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Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym of
Limnochromini Poll (Perciformes: Cichlidae)

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Running headline: SYNONYM OF A LAKE TANGANYIKA CICHLID TRIBE

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The infraorbitals (IOs) of four species endemic to Lake Tanganyika were examined. Based on the examination of the IOs and previous morphological and molecular studies, the tribe Greenwoodochromini is synonymised with the tribe Limnochromini, and a new combination for *Limnochromis abeelei* and *Limnochromis staneri* is proposed: *Greenwoodochromis abeelei* and *Greenwoodochromis staneri*. The revised tribe Limnochromini, which consists of ten species belonging to seven genera, is characterised by IOs representing types G and I, and the revised genus *Greenwoodochromis*, which consists of four species, is characterised by IOs representing type I.

Key words: infraorbitals; tribes; genus; classification; taxonomy.
In Lake Tanganyika, about 200 endemic cichlid species are currently considered valid, and new species continue to be described (e.g., Takahashi & Hori, 2006; Schelly et al., 2007; Verburg & Bills, 2007; Takahashi, 2008; Burgess, 2012; Kullander et al., 2012). These fishes are morphologically, ecologically and genetically diverse, and represent a well-established model system for the study of adaptive radiation (e.g., Fryer & Iles, 1972; Kornfield & Smith, 2000; Turner et al., 2001; Kocher, 2004; Seehausen, 2006; Turner, 2007; Koblmüller et al., 2008; Salzburger, 2009; Sturmbauer et al., 2011; Takahashi & Koblmüller, 2011; Gante & Salzburger, 2012).

Poll (1986) first classified the Lake Tanganyika cichlid fishes into 12 tribes based on morphological features. Some molecular and morphological studies pointed out that this classification was essentially reasonable but needed a few minor changes (Nishida, 1991; Kocher et al., 1995; Lippitsch, 1998; Salzburger et al., 2002). Subsequently, Takahashi (2003a) constructed a cladogram of these fishes based on anatomical data and proposed a new classification that recognised 16 tribes. Takahashi’s (2003a) classification resolved some taxonomic problems with Poll’s (1986) classification, but some molecular phylogenetic studies have highlighted the need for further minor changes (reviewed by Koblmüller et al., 2008). One of the contradictions between Takahashi’s (2003a) classification and molecular phylogenetic studies raised a problem on the validity of the tribe Greenwoodochromini Takahashi, 2003 (Duftner et al., 2005).

In Takahashi’s (2003a) classification, the genus Greenwoodochromis Poll, 1986 was
isolated from the tribe Limnochromini Poll, 1986 and Greenwoodochromini was established for this genus [at the same time, Takahashi (2003a) also isolated the genus *Benthochromis* Poll, 1986 from Limnochromini and established the tribe Benthochromini Takahashi, 2003 for this genus, and moved *Gnathochromis pfefferi* (Boulenger, 1898) from the Limnochromini to the Tropheini Poll, 1986, although *Gnathochromis permaxillaris* (David, 1936), which is the type species of *Gnathochromis* Poll, 1981, remained in Limnochromini]. However, the Greenwoodochromini is nested within Takahashi’s (2003a) Limnochromini in a molecular phylogeny resulting in a polyphyletic Limnochromini (Duftner et al., 2005; and see Discussion).

Takahashi (2003a) used a morphological difference in the infraorbitals (IOs), which are bones surrounding the lower half of the eye, to distinguish the Greenwoodochromini from the Limnochromini. The IO series of the type species of *Greenwoodochromis*, *Greenwoodochromis christyi* (Trewavas, 1953), were identified as type A (according to the definitions of Takahashi, 2003b), which is the most common of the Lake Tanganyika cichlid flock; whereas the IOs of species of Limnochromini were identified as type G [Fig. 1(a); Takahashi, 2003a, b]. However, the number of specimens of *G. christyi* used by Takahashi (2003a, b) was small, and IOs from *Greenwoodochromis bellcrossi* (Poll, 1976), *Limnochromis abeelei* Poll, 1949, and *Limnochromis staneri* Poll, 1949 were not examined. Although few samples are available because of the difficulties in collecting from deep-water habitats, the present study examined the IO series of six to 20 specimens from these four species. By combining the present anatomical data with previously published molecular phylogenetic and morphological studies, the validity of
Greenwoodochromini and the extent of Greenwoodochromis are discussed.

MATERIALS AND METHODS

Greenwoodochromis christyi, G. bellcrossi, L. abeelei, and L. staneri were collected off Mtondwe Island, Mpulungu, Zambia, with gill nets at 42–140 m depth between August 1995 and December 2007 and in October 2013 (N = 57). Fish were fixed in 10% formalin. The standard length (Ls) of each fish was measured with digital callipers. The IOs on the left side of the head were observed. The IOs of some specimens were removed from the head and stained with Alizarin Red-S in 70% ethyl alcohol, and sketches were made under a binocular microscope (Nikon SMZ 1000). A likelihood ratio test on a logistic regression was used to test any association between intra-specific variation in IOs and fish size. The term "anteriormost bone" is used instead of lacrimal.

RESULTS

The number of bones in the IO series ranges from four to six in G. christyi, G. bellcrossi, L. abeelei, and L. staneri (Table I). The anteriormost bone is larger than the others and has a sensory canal that opens through five or six sensory pores. Four of the pores are large; the widths of these pores are wider than the intervals between the pores [arrows in Fig. 2(a-c)]. The remaining small, tube-like bones have two to five (usually two) sensory pores each.
Greenwoodochromis christyi has two major variations in IO configuration (Table I). Thirteen out of the 20 individuals examined have six IOs with 6-2-2-2-2-2 sensory pores [Fig. 2(b)], whereas five individuals have five IOs with 6-2-3-2-2 sensory pores [Fig. 2(a)]. The third bone of the latter variation (6-2-3-2-2) is elongated and has three sensory pores; two pores are located at the ends of the bone, and the other pore is located anterior to the midpoint of the bone. The individuals that have five IOs with 6-2-3-2-2 sensory pores are significantly larger than the individuals that have six IOs with 6-2-2-2-2-2 sensory pores (Fig. 3).

In G. bellcrossi and L. abeelei, the IO series varies greatly in numbers of bones and sensory pores, although smaller numbers of samples were examined compared to the other species (Table I). The major variation accords with the IO configuration of the large individuals of G. christyi (6-2-3-2-2). One individual of L. abeelei exhibits the same configuration as the small individuals of G. christyi (6-2-2-2-2-2).

In L. staneri, the IO series varies somewhat in numbers of bones and sensory pores (Table I). Fourteen of the 20 individuals examined have six bones with 5-2-2-3-2-2 sensory pores [Fig. 2(c)]. The fourth bone is elongate and has a pore located at each end, and the other pore is located anterior to the midpoint of the bone. One individual exhibits the same condition as the small individuals of G. christyi (6-2-2-2-2-2).

**DISCUSSION**
The IOs of *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* are distinguishable from those of the other Lake Tanganyika cichlids. In these species, four of the five or six sensory pores on the anteriormost bone are large and the widths of these four pores are wider than the intervals between the pores [Fig. 2(a-c)], whereas the widths of all the sensory pores on the anteriormost bone are small and the widths of the pores are narrower than the intervals between the pores in many other species (Takahashi, 2003b).

Species of *Trematocara* Boulenger, 1899 also have large pores on the anteriormost bone, but all the pores (not only four of the pores) are large (Takahashi, 2002, 2003b).

Several variations were found in IO configuration of *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* (Table I). Taking into account the size and number of sensory pores on the bones and distances between the pores, these variations may be a result of a few fusion events between certain bones. For example, the anteriormost and second bones of *L. staneri* [Fig. 2(c)] appear to be homologous with the anteriormost bone of *G. christyi* [Fig. 2(a, b)], and the third and fourth bones of small individuals of *G. christyi* [Fig. 2(b)] appear to be homologous with the third bone of the large individuals of *G. christyi* [Fig. 2(a)] and the fourth bone of *L. staneri* [Fig. 2(c)]. Accordingly, assuming a plesiomorphic IO configuration of 5-2-2-2-2-2-2, the most common condition of small individuals of *G. christyi* (6-2-2-2-2-2) can be considered a result of fusion between the first and second bones of the plesiomorphic IO configuration [Fig. 2(b)]. The most common condition of large individuals of *G. christyi* (6-2-3-2-2), which is also the most common condition of *G. bellcrossi* and *L. abeelei*, can be considered a result of fusion between the first and second bones and between the fourth and fifth bones of the
plesiomorphic IO configuration [Fig. 2(a)]. Similarly, the most common condition of *L. staneri* (5-2-3-2) can be considered a result of fusion between the fourth and fifth bones of the plesiomorphic IO configuration [Fig. 2(c)]. Other minor variations can be explained by an irregular increase in the number of sensory pores on a tube-like bone, an increase or decrease of the number of tube-like bones, or unusual fusion event (see Table I foot notes).

Takahashi (2003b) described IOs of Lake Tanganyika cichlids and classified them into eight types: types A to H. Accordingly, the condition of the IOs unique to *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* is named ‘type I’, the ninth type. Type I can be defined as IOs that have four large sensory pores on the anteriormost bone, and usually consist of seven IOs with 5-2-2-2-2-2 sensory pores, of which the first and second IOs and/or the fourth and fifth IOs are fused. Takahashi (2003b) considered the IOs of small individuals of *G. christyi* (6-2-2-2-2) to be a variation of type A [Fig. 2(e) shows the typical condition of type A, which has six bones with 5-2-2-2-2 sensory pores and the sensory pores on the anteriormost bone are small]; however, the 6-2-2-2-2 condition is regarded as type I in the present study.

In their phylogenetic analysis of Lake Tanganyika cichlids using mitochondrial DNA (mtDNA) sequences, Duftner *et al.* (2005) inferred that nine of the ten species in Takahashi’s (2003a) Greenwoodochromini and Limnochromini formed a monophyletic group [Fig. 1(a)] (*Tangachromis dhanisi* (Poll, 1949) was not examined]. The monophyly of this group is strongly supported by various statistical tests (*e.g.*, 100% Bayesian posterior probability). A clade of Takahashi’s (2003a) Greenwoodochromini
nested within the Limnochromini resulted in the Limnochromini and the genus *Limnochromis* Regan 1920 [the type species is *Limnochromis auritus* (Boulenger, 1901)] being polyphyletic [Fig. 1(a)]. Although phylogenetic inference based on mtDNA can be heavily affected by incomplete lineage sorting and introgression (e.g., Rueber et al., 2001; Koblmüller et al., 2007a, b, 2010; Sturmbauer et al., 2010), the morphology of the IOs supports the monophyly of a clade including *G. christyi*, *G. bellcrossi*, *L. abeelei* and *L. staneri* [Fig. 1(b)], which is supported by 100% Bayesian posterior probability in the mtDNA tree (Duftner et al., 2005). To resolve the disagreements between systematic classification and phylogeny, it is proposed to synonymise Greenwoodochromini with Limnochromini and to make two new combinations: *Greenwoodochromis abeelei* and *Greenwoodochromis staneri* [Fig. 1(b)].

The IOs representing type G, which consists typically of three bones with the elongated second bone [Fig. 2(d); Takahashi, 2003b], and type I are unique to the revised Limnochromini. The species of Limnochromini inhabit bottoms at depths greater than 30 m (Poll, 1956). The large sensory pores on the anteriormost bone (type I) may possibly improve noise sensitivity in deep, dark environments, and fusion of bones (type G, type I) may possibly strengthen the structure.

In conclusion, the tribe Greenwoodochromini is synonymised with the tribe Limnochromini. The revised Limnochromini consists of seven genera: *Baileychromis*, *Gnathochromis* (excluding *G. pfefferi*, which is included in the tribe Tropheini), *Greenwoodochromis, Limnochromis, Reganochromis, Triglachromis* and presumably *Tangachromis* (although it’s phylogenetic position has not been directly examined here...
of by Duftner et al., 2005). This Limnochromini is characterised by having the IOs representing type G [Fig. 2(d)] and type I [Fig. 2(a-c)]. The genus Greenwoodochromis is revised to include four species: G. abeelei (new combination), G. bellcrossi, G. christyi (type species of this genus), and G. staneri (new combination). This genus is characterised by having IOs representing type I.

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References


Figure legends

FIG. 1. Schematic molecular phylogeny of nine of the ten species composing Takahashi’s (2003a) Limnochromini and Greenwoodochromini presented by Duftner et al. (2005). Phylogenetic position of Tangachromis dhanisi remains unknown. Letters on the right of the scientific names indicate types of infraorbitals. Boxes indicate tribal classification. (a) The infraorbital types (Takahashi, 2003b), generic classification (Poll, 1986), and tribal classification (Takahashi, 2003a) of previous studies. (b) The infraorbital types, generic classification, and tribal classification according to the present study.

FIG. 2. Infraorbital (IO) series on the left side of the head representing (a-c) type I, (d) type G and (e) type A. The bones are numbered from anterior to posterior (figures above the bones). Arrows indicate four large sensory pores on the anteriormost bone. Characters connected by pluses under the bones indicate suggested fusion patterns of IOs (see text for details). (a, b) Greenwoodochromis christyi (110.6 mm Ls and 70.3 mm Ls, respectively), (c) Limnochromis staneri (130.1 mm Ls), (d) Limnochromis auritus (114.4 mm Ls), and (e) Simochromis diagramma (Günther 1894) (130.0 mm Ls). Bars indicate 5 mm.

FIG. 3. Relationship between body size and configuration of IOs (six bones with 6-2-2-2-2-2 sensory pores or five bones with 6-2-3-2-2 sensory pores) in Greenwoodochromis christyi.
(a) Previous studies

- *Balteochromis centropomoides* G
- *Raganochromis callurus* G
- *Gnathochromis permascillaris* G
- *Limnochromis auritus* G
- *Triglachromis otostigma* G
- *Greenwoodochromis christyi* A
- *Greenwoodochromis bellcrossi* ?
- *Limnochromis stansyi* ?
- *Limnochromis abecele* ?
- *Tangachromis dharinii* G

(b) Present study

- *Balteochromis centropomoides* G
- *Raganochromis callurus* G
- *Gnathochromis permascillaris* G
- *Limnochromis auritus* G
- *Triglachromis otostigma* G
- *Greenwoodochromis christyi* I
- *Greenwoodochromis bellcrossi* I
- *Greenwoodochromis stansyi* I
- *Greenwoodochromis abecele* I
- *Tangachromis dharinii* G

Fig. 1
$Y = (1 + e^{-0.119X + 11.9})^{-1}$

$\chi^2 = 8.55$

$N = 13, 5$

$P = 0.003$