraethmoid; 10–12 olfactory lamellae on each half of the nasal rosette; 4–7 short gill rakers on the 1<sup>st</sup> ceratobranchial; a small pectoral axial scale with a fleshy ventral border; small scales; and a lateral line that decurves posteriorly, running close to the ventral margin of the body. Tang *et al.*'s (2010) molecular study suggests that all the diversity of Subfamily Chedrinae on the African Continent is descended from Eurasian chedrines that invaded Africa sometime after contact was established between the two continents (early Miocene).



**FIGURE 6.** Map showing sites of capture of *Neobola kinondo* (black star = type locality) in the Tana River Basin and *N. fluviatilis* (white star = type locality) in the Athi River. White circles are sites sampled during the IRES project that failed to yield specimens.

All recognized species of *Neobola* have distributions confined to East Africa (e.g., Lake Mweru in D.R. Congo, Lake Turkana of Ethiopia and Kenya, and rivers drainages of the Indian Ocean in Ethiopia, Kenya and Somalia). We regard *N. kinondo* as the Tana River sister taxon to *N. fluviatilis*, which we confine to the Athi River system. The two species presumably diverged morphologically after their common ancestor established populations in the two adjacent rivers. Cytochrome b (cyt b) sequence data we produced for *N. kinondo* (GenBank Accession numbers MK414480 - MK414481) is 2.8% divergent from published cyt b data of *N. bottegi* from Ethiopia (Tang *et al.* 2010). We have not been able to produce genetic data to confirm the hypothesis that *N. kinondo* and *N. fluviatilis* are sister taxa because of our inability to collect tissues from fresh specimens of *N. fluviatilis*. Howes (1984) conducted the most comprehensive study of morphological variation of species of *Neobola*, including skeletal anatomy, but provided no hypothesis of relationships of the three species included in his study.

Sister relationships between Athi River and Tana River populations have been observed in three other groups of fishes: the *Enteromius kerstenii* complex (Schmidt *et al.* 2017), the *Chiloglanis brevibarbus* species complex (Schmidt *et al.* 2014), and mountain catfishes, *Amphilius* (Thomson & Page 2013). There have likely been numerous instances of headwater capture within the upper Athi and Tana River drainages that could have facilitated biotic dispersal and subsequent divergence.



**FIGURE 7.** Pen and ink enhanced head of Holotype of *Neobola kinondo*, showing breeding tubercles on tip of snout, top of head, underside of jaws, cheek and operculum.

The distribution of *Neobola kinondo* covers a broad area of the middle and lower Tana River Basin, and encompasses a wider range of stream sizes than is the case with the known distribution of *N. fluviatilis*. Sampling for the IRES project (2010-2012) revealed viable populations of *N. kinondo* in three rivers confluent with the Tana River in Meru National Park. The viability of these populations is likely a consequence of the protected environment of the park. Very little sampling has been conducted in the Tana River drainage in the area between Meru National Park and the site on the lower Tana where the juvenile *N. kinondo* specimens were collected. Semi-arid and with few permanent streams other than the Tana River proper, this area will have to be further sampled to better understand the full Tana River drainage distribution of *N. kinondo*.

*Neobola fluviatilis*, in contrast, is only known from two nearby localities on the Athi River proper in the Yatta Region of Kenya. The species has not been collected since 1961. The Athi River below Nairobi is heavily polluted by municipal and industrial wastes from the city (Kinyua & Pacini 1991; Muiruri *et al.* 2013). Water quality doesn't improve until Kitui County, a considerable distance downstream. *Amphilius athiensis* was recently described from the upper Athi River based on collections from the early 1900s (Thomson 2013); this species was also not observed during our recent collecting efforts. Eleven sites in the Athi River system above and below Nairobi were sampled during the IRES Project, including three sites on the Athi River proper, and three sites in Nairobi National Park. We deliberately sampled habitats similar to those favored by *N. kinondo* in the Tana River Basin in an effort to find *N. fluviatilis*. The effort failed to yield any specimens. Additional, more concerted, sampling efforts in these and other areas of the Athi River system are needed to determine if the species still survives.

The new species described herein complements other recent discoveries in the region and highlights the diversity and endemism in the area (Thomson 2013; Schmidt *et al.* 2015; Schmidt *et al.* 2018). *Neobola fluviatilis* and *N. kinondo* are each endemic to a single drainage and have restricted ranges. Since *N. fluviatilis* has not been collected in over 50 years, despite our concerted recent sampling effort, this species should be listed as critically endangered (IUCN 2012). *Neobola kinondo* should be considered vulnerable as the full extent of its range in the Tana River Basin and the health of populations in these areas are unknown (IUCN 2012). The discoveries resulting from the NSF funded IRES expeditions underscore the importance of continued biodiversity research in freshwater environments in East Africa.

### **Additional Material Examined**

*Neobola kinondo* NMK FW/2687/1-8, Ura River at Ura Gate; BMNH 1966.8.25.6, Tana River at Hola Concentration Camps.

*Neobola fluviatilis* BMNH 1961.5.3.1, Athi River, at Kithimani, Athi/Galena River Basin, Kenya, HOLOTYPE; BMNH 1961.5.3.2-6, Athi River at Kithimani, Athi/Galena River Basin, Kenya, PARATYPES; BMNH 1966.7.5.29-42, Athi River at Yatta, Kenya, Athi/Galena River Basin, Kenya.

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### **References Cited**

- Boulenger, G.A. (1899) Matériaux pour la faune du Congo. Poissons nouveaux du Congo. Cinquième Partie. Cyprins, Silures, Cyprinodontes, Acanthoptérygiens. Annales du Musée du Congo (Ser. Zoology) v. 1 (fasc. 5), 97–128., pls. 40–47.
- Boulenger, G.A. (1915) Diagnoses de poissons nouveaux. II. Mormyrides, Kneriides, Characinides, Cyprinides, Silurides. (Mission Stappers au Tanganika-Moero.). *Revue de Zoologie Africaine* v. 4 (fasc. 2), 162–171.
- Fricke, R., Eschmeyer, W.N. & Van der Laan, R. (eds.) (2019) Eschmeyer's Catalog of Fishes: Genera, Species, References. Available form: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> (accessed 13–15 May 2019)
- Fowler, H.W. (1936) Zoological results of the George Vanderbilt African Expedition of 1934. Part III,--The fresh water fishes. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 88, 243–335.
- Günther, A. (1894) Second report on the reptiles, batrachians, and fishes transmitted by Mr. H. H. Johnston, C. B., from British Central Africa. *Proceedings of the Zoological Society of London*, 1893 (pt 4), 616–628., pls. 53–57.
- Howes, G.J. (1980) The anatomy, phylogeny and classification of bariliine cyprinid fishes. *Bulletin of the British Museum (Natural History). Zoology*, 37 (3), 129–198.
- Howes, G.J. (1983) Additional notes on bariliine cyprinid fishes. *Bulletin of the British Museum (Natural History). Zoology*, 45 (2), 95–101.
- Howes, G.J. (1984) A review of the anatomy, taxonomy, phylogeny biogeography of the African neoboline cyprinid fishes. *Bulletin of the British Museum (Natural History). Zoology*, 47 (3), 151–185.
- IUCN. (2012) *IUCN Red List Categories and Criteria: Version 3.1*. Second edition. Gland, Switzerland and Cambridge, UK: IUCN, iv + 32 pp.
- Kinyua, A.M. & Pacini, N. (1991) The impact of pollution on the ecology of the Nairobi-Athi River system in Kenya. *International Journal of Biochemophysics*, 1 (1), 5–7.
- Lévêque, C. & Daget, J. (1984) Cyprinidae In. Daget, J., Gosse, J.-P. & Thys van den Audenaerde, D.F.E. (Eds.), *Check-list of the freshwater fishes of Africa. CLOFFA*. ORSTOM Paris, MARC Tervuren, v. 1, pp. i-xviii + 1–410.
- Muiruri, J.M., Nyambaka, H.N. & Nawiri, M.P. (2013) Heavy metals in water and tilapia fish from Athi-Galana-Sabaki tributaries. *Kenya. International Food Research Journal*, 20 (2), 891–896.

- Seegers, L., DeVos, L.D.G. & Okeyo, D.O. (2003) Annotated checklist of the freshwater fishes of Kenya (excluding the lacustrine haplochromines from Lake Victoria). *Journal of East African Natural History*, 91, 11–47.
- Skelton, P.H., Jubb, R.A. & Bruton, M.N. (1980) Additions to the checklist and recent changes to the scientific names of Southern African freshwater fishes. *Journal of the Limnological Society of Southern Africa*, 6 (2), 109–112.
- Schmidt, R.C., Bart Jr., H.L., Nyingi, D.W. & Gichuki, N.N. (2014) Phylogeny of suckermouth catfishes (Mochokidae: Chiloglanis) from Kenya: The utility of Growth Hormone introns in species level phylogenies. *Molecular Phylogenetics and Evolution*, 79, 415–421.
- Schmidt, R.C., Bart Jr., H.L. & Nyingi, W.D. (2015) Two new species of African suckermouth catfishes, genus *Chiloglanis* (Siluriformes: Mochokidae), from Kenya with remarks on other taxa from the area. *Zootaxa*, 4044, 45–64.
- Schmidt, R.C., Bart Jr., H.L. & Nyingi, W.D. (2017) Multi-locus phylogeny reveals instances of mitochondrial introgression and unrecognized diversity in Kenyan barbs (Cyprininae: Smiliogastrini). *Molecular Phylogenetics and Evolution*, 111, 35–43.
- Schmidt, R.C., Bart Jr., H.L. & Nyingi, W.D. (2018) Integrative taxonomy of the red-finned barb, *Enteromius apleurogramma* (Cyprininae: Smiliogastrini) from Kenya, supports recognition of *E. amboseli* as a valid species. *Zootaxa*, 4482 (3), 566–578.
- Schmidt, R.C., Dillon, M.N., Kuhn, N.M., Bart Jr, H.L. & Pezold, F. (2019) Unrecognized and imperiled diversity in an endemic barb (Smiliogastrini, *Enteromius*) from the Fouta Djallon highlands. *Zoologica Scripta*, 00, 1–9.  
<https://doi.org/10.1111/zsc.12362>
- Sidlauskas, B.L., Mol, J.H., & Vari, R.P. (2011) Dealing with allometry in linear and geometric morphometrics: a taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. *Zoological Journal of the Linnean Society*, 162 (1), 103–130.
- Tan, M. & Armbruster, J.W. (2018) Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). *Zootaxa*, 4476 (1), 6–39.  
<https://doi.org/10.11646/zootaxa.4476.1.4>
- Tang, K.L., Agnew, M.K., Hirt, M.V., Sado, T., Schneider, L.M., Freyhof, J., Sulaiman, Z., Swartz, E., Vidhayanon, C., Miya, M., Saitoh, K., Simons, A.M., Wood, R.M. & Mayden, R.L. (2010) Systematics of the subfamily Danioninae (Teleostei: Cypriniformes: Cyprinidae). *Molecular Phylogenetics and Evolution*, 57, 189–214.
- Thomson, A.W. & Page, L.M. (2010) Taxonomic revision of the *Amphilius uranoscopus* group (Teleostei: Siluriformes) in Kenya, with the description of a new species from the Athi River. *Bulletin of Florida Museum of Natural History*, 49 (2), 45–66
- Werner, F. (1919) Wissenschaftliche Ergebnisse der mit Unterstützung der Kaiserlichen Akademie der Wissenschaften in Wien aus der Erbschaft Treitl von F. Werner unternommenen zoologischen Expedition nach dem Anglo-Ägyptischen Sudan (Kordofan) 1914. IV. Bearbeitung der Fische, Amphibien und Reptilien. *Denkschriften, Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Klasse*, 96, 437–445.
- Whitehead, P.J.P. (1962) Two new river fishes from eastern Kenya. *Annali del Museo Civico di Storia Naturale 'Giacomo Doria'*, 73, 98–108.
- Worthington, E.B. (1932) Scientific results of the Cambridge Expedition to the East African lakes, 1930-1. --2. Fishes other than Cichlidae. *The Journal of the Linnean Society of London. Zoology*, 38 (258), 121–134.