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Mitochondrial Phylogenetics, Biogeography, and Evolution of Parental Care and Mating Systems in *Gymnogeophagus* (Perciformes: Cichlidae)

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Abstract. The Neotropical cichlid genus *Gymnogeophagus* is found in southern Brazil, Paraguay, Uruguay, and northern Argentina. Fishes in the genus display a variety of parental care and mating system behavior ranging from mouthbrooding and polygyny to substrate spawning and monogamy. We sequenced 713 base pairs of mitochondrial DNA from cytochrome b, NADH subunit 2, methionine and glutamine tRNAs to estimate relationships, and to examine biogeography and behavioral evolution. There appears to have been a single origin of mouthbrooding and sexual dimorphism in the genus. Different populations of two of the substrate spawning species (*G. rhabdotus* and *G. meridionalis*) do not cluster together as distinct species suggesting that there may be multiple cryptic species within these two taxa. Biogeographic analysis suggests that there was an initial early split between the major drainages of the La Plata basin. There is also well supported separation between species in the upper and lower rio Uruguai. The rate of molecular evolution is significantly more rapid in the polygynous, mouthbrooding species.

Resumo. As espécies do gênero *Gymnogeophagus* são encontradas no sudeste do Brasil, Paraguai, Uruguai e norte da Argentina. Os peixes deste gênero apresentam uma variedade de cuidados parentais e comportamentos reprodutivos, com espécies poligínicas com incubação bucal e outras monogâmicas com incubação no substrato. Foram sequenciados 713 pares de bases de DNA mitocondrial do citocromo b, subunidade 2 do NADH, e tRNAs da metionina e glutamina para estimar as relações filogenéticas, examinar a biogeografia e a evolução comportamental. Aparentemente houve uma única origem de incubação bucal e dimorfismo sexual neste gênero. Diferentes populações de duas espécies incubadoras no substrato (*G. rhabdotus* e *G. meridionalis*) não ficaram agrupadas, sugerindo que múltiplas espécies crípticas podem estar envolvidas nestes dois táxons. A análise biogeográfica sugere que houve uma separação inicial antiga entre as principais drenagens da bacia do Prata. Existem também evidências de separação entre as espécies do baixo e alto rio Uruguai. A taxa de evolução molecular é significativamente mais rápida nas espécies poligínicas de incubação bucal.

Introduction

The cichlid fishes, comprised of over 2400 species, are among the largest vertebrate families (Cichocki, 1976; Stiassny, 1981, 1987, 1991; Zihler, 1982; Gaemers, 1984; Kaufmann & Liem, 1982; Stiassny & Jensen, 1987; Kullander, this volume). Cichlid fishes are distributed throughout the tropics and are best known for their spectacular evolutionary radiations in the African Rift Valley lakes (Fryer & Iles, 1972; Greenwood, 1981, 1984). Less well known is the radiation of diverse neotropical forms. Over 500 cichlid species are native to South and Central America (Kullander, this volume) and their morphological diversity rivals that of the more famous African cichlids. Clearly, the neotropical radiation has had a much longer time to take place than the spectacular Rift Valley lake radiations. Thus, it is not surprising to see such morphological diversity.

Cichlids are also remarkably behaviorally diverse. They are highly unusual fishes, in that all species provide parental care that extends past the egg stage. In some species both males and females provide care, while in other species only females provide care. In two species only males provide care (for reviews see Barlow 1974, 1984, 1991; Clutton-Brock, 1991; Gross & Sargent, 1985; Keenleyside, 1991; Loiselle, 1985). Many species are substrate spawners where the females deposit adhesive eggs on a cleaned surface and one or both parents guard and tend them. However, the majority of species are mouthbrooders where one or both parents orally brood the eggs and young. An intermediate form of mouthbrooding (delayed mouthbrooding) occurs when the parents initially spawn on a substrate, pick up the eggs just prior to their hatching, and then orally brood the young (Barlow, 1991; Keenleyside, 1991; Loiselle, 1985). Mating systems are also variable among cichlids and often correlated with parental care mode. Most substrate spawners are monogamous and have biparental care, whereas most mouthbrooders are polygynous with female only care (Barlow, 1991; Keenleyside, 1991). Sexual dimorphism is slight in monogamous species, whereas it is more pronounced in polygynous species, with males often being more brightly colored, larger and, in some species, developing fatty, tumescent nuchal humps.

The genus *Gymnogeophagus* belongs to the geophagine cichlid group (Kullander, 1983; this volume) which are characterized by a series of synapomorphies including an expanded epibranchial lobe. *Gymnogeophagus*, one of the best characterized geophagine genera (Reis & Malabarba, 1988) was first erected by Miranda-Ribeiro in 1918 and included a single species (*G. cyanopterus* = *G. balzanii*). Gosse (1976) included an additional three species in the genus (*G. gymnogenys*, *G. rhabdotus* and *G. australis*). *Gymnogeophagus* are distinguished by the presence of a forward directed spine on top of the first pterygiophore and the absence of bony supraneurals. In a major revision, Reis & Malabarba (1988) confirmed the monophyly of the genus and significantly rearranged the species. In their revision they recognized eight species, all of which we include in this analysis, with the ex-

ception of *G. australis*. One new species, *G. setequedas* has been added recently (Reis *et al.*, 1992), and there are at least six undescribed species (Malabarba and Reis, unpubl. data).

Gymnogeophagus is restricted to the La Plata basin, which includes the río Paraguay, lower río Paraná and río Uruguay, and the smaller coastal drainages in southern Brazil and Uruguay. *Gymnogeophagus balzanii*, a species relatively common in the río Paraguay, is also found in the río Guaporé, a tributary to the río Madeira, which has had recent connections with the Paraguay headwaters. Species of *Gymnogeophagus* were originally absent from the upper río Paraná above the former Sete Quedas Falls, today drowned by the Itaipu hydroelectric dam. Since its construction, *Gymnogeophagus* species can now disperse upstream. The greatest species diversity of *Gymnogeophagus* is found in the río Uruguay and coastal drainages of southern Brazil. Various species occur in the Uruguai system and a few others in the very recently formed coastal lagoon system of Rio Grande do Sul. *Gymnogeophagus lacustris* is endemic to the coastal lagoons of northeastern Rio Grande do Sul, while its sister-species, *G. labiatus*, is more widespread in the fast-flowing streams of the laguna dos Patos and other coastal drainages. The further resolution of *Gymnogeophagus* phylogenetic relationships described in the present paper, along with comparisons with other fish groups inhabiting the same area, will provide a solid basis for investigating the biogeography of the La Plata basin.

Gymnogeophagus is one of few cichlid genera that exhibit a diversity of parental care and mating systems. Some *Gymnogeophagus* species are substrate spawners while others are mouthbrooders (Table 1). Aquarium observations of *G. balzanii* (Loiselle, 1985), and *G. sp. "B"*, and *G. sp. "C"* (Santos, 1997), show these species to be delayed mouthbrooders. Other species we classify as mouthbrooders have been caught orally brooding young (Reis & Malabarba, 1988; Wimberger and Reis, unpublished data; Malabarba and Reis, unpublished data), but none have been observed orally brooding eggs. All mouthbrooders are strikingly dimorphic and males of these species develop fatty nuchal humps. Aquarium observations of *G. meridionalis*, *G. rhabdotus*, and *G. setequedas* show that they are monogamous, substrate spawners (Loiselle, 1985; Loiselle & Hougen, 1997; J. Casciotta, pers. comm.). These three species are only slightly sexually dimorphic.

Because cichlids possess a diverse array of parental care behaviors they have been the focus of many studies examining the evolution of parental care (Barlow, 1974, 1984; Perrone & Zaret 1979; Noakes & Balon, 1982; Gross & Sargent, 1985; Stiassny & Gerstner, 1992; McLennan, 1994). These studies all assume that biparental care is the ancestral care state for cichlids and that it presumably arose from male care. Stiassny & Gerstner (1992) point out that only one of the three possible sister families to cichlids has male care (Pomacentridae), one of the others has no care (Labridae), and the other exhibits viviparity and no care of larvae (Embiotocidae), a different type of female care. As a result, unraveling the evolutionary dynamics of parental care in cichlids depends on further phy-

logenetic and behavioral studies within cichlids (Stiassny & Gerstner, 1992; McLennan, 1994). *Gymnogeophagus* provides a sterling opportunity to add to our knowledge of cichlid behavioral evolution.

With the advent of molecular tools for systematics (Hillis *et al.*, 1996) and the development of comparative methods (Brooks & McLennan, 1991; Harvey & Pagel, 1991; Martins, 1996) it is possible to examine the dynamics of character evolution on independently derived phylogenies. We will use mitochondrial DNA (mtDNA) sequence data to estimate the relationships among *Gymnogeophagus* species, compare the molecular phylogeny to that derived from morphological characters, examine the biogeography of the genus, and estimate the evolution of behavioral characters.

Table 1. Parental care behavior, extent of sexual dimorphism and mating system for *Gymnogeophagus* species. Where there is no specific information, the behavior is inferred based on the extent of dimorphism and we have placed a question mark beside it. Parental Care: S - substrate spawning, Mb - mouth-brooding. Mating System: Mg - monogamous, Pg - polygynous. Source of observation: 1 - Reis & Malabarba (1988); 2 - Reis *et al.* (1992); 3 - Wimberger & Reis, unpubl. field notes; 4 - Santos (1997); 5 - Kullander (1983); 6 - Loiselle (1985); 7 - Loiselle & Hougen (1997); 8 - Casciotta, pers. comm. ; 9 - Malabarba & Reis, unpubl. field notes.

Species	Parental care	Dimorphism	Mating system
<i>G. meridionalis</i>	S ¹	slight ³	Mg?
<i>G. rhabdotus</i>	S ^{5,6,7}	slight ³	Mg ⁶
<i>G. setequedas</i>	S ⁸	slight ^{2,3}	Mg?
<i>G. balzanii</i>	Mb ^{1,6}	distinct ³	Pg ⁶
<i>G. gymnogenys</i>	Mb ^{1,3,6}	distinct ³	Pg ⁶
<i>G. labiatus</i>	Mb ⁹	distinct ³	Pg?
<i>G. lacustris</i>	Mb ¹	distinct ³	Pg?
<i>G. sp. "A"</i>	Mb ³	distinct ³	Pg?
<i>G. sp. "B"</i>	Mb ⁴	distinct ³	Pg?
<i>G. sp. "C"</i>	Mb ^{3,4}	distinct ³	Pg?
<i>G. sp. "D"</i>	Mb ³	distinct ³	Pg?
<i>G. sp. "E"</i>	Mb ³	distinct ³	Pg?

Materials and Methods

We collected representatives of all described and undescribed *Gymnogeophagus* species known to us except *G. australis* (an Argentinian species) and *G. setequedas*. *Gymnogeophagus setequedas* sequences represent DNA successfully extracted from two *G. setequedas* paratypes previously collected from the río Iguazú in Paraguay. Outgroup taxa were collected from the wild (*Geophagus brasiliensis*) or were obtained through the aquarium trade (*Geophagus steindachneri*). Kullander (1983, this volume) suggested that the two species groups from which these two species come, belong in different genera. Voucher specimens are deposited at the Museu de Ciências e Tecnologia, Porto Alegre (MCP) and University of Michigan, Museum of Zoology (UMMZ), and are listed in Appendix A. Heart, liver, and muscle tissue were

collected and preserved in liquid nitrogen or 95% ethanol. At least two individuals from each species and population were sequenced for a cytochrome *b* (*cytb*) fragment. For many populations, five individuals were sequenced for *cytb*. If the haplotypes were identical, then only one of the individuals was sequenced for a fragment of NADH subunit 2 (ND2) and methionine and glutamine tRNAs.

Total genomic DNA was extracted from liver, heart or gill filament tissue using a slight modification of Doyle's (1990) protocol described by Collins *et al.* (1996). A 425 base pair (bp) piece of *cyt b* and a fragment spanning ND2 from the isoleucine to asparagine tRNA genes were PCR amplified. The *cyt b* primers were GLUDG-L (Palumbi *et al.*, 1991) and H15149 (Kocher *et al.*, 1989) The ND2 primers ILE (5' - CCGGATCACTTTGATAGAGT - 3'; T.D. Kocher pers. comm.) which lies in the isoleucine tRNA and ASN (Kocher *et al.*, 1995) which lies in the asparagine tRNA were used to generate the ND2 fragment. An additional internal primer, ND2A (5' - CACTGACTCCTTGCTGAAT - 3') was used for sequencing. Double stranded fragments were generated in 25 to 100 uL volumes containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 to 2.5 mM MgCl₂, .01% gelatin (Difco), 0.5 uM of each primer, 200 uM of each dNTP, 50-1000 ng of template DNA and 1 to 2 units Taq polymerase (Perkin Elmer Cetus). The PCR profiles for the double stranded amplification were 30 sec to 1 min at 95°C, 45 sec to 1.5 min at 50-55°C, and 1.25 to 2 min at 72° for 30-35 cycles. The double stranded products were chloroform extracted, precipitated, resuspended in TE, electrophoresed on 3% NuSieve TAE agarose gels (FMC Bioproducts) and stained with ethidium bromide. DNA bands were visualized using long wavelength UV, excised with glass cover slips and purified with Gene-Clean (Bio 101). One-third of the double stranded product was then used in a single-stranded amplification identical to the first amplification except for the addition of a single primer and only 20 to 23 cycles were used. The single-stranded product was chloroform extracted concentrated and purified with Ultrafree-MC UFC3 TTK 30,000 NMWL microconcentrator tubes (Millipore). Single-stranded PCR products were sequenced using Sequenase 2.0 (USB) according to manufacturer's protocols with the PCR primer not used in the single-stranded amplification.

Sequences were initially aligned using GeneWorks 2.0 (by Intelligenetics, 1991) and the final alignment was done by eye. The aligned sequences were analyzed using parsimony (PAUP 3.1.1, Swofford, 1991, PAUP* 4.0d55-61 written by D. Swofford, 1997), neighbor joining (NJ) (MEGA, Kumar *et al.*, 1993 and PAUP* 4.0d55-61 written by Swofford, 1997) and maximum likelihood (PAUP* 4.0d55-61 written by Swofford, 1997). Individuals with identical haplotypes were lumped for the analysis. We tested the gene regions for conflicting phylogenetic signal using the partition test (Farris *et al.*, 1994) implemented in PAUP*4.0d61. Parsimony analyses were done using branch and bound searches with all sites weighted equally, and 1:1, 5:1 and a 10:1 transition: transversion (ts:tv) ratio. Bootstrap values were assigned to nodes by

doing 500 pseudoreplicates (heuristic option) using equal weights. Decay indices (Bremer, 1988; Donoghue *et al.*, 1992) were calculated to indicate the relative character support for the clades found in the most parsimonious tree. Neighbor-joining analyses were done using the Kimura 2-parameter correction. Maximum likelihood analysis was done using empirical nucleotide frequencies, ts:tv ratios of 2:1, 5:1 and 10:1, equal rates and no molecular clock enforced.

To examine the evolution of behavioral characters we mapped parental care and extent of sexual dimorphism on the phylogenies (since the two behaviors are perfectly correlated, one optimization sufficed). Table 1 documents observations of parental care and sexual dimorphism for the species included in this analysis. There are no data on the parental care or breeding behavior of *G. australis*, which is not included in the molecular analysis, but was included in the morphological analysis of Reis & Malabarba (1988). Extent of sexual dimorphism has been used to suggest differences in mating systems in some studies (e.g. Barrowclough *et al.*, 1995). The extent of sexual dimorphism corresponds to mating system in the three *Gymnogeophagus* species for which it has been described: *G. balzanii* (dimorphic and polygynous), *G. gymnogenys* (dimorphic and polygynous) and *G. rhabdotus* (nearly monomorphic and monogamous) (Loiselle, 1985; Loiselle & Hougen, 1997). We await further observations to rigorously examine the correlation.

Results

We collected a total of 713 bp of mtDNA sequence: 390 bp of data from *cytb*, 141 bp from the two tRNAs - methionine and glutamine, and 182 bp of ND2 from the 5' end of that protein. For two individuals, *G. setequedas* and *G. meridionalis* (locality 4, Table 2) we have only the *cyt b* data. There were no indels in any of the regions with the exception of a single A addition just 5' of the ND2 start codon in *G. balzanii*. In addition, the ND2 start codon for *G. balzanii* was TTG. Base composition was fairly typical of vertebrate mtDNA (Table 3) with all bases 27-29% of the total with the exception of G (15%) (Irwin *et al.*, 1991; Kocher *et al.*, 1995). The tRNA genes were slightly higher in A and lower in T than were the protein fragments. The frequency of G and C was similar across all three regions. When base composition was analyzed by position in the protein coding fragments differences between the two fragments became apparent. One of the major differences was a dearth of T's and an excess of C's in ND2 first positions relative to *cyt b* first positions (.14 vs. .24, and .32 vs. .24). T's were the most common base at second positions but there were somewhat more C's and fewer A's in ND2 second positions relative to *cyt b*, whereas this relationship was reversed for third positions. There were virtually no G's at third positions in either gene, a pattern typical of fish and cichlid mtDNA (Kocher *et al.*, 1995). Taxa did not significantly differ in base sequence composition.

Uncorrected total sequence divergences ranged from 0-15.9% (18.2% when corrected for multiple hits using the

Table 2. Collecting localities and *Gymnogeophagus* species collected. The numbers refer to localities marked in Fig. 4 and all localities are in Rio Grande do Sul with the exception of number 11.

Map number	Locality	Species
1A	rio Negro near Bagé	<i>G. meridionalis</i> <i>G. sp. "A"</i>
1B	rio Piraizinho	<i>G. rhabdotus</i> <i>G. sp. "A"</i>
2	rio Santa Maria near Rosário do Sul	<i>G. sp. "B"</i>
3	arroyo Sarandi, tributary of rio Quaraí	<i>G. sp. "C"</i> <i>G. rhabdotus</i>
4	rio Uruguai near Uruguaiana	<i>G. balzanii</i> <i>G. meridionalis</i>
5	arroyo Ximbocuzinho near São Luiz Gonzaga	<i>G. meridionalis</i> <i>G. sp. "D"</i>
6	rio Dourados near Severiano de Almeida	<i>G. sp. "E"</i>
7	rio Guafba near Porto Alegre	<i>G. gymnogenys</i>
8	arroyo Avipal, tributary of rio Guafba near Porto Alegre	<i>G. rhabdotus</i>
9	upper rio dos Sinos near Santo Antônio da Patrulha	<i>G. labiatus</i>
10	canal Cornélios between lagoa Itapeva and lagoa dos Quadros	<i>G. lacustris</i>
11	rio Iguazú, Alto Paraná, Paraguay	<i>G. setequedas</i>

Kimura 2-parameter correction). The distances between the outgroup and ingroup taxa ranged from 11.3-15.9%. The maximum divergence among the dimorphic species was 11.1% and while within the monomorphic species it was only 7.1%. Of the 713 bp used in the study, 214 sites were variable and of those 136 were polymorphic. An average of 33% of the protein coding sites were variable, while only 17% of the tRNA bp were variable, and of those only half were informative at this level of analysis.

Partition tests of the different gene regions yielded no significant differences, thus we analyzed all nucleotides as a single data set. Maximum parsimony using all the characters (equal weighting) yielded two shortest trees (362 steps, CI excluding uninformative characters = 0.60, RI = 0.73). The two trees only differed in the placement of *G. gymnogenys*. (Fig. 1). All of the dimorphic, mouthbrooding species formed one clade with the substrate spawner from the rio Paraná, *G. setequedas* at the base, and haplotypes of the monogamous, substrate spawning species, *G. rhabdotus* and *G. meridionalis* forming a second clade. It is striking that haplotypes of *G. rhabdotus* and *G. meridionalis* did not cluster as separate species but were thoroughly intermixed at the branch termini. In each of the terminal clades of substrate spawning species, a *G. rhabdotus* haplotype was sister to a *G. meridionalis* haplotype.

In further parsimony analyses where 5:1 and 10:1 transition:transversion ratios were implemented, four and three shortest trees were found. As in the trees resulting from the equal Ts:Tv ratio analysis, the position of *G. gymnogenys* was unstable in the same way as in the first parsimony analysis.

Table 3. Base composition by gene region (methionine and glutamine tRNAs, cyt *b* and ND2) and codon position for the protein coding genes.

Gene Region	A	C	G	T	number of sites
tRNA	0.32	0.29	0.15	0.25	140
ND2 total	0.29	0.30	0.14	0.28	182
1 st position	0.31	0.32	0.23	0.14	61
2 nd position	0.16	0.29	0.15	0.40	61
3 rd position	0.37	0.29	0.04	0.30	60
Cyt <i>b</i> total	0.26	0.29	0.16	0.29	390
1 st position	0.27	0.24	0.26	0.24	130
2 nd position	0.22	0.23	0.16	0.39	130
3 rd position	0.30	0.40	0.05	0.25	130

Using the 5:1 Ts:Tv ratio, *G. rhabdotus* from locality 1B (Table 2) emerged as the sister taxon to either of the other *rhabdotus/meridionalis* pairs (647 steps) in the different trees. Its position differed from the equal ts:tv ratio analysis where it formed a pair with the *meridionalis* (4) from the main branch of the rio Uruguai. The three trees resulting from a 10:1 Ts:Tv (998 steps) differed only in the placement of *G. gymnogenys*, two of these were similar to the two shortest trees (1:1 Ts:Tv) described above and in the third *G. gymnogenys* was sister to a clade that consisted of all of the undescribed species. We will refer to *G. gymnogenys* and the undescribed species included in this study as the "gymnogenys-like" group.

A bootstrap analysis of the data using equal Ts:Tv ratios, heuristic searches, and 500 pseudoreplicates yielded a tree with strong support for most of the basal branches and less resolution for some of the branch termini (Fig. 2). The dimorphic, mouthbrooding group was monophyletic in 96% of the pseudoreplicates. Other groups with over 80% support were *G. setequedas* + dimorphic mouthbrooders, the two coastal species, *G. labiatus* + *G. lacustris*, which in most replicates emerged as the sister group to the rest of the dimorphic mouthbrooders, a large group of the "gymnogenys-like" species in which the unstable *G. gymnogenys* from the rio Jacuf was placed, and one terminal pair of *rhabdotus/meridionalis* haplotypes. Decay indices show particularly good support for the monophyly of the mouthbrooding clade, the coastal *lacustris/labiatus* pair, and the upper rio Uruguai *G. sp.*"D"/*sp.*"E" pair.

Neighbor-joining trees estimated using the Kimura 2-parameter correction with 1:1, 5:1 and 10:1 Ts:Tv ratios, empirical base composition and either equal substitution rates or a gamma correction ($\alpha = 0.5$, 4 rates) all resulted in trees with the same topology (Fig. 3). The major difference between the distance and parsimony trees was the placement of *G. balzanii* at the base of the dimorphic, mouthbrooding clade, *G. gymnogenys* at the base of the "gymnogenys-like" species, and *G. meridionalis* (4) at the base of the *rhabdotus/meridionalis* clade. However when alpha is decreased to 0.2 in the neighbor joining estimates, the monophyly of *rhabdotus/meridionalis* clade breaks down and some haplotypes (e.g. *G. meridionalis* (4)) join the base of the *G. setequedas* + mouthbrooder clade. Maximum likelihood trees estimated

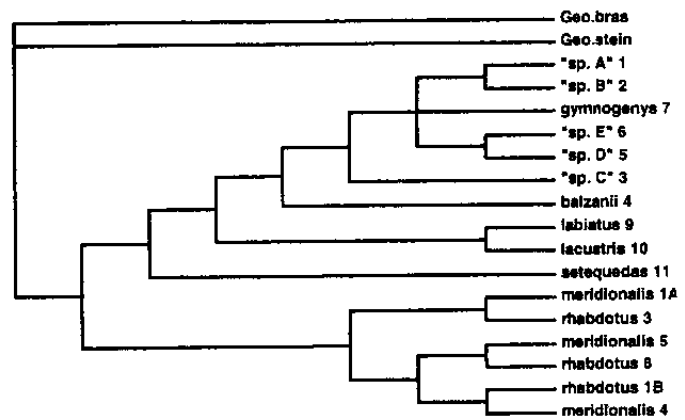


Figure 1. *Gymnogeophagus* consensus phylogeny (2 trees) from maximum parsimony analysis (Geo. bras = *Geophagus brasiliensis*; Geo. stein = *Geophagus steindachneri*). All characters equally weighted, 1:1 transition-transversion ratios. 362 steps, CI excluding uninformative characters = 0.60, RI = 0.73. The numbers next to each species name correspond to the collection localities reported in Table 2 and Fig. 4.

with Ts:Tv ranging from 2:1 to 10:1 were identical to one of the two shortest 1:1 Ts:Tv MP trees. ML trees using a maximum-likelihood determined alpha ($\alpha = 0.27$) and transition:transversion ratio (5.2) didn't alter the above maximum-likelihood results.

It is apparent when examining the neighbor-joining tree that branch lengths leading to the dimorphic, mouthbrooding species are all longer than the branches leading to monogamous substrate-spawners. Using the two-cluster relative rate test (Takezaki *et al.*, 1995), designed to test rate differences between groups, branch lengths of the dimorphic mouthbrooders were significantly longer than the branches leading to the substrate spawners ($p < 0.02$). Thus, not only is there the usual among-site variation, but there is significant among-taxon variation. This complex pattern of rate variation makes implementing rate variation corrections problematic since corrections generally address differences among sites, not taxa.

Discussion

The molecular results unequivocally support the monophyly of *Gymnogeophagus*, and thus provide independent corroboration of the morphological characters used to distinguish the genus. The analyses provide strong support for a basal split between a group including all the individuals identified as *G. rhabdotus* and *G. meridionalis*, and those that are dimorphic mouthbrooders. Support for the placement of *G. setequedas*, a substrate spawner placed at the base of the mouthbrooding group, was less strong. The placement of *G. setequedas* suggests the possibility of an early event that led to the simultaneous divergence of lineages in all of the major drainages (Paraguay, Paraná, Uruguai and coastal drainages). If the splitting of major drainages in the La Plata basin separated taxa in each of those basins and those splits occurred