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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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25 The infraorbitals (IOs) of four species endemic to Lake Tanganyika were examined.
26 Based on the examination of the IOs and previous morphological and molecular studies,
27 the tribe Greenwoodochromini is synonymised with the tribe Limnochromini, and a
28 new combination for *Limnochromis abeelei* and *Limnochromis staneri* is proposed:
29 *Greenwoodochromis abeelei* and *Greenwoodochromis staneri*. The revised tribe
30 Limnochromini, which consists of ten species belonging to seven genera, is
31 characterised by IOs representing types G and I, and the revised genus
32 *Greenwoodochromis*, which consists of four species, is characterised by IOs
33 representing type I.
34
35 Key words: infraorbitals; tribes; genus; classification; taxonomy.
36

INTRODUCTION

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

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

71

72 Takahashi (2003a) used a morphological difference in the infraorbitals (IOs), which are
73 bones surrounding the lower half of the eye, to distinguish the Greenwoodochromini
74 from the Limnochromini. The IO series of the type species of *Greenwoodochromis*,
75 *Greenwoodochromis christyi* (Trewavas, 1953), were identified as type A (according to
76 the definitions of Takahashi, 2003b), which is the most common of the Lake Tanganyika
77 cichlid flock; whereas the IOs of species of Limnochromini were identified as type G
78 [Fig. 1(a); Takahashi, 2003a, b]. However, the number of specimens of *G. christyi* used
79 by Takahashi (2003a, b) was small, and IOs from *Greenwoodochromis bellcrossi* (Poll,
80 1976), *Limnochromis abeelei* Poll, 1949, and *Limnochromis staneri* Poll, 1949 were not
81 examined. Although few samples are available because of the difficulties in collecting
82 from deep-water habitats, the present study examined the IO series of six to 20
83 specimens from these four species. By combining the present anatomical data with
84 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 (L_S) 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.

109

110 *Greenwoodochromis christyi* has two major variations in IO configuration (Table I).

111 Thirteen out of the 20 individuals examined have six IOs with 6-2-2-2-2 sensory

112 pores [Fig. 2(b)], whereas five individuals have five IOs with 6-2-3-2-2 sensory pores

113 [Fig. 2(a)]. The third bone of the latter variation (6-2-3-2-2) is elongated and has three

114 sensory pores; two pores are located at the ends of the bone, and the other pore is

115 located anterior to the midpoint of the bone. The individuals that have five IOs with

116 6-2-3-2-2 sensory pores are significantly larger than the individuals that have six IOs

117 with 6-2-2-2-2 sensory pores (Fig. 3).

118

119 In *G. bellcrossi* and *L. abeelei*, the IO series varies greatly in numbers of bones and

120 sensory pores, although smaller numbers of samples were examined compared to the

121 other species (Table I). The major variation accords with the IO configuration of the

122 large individuals of *G. christyi* (6-2-3-2-2). One individual of *L. abeelei* exhibits the

123 same configuration as the small individuals of *G. christyi* (6-2-2-2-2).

124

125 In *L. staneri*, the IO series varies somewhat in numbers of bones and sensory pores

126 (Table I). Fourteen of the 20 individuals examined have six bones with 5-2-2-3-2-2

127 sensory pores [Fig. 2(c)]. The fourth bone is elongate and has a pore located at each end,

128 and the other pore is located anterior to the midpoint of the bone. One individual

129 exhibits the same condition as the small individuals of *G. christyi* (6-2-2-2-2).

130

131

132 **DISCUSSION**

133

134 The IOs of *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* are distinguishable from
135 those of the other Lake Tanganyika cichlids. In these species, four of the five or six
136 sensory pores on the anteriormost bone are large and the widths of these four pores are
137 wider than the intervals between the pores [Fig. 2(a-c)], whereas the widths of all the
138 sensory pores on the anteriormost bone are small and the widths of the pores are
139 narrower than the intervals between the pores in many other species (Takahashi, 2003b).
140 Species of *Trematocara* Boulenger, 1899 also have large pores on the anteriormost bone,
141 but all the pores (not only four of the pores) are large (Takahashi, 2002, 2003b).

142

143 Several variations were found in IO configuration of *G. christyi*, *G. bellcrossi*, *L.*
144 *abeelei*, and *L. staneri* (Table I). Taking into account the size and number of sensory
145 pores on the bones and distances between the pores, these variations may be a result of a
146 few fusion events between certain bones. For example, the anteriormost and second
147 bones of *L. staneri* [Fig. 2(c)] appear to be homologous with the anteriormost bone of *G.*
148 *christyi* [Fig. 2(a, b)], and the third and fourth bones of small individuals of *G. christyi*
149 [Fig. 2(b)] appear to be homologous with the third bone of the large individuals of *G.*
150 *christyi* [Fig. 2(a)] and the fourth bone of *L. staneri* [Fig. 2(c)]. Accordingly, assuming a
151 plesiomorphic IO configuration of 5-2-2-2-2-2, the most common condition of small
152 individuals of *G. christyi* (6-2-2-2-2-2) can be considered a result of fusion between the
153 first and second bones of the plesiomorphic IO configuration [Fig. 2(b)]. The most
154 common condition of large individuals of *G. christyi* (6-2-3-2-2), which is also the most
155 common condition of *G. bellcrossi* and *L. abeelei*, can be considered a result of fusion
156 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* (5-2-2-3-2-2) 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* (6-2-2-2-2-2) to be a variation of type A [Fig. 2(e) shows the typical condition of type A, which has six bones with 5-2-2-2-2-2 sensory pores and the sensory pores on the anteriormost bone are small]; however, the 6-2-2-2-2-2 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

181 nested within the Limnochromini resulted in the Limnochromini and the genus
182 *Limnochromis* Regan 1920 [the type species is *Limnochromis auritus* (Boulenger,
183 1901)] being polyphyletic [Fig. 1(a)]. Although phylogenetic inference based on
184 mtDNA can be heavily affected by incomplete lineage sorting and introgression (*e.g.*,
185 Rüber *et al.*, 2001; Koblmüller *et al.*, 2007*a, b*, 2010; Sturmbauer *et al.*, 2010), the
186 morphology of the IOs supports the monophyly of a clade including *G. christyi*, *G.*
187 *bellocrossi*, *L. abeelei* and *L. staneri* [Fig. 1(b)], which is supported by 100% Bayesian
188 posterior probability in the mtDNA tree (Duftner *et al.*, 2005). To resolve the
189 disagreements between systematic classification and phylogeny, it is proposed to
190 synonymise Greenwoodochromini with Limnochromini and to make two new
191 combinations: *Greenwoodochromis abeelei* and *Greenwoodochromis staneri* [Fig. 1(b)].
192
193 The IOs representing type G, which consists typically of three bones with the elongated
194 second bone [Fig. 2(d); Takahashi, 2003*b*], and type I are unique to the revised
195 Limnochromini. The species of Limnochromini inhabit bottoms at depths greater than
196 30 m (Poll, 1956). The large sensory pores on the anteriormost bone (type I) may
197 possibly improve noise sensitivity in deep, dark environments, and fusion of bones
198 (type G, type I) may possibly strengthen the structure.
199
200 In conclusion, the tribe Greenwoodochromini is synonymised with the tribe
201 Limnochromini. The revised Limnochromini consists of seven genera: *Baileychromis*,
202 *Gnathochromis* (excluding *G. pfefferi*, which is included in the tribe Tropheini),
203 *Greenwoodochromis*, *Limnochromis*, *Reganochromis*, *Triglachromis* and presumably
204 *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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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 L_S and 70.3 mm L_S , respectively), (c) *Limnochromis staneri* (130.1 mm L_S), (d) *Limnochromis auritus* (114.4 mm L_S), and (e) *Simochromis diagramma* (Günther 1894) (130.0 mm L_S). Bars indicate 5 mm.

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

(a) Previous studies

(b) Present study

Fig. 1

Fig. 2

Fig. 3