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

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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 respected to *A. transilis*. These populations

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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 sinonímia 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 Tocuyo in the vicinity of Tucacas, Falcón State, Venezuela; and a

population from the upper Tacutu (Branco-Amazon) River drainage in the Rupununi Savannah of Guyana (Fig. 1). Based on the apparent availability of suitable habitat shown on topographic maps, we suspect that the Guyanese population likely extends further south to the savannahs around Boa Vista, Brazil, however, currently no specimens are known from this area.

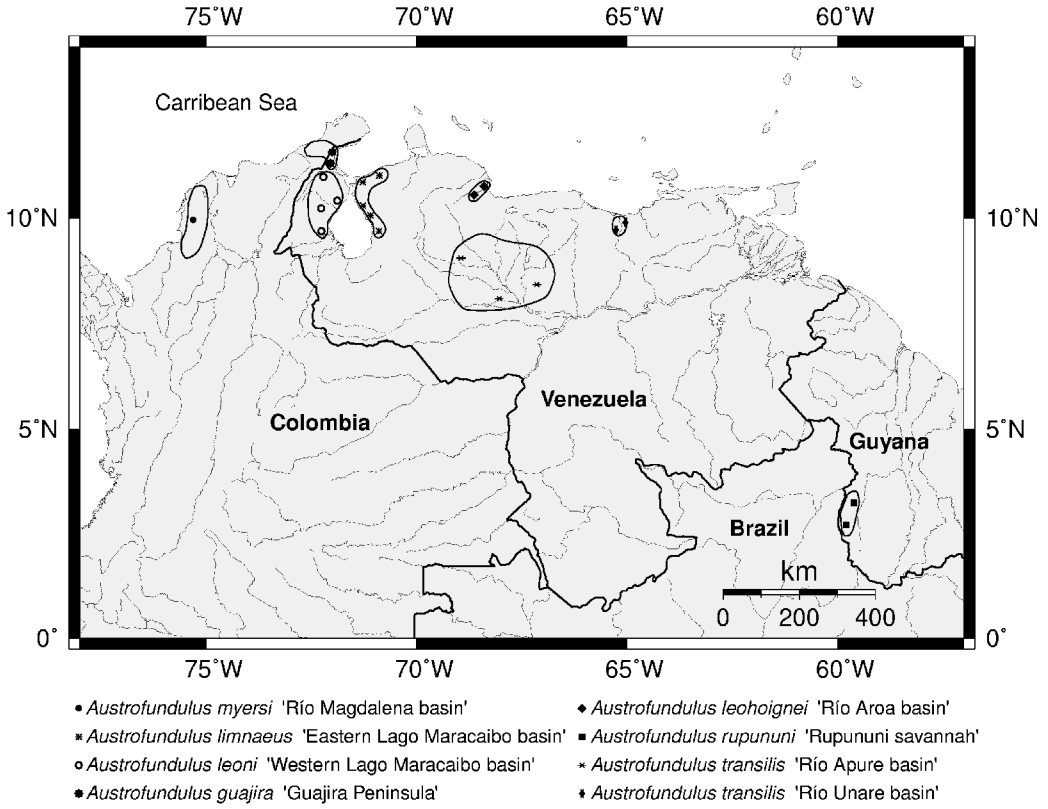

FIGURE 1. Distribution of *Austrofundulus* species and populations. Symbols represent localities from which animals were sampled, while outlines represent approximate areas of distribution.

Taphorn and Thomerson had initiated the 1978 study thinking that several of these populations of *A. limnaeus*, distinguishable by male color patterns, might prove to be valid species. However, because of considerable morphological variation among males of *A. limnaeus* within populations, few significant differences in meristic and morphometric characters, and great similarity among females of all *Austrofundulus* populations, Taphorn and Thomerson (1978) did not give formal taxonomic recognition to any of these populations. Subsequent popular articles (Thomerson and Taphorn 1992a; b) included updated distribution maps, photographs of fish from various populations to document differences in male color patterns, and comments on natural history and aquarium culture, but they also suggested no further taxonomic changes.

In recent years molecular analyses have been applied as an additional tool in identifying species, and in inferring phylogenetic relationships among species (e.g. Avise 1994; Avise 2000; Templeton 2001). Mitochondrial DNA (mtDNA) in particular has found a widespread use as a molecular marker. Although mtDNA is non-recombining and is maternally inherited, and thus it cannot reflect the tokogenetic relationships among individuals within a sexual species, it is useful for inferring phylogenetic relationships among species, as well as in identifying clusters of individuals that are significantly differentiated from other individuals. This naturally requires the caveat that the evolutionary history of the mtDNA is the same as the evolutionary history of the individuals bearing them. This caveat applies to any character, whether molecular or morphological. Allowing for these assumptions, we proceed to investigate phylogenetic relationships among populations of the annual killifish genus *Austrofundulus* Myers1932, known from Colombia, Venezuela and Guyana.

Molecular phylogenetic analysis allowed us to reassess former conclusions regarding these different populations of *Austrofundulus*. Assuming that the evolutionary history of mtDNA reflects the evolutionary history of the different populations of *Austrofundulus*, reconstructing phylogenetic relationships among mtDNA haplotypes originating from different areas should amount to reconstructing phylogenetic relationships among these areas. Phylogenetic analysis of mtDNA sequence data suggests that all *A. limnaeus* populations recognized by Taphorn and Thomerson (1978) except "*A. stagnalis*" are monophyletic. Thus geographic areas are inhabited by clades of individuals (Figs. 1, 2, 3). *Austrofundulus transilis* is also monophyletic (Figs. 2, 3). However, *A. transilis* is sister to the *A. limnaeus* population from the Rupununi savannah, and together they are sister to *A. limnaeus* populations from the Río Aroa basin. *Austrofundulus transilis* is nested within *A. limnaeus* as defined Taphorn and Thomerson (1978) making *A. limnaeus* a paraphyletic entity. It is unlikely this paraphyly would result from a process such as incomplete lineage sorting. In this case we would not expect monophyly of sampled geographical areas, or male color pattern differences diagnostic for these same geographical areas. Furthermore, we would not expect to observe spatial and temporal concordance of phylogenetic relationships with the geological history of northern South America. Combined evidence suggests that these geographically restricted, monophyletic entities represent species.

Based on the combined phylogenetic, geographic-distributional, male color pattern, and hybridization (see discussion), we propose a revision of the genus *Austrofundulus*. We propose to remove *A. myersi* from synonymy with *A. limnaeus*, restrict *A. limnaeus* to populations occurring on the eastern side of Lake Maracaibo but retain within *A. limnaeus* "*A. stagnalis*" from the southeastern side of Lake Maracaibo, and to describe the populations from the Guajira peninsula, from the western side of Lake Maracaibo, from the Tucacas region, and from the Rupununi Savannah as new species.

Costa (1990) synonymized *Austrofundulus* Myers 1932 with *Rachovia* Myers 1927, but in a later publication (Costa 1998) resurrected *Austrofundulus* as a separate genus

without justification. We follow Taphorn and Thomerson (1978) in recognizing both genera. Although there are no universally accepted definitions as to what constitutes a genus, it is generally agreed that generic designations should encompass not only monophyletic units, but also a morphologically and ecologically distinct groups, thus conveying additional information above and beyond the species level. Since this analysis shows *Austrofundulus* species to be a monophyletic group, and *Austrofundulus* and *Rachovia* form morphologically distinct units (Taphorn and Thomerson 1978), the retention of *Austrofundulus* as a separate genus is justified.

MATERIALS AND METHODS

Taxon Sampling. We list all species and locations used for DNA analysis in this study in Table 1. Venezuelan species and populations used in the molecular analysis were collected in 1994 and 1995 summer field seasons. Guyanese populations were collected in the summer of 1996, and representatives of the Colombian *Austrofundulus limnaeus* from Sincelejo were from the original 1958 collections of Dahl. Due to large phenotypic differences among Maracaibo populations, representatives from this region were selected to represent extreme and intermediate phenotypes, and to sample the whole region. Thus a total of 11 populations from both the eastern and the western shores of Lake Maracaibo and the Guajira peninsula were included in this study. From the Río Aroa basin the only two known populations were included. In the Río Apure basin three populations in the extreme and middle portions of the distribution of A. *transilis* were analyzed. Two populations from within the very narrow distribution range of *A. transilis* in Río Unare basin were also included. Guyanese *A. limnaeus* were represented by two populations collected between the towns of Lethem and Good Hope in the Rupununi savannah. Colombian *A. limnaeus* were represented by a 1958 collection from the vicinity of the town Sincelejo (paratypes of *A. myersi*).

TABLE 1. Species and localities included in this study. All examined individuals except for *Austrofundulus myersi* from Colombia were collected by the first author. Numbers associated with general area descriptions correspond to numbers indicated on Figure 1. Associated GenBank accession numbers are listed next to individual species.

Genus	Species	Population	GenBank #
***Rachovia* species:**			**12S ND2**
Rachovia	*maculipinnis*	Papelón, Venezuela	AY850664, AY850639
Rachovia	*brevis*	Carrasquero, Venezuela	AY850665, AY850640

.....continued on the next page

TABLE 1 continued

Genus	Species	Population	GenBank #
Rachovia	*pyropunctata*	Palmarito, Venezuela	AY850666, AY850641
Rachovia	*hummelincki*	Quisiro, Venezuela	----------, AY850642
***Austrofundulus* species:**			
Río Magdalena basin, Carribean lowlands of Colombia			
Austrofundulus	*myersi*	Sincelejo, Colombia	----------, AY850643
Eastern Lago Maracaibo basin, Venezuela			
Austrofundulus	*limnaeus*	(1) Quisiro, Venezuela	AY850667, AY850644
Austrofundulus	*limnaeus*	(2) Cato Cato Ocho, Venezuela	AY850668, AY850645
Austrofundulus	*limnaeus*	(3) Lagunita, Venezuela	AY850669, AY850646
Austrofundulus	*limnaeus*	(4) Bachaquero, Venezuela	AY850670, AY850647
Austrofundulus	*limnaeus*	(5) La Ceiba, Venezuela	AY850671, AY850648
Guajira Peninsula, Venezuela and Colombia			
Austrofundulus	*guajira*	(1) Guarero, Venezuela	AY850672, AY850649
Austrofundulus	*guajira*	(2) El Carretal, Venezuela	AY850673, AY850650
Western Lago Maracaibo basin, Venezuela			
Austrofundulus	*leoni*	(1) Río Cachirí, Venezuela	AY850674, AY850651
Austrofundulus	*leoni*	(2) Los Claros, Venezuela	AY850675, AY850652
Austrofundulus	*leoni*	(3) Sartanejo, Venezuela	AY850676, AY850653
Austrofundulus	*leoni*	(4) Campo Altamira, Venezuela	AY850677, AY850654
Río Aroa basin, Venezuela			
Austrofundulus	*leohoignei*	(1) Palma Sola, Venezuela	AY850678, AY850655
Austrofundulus	*leohoignei*	(2) Sanare, Venezuela	AY850679, AY850656
Río Tacutu basin, Rupununi savannah, Guayana			
Austrofundulus	*rupununi*	(1) Pirara, Guyana	AY850680, AY850657
Austrofundulus	*rupununi*	(2) near Manari, Guyana	AY850681, AY850658
Llanos, Río Apure basin, Venezuela			
Austrofundulus	*transilis*	(4) Guanarito, Venezuela	AY850682, AY850659
Austrofundulus	*transilis*	(5) Achaguas, Venezuela	AY850683, AY850660
Austrofundulus	*transilis*	(1) Punta Fleitera, Venezuela	AY850684, AY850661
Río Unare basin, Venezuela			
Austrofundulus	*transilis*	(2) San Miguel, Venezuela	AY850685, AY850662
Austrofundulus	*transilis*	(3) Onote, Venezuela	AY850686, AY850663

Morphological Methods. Counts and measurements were recompiled from the data matrix on which Taphorn and Thomerson (1978) based their taxonomic revision of *Austrofundulus*. Additionally, numerous other specimens were studied for comparison, and new observations on color patterns were made. Counts and measurements follow Hubbs and Lagler (1964) unless noted otherwise. Institutional abbreviations are as listed in Levinton *et al*. (1985) with the addition of Museo de Ciencias Naturales de la UNELLEZ-Guanare (MCNG).

Molecular Methods. Total genomic DNA was extracted from muscle tissue of the right caudal peduncle of specimens preserved in 95% ethanol or frozen in liquid nitrogen. Muscle tissue was dissolved and digested with a proteinase K/SDS solution, followed by phenol and chloroform extraction, the addition of 5M NaCl followed by 70% ethanol precipitation of DNA product. Amplification and sequencing primers were taken from the literature (Hrbek and Larson 1999). DNA sequences used in this study consisted of genes encoding part of 12S rRNA, partial NADH1 and CO1 genes, and complete sequences of NADH2, transfer RNAs for valine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine and tyrosine, and the light-strand replication origin. Mitochondrial DNA regions were amplified via the Polymerase Chain Reaction (PCR). Amplifications were done in 25 µl volume containing: 10mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.1 µM each primer and 0.5 units of Gibco *Taq* polymerase. An additional 1 µl of DNA extract was added to the reaction tubes. The temperature profile for the 30 cycle amplification reaction consisted of 94°C for 35 seconds (denaturation), 52ûC for 35 seconds (annealing), and 72°C for 150 seconds (extension). The duration of extension was increased by 4 seconds after each cycle. Purification was done on a 4% acrylamide minigel, followed by electro-elution of DNA product from the acrylamide band. Amplified mtDNA segments were sequenced from the 5' end, with at least one individual per population also sequenced from the 3' end. Sequencing followed standard Promega *fmol* double stranded cycle sequencing protocol incorporating S^{35} labeled dATP, and resolved on 6% LongRanger™ polyacrylamide gel. Polyacrylamide gels were transferred onto chromatography grade blotting paper, dried and autoradiographed. Manual sequencing results were verified by resequencing these individuals with Amersham BigDye v3.1 chemistry, and resolved on MJ Research Megabase automatic DNA sequencer.

Phylogenetic analyses. Orthologous protein-coding regions (NADH1, NADH2 and CO1) were aligned manually and confirmed by translating DNA data into amino acid sequences in BioEdit (Hall 1999). Alignments of ribosomal and transfer RNAs were constructed manually based on secondary structural models (Kumazawa and Nishida 1993; Springer and Douzery 1996). All regions whose alignment is ambiguous (31 b.p. of 12S rRNA loop structure #49, 6 b.p. of $tRNA^{Cys}$ T loop, and 7 b.p. of $tRNA^{Trp}$ D loop) were excluded from phylogenetic analyses. A total of 2653 alignable characters representing 25 taxa were scored; 796 of these characters were parsimony informative. Furthermore, all sequences were tested for an anti-G bias characteristic of the mitochondrial DNA genes,

but not of the nuclear genome, to support our conclusion that we have collected genuine mitochondrial DNA data (Zhang and Hewitt 1996).

The most parsimonious phylogenetic tree was estimated using the program PAUP* 4.10b (Swofford 2001) with 100 heuristic searches using random addition of sequences, and implementing the tree bisection and reconnection (TBR) algorithm. Equal weight was given to all characters. Bootstrap resampling (Felsenstein 1985) was applied to assess support for individual nodes using 1000 bootstrap replicates with 25 random additions and TBR branch swapping. A nexus file containing a constraint tree for each node of the maximum parsimony tree and directives for a heuristic search with 50 random additions and TBR branch swapping was used to find the minimum-lengths of alternative trees not satisfying each constraint. Bremer branch-support values (Bremer 1994) were calculated by subtracting the length of the shortest tree from the shortest tree constrained not to include the branch being analyzed. Topologies were rooted with species of the genus *Rachovia*, the sister group of *Austrofundulus* (Hrbek and Larson 1999; Murphy *et al.* 1999).

Maximum likelihood trees were estimated in PAUP* v4.10b (Swofford 2001). The simplest maximum likelihood model that best explains the data was estimated using MODELTEST (Posada and Crandall 1998). Results of MODELTEST (Posada and Crandall 1998) indicated that the HKY85 model (Hasegawa *et al.* 1985) of evolution with rate heterogeneity, rates for variable sites assumed to flow the gamma distribution with shape parameter estimated by the maximum likelihood method was the most appropriate model of molecular evolution. Bootstrap resampling (Felsenstein 1985) was applied to assess support for individual nodes using 100 bootstrap replicates with 10 random additions and TBR branch swapping. A file with the aligned data is available directly from the first author.

RESULTS

Characteristics of mtDNA Data

Of the 2653 characters included in the analysis, there were 1425 constant characters, 1228 characters were variable and 796 characters were parsimony-informative including the outgroup. Maximum likelihood sequence divergences based on the HKY85 model (Hasegawa *et al.* 1985) of evolution with rate heterogeneity, rates for variable sites assumed to flow the gamma distribution with shape parameter estimated by the maximum likelihood method ranged from 0.00041 to 0.16005 within *Austrofundulus*. Values between the ingroup and outgroup taxa ranged from 0.20441 to 0.25168.

We conducted maximum likelihood and maximum parsimony analyses of the data. The results of the two analyses are nearly identical. In maximum likelihood analysis, the Colombian *Austrofundulus* are sister to all other *Austrofundulus* (Fig. 2), while in maximum parsimony analysis the Colombian *Austrofundulus* are sister to all other *Austrofundulus* and three *Rachovia* species from the Maracaibo basin (*R. hummelincki*, *R. pyropunctata* and *R. brevis*) (Fig. 3). In both analyses the Guajira *Austrofundulus* are sister to all other Lago Maracaibo basin *Austrofundulus*. *Austrofundulus transilis* is the sister taxon to *A. limnaeus* from the Rupununi, Guyuana, and in turn this clade formed the sister clade to the Tucacas population of *A. limnaeus*. These two *A. limnaeus* and *A. transilis* populations form the sister clade to the Lago Maracaibo populations of *A. limnaeus* (Fig. 2). Likelihood of the maximum likelihood topology is Πln L = 13502.97562 with ‡ = 0.465919. Tree length of the maximum parsimony topology is 2422 steps, the consistency index is 0.689 and retention index is 0.792. Monophyly of all populations is strongly supported statistically, as are majority of among population relationships (Figs. 2, 3). Further evidence supporting monophyly and distinctiveness of these populations are unique length variations at positions 430 to 475 of the 12S rRNA (Table 2).

TABLE 2. Diagnostic pattern of length variation and base-pair composition at positions 430 to 475 of the 12S rRNA. Intraspecific as well interspecific variation is shown.

R. maculipinnis	TTAAAC--------ATTAATAAAA–CCCAACATCACTTA--AAGG
R. pyropunctata	TTAAACC-------ATTAATAAGCCACATGGATCATCAAC-AGGG
R. hummelincki	TTAAATC-------ATTAATAAGTCACATGGATCATCAAC-AGGG
R. brevis	TTAAAAT-------ATTAATAAAT--CCTTAAATTAACTA-AAGG
A. transilis	TCAAAT-----TACATATATATAAACTTCTATAACTCTCA-AAGG
A. rupununi	TCAAA--------TATATATATAAACTTCTGTAACTCTCA-GAGG
A. rupununi	TCAAA----TATATATATATATAAACTTCTGTAACTCTCA-GAGG
A. leohoignei	TCAAAA----TTATTCATATATAAACTTCTATAACTCTTA-AAGG
A. limnaeus	TTAAAT---------TATAAATAAACCCCTATAACTATTA-AAGG
A. limnaeus	TTAAAC---------TATAAATAAATCCCTATAACTATTA-AAGG
A. guajira	TCAAAC---------TATATATAAATCTCTATAACTCTTA-AAGG
A. leoni	TCAAAC---------TATAAATAAACCCCTATAACTATTA-AAGG
A. leoni	TCAAAC---------TATAAATAAACCCCTATAACAATTA-AAGG
A. leoni	TCAAAC---------TATAAATAAACCCCGATAACAATTA-AAGG
A. leoni	TAAAAC---------TATAAATAAACCCCTATAACTATTA-AAGG

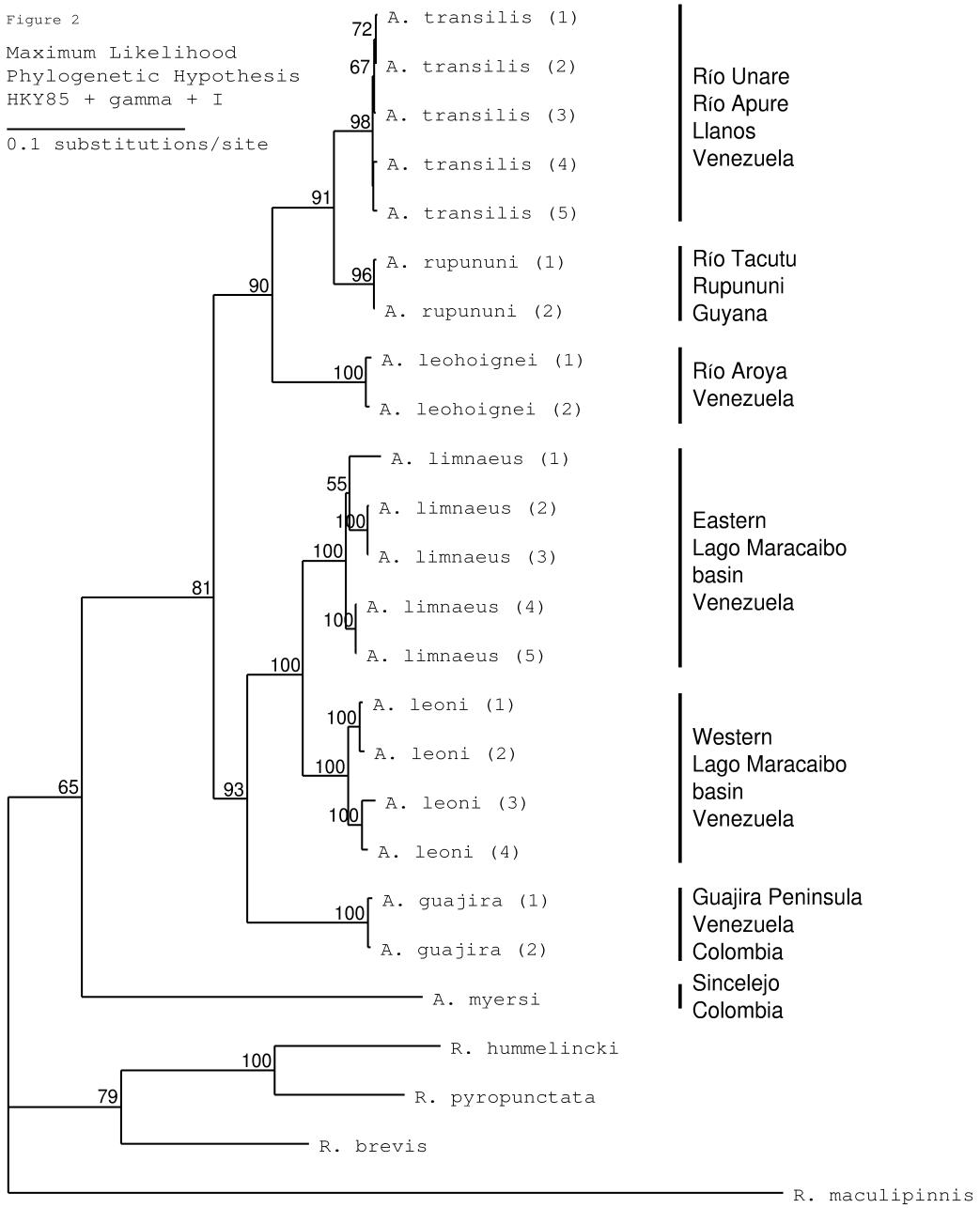

FIGURE 2. Maximum likelihood phylogenetic tree on 2653 aligned mtDNA base pairs. Likelihood of the topology is –ln L = 13502.97562 with ‡ = 0.465919. Numbers above nodes are bootstrap values (Felsenstein, 1985). In general, divergences are well supported. Key to genera: *A.* = *Austrofundulus*; *R.* = *Rachovia*.

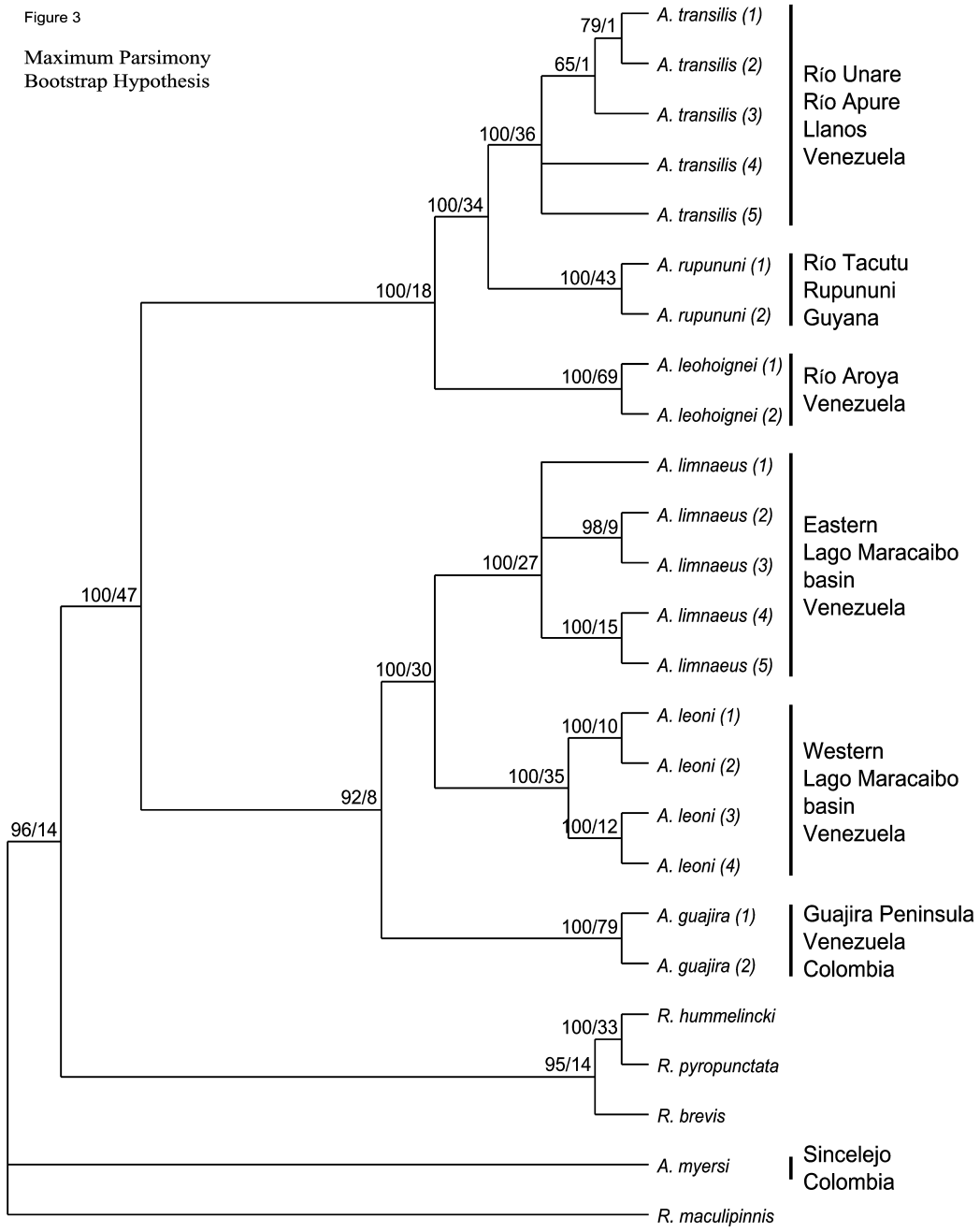

FIGURE 3. A strict consensus of four single most parsimonious mtDNA trees based on 2653 aligned base pairs, 796 of which were parsimony informative. The length of the trees is 2422 steps. Numbers above nodes are bootstrap values (Felsenstein, 1985) followed by Bremer support values (Bremer, 1994). In general, divergences are well supported. Key to genera: *A.* = *Austrofundulus*; *R.* = *Rachovia*.

Austrofundulus Myers 1932

Austrofundulus Myers 1932: 159–162 (original description, type species: *A. transilis* Myers, by original designation, based on single specimen). Hoedeman 1961: 89–91 (based on head scale patterns, *Austrofundulus* put in separate subfamily from *Rachovia*). Weitzman and Wourms 1967: 89–100 (generic characters discussed, validity of *Austrofundulus*, *Rachovia* and *Pterolebias* is doubted). Vaz-Ferreira and Sierra de Soriano 1972: 38–40 (attempt to distinguish *Austrofundulus* and *Rachovia* based on neuromast and lateral pore patterns, caudal and pelvic-fin morphology). Thomerson 1979: (comments on generic name). Elder Jr. *et al.* 1991: (karyotypes, comments on generic names). Costa 1990: (based primarily on osteological and meristic characters, *Austrofundulus* placed into synonymy of *Rachovia*). Costa 1998: (*Austrofundulus* revalidated but without explanation). Huber 1999: (morphometric relationships to other rivulin genera). Hrbek and Larson 1999: (phylogeny based on mitochondrial DNA data). Murphy *et al.* 1999: (phylogeny based on mitochondrial DNA data).

Diagnosis. *Austrofundulus* are Neotropical, northern South American, annual rivulid fishes that are distinguished from all new world rivulids except *Rachovia* in having at least the basal 45% of the caudal fin scaled. They differ from the closely related genus *Rachovia* (see Fig. 4) in having: more dorsal rays, usually 14 or more (range 12–18) vs. 13 or fewer (range 9–14); a longer dorsal fin base, more than 16% SL (12–25%) vs. less than 16% (10–18%); more lateral scales, usually 32 or more (28–38) vs. 32 or fewer (27–33); and more transverse scales, usually 11 or more (9–16) vs. usually 10 or less (8–11). Many males of *R. brevis* and *R. maculipinnis* have a dark blotch around white spots on the dorsal fin, a pattern never seen in *Austrofundulus*, which never has white spots on the dorsal fin of males. The anal and genital papillae are usually heavily pigmented in *Austrofundulus* but only lightly pigmented or unpigmented in *Rachovia* and other new world rivulids from northern South America.

FIGURE 4. Photo of a male *Rachovia maculipinnis.*

Austrofundulus is distinguished from *Terranatos* (see Fig. 5) in having: shorter fins, the dorsal fin less than 55% SL (range 23–47%) in males, and less than 40% SL (range

22–40%) in females, versus more than 55% (55–124%) and more than 37% (37–47%) in males and females of *Terranatos*, respectively; caudal fin scaled for more than one third of its length vs. unscaled except at base; female without extensions of caudal fin rays vs. filamentous rays on the dorsal and ventral margins; maximum standard length more than 30 mm vs. less than 30 mm SL; anal actinosts articulated with hemal spines rather than ribs.

FIGURE 5. Photo of a male *Terranatos dolichopterus*.

***Austrofundulus transilis* Myers 1932**
Figure 6

Austrofundulus transilis Myers 1932: 159–162 (original description of genus *Austrofundulus*, and *A. transilis*). de Beauford 1940: 110 (specimens of *A. guajira* from Guajira peninsula listed). Myers 1942: 110–112 (based on specimens of *A. limnaeus* and possibly other species from Lake Maracaibo Basin). Schultz 1949: 82, 85–89 (description of subspecies *A. t. transilis*, and *A. t. limnaeus*; key, data on holotype of *A. t. transilis*). Myers 1952: 135, 138–139 (life cycle, figure of *A. limnaeus* {labeled as *A. transilis*} from near Lake Maracaibo). Hoedeman 1958: 25, 27 (frontal head scale pattern for "*Austrofundulus transilis*" but author does not identify material used to prepare figure). Weitzman and Wourms 1967: 89–100 (discussion of annual killifish genera, refers to *A. transilis* {=*A. limnaeus* or one of the new species described here} figure 3 shows a fish of the *A. limnaeus* group identified as *A. transilis*). Turner 1967: 845 (distribution of *Rachovia hummelincki* and "*A. transilis*" compared, but *Austrofundulus* sites refer to species of the *A. limnaeus* group). Scheel 1969: 11–16 (frontal scale pattern and caudal scales discussed for "*A. transilis*" but material not identified). Thomerson 1971: 23 (discussion of distribution of *A. transilis limnaeus* in relation to that of *R. hummelincki*). Goldstein 1972:51 (color photo identified as *A. transilis* is probably *A. leohoignei*). Thomerson and Turner 1973: 786 (*A. transilis* listed as syntopic with other annual form Caño Benito area of the Venezuelan llanos). Vaz-Ferreira and Sierra de Soriano 1972: 36–40 (lateral line and head pore patterns for *A. transilis* = *A. limnaeus* species group). Taphorn and Thomerson 1975: 67–73 (photo, key, color description, distribution).

Holotype. USNM 92191. An adult male (40.0 mm) from a pond in the state of Guarico, Río Orinoco basin, Venezuela; collected by F. F. Russell in 1928 (precise date is unknown).

Paratypes. None.

Diagnosis. *Austrofundulus transilis* is the smallest of all species of *Austrofundulus*. It is distinguished from all other species of *Austrofundulus* by pastel light tan to pink-grey background body color, and the lack of any black markings on the body. Males of *A. transilis* have pink to red background color on all fins with occasional blue-green highlights between the fin rays; this fin color pattern is also diagnostic.

FIGURE 6. Photo of a male *Austrofundulus transilis*.

Description. A small proportion of males also have an iridescent blue-green or black ocellus on the dorsal fin. The caudal fin is rounded and without "lyre-tail" extensions (usually present in *A. rupununi*, morphologically the most similar species). Pelvic and pectoral fins are lighter than the unpaired fins. Females have clear fins. The sides of the body of males are unpatterned. The dorsum of the body is light tan anterior to the dorsal fin, and gets progressively pinker posterior to the dorsal fin, and on the sides of the body. Each scale is lightly outlined with white or lighter pink. The abdomen is pale, almost white. In females, the basic color is plain light tan. As in males, the abdomens of females are lighter than the rest of the body. The iris of the eye is silver. A faint eye bar is usually visible; however, this character varies with the temperament of the fish. For additional characters see Myers (1932), Taphorn and Thomerson (1978) and Table 3.

Distribution. Known from the Llanos of the Río Apure drainage, and from the lower Río Unare basin (Fig 1).

Remarks. *Austrofundulus transilis* are small, relatively non-aggressive fish when compared to *A. limnaeus* and the other species described in this paper. Although there is sexual differentiation in the color pattern, it is subtle compared to the other *Austrofundulus* species.

TABLE 3. Meristics and morphometrics of *Austrofundulus transilis*.

		males, n = 24			females, n = 9		
	H	low	high	mean	low	high	mean
Standard Length (mm)	40.0	20.5	38.3	28.9	20.8	33.3	27.2
Meristics							
Dorsal rays		12	15	13.5	12	14	13.1
Anal rays		14	17	15.8	14	17	15.6
Pectoral rays		14	16	14.8	14	16	14.6
Lateral scales		28	33	31.2	30	31	30.4
Transverse scales		10	12	11.4	10	12	11.4
Caudal peduncle scales		16	18	16.3	16	18	16.4
Breast scales		6	11	8.6	7	11	8.8
Thousandths of standard length							
Greatest body depth		.262	.312	.292	.261	.333	.286
Caudal peduncle depth		.134	.166	.149	.125	.169	.141
Caudal peduncle length		.191	.241	.212	.190	.241	.211
Head width		.155	.204	.184	.163	.205	.184
Head depth		.184	.225	.203	.173	.232	.194
Head length		.373	.373	.325	.316	.357	.337
Snout length		.011	.050	.037	.010	.059	.030
Eye diameter		.079	.012	.096	.086	.012	.098
Predorsal length		.627	.695	.660	.639	.722	.675
Preanal length		.572	.627	.599	.598	.654	.626
Dorsal fin base length		.151	.201	.310	.127	.189	.298
Anal fin base length		.165	.237	.191	.152	.219	.194
Pelvic fin length		.094	.141	.110	.109	.137	.121
Dorsal fin length		.257	.346	.600	.228	.321	.625
Anal fin length		.274	.345	.175	.270	.329	.166
Pectoral fin length		.164	.217	.191	.168	.224	.174

Austrofundulus rupununi new species

Figures 7 and 8

Austrofundulus limnaeus Schultz 1949. Taphorn and Thomerson 1978:415, 420–421 as "Guyanan Populations", Table 13.

Holotype. FMNH 108226 (ex FMNH 92580). Adult male (38.2 mm) collected in an Ara-

pari area on Louis Orella's Manari Ranch, 9th District, Guyana, approximately 4 miles due west of ranch house by L. Orella, D. and A. Melville, J. E. Thomerson, D. C. Taphorn and D. Hicks on 12 August, 1975.

Paratypes. FMNH 92580, twenty three additional specimens collected at the type locality together with the holotype. MCNG 52001, four specimens collected at the type locality together with the holotype. UMMZ uncatalogued (2 specimens), Rupununi, flooded roadside cut south of Grami Pond. 15 July 1971, F. Cichocki, B. Carlson. UMMA uncatalogued (1 specimen) Rupununi, ditch 1.5 miles south of Pirara Ranch, 22 July 1971, F. Cichocki, B. Carlson.

FIGURE 7. Photo of *Austrofundulus rupununi* FMNH108226 (male holotype).

FIGURE 8. Photo of a male *Austrofundulus rupununi*.

Diagnosis. *Austrofundulus rupununi* is distinguished from all other species of *Austrofundulus* by the presence of black to dark brown humeral spots above the pectoral fin, iridescent blue-green dorsal fin with black edges, showing approximately five curved rows of dark spots, and iridescent blue-green caudal fins, with scattered black pigment. Com-

pared with other species of the "*limnaeus*" group, *A. rupununi* have larger scales reflected in the counts as significantly fewer lateral, transverse, caudal peduncle and breast scales; they also have fewer dorsal, and pectoral fin rays than other *Austrofundulus* species; as well as the greatest caudal peduncle depth of any *Austrofundulus* species.

Description. The males have an iridescent blue-green dorsal fin with black edges, showing approximately five curved rows of dark spots. Females have clear dorsal fins with light gray spots. Males have iridescent blue-green caudal fins, with scattered black pigment. The color is more intense proximally; distally the fin is dark gray. Both the dorsal and the ventral edge of the caudal fin extend to form a "lyre-tail". Females have clear to light brown caudal fin. The anal fin of males is similar to the dorsal and caudal fins. A background of iridescent blue-green near the base is broken with about three bars of dark brown, with start at the base and curve out anteriorly, away from the body. The distal third of the anal fin is sometimes brown or sooty gray, but the proximal blue-green background may extend all the way to the distal edge. Females have clear light brown anal fins, with four to five brown spots. Both the pectoral and pelvic fins of males are reddish-brown, with easily visible rays. The pectoral and pelvic fins of females are clear or light gold. The sides of the body of males are complexly patterned. The reddish-brown background gets progressively darker from ventral to dorsal surface. Behind and above the pectoral fin are several (6–20) irregularly shaped spots of black, dark brown or maroon color. On the sides of the body, just anterior of the dorsal fin, there are vertical rows of iridescent blue-green scales. In some individuals these align to form vertical bars of blue-green alternating with reddish-brown. These colorful scales continue out onto the caudal peduncle. In females, the basic color is plain light brown, but a few individuals have an occasional blue-green scale. As in males, the abdomens of females are lighter than the rest of the body. Morphometric data are given in Table 4. Largest *Austrofundulus rupununi* male: 41.2 mm SL, mean: 36.6 mm SL. Females attain about equal size. Dorsal profile of head strongly convex, rapidly increasing posteriorly. Head smaller and less deep than in other species of the *A. limnaeus* group. Convex dorsal profile continued through dorsal fin base. Ventral profile of head convex, straightening posteriorly to anal fin. The double convexity makes the body robust with greatest body depth between pectoral fin base and dorsal fin origin. Caudal peduncle very deep, the deepest seen in the "*A. limnaeus"* species group. Lower jaw prominent, extended beyond upper jaw. Dorsal and anal fins slightly pointed in males, rounded in females. Caudal fin with extensions of upper and lowermost rays forming a slight "lyretail". Head scale pattern variable, but most individuals show the "E" pattern (Taphorn and Thomerson 1978, figure 2H, pg. 444). Neuromasts on top of the head arranged in opposing pairs in the form of a lyre. Lateral line is complete.

Etymology. Named after the Rupununi savannah, Guyana.

Distribution. Known only from the Rupununi savannah in Guyana, of the upper Tacutu River drainage which flows into the Branco and is part of the Amazon River Basin. This species is expected to occur to the south in the savannahs surrounding Boa Vista,

Roraima, Brazil, but to date no *Austrofundulus* has been confirmed in this region. It is the only *Austrofundulus* known from an Amazon Drainage system.

TABLE 4. Meristics and morphometrics of *Austrofundulus rupununi*.

		males, n = 13			females, n = 15		
	H	low	high	mean	low	high	mean
Standard Length (mm)	41.0	33.0	41.2	36.6	30.3	41.7	33.0
Meristics							
Dorsal rays		13	15	13.9	13	15	13.8
Anal rays		15	17	15.6	15	16	15.3
Pectoral rays		14	15	14.7	14	16	14.8
Lateral scales		29	33	30.9	29	32	30.7
Transverse scales		11	11	11.0	11	11	11.0
Caudal peduncle scales		16	18	16.9	16	18	16.8
Breast scales		7	10	8.3	7	10	8.8
Thousandths of standard length							
Greatest body depth		.286	.320	.303	.277	.309	.288
Caudal peduncle depth		.159	.175	.170	.143	.166	.156
Caudal peduncle length		.180	.221	.202	.195	.222	.204
Head width		.177	.195	.186	.184	.207	.197
Head depth		.214	.238	.224	.178	.220	.200
Head length		.298	.318	.327	.312	.331	.322
Snout length		.034	.051	.042	.035	.050	.041
Eye diameter		.086	.100	.093	.089	.108	.099
Predorsal length		.637	.681	.661	.664	.710	.686
Preanal length		.579	.625	.606	.623	.656	.635
Dorsal fin base length		.175	.209	.190	.158	.185	.170
Anal fin base length		.190	.218	.202	.153	.175	.163
Dorsal fin length		.295	.363	.335	.273	.310	.286
Anal fin length		.303	.388	.332	.271	.309	.290
Pectoral fin length		.182	.238	.214	.187	.234	.216
Pelvic fin length		.104	.149	.131	.117	.142	.132

Remarks. In general, individuals of *Austrofundulus rupununi* are phenotypically "intermediate" between the most gracile or delicate species of the genus, *A. transilis*, of the Venezuelan llanos and the rest of the species previously known as *A. limnaeus* which

attain a larger size and are robust predators. Similarly to *A. transilis* they have relatively small body size, and do not appear to be very aggressive. As in the *A. limnaeus* species group this species also have highly developed male coloration, as well as dorsal and ventral extensions on the caudal fin. In general, the over all tone of the body coloration is pastel, rather than the more gaudy color patterns seen in other *Austrofundulus* species.

Austrofundulus leohoignei **new species**

Figures 9 and 10

Austrofundulus limnaeus Taphorn and Thomerson 1978:415, 418–420 as "Tucacas Populations", Table 12;

Austrofundulus transilis Goldstein 1972: 51 (color photo, no locality given, but fish are very similar to phenotypes from Tucacas).

Holotype. FMNH 108224 (ex FMNH 85266). Adult male (65.3 mm) collected in a small temporary pond 4 km north of Sanare on the road to San Juan de Los Cayos by J. E. Thomerson and L. Hoigne on 20 August 1969.

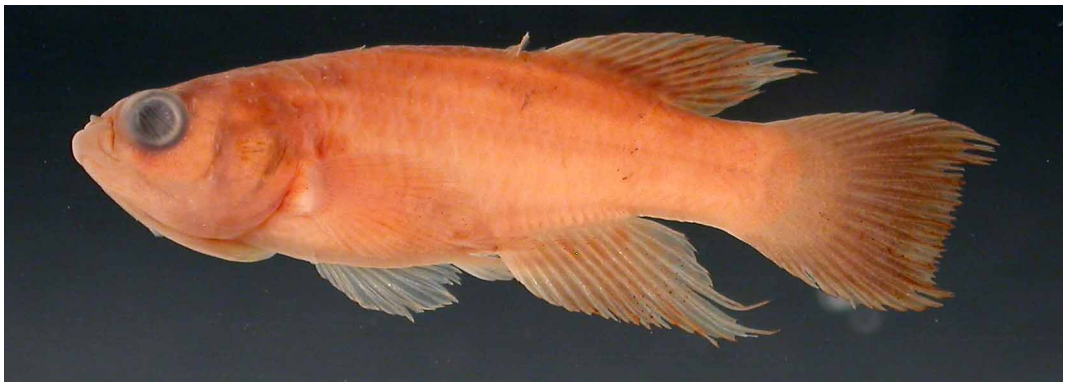

FIGURE 9. Photo of *Austrofundulus leohoignei* FMNH108224 (male holotype).

FIGURE 10. Photo of a male *Austrofundulus leohoignei*.

Paratypes. FMNH 85266, nine additional specimens collected at the type locality together with the holotype. FMNH 92574, thirty three additional specimens collected at the type locality together with the holotype. MCNG 32100, one additional specimen collected in a rain pool (Río Aroa drainage) about 10 km east of Palma Sola in the direction of Tucacas by D. Taphorn, L. Page, K. Cummings, C. Meyer, P. Ceas and J. Armbruster on Jan. 21, 1995.

Diagnosis. *Austrofundulus leohoignei* are phenotypically "intermediate" between the gracile *A. transilis* and the robust *A. limnaeus*, and the other new species described here. They have relatively small body size, and do not appear to be very aggressive; however, they do have highly developed male coloration and sexual dimorphism, including the red subterminal ring seen in *A. guajira*, as well as short dorsal and ventral extensions on the caudal fin. In general, the overall tone of the body coloration is somber gray-green, more akin to the pastel colors of *A. transilis* than the more gaudy color patterns seen in other *Austrofundulus* species.

Description. Males have a dull steel-gray-blue, sky-blue or aqua colored background in the dorsal fin over which approximately six irregularly curved rows of black, brown or maroon spots are overlaid. Proximal spots are larger, and are often so indistinct so as to form irregular blotches. The edge of the dorsal fin is black. Females have clear or dusky gray dorsal fins with a few faint gray spots. Males have dull steel-gray-blue caudal fins. Subtending the distal margin, there is often an orange-red or blue subterminal band, which may be continuous from the top to the bottom of the fin or broken in several sections by an intervening black or other shades of blue. Frequency of the subterminal band varies significantly from year to year, however. There are sometimes a few diffuse spots at the upper edge of the fin near the caudal peduncle. Both the dorsal and the ventral edge of the caudal fin extend to form a "lyre-tail". Females have clear to light golden caudal fin, with some individuals exhibiting melanistic blotches. The anal fin of males consists typically of light background overlaid with darker spots. However, especially the basal spots are not arranged in distinct row. The background is light gray or blue, with dark gray spots. Distally the fin is darker, almost becoming black. Females have tan or gray, with similarly arranged spots. In some females, all anal pigment is lacking. Both the pectoral and pelvic fins of males are dusky-gray to maroon, and often have faint gray spots. The pectoral and pelvic fins of females are clear or dusky-gray. The sides of the body of males are complexly patterned. The background color is gray or brown, with black or dark brown spots just behind the opercle, and reddish-brown to maroon spots further back, often arranged in a line pattern. Posterior to the dorsal fin, the body is darker, and the sides may have few scattered blue scales. In some individuals, the blue scales and brown background alternates to form diagonal zig-zag rows. The ventrum is lighter, but similarly patterned. In females, the basic color is plain light gray, with scales near the middle of the sides having dark centers with lighter margins. As in males, the abdomens of females are lighter than the rest of the body. The color patterns of males are heterogeneous, but do not overlap with

those of any other species. Morphometric data are given in Table 5, see also Table 12 of Taphorn and Thomerson (1978). *Austrofundulus leohoignei* attains up to 68.0 mm SL in males, 32.0 mm in females.

TABLE 5. Meristics and morphometrics of *Austrofundulus leohoignei*.

		males, n = 24			females, n = 10		
	H	low	high	mean	low	high	mean
Standard Length (mm)	27.0	25.4	68.0	34.7	25.8	32.0	28.5
Meristics							
Dorsal rays		14	16	15.5	15	16	15.1
Anal rays		16	18	16.7	16	18	16.8
Pectoral rays		14	17	15.3	13	16	15.3
Lateral scales		30	33	31.3	30	33	31.0
Transverse scales		10	13	11.0	11	13	12.0
Caudal peduncle scales		15	19	16.1	15	16	15.9
Breast scales		6	10	7.4	7	9	7.5
Thousandths of standard length							
Greatest body depth		.288	.378	.318	.280	.296	.287
Caudal peduncle depth		.146	.172	.157	.131	.154	.143
Caudal peduncle length		.168	.215	.194	.181	.212	.194
Head width		.184	.228	.203	.197	.215	.268
Head depth		.186	.258	.218	.165	.210	.191
Head length		.286	.367	.329	.319	.341	.329
Snout length		.026	.059	.038	.020	.041	.033
Eye diameter		.076	.108	.097	.096	.108	.101
Predorsal length		.642	.764	.668	.626	.712	.684
Preanal length		.591	.716	.621	.615	.672	.642
Dorsal fin base length		.177	.253	.212	.165	.203	.188
Anal fin base length		.188	.242	.213	.153	.178	.170
Dorsal fin length		.294	.466	.376	.306	.396	.341
Anal fin length		.342	.478	.412	.298	.363	.330
Pectoral fin length		.164	.256	.219	.207	.240	.221
Pelvic fin length		.110	.208	.165	.108	.143	.127

Etymology. We take great pleasure in naming this species in honor of the late Mr. Leo Hoigne who discovered this species, and many other annual killifishes in Venezuela. It was our privilege to know him and share his delight in discovering and keeping annual killifishes.

Distribution. Endemic to the Río Aroa drainage, Falcón state Venezuela. This species is known from only two lowland localities near Sanare, and its survival is threatened by agriculture.

***Austrofundulus limnaeus* (Schultz 1949)**
Figure 11

Austrofundulus transilis Myers 1942: 110–112 (in part; specimens from Lake Maracaibo Basin described, some of which were later types of *A. t . limnaeus* Schultz 1949). Myers 1952: 135, 139 (figure and discussion of annual life cycle). Hoedeman 1958: 26–27 (head scales illustrated; no locality given). Weitzman and Wourms 1967: 89, 100 (generic characters; photo of *A. t. limnaeus* paratypes). Thomerson 1971: 21–28 (in part; several Venezuelan localities listed). Vaz-Ferreira and Sierra de Soriano 1972: 38–40 (head and lateral neuromast patterns for specimens from Lagunillas, Venezuela are given).
Austrofundulus transilis limnaeus Schultz 1949: 85–88 (original description; holotype illustrated).
Austrofundulus stagnalis Schultz 1949: 82–89 (original description). Taphorn and Thomerson 1978: 414: (synonymized with *A. limnaeus*).

Holotype. UMMZ 141916: An adult male (61.0 mm) in good condition from the lower Río Cocuiza drainage 15 km west of San Felix, on the western border of Falcón state, Venezuela; collected by F. Bond on 21 March 1938.

Paratypes. UMMZ 141917, thirty two of the 34 paratypes were collected with the holotype, and of these nine were members of the species *Rachovia pyropunctata* (Taphorn and Thomerson 1978). The two other paratypes were collected by P. Wagner Hummelick in Pozo de Arroyo de Arara, El Cardon, Guajira; according to the here-proposed classification, these specimens are transferred to the paratype series of the species *Austrofundulus guajira*.

FIGURE 11. Photo of a male *Austrofundulus limnaeus*.

Diagnosis. This species is distinguished from all species of *Austrofundulus* by the presence of iridescent blue-green spots present on its caudal peduncle and the basal por-

tion of its caudal fin. This character is present in all sexually mature males. Intensity of the iridescent spots varies along a north-to-south gradient. Individuals from the northern coastal deserts occur in very turbid environments, and have corresponding more intense iridescent colors, while individuals from more southern, less turbid habitats have less intense colors.

TABLE 6. Meristics and morphometrics of *Austrofundulus leoni*.

		males, n = 16			females, n = 16		
	H	low	high	mean	low	high	mean
Standard Length (mm)	28.8	27.9	29.4	28.6	19.7	30.7	25.2
Meristics							
Dorsal rays		14	15	14.5	14	15	14.5
Anal rays		16	18	17.0	16	17	16.5
Pectoral rays		14	16	15.0	16	16	16.0
Lateral scales		31	33	32.0	32	33	32.5
Transverse scales		11	13	12.0	12	13	12.5
Caudal peduncle scales		17	19	18.0	16	20	18.0
Breast scales		8	8	8.0	9	9	9.0
Morphometrics							
Greatest body depth		.259	.296	.274	.236	.290	.260
Caudal peduncle depth		.127	.153	.142	.122	.147	.138
Caudal peduncle length		.180	.228	.199	.199	.236	.212
Head width		.186	.234	.210	.180	.218	.202
Head depth		.171	.216	.195	.165	.203	.186
Head length		.333	.366	.352	.327	.366	.350
Snout length		.041	.060	.048	.039	.059	.049
Eye diameter		.090	.116	.098	.092	.124	.106
Predorsal length		.636	.700	.670	.635	.717	.672
Preanal length		.578	.640	.602	.583	.654	.620
Dorsal fin base length		.140	.196	.174	.148	.198	.173
Anal fin base length		.161	.216	.198	.166	.204	.186
Dorsal fin length		.254	.386	.329	.294	.349	.324
Anal fin length		.271	.421	.361	.294	.390	.336
Pectoral fin length		.141	.286	.229	.199	.268	.228
Pelvic fin length		.111	.153	.130	.091	.142	.126

Description. See also: (Schultz 1949; Taphorn and Thomerson 1978). In general, the color seems to reflect a gradation that seems correlated to the amount of yearly rainfall and the resultant variety in habitat. In the northern end of the distribution, the extremely arid coastal deserts near Quisiro, the males have bright blue-green caudal fins and numerous iridescent blue spots on the body, particularly on the caudal peduncle. The over all background body color is dominated by a strong blue-green hue. Sides of the body near the head are light with few darker spots. Proceeding south, where populations inhabit progressively less xeric habitats, the background body color becomes darker, and the number of iridescent spots decreases. These populations were originally described as *A. stagnalis* by Schultz. In the southern-most portions of the species distribution, the background body color becomes rich golden brown with very few blue spots on the body. The southern-most populations inhabit clear, tea colored waters, while the northern-most populations inhabit heavily turbid waters, laden with light-colored inorganic silt (Podrabsky *et al.* 1998).

Specifically, the males have a light bluish, gray-bluish, grayish or gray-greenish colored background in the dorsal fin with several curved rows of darker spots, the proximal ones larger, less numerous, and arranged more regularly than the distal ones. Females have clear dorsal fins with a few gray, golden or light green spots. Males have intricately colored caudal fins, combining flecks and spots over variously colored background. Shiny iridescent green and/or blue are the dominant background colors. The basal two thirds of the caudal fin are scaled, ranging from iridescent blue-green to bronze with light yellow tint as one proceeds from north to south, while iridescent blue-green is the dominant color of the unscaled portion of the caudal fin. The iridescent blue spots present on the caudal peduncle extend into the caudal fin, and are especially prominent in the scaled portion of the caudal. The spots are often joined in an irregular line-like pattern. Similar to the body, the density of iridescent spots decreases as one proceeds south. The whole fin is mottled with black specks. The posterior edge of the caudal fin is often grayish. Both the dorsal and the ventral edge of the caudal fin extend to form a “lyre-tail”. Females have a drab brown to gray caudal fin, with few gray spots. The anal fin of males has a basic plan of light background with curved rows of darker spots. The base color is reddish-brown or golden with light blue to dark brown or gray spots. The basal spots are often diffused, more like amorphous blotches. The area surrounding these blotches is often white or light brown. The anal fin of females is golden near the base, clear or light gray distally, with a few faint basal spots. The pectoral fins of both sexes are clear or dusky-gray, with a few faint spots. Pectoral fin rays are easily visible. The pelvic fins of males have a similar color pattern to the anal fin, however, are much less intense. The basic background color tends to be reddish-brown, with few faint spots. The pelvic fins of females are clear to light gold, with faint spots. The sides of the body of males are complexly patterned. The background color is light gray to brown, with dark brown spots or flecks in the anterior portion of the body. In the middle and posterior portion of the body, the spots become lighter, changing into numerous white, light yellow or iridescent blue spots. The ventrum is lighter brown or

cream, without spots, while the dorsum is darker than the rest of the body. In females, the sides are gray or brown, occasionally with darker outline of each scale. The body is not spotted, or only rarely a few gray spots are present behind the pectoral fin base. As in males, the abdomen of females is lighter than the rest of the body, while the dorsum is darker. In both sexes a dark brown or black bar passes obliquely through the eye onto the head posteriorly above and anteriorly below the eye. The iris is usually silver, sometimes golden. In males, the intensity of this pattern decreases as one moves south, while in females this pattern is generally less pronounced. An outstanding feature of the head is the yellow or gold opercle. Rear portion of the opercle is covered with large shiny scales that change color with the angle of reflected light. The upper portion of the hear is brown or tan, the lower cream or tan. In females the head is predominantly gray or olive dorsally, and lighter ventrally. For meristics see Table 6.

Distribution. We here restrict the type locality to the eastern shores of the Lake Maracaibo; from the far western coastal border of Falcón State south through much of eastern Zulia to Santa Apolonia, Trujillo State. Despite intensive collecting, neither *A. limnaeus* nor any other *Austrofundulus* species, have been found in the southern region of Lake Maracaibo.

Austrofundulus leoni new species

Figures 12 and 13

Holotype. FMNH 108225 (ex FMNH 85268). Adult male (28.2 mm) collected in a small temporary pond approximately 5 km from the junction of the road to Machiques between Macoa and Río Yasa; collected by J. Thomerson and F. Mago on 26 June 1972.

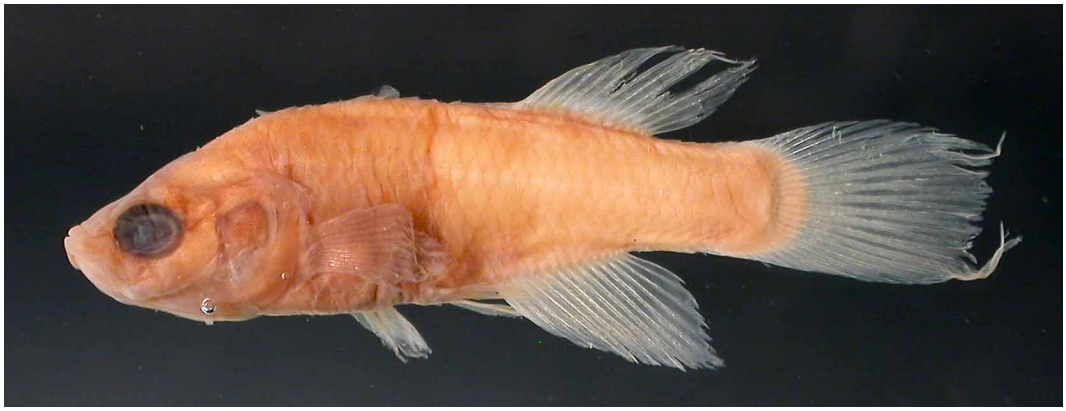

FIGURE 12. Photo of *Austrofundulus leoni* FMNH108225 (male holotype).

FIGURE 13. Photo of a male *Austrofundulus leoni*.

Paratypes. FMNH 85268, ten additional specimens collected with the holotype. FMNH 85724, twenty six specimens Venezuela, Zulia collected in a temporary pond 5 km north of Campo Mara, 25 June 1972, J. E. Thomerson. MCNG 33448, six specimens Venezuela, Zulia, Libertad, Caño antes de la Hacienda Las Mercedes, 22 July 1974, C. Lilyestrom, D. Spiers, V. Sabril, DISCA74-128. MCNG 39145, one specimen Venezuela, Zulia, Perijá, Saliendo de la población de Sartaneja, 10° 6´ 35" N, 72° 19´ 7" W, O. Leon. MCNG 39146, six specimens, same data as 39145. MCNG 39147, four specimens Venezuela, Zulia, Préstamo cerca de la Hacienda el Japon, 30 June 1978, D. Taphorn, E. Sutton.

Diagnosis. This species is distinguished from all species of *Austrofundulus* by strong reddish-brownish background body color observed in all sexually mature males. Relative to other species, the caudal peduncle is slender. The caudal fin is also large and slender with long extensions.

Description. Males have a reddish-brown colored background in the dorsal fin with several curved rows of dark brown spots. Basal spots are large, often joining into irregular or oblong flecks. The dorsal often has long fin ray extensions. The dorsal fin of females is unpatterned, with clear or light gray background. The background color of the males' caudal fin is reddish, with grayish overtones towards the posterior section of the fin. There are numerous indistinct dull gray spots randomly dispersed on the caudal fin. The distal edge has a weak blue-black terminal edge. Both the dorsal and the ventral edge of the caudal fin extend to form a long "lyre-tail". Females have a light grayish translucent caudal fin. The anal fin of males is patterned similarly to the dorsal fin. The background color is brown-reddish with a few large indistinct gray flecks in the basal half of the anal fin. The large gray flecks coalesce and become darker to form a blue-black distal margin. The base of the anal fin is creamy white, grading into this same color on the belly. The anal fin often has a long extension. The anal fin of females is clear or light gray, with a few faint basal spots. The pectoral fins of males are uniformly translucent brownish-red with a grayish edge. The pelvic fins of males are similar to the pattern found on the anal fin. The background color is brownish-red with a few large dull gray spots in the basal portion of the fin,

although in some males the spots are absent. The base of the pelvic fin is almost white, and blends with the color of the belly. Females have translucent, light gray pectoral and pelvic fins. The basic background color is brownish-red with gray overtones. Few dull light gray to almost white spots are present in the posterior two thirds of the body, while the anterior one third of the body has several dark brown to black colored spots. Just above, and across the opercle, the spots are arranged into several diagonal rows. The ventrum is light gray to almost white, without spots, while the dorsum is darker than the rest of the body. In females, the sides are light brown to light gray colored. The body is not spotted. As in males, the abdomen of females is lighter than the rest of the body, while the dorsum is darker. An indistinct black bar passes through the eye of both sexes, passing obliquely through the eye onto the head posteriorly above and anteriorly below the eye. The iris is silver. The upper portion of the head is dark gray, the lower light gray to cream. In females the head is predominantly gray or olive dorsally, and lighter ventrally. A few iridescent golden scales are present on the males' opercle, which has an overall yellowish tone. For meristics and morphometrics see Table 7. *Austrofundulus leoni* attains up to 29.4 mm. SL with a mean length of 26.0 mm SL. Females reach up to 33.6 mm. SL with a mean length of 25.9 mm SL.

TABLE 7. Meristics and morphometrics of *Austrofundulus limnaeus*.

		males, n = 45			females, n = 24		
	H	low	high	mean	low	high	mean
Standard Length (mm)	61.0	18.2	86.9	43.5	15.6	61.3	35.1
Meristics							
Dorsal rays		13	16	14.5	13	15	14.1
Anal rays		16	18	16.8	16	18	16.7
Pectoral rays		14	17	15.4	15	18	15.5
Lateral scales		30	38	32.6	30	35	32.3
Transverse scales		10	15	12.1	10	14	12.2
Caudal peduncle scales		15	21	18.0	17	20	18.7
Breast scales		7	17	11.5	7	14	10.2
Thousandths of standard length							
Greatest body depth		.259	.406	.314	.250	.335	.298
Caudal peduncle depth		.132	.203	.157	.128	.169	.150
Caudal peduncle length		.184	.244	.212	.189	.246	.218
Head width		.162	.243	.206	.186	.249	.212
Head depth		.187	.292	.272	.179	.225	.205
Head length		.287	.388	.341	.300	.383	.353

.....continued on the next page

TABLE 1 continued

		males, n = 45			females, n = 24		
	H	low	high	mean	low	high	mean
Snout length		.025	.082	.051	.039	.064	.050
Eye diameter		.063	.109	.089	.080	.119	.099
Predorsal length		.628	.718	.672	.641	.713	.688
Preanal length		.566	.648	.614	.596	.654	.632
Dorsal fin base length		.157	.216	.188	.151	.194	.175
Anal fin base length		.174	.239	.202	.141	.192	.172
Dorsal fin length		.259	.381	.330	.254	.333	.296
Anal fin length		.256	.418	.335	.194	.336	.287
Pectoral fin length		.138	.260	.207	.199	.266	.225
Pelvic fin length		.104	.148	.125	.090	.147	.125

Etymology. From Latin *leo* (lion) for its large size and majestic nature, and for the family León Mata who has been instrumental to conducting research in the Maracaibo basin.

Distribution. This species is distributed in the western and southwestern lowlands of the Lake Maracaibo basin. In the north it is replaced by *A. guajira* which occurs in Venezuela only near the border with Colombia and across the Guajira peninsula. Specimens collected from the drainage of the Río Limón have all proved to be *A. leoni*.

Austrofundulus guajira new species

Figures 14 and 15

Austrofundulus transilis de Beauford 1940: 110 (specimens listed from Guajira Peninsula, Colombia probably refer to this new species).

Holotype. FMNH 108223 (ex FMNH 85252). Adult male (55.1 mm) collected in a temporary pond 34 km west of Maicao, Colombia on road to Río Hacha, Colombia by J. E. Thomerson and P. Cala C. on 26 August 1972.

Paratypes. FMNH 85252, five additional specimens collected at the type locality together with the holotype. FMNH 85251, four additional specimens collected in a borrow pit approximately 8 km south of Río Hacha on road to Santa Marta, Colombia by J. E. Thomerson and P. Cala C. on 25 August 1972. FMNH 97709, three additional specimens collected in a temporary pond 7 km west of Maicao, Colombia on road to Río Hacha, Colombia by J. E. Thomerson and P. Cala C. on 26 August 1972. MCNG 17177, fifty three additional specimens collected about 2 km west of the Y fork in the road, going from

Guarero, Venezuela to Maicao, Colombia; collection by J. E. Thomerson, O. Leon M. and N. Montilla M. on 6 July 1987. MCNG 17180, seventy nine additional specimens collected about 12 km southwest of Y fork in the road, going from Guarero to Carrasquero, Venezuela; collection by J. E. Thomerson, O. Leon M. and N. Montilla M. on 6 July 1987. Additionally, the two other paratypes (ex UMMZ 141917) collected by P. Wagner Hummelick in Pozo de Arroyo de Arara, El Cardon, Guajira are removed from the paratypes of *A. limnaeus* and transferred to paratypes of *A. guajira*.

FIGURE 14. Photo of *Austrofundulus guajira* FMNH108223 (male holotype).

FIGURE 15. Photo of a male *Austrofundulus guajira*.

Diagnosis. This species is distinguished from all species of *Austrofundulus* by its robust size and by its dusky-gray to black background body color in all sexually mature males. The caudal fin of this species is without extensions, and it sometimes contains a bright red subterminal band, edged by a black terminal band. All fins are very round, without extensions, with the exception of the anal fin which in some large adult males has a small lance-like extension.

TABLE 8. Meristics and morphometrics of *Austrofundulus guajira*.

		males, n = 43			females, n = 41		
	H	low	high	mean	low	high	mean
Standard Length (mm)	49.0	37.0	60.2	46.7	19.4	71.6	40.1
Meristics							
Dorsal rays		14	18	15.6	14	18	15.7
Anal rays		15	19	16.9	16	19	17.0
Pectoral rays		13	17	15.8	14	17	15.9
Lateral scales		30	38	33.2	30	36	33.0
Transverse scales		11	15	13.2	11	16	13.2
Caudal peduncle scales		16	23	19.2	16	22	19.1
Breast scales		7	14	9.9	8	15	10.1
Morphometrics							
Greatest body depth		.284	.381	.321	.225	.361	.281
Caudal peduncle depth		.141	.185	.162	.128	.233	.146
Caudal peduncle length		.153	.225	.193	.162	.237	.203
Head width		.180	.238	.206	.185	.232	.208
Head depth		.192	.289	.244	.167	.228	.202
Head length		.308	.377	.348	.313	.375	.345
Snout length		.031	.055	.043	.029	.062	.045
Eye diameter		.078	.105	.091	.081	.107	.096
Predorsal length		.463	.681	.651	.639	.708	.669
Preanal length		.585	.647	.618	.617	.728	.651
Dorsal fin base length		.189	.252	.215	.175	.214	.193
Anal fin base length		.192	.250	.216	.139	.197	.173
Dorsal fin length		.295	.448	.381	.258	.364	.325
Anal fin length		.310	.429	.372	.243	.394	.313
Pectoral fin length		.108	.261	.199	.072	.243	.181
Pelvic fin length		.125	.255	.177	.108	.246	.171

Description. The males have a steel-blue to gray colored background in the dorsal fin with several curved rows of dark gray to black spots. Basal spot are large, often joining into irregular or oblong flecks. Distal spots are smaller, and are arranged in a concentric pattern. In large individuals, the dorsal fin has a lance-like extension, but the overall shape of the fin is round. Females have clear dorsal fins with a few gray spots. The background color of the caudal fin is greenish, with grayish overtones. There are numerous small dull greenish-yellow spots that are arranged in irregular rows radiating from the base of the

caudal fin. The distal edge has a black terminal edge. Many individuals also have a bright red subterminal band, which is anteriorly edged by a black edge. The caudal fin is very round, almost circular, and has neither dorsal nor ventral extensions. Females have a light grayish caudal fin, with few gray spots. The anal fin of males is patterned similarly to the dorsal fin. The background color is steel-blue to light gray with a few large gray flecks in the basal half of the anal fin. The large gray flecks transition into a black terminal margin. Large males possess lance-like extensions on the anal fin, but the overall shape of the fin is very round. The anal fin of females is clear or light gray, with a few faint basal spots. The pectoral fins of males are uniformly colored dusky steel-blue with a grayish edge, and some individuals have black specks scattered throughout the fin. The pelvic fins of males are similar to the pattern found on the anal fin. The background color is dusky steel-blue with a few large dull gray spots in the basal portion of the fin. The base of the pelvic fin is almost white, and blends with the color of the belly. Females have translucent, light gray pectoral and pelvic fins. The basic background color is steel-blue to gray, with numerous dull light gray to almost white spots. The light spots are predominant in the central portion of the body, with the caudal peduncle containing relatively few spots. The ventrum is light gray to almost white, without spots, while the dorsum is darker than the rest of the body. In females the sides are grayish to dark gray, the body is not spotted. As in males, the abdomen of females is lighter than the rest of the body, while the dorsum is darker. A black bar passes obliquely through the eye onto the head (posteriorly above and anteriorly below the eye) in both sexes. The iris is silver. The upper portion of the head is dark gray, the lower light gray to cream. In females the head is predominantly gray or olive dorsally, and lighter ventrally. For meristics and morphometrics see Table 8. *Austrofundulus guajira* attains up to 71.6 mm SL with a mean length of 43.4 mm SL. Females are of equal size as males.

Etymology. This species is named for the Guajira peninsula of Venezuela and Colombia, an area where it occurs.

Distribution. Known from the xeric regions of the Guajira peninsula of NW Venezuela and NE Colombia, north - northwest of the city of Maracaibo, Venezuela.

***Austrofundulus myersi* Dahl 1958**
Figure 16

Austrofundulus myersi Dahl 1958a: 1–58 (original description). Dahl 1958b: 42–44 (English translation of description). Weitzman and Wourms 1967: 96, fig 4 (photo). Wourms 1972: 142–168 (embryology).

Austrofundulus limnaeus Taphorn and Thomerson 1978: (proposed synonymy with *A. limnaeus*, description and morphometrics).

Austrofundulus transilis Turner 1967: 843–846 (reference to Colombian populations).

Holotype. CAS 149513 (original SU 49513). Adult male collected in a seasonal pond near Sincelejo, Colombia on 31 July 1950 by G. Dahl.

Paratypes. CAS 149513 (original SU 49513). Additional 7 specimens collected together with the holotype.

FIGURE 16. Photo of a male *Austrofundulus myersi.*

Diagnosis. This species is most similar to the *Austrofundulus leoni*. However, it is distinguished from all species of *Austrofundulus* by strong dark yellowish background body color in sexually mature males, and a robust body similar to *A. guajira*. *Austrofundulus myersi* has a short caudal peduncle, and a stocky caudal fin with medium sized extensions.

Description. The males have a dirty brown-yellowish colored background in the dorsal fin with several curved rows of dark brown spots. The dorsal can has long extensions. The dorsal fin of females is unpatterned, with clear or light gray background. The background color of the males' caudal fin is yellowish, with grayish overtones towards the posterior section of the fin. There are numerous indistinct dull brown spots randomly interspersed throughout the caudal fin. The distal edge has a weak blackish-brown terminal edge. Both the dorsal and the ventral edge of the caudal fin extend to form a long "lyre-tail". Females have a light grayish caudal fin. The anal fin of males is patterned similarly to the dorsal fin. The background color is brown-yellowish with a few large indistinct brown flecks in the basal one half of the anal fin. The large brown flecks transition into a blackish-brown terminal margin. The base of the anal fin is light, and transitions into the light colored belly. The anal fin often has a long extension. The anal fin of females is clear, with a few faint basal spots. The pectoral fins of males are uniformly translucent brownish-yellow. The pelvic fins of males are similar to the pattern found on the anal fin. The background color is dirty brownish-yellow. Few males have indistinct brown spots near the basal portion of the fin. The base of the pelvic fin is almost white, and blends with the color of the belly. Females have translucent, light gray pectoral and pelvic fins. The basic background color is dirty brownish-yellow with gray overtones. Few dull light blue to almost white spots are present in the posterior two thirds of the body, while the anterior one third of the body has several light brown colored spots. Above the opercle, the spots are arranged into a diagonal pattern. The ventrum is light gray to almost white, without spots, while the dorsum is darker than the rest of the body. In females, the sides are light brown colored. The body is not spotted. As in males, the abdomen of females is lighter

than the rest of the body, while the dorsum is darker. An indistinct black bar passes obliquely through the eye of both sexes, onto the head posteriorly above and anteriorly below the eye. The iris is silver. The upper portion of the head is dark gray, the lower light gray to cream. In females the head is predominantly gray or olive dorsally, and lighter ventrally. A few iridescent golden scales are present on the males' opercle, which has an overall yellow tone. For meristics see Dahl (1958b), and Table 9.

TABLE 9. Meristics and morphometrics of *Austrofundulus myersi*.

		males, n = 7			females, n = 9		
	H	low	high	mean	low	high	mean
Standard Length (mm)	59.0	42.4	63.9	52.1	37.9	54.7	49.0
Meristics							
Dorsal rays		15	19	16.4	15	17	16.2
Anal rays		17	19	18.0	17	19	18.0
Pectoral rays		15	17	16.0	16	17	16.1
Lateral scales		30	35	32.8	32	36	33.5
Transverse scales		12	13	12.8	12	14	13.0
Caudal peduncle scales		19	20	19.3	18	19	18.3
Breast scales		8	16	11.3	8	14	10.5
Morphometrics							
Greatest body depth		.284	.312	.299	.258	.305	.277
Caudal peduncle depth		.160	.181	.170	.151	.172	.160
Caudal peduncle length		.183	.235	.220	.219	.383	.251
Head width		.196	.210	.205	.190	.220	.202
Head depth		.197	.234	.216	.204	.230	.211
Head length		.318	.353	.341	.346	.364	.354
Snout length		.042	.056	.051	.055	.072	.063
Eye diameter		.071	.091	.080	.079	.089	.084
Predorsal length		.624	.647	.636	.585	.669	.645
Preanal length		.555	.629	.600	.633	.671	.652
Dorsal fin base length		.200	.237	.217	.166	.212	.197
Anal fin base length		.200	.240	.222	.153	.172	.162
Dorsal fin length		.347	.402	.380	.310	.357	.333
Anal fin length		.329	.440	.406	.287	.323	.304
Pectoral fin length		.224	.252	.241	.209	.227	.218
Pelvic fin length		.129	.188	.162	.135	.152	.144

Etymology. Dahl named this species to honor Dr. George Sprague Meyer, an eminent ichthyologist from Stanford University.

Distribution. *A. myersi* is known from the floodplain savannahs of the lower Río Magdalena between Cartagena and Barranquilla Colombia. Its distribution extents inland up the Río Magdalena valley (Fig. 1), however, the extent of its distribution is poorly known.

DISCUSSION

In the presented taxonomic revision of the genus *Austrofundulus*, we describe four new species and remove from synonymy an additional species. Some of the here presented taxonomic revisions have been anticipated in the most recent, but non-phylogenetic revision of the genus. In their revision, Taphorn and Thomerson (1978) predicted a close relationship of the Río Aroa populations with those of the Rupununi. Due to the overall smaller body size of individuals from these populations, the authors furthermore predicted a close relationship to *A. transilis*. However, body shape and caudal fin morphology of the males suggested a relationship with *A. limnaeus*. Due to the sharing of features with both *A. transilis* and the other *A. limnaeus* populations, and the inability of morphometric and meristic data to distinguish the Río Aroa and Rupununi populations from the other *A. limnaeus* populations, the Río Aroa and Rupununi populations were conservatively assigned to *A. limnaeus*. However, these populations were considered an evolutionary transition between *A. transilis* and the other *A. limnaeus* populations. After the publication of Taphorn and Thomerson (1978), additional clues suggesting distinctness and possible species status of some of the Maracaibo populations came from difficulties of hybridization of the Guajira fish (*A. guajira*) with Río Machango fish (*A. limnaeus*). Despite repeated attempts to hybridize these fish, only two F_1 hybrid offsprings of unknown fertility were produced (JET, pers. obs.).

Due to rampant non-informative morphometric and meristic variation, a fine-scaled phylogenetic analysis of *Austrofundulus* was possible only with the advent of modern molecular methods, resulting in a well supported phylogeny of the genus. The molecular phylogeny confirmed some, but not all of the previous revisers' (Taphorn and Thomerson 1978) predictions. Additionally, the maximum likelihood molecular phylogeny is highly concordant with the geologic history of northern South America. The maximum parsimony phylogeny differs, but only in the phylogenetic position of *A. myersi*. Differences are most likely due to reconstruction artifacts associated with incomplete sequence data obtained from the 1958 *A. myersi* paratypes. Final resolution of the phylogenetic position of *A. myersi* will require specimens more suitable for molecular analyses.

Short Overview of northern South American Geology.— Although complex, the orogeny of northern South America is well documented and can be used as an additional source of support for the here proposed taxonomic revision of *Austrofundulus*.

During the middle Eocene orogeny of Colombia, subduction of the Caribbean crust beneath the South American plate at the newly formed Sinu trench, caused a rapid uplift of the Cordillera Central and Cordillera Oriental respectively. This initiated the separation of the Colombian lowland, which, however, was not completed until the late Oligocene when major changes in direction of movement of the South American and Maracaibo plate with respect to one another caused the uplift of the Santa Marta massif, and the formation of the Sierra de Perijá (Kellogg 1984). Uplift of the Sierra de Perijá concluded at the early Pliocene. Further clockwise rotation of the Maracaibo basin initiated the orogeny of the Venezuelan Andes in the late Pliocene (Mattson 1984), resulting in the rapid rise of the Venezuelan Andes, and the separation of the Maracaibo basin from the present day Orinoco Llanos (Macallari 1984). Continuing clockwise rotation of the Maracaibo block together with the movement of the Caribbean and South American plates caused the rise of the Cordillera de la Costa and El Tigre highlands, respectively, effectively isolating the Tucacas lowlands and the Río Unare basin (Mattson 1984). A secondary uplift of the Guyana shield during the late Pleistocene isolated the Venezuelan Llanos from the Rupununi Savannah (Gibbs and Barron 1993). Based on this series of geologic events, the Colombian lowlands should have become isolated first, while the Río Unare basin should have separated from the Río Orinoco basin last.

This orogenic series is concordant with the phylogenetic relationships within the genus *Austrofundulus* (Fig. 3). Geological areas that became separated more recently also contain species which branched off more recently from the ancestral *Austrofundulus* population. The maximum likelihood phylogeny of *Austrofundulus* is thus highly concordant with the geological history of northern South America. Geological evidence there provides additional support for the description of four new species of the genus *Austrofundulus*, and the resurrection of a fifth species from synonymy with *A. limnaeus*. Populations of *A. transilis* from the Río Unare basin are not recognized as a distinct species, since the separation of the Orinoco and Unare basins is very recent (Holocene), and populations from these two areas are morphologically indistinguishable and not reciprocally monophyletic. This also applies to male coloration which otherwise clearly differentiates males of other species of *Austrofundulus*. Detailed biogeographical analysis will be presented elsewhere.

When evaluated by traditional morphological criteria of species recognition and discrimination, our studies suggest that in areas of active orogeny that provide opportunities for allopatric speciation, much of the extant biodiversity remains and will remain unrecognized under many species concept criteria (Mayden 1997). However, the species in these different areas are not genetically interchangeable (Templeton 1981), although they may be ecologically interchangeable and morphologically indistinguishable simply due to stabilizing selection on morphological characters driven by the same set of ecological/envi-

ronmental variables. This is the pattern we observe in northern South America. Moreover, this pattern is also prominent in the geologically active central Turkey, where, for example at least seven cryptic lineages of the killifish genus *Aphanius* occur (Hrbek *et al.* 2002). These lineages show complete or partial reproductive isolation (Villwock 1964), but only three species are scientifically recognized (Wildekamp *et al.* 1999; Hrbek and Wildekamp 2003). Thus, these species, whether occurring in northern South America or Anatolia, are real in that they embody an evolutionary process and form independent evolutionary lineages, but do not necessarily demonstrate a clear pattern of morphometric and meristic differentiation. It is important to remember that real evolutionary groups need not be morphologically distinct, whereas morphological categories are created as a direct function of their perceived distinction (Hey 2002). Therefore lack of morphological distinctness does not imply lack of real evolutionary lineages, i.e. species. Evolutionary lineages and morphological categories are not the same.

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