

## Molecular phylogeny of *Austrofundulus* Myers (Cyprinodontiformes: Rivulidae), with revision of the genus and the description of four new species

TOMAS HRBEK<sup>1‡</sup>, DONALD C. TAPHORN<sup>2</sup> & JAMIE E. THOMERSON<sup>3</sup>

<sup>1</sup> Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, USA; hrbek@pcg.wustl.edu

<sup>2</sup> Museo de Ciencias Naturales de la UNELLEZ-Guanare, Guanare, Estado Portuguesa, Venezuela; taphorn@cantv.net

<sup>3</sup> 13030 Nutty Brown Road, Austin, Texas, 78737, USA; jthomerson@austin.rr.com

‡ Current address of corresponding author: Department of Biology, University of Puerto Rico – Rio Piedras, San Juan, PR 00931, Puerto Rico

### TABLE OF CONTENTS

ABSTRACT .....	1
INTRODUCTION .....	2
MATERIALS AND METHODS .....	5
RESULTS .....	8
Characteristics of mtDNA Data .....	8
<i>Austrofundulus</i> Phylogeny .....	9
<i>Austrofundulus</i> Myers 1932 .....	12
<i>Austrofundulus transilis</i> Myers 1932 .....	13
<i>Austrofundulus rupununi</i> new species .....	15
<i>Austrofundulus leohoignei</i> new species .....	19
<i>Austrofundulus limnaeus</i> (Schultz 1949) .....	22
<i>Austrofundulus leoni</i> new species .....	25
<i>Austrofundulus guajira</i> new species .....	28
<i>Austrofundulus myersi</i> Dahl 1958 .....	31
DISCUSSION .....	34
ACKNOWLEDGMENTS .....	36
LITERATURE CITED .....	37

### ABSTRACT

Phylogenetic analysis of 13 mitochondrial DNA genes of *Austrofundulus* Myers 1932 indicates that as presently recognized, *A. limnaeus* is composed of several populations with monophyletic haplotype lineages, which together are paraphyletic with respect to *A. transilis*. These populations

were previously united based on shared plesiomorphic morphometric characters. *Austrofundulus myersi* is removed from synonymy; four new species: *A. rupununi*, *A. leohoignei*, *A. guajira*, and *A. leoni* are described; and *A. limnaeus* is restricted to populations along the eastern side of Lake Maracaibo. In contrast, populations of *A. transilis* from the Río Apure Llanos and the lower Río Unare basin show little divergence. The proposed phylogeny: (*A. myersi* (*A. leoni* (*A. limnaeus* (*A. guajira* (*A. leohoignei* (*A. rupununi* (*A. transilis*)))))) is strongly supported by high bootstrap values and Bremer decay indexes, and unique length variations in the 12S ribosomal DNA (rDNA) gene. The distributions and the relationships of the newly erected species are concordant with the geological history of northern South America.

Un análisis filogenético de 13 genes del ADN mitocondrial de *Austrofundulus* muestra que como actualmente este configurada, la especie *Austrofundulus limnaeus* es parafilética, y consiste de varias linajes monofiléticas que estuvieron unidas en base de características morfométricas plesiomórficas que comparten. Se remueve *Austrofundulus myersi* de la sinonimia de *A. limnaeus*, se describen cuatro especies nuevas: *A. rupununi*, *A. leohoignei*, *A. guajira* y *A. leoni*, y se restringe *A. limnaeus* a las poblaciones del lado este del Lago de Maracaibo. Muy distinta la situación de las diferentes poblaciones de *A. transilis* de las cuencas del Río Apure y Unare, que muestra poca divergencia genética. La filogenia propuesta es: (*A. myersi* (*A. leoni* (*A. limnaeus* (*A. guajira* (*A. leohoignei* (*A. rupununi* (*A. transilis*)))))) se apoya fuertemente por los altos valores bootstrap y los índices Bremer de descomposición y por variaciones únicas en la longitud del gen 12S de la ribosoma de RDN (rRDN). Las distribuciones zoogeográficas y las relaciones filogenéticas de las especies nuevas descritas concuerdan bien con la historia geológica del norte de Sudamérica.

**Key words:** *Austrofundulus* sp. complex, Andean Orogeny, PCR, mtDNA, speciation, molecular phylogeny

## INTRODUCTION

*Austrofundulus* was last revised by Taphorn and Thomerson (1978). In that study Taphorn and Thomerson (1978) recognized only two species: *Austrofundulus transilis* and *A. limnaeus*, and placed the other two then described species, *A. myersi* and *A. stagnalis* into synonymy with *A. limnaeus*. *Austrofundulus transilis* was at that time only known from the Río Apure basin of Venezuela, while *A. limnaeus* had a very wide and disjunctive distribution.

The type species, *Austrofundulus transilis* Myers 1932, is known from the Venezuelan Llanos north of the Orinoco mainstream and from the lower Río Unare Basin (Thomerson *et al.* 1990). Taphorn and Thomerson (1978) recognized seven distinctive populations of *A. limnaeus* Schultz, 1949: the Colombian population found on the coastal lowlands between Cartagena and Sincelejo previously described as *A. myersi* Dahl, 1958; a population from the Guajira Peninsula; three populations from the Lake Maracaibo basin and the adjacent coastal desert including a population from the southeastern Maracaibo basin bearing the name *A. stagnalis*; a population from the coastal Caribbean drainages of Río Aroa and probably also Río Tucuyo in the vicinity of Tucacas, Falcón State, Venezuela; and a