PRIMARY RESEARCH PAPER

The remarkable species complex *Mimagoniates microlepis* (Characiformes: Glandulocaudinae) from the Southern Atlantic Rain forest (Brazil) as revealed by molecular systematic and population genetic analyses

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Abstract The aim of the present study is to test by molecular DNA data a hypothesis concerned to speciation by allopatry occurring in *Mimagoniates microlepis*, associated to the Serra do Mar mountain chain (Atlantic Rain Forest hotspot) in Southern Brazil. Overall genetic diversity and mean genetic distances were high, demonstrating both good conservation status and genetic differentiation. Neighbor-Joining (NJ) and parsimony analyses, together with population genetic parameters (Φ_{ST}, N_m, G_{ST}, and AMOVA), identified two main vicariant genetic/evolutionary stocks dividing the upper Iguaçu River samples from those of the coastal plains. Other well-supported intrinsic monophyletic clades were also identified, suggesting fast and remarkable speciation processes. In addition, the genetic, evolutionary, geographic, and phylogeographic evidences reinforced an occurring species complex. Moreover, these evolutionarily significant units (species complex) seem to be inside four natural biogeographic areas. Thus, the genesis and evolution of the Serra do Mar complex might be associated to diversification processes of *M. microlepis*. Such a consideration suggests that the areas including the upper Iguaçu River and the coastal plains of the states of São Paulo, Paraná, and Santa Catarina require distinct conservation policies involving one of the global biodiversity hotspots, namely, the Brazilian Atlantic Rain Forest.

Keywords *Mimagoniates microlepis* · Molecular systematics · Neotropical fish conservation · Neotropical fish diversity · Neotropical fish genetics

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Introduction

The Brazilian Atlantic rain forest is one out of the 25 biodiversity hotspots worldwide. It has lost around 70% of its original plant covering, but still hosts about 60% of the total terrestrial species and has the greatest richness of freshwater fish occurring on the planet (Menezes, 1996; Myers et al., 2000; Mittermeier et al., 2004; Galindo-Leal & Câmara, 2005;

Martini et al., 2007). As the most important biological component for species preservation is their genetic variability and the historical processes that sustain it (Moritz 2002), evolutionary studies on both intra-specific and/or assemblage relationships should be a primary activity for conservation programs.

Molecular markers may yield interesting evolutionary patterns, such as relationships between congeneric species or populations of a species. These genetic characters can provide additional tools for understanding taxonomy, cryptic species phenomena, and population isolation (Torres et al., 2004b; Paiva et al., 2006; Sofia et al., 2006; Lakra et al., 2007). Specially, historical genetic connectivity approaches contribute to a wide-range of ecology and evolution studies, such as the identification of evolutionarily independent regions, and provide evolutionary/geographic contexts for populations and species, thereby contributing to the understanding of both spatial and historical influences over ecological assemblages (Ricklefs & Schluter, 1993; Bermingham & Moritz, 1998; Avise, 2000). Yet, dominant DNA markers have been used as the basis for delineating fish populations and species identification (Brahmane et al., 2006; Sofia et al., 2006; Lakra et al., 2007).

Along the Atlantic Rain Forest, studies on historical genetic connectivity have revealed a bridge between the Amazon and Atlantic Rain Forests based on the patterns of small mammals (Costa, 2003), subdivisions of the Brazilian pit viper (*Bothrops jararaca*) into two monophyletic lineages (Grazziotin et al., 2006), subdivisions of the Brazilian lizard *Gymnodactylus darwinii* into three clades (Pellegrino et al., 2005), and population genetic structuring in blue manakin (*Chiroxiphia caudata*) along a continuous habitat even in the absence of geographical barriers (Francisco et al., 2007). Studies on genetic connectivity have also been carried out involving evolution of large aquatic areas, including studies on fish from the northern boundaries of North America (Bernatchez & Wilson, 1998) and Central and South America (Bermingham & Martin, 1998; Montoya-Burgos, 2003; Perdices et al., 2002) as well as on the diversification of Atlantic reef fish (Rocha et al., 2005) and ring speciation among *Allostomus* spp. (Bowen et al., 2001). In general, all these studies, such as the present research, highlight the relevance of combining molecular DNA markers with geographic evidence for discerning on the limits of

populations and species as a function of their environment histories.

The Serra do Mar formation is a prominent 1000-km mountain chain parallel to the Brazilian coastline and covers a large part of southeastern and southern Brazil, reaching heights of 1800 m (Almeida & Carneiro, 1998). According to Asmus & Ferrari (1978), this mountain chain formed as a consequence of vertical tectonic processes occurring in the Cenozoic era. Based on its physiographic pattern, this mountain complex seems to be a divisor between continental and coastal freshwater courses. This allows the investigations into formation of the Serra do Mar chain as a possible geographic barrier to genetic flow between populations of *Mimagoniates microlepis* from Iguaçu River headwaters (located on the inland border of the Serra do Mar chain) and those from the coastal plains.

Mimagoniates microlepis is one out of the seven species of the genus, in which it is the sister taxon of *Glandulocauda*. The genera *Mimagoniates*, *Glandulocauda*, and *Lophiobrycon weitzmani* compose the Neotropical freshwater subfamily Glandulocaudinae that comprehends fishes with a hypertrophied caudal-fin gland derived from modified "club cells" for pheromone synthesis (Weitzman et al., 2005). *M. microlepis* is widely distributed from the northern portion of the state of Espírito Santo to the northwestern portion of the state of Rio Grande do Sul (Weitzman et al., 1988). In the state of Paraná also (southern Brazil), the species occurs throughout the main course and headwaters of the Iguaçu River. Very recently, the species was also found in the headwaters of the Tibagi River (Sant'anna et al., 2006).

On the basis of its wide distribution *M. microlepis* not seems to be an endangered species. However, wide distributions and/or species cohesiveness not seem to be related to genetic variation (conservation status) (Vendramin et al., 2008; Thuesen et al., 2008). Thus, such as the focus of the present research, to diagnose the degree of genetic variation in a species, and how such putative variations are spatially related should be a prerequisite for preserving the biota and its environments.

In genetic terms, *M. microlepis* exhibits conservation in diploid number (52 chromosomes). Contrarily, striking differences in the chromosome formula as

well as the number and location of ribosomal cistrons have been documented by comparing samples from the southern and southeastern coastal plains with those native to Iguaçu headwaters (Guimarães et al., 1995; Torres et al., 2008). It is presumed that variations in the number and location of ribosomal cistrons (nucleolar organizer regions—NORs) are diagnostic markers for species level (Galetti et al., 1984; Amemyia & Gold, 1988; Venere & Galetti, 1989; Torres et al., 1998, 2004a, b).

The occurrence of geographic barriers may isolate populations, thereby fixing genetic variations from generation to generation. Evidence of genetic discontinuities in *M. microlepis* associated with geographic isolation may be a sign of the occurrence of evolutionarily significant units, as mentioned by Fraser & Bernatchez (2001). One such evidence of this phenomenon was shown in the revisited biogeography of Glandulocaudinae, in which *M. microlepis* was a study case (Menezes et al., 2008). Thus, the present research focused on yielding the first robust molecular evidence for understanding the evolutionary and ecological histories of *M. microlepis* from the Southern Atlantic Rain Forest in Brazil. Additionally we have tested for a species complex occurring in the *M. microlepis* along part of its geographical range.

Materials and methods

A hundred specimens of both sexes of *M. microlepis* were sampled from 10 streams located in two main regions: Iguaçu headwaters and coastal plains of the states of Paraná, São Paulo and Santa Catarina (Table 1, Fig. 1). Voucher specimens were deposited in the collection of Museu de História Natural do Capão da Imbuia (MHNCI), Curitiba, Brazil.

DNA isolation and PCR-RAPD procedures

DNA was isolated from muscle samples from 10 specimens of each stream sampled, using the methodology described by Sambrook & Russel (2001) with some suggestions made by Almeida et al. (2001).

About 45 primers (Operon Technologies series) were first tested in order to identify those with good amplification performance in terms of molecular

variation and band clarity. Of those with good performances, the best six primers were selected (Table 2), also focusing on minimizing lab procedures for achieving analytical robustness, such as that suggested by Telles et al. (2001) and Hollingsworth & Ennos (2004). RAPD reactions were performed at least twice on different days and with different reagents in a final volume of 15 μl, using a *Personal Eppendorf* thermocycler, following procedures described by Almeida et al. (2003). Reactions were carried out with an initial denaturation step of 4-min at 92°C, followed by another 40 cycles of 40 s of denaturation at 92°C, 1 min 30 s, annealing at 40°C, 2 min, extension at 72°C and a final elongation step of 5 min at 72°C. PCR products were resolved by electrophoresis in 1.4% agarose gel immersed in TBE buffer and stained with ethidium bromide. After runs of approximately 4/5 h (80 V), the results were documented using an image capture system (Vilber Lourmat). The molecular weight of the fragments was estimated with a 100-bp molecular marker ladder (Invitrogen Inc.).

Data analysis

The results were converted into a binary matrix for which 0 was attributed to the absence of a band and 1 was attributed to the presence of a band. Overall genetic diversity was measured manually based on the percentage of polymorphic loci. The genetic diversity of each sample was also measured based on the percentage polymorphic loci in the total loci identified for each sample.

The binary matrix was analyzed using Neighbor-Joining (NJ) and Maximum Parsimony (MP) algorithms, using Paup v.4.0 b10 (Swofford, 2000) through its graphic interface PaupUp (Calendini & Martin, 2005), with the co-generic species of *M. laterallis* as the out group. NJ analysis provided the genetic distance (Nei, 1972 distance) between the terminals. Based on these distances, we estimated the pairwise mean genetic distance between the samples. MP analysis was performed through heuristic searches, with the characters designated as "not-ordered" and with equal weights. The MaxTrees number of trees analyzed was 100,000 with 5000 random replications by random addition of terminals and the tree-bisection-reconnection (TBR) algorithm for branch swapping. Strict consensus was computed

Table 1 Rivers and sample sites of *Mimagoniates microlepis*

Sample codes	Site number	River/Site	Region	Geographic coordinates
PIR	1	Piraquara; Piraquara—PR	IGH	25°29′59″S–49°02′58″W
CAE	2	Caerana; Piraquara—PR	IGH	25°26′59″S–49°02′31″W
PEQ	3	Pequeno; São José dos Pinhais—PR	IGH	25°33′32″S–49°07′41″W
VAR	4	Várzea; Lapa—PR	IGH	25°52′57″S–49°43′26″W
AGU	5	Água Verde; Canoinhas—SC	IGH	26°12′44″S–50°23′35″W
MER	6	Mergulhão; Antonina—PR	CP	25°17′58″S–48°44′39″W
RIB	7	Ribeirão; Paranaguá—PR	CP	25°35′21″S–48°36′40″W
MON	8	Mongaguá; Mongaguá—SP	CP	24°05′30″S–47°06′46″W
ITA	9	Caixa d'Água; Itariri—SP	CP	24°17′54″S–47°06′46″W
ACA	10	Tributary of Acarai River; S. Francisco do Sul—SC	CP	26°17′33″S–48°35′20″W

From left to right, the first column indicates the sample codification in terms of location: PIR = Piraquara River; CAE = Caerana River; PEQ = Pequeno River; VAR = Várzea River; AGU = Água Verde River; MER = Mergulhão River; RIB = Ribeirão River; MON = Mongaguá River; ITA = Caixa d'Água River; ACA = Acarai Basin. IGH = Iguaçu headwater; CP = Coastal plain

SC—state of Santa Catarina, southern Brazil; SP—state of São Paulo, southeastern Brazil; PR—state of Paraná, southern Brazil
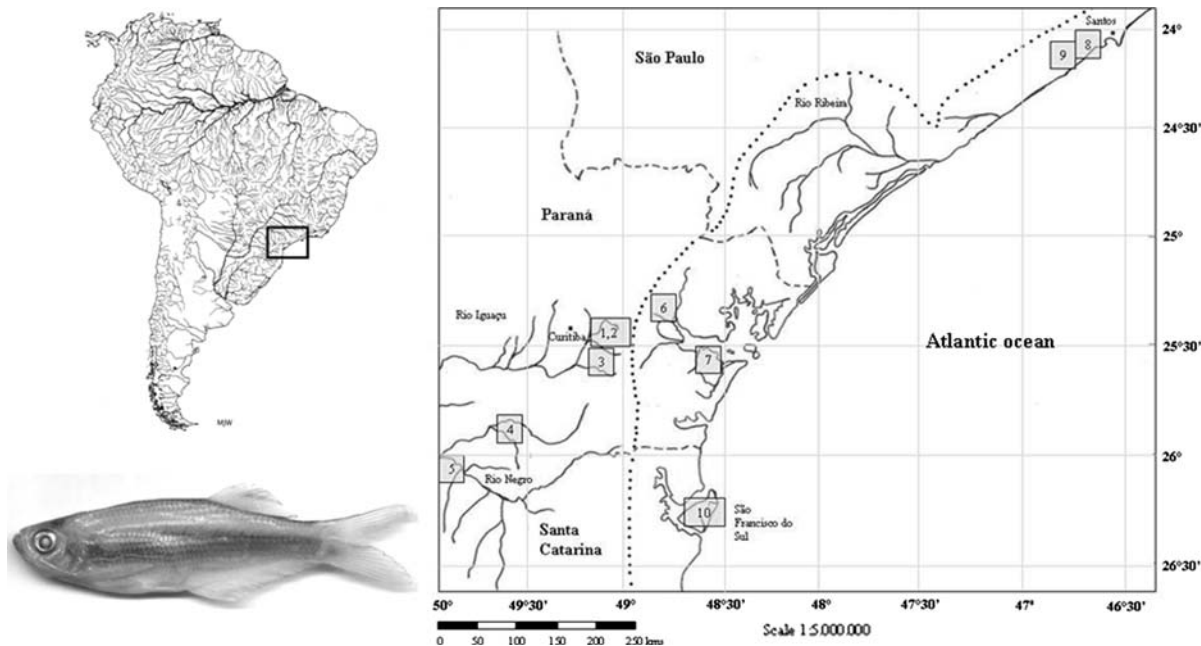

Fig. 1 Composite plate showing maps of the study area and sample locations (1–10; see Table 1 for details) in southern Brazil. Dotted line shows the physiographic profile of the Serra do Mar mountain chain along the states. The line also defines the two main areas sampled: Iguaçu headwaters (at left) and coastal plains (at right). Dashed lines indicate the state boundaries in the study area. Below the map, an 8-cm specimen of *Mimagoniates microlepis* is shown. SP = State of São Paulo; PR = State of Paraná; SC = State of Santa Catarina

and the robustness of the resulting topology was assessed through bootstrap and jackknife analyses with 1000 pseudo replicates as well as through heuristic searches using the fast stepwise addition of terminals and TBR for branch swapping.

AMOVA was performed using the ARLEQUIM v.3.01 software (Schneider et al., 2000). This analysis is based on obtaining the null distribution of the molecular variation, considering a priori the entire set of the observed diversity (binary matrix) as from a

Table 2 List of the primers used and their sequences

Primers	Sequences
OPA2	5′ GTTGCGATCC 3′
OPAM07	5′ AACCGCGGCA 3′
OPAM13	5′ CACGGCACAA 3′
OPW5	5′ GGCGGATAAG 3′
OPX04	5′ CCGCTACCGA 3′
OPX18	5′ GACTAGGTGG 3′

single population (Excoffier et al., 1992). The components of variation were estimated from 1000 permutation matrices, following the procedure described by Excoffier et al., (1992), and Φ_{ST} was then estimated. Global and pairwise number of migrants per generation (N_m) and G_{ST} values was determined using the PopGene v.1.32 software (Yeh et al., 1999).

Results

The six primers used provided a total of 111 markers among the *Mimagoniates microlepis* samples (Fig. 1, Table 1). Fragments ranged from 250 to 3000 bp. Of the 111 markers, 5 were constant and 106 were variable. This revealed an overall genetic diversity of 95.4% for the species, whereas the genetic diversity of each sample ranged from 44.4 to 80.3% (Table 3).

The NJ analysis provided mean genetic distances among the *M. microlepis* samples ranging from 0.221 to 0.461 (Table 4). Both grouping analyses (NJ and MP) revealed two well-separated genetic/evolutionary stocks for *M. microlepis* (Fig. 2a, b). One comprises samples living in continental streams (Iguaçu River headwaters) and the other stock is comprised of samples from streams of the coastal plains. Indeed, both topologies revealed similar patterns of local genetic structuring and a striking tendency toward evolutionary diversification in the species.

The NJ analysis has also revealed a closer genetic relationship between samples of *M. microlepis* from the Água Verde and Pequeno Rivers (Iguaçu headwaters). Such unit is closely genetically related to specimens from the Piraquara River. Specimens from the Várzea River were the most genetically distant from the others (Fig. 2a). Thus, there are five distinct genetic groupings for *M. microlepis* along the Iguaçu River headwaters. Throughout the coastal plains, the closest genetic relationship was detected between samples living at in the Mongaguá and Caixa d'água Rivers (coastal region of the state of São Paulo; Fig. 1) and between specimens from the Mergulhão and Ribeirão Rivers (coast of the Paraná). The latter grouping was closely genetically related to the genetic unit composed by specimens of *M. microlepis* from the Acarai River (coast of the Santa Catarina) and with the entire set of samples from the Iguaçu headwaters. Thus, the sample from the Acarai River may constitute a third coastal stock (Fig. 2a).

Parsimony analysis (Fig. 2b) yielded 1248 equally parsimonious trees (L = 894; Ci = 12; Ri = 69).

Table 3 Genetic diversity of *M. microlepis* in the study area

Location	Sample codes	Tnl	Tvl	Gd (%)	Average genetic diversity (%)
Iguaçu headwaters	PIR	69	32	46.37	60.1
	CAE	64	50	78.10	
	PEQ	61	41	67.21	
	VAR	37	23	62.16	
	AGU	51	24	47.05	
Coastal plains	MER	51	41	80.39	66.5
	RIB	62	44	70.96	
	MON	53	35	66.03	
	ITA	58	41	70.68	
	ACA	54	24	44.44	

Tnl = total number of loci in each sample; Tvl = total number of variable loci in each sample; Gd = genetic diversity. For abbreviations in the second column, see Table 1

Table 4 Pairwise mean genetic distances (Nei, 1972) of *M. microlepis* in the study area (above diagonal)

	PIR	CAE	PEQ	VAR	AGU	MER	RIB	MON	ITA	ACA
PIR	–	0.284	0.264	0.261	0.224	0.349	0.431	0.448	0.435	0.381
CAE	2.2	–	0.273	0.275	0.261	0.338	0.441	0.420	0.422	0.363
PEQ	13.2	11	–	0.254	0.228	0.364	0.419	0.451	0.450	0.387
VAR	71.5	69.3	62.7	–	0.255	0.271	0.349	0.390	0.393	0.355
AGU	128.7	126.5	121	60.5	–	0.333	0.401	0.461	0.439	0.392
MER	24.2	24.2	37.4	88	146.3	–	0.243	0.316	0.292	0.313
RIB	38.5	38.5	35.2	93.5	154	16.5	–	0.361	0.338	0.413
MON	236.5	238.7	242	304.7	357.5	161.7	214.5	–	0.221	0.352
ITA	189.2	191.4	198	255.2	314.6	170.5	170.5	49.5	–	0.373
ACA	82.5	80.3	71.5	99	148.5	71.5	60.5	253	212.3	–

Pairwise relative geographic distances (km) of *M. microlepis* in the study area (below diagonal). For abbreviations, see Table 1
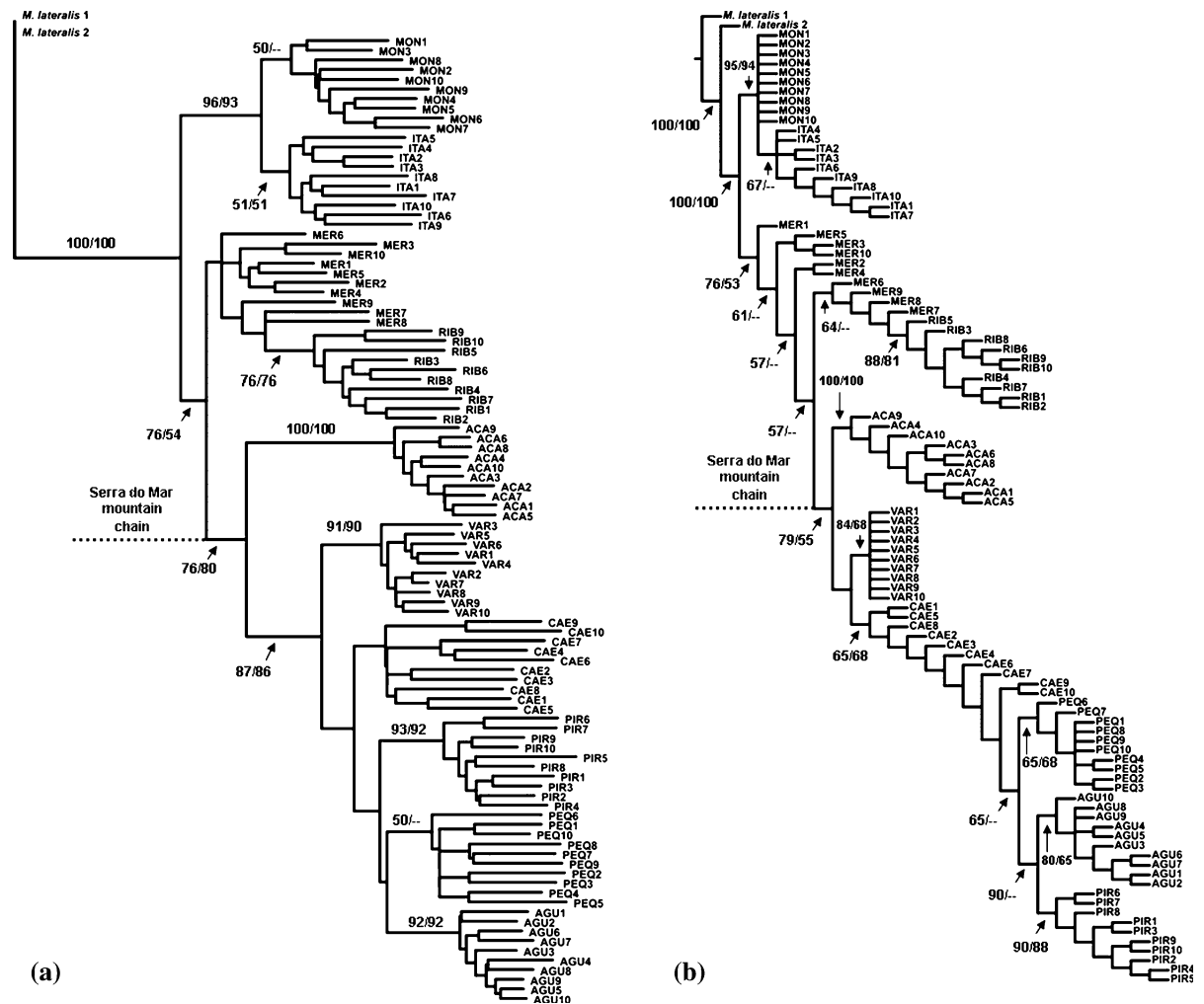

Fig. 2 Grouping results obtained from the entire set of samples of *M. microlepis* in the study area. (**a**) Neighbor-Joining topology; (**b**) Maximum parsimony topology. Numbers above or below the branches indicate the bootstrap/jackknife supports

The strict consensus topology had 989 steps with CI = 10 and RI = 66. As mentioned above, the analysis also allowed the identification of two well-separated evolutionary groups: one from the Iguaçu headwaters and the other from the streams of the coastal plains. The sample of *M. microlepis* from the Acaraí River emerged as the putative sister group of samples from the Iguaçu headwaters. *M. microlepis* from the Várzea River was the most basal sample. Besides the specimens from the Caerana River in the Iguaçu headwaters, four monophyletic groups were observed in the Água Verde, Pequeno, Piraquara, and Várzea Rivers. Along the coastal plains, four mono-phyletic groups were also observed occurring in the Acarai, Ribeirão, Caixa d'água, and Mongaguá Rivers. *Mimagoniates microlepis* from the Caixa d'água and Mongaguá Rivers are potentially sister groups. This grouping seems to be the most basal lineage along the coastal plains (Fig. 2b). Indeed, a global analysis in terms of broad coastal areas revealed three well-defined evolutionary lineages throughout southern coastal plains along the coast of the states of São Paulo, Paraná and Santa Catarina.

AMOVA revealed that 61.32% of the total variance is between samples and 38.7% of this occurs within the samples (Table 5) of *M. microlepis* studied (Table 5). Global Φ_{ST} was 0.597, with high statistical robustness ($P = 0.00039$). Global Wright population parameters revealed the global N_m as 0.3235 and global G_{ST} as 0.6071. Among samples from the Iguaçu River, these parameters were 0.5804 and 0.4828, respectively, whereas among samples from the coastal plains, values were 0.5544 and 0.4019, respectively. Pairwise N_m and G_{ST} values ranged from 0.287 to 1.557 and 0.243 to 0.635, respectively (Table 6).

Discussion

Genetic diversity and conservation status

The genetic diversity of a species is the most important component to maintain evolutionary potential, pro-viding the core for the plasticity and adaptation of the species (Amos & Hardwood, 1998; Moritz, 2002; Torres, 2003).

Table 5 AMOVA results for *M. microlepis* in the study area

Source of variation	df	Ssq	% Variation	Ms	F	Vc	Φ_{ST}	P
Between samples	9	1,111,870	61.32	123,541		11.5748888889	59.7	
Within samples	90	701,300	38.68	7,792		7.79222222222	40.3	
Total	99	1,813,170	100		15,885			0.00039

df = degrees of freedom; Ssq = sum of squares; Ms = mean square; F = Fisher's exact test; Vc = variance components; P = statistical significance

Table 6 Pairwise G_{ST} (above diagonal) and N_m (below diagonal) values for *M. microlepis* in the study area

	PIR	CAE	PEQ	VAR	AGU	MER	RIB	MON	ITA	ACA
PIR	–	0.320	0.329	0.434	0.394	0.493	0.539	0.559	0.538	0.573
CAE	1.062	–	0.246	0.298	0.326	0.345	0.438	0.416	0.413	0.456
PEQ	1.018	1.532	–	0.342	0.336	0.425	0.465	0.487	0.479	0.521
VAR	0.649	1.172	0.961	–	0.511	0.407	0.488	0.531	0.530	0.596
AGU	0.766	1.032	0.984	0.477	–	0.521	0.551	0.598	0.567	0.635
MER	0.514	0.946	0.675	0.725	0.459	–	0.270	0.409	0.359	0.506
RIB	0.427	0.639	0.575	0.523	0.406	1.345	–	0.432	0.396	0.571
MON	0.393	0.699	0.526	0.440	0.335	0.720	0.656	–	0.243	0.529
ITA	0.429	0.708	0.543	0.442	0.380	0.891	0.759	1.557	–	0.535
ACA	0.372	0.596	0.459	0.337	0.287	0.488	0.375	0.444	0.433	–

For abbreviations, see Table 1

Mimagoniates microlepis exhibited 95.4% overall genetic diversity in the area studied. Such results are very similar to those found for *Astyanax altiparanae* (Characidae) from the Iguaçu River (90%) and *Astyanax scabripinnis* from the Tibagi River (81.3%) (Prioli et al., 2002; Sofia et al., 2006). On the other hand, *Pseudoplatystoma corruscans* (Pimelodidae) from the Paraná River exhibited 27% overall genetic diversity and *Brycon lundii* (Characidade) from the São Francisco River exhibited 34.7% genetic diversity (Sekine et al., 2002; Wasko & Galetti, 2002). Comparisons between these estimates indicate that *M. microlepis* exhibits high genetic diversity. Additional evidences supporting this conclusion may be gathered from Table 3, which displays the genetic diversity of each sample studied. Both large areas studied (Iguaçu headwater and Costal plains) showed similar and satisfactory average genetic diversities (60%). These evidences might suggest two hypotheses: (1) the species retains good conservation status by presenting high genetic diversity; and (2) a significant part of the observed genetic diversity represents genetic differences between distinct species.

Genetic distances, genetic/evolutionary relationships

Iguaçu headwaters

Historical aspects such as dispersion and/or vicariance may have influence over the distribution of the breeders and over their genetic pool (Amos & Hardwood, 1998; Avise, 2000). Although *M. microlepis* samples from the Caerana River (Iguaçu headwaters) and Mergulhão River (coastal plain) exhibited a lesser degree of cohesiveness than the other samples (Fig. 2a, b), both genetic and phylogenetic relationships suggest that *Mimagoniates microlepis* is in a true process of diversification. Such a conclusion is supported by the occurrence of nine local genetic units (Fig. 2a) and the seven monophyletic clades, as determined through parsimony analysis (Fig. 2b). Additional support for this diversification may be recruited from the global and pairwise N_m and G_{ST} values as well as the Φ_{ST} value and AMOVA results (Tables 5, 6). According to Wright (1978), Hartl & Clark (1997), and Hedrick (1999), G_{ST} and Φ_{ST} equal to or higher than 0.25

indicate a striking evolutionary structuring. The values of $N_m < 1$ point to differentiation (c.f. Almeida et al., 2003). Thus, a large part of the evidence suggests that *M. microlepis* may be a species flock, as supposed (Guimarães et al., 1995).

The Iguaçu River headwaters seem to be a biogeographic province based on the evolution of the region. For instance, this is the hypothesis for the prior relationship between the headwaters of the Iguaçu River and some coastal drainage (Abilhoa & Boscardim 2004; Abilhoa, 2005). The samples of *M. microlepis* from the Iguaçu headwaters exhibited significant genetic and evolutionary structuring (Fig. 2a, b; $N_m = 0.5804$; $G_{ST} = 0.48828$), possibly achieving metapopulation conditions that could had driven this fauna to events of local speciation (Levin, 1995; Baguette, 2004).

Additional evidence for defining inter-population/species relationships could be gathered from genetic distances (Table 4), given that genetic distances more than 0.3 might be associated with inter-species relationships (Thorpe, 1982). However, the mean of the genetic distances between *M. microlepis* samples from the Iguaçu headwaters are, on the whole, lower than 0.3. Therefore, the mean genetic distances have failed to detect the real genetic structure evidenced by N_m and G_{ST} values.

Analyzing specific pairwise genetic distances and N_m data (Tables 4, 6), Piraquara and Pequeno Rivers exhibited a genetic distance of 0.264 and 1.018 migrants per generation, which suggests a single population occurring throughout these streams. This hypothesis seems to be reinforced by chromosome data, given that the samples shared the same diploid numbers, karyotypical formula, and NOR features (ribosomal cistrons) (Torres et al., 2008). Contrarily, the pairwise G_{ST} (0.329) value suggests a high degree of genetic structuring for the streams sampled (Hartl & Clark, 1997; Hedrick, 1999). A possible explanation would be a very recent isolation event that occurred between these samples, not yet associated to karyotype rearrangements, but strongly evidenced molecular population parameters. Additional support for this putative explanation may be observed in the genetic and phylogenetic topologies (Fig. 2a, b) as well as in the Bayesian population phylogeny provided by Menezes et al. (2008), which suggest that these samples are genetically and evolutionarily different.

Coastal plains

The inexistence of evident physical barriers to gene flow suggests a higher tendency to homogeneity among the members of a species, whereas physical barriers normally enhance the evolutionary processes by fractioning a gene pool that was maintained cohesive during a period. The genetic/ evolutionary scenarios (Fig. 2a, b) presented herein for the samples of *M. microlepis* from coastal plains suggest that they may have reached the status of completely isolated populations. They might also constitute evolutionarily significant units (Ryder, 1986; Moritz, 1994; Eizirik, 1996). Such a hypothesis is supported by the fact that the streams assessed are intra- and inter-regionally isolated. It also reinforces the idea of the remarkable endemism of Neotropical fish fauna in coastal freshwater environments in southern Brazil as a consequence of isolated lineages (Menezes, 1996).

Several studies on molecular data (genetic distances) from fish species have shown the genetic distance ranging from 0.32 to 0.45 for species level comparisons (Mullidae: Mamuris et al., 1998; Serranidae: Govindaraju & Jayasankar, 2004). These evidences reinforce the hypothesis of endemism of *M. microlepis* throughout the coastal plains assessed, given that most of the mean genetic distances observed among *M. microlepis* that were sampled from the coastal plains (Table 4) are very similar to those of the aforementioned studies and are also higher than 0.3. According to Thorpe (1982), genetic distances above 0.3 normally differentiate most species.

Moreover, chromosome data indicate that *M. microlepis* from the coastal plains of the states of São Paulo and Santa Catarina may represent distinct taxa, given the wide diversity in number and position of ribosomal cistrons (Guimarães et al., 1995). The global and pairwise G_{ST} and N_m values (0.4019/ 0.5544 and Table 6) also indicate strong population differentiation and a low number of migrants per generation. The pairwise N_m and G_{ST} values (Table 6) may also be considered as evidences for the aforementioned hypothesis. About 90% of the G_{ST} comparisons show values higher than 0.27 and 80% of these N_m comparisons show values smaller than 1 migrant per generation. Figure 2a and b illustrate additional support for the genetic and

evolutionary differentiation of *Mimagoniates microlepis*, given the occurrence of four distinct genetic units, as determined through NJ analysis and corresponding to four monophyletic clades, as determined through MP analysis (Acaraí, Ribeirão, Mongaguá, and Caixa d'Água).

Phylogeographic inferences

A geographic barrier is any feature of the terrestrial environment that exercises a primary influence on the spatial distribution and secondary influence on the gene flow of organisms. A question arises as to whether the Serra do Mar mountain chain could be a cause of the potential ichthyofaunistic diversification in its continental and coastal parts. We suppose this to be true, as the Serra do Mar mountain chain seems to be associated to species divisions and their potential diversification. The fact of 16 freshwater fish species occurring at both sides of the mountains chain (west to the Iguaçu headwaters and east to the Atlantic coast; Abilhoa & Boscardim, 2004; Abilhoa, 2005) is a strong evidence of this. Furthermore, such shared distribution suggests a close biogeographic relationship between these areas.

The genetic and evolutionary relationships assessed herein (Fig. 2a, b) offer strong evidence that the Serra do Mar must be associated to the diversification of *M. microlepis*. Such diversification is supported by the occurrence of nine distinct genetic units: (1) Água Verde River, (2) Pequeno River, (3) Várzea River, (4) Piraquara River, (5) Caerana River, (6) Acarai River, (7) Ribeirão River, (8) Mongaguá River, and (9) Caixa d'Água River. This hypothesis is also supported by the seven monophyletic clades occurring in *M. microlepis*: (1) Água Verde River, (2) Pequeno River, (3) Várzea River, (4) Piraquara River, (5) Acarai River, (6) Ribeirão River, and (7) Mongaguá River + Caixa d'Água River. Table 6 displays additional support to the speciation hypothesis mentioned above, as 90% of the comparisons reveal $N_m < 1$ and $G_{ST} > 0.25$, suggesting an intense genetic–evolutionary structuring of the *M. microlepis* samples studied.

The 9/7 distinct lineages also seem to be inserted into four biogeographic areas: (1) Iguaçu headwaters, (2) coastal plain of Paraná, (3) northern coastal plain of Santa Catarina, and (4) southern coastal plain of São Paulo (Fig. 3). In support of such a hypothesis,

Fig. 3 Composite plate showing maps, sample locations (1–10; see Table 1, Fig. 1 for details) and satellite images (Google Earth) from study area in southern Brazil (**a**–**c**). Numbers 1–4 show the regions hosting the four proposed biogeographic areas and the 9/7 distinct genetic/evolutionary lineages of *M. microlepis*. The white arrows indicate the mountain barriers isolating these biogeographic areas
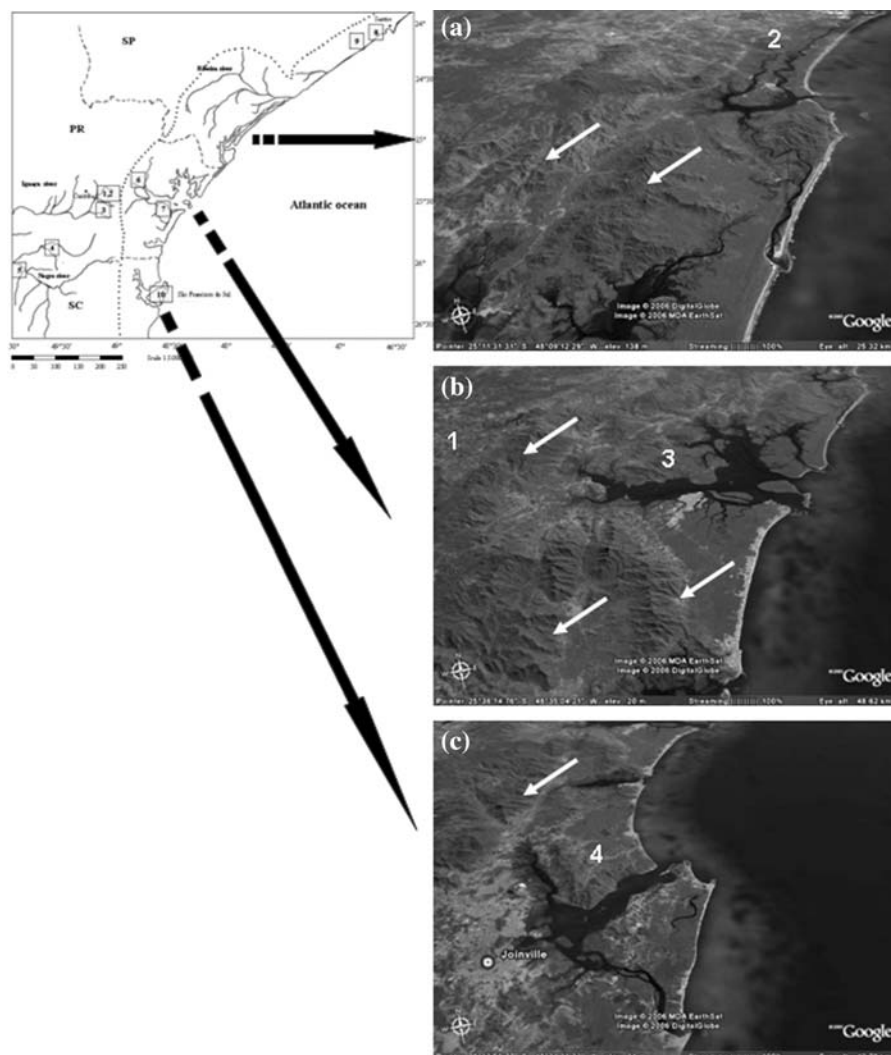
the following barriers are identified: the main axis of Serra do Mar separating coastal and continental regions; and its dendrite extensions dividing the entire coastal plains assessed (Fig. 3).

Based on the temporal diversification, the topology suggests that *M. microlepis* might have colonized the region of Iguaçu headwaters from the Acaraí River lineage. Such a hypothesis could be supported by events of headwater captures involving original Iguaçu headwaters and any primitive coastal river. Ribeiro (2006) reviewed the geological and tectonic histories of the Brazilian coastal region. The author discussed in detail the upper Iguaçu area, demonstrating that this region might have suffered faunal exchange with adjacent drainage systems by

pertaining to a Quaternary tectonic fault/uplift system: the Ponta Grossa Arch. Events of headwater captures occurring along these areas are considered to be robust evidence explaining genetic relationships and the origin of *Hoplias malabaricus* (Erythrynidae) in the region (Lemos et al. 2002; Vicari et al. 2006). Another alternative and equally consistent explanation for that could be the occurrence of a wide-ranging common ancestral lineage of the Acaraí and Iguaçu headwaters, given the samples are sister lineages (Fig. 2a, b).

In terms of temporal colonization, the topologies (specially the parsimony topology) suggest that *Mimagoniates microlepis* expanded to the southern coastal areas and reached the upper Iguaçu in an

earlier moment. At this sense, the lineages from Mongaguá and Caixa d'Água rivers seem to be older than the others, given their basal position (branching supports of 95/94; Fig. 2b). Subsequent genetic structuring and cladogenetic events occurred in coastal parts from Paraná and Santa Catarina, respectively, before reaching the Iguaçu headwaters (Fig. 2a, b). Such a perspective is supported by two pieces of evidence: (1) *Mimagoniates lateralis* and *Mimagoniates reocharis* only occur in coastal areas; and (2) they are sister species of *M. microlepis*. Patterns of phylogenetic relationships based on molecular data that corroborate geographic distribution have been extensively reported for fish species, especially in Glandulocaudinae (Menezes et al., 2008).

Conclusion

Mimagoniates microlepis exhibited a high genetic diversity (∼95%) concluding that the species might host a good conservation status in terms of genetic variation or most of the large part of the diversity might be from different cryptic species.

In terms of the putative historical differentiation in the species both genetic and phylogenetic relationships suggest that *Mimagoniates microlepis* is in a true process of diversification. Thus, a large part of the evidence indicates *M. microlepis* as a species flock, as supposed. In both studied regions (Iguaçu headwaters and Coastal plains) the species exhibited significant genetic and evolutionary structuring. Such conclusion indicates that the paleogeographic patterns of the evolution of the Serra do Mar mountain chain has played an important role in the diversification of the species by separating the three southern Brazilian coastal plains studied and the Iguaçu headwaters from each other.

Based on the evidences on the sea level fluctuations (Suguio et al., 2005), the genetic/evolutionary scenarios demonstrated herein might have comprised several events of population contraction and demographic expansions of *Mimagoniates microlepis* on the coastal plains. Yet, regarding the principles of the phylogeography the observed evolutionary scenario indicated that *Mimagoniates microlepis* seems to have expanded to the southern coastal areas and the upper Iguaçu lineages would possibly be the youngest populations.

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