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Phylogenetic Relationships of African Killifishes in the Genera *Aphyosemion* and *Fundulopanchax* Inferred from Mitochondrial DNA Sequences

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We have analyzed the phylogenetic relationships of 52 species representing all defined species groups (J. J. Scheel, 1990, Atlas of Killifishes of the Old World, 448 pp.) of the African aplocheiloid fish genera *Aphyosemion* and *Fundulopanchax* in order to examine their interrelationships and to reveal trends of karyotypic evolution. The data set comprised 785 total nucleotides from the mitochondrial 12S rRNA and cytochrome *b* genes. The molecular-based topologies analyzed by both maximum parsimony and neighbor-joining support the monophyly of most previously defined species groups within these two killifish genera. The genus *Aphyosemion* is monophyletic except for the nested position of *Fundulopanchax kunzi* (*batesi* group; subgenus *Raddaella*) within this clade, suggesting that this taxon was improperly assigned to *Fundulopanchax*. The remaining *Fundulopanchax* species sampled were supported as being monophyletic in most analyses. Relationships among the species groups in both genera were not as strongly supported, suggesting that further data will be required to resolve these relationships. Additional sampling from the 16S rRNA gene allowed further resolution of relationships within *Fundulopanchax*, more specifically identifying the nonannual *scheeli* group as the basal lineage of this otherwise annual genus. Chromosomal evolution within *Aphyosemion* has been episodic, with the evolution of a reduced $n = 9–10$ metacentric complement having occurred in multiple, independent lineages. Polarity of chromosomal reductions within the *elegans* species group appears to support previous hypotheses concerning mechanisms of karyotypic change within the genus *Aphyosemion*.

INTRODUCTION

African aplocheiloid killifishes are currently assigned to four speciose genera (*Aphyosemion*, *Epiplatys*, *Fundulopanchax*, and *Nothobranchius*) and six monotypic genera (*Adamus*, *Foerschichthys*, *Fundulosoma*, *Pronothobranchius*, *Aphyoplatys*, and *Episemion*). The composition and relationships of these genera have undergone numerous changes as our knowledge of these fishes has grown. The greatest number of changes have occurred with regard to the genus *Aphyosemion* Myers 1924.

The genus *Aphyosemion* was originally divided into three subgenera: *Aphyosemion*, *Fundulopanchax*, and *Adinops*. Those species assigned to *Adinops* were from east Africa and were later removed to the genus *Nothobranchius*. The remaining species and subsequent taxa assigned to these subgenera could be divided by distributional criteria and independently by phenotypic criteria. The vast majority of these fishes are found in small streams in the understory of the rainforest (Scheel, 1990). The rainforest of equitorial Africa is cleanly divided into western and eastern blocks by the Dahomey Gap, a strip of savanna habitat that extends to the coast in Benin, Togo, and eastern Ghana. In 1966, Clausen recognized the distinctiveness of those species west of the Dahomey Gap. Subsequent workers have identified additional morphological characters that distinguish these western taxa (Zee and Wildekamp, 1995) and recent DNA sequence data (Murphy, 1997, Murphy and Collier, 1997, 1999) clearly identify the western forms as a distinct clade not closely related to the eastern taxa. Thus the remaining problem with the genus *Aphyosemion* involves those eastern species formerly assigned to the subgenera *Aphyosemion* and *Fundulopanchax*.

The subgenus *Fundulopanchax* was elevated to generic level by Parenti (1981) based on two characters. Zee and Wildekamp (1995) dispute the diagnostic value of one of these characters but added four new characters defining *Fundulopanchax*. Prominent among the life history traits that distinguish *Aphyosemion* and *Fundulopanchax* is annualism. Annual fishes (Myers 1942, 1955) are those that deposit their eggs in the substrate where they withstand the dessication of an annual dry season to hatch once the rains resume.

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Members of *Fundulopanchax* are believed to be annual while members of *Aphyosemion* are not (Parenti, 1981; Wildekamp, 1993).

Chromosome complements are relatively well conserved in teleostean fishes, particularly within the immense acanthomorph clade (*sensu* Johnson and Patterson, 1993) in which the predominant diploid karyotype is $2n = 48$ (Sola *et al.*, 1981). Significant deviations from this number have occurred in only a handful of these fish species. One order in particular, the Cyprinodontiformes (killifishes), displays a striking propensity for clade-specific karyotypic rearrangement. Perhaps the best example is the aplocheiloid genus *Aphyosemion*, which shows more inter- and intraspecific chromosomal rearrangements (Scheel, 1990) than perhaps any other fish genus. Our knowledge of the types of mechanisms behind karyotypic evolution, and its potential contribution to speciation within this genus, have been hampered by the lack of a phylogenetic framework for this diverse group.

The specific aim of this work was to use mitochondrial DNA sequences to assess the monophyly and composition of *Aphyosemion* and *Fundulopanchax*, to determine the monophyly of recently proposed subgenera and species groups (Scheel, 1990; Table 1) within these genera, and to determine the polarity of chromosomal rearrangements within the molecular phylogeny. Further, this enlarged data set has allowed further consideration of the origin of annualism (Murphy and Collier, 1997) within these genera. We sampled 36 populations of 32 described and 4 undescribed species of *Aphyosemion* and 16 species of *Fundulopanchax*. In total these represent 14 of Scheel's (1990) 15 species groups. The 15th group, composed of a single species, *Pronothobranchius kiyawensis*, has been subsequently excluded from *Aphyosemion* on the basis of both morphological and molecular characters (Parenti, 1981; Murphy, 1997).

TABLE 1

Taxa Under Study, Proposed Species Groups and Their Abbreviations Used in Figures (Scheel, 1990), and Various Subgeneric Names Assigned to Specific Taxa

Species group[1]/ species sampled	Subgenus
Genus *Aphyosemion*	
bivittatum group (BIV)	*Chromaphyosemion*[2]
bivittatum,* *volcanum*, sp. LEC 93/27	
calliurum group (CAL)	*Mesoaphyosemion*[3]
ahli, australe,* *calliurum, celiae*	
cameronense group (CAM)	*Mesoaphyosemion*
cameronense, maculatum, mimbon	
coeleste group (COL)	*Mesoaphyosemion*
aureum, citrinepinnis, coeleste, occelatum	
elegans group (ELE)	*Mesoaphyosemion*
christyi, cognatum (2), *decorsei, elegans* (3), *lamberti, melanopteron, punctatum, rectogoense, wildekampi*	
exiguum group (EXI)	*Kathetys*[4]
bualanum, exiguum	
georgiae group (GEO)	*Diapteron*[5]
cyanostictum	
striatum group (STR)	*Mesoaphyosemion*
exigoideum, gabunense, louessense, ogoense, primigenium, striatum	
ungrouped (UG)	
labarrei	*Mesoaphyosemion?*
Genus *Fundulopanchax*	
arnoldi group (ARN)	*Paludopanchax*[6]
filamentosum, robertsoni, walkeri	
batesi group (BAT)	*Raddaella*[7]
kunzi	
gardneri group (GAR)	*Paraphyosemion*[8]
*cinnamomeum, gardneri, mirabile**	
gulare group (GUL)	*Gularopanchax*[9]
*deltaense, fallax, gulare, schwoiseri, sjoestedi**	
ndianum group (NDI)	*Paraphyosemion*[10]
amieti, ndianum	
scheeli group (SCE)	*Paraphyosemion*[8]
santaisabellae, scheeli	

Note. Generic divisions follow those of Parenti (1981). Numbers in parentheses denote number of populations sampled. Data from taxa marked with asterisks have previously been reported (Murphy and Collier, 1997).

[1] Scheel, 1990.
[2] Radda, 1971.
[3,6,9,10] Radda, 1977.
[4,7] Huber, 1977.
[5] Huber and Seegers, 1977.
[8] Kottelat, 1976.

MATERIALS AND METHODS

A list of the taxa examined and their sources is in the Appendix. Mitochondrial DNA was extracted from muscle or liver tissues. Mitochondrial DNA extractions and amplification protocols were performed as previously described (Murphy and Collier, 1996). Some of the sequences have been previously reported (see Table 1). We sequenced a 360-bp region of the cytochrome *b* (cyt*b*) gene and a 425-bp region of the 12S rRNA gene. The primers used were L14724 and H15149 (Kocher *et al.*, 1989; Meyer *et al.*, 1990) for the cyt*b* segment and L1091 and H1478 (Kocher *et al.*, 1989) for the 12S rRNA segment. Primers 16Sar-L and 16Sbr-H (Palumbi *et al.*, 1991) were used to amplify a region of the 16S rRNA gene for *Fundulopanchax* taxa. The new DNA sequences were generated with an automated sequencer (ABI 373 Stretch). Symmetric amplification products were purified with 30,000 MW regenerated cellulose filter devices (Millipore Inc.). Cycle sequencing using fluorescent-labeled terminators was performed using Ampli-*Taq* FS DNA polymerase (Applied Biosystems Inc.). The reactions were purified free of fluorescent terminators using Centri-Sep columns (Princeton Separations) before loading onto a sequencing gel (6% Long-Ranger acrylamide, FMC).

Sequences were initially aligned using the program CLUSTAL W (Thompson *et al.*, 1994). Manual adjustments were made to the preliminary alignment of the rRNA segments. Regions of length variation due to insertions or deletions were omitted from the analyses when they could not be aligned without making assumptions concerning homology. The complete aligned data sets were analyzed by maximum parsimony (MP) and neighbor-joining (NJ; Saitou and Nei, 1987) methods. Parsimony analyses were done with PAUP vers. 3.1.1 (Swofford, 1993). In all cases heuristic searches were used (50 replicates, random addition of taxa, TBR branch-swapping). A series of different weighting schemes was applied to the parsimony analyses to adjust for transitional saturation in increasingly divergent comparisons. This phenomenon is well documented in most animal groups, including previous studies of aplocheiloid phylogeny using mitochondrial DNA (Murphy and Collier, 1996, 1997). We employed the following weighting strategies: (1) all sites given equal weight; (2) all sites given equal weight, while excluding first-codon-position leucine transitions and all third-codon-position transitions in the cytochrome *b* segment (conservative substitution, CS parsimony; Irwin *et al.*, 1991); and (3) all transversions weighted five times transitions (5:1 parsimony). These last two weighting schemes attempt to resolve deeper divergences due to the greater conservation of the substitutions analyzed. Bootstrap values for all parsimony analyses were based on 100 heuristic replicates generated in PAUP.

Neighbor-joining analyses were generated in MEGA (vers. 1.01; Kumar *et al.*, 1993) with indels or ambiguities deleted from the analyses. The Kimura two-parameter correction was used to account for transition bias (Kimura, 1980). Confidence probabilities (P_C; Rzhetsky and Nei, 1992) for branches of the NJ tree were assessed with the interior-branch test implemented in MEGA (vers. 1.01, Kumar *et al.*, 1993). We opted to use this method based on recent data suggesting that the bootstrap conservatively underestimates the statistical support for groupings within a topology, particularly when large numbers of taxa are being analyzed (Sitnikova *et al.*, 1995). All trees were rooted with members from the genus *Nothobranchius* (*N. kirki* and the monotypic subgenus *Pronothobranchius kiyawense*) and the allied monotypic genus *Fundulosoma thierryi* (Parenti, 1981). A recent molecular analysis of the major aplocheiloid genera demonstrated the sister-group status of *Nothobranchius* to a monophyletic *Aphyosemion* + *Fundulopanchax* clade (Murphy and Collier, 1997), justifying its use here as an outgroup.

RESULTS

Sequences obtained for this study have been deposited in GenBank under Accession Nos. AF002284–AF002401. The total analyzed data set consisted of 763 bp, following removal of 22 bp of unalignable regions from the 12S rRNA segment. This resulted in 317 variable sites, 265 being parsimony informative. Nucleotide frequencies for the entire data set were A = 31%, T = 29%, C = 23%, and G = 17% and did not differ significantly between taxa. Transition/transversion ratios varied from 1.1 to 19.0 in ingroup comparisons, with many of the higher ratios (particularly among closely related taxa) ranging between 5.0 and 10.0.

Parsimony analysis of the data set when all sites were given equal weight resulted in eight 1806-step trees having consistency indices of 0.271 and retention indices of 0.565. Figure 1 shows the strict consensus of these eight trees. The consistency and retention indices are relatively low, most likely attributable to the large number of taxa analyzed and the resulting increased probability for homoplasious substitutions at rapidly evolving sites. The members of the genus *Aphyosemion* form a strongly supported group (94% of bootstrap replications) which includes *Fundulopanchax kunzi* nested within this clade. Bootstrap values are also very high for nodes defining most of Scheel's species groups within *Aphyosemion* (Fig. 1). The remaining species of *Fundulopanchax* form a monophyletic group, though this clade is weakly supported by the bootstrap results (50%). The relationships within *Fundulopanchax* are also poorly resolved by these data, with the exception of a few interspecific relationships, which corresponded to some of Scheel's (1990) species groups.

Weighted parsimony analyses were employed to resolve deeper relationships which might be obscured by transitional saturation. Both weighting schemes (conservative substitutions and transversions weighted greater than transitions) produced trees in general agreement with the equal-weighted results (Fig. 1b), with most of the species groups within *Aphyosemion* being monophyletic, though the relationships between species groups differed somewhat (see Discussion). The 5:1 parsimony resulted in two trees of 3520 steps (Fig. 1b). This weight was derived from the higher transition rate among closely related taxa (see above). Results based on weighting transversions between 5 and 10 times transitions were equivocal. The analysis based on CS parsimony (eight trees, CI = 0.442, RI = 0.709) differed primarily from all other analyses in that most of the *Fundulopanchax* taxa (exclusive of *F. kunzi*) were not resolved as being monophyletic, collapsing into a basal polytomy in the consensus tree (not shown). The bootstrap trees for these latter two weighted analyses gave similar results (shown in Fig. 1b), strongly supporting most of the species groups within *Aphyosemion*, with less resolution between species groups.

The neighbor-joining tree based on Kimura-corrected distances (Fig. 2) was congruent with the strongly supported aspects of the parsimony analyses, with

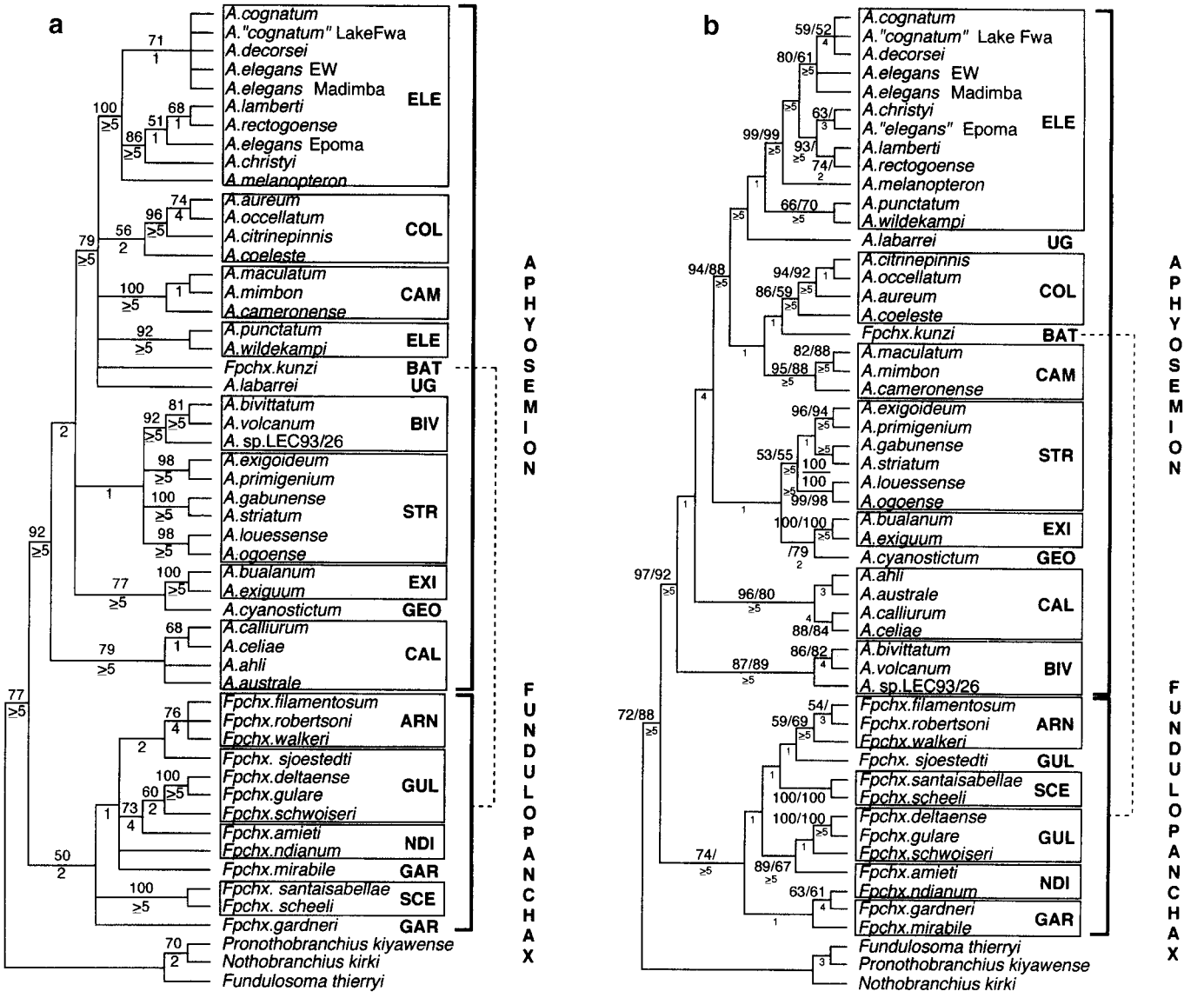

FIG. 1. (a) Consensus tree of eight equal-length cladograms (TL = 1806; CI = 0.271, RI = 0.565) produced when all sites are given equal weight. Numbers above branches are bootstrap values (Felsenstein, 1985) based on 100 replications. Values below 50% are not shown. Boxes denote taxa sampled from each of Scheel's (1990) species groups. Abbreviations for species groups are given in Table 1. (b) Consensus tree of two equal-length cladograms (TL = 3519) produced when all transversions were weighted five times greater than transitions. Numbers above branches are bootstrap values (Felsenstein, 1985) based on 100 replications. Values in front of the slash are produced by the 5:1 transversion/transition weighting scheme. Values following the slash are produced by the bootstrap analysis using conservative substitutions (See text for discussion). Values below 50% are not shown. Boxes denote taxa sampled from each of Scheel's (1990) species groups. Abbreviations for species groups are given in Table 1.

most of the differences revolving around branches with low statistical support. Similar to parsimony, the species groups within *Aphyosemion* are strongly supported (P_C values), while the relationships between the groups are weaker. Monophyly of *Fundulopanchax,* exclusive of *F. kunzi,* received robust support from the interior-branch test (Fig. 2). Complete deletion of sites with gaps or the use of different distance corrections changed the topology very little, these differences again being observed among branches with low statistical support.

To further resolve the relationships with *Fundulopanchax,* an additional 472 bp of sequence data was obtained from the 16S rRNA gene for each taxon. In addition, we determined DNA sequences from all three gene segments for two *Fundulopanchax* species not sampled in the initial portion of this study—*F. cinnamomeum* and *F. fallax.* Some of the 16S rRNA sequences have been previously reported (Murphy and Collier, 1997). Trees were rooted with two *Aphyosemion* species, and the combined data set (1221

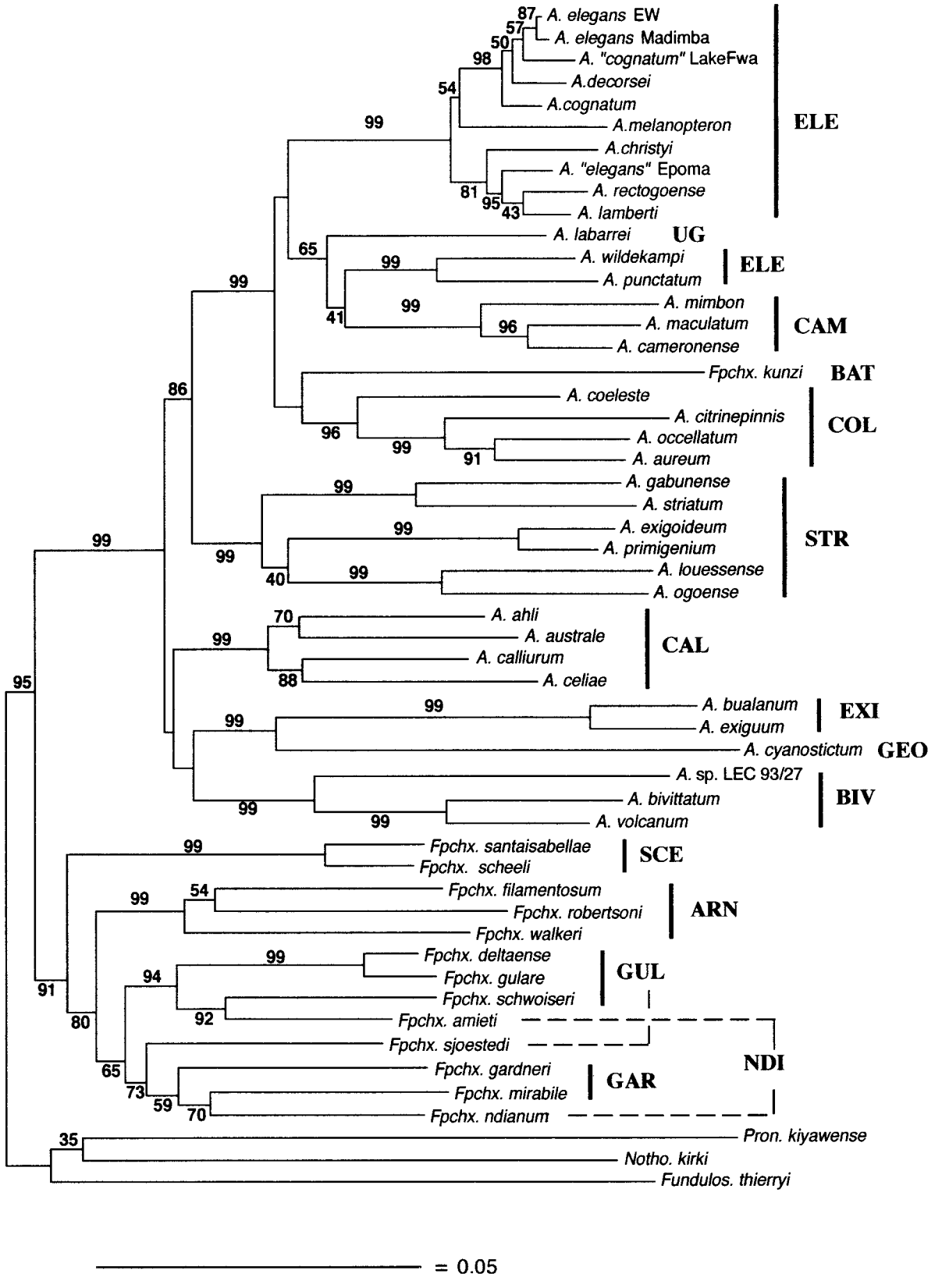

FIG. 2. Neighbor-joining tree based on Kimura-corrected distances (Kimura, 1980) with pairwise deletion of gaps. Numbers above the branches are confidence probabilities based on the interior-branch test implemented in MEGA vers. 1.01 (Kumar *et al.*, 1993). Bars span taxa assigned to Scheel's (1990) species groups. Dashed lines connect nonadjacent members of these groups. Abbreviations are given in Table 1. A distance scale is represented at the bottom.

bp, 330 variable sites, 220 of these parsimony informative) was analyzed by three parsimony methods (all sites equal weight, conservative substitutions only, and transversions weighted five times transitions), neighbor-joining, and, as allowed by the smaller size of the data set, maximum likelihood (fastDNAML; Olsen *et al.*, 1994). A 6-bp region of the 12S rRNA segment was deleted due to ambiguity in alignment. Weighted parsimony, neighbor-joining, and maximum likelihood analyses all produced the topology shown in Fig. 3. Equal-weighted parsimony generated a single tree congruent with Fig. 3, with the exception of *F. amieti* being placed basal in the *gulare*-group clade. The conservative substitution parsimony tree placed the *scheeli* group sister to the *gardneri*-group/*F. ndianum* clade.

DISCUSSION

Phylogenetic Relationships within Aphyosemion

These molecular results are highly concordant with previous species-group definitions of the genus *Aphyosemion*, created on the basis of karyology, meristics, and geographical distributions (Scheel, 1990). Initial sampling from the following species groups appear to define monophyletic lineages which support Scheel's groupings: *bivittatum*, *calliurum*, *cameronense*, *coeleste*, *exiguum*, and *striatum* groups.

The *georgiae* species group [the subgenus *Diapteron* of Huber and Seegers (1977)] is represented in the combined dataset by a single taxon—*A. cyanostictum*. Two additional species from this group, *abacinum* and *georgiae*, were sampled; however, the cytochrome *b* primers were unsuccessful with DNA from these taxa. The results from 12S rRNA gene alone (not shown) demonstrated that all three species comprise a monophyletic group, with *abacinum* being basal to the other two. These data also resolved the *georgiae* group as the sister taxon to the *exiguum* group, as did the combined dataset. The nested placement of this clade within, and not outside of, *Aphyosemion* does not support Seeger's (1980) suggestion of full generic rank for this group.

Two other *Aphyosemion* subgenera [*Chromaphyosemion* (Radda, 1977) and *Kathetys* (Huber, 1977)] are also supported here by the apparent monophyly of the corresponding *bivittatum* and *exiguum* species groups. The subgenus *Mesoaphyosemion* (Radda, 1977), which includes the *cameronense*, *calliurum*, *coeleste*, *elegans*, *scheeli*, and *striatum* groups, is clearly not a monophyletic group based on these data.

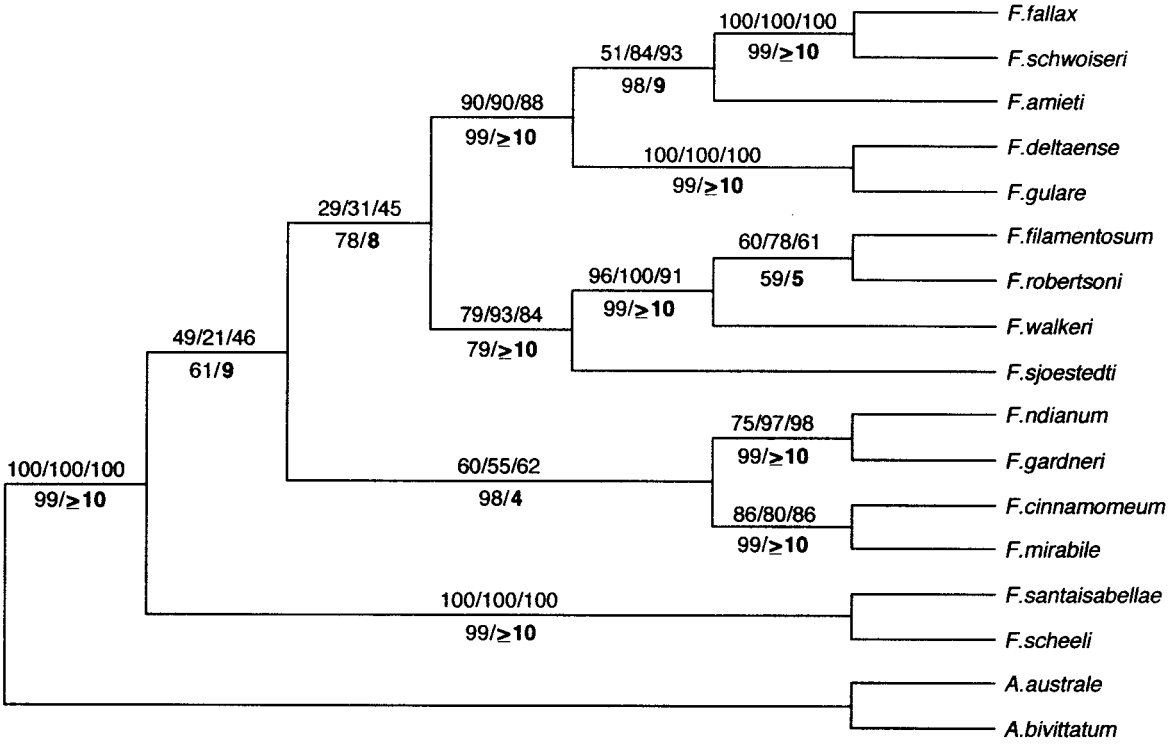

FIG. 3. Phylogenetic hypothesis for the genus *Fundulopanchax* based on the expanded (cytochrome *b* + 12S rRNA + 16S rRNA) data set, using *Aphyosemion* as an outgroup. Weighted parsimony (TL = 1682), neighbor-joining with Kimura (1980) distances, and maximum likelihood (−ln Likelihood = −6010.09303) analyses all produced this same topology. The equal-weighted and conservative substitution parsimony trees are discussed in the text. Numbers above the branches are bootstrap values (500 replicates) compatible with equal-weighted parsimony, conservative substitution parsimony, and 5:1 (Tv:Ts) weighted parsimony. Numbers below the branches are the results of the interior-branch test (Rzhetsky and Nei, 1992) of the neighbor-joining tree, implemented in MEGA (Kumar *et al.*, 1993).

The placement of the annual *Fundulopanchax kunzi* within a larger clade of nonannual *Aphyosemion* species is quite unexpected. This species is part of a small group (*batesi* group) (Scheel, 1990) distributed in upland habitats of eastern Cameroon and western Gabon disjunct from the usual coastal ranges of the remaining species of *Fundulopanchax*. The *batesi* group is, however, sympatric in some areas with the groups for which the molecular data suggest phylogenetic similarity.

Recently, Zee and Wildekamp (1994) presented new morphological characters defining *Fundulopanchax* which are absent in members of the *batesi* group (subgenus *Raddaella*). Chorionic puncti, present on the surface of eggs of all annual *Fundulopanchax* species, are lacking in the *batesi* group. All species of *Fundulopanchax* (with the exception of the *arnoldi* group) have a mean of 16 or more circumpeduncular scales (cp scales), while all species of *Aphyosemion* sampled (including the *batesi* group) have a mean of 14.2 or fewer cp scales. Together, these two morphological characters support the placement of the *batesi* group within *Aphyosemion*.

The interrelationships between the *Aphyosemion* species groups are less apparent with these data. Our results distinguish two major components within the genus: (1) a strongly supported monophyletic clade containing the *batesi*, *cameronense*, *coeleste*, and *elegans* groups, plus the ungrouped *A. labarrei;* and (2) a basal grade composed of the *bivittatum*, *calliurum*, *exiguum*, *georgiae*, and *striatum* groups. The former group is centered primarily around the Congo River system and its neighboring drainages in Gabon while the latter group is centered primarily in Cameroon and western Gabon.

The neighbor-joining tree also supports a basal bifurcation between the *calliurum/bivittatum/exiguum/georgiae* groups and the remaining species groups, though the monophyly of the former group is supported by a very short internode, which receives no support from the interior-branch test. However, when members of *Fundulopanchax* are used as an outgroup for the NJ analysis (data not shown) the *calliurum* group is found as the most basal *Aphyosemion* clade, similar to parsimony analyses.

Phylogenetic Relationships within Fundulopanchax

In contrast to *Aphyosemion*, only two of Scheel's *Fundulopanchax* species groups are supported by the current molecular data. One of these, the *scheeli* group, is the lone nonannual taxon within *Fundulopanchax*, inhabiting a restricted range in Cameroon and southeastern Nigeria. The monophyly of this distinctive set of species is strongly supported (bootstrap = 100% all analyses, $P_C = 0.99$) and the addition of the 16S rRNA segment stabilizes the basal position of the *scheeli* group in weighted parsimony, neighbor-joining, and maximum likelihood analyses. The elevated number of circumpeduncular scale rows characteristic of most other *Fundulopanchax* is also characteristic of the *scheeli* group (Zee and Wildekamp, 1995). Given our topology for *Fundulopanchax* (Fig. 3) it would appear that 16 or more cp scales is diagnostic for *Fundulopanchax*, being secondarily reduced in the derived *arnoldi* group.

Biogeography

Basal members of both *Aphyosemion* (*calliurum* group) and *Fundulopanchax* (*scheeli* group) occupy the eastern region of Cameroon, suggesting that this region may have been the center of diversification of these two genera. The genus *Aphyosemion* subsequently diversified to the east with the terminal *elegans* group relatively recently filling the Zaire basin. The genus *Fundulopanchax* diversified westward in essentially coastal habitats with the *gardneri* group expanding inland to fill much of the area of Nigeria. *Fundulopanchax walkeri* is the only member of the group to have crossed the Dahomy Gap to occupy western habitat in Ghana and Togo. Given its rather terminal position within the combined *Aphyosemion/Fundulopanchax* clade, this has been a relatively recent event. As such, it does not invalidate the presumption of the epicontinental seas being the viacariant event separating the eastern and western aplocheiloid taxa (Murphy and Collier, 1997; Murphy *et al.*, 1999).

Annualism

The suite of traits referred to as annualism includes behavioral components (bottom spawning), morphological components (enlarged dorsal and anal fins of males and features of chorion structure), and developmental components (embryonic diapauses). Within the suborder Aplocheiloidei, annualism has been hypothesized to have arisen once and then to have been subsequently lost and regained (Murphy and Collier, 1997). The more detailed phylogenetic analysis presented here, with a unique annual species (*F. kunzi*) nested within an otherwise nonannual clade and the nonannual *scheeli* group being the basal group of the otherwise facultatively annual *Fundulopanchax*, suggests that annualism is evolutionarily more plastic than once thought.

Chromosomal Evolution

Acanthomorph fishes have extremely conserved karyotypes, with the vast majority of taxa having a diploid number of 44–48 chromosomes (Sola *et al.*, 1981). This number is particularly well conserved within the speciose Percomorpha. While there are a few scattered examples of significant reductions in number throughout the acanthomorph fishes, the Order Cyprinodontiformes exhibits more documented inter- and intraspecific variability than perhaps any other fish group of equal phylogenetic diversity. The suborder

Cyprinodontoidei has well-conserved chromosome numbers, with the only significant examples of reduction coming from the Goodeidae (Turner *et al.*, 1985). Within the sister-suborder Aplocheiloidei we see an increased propensity for karyotypic reduction, the Neotropical clade Rivulidae showing a few sporadic cases ($n = 10$ for *Pterolebias longipinnis*, $n = 16$–17 in a handful of taxa) amid a larger trend of $n = 22$–24 chromosomes (Scheel, 1972, 1990; Garcia *et al.*, 1993; Collier, unpublished data).

The African genera show by far the greatest karyological variability—the most striking case found within the genus *Aphyosemion* (Scheel, 1990). Of the eight defined species groups, three (*bivittatum*, *calliurum*, and *elegans*) contain taxa with karyotypes ranging from $n = 18$ to $n = 9$ or 10 (Fig. 4). The remaining species groups show much less variability in chromosome number and morphology. One of the more notable findings from this study is that the species groups showing reduced karyotypes are not phylogenetically restricted. Rather, this propensity toward reduced karyotypes has occurred multiple times in *Aphyosemion*. A similar extreme reduction in haploid number has occurred in *Nothobranchius rachovii* ($n = 9$/18 arms; Scheel, 1972, 1990). This trend is particularly striking because the populations having $n = 9$ to 10 in *Aphyosemion* (14) outnumber all other nonaplocheiloid teleosts having

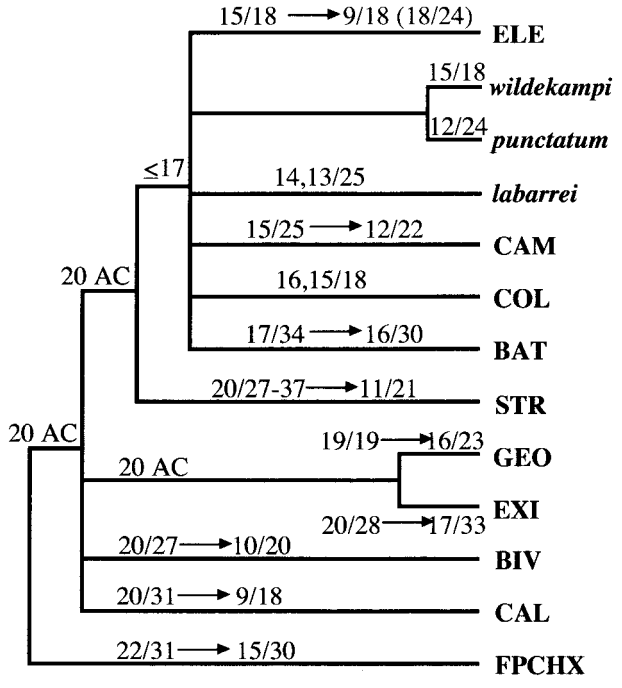

FIG. 4. Hypothesis for chromosomal evolution within the genus *Aphyosemion*. Numbers preceding the slash are haploid chromosome numbers; numbers following the slash indicate the number of chromosome arms in the haploid complement. The range of values for each terminal taxon are given on the respective branches. Karyological data are derived from Scheel (1990). See text for discussion.

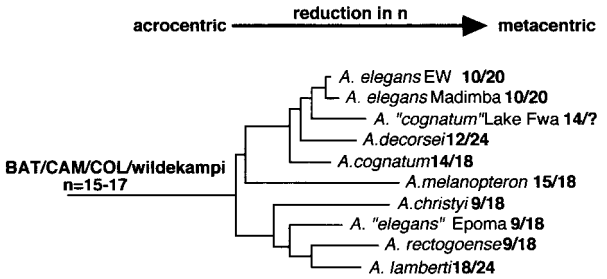

FIG. 5. Distribution of karyotypic data onto the phylogeny for the monophyletic Zaire-basin components of the *elegans* group. Karyotypic information for each species is from Scheel (1990), except "*elegans*" Epoma and "*cognatum*" Lake Fwa (Collier, unpublished data).

such reduced chromosome numbers: the bristlemouth *Gonostoma bathyphilum* (Gonostomatidae), $n = 6$; the gouramie *Sphaerichthys osphromonoides* (Belontiidae), $n = 8$; (Sola *et al.*, 1981).

Within *Aphyosemion* a consensus topology can be constructed based on the relationships depicted in both parsimony and neighbor-joining analyses (Fig. 4). One observation is that a karyotype of 19–20 haploid chromosomes is found in all of the basal species groups (*bivittatum*, *calliurum*, *exiguum*, *georgiae*, and *striatum*), while the monophyletic "eastern" groups (*batesi*, *cameronense*, *coeleste*, and *elegans*) have an apparently ancestral upper limit of $n = 17$ chromosomes. The presence of $n = 20$ chromosomes in the basal groups suggests that it might be the ancestral karyotype for the genus and that reduction has occurred in each group. Determining the polarity of chromosome changes within most of the species groups is currently not possible without more complete sampling. However, Scheel's study of the *calliurum* group suggests that the Nyong-North population of *A. ahli* ($n = 20$, acrocentric elements) is ancestral and gave rise to the remaining karyotypes of reduced number in the group (Scheel, 1990). The more derived taxon-pair *calliurum* and *celiae* have reduced karyotypes (10 populations, all $n \leq 12$), and the grouping of these two taxa in all analyses, together with some analyses showing *ahli* and *australe* in basal positions, supports this general trend towards reduced karyotypes.

Scheel hypothesized that aplocheiloid karyotypes have evolved via two major mechanisms: pericentric inversions and centric fusions (Scheel, 1972, 1990). Pericentric inversions would move metacentric centromeres into terminal positions. Acrocentric chromosomes could then undergo centric fusions to return the complement to one of oversized metacentric elements and a reduced number of chromosomes. If this hypothesis is correct, we would expect to see basal taxa having higher chromosome numbers and more acrocentric elements, while terminal, derived taxa would have lower haploid numbers with symmetrical (metacentric)

complements. Our sampling of several described *elegans* group members presents us with a template for testing this hypothesis of chromosomal evolution within *Aphyosemion*. Figure 5 shows the NJ topology of the *elegans* group. Analysis of this group alone produces a similar topology with both parsimony (all sites equal weight) and NJ methods. The distribution of karyotype information onto this tree shows a gradual transition from $n = 15$, mostly acrocentric elements in *A. melanopteron*, to both *elegans* populations having $n = 10/18$ arms in the upper lineage. All taxa in the bottom lineage exhibit completely symmetrical complements of $n = 9/18$ arms, though there appears to be a rare exception of an increase to 18 chromosomes/24 arms in *A. lamberti*. This single example supports Scheel's hypothesis; however, more extensive sampling from within other groups will be necessary to determine the generality of this pattern of chromosomal evolution.

APPENDIX

The following is a list of specimens and their various sources: *Aphyosemion ahli*, Kribi, Cameroon; *A. aureum*, GEB 94/9, Gabon; *A. australe*, chocolate Aquarium strain (AS); *A. bivittatum*, Funge, Cameroon; *A. bualanum*, NDOP, Cameroon; *A. calliurum*, Epe, Cameroon; *A. cameronense halleri*, EMS 90/6 Bikong, Gabon; *A. celiae winifrediae*, New Butu, Cameroon; *A. christyi*, HZ 85/8, Zaire; *A. citrinepinnis*, GEB 94/1, Gabon; *A. coeleste*, RPC/5, Congo Republic; *A. cognatum*, Bandundu, Zaire; *A. "cognatum,"* Lake Fwa, Zaire; *A. cyanostictum*, Makouko, Gabon; *A. decorsei*, RCA 91/3, Central African Republic; *A. elegans*, "EW," Naoimda, Zaire; *A. elegans*, Epoma, Congo Republic; *A. elegans*, Madimba, Zaire; *A. exigoideum*, N'goudoufola, Gabon; *A. exiguum*, GKCAR 90/4, Central African Republic; *A. gabunense boehmi*, AS, Gabon; *A. labarrei*, AS, Zaire; *A. lamberti*, NRSC, Gabon; *A. louessense*, Sibiti, Congo Republic; *A. maculatum*, LEC93/4, Gabon; *A. melanopteron* (=*congicum*), AS, Zaire; *A. mimbon*, LEC93/19, Gabon; *A. occellatum*, G-20, Gabon; *A. ogoense*, RPC/206, Gabon; *A. primigenium*, 88/6, Gabon; *A. punctatum*, LEC, Gabon; *A. rectogoense*, GAB 90/ABB, Gabon; *A. striatum*, Cape Esterias, Gabon; *A. volcanum*, Kumba, Cameroon; *A. wildekampi*, AS; *A.* sp. LEC93/27, Gabon; *Fundulopanchax amieti*, AS, Cameroon; *F. cinnamomeum*, AS, Cameroon; *F. deltaense*, AS, Nigeria; *F. fallax*, Mamou, Ghana; *F. filamentosum*, AS, Nigeria; *F. gardneri*, Akure, Nigeria; *F. gulare*, AS, Nigeria; *F. kunzi*, CGE/91, Gabon; *F. mirabile moense* Takwai, Cameroon; *F. ndianum*, AS, Nigeria; *F. robertsoni*, AS, Cameroon; *F. oeseri* (=*santaisabellae*), AS, Bioko Island; *F. scheeli*, AS, Nigeria; *F. schwoiseri*, AS, Cameroon; *F. sjoestedi*, AS, Nigeria; *F. walkeri*, AS; *Fundulosoma thierryi*, AS; *Nothobranchius kirki*, Chilwa, Malawi; *Pronothobranchius kiyawense*, AS. Collection codes are detailed in Langton (1996).

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