Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 46 (2008) 659–672
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae)

Zuzana Musilová [a,b,*], Oldřich Říčan [b,c], Karel Janko [b], Jindřich Novák [c]

[a] *Department of Zoology, Charles University in Prague, Viničná 7, 128 44 Praha, Czech Republic*
[b] *Institute of Animal Physiology and Genetics of the Academy of Sciences of the Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic*
[c] *Department of Zoology, Faculty of Sciences, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic*

Received 31 May 2007; revised 5 October 2007; accepted 11 October 2007

Abstract

We have conducted the first comprehensive molecular phylogeny of the tribe Cichlasomatini including all valid genera as well as important species of questionable generic status. To recover the relationships among cichlasomatine genera and to test their monophyly we analyzed sequences from two mitochondrial (16S rRNA, cytochrome *b*) and one nuclear marker (first intron of S7 ribosomal gene) totalling 2236 bp. Our data suggest that all genera except *Aequidens* are monophyletic, but we found important disagreements between the traditional morphological relationships and the phylogeny based on our molecular data. Our analyses support the following conclusions: (a) *Aequidens* sensu stricto is paraphyletic, including also *Cichlasoma* (CA clade); (b) *Krobia* is not closely related to *Bujurquina* and includes also the Guyanan *Aequidens* species *A. potaroensis* and probably *A. paloemeuensis* (KA clade). (c) *Bujurquina* and *Tahuantinsuyoa* are sister groups, closely related to an undescribed genus formed by the '*Aequidens*' *pulcher*–'*Aequidens*' *rivulatus* groups (BTA clade). (d) *Nannacara* (plus *Ivanacara*) and *Cleithracara* are found as sister groups (NIC clade). *Acaronia* is most probably the sister group of the BTA clade, and *Laetacara* may be the sister group of this clade. Estimation of divergence times suggests that the divergence of Cichlasomatini started around 44 Mya with the vicariance between coastal rivers of the Guyanas (KA and NIC clades) and remaining cis-andean South America, followed by evolution of the *Acaronia*–*Laetacara*–BTA clade in Western Amazon, and the CA clade in the Eastern Amazon. Vicariant divergence has played importantly in evolution of cichlasomatine genera, with dispersal limited to later range extension of species within genera.

Keywords: Molecular phylogeny; Cichlids; Cichlidae; Cichlasomatini; South America; Biogeography; Penalized likelihood; Partitioned Bremer support; Cytochrome *b*; 16S rRNA; S7; DIVA; Vicariance; Dispersal

---

1. Introduction

Neotropical cichlids are extremely varied in morphology, behaviour and ecology (Lowe-McConnell, 1991) despite comprising fewer species than their relatives in African lakes. Since the early 20th century, when Regan (1905a,b,c, 1906a,b) completely revised the group, the number of species and genera has increased considerably. Kullander (2003) summarizes the cichlid diversity in the Neotropics as including 54 genera and 407 species. These numbers are likely to increase significantly, as there still are many undescribed species and genera (e.g. Kullander and Ferreira, 2006; Kullander and Lucena, 2006; López-Fernández et al., 2006; Říčan and Kullander, 2006).

The cichlid subfamily Cichlasomatinae has one of the most complex taxonomic histories among cichlid groups in the Neotropics and no other group of Neotropical cichlids has witnessed so many taxonomic changes and new descriptions at the genus level. The subfamily Cichlasomatinae was formally diagnosed by Kullander (1998) and today includes species placed during the 19th and 20th cen-

---

[*] Corresponding author. Address: Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic.
E-mail address: zuzmus@email.cz (Z. Musilová).

1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2007.10.011

tury mainly in the genera *Cichlasoma* and *Aequidens*. Kullander (1998) also divided the subfamily into two tribes, the Heroini and the Cichlasomatini. Since this seminal work, both the monophyly and division of the subfamily have been supported by independent data (Farias et al., 1999, 2000, 2001; Sparks, 2004; Marescalchi, 2005). Cichlasomatine species have mostly been associated with the genus *Aequidens*, while most heroines have been placed in the catchall genus *Cichlasoma*. Since Kullander (1998) the genus *Cichlasoma* has been placed in the tribe Cichlasomatini, while most of the species previously constituting *Cichlasoma* are now in the tribe Heroini (type genus *Heros* Heckel 1840). Kullander's studies (1983a, 1986; Kullander and Nijssen, 1989) rejected the classification of Regan (1905a,b) based predominantly on the number of anal fin spines. Regan (op. cit.) placed Neotropical cichlids into two groups, one with more than three spines in the anal fin (nearly all placed in former *Cichlasoma* and most of them distributed in Central America), and the other one with three anal fin spines, distributed in many genera (and subfamilies according to current classification).

At present, the Neotropical cichlid fish tribe Cichlasomatini Kullander, 1998 comprises 69 valid species placed in 10 valid genera (*Aequidens*, *Bujurquina*, *Cichlasoma*, *Cleithracara*, *Ivanacara*, *Krobia*, *Laetacara*, *Nannacara*, *Tahuantinsuyoa* and *Acaronia*). The generic assignment of several species is questionable (e.g. '*Aequidens*' *potaroensis*, '*Aequidens*' *paloemeuensis*, '*Aequidens*' *hoehnei*; Kullander, 1998) and some species groups likely represent unnamed genera (i.e. the '*Aequidens*' *pulcher* group and '*Aequidens*' *rivulatus* group; Kullander, 1998). Species of the genera *Aequidens*, *Bujurquina*, *Krobia*, *Cleithracara* and *Laetacara* were formerly placed in the catchall genus *Aequidens* previous to the taxonomic revisions of Kullander (1986) and Kullander and Nijssen (1989), which excluded them from *Aequidens.* In addition to its current twelve species, *Cichlasoma*, another catchall group, contained also the majority of Mesoamerican cichlids according to Regan (1905b). These have been later classified as the tribe Heroini (Kullander, 1998). A revision of the Mesoamerican Heroini formerly placed in *Cichlasoma* is still pending, but recent studies have contributed to that goal (Roe et al., 1997; Martin and Bermingham, 1998; Hulsey et al., 2004; Chakrabarty, 2006; Říčan and Kullander, 2006; Concheiro Pérez et al., 2007). The South American ex-*Cichlasoma* (now Heroini) have a stable genus-level taxonomy (*Caquetaia* and *Heroina*—Kullander, 1996; *Australoheros*—Říčan and Kullander, 2006). The genus *Nannacara* established by Regan (1905a) remained without intrageneric changes until 2007, when Römer and Hahn (2007) described and separated the genus *Ivanacara* for two species of *Nannacara*. The genus *Acaronia* has been variously assigned to Heroini (Stiassny, 1991) or a separate tribe Acaroniini (Kullander, 1998). These placements based on morphology are refuted by all molecular analyses, which clearly place *Acaronia* among the Cichlasomatini (Farias et al., 1999, 2000, 2001; Sparks and Smith, 2004; Marescalchi, 2005; Concheiro Pérez et al., 2007).

The distribution of Cichlasomatini covers most of cis-andean South America and to a lesser extent also trans-andean South America including lower Central America. Therefore, their distribution area covers biogeographically crucial regions such as both Andean slopes, the Amazon basin and the old geological formations (the Guyana and Brazilian shields). Cichlasomatini are thus an ideally suited model group for the study of historical biogeography and evolutionary processes in the Neotropics, especially in combination with their sister group, the predominantly mesoamerican Heroini.

Kullander (1998) presented the first morphology-based phylogeny which was not able to convincingly determine relationships between and within the genera of Cichlasomatini. No robust tests of the relationships among genera or their monophyly have been performed to date. Using molecular markers and extensive taxonomical sampling we evaluated for the first time the monophyly of cichlasomatine genera and their relationships. Our results contribute to the ongoing investigation of the World's richest (Neotropical) freshwater biota, its diversity and biogeographic history.

2. Materials and methods

2.1. Taxon sampling

Representative species of all valid genera of the tribe Cichlasomatini as well as the genus *Acaronia* were included in this study. We have strived to include multiple morphologically and geographically distant species for each genus to have as representative sampling as possible. The taxon sampling includes 47 OTUs representing 41 species for all three genes studied, i.e. the mitochondrial genes for cytochrome *b* (cyt *b*) and 16S rRNA and the nuclear intron in the ribosomal S7 gene. Specimens were wild-caught and obtained from ornamental-fish importers with reliable locality data, aquarium populations were used as well (Table 1). Representative species of Heroini and Geophagini were used as outgroup taxa.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from a fin clip (approx. 5 × 5 mm) using Dneasy® Tissue Kit (Qiagen), following the manufacturer's protocol. The polymerase chain reactions (PCRs) used 1 μl of DNA as templates. Primers used in the study are the following: for the 16S rRNA gene we used the forward mtD-32 (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) and the reverse mtD-34 (5′-CGC CTG TTT AAC AAA AAC AT-3′; both Marescalchi, 2005). The S7 intron primers S7RPE-X1F (5′-TGG CCT CTT CCT TGG CCG TC-3′) and S7RPE-X2R (5′-AAC TCG TCT GGC TTT TCG CC-3′) were from Chow and Hazama (1998). The following combinations of cyt *b* prim-