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Estudo da evolução dos padrões de comportamento reprodutivo de Cynolebiatini (Cyprinodontiformes – Rivulidae)

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Resumo

A tribo Cynolebiatini pertence à família Rivulidae e possui cinco gêneros (*Nematolebias, Simpsonichthys, Cynolebias, Austrolebias* e *Megalebias*) com aproximadamente sessenta e uma espécies de peixes de água doce. As espécies deste grupo habitam ambientes lênticos, rasos e temporários (mantidos pelas águas das chuvas) distribuídos pela América do Sul sendo amplamente representados no território brasileiro, e apresentam elaborados comportamentos reprodutivos associados ao ciclo de vida anual característico da maioria das espécies da família.

Neste estudo são descritos os comportamentos reprodutivos de dezenove espécies de Cynolebiatini. As informações obtidas, somadas a informações da literatura e de comunicações pessoais, geraram alguns padrões etológicos que foram otimizados a partir de robustas árvores filogenéticas baseadas principalmente em caracteres morfológicos, a fim de se obter a hipótese mais parcimoniosa da evolução destes padrões ao nível de espécie entre gêneros. Inicialmente o comportamento reprodutivo foi dividido em cinco fases distintas: 1) "Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato), dentro dos quais alguns padrões foram estabelecidos e otimizados em filogenias pré-existentes. As nadadeiras ímpares fechadas exibidas pelos machos de *S. similis* + *S. stellatus* + *Simpsonichthys sp.* 2 durante a corte é sinapomorfico para este grupo dentro de *Simpsonichthys*, mas analisando esta condição para Cynolebiatini esta se torna uma homoplasia pois também ocorre em *Nematolebias myersi*. Outras sinapomorfias para *Simpsonichthys* são: a ausência de convite para *S. similis* + *S. stellatus* + *Simpsonichthys sp.* 2 e a ausência de tremores durante o convite para *S.*

notatus + *S. trilineatus*. A presença de movimentos da cabeça, a exibição de movimentos ondulantes laterais lentos (ou até a ausência destes), a seleção de uma fêmea dominante pelo casal e a fêmea esfregando o macho são considerados sinapomórficos para *Cynolebias*. Uma sinapomorfia de *Nematolebias* é a ausência de brigas entre as fêmeas. Algumas autapomorfias e apomorfias indefinidas também foram observadas.

Abstract

The tribe Cynolebiatini belongs to the family Rivulidae and contains five genera (*Nematolebias, Simpsonichthys, Cynolebias, Austrolebias* e *Megalebias*) with about sixty-one freshwater fish species. The species of this group are widespread through the South America, inhabiting lentic, shallow and temporary environments (maintained by the rains), and are well represented through the Brazilian territory. They present elaborated reproductive behaviors associated to their characteristic annual life cycle shared by the greatest part of the species of the family.

The reproductive behaviors of nineteen species of the Cynolebiatini are described in this study. The obtained data added to the literature data plus personal communications provided some ethological patterns. These patterns were optimized through strong phylogenetic trees based principally on morphological characters, in order to obtain the most parsimonious hypothesis of the evolution of these patterns at the species level and among genera. Initially, the reproductive behavior was divided into five distinct stages: 1) Courtship displays, 2) Invitation, 3) Submerging (to . dig the substrate), 4) Spawning/fertilization, 5) Emerging (comes back from the substrate), where some patterns were established and optimized into preexistent phylogenies. The closed unpaired fins of the males during the courtship showed by *S. similis* + *S. stellatus* + *Simpsonichthys sp.* 2 is synapomorphic for this group into the genus *Simpsonichthys*, but analyzing this condition for Cynolebiatini it becomes homoplasic because also occurs in *Nematolebias myersi*. Other synapomorphies for *Simpsonichthys* are: the absence of invitation for *S. similis* + *S. stellatus* + *Simpsonichthys sp.* 2 and the absence of trembling during the invitation for *S. notatus* + *S. trilineatus*. The presence of head-

shakings, the exhibition of slow lateral waving movements (or even the absence of it), the selection of a dominant female by the couple and the female scrubbing the male are considered synapomorphic for *Cynolebias*. A synapomorphy of *Nematolebias* is the absence of fight among the females. Some autapomorphies and undefined apomorphies were also observed.

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Capítulo 1: Introdução Geral

Introdução Geral

A tribo Cynolebiatini possui cerca de 61 espécies válidas de peixes de água doce (medindo entre 20 mm e 150 mm de comprimento total quando adultos) atualmente distribuídas em cinco gêneros: *Nematolebias* Costa, *Simpsonichthys* Carvalho, *Cynolebias* Steindachner, *Austrolebias* Costa e *Megalebias* Costa (Costa, 1998).

Os Cynolebiatini são conhecidos como "peixes anuais", assim como a grande maioria das espécies da família Rivulidae (excetuando o gênero *Rivulus*) (Costa, 1998), devido aos seus curtos períodos de vida e por viverem em biótopos lênticos, rasos e temporários cujo nível de água é dependente das chuvas (Myers, 1942, 1952). Com o fim do período chuvoso as poças secam gradativamente ocasionando a morte dos peixes adultos (Costa, 1998). Porém, vários ovos são enterrados no substrato durante a reprodução (o que faz parte de seus elaborados comportamentos reprodutivos), suportam períodos de seca. Durante o desenvolvimento embrionário várias transformações ocorrem com os ovos, dentre elas, movimentos celulares e períodos de diapausa (Wourms, 1972 a,b,c). Após nova cheia do biótopo no próximo período de chuvas acontece a eclosão dos ovos.

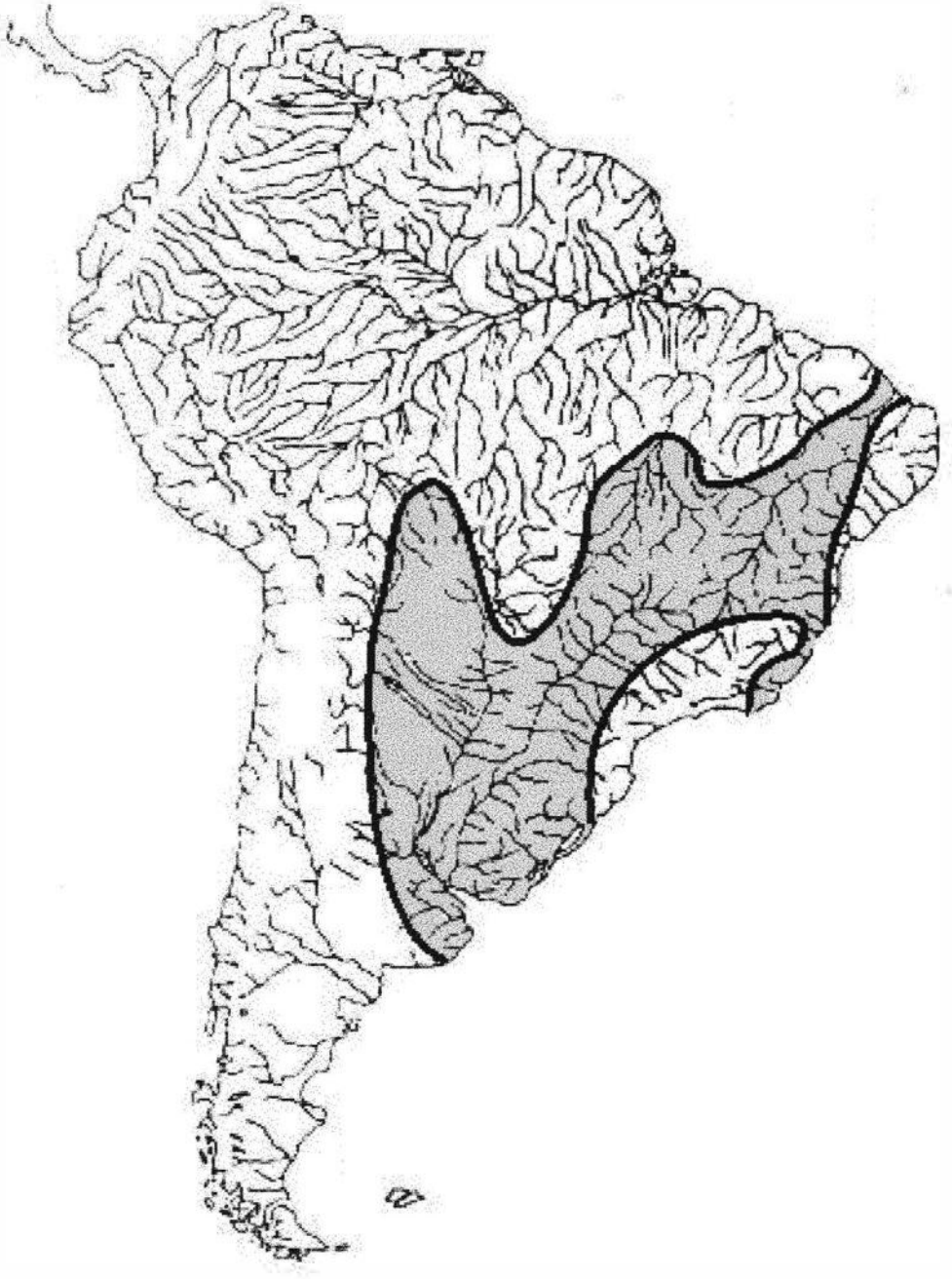
Os Cynolebiatini estão distribuídos na América do Sul, sendo que grande parte das espécies é encontrada no Brasil (Costa, 1998). Os biótopos são encontrados nos mais variados ambientes como na caatinga, no cerrado, na restinga ou em matas (Costa, 1995; Costa & Brasil, 1991) tais biótopos, que podem estar ao nível do mar como os de *Nematolebias whitei* (Myers) ou até a 1000 m de altitude como a localidade tipo de *Simpsonichthys boitonei* Carvalho (Costa, 1995).

A distribuição dos Cynolebiatini na América do Sul está contida na tabela 1 e ilustrada no mapa da figura 1, de acordo com Costa (1995, 1998).

Tabela 1: Distribuição dos gêneros da tribo Cynolebiatini na América do Sul, segundo Costa (1998; 1996).

Gêneros	**Distribuição**
Nematolebias	Bacias costeiras nos estados do Rio de Janeiro, Espírito Santo e Bahia
Simpsonichthys	Bacias dos rios Araguaia, Tocantins, Paraná, São Francisco, Jaguaribe, Mossoró, Cachoeira bacias menores no nordeste e no sudeste do Brasil, além de poças temporárias no Chaco (Paraguai) e Beni (Bolívia).
Cynolebias	Bacias dos rios São Francisco, Jaguaribe, Mossoró, Itapicurú, Vaza-Barris e alto Tocantins
Austrolebias	Bacias dos rios Iguaçú (Brasil), Uruguai (Argentina, Brasil e Uruguai), Plata (Argentina e Uruguai), Chaco paraguaio e argentino, além de bacias costeiras menores no sul do Brasil e Uruguai.
Megalebias	Bacias dos rios Paraná e Baixo Paraguai (Argentina), bacias costeiras no Rio Grande do Sul (Brasil) e nordeste do Uruguai, e Chaco urugauio, paraguaio e argentino

Figura 1 - Distribuição geográfica de Cynolebiatini na América do Sul, segundo Costa (1995, 1996; 1998).



Pela beleza dos padrões de colorido apresentados na maioria dos Cynolebiatini, e relativa facilidade de criação em cativeiro, este grupo é de grande interesse dentro da aquariofilia. Apesar da facilidade de reprodução em cativeiro, o que possibilita a realização de trabalhos etológicos sobre a reprodução, somente poucos trabalhos sobre este tema podem ser encontrados na literatura, como exemplo Costa e Brasil 1991, Carvalho, 1957. Quando encontrados, são na maioria publicações em revistas não especializadas, pouco detalhados, prendendo-se somente a descrições ou apenas citações da corte (e.g., Ostrow, 1976; Costa, Lacerda & Brasil, 1988). Nenhum trabalho com intenção de entender a evolução dos padrões reprodutivos deste grupo é encontrado na literatura sendo este um dos motivos da importância da realização deste trabalho.

Trabalhos etológicos com perspectivas evolutivas fazem parte da etologia comparada, ciência cujos fundamentos tem origem em Darwin (1872), mas que ganhou independência como um ramo da ciência somente no início do século XX quando pesquisadores começaram a estudar padrões de comportamento dentro de uma proposta evolucionária, e a propor que padrões de comportamento são úteis para análises sistemáticas. Charles Otis Whitman e Oskar Heinroth foram os dois nomes mais importantes no início dos estudos em etologia comparada (Lorenz, 1950; McLennan, Brooks & McPhail, 1988; de Queiroz & Wimberger, 1993), sendo chamados de "pais fundadores" da etologia (Brooks & McLennan, 1991), ambos compartilhando as idéias de Darwin (1859) de que o instinto (comportamentos com base genética) evolui e que padrões comportamentais podem ser utilizados como indicadores de ancestralidade comum, ainda, aplicando comportamentos no estudo de sistemática de aves (Whitman, 1899; Heinroth, 1911). As aves e os artrópodes foram os grupos preferidos dos pesquisadores que

relacionavam comportamentos e filogenética no início do século (Wheeler, 1919; Friedmann, 1929, Emerson, 1938). No entanto, só nas décadas de 40 e 50 com Niko Tinbergen, que citou os objetivos da etologia (Tinbergen, 1963), e com Konrad Lorenz, é que os estudos comparativos de comportamento começaram a ter destaque; ambos também defendiam que os padrões de comportamento são tão úteis em análises filogenéticas quanto padrões morfológicos (McLennan, Brooks & McPhail, 1988; Queiroz & Wimberger, 1993). A idéia defendida por Tinbergen e Lorenz foi mais tarde suportada por de Queiroz & Wimberger (1993) que testaram a plasticidade de caracteres comportamentais analisando os índices de consistência (CIs, que testa o nível de homoplasia) de caracteres comportamentais e morfológicos, chegando a conclusão de que não há razão para acreditar que comportamentos são menos úteis ou inadequados na formulação de filogenias. Mas alguns autores tinham dúvidas sobre o uso de tais caracteres, mantendo as idéias de que comportamentos são lábeis, fortemente influenciados pela seleção natural, dificeis de determinar homologias e dificeis de caracterizar (Atz, 1970; Hodos, 1976; Aroson, 1981; Urbani, 1989). Apesar das críticas, seguindo as idéias deixadas por Tinbergen, considerado o pai da ecologia comportamental (de Queiroz & Wimberger, 1993), vários autores vêm estudando comportamento dentro de uma óptica evolucionária, tentando entender a evolução dos padrões comportamentais com base em árvores filogenéticas preexistentes (Dobson, 1984; Brooks & McLennan, 1991; Greene, 1994; Belote, 1998), propondo hipóteses filogenéticas baseadas unicamente em caracteres etológicos (McLennan, Brooks & McPhail, 1988; McLennan, 1993), ou utilizando caracteres comportamentais aliados a caracteres morfológicos e outros na obtenção de filogenias (Costa, 1998).

Neste trabalho são descritos os comportamentos reprodutivos de dezenove espécies de Cynolebiatini baseadas em observações de laboratório, sendo duas espécies de *Nematolebias* (*N. whitei* Myers, *N. myersi* Carvalho), treze de *Simpsonichthys* (*S. constanciae* (Myers), *S. stellatus* (Costa & Brasil), *S. alternatus* (Costa & Brasil), *S. notatus* (Costa, Lacerda & Brasil), *S. flavicaudatus* (Costa & Brasil), *S. costai* (Lazara), *S. similis* Costa & Hellner , *S. trilineatus* (Costa & Brasil), *Simpsonichthys sp.*1, *Simpsonichthys sp.*2, *Simpsonichthys sp.* 3, *Simpsonichthys sp.* 4), três de *Austrolebias* (*A. nigripinnis* (Regan), *A. cyaneus* (Amato), *Austrolebias* sp. 1) e uma de *Cynolebias* (*C. albipunctatus* Costa & Brasil). Inicialmente o comportamento reprodutivo foi dividido em cinco fases distintas: 1) "Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato) e dentro de cada fase são observados alguns padrões de comportamento. Os padrões observados foram otimizados a partir de árvores filogenéticas bem suportadas, disponíveis na literatura (Costa, 1996) e outras árvores ainda não publicadas cedidas por Wilson J. E. M. Costa (Costa, no prelo a, b), para obter a hipótese mais parcimoniosa sobre a evolução destes caracteres. Informações obtidas na literatura sobre comportamento reprodutivo e por comunicação pessoal sobre as espécies estudadas e sobre outras espécies do grupo também são utilizadas. A otimização dos caracteres foi feita pelo método conhecido como "Otimização de Farris" (Brooks & McLennan, 1991) através do software MacClade (Maddison & Maddison, 1992).

Algumas sinapomorfias ao nível de gênero são hipotetizadas como na forma das nadadeiras ímpares de *Simpsonichthys boitonei* + *S. zonatus*, que é vista como homoplasia quando se estende à análise para o nível de tribo. Outras sinapomorfias são *a priori* identificadas para *Cynolebias* e *Austrolebias*, como: os movimentos ondulatórios sutis, ou a

ausência destes, os movimentos da cabeça e designação de uma fêmea dominante em *Cynolebias*; e a ausência de brigas entre as fêmeas de *Austrolebias*, ainda foram identificadas algumas sinapomorfias.

Os capítulos 3, 4, 5 e 6 estão escritos no formato de artigos científicos segundo as novas normas para formato de dissertações e teses do programa de pós-graduação em zoologia do Museu Nacional/UFRJ. Nestes capítulos são encontradas as descrições dos comportamentos separadas por gênero. No caso do capítulo 4 ainda foi feita a análise evolutiva dos padrões de comportamento das espécies através da otimização de *Simpsonichthys*.

Capítulo 2: Materiais e Métodos Gerais

Materiais e Métodos Gerais

Este estudo foi conduzido tanto com espécimes selvagens como com espécimes nascidos em aquário. Em ambos os casos os exemplares foram mantidos em aquários separados, sendo que os casais só se encontravam nas ocasiões das observações. Sempre que possível os encontros eram proporcionados com casais diferentes. Os encontros foram filmados para se obter uma videoteca sobre comportamento de cada espécie favorecendo a observação mais detalhada dos padrões de comportamento. Durante o período pré-encontro, os peixes eram alimentados com larvas de culicídeos, quironomídeos, coleópteros, enquitréias e principalmente *Artemia salina*. *Sphagnum sp*. (seco, picado e fervido) e *Vesicularea dubyana* (picada) foram utilizados como substrato de desova.

Devido a repetição dos experimentos em várias ocasiões ocorre a perda de exemplares por morte. Devido a este fato vários exemplares não puderam ser fixados. Os espécimes serão depositados na coleção do Laboratório de Ictiologia Geral e aplicada da UFRJ.

Um dos objetivos da etologia comparada é dar um enfoque evolutivo aos estudos de comportamento e um dos métodos utilizados para formular hipóteses sobre a evolução de características comportamentais é o método criado por Farris (1970), conhecido como otimização de Farris (Brooks & McLennan, 1991), através do qual as características alvo são locadas em uma filogenia pré-existente para se obter a hipótese evolutiva mais parcimoniosa sobre a evolução destas características. Para uma explicação detalhada do método, ver Brooks & McLennan (1991). O programa MacClade (Maddison & Maddison, 1992) foi utilizado para realização da otimização e obtenção dos cladogramas indicando o

caminho evolutivo de cada um dos caracteres, além de fornecer também o índice de concistência (CI) de tais caracteres. Neste estudo foram utilizadas árvores filogenéticas, baseadas principalmente em dados morfológicos, disponíveis na literatura no caso de *Nematolebias* + *Simpsonichthys* (Costa, 1996) e também em árvores ainda não publicadas como as aqui apresentadas para as espécies de *Austrolebias*, *Cynolebias*, *Megalebias* e para os gêneros de Cynolebiatini (Costa, no prelo a e b). Para a otimização dos caracteres foi construída uma árvore contendo somente as espécies estudadas (Figura), respeitando o grau de parentesco das filogenias utilizadas (Costa, 1996, noprelo a, b). Os caracteres otimizados e seus estados estão citados na Tabela 2.

Capítulo 3: Artigo 1

Reproductive behavior patterns in the genus *Nematolebias* Costa (Cyprinodontiformes: Rivulidae)

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Abstract

The fishes of the genus *Nematolebias* Costa 1998 are placed within the family Rivulidae, and they are known as "annual fishes" because of their specialized life cycle. The reproductive behaviors of the two species of *Nematolebias* (*N. whitei* Myers and *N. myersi* Carvalho) are stated in this study, and compared with the literature data sets of other rivulids. Some differences and likeness were found when the reproductive behavior of both was compared. Among the differences, the fins shape of the male during the waving movements and some attitudes of female, can be outstanding, while the presence of male interrupted waving movements around the female and how the male invites the female to dig under the substrate are shared with both species. Thus, both species present the five distinct stages forming a cycle of reproductive behavior like other rivulids: 11) Courtship displays, 2) Invitation to dive, 3) Diving (to dig the substrate), 4) Spawning \ fertilization, 5) Emerging (comes back from the substrate)

Resumo

Os peixes do gênero *Nematolebias* Costa, 1998, pertencem à família Rivulidae, e são conhecidos como "peixes anuais" pelos seus especializados ciclos de vida. Os comportamentos reprodutivos das duas espécies do gênero (*N. whitei* e *N. myersi*) são descritos neste estudo e comparados com as informações sobre outros rivulídeos obtidas na literatura. Algumas diferenças e semelhanças foram encontradas quando o comportamento reprodutivo das duas espécies foi comparado. Entre as diferenças, a forma das nadadeiras dos machos durante o movimento ondulante, e algumas atitudes das fêmeas, podem ser destacadas, enquanto a presença de movimentos ondulantes interrompidos do macho ao redor da fêmea e a forma do macho convidar a fêmea para cavar o substrato são compartilhados pelas duas espécies. E ainda, ambos apresentam as cinco fases distintas formando um ciclo no comportamento reprodutivo como em outros rivulídeos: 1) "Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato).

Introduction

The genus *Nematolebias* was recently described by Costa (1998) and comprises two valid species, *N. whitei* (Myers, 1942) and *N. myersi* (Carvalho, 1971). Both were first described and placed in *Cynolebias* Steindachner until recent years. *Nematolebias* is currently defined by a broad rostral cartilage, a strongly widened ventral process of angulo-articular, absence of teeth on the second pharygnobranchial, reduced neural spine on the first vertebra and a dark red anteriorly directed suborbital bar (Costa, 1998). The species are

distributed in restricted areas of three Brazilian states: Rio de Janeiro, Espírito Santo and Bahia.

In this study, the reproductive behaviors of *N. whitei* and *N. myersi* are described and compared among themselves and with other rivulids.

Material and Methods

Studies were conducted both in wild and born in aquaria specimens. The wild specimens of *N. whitei* were collected from a temporary pool in Município de Barra de Maricá, state of Rio de Janeiro, Brazil, and the wild specimens of *N. myersi* from a temporary pool in the state of Espirito Santo, Brazil. The specimens were maintained in individual aquaria, and they only meet in the observation aquarium. Some of the studied specimens were preserved and deposited in the LIGA/UFRJ (Lab. de Ictiologia Geral e Aplicada, Universidade Federal do Rio de Janeiro).

Observations were made in aquaria with 20 × 20 × 20 cm or 30 × 20 × 20 cm, containing *Sphagnum* sp. as spawning substrate. The reproductive behavior was recorded in view to provide a more detailed description of the behavior. The number of observed specimens, their sizes, the time of recordings and the number of male/female meetings for observations can be found in the table 1.

Results

Nematolebias whitei:

1) **Courtship displays**: The courtship begins about 10 minutes, after the couple is put together. At first, colors intensify, and the male makes a courtship dance of lateral

waving movements of the body around the female. During these movements, the male exhibits the unpaired fins expanded, by the removal of the anterior and posterior rays, while the female observes him (Figure 1). Two responses of the female were observed: or she turns toward and follows, or she remains indifferent. If more than one female is present, they (females) fight, what can result in injuries.

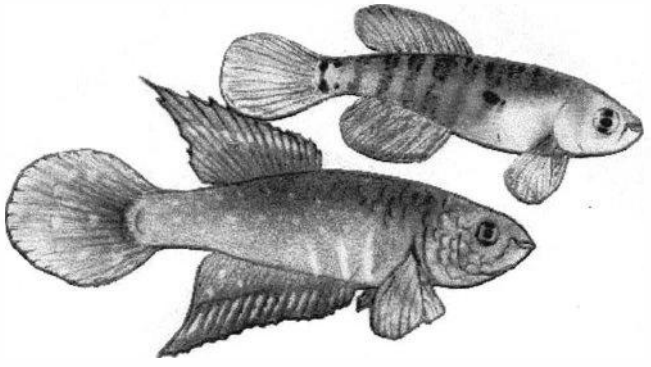

Figure 1 – Male of *N. whitei* exhibiting the unpaired fins expanded during the courtship displays. (Figure by the author and A.Aranda)

2) **Inviting to submerge**: Sometimes, the male gets into an upright position with the head down in the substrate to pick the site to submerge, making an angle between 30 or 90° (Figure 2), and then vibrates the body, as if inviting the female to dig under the substrate.

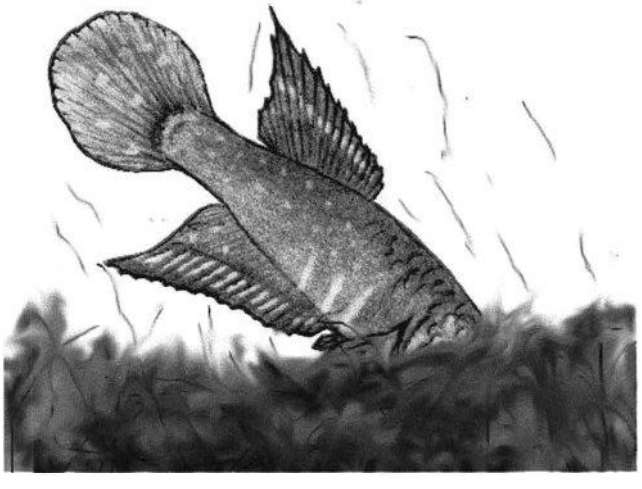

Figure 2 – The invitation of *N. whitei*. A male of *S. whitei* looking for a place to submerge, he puts the head into the substrate and trembles the body and fins calling the female. (Figure by the author)

3) **Submerging** + 4) **Spawning/fertilization**: If the female accepts, she couples him at his right or left side (Figure 3). So, both dig under the substrate together, conjugating vibrations. Into the substrate, the male goes over the female and presses her against the bottom (Figure 4). Subsequently spawning and external fertilization occurs.

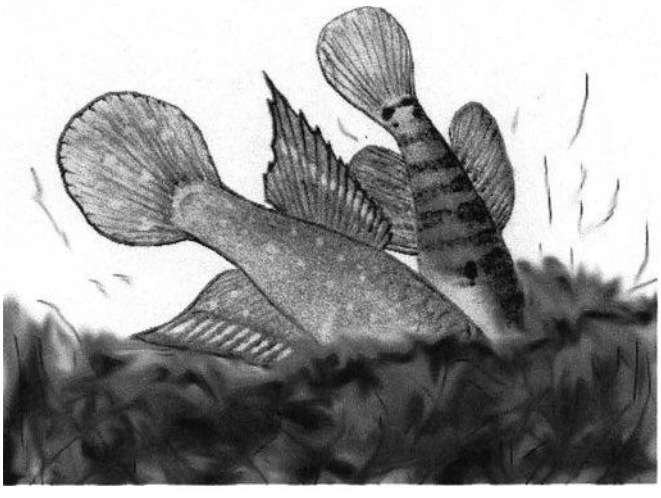

Figure 3 – When the female accepts the invitation, she couples the male and both dig under the substrate together (stage 3). (Figure by the author and A. Aranda)

Figure 4 – The spawning\ fecundation. The male lies over the female with the body curved, and presses her against the bottom, happening the spawning and fecundation. (Figure by A. Aranda)

5) **Emerging**: After the spawning, they stay completely submerged for some seconds, until they emerge randomly at different times and in different places (Figure 5).

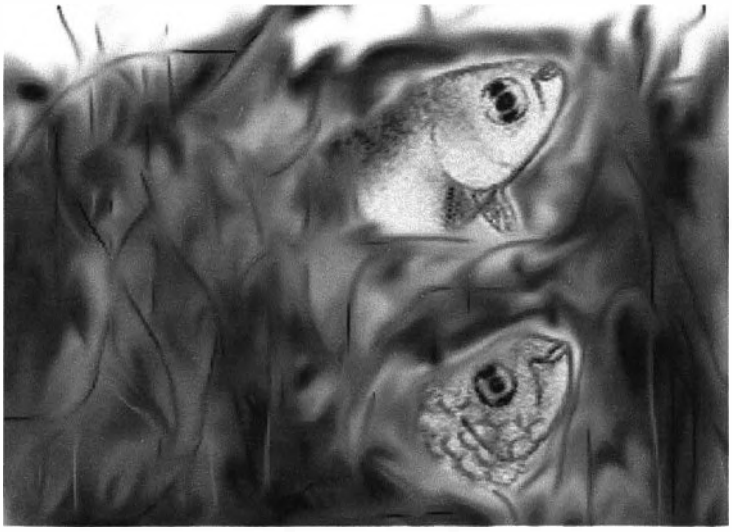

Figure 5 – Male and female emerging randomly. (Figure by the author)

New courtship displays are repeated for many times during hours until the disinterest of the female, which stops following him. This fact can be closely related to the end of mature ovules to spawn. If the couple stays together, the male will courtship her occasionally until a new acceptance, which can delay some days, occurs.

Sometimes, the male touches with the snout some regions of the body of the female such as flanks, caudal fin and opercles without aggressiveness (figure 6).

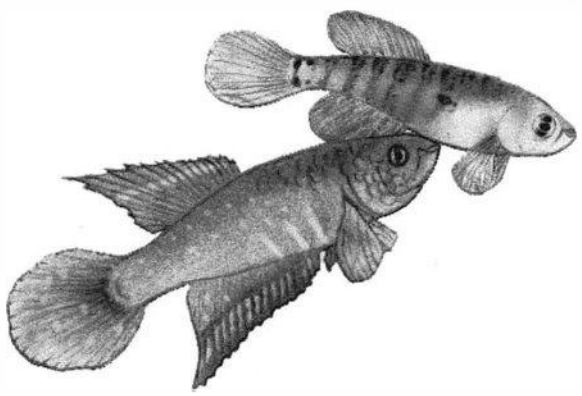

Figure 6 – The male of *N. whitei* touching the female without aggressiveness. (Figure by the author and A. Aranda)

Nematolebias myersi: (stages 1 to 5)

1) Courtship displays: After the male identifies the presence of the female in the aquarium, he begins the courtship displays showing waving movements with the body in a tilt position (with head down) around the female. During these movements the male exhibits the unpaired fins contracted (figure 7), although sometimes it can be opened (during the displays or during the submerge), and opercle slightly expanded. **2) Inviting to submerge**: The male invites the female as stated here to *N. whitei*. **3) Submerging + 4) Spawning/fertilization**: After picking the site, he gets the head down the substrate, she goes to his side, and both dig under with interrupted vibrations. However, sometimes she begins to dig under and is followed by him through the substrate. They prefer to dig under through holes that are formed in the substrate after several attempts of submerging. While he presses against the female, the spawning occurs followed by fertilization. **5) Emerging**: After this, they emerge and a new cycle of courtship displays begins. The time of submerging is brief and usually the female emerges first.

Figure 7 - Male of *N. myersi* exhibiting the dorsal and anal fins twisted andclosed caudal fin during the courtship displays. (Figure by the author)

Discussion

It is common to both species the presence of five distinct stages in the reproductive behavior: 1) Courtship displays, 2) Invitation to submerge, 3) Submerging, 4) Spawning/fertilization and 5) Emerging.

In spite of several likenesses, some differences could be observed among the presented behavior of those two species. At first, while *N. whitei* shows the unpaired fins expanded during the courtship dance, in *N. myersi* the male presents twisted dorsal and anal fins and contracted caudal fin, as described by Costa (1998).

According to many authors (*e.g.*, Belote, 1998; Costa, 1998; Carvalho, 1957, Vaz-Ferreira, Sierra & Paulete, 1964 a; Vaz-Ferreira & Sierra, 1973), the majority of the species of the Cynolebiatini, including the genera (*Nematolebias, Simpsonichthys, Austrolebias, Megalebias* and *Cynolebias*), presents expanded unpaired fins during the male courtship display. Only two species of *Simpsonichthys* show the fin behavior pattern as reported here to *N. myersi*. They are *S. boitonei* Carvalho (Costa, 1998) and *S. zonatus* (Costa & Brasil) (Belote, 1998). However, a similar pattern also occurs in rivulids of the Cynopoecilidi: *Campellolebias* Vaz-Ferreira & Sierra (*e.g.*, Vaz-Ferreira & Sierra, 1974, Costa *et al.* 1989:figure 19, 1990:figure 5a) and *Leptolebias* Myers (*e.g.*, Foersch, 1958; Costa, 1988b; Costa et al., 1989); this pattern was considered as synapomorphic for Cynopoecilidi (*e.g.*, Costa, 1990a; 1998).

Carvalho (1957) reported to *N. whitei* that if the female accepts the invitation, she couples the male by putting the snout through his pectoral fin axil before digging. Later, Vaz-Ferreira & Sierra (1973) supported this behavior to other rivulids. The presence of the snout of the female in the pectoral axil of the male can be considered as a tactile sign of

accepting to submerge and spawn, based on the presence of pectoral fins papillae in males (Carvalho, 1957; Costa, 1996). However, this behavior was not clearly observed here.

In both studied species, before the couple digs under the substrate, the male picks the site, gets head down in the substrate, and then invites the female to dig under the substrate by vibrating the body. This sequence of behaviors is also shared with several rivulids (Belote, 1998; Foersch, 1956; Vaz-Ferreira & Sierra, 1973). However, sometimes the female of *N. myersi* submerges alone and is followed by the male. Curiously, though, the spawning was never observed when it happened.

The males of the two species were not aggressive to the females, and no aggressive behavior was observed among females of *N. myersi* placed together in the spawning aquaria, but females of *N. whitei* were very aggressive among them.

It was observed the males of *N. whitei* touching the flanks of the female or fins with the dorsal surface of the head or with the snout without aggressiveness. This behavior was not described to *N. myersi* or other rivulids.

Acknowledgements

I am grateful to A. Aranda for the figures, F. Moutinho for providing specimens, to A. Belote, J. Freitas, F. Belote and T. Borges for their general support. Collecting trips were linked to a project of W. Costa supported by the Fundação O Boticário de Proteção à Natureza, and were authorized by IBAMA. The manuscript benefited from the criticisms of W. Costa.

Table 1 – The number of analyzed specimens (male/female) and their sizes (total length), the time of recordings after published and the number of meetings male/female in the observation aquaria.

Species	Number of specimens (male/female)	Sizes (males/females)	Time of recordings	Meetings
N. whitei	4/6	(3,6-5,9 cm/ 3.0-4.3cm)	4 hours	8
N. myersi	3/3	(3,9-4,7 cm/ 3,0-3.4cm)	4 hours	4

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Capítulo 4: Artigo 2

Description and evolution of the reproductive behavior patterns in the genus *Simpsonichthys* Carvalho (Cyprinodontiformes: Rivulidae)

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Abstract

The reproductive behaviors of twelve species of *Simpsonichthys* are described in this study (*S. constanciae* (Myers), *S. stellatus* (Costa & Brasil), *S. alternatus* (Costa & Brasil), *S. notatus* (Costa, Lacerda & Brasil), *S. flavicaudatus* (Costa & Brasil), *S. costai* (Lazara), *S. similis* Costa & Hellner, *S. trilineatus* (Costa & Brasil), *Simpsonichthys sp.*1, *Simpsonichthys sp.*2, *Simpsonichthys sp.* 3 and *Simpsonichthys sp.* 4), besides notes of the first steps of the reproductive behavior of *S. zonatus* (Costa & Brasil). It is defined a cycle of reproductive behaviors with five distinct stages: 1) Courtship displays, 2) Invitation to submerge, 3) Submerging (to dig under the substrate), 4) Spawning/fertilization, 5) Emerging (come back from substrate). The observed patterns are compared with the most recent and parsimonious morphological phylogenetic tree of the genus to provide an evolutionary view. Some shared patterns of courtship are considered plesiomorphic, such as: male making lateral waving movements of the body, male with the unpaired fins expanded during the waving movements, male invitation, and the couple digging under the

substrate to spawn. The shape of the unpaired fins during the courtship displays of the males of *S. zonatus* plus *S. boitonei* was interpreted as synapomorphic, as the absence of the invitation by the male of *S. stellatus* + *S. similis* + *Simpsonichthys sp.* 2, when the male submerges first and is followed by the female. Some unique behavioral patterns were identified and defined as autapomorphies.

Resumo

Os comportamentos reprodutivos de doze espécies de *Simpsonichthys*: *S. constanciae* (Myers), *S. stellatus* (Costa & Brasil), *S. alternatus* (Costa & Brasil), *S. notatus* (Costa, Lacerda & Brasil), *S. flavicaudatus* (Costa & Brasil), *S. costai* (Lazara), *S. similis* Costa & Hellner , *S. trilineatus* (Costa & Brasil), *Simpsonichthys sp.*1, *Simpsonichthys sp.*2 e *Simpsonichthys sp.* 3) são descritos neste estudo, além de notas dos primeiros estágios do comportamento de *S. zonatus* (Costa & Brasil). Um ciclo de comportamento reprodutivo com cinco estágios distintos é definido: 1) "Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato). Os padrões observados foram comparados com a mais recente e parcimoniosa árvore filogenética proposta para o grupo, a fim de obter uma visão evolutiva. Alguns padrões de corte compartilhados entre as espécies estudadas são considerados plesiomórficos (macho realizando movimentos ondulantes do corpo; nadadeiras ímpares do macho expandidas durante os movimentos ondulantes, convite do macho, e o casal cavando o substrato para desovar juntos). A forma das nadadeiras ímpares do macho durante a corte em *S. zonatus* e *S. boitonei* é interpretada como uma sinapomorfia, assim como a ausência do convite do macho para submergir em *S. stellatus* + *S. similis* + *Simpsonichthys sp.* 2, onde o macho

submerge primeiro e é seguido pela fêmea. Alguns padrões únicos de comportamento são identificados e definidos como autapomorfias.

Introduction

Tinbergen (1963) stated the goals of ethology, and later, the use of behavior patterns in phylogenetic studies was discussed (e.g., Atz, 1970). Recently, researches suggest that behavior patterns may be so useful as other phenotypic characters to predict phylogenetic hypotheses (e.g., de Queiroz & Wimberger, 1993). Thus, the behavioral evolution can be more understanding based on pre-existent phylogenetic trees (e.g., Greene, 1994).

The genus *Simpsonichthys* described by Carvalho in 1959 was considered a synonym of *Cynolebias* Steindachner until recent years (e.g., Costa 1995). However, the monophyly of both genera is supported in most recent phylogenetic analyses (*e.g.*, Costa 1996, 1998), and both are placed within the family Rivulidae (Cyprinodontiformes). *Simpsonichthys* is currently defined by a prominent process on the dorsal tip of autopalatine and unbranched dorsal and anal-fin-rays (Costa, 1998), it comprises about 25 valid species distributed over a major area of South America (Costa, 1996a),

This genus is well studied taxonomically, with available data sets for phylogenetic and biogeographic hypotheses (e.g., Costa 1996a; 1998), but only short ethological notes of *Simpsonichthys* are available in the literature (e.g., Costa 1998). Among the few ethological works and notes about the Rivulidae species, Meder (1955) stated the occurrence of spawning down the substrate, and the female picking the site in the substrate (a change of male invitation to female invitation) in *Pterolebias longipinnis* Garman; Vaz- Ferreira & Sierra (1972a) studied the reproductive behavior of *Austrolebias bellottii* and compared to

other Cynolebiatinae; Costa (1990a, 1998) established reproductive behavior patterns to define rivulid clades, setting up the evolution of these patterns. Even so, these studies represent just a little part of the ethological data sets of ichthyological groups compared with poeciliids or cichlids (Baerends & Van Roon, 1950; Baerends, Brouwer & Waterbolk, 1955).

In this study, the reproductive behaviors of twelve species of *Simpsonichthys* are described followed, by notes on the first steps of the reproductive behavior of *S. zonatus*. The behavior patterns were compared in view to provide new phylogenetic informative character sets, and optimized through previous hypothesis about the relationships within the genus (e.g., Costa 1996, 1998), permitting to erect hypotheses for evolution of the reproductive behavior.

Material and Methods

Studies were conducted both in wild specimens and born in aquaria specimens. The wild specimens of *S. alternatus*, *S. similis*, *S. costai*, *S. flavicaudatus*, *S.* sp1, *S.* sp.2, *S. sp.* 3, *S. sp.* 4 and *S. notatus* were collected from the type locality (Costa, 1995a; Costa & Hellner, 1999), the wild *S. constanciae* were collected at Rio das Ostras, R.J.. The born in aquaria studied species were *S. flavicaudatus*, *S. constanciae*, *S. stellatus*, *S. similis*, *S. zonatus*, *S. alternatus*, *S. trilineatus*. Some of the studied specimens were deposited in the Laboratório de Ictiologia Geral e Aplicada - Universidade Federal do Rio de Janeiro.

The fishes were maintained in individual aquaria. Observations and videotape records were made in aquaria with 20 × 20 × 20cm and 30 × 20 × 20cm containing *Sphagnum* sp. or *Vesicularea dubyana* as spawning substrate. The recordings started after the pair union.

The optimization method used follows Farris (1970) using the program MacClade (Maddison & Maddison, 1992). The number of observed specimens, their sizes, the time of recordings and the number of meetings (male/female) for observations can be found in the Table 1.

Results

The general patterns of reproductive behavior of *Simpsonichthys* through the five stages.

1) **Courtship displays**: On average ten minutes after the meeting in the observation aquarium the male begins the courtship displays with lateral waving movements of the body around and in front of the female. At first, in the majority of species the colors intensify. During the displays, the male exhibits the unpaired fins expanded (figure 1) while the female observes. If the female is receptive she follows the male, and if not, she gets away from him. When more than one female is present, they fight until one dies.

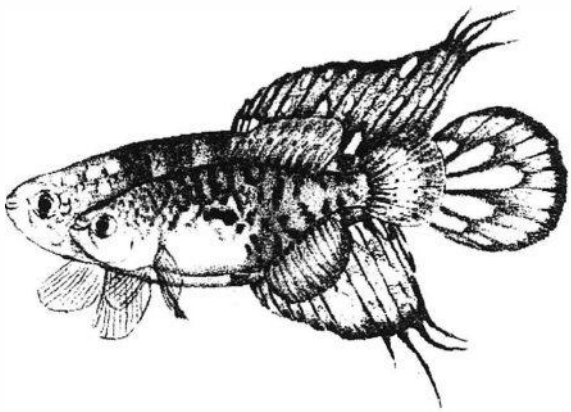

Figure 1 – Male of *S. similis* exhibiting the unpaired fins expanded during the courtship displays (plesiomorphic). This picture represents this pattern for the whole studied *Simpsonichthys*. (Figure by A. Aranda)

2) **Invitation to submerge**: The male puts his snout down in the substrate picking the site to dig under. To invite the female for spawning, he gets into an upright position

with the snout down in the substrate, making an angle between 30 and 90° and then vibrates the body and fins (Figure 2).

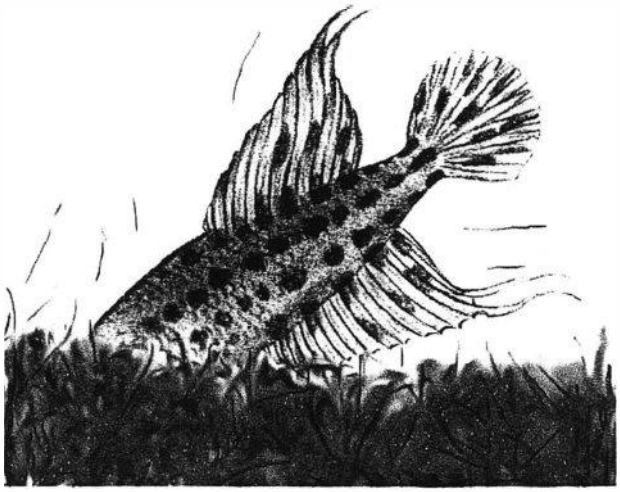

Figure 2 – The invitation represented by *S. constanciae*. In this position, the male trembles the body and fins calling the female. (Figure by the author and A. Aranda)

3) **Submerging**: If she accepts the invite, she couples him at any side, and both dig under the substrate coupled making interrupted body vibrations (figure 3).

Figure 3 – When the female accepts the invitation, she couples the male and both dig under the substrate together (stage 3). (Figure by the author)

4) **Spawning/fertilization**: Submerged in the substrate, the spawning and fertilization occurs while the male presses the female with his body "S" shaped" (figure 4).

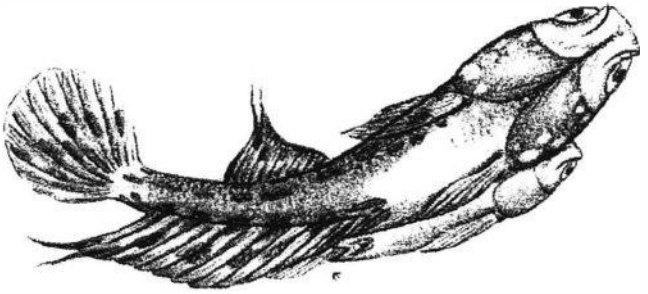

Figure 4 – The spawning\ fecundation. The male lies over the female with the body curved, and presses her against the bottom. (Figure by A. Aranda)

After the spawning, they can remain submerged for some seconds or minutes, until emerge together or they emerge randomly at different times and in different places (figure 5). New courtship displays are repeated until the disinterest of the female who stops to follow him. If the couple remains together, the male will continue the displays occasionally, then he can become aggressive or indifferent.

Figure 5 - After the spawning, having been submerged fore some seconds, they emerge. The male usually emerges first. (Figure by the author and A. Aranda)

Some modifications of the common pattern were observed in each species:

S. notatus + *S. trilineatus* - When these males were inviting the females to dig the substrate, they do it often with half body entered, and the trembling was not observed.

S. stellatus + *S. similis* + *Simpsonichthys sp.* 2 – The stage 2 (Invitation to submerge) is absent in this group. In these species, the male submerges first and is followed by the female (figure 6).

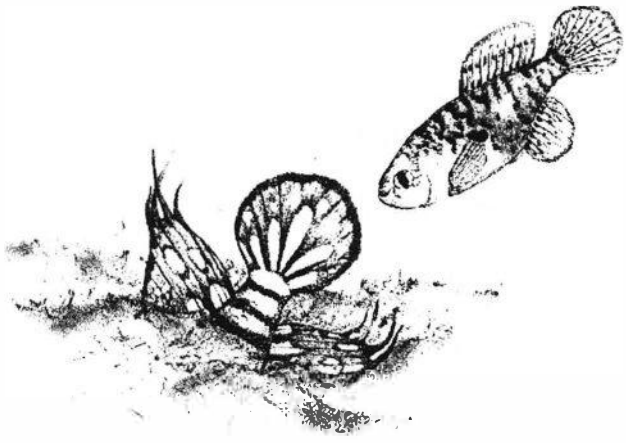

Figure 6 – Male of *S. similis* submerging first followed by the female, characterizing the absence of the invitation. This behavior is also shared by *S. stellatus* and *Simpsonichthys sp.* 2. (Figure by A. Aranda)

S. stellatus - After the spawning, either the couple emerge like the other species, or the male begins new displays of waving movements submerged, in an oblique position with head aimed up, and turns down digging a little more followed by the female to a new spawn.

S. alternatus - Sometimes after the invite and before the coupling, the female touches the flanks of the male with the snout. Beyond the common pattern, another one was observed in *S. alternatus:* when both are coupled, the male lays down over her slowly, undulating the caudal fin and peduncle. The anal, dorsal and caudal fins folds over her, and the

spawning and fecundation occur, so he pushes himself away from her with a stronger waving. This pattern happens on surface of the substrate without submerging, like species of *Nothobranchius* (personal observations).

S. costai – The color of male shows the most intensification among the studied species, from a pallid brown to a dark black. The male remains distant from the female between 3 and 5 cm, he makes few waving movements in front and on the sides of the female. He stops waving and approaches her beating the pectorals and vibrating the body that is tilt with head down and "S" shaped. Close the female he stops these movements and picks a site to dig under. During the waving movements and the vibrations the fins are expanded. This kind of approach is unique for this species.

S. trilineatus, *S. flavicaudatus*, *S. constanciae*, *Simpsonichthys sp.* 1, *Simpsonichthys sp.* 3 and *Simpsonichthys sp.* 4 – These species present no difference from the general pattern.

Note of reproductive behavior of *S. zonatus* - Only the beginning of courtship displays could be observed because the disinterest of the female. During the courtship displays and the invitation, the male always near the bottom makes interrupted waving movements around the female, with the dorsal and anal fins twisted and the anal fin closed (figure 7).

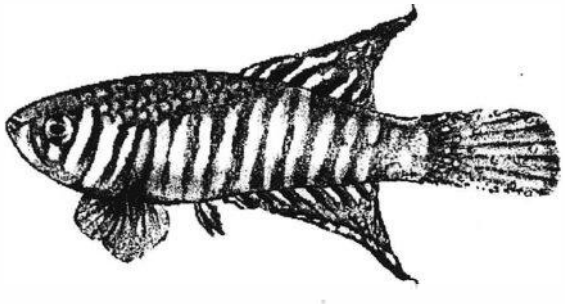

Figure 7 - Male of *S. zonatus* exhibiting the dorsal and anal fins twisted and closed caudal fin. (Figure by the author and A. Aranda)

Discussion

The evolutionary view is based on the optimization of the patterns within the phylogenetic tree proposed to *Simpsonichthys* by Costa (1996) (Figure 8), excluding the *Nematolebias* Costa species, and in recent relationship information about *S. similis* and *Simpsonichthys sp.* 2 (Costa, pers. comm.). A new tree was made using only the studied species and the program MacClade (Maddison & Maddison, 1992) provided the character optimization.

All studied males of *Simpsonichthys* share primitive patterns of reproductive behavior: courtship displays with lateral waving movements around and in front of the female, unpaired fins expanded during the waving movements, and invitation. The couple digging under the substrate together, and the spawning happening into the substrate are also shared and primitives.

The males of *S. zonatus* and *S. boitonei* (see Costa, 1998) present the unpaired fins twisted and closed during the displays, this change probably occurred in the common ancestor of these two species as showed by the Figure 9. This behavior is interpreted as a synapomorphy since they showed as close related species. Observing the real relationship hypothesis (Figure 8), we can see that *S. zonatus* is closely related to *S. santanae* and they form the sister group of *S. boitonei*. Since *S. santanae* was not available, it is not possible to establish the exactly point where the change occurred, but it is more parsimonious to believe that it has occurred in the common ancestor of these three species.

With the *S. notatus* + *S. trilineatus* the males do not tremble their bodies and fins during the invitation. In *S. stellatus*, *S. similis* and in *Simpsonichthys sp.* 2 the invitation is

absent (see discussion below), so they also do not tremble their bodies. The presence of this behavior is considered plesiomorphic for *Simpsonichthys*, and the absence is considered homoplasic. The branch where the change happened appears as equivocal represents uncertain origin of this condition (Figure 10).

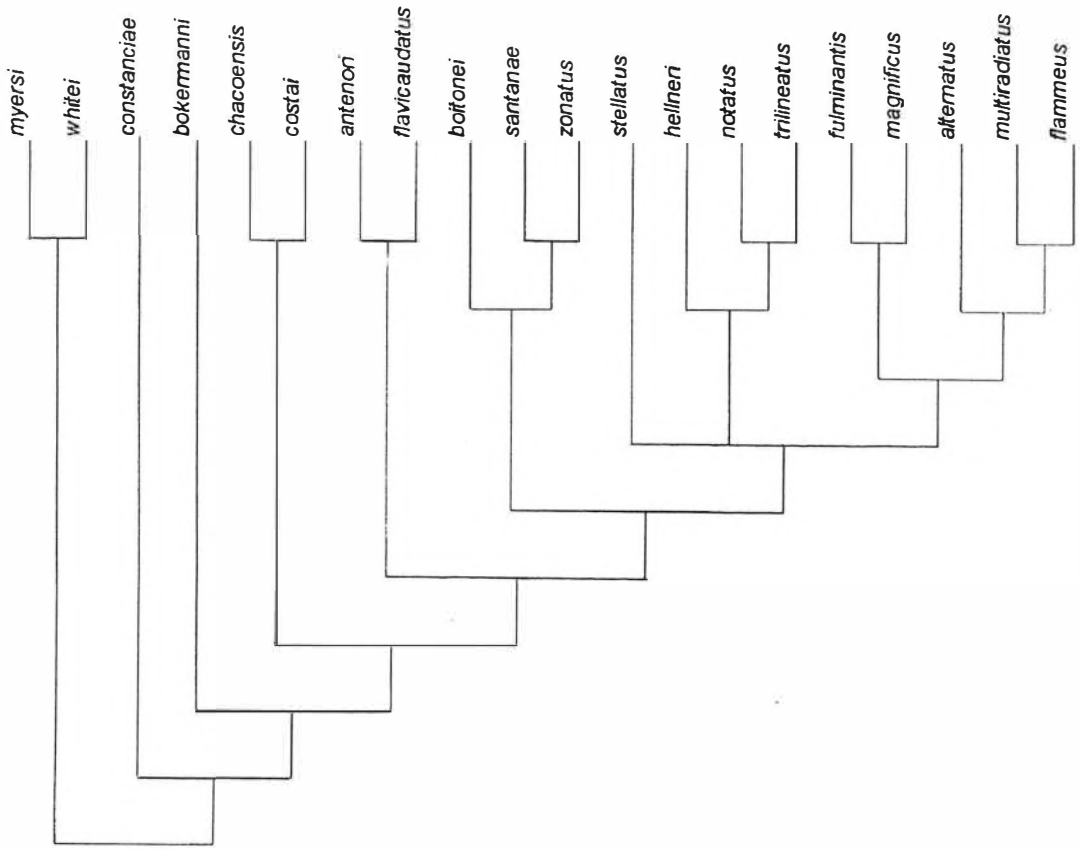

Figure 8 – Phylogenetic tree representing the relationships among the species of Simpsonichthys (Costa, 1996).

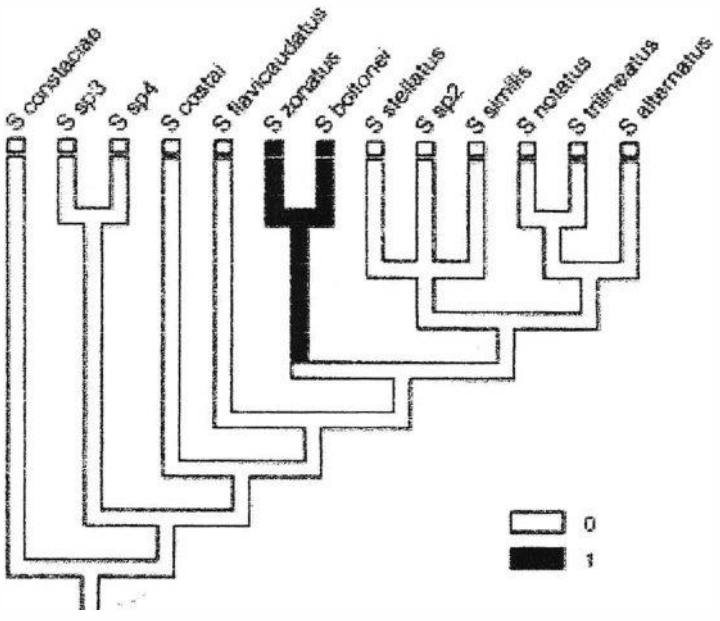

Figure 9 – The unpaired fins closed (1) represents a synapomorphy for *S. boitonei* + *S. zonatus*.

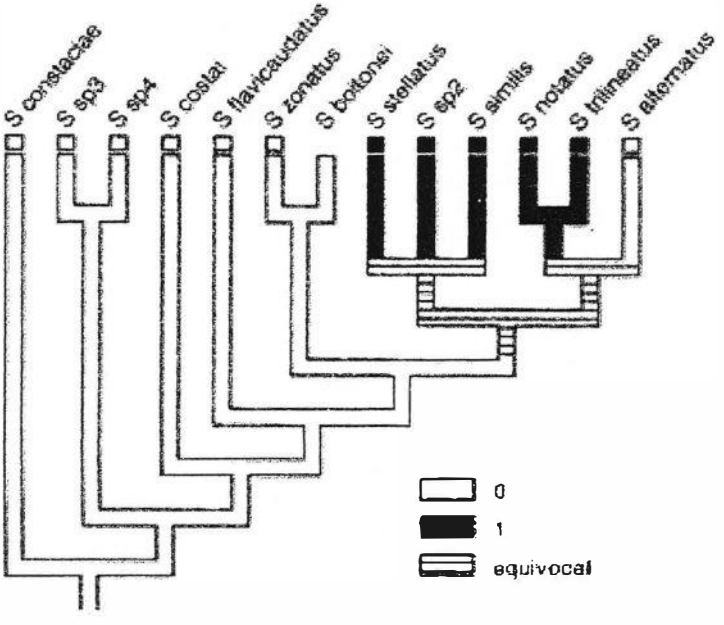

Figure 10 – The absence of trembling (1) is considered homoplasic. The equivocal area represents uncertain origin of this condition.

In *S. alternatus* the couple digging under the substrate for spawning is facultative, because some times the spawning occurred over the substrate. Thus, the presence of these two behavior patterns is an apomorphy for this species. Since closely related species as *S. multiradiatus* (Costa & Brasil) and *S. flammeus* (Costa) were not available to this study, it was not possible to establish the extension of this apomorphic condition. Similarly, the female touching the flanks of the male before the spawning is considered another apomorphy for *S. alternatus*.

The studied species share the presence of male invitation and the couple digging under together, both behavioral patterns considered primitive. These patterns are similarly changed in *S. stellatus, S. similis* and in *Simpsonichthys sp.* 2, where the male digs under first without inviting the male. According to Costa (personal communication) these three species seems to be closely related, thus the two patterns are synapomorphic for them (Figure 11).

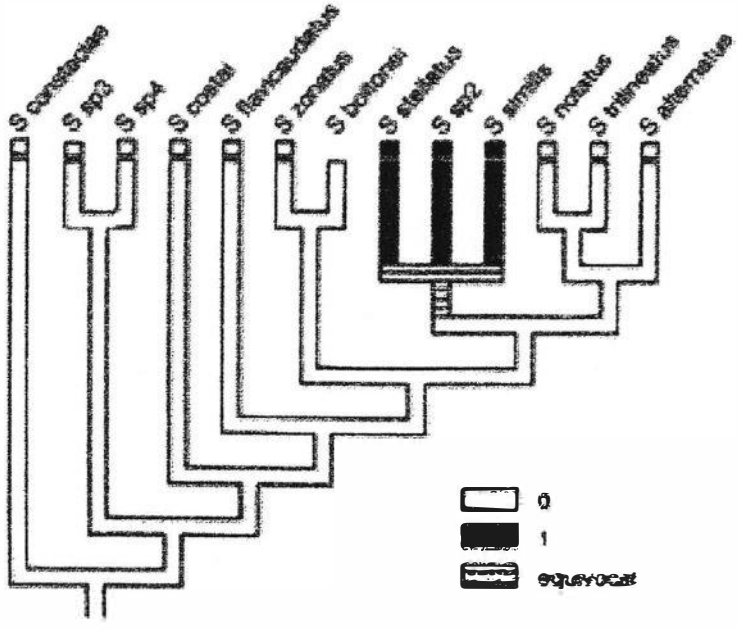

Figure 11 – Evolution of the invitation The absence (1) is synapomorphic to *S. similis* + *S. stellatus* + *S. sp.* 2. The origin of this condition is uncertain (equivocal area).

Some other autapomorphies are: the male of *S. stellatus* making courtship display into the substrate and the different display showed by the male of *S. costai* which approaches the female vibrating the body "S" shaped in a tilt position.

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Table 1: Number and sizes (total length) of studied specimens (by sex), the approximate time of recordings and the number of meetings (male/female) of each species. (?) indicates waste of specimens by aggressiveness, diseases or any problems that impeded the measurement and (0) indicates problems with the tape.

Species	**Number of specimens (male/female)**	**Sizes (male/female)**	**Time of recordings**	**Meetings**
S. stellatus	2/2	?	2 hours	4
S. similis	4/6	(3,4-4,1 cm/ 3,0-3,5 cm)	3 hours	6
S. notatus	1/2	(4,0 cm/3,3-3,4cm)	2 hours	2
S. constanciae	3/4	?	3 hours	3
S. trilineatus	1/1	(4,0 cm/?)	2 hours	2
S. costai	1/1	(2,5 cm/2,0 cm)	4 hours	2
S. alternatus	2/2	?	3 hours	2
S. zonatus	1/1	(3,5 cm/ 3,0 cm)	2 hours	1
S. flavicaudatus	3/5	(3,5-4,8 cm/ 3,0-4,3 cm	3 hours	3
Simpsonichthys. sp. 1	5/5	(3,5-3,8 cm/ 3,2-3,5)	3 hours	6
Simpsonichthys. sp. 2	1/2	?	0	2
Simpsonichthys. sp. 3	2/2	(3,8 cm/ 3,1 cm)	2 hours	3
Simpsonichthys. sp. 4	1/1	(3,9 cm/ 3,1 cm)	0	2

Capítulo 5: Artigo 3

The reproductive behavior of three species of *Austrolebias* (Cyprinodontiformes – Rivulidae)

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Abstract

Austrolebias is a genus of the Rivulidae that is endemic to in the southern Brazil, Argentina, Uruguay and Paraguay. In this study, the reproductive behaviors of the three species are described: *A. nigripinnis* (Regan), *A. cyaneus* (Amato) and *Austrolebias* sp. 1. The reproductive behaviors of these species are homogeneous even when compared with other *Austrolebias*. It can be divided into five distinct phases like proposed to other rivulids, 1) Courtship displays, 2) Invitation to dive, 3) Diving (to dig the substrate), 4) Spawning \ fertilization, 5) Emerging (comes back from the substrate). In spite of the homogeneity of patterns between the studied species and the literature data of other species, more detailed studies need to be done with other species of the genus.

Resumo

Austrolebias é um gênero de Rivulidae endêmico do sul do Brasil, Argentina, Uruguai e Paraguai. Neste estudo os comportamentos reprodutivos das três espécies são descritos: *A. nigripinnis* (Regan), *A. cyaneus* (Amato) e *Austrolebias sp.* 1. Os comportamentos reprodutivos destas espécies são homogêneos até quando comparados com outras *Austrolebias*, e pode ser dividido em cinco fases distintas como proposto para outros

rivulídeos, 1) "Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato). Apesar da homogeneidade dos padrões entre as observações das espécies estudadas e entre as informações da literatura sobre *A. nigripinnis* e outras espécies, mais estudos detalhados precisam ser feitos sobre outras espécies do gênero.

Introduction

The genus *Austrolebias* is widespread in the southern Brazil including the states of Paraná, Santa Catarina and Rio Grande do Sul, besides Paraguay, Argentina and Uruguay. This taxon was recently designed as monophyletic, comprising about 24 species (Costa, 1998). *Austrolebias* belongs to the tribe Cynolebiatini and is diagnosed by a deep and short urohyal, an expanded cartilaginous portion of basihyal and an anterior frontal scale with all borders free (Costa, 1998). Among the rivulids, *Austrolebias* is the group, where more behavioral studies can be found (e.g., Foersch, 1956; Vaz Ferreira & Sierra, 1973), in spite of few detailed studies. Here, the reproductive behaviors of three *Austrolebias* are described (*A. nigripinnis*, *A. cyaneus* and *Austrolebias sp.* 1) and compared with the literature data about this genus and other rivulids, in view to provide the reproductive behavior patterns within the genus.

Material and Methods

It was analyzed three wild couples of *A. cyaneus* sizing between 3,1 – 3,5 cm (males) and 2,8 – 3,4 cm (females) that were collected at the type locality (Arroyo Dom Marcos, state of Rio Grande do Sul, Brazil). The wild *Austrolebias sp.* 1 (locality not

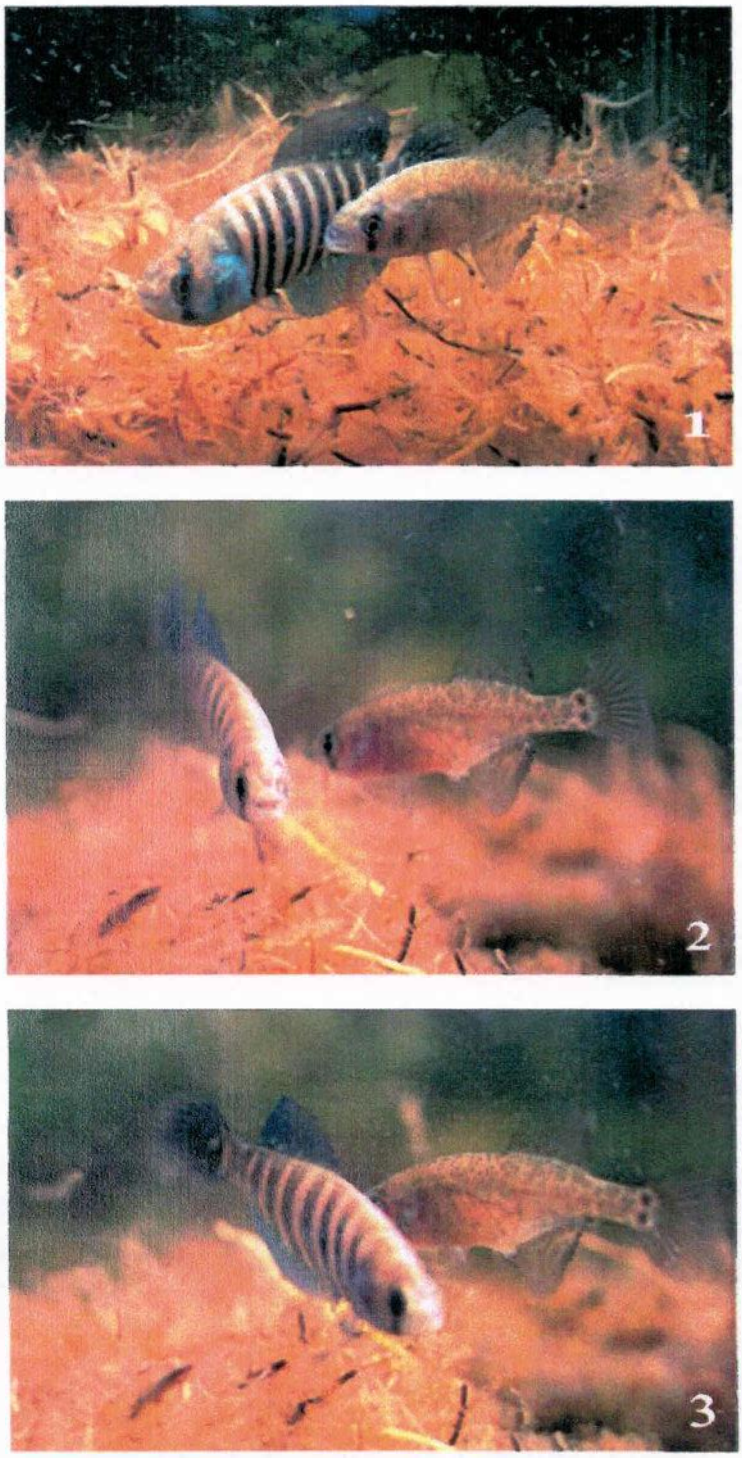
available) sizing between 4,1 – 5,0 cm (males) and 3,3 – 4,7 cm (females) were four males and five females, whereas two couples of *A. nigripinnis* lived in aquaria sized 3,2 and 3,7 cm (males), 2,5 and 3,3 mm (females). The specimens were maintained in individual aquaria, and the couples only meet in the observation aquarium. Observations were made in aquaria with 20 × 20 × 20 cm or 30 × 20 × 20 cm, containing *Sphagnum* sp. as spawning substrate. The reproductive behavior was recorded since the first meeting of the fishes in the observation aquaria, in view to provide a more detailed description and some photographs were also taken. It was obtained about ten hours of video tape recordings. A comparison is made among the reproductive patterns of these three species and other *Austrolebias* obtained from the literature, besides some personal communications.

Results

The reproductive behavior patterns of the three studied species remains the same in the general aspects; because of this, the description below represents the three studied *Austrolebias*. It presents a cycle of behaviors with the same five distinct phases as related to other rivulids: 1) Courtship displays, 2) Invitation to dive, 3) Diving (to dig the substrate), 4) Spawning \ fertilization, 5) Emerging (comes back from the substrate).

1) **Courtship displays**: After few minutes of the initial male/female meeting, the male begins to show courtship displays with intense lateral waving movements of the body around the female, exhibiting expanded fins and color intensification, while the female observes (Figures 1-3). If the female accepts the displays, she approaches and begins to follow the male that swims near the bottom trying to find a place to dive. When more than

Figures 1-3 – Male of *Austrolebias sp.* 1 exhibiting the expanded fins during the courtship.



one female is present, they alternate following and spawning. No fight was observed among the females.

2) **Inviting to dive**: Swimming near the substrate, the male puts the head into the substrate, making an angle between 30° and 90°, and vibrates the body calling the female to dive and spawn (Figure 4). He repeats this action until the female couples him to dive (an approval) (Figure 5).

Figure 4 – The invitation. The male putting the head through the substrate, and vibrates the body and fins waiting for the female. (Photo by the author)

Figure 5 – The female coupled with the male. They are prepared to dive. (Photo by the author)

3) **Diving**: After the approval, the female couples with the male (which is with the head interred) on any side, and both vibrate their bodies and fins, digging under the substrate (Figure 6). Sometimes when two females were present, the male dived with one and the other followed back. The used spawning substrate was *Sphagnum sp.* but in some observations of *Austrolebias sp.* 1 it was used pricked or natural *Vesicularea dubyana*. In this case the couple presented difficulty to dive and tried to do it through the gravels. A different event happened with some females of *Austrolebias* sp. 1, after the first dive two of the females spawned at about 4 centimeters over the substrate (one spawned 2 ovules and the other 3, one by one). In this behavior the female with expanded fins, outstanding urogenital region and body "S"-shaped, gave a strong caudal shake to release an ovule. After the ovules liberation, they continued the cycles as usual.

Figure 6 – The couple digging under the substrate. (Photo by the author)

4) **Spawning \ fertilization**: Into the substrate, the male goes over the female, presses her against the bottom (on an average three seconds) with the body curved, occurring the spawning and external fertilization.

5) **Emerging**: After the spawning, they emerge randomly at different times and in different places (Figure 7), and a new courtship cycle begins.

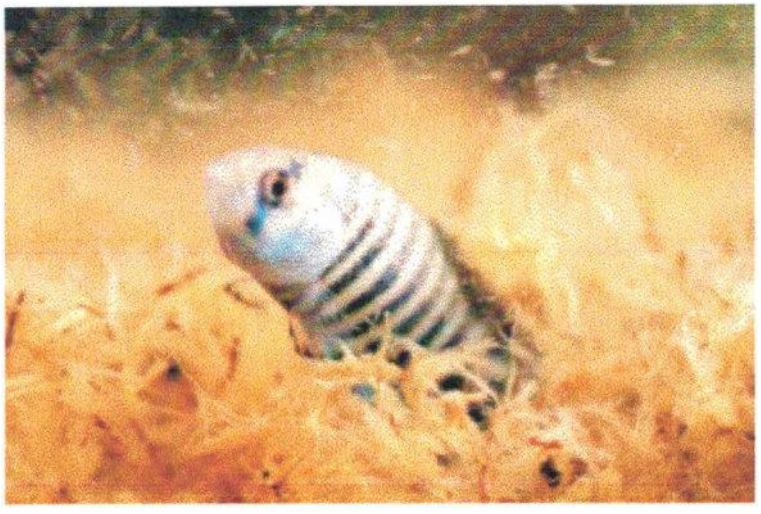


Figure 7 - The male emerging. (Photo by the author)

Discussion

The reproductive behavior of *Austrolebias* follows the patterns of other Cynolebiatini (Belote, 1998). The cycle of spawning behavior can be easily divided into five distinct phases as mentioned above. The males show intense courtship displays like in *Simpsonichthys* (Belote, 1998) but there is no fight among the females, what is corroborated by Belote (1997); Axelroad & Shaw, 1967 like in others (*e.g.*, Carvalho, 1957; Belote, 1998). The absence of fight among the females is a pattern shared among the *Austrolebias* and can be interpreted as synapomorphic for this group, but more species need

to be analyzed to confirm this. Other authors had described that during the matching the female inserts the snout in the axillary angle of the pectoral fin of the male and than they submerge (Carvalho, 1957; Axelroad & Shaw, 1967; Vaz-Ferreira & Sierra, 1973). However, I do not consider this behavior as a pattern, just because it was not clear all the time. It is right that the male needs some incentive to continuous to dig under the substrate, and this incentive might be the female's contact that happens near this area (flanks near pectoral fins). I think that this behavior happens by chance, not necessarily. This behavior is also related to *Nematolebias whitei* (Carvalho, 1957) and I think the same happens, in spite of the fact that species presents large papillae in inner surface of pectoral fins. The presence of these papillae may suggest its purpose of stimulator in the reproductive behavior when the behavior cited above is observed, but when this behavior clearly does not occur and even so the couple digs the substrate until the spawning, the objective of the papillae is still uncertain.

Vaz Ferreira & Sierra performed experiments using heterospecific couples in 1973. They observed males of *A. bellottii* (Steindachner) with females of *A. nigripinnis* and the opposite, males of *A. viarius* (Vaz-Ferreira, Soriano & Paulete) with females of *A. luteoflammulatus* (Vaz-Ferreira, Soriano & Paulete) and the opposite and also males of *Cynopoecilus melanotaenia* (Regan) with females of *Megalebias elongatus* (Steindachner) and reported that the cycle of behaviors was interrupted at any phase by the disinterest of the female and the stop of the displays by the male, without happening the dive through the substrate. The dive only occurred with a female of *M. elongatus* of 150 mm and a male of *C. melanotaenia* of 45 mm. In this study I observed the behavior of a heterospecific couple

with males of *Austrolebias sp.* 1 and a female of *A. nigripinnis* and the phases of the reproductive cycle happened until the end without problems (Figure 8), but I can not confirm the fecundation. Adding a female of *Austrolebias sp.* 1 with the couple, they alternated the spawning and had no fight. Sometimes both females tried to dive at the same time with the male; Kadlec (1990) reported for *A. affinis* a similar case of diving with two females.

Figure 8 – Male of *Austrolebias sp.* 1 coupled with the female of *A. nigripinnis.* The arrow indicates the female of *Austrolebias sp.* 1. (Photo by the author)

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Capítulo 6: Artigo 4

Reproductive behavior of *Cynolebias albipunctatus* (Cyprinodontiformes – Rivulidae)

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Abstract

The reproductive behavior of *Cynolebias albipunctatus* presents a cycle of reproductive behaviors (spawning cycle) with five coincident phases proposed for other rivulids: 1) Courtship displays, 2) Invitation to dive, 3) Diving (to dig the substrate), 4) Spawning \ fertilization, 5) Emerging (comes back from the substrate). Other patterns are shared with several species of annual fish such as: courtship displays with lateral waving movements of the body (in spite of fewer and less intense movements in this species, and they can even become absent) and fins expanded during the waving movements. Some unique behaviors are still observed in this species (comparing to other Cynolebiatini): movements of the head of the male (head-shaking) as a replacement of the lateral waving movements, determination of a dominant female with exclusion of any other by the couple and some exhibitions from dominant female that scrubs her head or the snout in the anal and caudal fins of the male and exhibits brief waving movements for the male. It was also observed the preference of *Sphagnum* as substrate for spawning.

Resumo

O comportamento reprodutivo de *Cynolebias albipunctatus* apresenta um ciclo de comportamentos com cinco fases coincidentes propostas para outros rivulídeos: 1)

"Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato). Outros padrões são compartilhados com várias espécies de peixes anuais como: corte com movimentos ondulantes laterais do corpo (apesar de poucos e menos intensos nesta espécie, podendo até deixar de ocorrer) e nadadeiras expandidas durante os movimentos ondulantes. Alguns comportamentos únicos são ainda observados nesta espécie: movimentos articulados da cabeça do macho ("head-shaking") como uma substituição dos movimentos ondulantes laterais, determinação de uma fêmea dominante com exclusão de qualquer outra pelo par e algumas exibições da fêmea dominante que esfrega a cabeça ou o focinho nas nadadeiras anal e caudal do macho e exibe breves movimentos ondulantes para ele. Também foi observada a preferência de desova tendo *Sphagnum* como substrato.

Introduction

Cynolebias is a genus of the family Rivulidae that currently includes seven species (*C. porosus* Steindachner, *C. microphthalmus* Costa & Brasil, *C. albipunctatus* Costa & Brasil, *C. perforatus* Costa & Brasil, *C. leptocephalus* Costa & Brasil, *C. gilbertoi* Costa and *C. griseus* Costa, Lacerda & Brasil), restricted to the northeast and southeast of Brazil (Costa, 1998). Costa (1998) redefined and identified some monophyletic groups within the family, including *Cynolebias*. This genus is diagnosed by a non reduced ventral process of angulo-articular, a long coronoid process of angulo-articular, elongate filamentous rays on the dorsal and anal-fins of males, absence of a transversal suborbital and supraorbital bars and dark brown to black blotches on the dorsoposterior portion of the head (see Costa, 1998). *Cynolebias albipunctatus* Costa & Brasil 1991 was described from the middle São

Francisco river floodplains in Juazeiro, Bahia, northeastern Brazil, and is diagnosed by frontal squamation about 20 small irregularly distributed scales, males reaching at least 60 mm SL (standard length), scales irregularly arranged in longitudinal series, blotches on dorso-lateral region of head, rounded head profile, anal fin base in oldest males 36.0-36.6% SL and body and unpaired fins with conspicuous white dots (Costa & Brasil, 1991). Studies about behavior of *Cynolebias, stricto sensu,* are almost unknown in the literature, in fact represented by a single short (e.g., Costa, Lacerda & Brasil, 1990). In the present study the reproductive behavior of *C. albipunctatus* is described and compared with other rivulids, principally with *C. perforatus*, providing some unique behavior patterns for the genus that can be used to give a better understanding of the evolution of reproductive behavior within the genus or contribute in future phylogenetic hypothesis.

Materials and Methods

Cynolebias albipunctatus was collected in the type locality and maintained in aquaria sizing 100 × 50 × 50 cm, containing gravels, logs and vegetation, besides a smaller aquarium (20 × 15 × 15 cm) with spawn substrate. Two kinds of spawning substrate were used, *Sphagnum sp.* (dry, pricked and boiled) and *Vesicularea dubyana* (natural). A male and two females remained together for three months, during this time they were monitored randomly. The reproductive behavior was recorded in view to provide a more detailed description of the behavior, producing six hours of tape recordings. The description was based on a male measuring 11,5 cm and two females measuring 8,5 cm each. Other rivulids data sets were obtained from the literature and by personal communication of breeders. The

studied specimens were deposited in the Laboratório de Ictiologia Geral e Aplicada da Universidade Federal do Rio de Janeiro (UFRJ).

Results

Reproductive behavior.

1) **Courtship displays**: The male presents courtship displays with few and slow lateral waving movements of the body (comparing with other rivulids) showing the fins expanded during it. This behavior can be absent for a long time, or can be present only at the first meeting of the couple. The female remains near the male, following him through the aquarium. The female also presents some exhibiting behaviors; she often scrubs her head or snout in the anal and caudal fins of the male (Figures 1 and 2) and, not so frequently, exhibits brief and subtle waving movements. When more than one female is present, one of them becomes the dominant, and the other one is excluded by the dominant one and by the male. The exclusion is characterized with the disinterest of the male and some aggressive behaviors by the couple against her (chases and bites), but specially by the dominant female which shows courtship-like aggressive waving movements and swims toward the other with the body "S"-shaped and expanded opercle then bites and chases the other one that gets out.

2) **Invitation to dive**: The male swims near the bottom trying to find a place to dive, always followed and scrubbed by the female. He puts his head into the substrate (or tries to do it in the gravels when *Vesicularea dubyana* was used as spawning substrate) making an angle between 30° and 90°, and vibrates the body.

Figures 1 and 2 – The female of *C. albipunctatus* scrubbing the head in the anal fin of the male. (Photos by the author).

3) **Diving**: The female couples him at one of his sides. So, both dig under the substrate together by vibrating their bodies.

4) **Spawning \ fertilization**: Into the substrate, the male goes over the female, presses her against the bottom causing the spawn and external fertilization. Two kinds of spawning substrate were used, but only the *Sphagnum* was approved. Using *Vesicularea dubyana* or without spawning substrate, this phase happens over the gravel, which provides more details. The male tries to put the head through the gravel to pick a site to dive and the female, which is following, couples him (figure 3). They try to dive by vibrating the body together, and after many unsuccessful attempts, the male lies over her with the body curved, with the fins a little folded against her, and presses against and vibrates the body with an open mouth (figure 5). After the spawning, the female looks for the eggs to eat them.

5) **Emerging**: After the spawning into the substrate, they emerge randomly at different times and in different places. So, the female continues to follow the male until a new cycle

begins, and in this new cycle the first stage can be absent. They can remain spawning for two days long without interruption and for more than a week without spawning.

Figure 3 - When the spawning substrate is absent, the male looks for a place to invite and submerge in the gravels while is followed and scrubbed by the female. (Photo by the author)

Figure 4 – The male of *C. albipunctatus* trying to lie over the female. (Photo by the author).

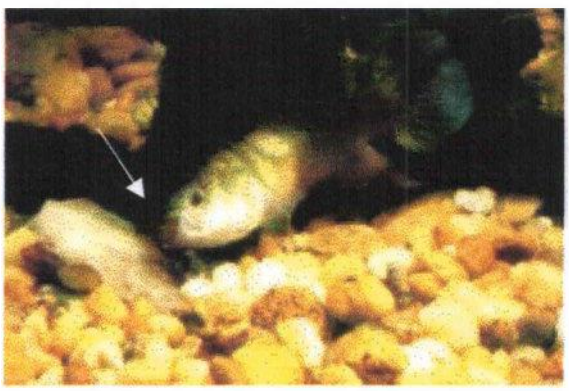

Figure 5 – When the male is pressing the female he often opens his mouth. (Photo by the author)

Figure 6 – Male over the female after the pressing. Photo by the author.

Discussion

The reproductive behavior of *C. albipunctatus* presents some different patterns if compared with other rivulids, but it seems to be exactly the same of *C. perforatus* (Carlos da Silva, personal communication). Thus, in both species the males present courtship displays with few and slow lateral waving movements, but only during the first spawning cycles after the initial meeting. In the whole of studied rivulids species, the males show the courtship displays with quickly lateral waving movements happening in each spawning cycle (e.g. Belote, 1998; Vaz-Ferreira & Sierra, 1973; Costa, Lacerda & Brasil, 1990), but slow waving movements are also related to *C. griseus* Costa, Lacerda & Brasil. These waving movements seem to be replaced by another behavior, the head shaking, (both happening in the phase 1 and apparently serving to attract the female). In this trait observed in *C. albipunctatus* and *C. perforatus* the males shake the head up and downwards for a second. When the head shaking happens, the females approach the male, as when the waving movements happen.

The females of the Rivulidae are often restricted to observe courtship displays and to follow the males to spawn if they are ready to do it, except the female of *Pterolebias longipinnis* Garman that picks the site to dive instead of the male (Foersch, 1956). However, the females of *Cynolebias* scrub the head or snout in the anal and caudal fins of the male, what might mean that she is inviting him to spawn or indicating that she is ready; and not so frequently exhibits brief and subtle lateral waving movements to him. After the spawning, the male leaves the female, and she looks for the egg to eat. This behavior was never related for other rivulids.

As it was reported about some of the other rivulids, when more than one female is present, they can fight like in *Simpsonichthys whitei* (Carvalho, 1957), they can be indifferent to each other like in *Austrolebias cyaneus* (Belote, 1998) and *Austrolebias sp.* 1 or even the two females try to dive with the male at the same time, one in each side like in *Austrolebias nigripinnis* (personal observation). In *Cynolebias* a dominant female is selected, this one jointly with the male chases and bites the other, impeding the approach thereby only the dominant female spawns. In no other rivulids the male excludes a female.

Between the two spawning substrates used with *C. albipunctatus*, only the *Sphagnum* sp. was accepted, using *Vesicularea dubyana* they preferred to try to dig under through the gravels, and unable to do this, the spawning happened over it. So, it could be observed that the spawning substrate is not a limiting factor to the spawning of this species, since they can do it over the gravels, for example. Other rivulids also do not have problems with the absence of a spawning substrate; *Nematolebias whitei* and *Simpsonichthys constanciae* can spawn over the glass aquarium bottom (personal observations) and *Austrolebias bellottii* until over the palm of the hand (Foersch, 1956).

The presence of the female's behaviors (scrubbing, egg eating and displays of lateral waving movements), the decrease and replacement of the lateral waving movements of male by the head-shaking and the male avoiding the excluded female can be considered, *a priori*, as synapomorphic for *Cynolebias* until new studies of the other species are done.

The whole data indicate that there is no effort of the male in being courting the female as it happens to the other related species, but that the male only exhibits himself at the beginning (moment when they meet for the first times) and he substitutes the exhibition of waving movements for head-shakings. On the other hand, the dominant female plays the

role of stimulating or inviting or simply informing the male that she is ready for the spawning, besides preserving her partner far from other females. Seemingly, all the observed innovations at the level of the reproductive behavior for *C. albipunctatus* are shared with *C. perforatus* (Silva, personal communication), but in spite of not doubting about the truthfulness of the information about this last one, it is necessary to analyze this species under the same conditions that were made for the *C. albipunctatus*.

Acknowledgements

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Capítulo 7: Conclusões Gerais

Conclusões Gerais

O comportamento reprodutivo das espécies da tribo Cynolebiatini analisadas neste estudo foi dividido em 5 fases distintas, sendo elas: 1) "Displays" de corte (onde os machos realizam movimentos ondulantes laterais geralmente com as nadadeiras expandidas), 2) Convite para submergir (neste comportamento, o macho enterra a cabeça no substrato e treme o corpo e nadadeiras), 3) Submersão (o casal cava o substrato), 4) Desova\ fertilização (mergulhados no substrato, o macho deita sobre a fêmea e a pressiona contra o fundo, ocorrendo assim a desova seguida da fecundação), 5) Emersão (volta do substrato após a desova). Dentro destas fases, alguns padrões foram definidos e estão citados na Tabela 2.

Após uma análise geral dos dados é possível estimar o possível comportamento reprodutivo do ancestral de Cynolebiatini. Neste caso, o macho provavelmente cortejava a fêmea com intensos movimentos ondulantes laterais exibindo as nadadeiras expandidas. Enterrava o focinho no substrato procurando um local para o mergulho. Após achar um local, ele enterrava a cabeça no substrato e tremia o corpo e nadadeiras, convidando a fêmea para o mergulho. Aceitando o convite, a fêmea se posicionava em um dos lados do macho e ambos mergulhavam no substrato conjugando vibrações. Dentro do substrato ocorria a desova e fecundação, após o macho comprimir a fêmea.

É visto também que o grupo que apresentou maior número de derivações de comportamento reprodutivo foi *Cynolebias*. Enquanto *Austrolebias* se demonstrou como o grupo com os padrões mais homogêneos dentro de Cynolebiatini. *Simpsonichthys* é o grupo onde ocorre o maior número de variáveis entre as espécies, porém é fato de que este foi o

grupo mais representado neste estudo. Por fim, *Nematolebias* apresenta uma divergência quanto à forma das nadadeiras ímpares dos machos durante a corte. Enquanto *N. whitei* se comporta de maneira primitiva, *N. myersi* apresenta uma derivação desta característica.
O índice de consistência igual a 0.75 e o índice de retenção 0.82 (Figura 6), ajudam a suportar as idéias de que caracteres comportamentais são bem aceitos em análises filogenéticas como de Queiroz & Wimberger (1993) e outros.

Após a otimização dos caracteres na filogenia montada com as espécies estudadas representadas na Figura 6 e nas outras filogenias (Figuras 2, 3 , 4, 5), as conclusões sobre a evolução dos caracteres foram as seguintes:

Característica 1 (C1)- Forma das nadadeiras ímpares dos machos durante a corte.

Analisando a otimização de C1 observa-se que apresentar estas nadadeiras expandidas (estado 0) é uma plesiomorfia para o gênero estando presente no ancestral do grupo, e o estado 1, onde os machos apresentam as nadadeiras contraídas, é uma sinapomorfia de *S. boitonei* + *S. zonatus*, tendo aparecido no ancestral comum destas duas espécies (Figura 8 [*Simpsonichthys*]), esta hipótese é a mais parcimoniosa. Porém a relação mais correta entre *S. boitonei* e *S. zonatus* se encontra na Figura 2, onde *S. boitonei* é grupo irmão de *S. zonatus* + *S. santanae*. Como não foi possível a análise desta última espécie se torna difícil estabelecer onde ocorreu a mudança no comportamento. Caso *S. santanae* se comporte como estas outras duas espécies, é visto que a mudança de 0 para 1 ocorreu no ancestral comum das três espécies, mas caso ela se comporte como a maioria dos *Simpsonichthys*, duas hipóteses são possíveis: 1) a mudança 0→1 ocorreu no ancestral

comum as três espécies, ocorrendo uma reversão (1→0) em *S. santanae*; 2) ocorrência de evolução paralela (0→1) em *S. boitonei* e em *S. zonatus* independentemente.

Otimizando C1 na filogenia de Cynolebiatini (Figuras 3, 6), surge à hipótese de homoplasia para o estado 1 desta característica, já que além de aparecer em dois *Simpsonichthys* estreitamente relacionados ele também aparece em *Nematolebias myersi*. Neste caso o estado 0 continua plesiomórfico para a tribo.

Característica 2 (C2) - Convite para mergulhar

O convite pra o mergulho no substrato ocorre enquanto o macho acha um lugar adequado para mergulhar e desovar, ele enterra a cabeça no substrato, (no caso de *S. notatus*, o macho pode enterrar até dois terços do corpo). Neste caso o estado 0 é considerado plesiomórfico para *Simpsonichthys* e para Cynolebiatini como um todo, quando otimizadas nas Figuras 3 e 6. Já a ausência do mergulho (estado 1) é uma sinapomorfia do grupo *S. stellatus* + *S. similis* + *Simpsonichthys sp.* 2 - segundo Dr. Wilson Costa estas três espécies estão (aparentemente) estreitamente relacionadas (comunicação pessoal) – tendo esta característica evoluído de 0→1 no ancestral comum a estas três espécies, já que estas formam um grupo monofilético. Porém na Figura 9, indica dúvidas quanto a o local de origem da mudança (região listrada), devido à falta de relação de parentesco exata sobre estas três espécies.

Característica 3 (C3) -. Vibrações no convite

A presença de tremidas durante o convite (0) é avaliada como plesiomorfia para Cynolebiatini (Figura 10). A ausência de tremida (1) é vista como uma homoplasia pois

aparece em *S. notatus* + *S. trilineatus* e em *S. stellatus* + *S. similis* + *Simpsonichthys sp.* 2. A origem deste estado aparece duvidosa (Figura 10 [área listrada]).

Característica 4 (C4) - Apresentação dos movimentos ondulantes laterais.

As duas espécies de *Cynolebias* citadas neste estudo apresentam poucos e menos intensos movimentos ondulantes (estado 1), que também é relatado para *C. griseus* Costa, Lacerda & Brasil, mas na maioria das vezes este comportamento é ausente ou só é presente no encontro inicial do casal, o que parece, *a priori*, uma plesiomorfia do gênero tendo sido herdada do ancestral de *Cynolebias* (Figura, 11 [*Cynolebias*]). Na análise de C4 nas Figuras 3 e 6, o estado 1 aparece como sinapomorfia de *Cynolebias*. Porém, estas hipóteses são fracas pois são baseadas em poucas espécies, e por isso, se faz necessário o estudo de outras espécies para suportá-las.

Característica 5 (C5) - Movimentos da cabeça - "head-shakings"

Os movimentos ondulantes servem aparentemente para exibição do macho e atração da fêmea. É possível que em *Cynolebias* este comportamento tenha sido substituído por movimentos da cabeça do macho. Neste comportamento o macho de *Cynolebias* para e move a cabeça para cima e para baixo por 1 ou 2 segundos mantendo as nadadeiras bem abertas. Após este comportamento a fêmea se aproxima. Otimizando esta característica na filogenia de *Cynolebias* (Figura4) , observa-se que o estado 1 (presença do comportamento) pode ter aparecido em A, em B ou no ancestral comum de *Cynolebias* como um todo, sendo as três hipóteses igualmente parcimoniosas. Neste último caso a característica é plesiomórfica para o gênero. Do contrário, caso todas as outras *Cynolebias* apresentem o

estado 0, a presença deste comportamento passa a ser uma homoplasia para *Cynolebias*, pois aparece em *C. albinpuctatus* e *C. perforatus* que não são estreitamentos relacionados (Figura 4).

Analisando C5 nos cladogramas das Figuras 3 e 6, a presença deste comportamento aparece como sinapomorfia de *C. albipunctatus* + *C. perforatus* (Figura 12). Segundo Costa (1998) *Megalebias* é grupo irmão de *Cynolebias*, neste caso, além o estudo de outras espécies de *Cynolebias* se faz necessário também o estudo de espécies de *Megalebias* para uma afirmação mais concreta da extensão do estado 0. Caso *Megalebias* também compartilhe destes movimentos, este caráter seria uma sinapomorfia de *Cynolebias* + *Megalebias* de acordo com a filogenia de Costa (1998). Porém, a hipótese mais atual do parentesco de Cynolebiatini indica que *Megalebias* é grupo irmão de *Austrolebias*, e *Cynolebias* como grupo irmão destes dois (Figura, 3). Neste caso, esta mesma situação teria duas hipóteses igualmente parcimoniosas: 1) surgimento da característica no ancestral comum a estas três espécies com reversão em *Austrolebias* ou 2) surgimento da característica independentemente em *Cynolebias* e em *Megalebias*.

Característica 6 (C6) – Designação de uma fêmea dominante

Esta característica observada em *C. albipunctatus* e *C. perforatus* que se comporta como a característica anterior sendo o estado 1 considerado, *a priori*, plesiomórfico quando otimizado no cladograma da Figura 5 e sinapomórfico para o gênero dentro de Cynolebiatini (Figura 13). Surgem dúvidas sobre sua origem real, devido à falta destas informações sobre *C. griseus* (Figura 13 [área listrada]).

Característica 7 (C7) – Exibições das fêmeas para os machos.

O estado 1 de C7 se apresenta como os estados 1 de C6 de C5 citados acima (Figura 14).

Característica 8 (C8) - Comportamento das fêmeas em relação a outras fêmeas durante a reprodução.

A análise de C8 indica que no ancestral de Cynolebiatini as fêmeas apresentavam agressividade entre si. Desta forma, para *Austrolebias* a indiferença entre as fêmeas (estado 1) é uma sinapomorfia surgindo no ancestral do gênero (Figura 7, 15). A ausência de conflitos entre fêmeas é bem caracterizada em *Austrolebias* aonde elas chegam a se alternarem nos mergulhos ou até chegam a mergulhar duas de uma vez, uma de cada lado do macho.

Características 9 (C9) e 10 (C10):

Algumas autapomorfias foram observadas: o cortejo dentro do substrato, antes mesmo de submergir, do macho de *S. similis* (Figura 16) e os toques dados nas fêmeas sem agressividade pelos machos de *N. whitei* (Figura 17). Nestes casos, estas características foram observadas unicamente nestas espécies e em nenhuma das espécies estreitamente relacionadas a elas (*S. similis* e *Simpsonichthys sp.* 2 ou *N. myersi*).

Características 11 (C11), 12 (C12) e 13 (C13):

O mergulho facultativo em *S. alternatus*, o comportamento de comer os ovos após a desova das fêmeas de *C. albipunctatus* e forma de cortejar do macho de *S. costai* também

são únicos a estas espécies até o momento. Porém como espécies relacionadas como *S. chacoensis* (grupo irmão de *S. costai*), *S. flammeus* e *S. multiradiatus* (relacionados com *S. alternatus*) e alguma outra relacionada com *C. albipunctatus* não estavam disponíveis para a análise deste estudo, não é possível estabelecer a extensão destas apomorfias. Porém na otimização a partir do cladograma da Figura 2, estas características aparecem como autapomorfias (Figuras 18, 19, 20).

Tabela 2 – Lista de caracteres e estados dos caracteres que foram otimizados.

Caracteres	Estados dos caracteres
1 – Forma das nadadeiras ímpares dos machos durante a corte.	0 - Expandidas 1 – Enroladas (contraídas) (característica apresentada somente pelos machos de *S. boitonei*, *S. zonatus* e *N. myersi*)
2 – Convite para mergulhar (Neste comportamento o macho enterra a cabeça no substrato esperando a fêmea)	0 - Presente 1 - Ausente (A ausência do convite é característica do grupo que inclui *S. stellatus*, *S. similis* e *Simpsonichthys sp.* 2; nestes, o macho mergulha primeiro e é seguido pela fêmea)
3 – Vibrações durante o convite (Neste comportamento quando o macho enterra a cabeça no substrato ele vibra o corpo e as nadadeiras)	0 – Presente 1 - Ausente
4 – Apresentação dos movimentos ondulantes laterais.	0 - Presença de intensos movimentos ondulantes laterais em todos os ciclos de corte 1 - Movimentos ondulantes laterais sutis, chegando a ser ausente (Característico dos machos de *Cynolebias* que exibe movimentos sutis restritos aos primeiros instantes de contato com as fêmeas)
5 – Movimentos da cabeça (Neste comportamento o macho para, e move a cabeça para cima e para baixo, exibindo as nadadeiras ímpares	0 - Ausentes 1 - Presentes (característico dos machos das espécies de *Cynolebias* estudadas)

expandidas).	
6 – Designação de uma fêmea dominante	0 - Ausente 1 - Presente (com exclusão da fêmea dominada pelo casal, característico das espécies de *Cynolebias*)
7 – Exibições das fêmeas para os machos	0 - Ausência de exibições 1 - Fêmea se exibindo com movimentos ondulantes e esfregando a cabeça e o focinho nas nadadeiras anal e caudal do macho. (Característico das fêmeas de *Cynolebias*)
8 – Comportamento das fêmeas em relação a outras fêmeas durante a reprodução	0 - Agressivas 1 – Indiferentes (Característico das fêmeas de *Austrolebias* que não são agressivas entre si)
9 – Corte dentro do substrato (este comportamento somente foi observado pelos machos de *S. similis* que realizavam movimentos ondulantes ainda submersos no substrato logo após a desova)	0 – Ausente 1 - Presente
10 – Toques nas fêmeas	0 – Ausente 1 – Presente (característica observada somente nos machos de *N. whitei* onde estes eventualmente tocavam o focinho nos flancos, nadadeira anal e região pélvica das fêmeas, sem demonstrar agressividade).
11 – Mergulho no substrato	0 – Presente 1 – Facultativo (ocasionalmente observado em *S. alternatus*)
12 – Comportamento das fêmeas em relação aos ovos após a desova.	0 –Indiferença 1 – A fêmea procura e come os ovos

	(característica claramente observada nas fêmeas de *C. albipunctatus*)
13 - Forma da corte apresentada pelos machos	0 – Corte com movimentos ondulantes laterais ao redor ou aos lados das fêmeas (padrão de corte na maioria das espécies estudadas). 1 – Além dos movimentos laterais, o macho para e se aproxima lateralmente da fêmea vibrando o corpo em forma de "S" e levemente inclinado com a cabeça para baixo (único de *S. costai*).

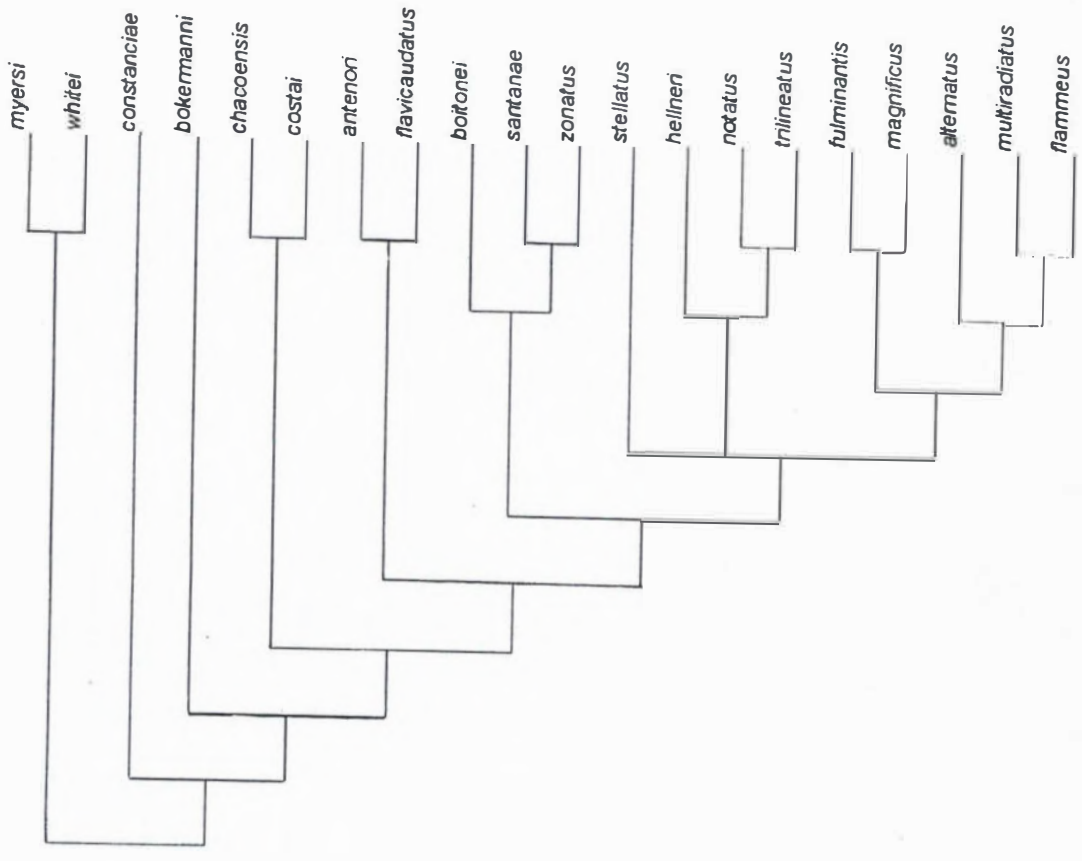

Figura 2 – Filogenia proposta por Costa (1996) representando a relação filogenética das espécies de *Simpsonichthys* e *Nematolebias*.

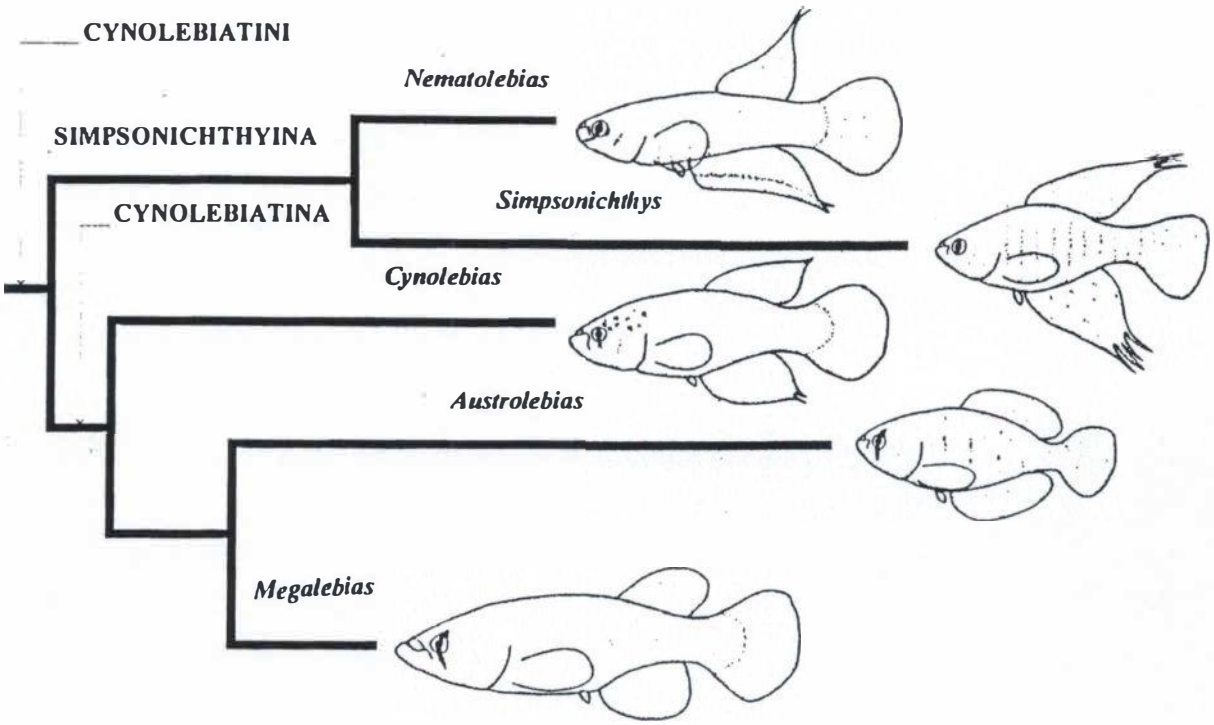

Figura 3 – Representação do parentesco entre os gêneros da tribo Cynolebiatini (Costa, no prelo).

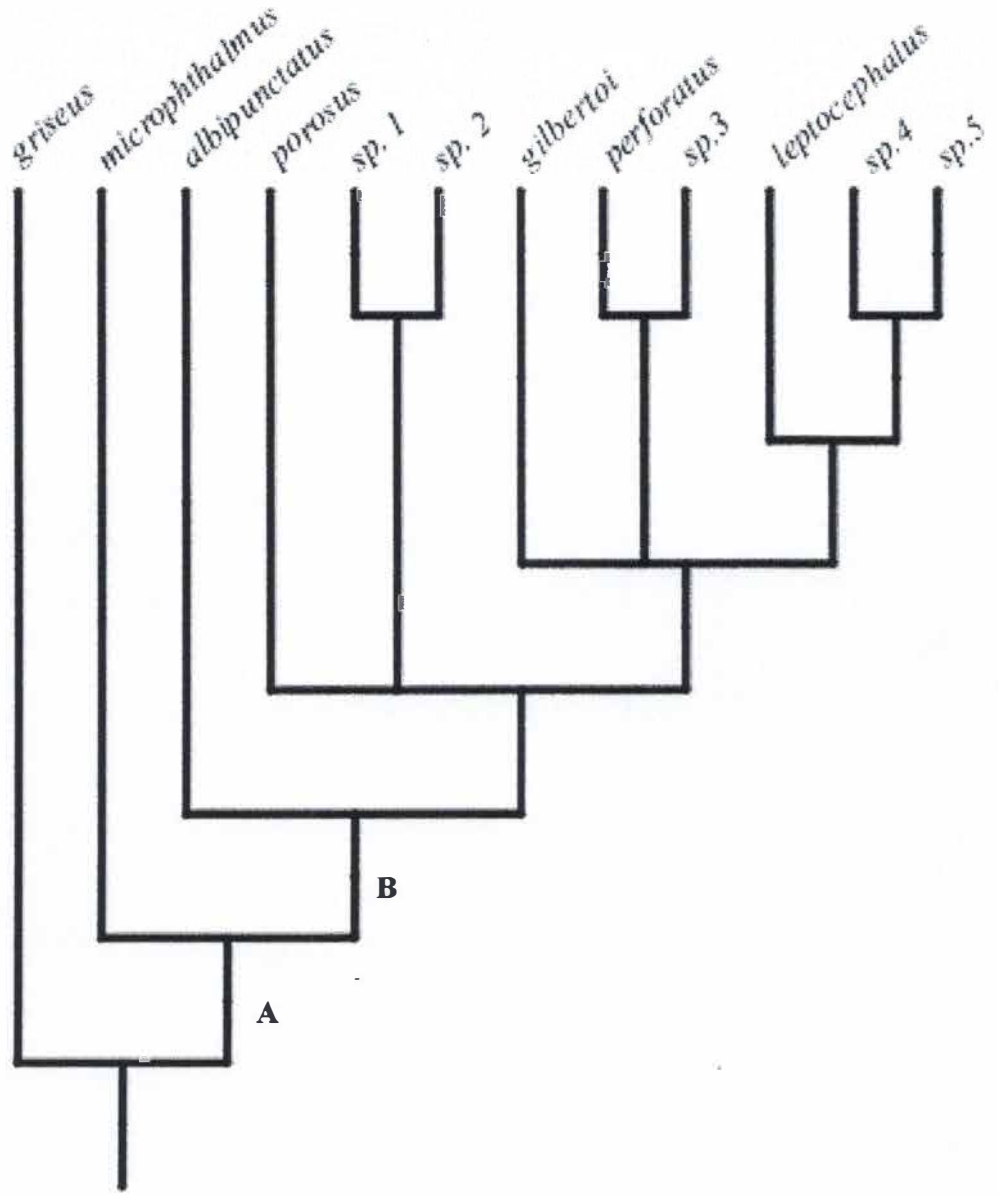

Figura 4 – Árvore filogenética representando a relação entre as espécies de *Cynolebias* (Costa, no prelo a). A e B indicam os braços onde o estado 1 pode ter aparecido além do ancestral comum de todas as *Cynolebias*.

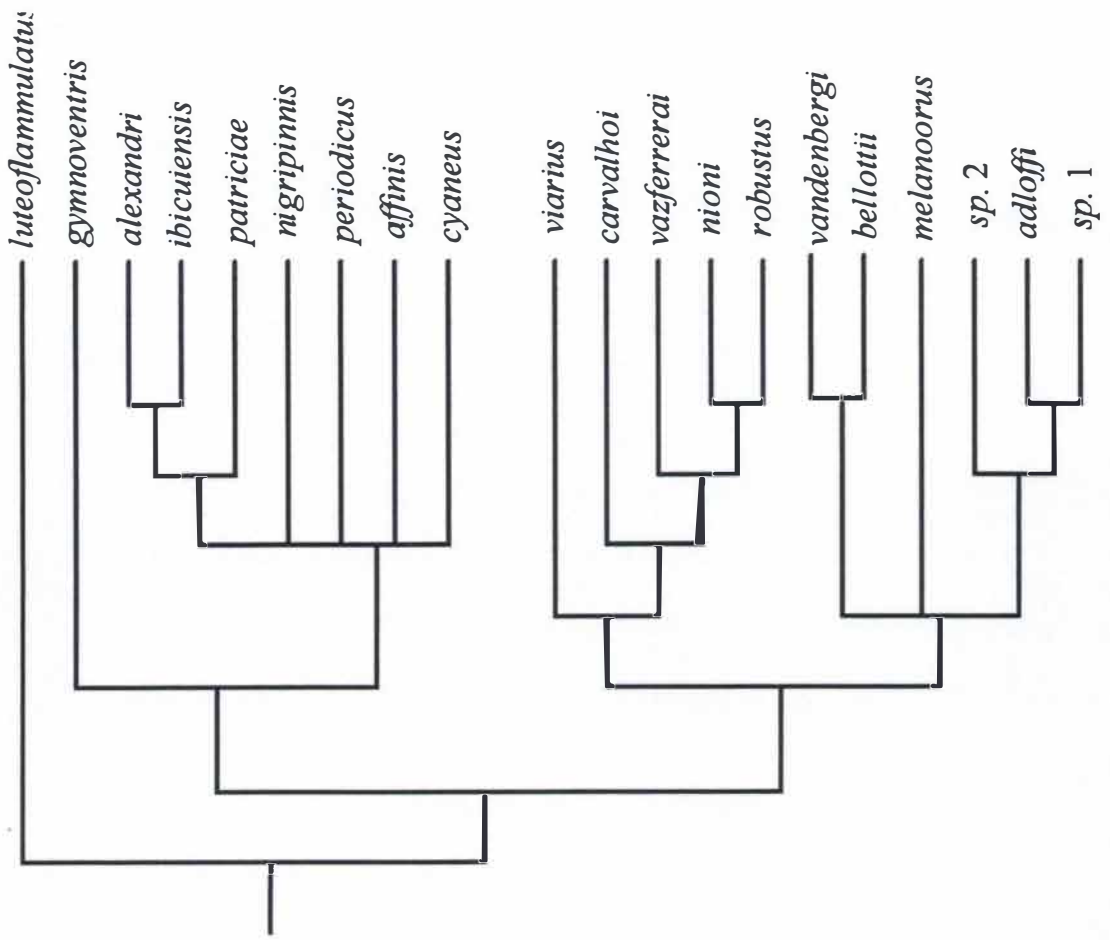

Figura 5 – Árvore filogenética das espécies de *Austrolebias* segundo Costa (no prelo b).

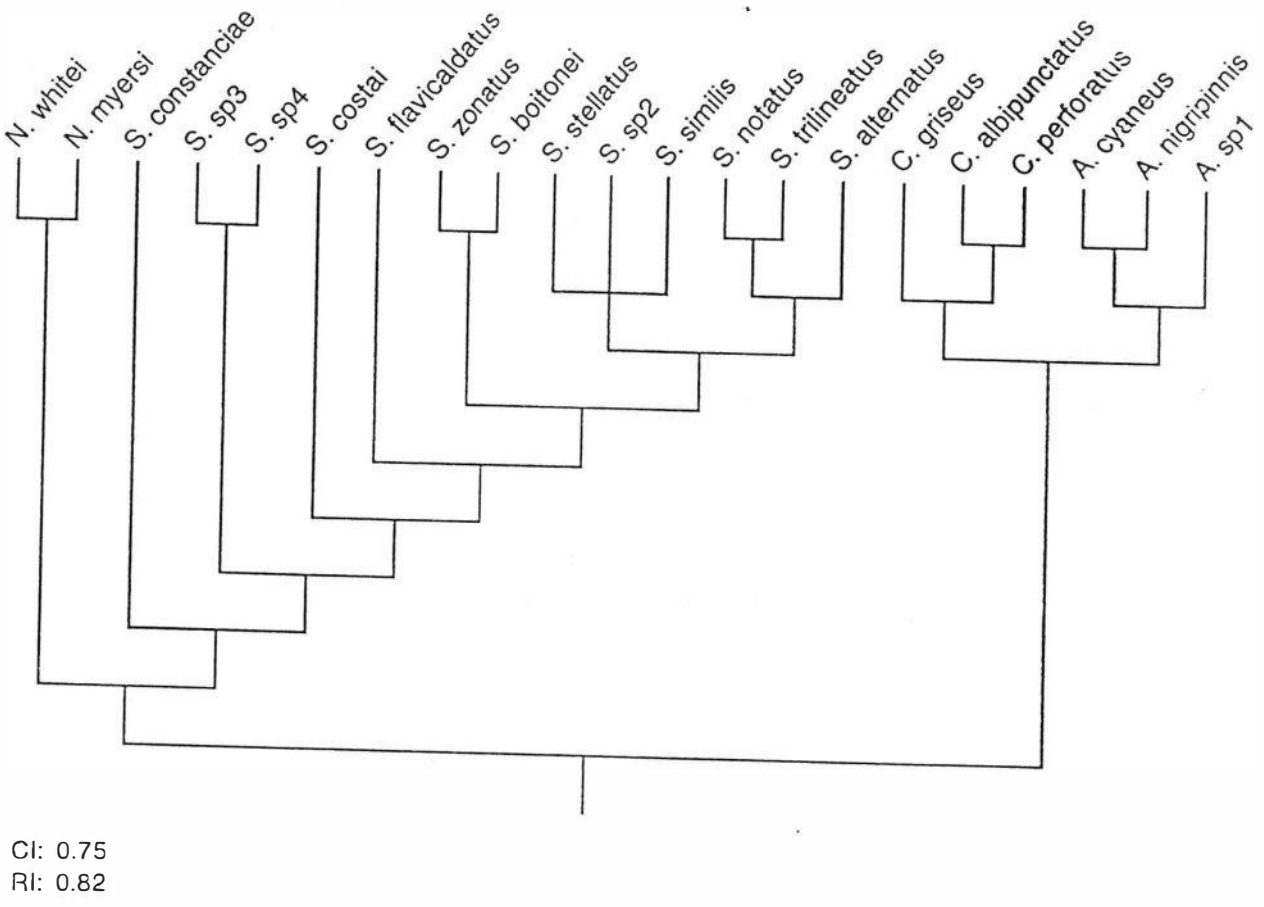

Figura 6 – Cladograma representando a filogenia das espécies estudadas, montada a partir das filogenias propostas por Costa (1996, no prelo a, b). São incluídas as espécies novas segundo orientações de W. Costa, exceto *Simpsonichthys sp.* 1, devido à falta de informação sobre o grau de parentesco com as outras espécies. São incluídas as espécies *S. boitonei*, *C perforatus* e *C. griseus*. O índice de consistência e o índice de retenção são apresentados.

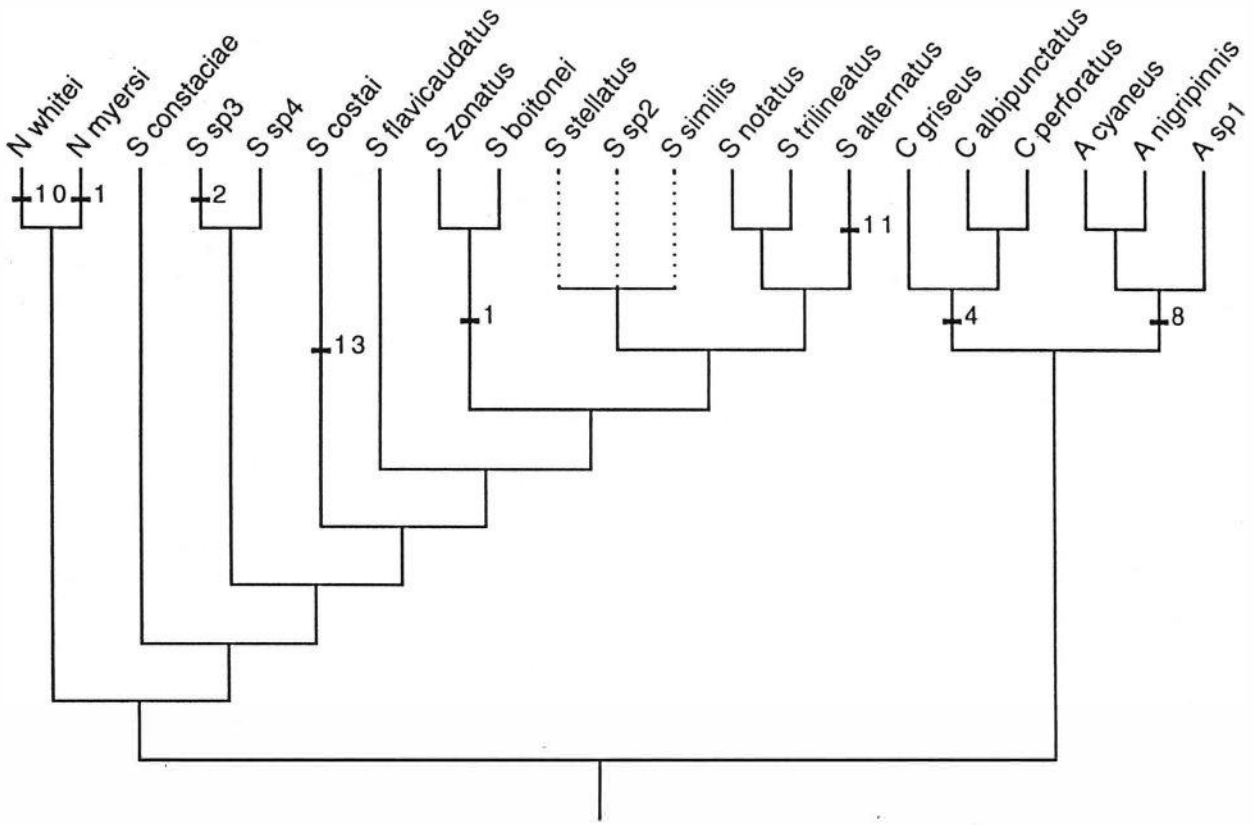

Figura 7 – Indicação dos locais onde ocorreram as mudanças de estado de C1, C2, C4, C8, C10, C11 e C13. O número do caráter é indicado no braço onde a mudança aconteceu.

Caráter 1 - Forma das nadadeiras ímpares dos machos durante a corte.

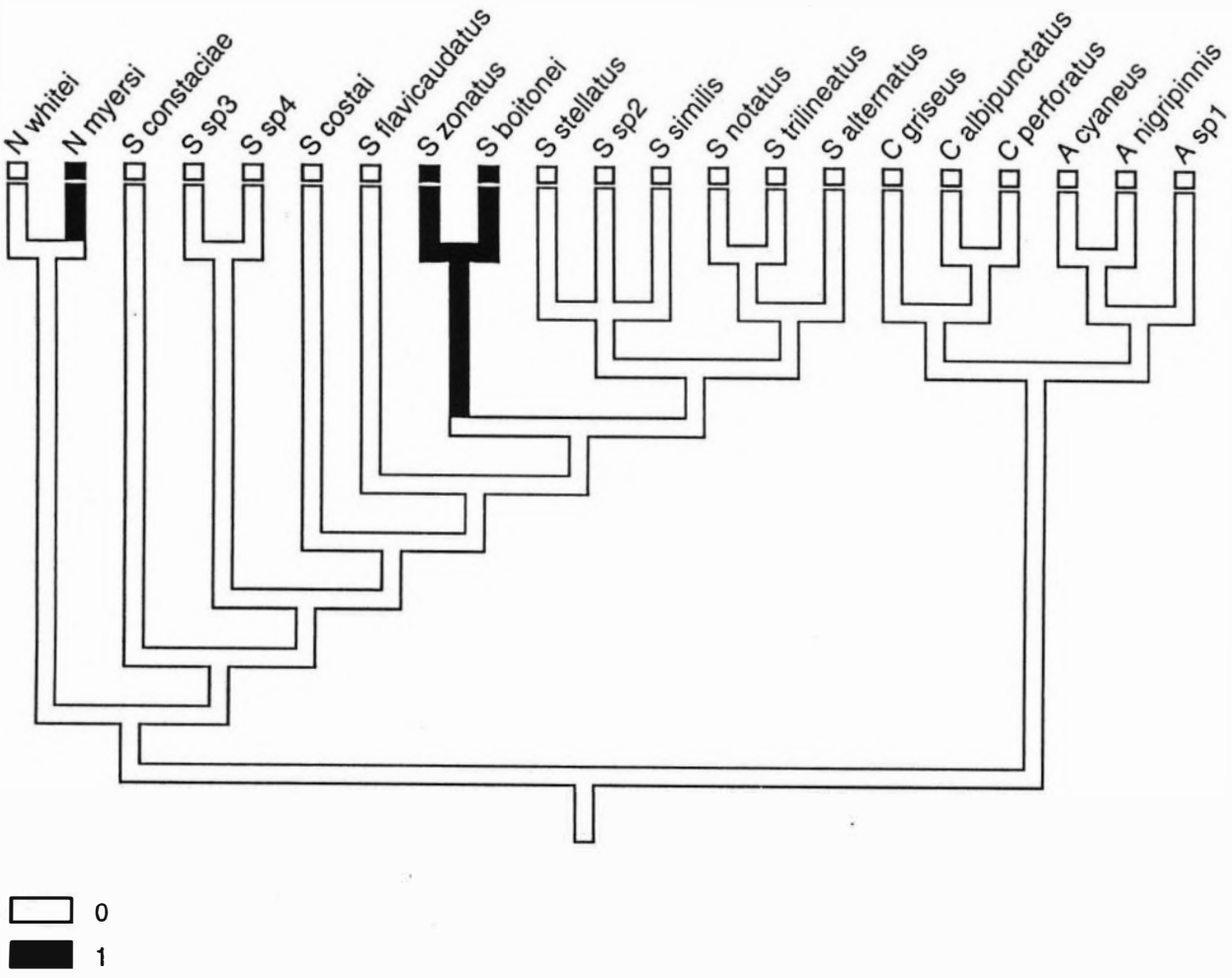

Figura 8 – Cladograma representado a evolução de C1 na tribo Cynolebiatini. Quando a característica é avaliada na tribo como um todo, o estado 1 (em preto) se apresenta como uma homoplasia, mas pode ser uma sinapomorfia de *S. zonatus* + *S. boitonei* quando analisada somente em *Simpsonichthys*. O estado 0 (em branco) é sempre plesiomórfico tanto para a tribo quanto para *Simpsonichthys* separadamente.

Caráter 2 - Convite para mergulhar.

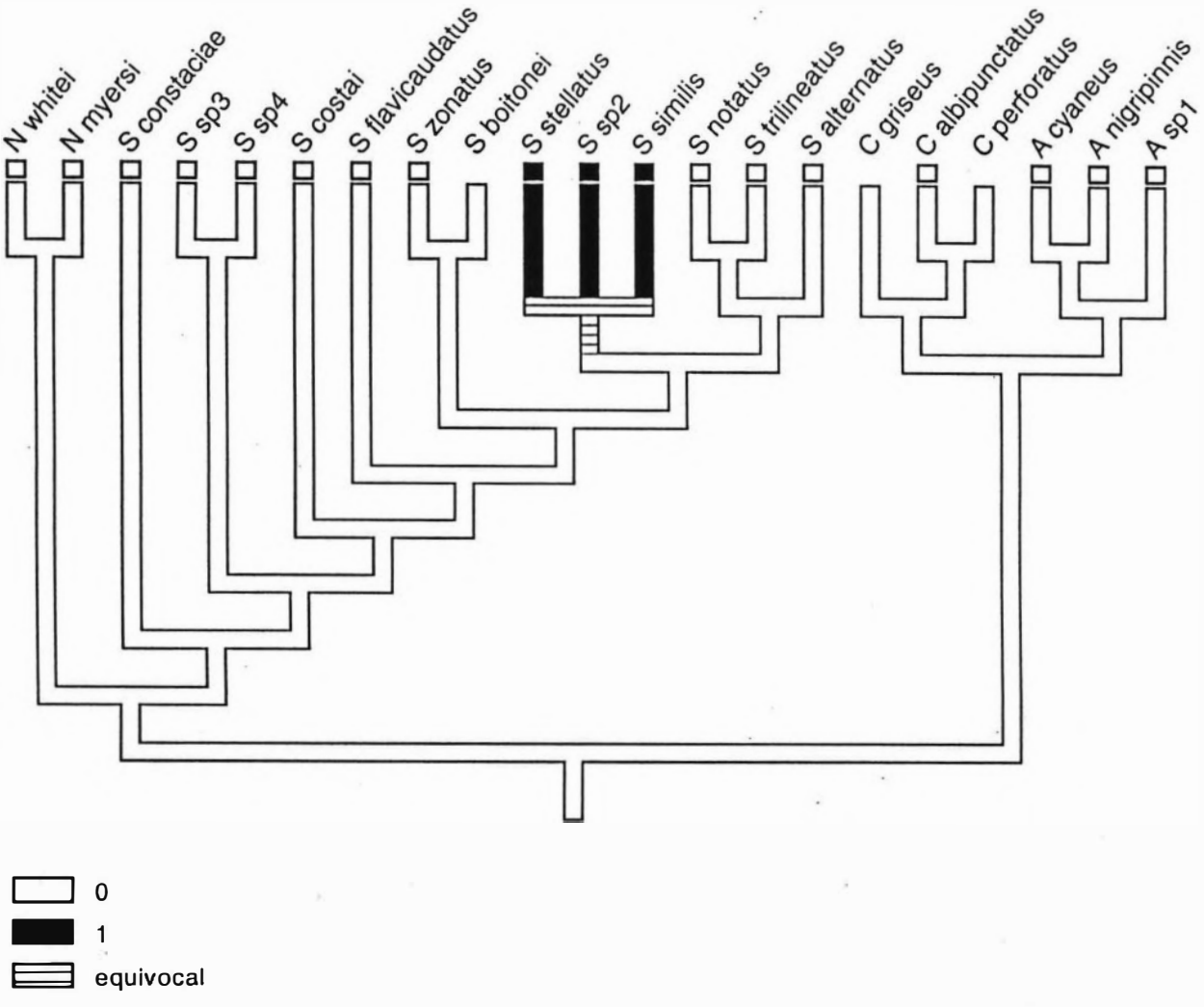

Figura 9 – Cladograma representado a evolução de C2. O estado 1 (em preto) indica uma sinapomorfia de *S similis* + *S. stellatus* + *Simpsonichthys sp. 2.* O cladograma indica dúvidas quanto a o local de origem da mudança.(região listrada).

Caráter 3 - Vibrações durante o convite.

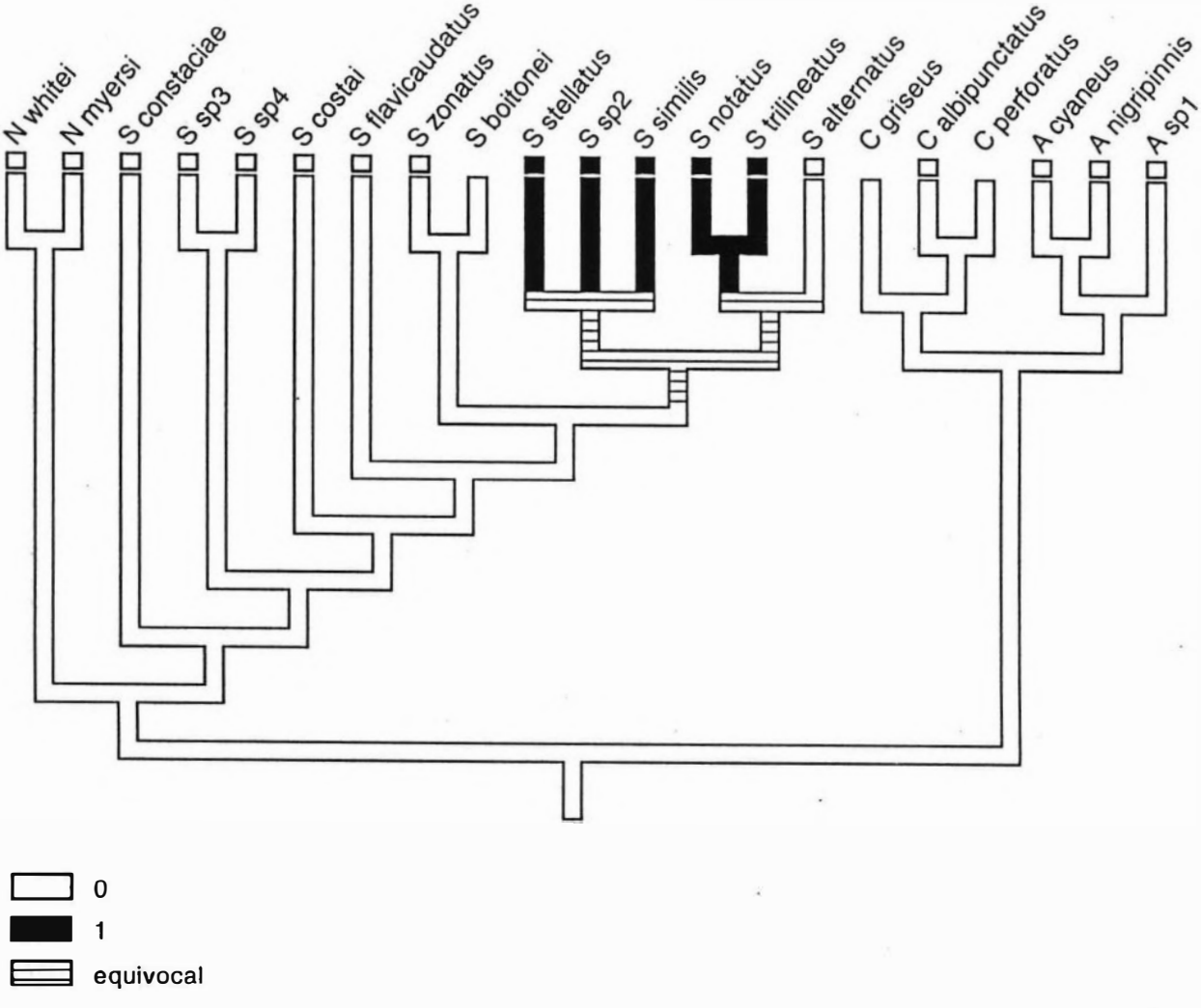

Figura 10 – Representação da evolução de C3. O estado 1 aparece como uma homoplasia em dois grupos distintos dentro de Simpsonichthys. E a origem da mudança de estados aparece duvidosa (região listrada).

Caráter 4 - Apresentação dos movimentos ondulantes laterais.

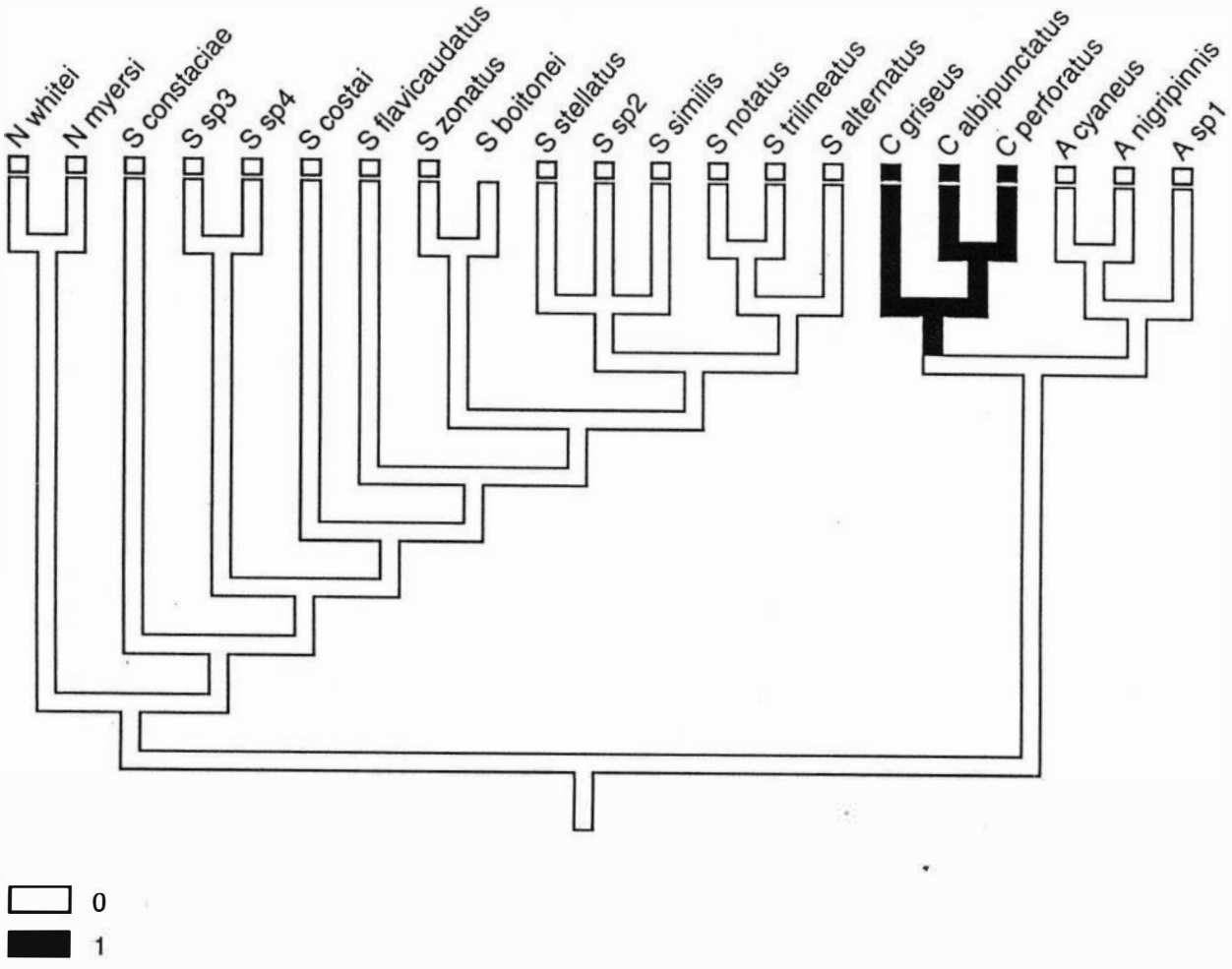

Figura 11 – Evolução de C4, onde a região em preto representa o estado 1 como uma sinapomorfia de *Cynolebias* tendo surgido no ancestral comum as três espécies representadas.

Caráter 5 - Movimentos da cabeça.

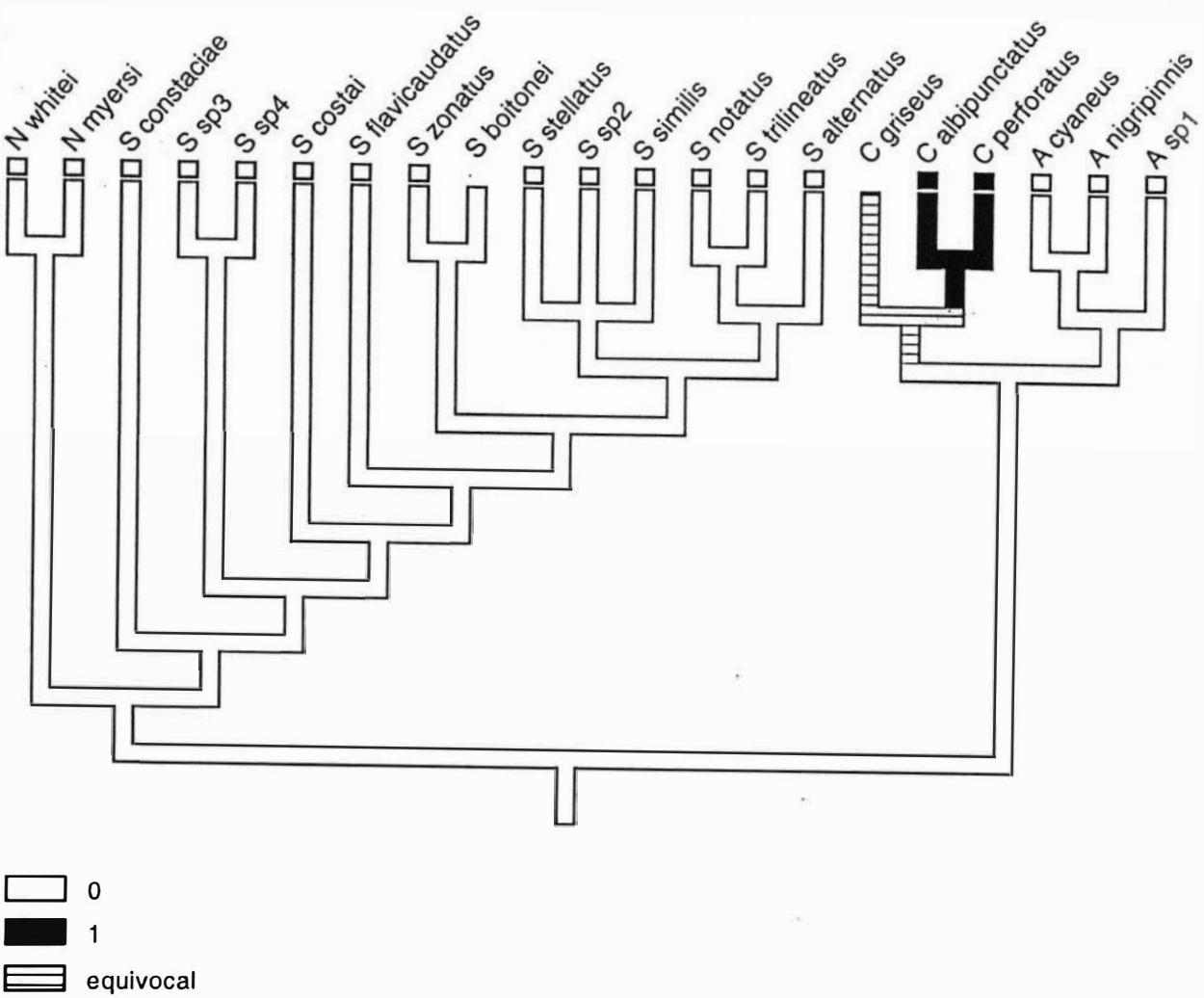

Figura 12 – O estado 1 (em preto) é visto como sinapomorfia de *C. albipunctatus* + *C. perforatus* que *a priori* surgiu no ancestral comum a estas duas espécies já que este caráter não pode ser checado em *C. griseus*. A área listrada indica dúvida, sobre o local correto onde a mudança de estado ocorreu, já que a informação sobre o caráter para *C. griseus* não é disponível.

Caráter 6 - – Designação de uma fêmea dominante.

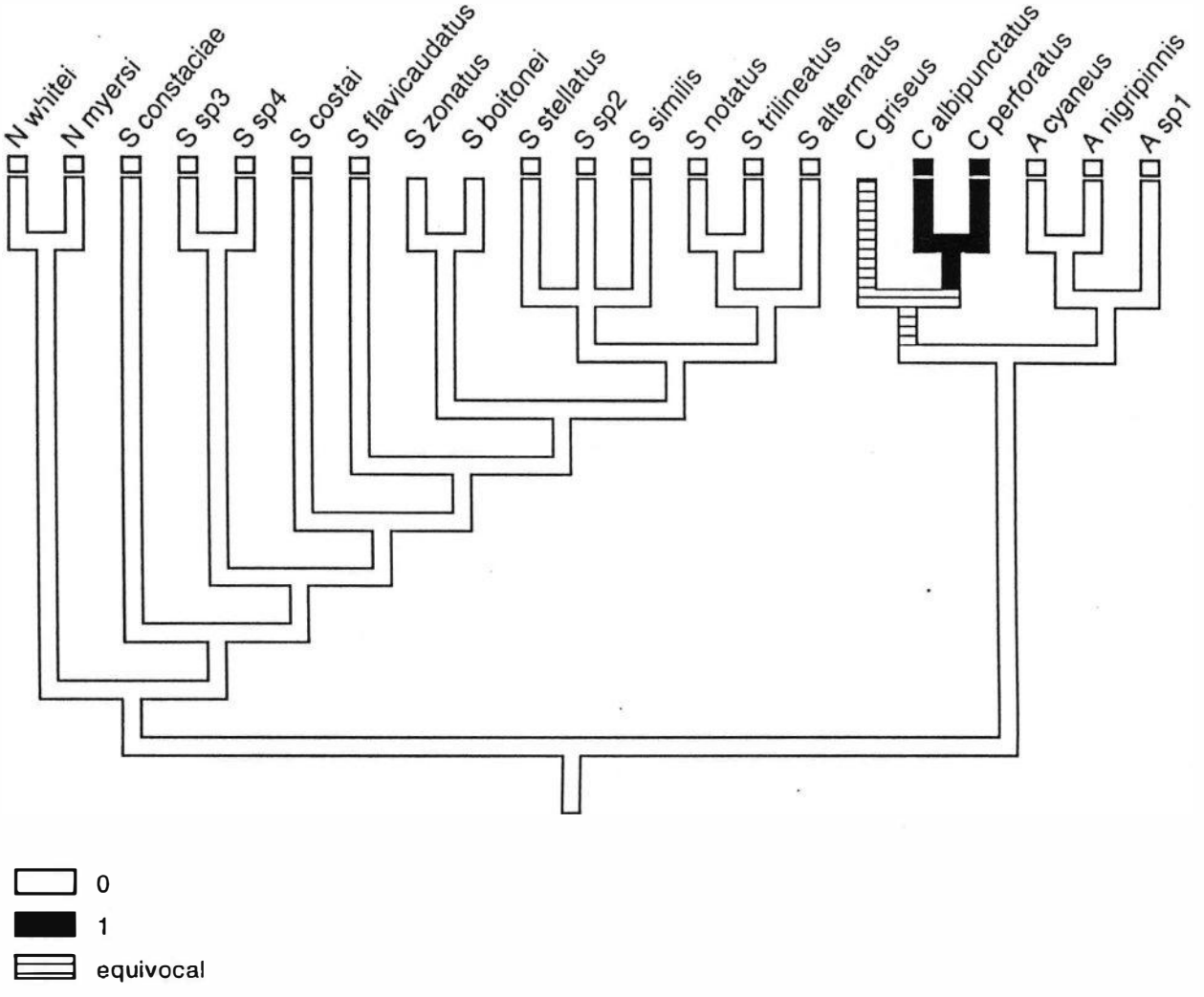

Figura 13 – Como no caráter C5, (Figura 12).

Caráter 7 - Exibições das fêmeas para os machos.

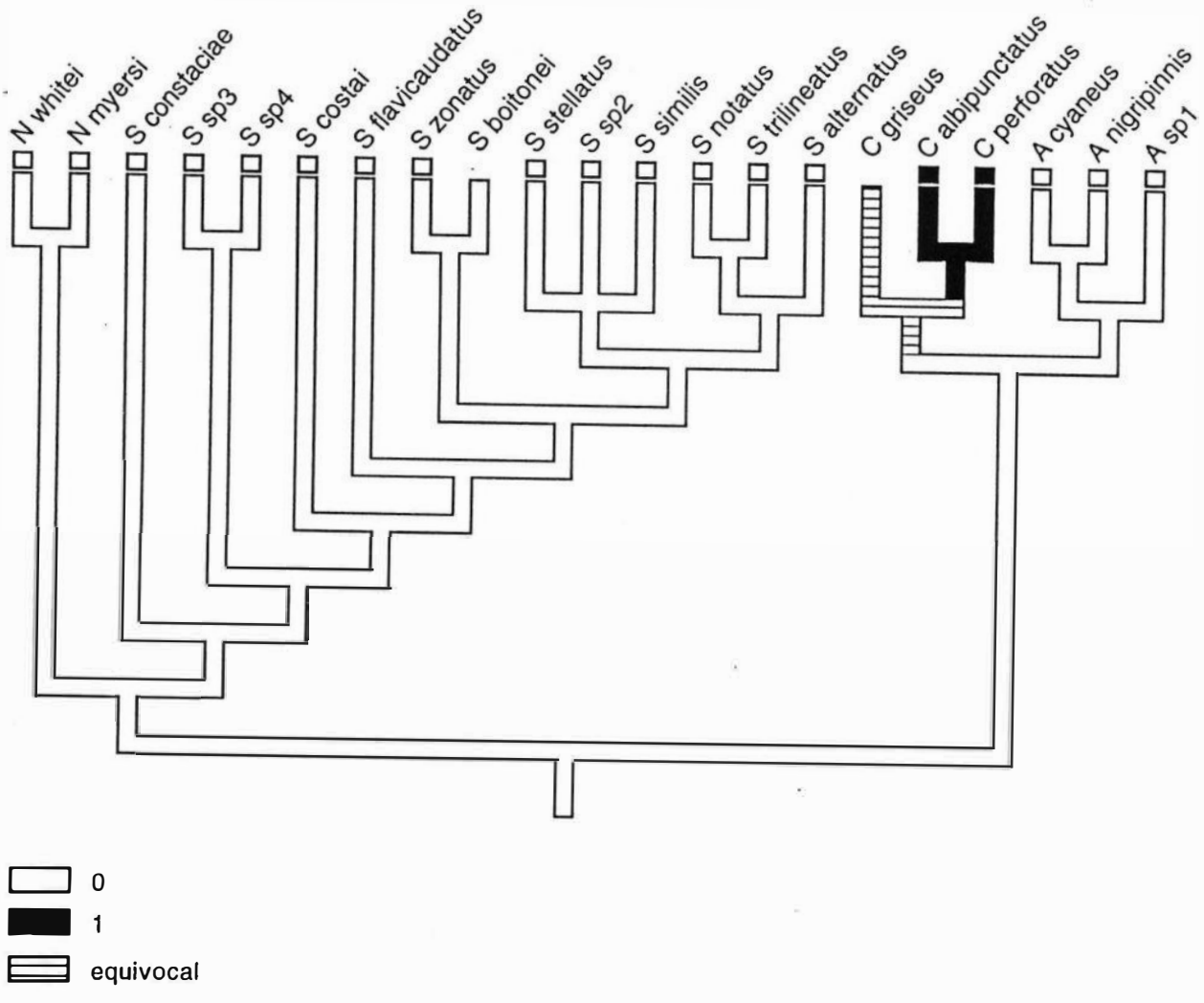

Figura 14 – Como no caráter C5, (Figura 12).

Caráter 8 - Comportamento das fêmeas em relação a outras fêmeas durante a reprodução.

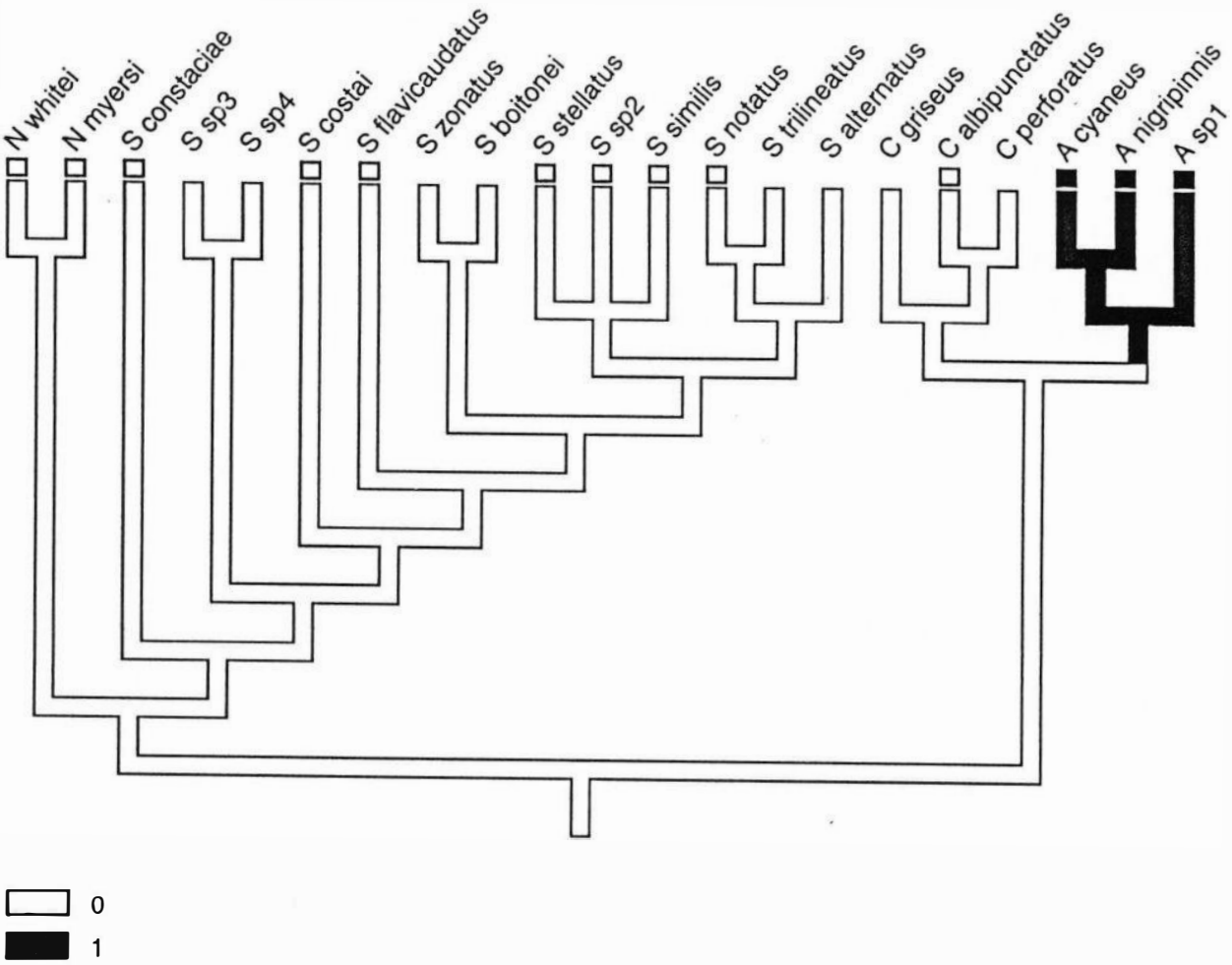

Figura 15 – Evolução de C8. A região em preto (estado 1) indica uma sinapomorfia para as espécies de *Austrolebias*. Para as espécies de *Simpsonichthys sp.* 3, *Simpsonichthys sp.* 4, *S. boitonei*, *S. alternatus* e *S. trilineatus* não se tem informações sobre esta característica.

Caráter 9 - Corte dentro do substrato.

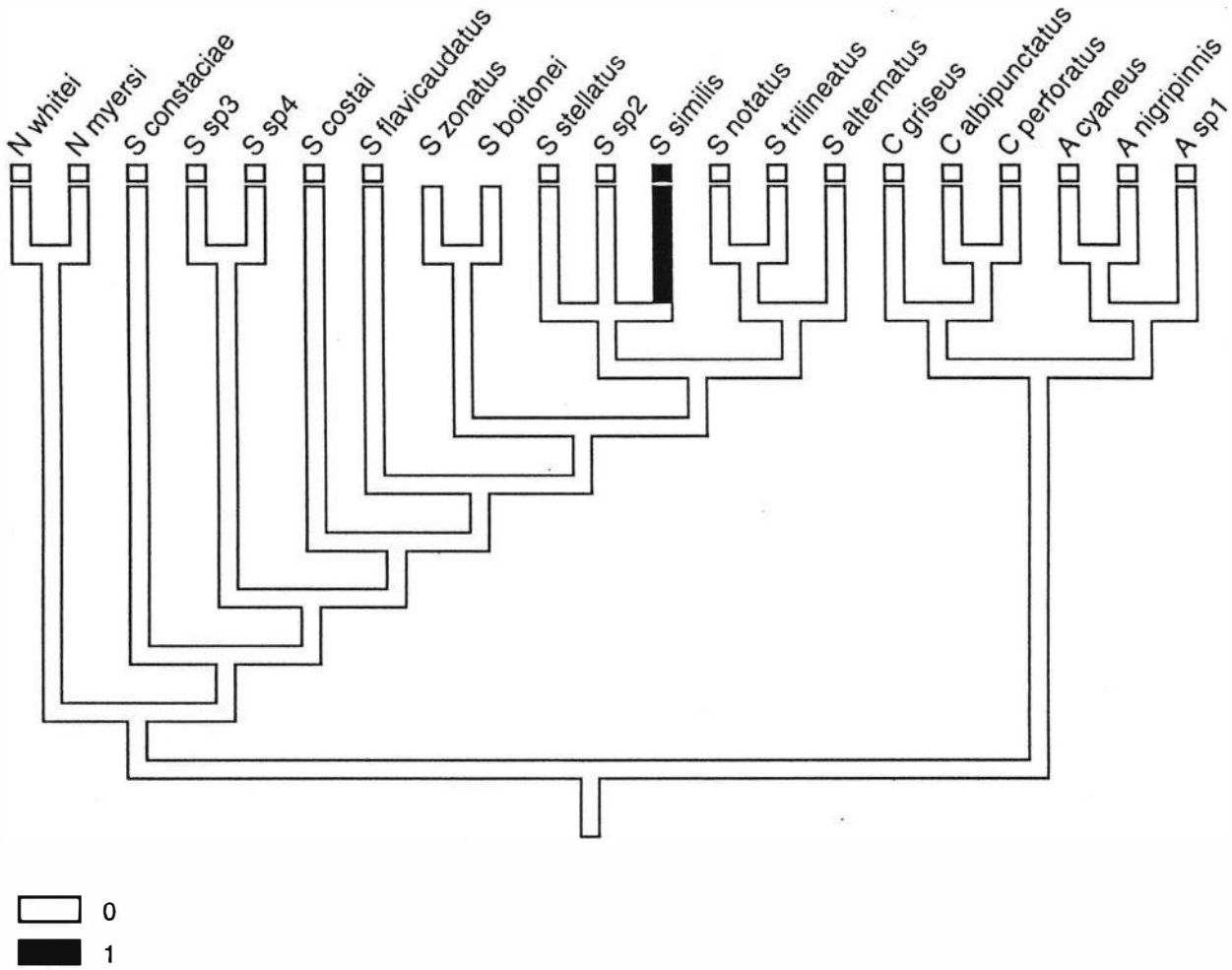

Figura 16 – O cladograma indica uma autapomorfia de *S. similis*.

Caráter 10 - Toques nas fêmeas.

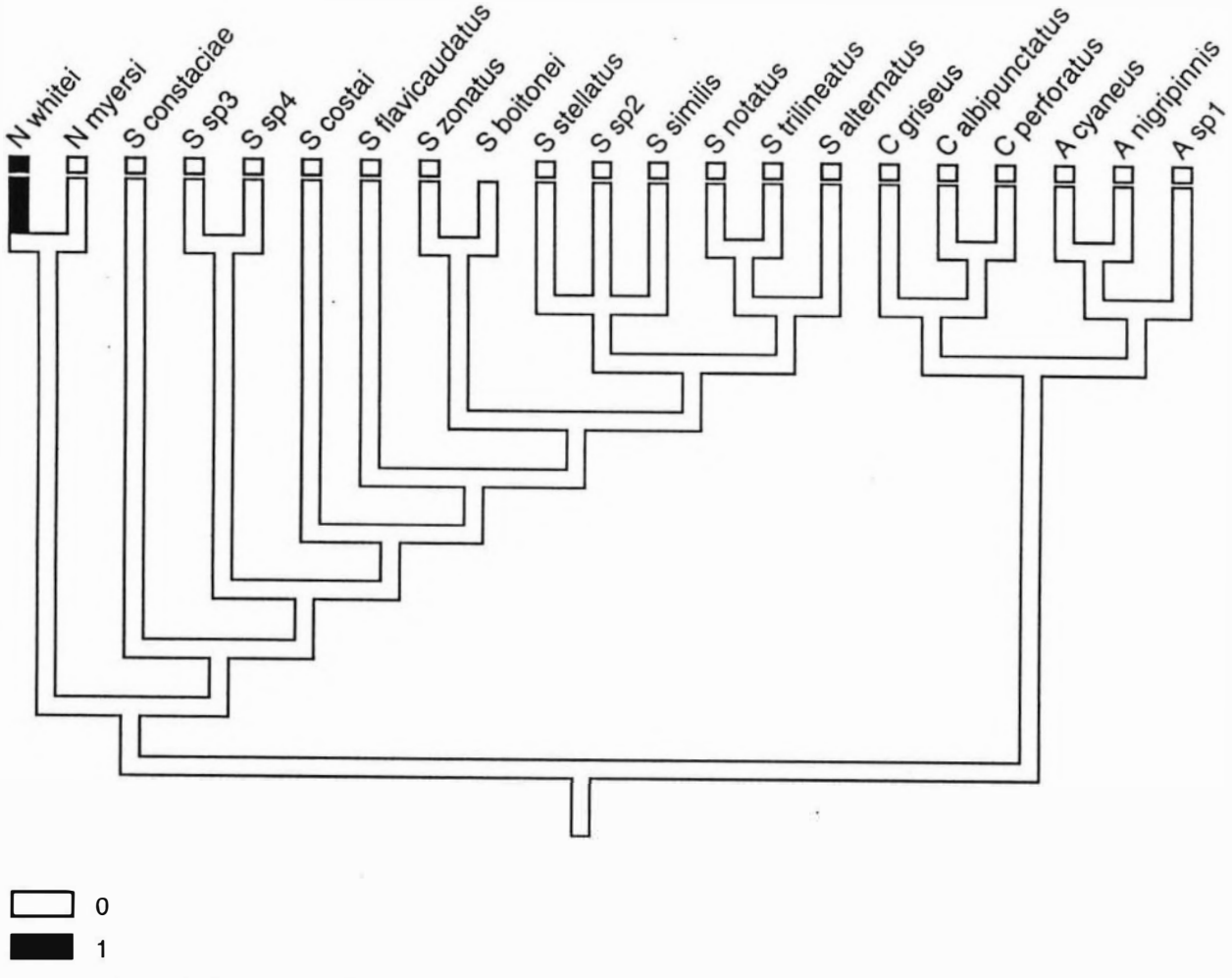

Figura 17– O cladograma indica uma autapomorfia de *N. whitei*.

Caráter 11 - Mergulho no substrato.

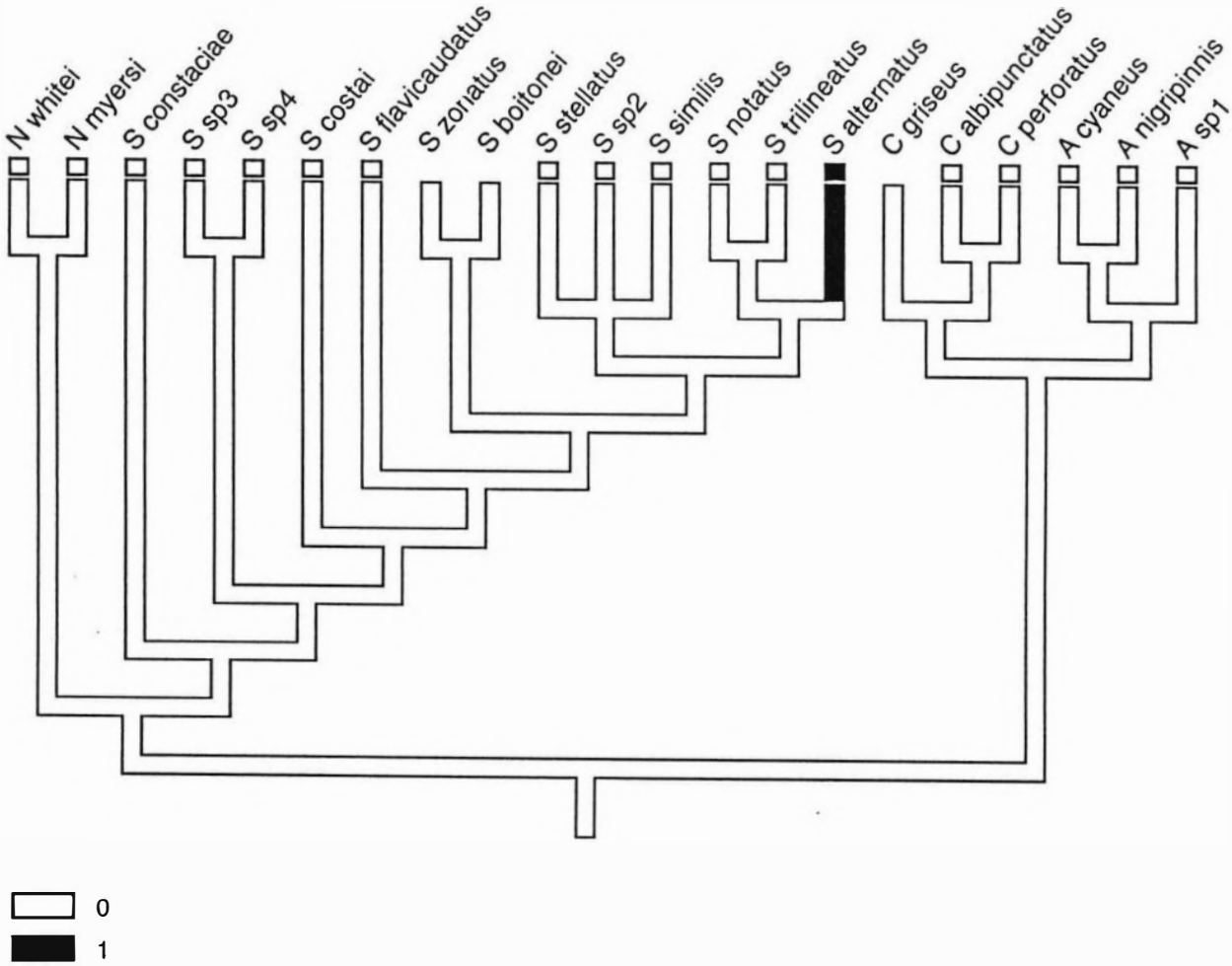

Figura 18 - O cladograma indica uma autapomorfia de *S. alternatus*.

Caráter 12 - Comportamento das fêmeas em relação aos ovos após a desova.

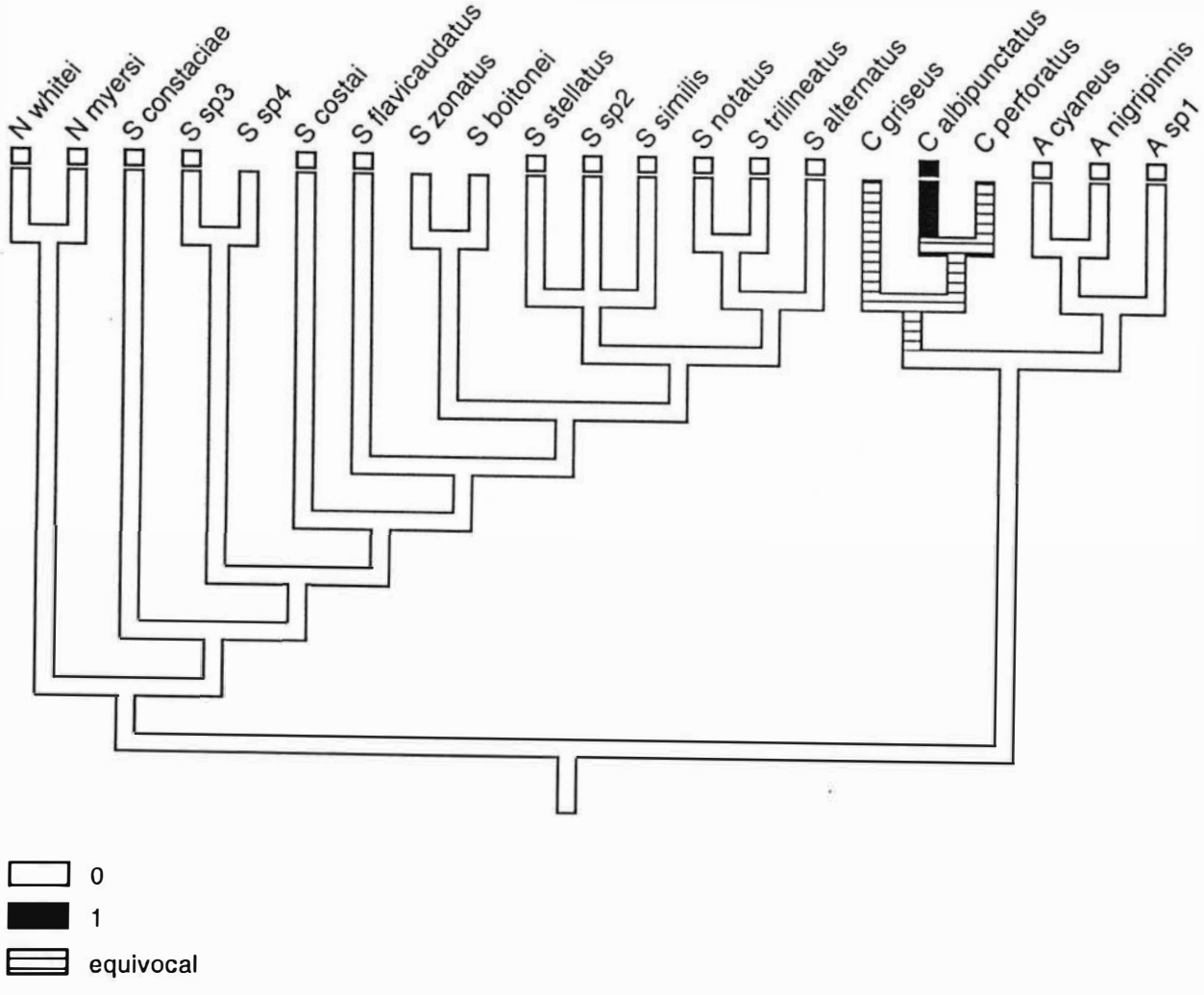

Figura 19 - O cladograma indica uma autapomorfia de *C. albipunctatus*, e como informações sobre esta característica não estão disponíveis para as outras espécies de *Cynolebias*, a região listrada indica dúvidas sobre a real extensão desta apomorfia.

Caráter 13 - Forma da corte apresentada pelos machos.

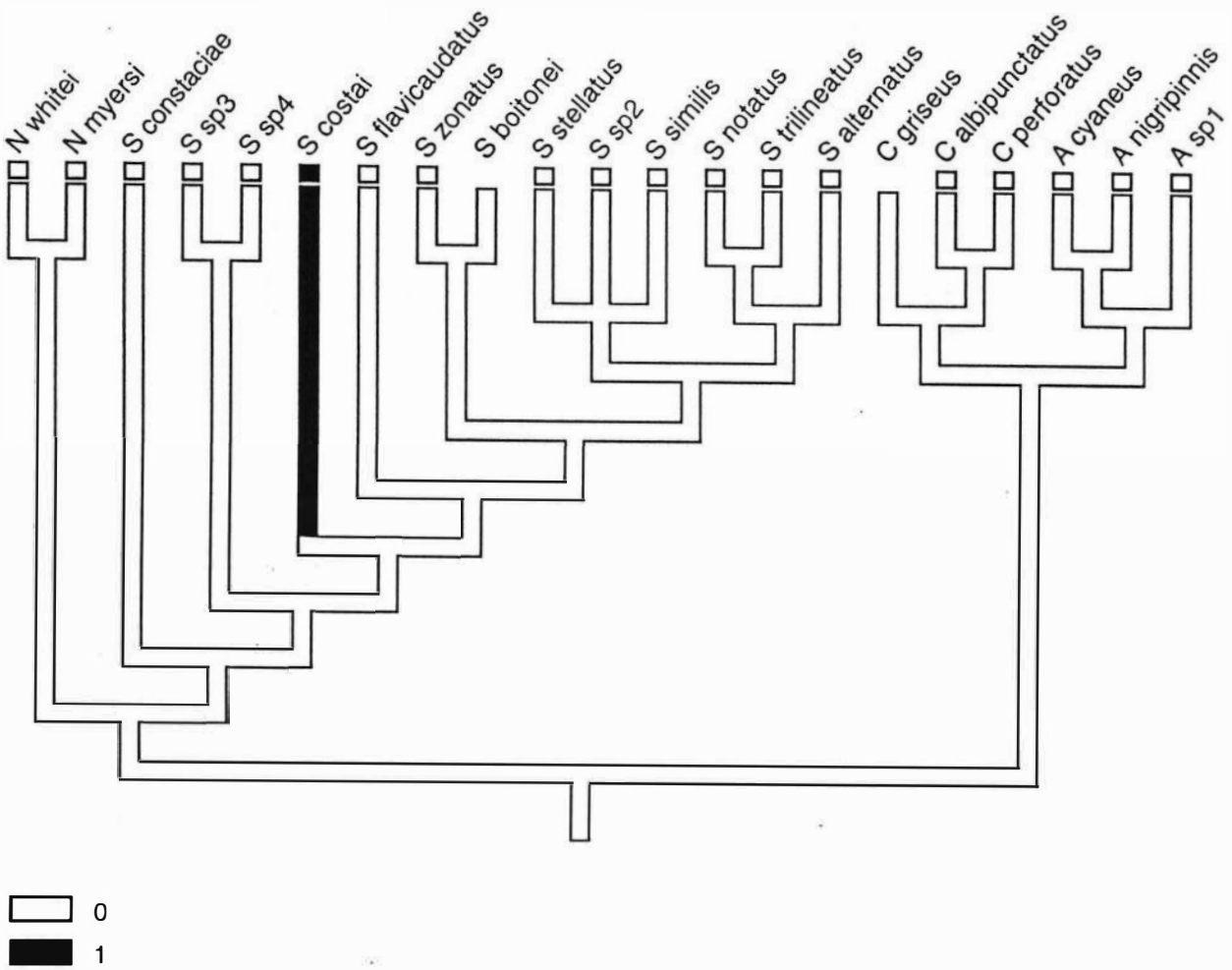

Figura 20 - O cladograma indica uma autapomorfia de *S. costai*.

Capítulo 8: Referências Bibliográficas

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4) **Spawning \ fertilization**: Into the substrate, the male goes over the female, presses her against the bottom (on an average three seconds) with the body curved, occurring the spawning and external fertilization.

5) **Emerging**: After the spawning, they emerge randomly at different times and in different places (Figure 7), and a new courtship cycle begins.

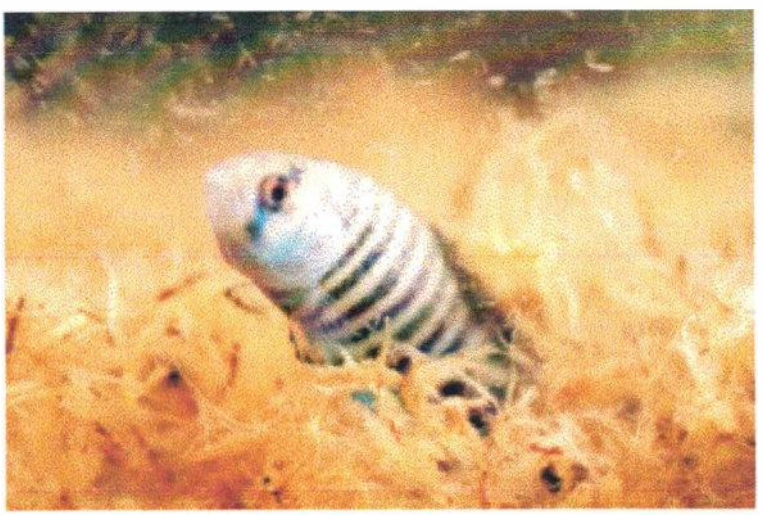

Figure 7 - The male emerging. (Photo by the author)

Discussion

The reproductive behavior of *Austrolebias* follows the patterns of other Cynolebiatini (Belote, 1998). The cycle of spawning behavior can be easily divided into five distinct phases as mentioned above. The males show intense courtship displays like in *Simpsonichthys* (Belote, 1998) but there is no fight among the females, what is corroborated by Belote (1997); Axelroad & Shaw, 1967 like in others (*e.g.*, Carvalho, 1957; Belote, 1998). The absence of fight among the females is a pattern shared among the *Austrolebias* and can be interpreted as synapomorphic for this group, but more species need

to be analyzed to confirm this. Other authors had described that during the matching the female inserts the snout in the axillary angle of the pectoral fin of the male and than they submerge (Carvalho, 1957; Axelroad & Shaw, 1967; Vaz-Ferreira & Sierra, 1973). However, I do not consider this behavior as a pattern, just because it was not clear all the time. It is right that the male needs some incentive to continuous to dig under the substrate, and this incentive might be the female's contact that happens near this area (flanks near pectoral fins). I think that this behavior happens by chance, not necessarily. This behavior is also related to *Nematolebias whitei* (Carvalho, 1957) and I think the same happens, in spite of the fact that species presents large papillae in inner surface of pectoral fins. The presence of these papillae may suggest its purpose of stimulator in the reproductive behavior when the behavior cited above is observed, but when this behavior clearly does not occur and even so the couple digs the substrate until the spawning, the objective of the papillae is still uncertain.

Vaz Ferreira & Sierra performed experiments using heterospecific couples in 1973. They observed males of *A. bellottii* (Steindachner) with females of *A. nigripinnis* and the opposite, males of *A. viarius* (Vaz-Ferreira, Soriano & Paulete) with females of *A. luteoflammulatus* (Vaz-Ferreira, Soriano & Paulete) and the opposite and also males of *Cynopoecilus melanotaenia* (Regan) with females of *Megalebias elongatus* (Steindachner) and reported that the cycle of behaviors was interrupted at any phase by the disinterest of the female and the stop of the displays by the male, without happening the dive through the substrate. The dive only occurred with a female of *M. elongatus* of 150 mm and a male of *C. melanotaenia* of 45 mm. In this study I observed the behavior of a heterospecific couple

with males of *Austrolebias sp.* 1 and a female of *A. nigripinnis* and the phases of the reproductive cycle happened until the end without problems (Figure 8), but I can not confirm the fecundation. Adding a female of *Austrolebias sp.* 1 with the couple, they alternated the spawning and had no fight. Sometimes both females tried to dive at the same time with the male; Kadlec (1990) reported for *A. affinis* a similar case of diving with two females.

Figure 8 – Male of *Austrolebias sp.* 1 coupled with the female of *A. nigripinnis.* The arrow indicates the female of *Austrolebias sp.* 1. (Photo by the author)

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Capítulo 6: Artigo 4

Reproductive behavior of *Cynolebias albipunctatus* (Cyprinodontiformes – Rivulidae)

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Abstract

The reproductive behavior of *Cynolebias albipunctatus* presents a cycle of reproductive behaviors (spawning cycle) with five coincident phases proposed for other rivulids: 1) Courtship displays, 2) Invitation to dive, 3) Diving (to dig the substrate), 4) Spawning \ fertilization, 5) Emerging (comes back from the substrate). Other patterns are shared with several species of annual fish such as: courtship displays with lateral waving movements of the body (in spite of fewer and less intense movements in this species, and they can even become absent) and fins expanded during the waving movements. Some unique behaviors are still observed in this species (comparing to other Cynolebiatini): movements of the head of the male (head-shaking) as a replacement of the lateral waving movements, determination of a dominant female with exclusion of any other by the couple and some exhibitions from dominant female that scrubs her head or the snout in the anal and caudal fins of the male and exhibits brief waving movements for the male. It was also observed the preference of *Sphagnum* as substrate for spawning.

Resumo

O comportamento reprodutivo de *Cynolebias albipunctatus* apresenta um ciclo de comportamentos com cinco fases coincidentes propostas para outros rivulídeos: 1)

"Displays" de corte, 2) Convite para submergir, 3) Submersão (cavar o substrato), 4) Desova\ fertilização, 5) Emersão (volta do substrato). Outros padrões são compartilhados com várias espécies de peixes anuais como: corte com movimentos ondulantes laterais do corpo (apesar de poucos e menos intensos nesta espécie, podendo até deixar de ocorrer) e nadadeiras expandidas durante os movimentos ondulantes. Alguns comportamentos únicos são ainda observados nesta espécie: movimentos articulados da cabeça do macho ("head-shaking") como uma substituição dos movimentos ondulantes laterais, determinação de uma fêmea dominante com exclusão de qualquer outra pelo par e algumas exibições da fêmea dominante que esfrega a cabeça ou o focinho nas nadadeiras anal e caudal do macho e exibe breves movimentos ondulantes para ele. Também foi observada a preferência de desova tendo *Sphagnum* como substrato.

Introduction

Cynolebias is a genus of the family Rivulidae that currently includes seven species (*C. porosus* Steindachner, *C. microphthalmus* Costa & Brasil, *C. albipunctatus* Costa & Brasil, *C. perforatus* Costa & Brasil, *C. leptocephalus* Costa & Brasil, *C. gilbertoi* Costa and *C. griseus* Costa, Lacerda & Brasil), restricted to the northeast and southeast of Brazil (Costa, 1998). Costa (1998) redefined and identified some monophyletic groups within the family, including *Cynolebias*. This genus is diagnosed by a non reduced ventral process of angulo-articular, a long coronoid process of angulo-articular, elongate filamentous rays on the dorsal and anal-fins of males, absence of a transversal suborbital and supraorbital bars and dark brown to black blotches on the dorsoposterior portion of the head (see Costa, 1998). *Cynolebias albipunctatus* Costa & Brasil 1991 was described from the middle São

Francisco river floodplains in Juazeiro, Bahia, northeastern Brazil, and is diagnosed by frontal squamation about 20 small irregularly distributed scales, males reaching at least 60 mm SL (standard length), scales irregularly arranged in longitudinal series, blotches on dorso-lateral region of head, rounded head profile, anal fin base in oldest males 36.0-36.6% SL and body and unpaired fins with conspicuous white dots (Costa & Brasil, 1991). Studies about behavior of *Cynolebias, stricto sensu,* are almost unknown in the literature, in fact represented by a single short (e.g., Costa, Lacerda & Brasil, 1990). In the present study the reproductive behavior of *C. albipunctatus* is described and compared with other rivulids, principally with *C. perforatus*, providing some unique behavior patterns for the genus that can be used to give a better understanding of the evolution of reproductive behavior within the genus or contribute in future phylogenetic hypothesis.

Materials and Methods

Cynolebias albipunctatus was collected in the type locality and maintained in aquaria sizing 100 × 50 × 50 cm, containing gravels, logs and vegetation, besides a smaller aquarium (20 × 15 × 15 cm) with spawn substrate. Two kinds of spawning substrate were used, *Sphagnum sp.* (dry, pricked and boiled) and *Vesicularea dubyana* (natural). A male and two females remained together for three months, during this time they were monitored randomly. The reproductive behavior was recorded in view to provide a more detailed description of the behavior, producing six hours of tape recordings. The description was based on a male measuring 11,5 cm and two females measuring 8,5 cm each. Other rivulids data sets were obtained from the literature and by personal communication of breeders. The

studied specimens were deposited in the Laboratório de Ictiologia Geral e Aplicada da Universidade Federal do Rio de Janeiro (UFRJ).

Results

Reproductive behavior.

1) **Courtship displays**: The male presents courtship displays with few and slow lateral waving movements of the body (comparing with other rivulids) showing the fins expanded during it. This behavior can be absent for a long time, or can be present only at the first meeting of the couple. The female remains near the male, following him through the aquarium. The female also presents some exhibiting behaviors; she often scrubs her head or snout in the anal and caudal fins of the male (Figures 1 and 2) and, not so frequently, exhibits brief and subtle waving movements. When more than one female is present, one of them becomes the dominant, and the other one is excluded by the dominant one and by the male. The exclusion is characterized with the disinterest of the male and some aggressive behaviors by the couple against her (chases and bites), but specially by the dominant female which shows courtship-like aggressive waving movements and swims toward the other with the body "S"-shaped and expanded opercle then bites and chases the other one that gets out.

2) **Invitation to dive**: The male swims near the bottom trying to find a place to dive, always followed and scrubbed by the female. He puts his head into the substrate (or tries to do it in the gravels when *Vesicularea dubyana* was used as spawning substrate) making an angle between 30° and 90°, and vibrates the body.

Figures 1 and 2 – The female of *C. albipunctatus* scrubbing the head in the anal fin of the male. (Photos by the author).

3) **Diving**: The female couples him at one of his sides. So, both dig under the substrate together by vibrating their bodies.

4) **Spawning \ fertilization**: Into the substrate, the male goes over the female, presses her against the bottom causing the spawn and external fertilization. Two kinds of spawning substrate were used, but only the *Sphagnum* was approved. Using *Vesicularea dubyana* or without spawning substrate, this phase happens over the gravel, which provides more details. The male tries to put the head through the gravel to pick a site to dive and the female, which is following, couples him (figure 3). They try to dive by vibrating the body together, and after many unsuccessful attempts, the male lies over her with the body curved, with the fins a little folded against her, and presses against and vibrates the body with an open mouth (figure 5). After the spawning, the female looks for the eggs to eat them.

5) **Emerging**: After the spawning into the substrate, they emerge randomly at different times and in different places. So, the female continues to follow the male until a new cycle

begins, and in this new cycle the first stage can be absent. They can remain spawning for two days long without interruption and for more than a week without spawning.

Figure 3 - When the spawning substrate is absent, the male looks for a place to invite and submerge in the gravels while is followed and scrubbed by the female. (Photo by the author)

Figure 4 – The male of *C. albipunctatus* trying to lie over the female. (Photo by the author).

Figure 5 – When the male is pressing the female he often opens his mouth. (Photo by the author)

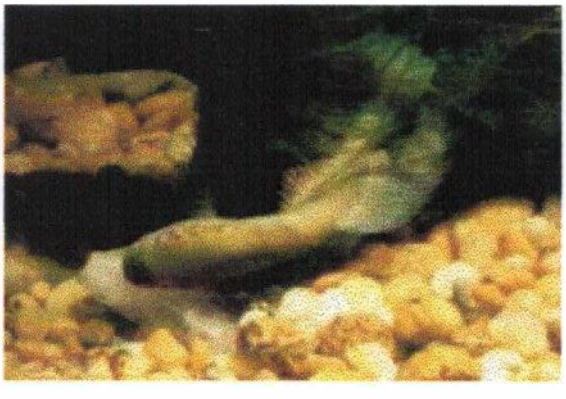

Figure 6 – Male over the female after the pressing. Photo by the author.

Discussion

The reproductive behavior of *C. albipunctatus* presents some different patterns if compared with other rivulids, but it seems to be exactly the same of *C. perforatus* (Carlos da Silva, personal communication). Thus, in both species the males present courtship displays with few and slow lateral waving movements, but only during the first spawning cycles after the initial meeting. In the whole of studied rivulids species, the males show the courtship displays with quickly lateral waving movements happening in each spawning cycle (e.g. Belote, 1998; Vaz-Ferreira & Sierra, 1973; Costa, Lacerda & Brasil, 1990), but slow waving movements are also related to *C. griseus* Costa, Lacerda & Brasil. These waving movements seem to be replaced by another behavior, the head shaking, (both happening in the phase 1 and apparently serving to attract the female). In this trait observed in *C. albipunctatus* and *C. perforatus* the males shake the head up and downwards for a second. When the head shaking happens, the females approach the male, as when the waving movements happen.

The females of the Rivulidae are often restricted to observe courtship displays and to follow the males to spawn if they are ready to do it, except the female of *Pterolebias longipinnis* Garman that picks the site to dive instead of the male (Foersch, 1956). However, the females of *Cynolebias* scrub the head or snout in the anal and caudal fins of the male, what might mean that she is inviting him to spawn or indicating that she is ready; and not so frequently exhibits brief and subtle lateral waving movements to him. After the spawning, the male leaves the female, and she looks for the egg to eat. This behavior was never related for other rivulids.

As it was reported about some of the other rivulids, when more than one female is present, they can fight like in *Simpsonichthys whitei* (Carvalho, 1957), they can be indifferent to each other like in *Austrolebias cyaneus* (Belote, 1998) and *Austrolebias sp.* 1 or even the two females try to dive with the male at the same time, one in each side like in *Austrolebias nigripinnis* (personal observation). In *Cynolebias* a dominant female is selected, this one jointly with the male chases and bites the other, impeding the approach thereby only the dominant female spawns. In no other rivulids the male excludes a female.

Between the two spawning substrates used with *C. albipunctatus*, only the *Sphagnum* sp. was accepted, using *Vesicularea dubyana* they preferred to try to dig under through the gravels, and unable to do this, the spawning happened over it. So, it could be observed that the spawning substrate is not a limiting factor to the spawning of this species, since they can do it over the gravels, for example. Other rivulids also do not have problems with the absence of a spawning substrate; *Nematolebias whitei* and *Simpsonichthys constanciae* can spawn over the glass aquarium bottom (personal observations) and *Austrolebias bellottii* until over the palm of the hand (Foersch, 1956).

The presence of the female's behaviors (scrubbing, egg eating and displays of lateral waving movements), the decrease and replacement of the lateral waving movements of male by the head-shaking and the male avoiding the excluded female can be considered, *a priori*, as synapomorphic for *Cynolebias* until new studies of the other species are done.

The whole data indicate that there is no effort of the male in being courting the female as it happens to the other related species, but that the male only exhibits himself at the beginning (moment when they meet for the first times) and he substitutes the exhibition of waving movements for head-shakings. On the other hand, the dominant female plays the

role of stimulating or inviting or simply informing the male that she is ready for the spawning, besides preserving her partner far from other females. Seemingly, all the observed innovations at the level of the reproductive behavior for *C. albipunctatus* are shared with *C. perforatus* (Silva, personal communication), but in spite of not doubting about the truthfulness of the information about this last one, it is necessary to analyze this species under the same conditions that were made for the *C. albipunctatus*.

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