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Discus fishes: mitochondrial DNA evidence for a phylogeographic barrier in the Amazonian genus *Symphysodon* (Teleostei: Cichlidae)

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Genetic relationships and variation in meristic counts, body shape and colour were examined in a large sample of *Symphysodon* collected from several locations in floodplain habitats along the length of the Amazon River. Surprisingly, mitochondrial DNA indicates no difference between the two historically described species, *Symphysodon discus* and *Symphysodon aequifasciatus*, but shows that non-clinal variation exists with a distinct lineage found in the western Amazon. This lineage is consistent with a colour form that is distinct from other *Symphysodon* lineages. This form has a parapatric distribution and is recognized as a distinct species, *Symphysodon tarzoo*. Adaptation to floodwater habitats supports genetic cohesion across a large range preventing fine scale regional diversification of the genus. Possible explanations for the unusual set of distributions for genetic and colour characters relate to the history of the Amazon basin and the probable division of lowland species when submerged geologic arches influence surface topology.
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Key words: Amazon; colour; discus; geographical variation; mitochondrial DNA; morphology.

INTRODUCTION

The discus fishes, genus *Symphysodon* Heckel, 1840, are one of the most intriguing and distinctive groups among the South American Cichlidae. At present, there are two recognized species within the genus, *Symphysodon discus* Heckel, 1840, and *Symphysodon aequifasciatus*, Pellegrin, 1904, which differ in the pattern of melanic bars and in some meristic characters. The first species is found in the central Amazon in the Rio Negro, Abacaxis and Trombetas drainages, while the latter is found along almost the entire length of the Amazon from 49° to 70° west longitude (Fig. 1). Species of *Symphysodon* are restricted to areas where seasonal flooding occurs and are therefore found only near the mainstem of the Amazon River itself and in the lower reaches of tributaries

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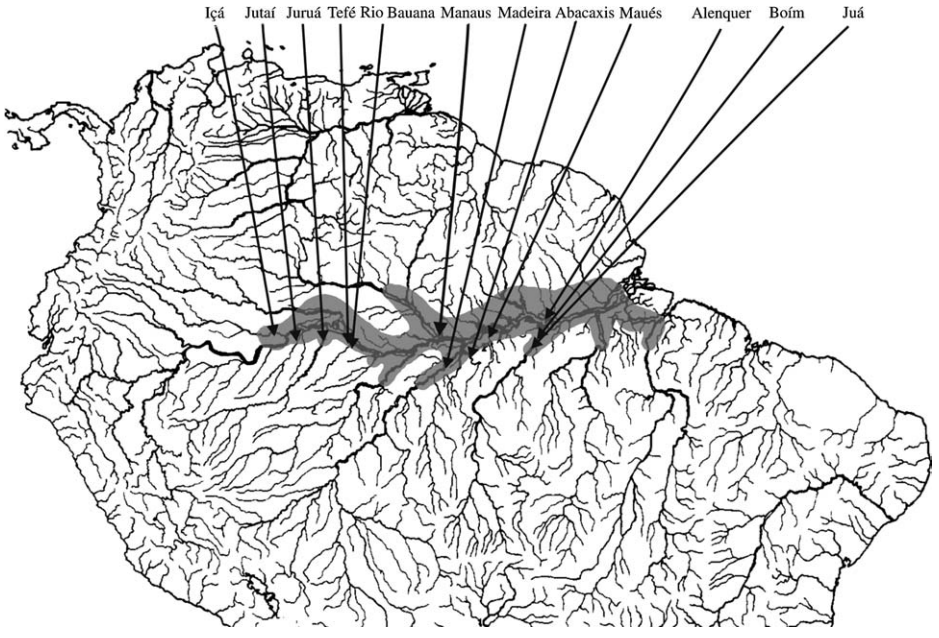

FIG. 1. Geographical distribution of *Symphysodon* (after Kullander 1996, modified) and sample locations.

on the Amazonian floodplain (Kullander, 1996). The combined distribution of the genus forms one large continuous range in which many environmental and historical factors could have been involved in isolating populations and driving speciation.

Symphysodon are part of the Heroini, a Neotropical clade of cichlid fishes (Kullander, 1998; Farias *et al.*, 2001). The exact relationship of *Symphysodon* within the Heroini is partially contentious, though morphological analysis apparently provides stronger support than molecular evidence, placing *Symphysodon* as sister taxon to a clade containing *Heros*, *Uaru, Mesonauta* and *Pterophyllum* (Kullander, 1998). These are all high-bodied, lowland fishes known mainly from the Amazon basin.

Colour patterns on living fish vary considerably in *Symphysodon*. There are normally nine dark bars on the sides. These are of equal width and intensity in *S. aequifasciatus*, but bars 1, 5 and 9 are intensified and wider in *S. discus*. Although the general colour of *S. aequifasciatus* is brown with some blue and red markings on the forehead and near the anal fin, some individuals in all populations have brighter colours, often including greater coverage or strength of bright red and blue stripes, spots or reticulations (pers. obs.). This colour variation enhances the value of these species in the ornamental pet trade where they are highly regarded and priced.

Studies of colour variation have resulted in the descriptions of several subspecies based on the purported prevalence of variants in different geographic regions and include *Symphysodon a. aequifasciatus, Symphysodon a. haraldi* Schultz, 1960*, Symphysodon a. axelrodi* Schultz, 1960, and *S. discus willischwartzi* Burgess, 1981. Unfortunately, descriptions are based on only a few samples,

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often of uncertain origin (Schultz, 1960; Burgess, 1981), and Kullander (1986; 1996; 2003) recognized only two diagnosable species, *S. aequifasciatus* and *S. discus*.

The present study includes a new larger set of specimens, with both colour photos and tissue samples for DNA analysis, to determine whether variation in colour, morphology or genetics is correlated with geography or environmental variation. Despite the confusion over the precise origins of colour forms obtained from ornamental fish importers, some populations are relatively easy to identify from preliminary inspection of photographs of recently collected material. For example, fish from Tefé, in the Western Amazon, can be recognised by very light coloured anterior sides with red spots on the anal fin and sides of the body.

Symphysodon aequifasciatus is one of very few cichlid species with an almost linear distribution along the entire lowland course of the Amazon River mainstream.

The Amazon River has a dramatic history, being diverted from a Pacific outlet to a northern outlet about 67 million years ago (MYA) and finally to an Atlantic outlet about 8 MYA (Lundberg *et al.*, 1998). *Symphysodon aequifasciatus* was selected to test for clinal variation in morphology and genetic characteristics along the Amazon River, as a possible explanation of phenotypic variation, and at the same time for possible breaks in continuity that could be correlated with hypotheses about the historical development of the Amazon River.

MATERIALS AND METHODS

Samples were collected at several localities (Fig. 1) in 1998. Specimens were deposited in the collections of INPA, Manaus, Brazil (INPA 14334–14345, INPA 14386 and INPA 25960). Samples from further east in the overall distribution could not be obtained as the area is less frequently visited by discus fishermen whose assistance was required to obtain samples. Additional material examined included the type series of *S. aequifasciatus* from the Muséum National d'Histoire Naturelle, Paris, France (MNHN 1902–130, MNHN 1902–134 and MNHN 1902–135). Analysis of variation includes molecular data (from 23 individuals), characters of colour pattern and body shape (assessed from 263 photographs of live fish) and morphological characteristics (meristics and rhomboid measures for 94 individuals).

Tissue samples were stored in ethanol before preservation of whole fish in formalin (except for Rio Bauana specimens). Genomic DNA was extracted using DNeasy extraction protocols and Kits (QIAGEN, through VWR International AB, Stockholm, Sweden). The sequences were then amplified using puReTaq Ready-To-Go PCR beads (Amersham Biosciences Europe, Uppsala, Sweden) and polymerase chain reaction cycles as follows: 1 denaturation cycle at 94° C for 4 min, 30 cycles with a denaturation temperature of 94° C for 30 s, an annealing temperature of 54° C (for all cytochrome *b* primers) or 62° C (for rhodopsin primers), and extension at 72° C for 30 s, all followed by 1 extension cycle at 72° C for 7 min. For cytochrome *b*, preliminary amplification used the primers FISCYTB-F (5′ ACC ACC GTT GTT ATT CAA CTA CAA GAA C 3′) and TRUCCYTB-R (5′ CCG ACT TCC GGA TTA CAA GAC CG 3′), and a secondary nested amplification used these with the internal primers CYTBi3-R (5′ GGG GTA AAG TTG TCT GGG TCT CC 3′) and CYTB1-F (5′ CGA TTC TTC GCA TTC CAC TTC CT 3′), respectively, to obtain sequences of two overlapping fragments. Amplification of rhodopsin used the primers RodF2w (5′ AGC AAC TTC CGC TTC GGT GAG AA 3′) and RodR4n (5′ GGA ACT GCT TGT TCA TGC AGA TGT AGA T 3′). All primers are from the European Union project FishTrace (M. Norén, pers. comm.). Reactions included negative controls. Sequencing was performed using the

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BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems Inc., through Applera Sweden, Stockholm, Sweden) on an automated DNA sequencer (Applied Biosystems 377) following manufacturer's instructions. Nucleotide sequences were deposited in GenBank under accession numbers DQ533587–DQ533606.

Sequences were aligned by eye using BioEdit v. 5·0·9 software (Hall, 1999). Cytochrome *b* gene sequences for several genera, which are potentially close relatives of *Symphysodon* (Kullander, 1998), were obtained from GenBank to root the phylogenies produced. These included *Acaronia* sp. Myers, 1940 (AF370666) and all available sequences for the genera of the Heroini: *Pterophyllum scalare* (Lichtenstein, 1823) (AF370676), *Hoplarchus psittacus* Kaup, 1860 (AF370673), *Hypselecara coryphaenoides* (Heckel, 1840) and *Hypselecara temporalis* (Günther, 1862) (AF370674 and AY050612, respectively), *Caquetaia kraussi* (Steindachner, 1878) and *Caquetaia spectabilis* (Steindachner, 1875) (AF009938 and AF370671, respectively), *Theraps maculicauda* (Regan, 1905)—now *Vieja maculicauda* (Regan, 1905) (U97165), *Mesonauta insignis* (Heckel, 1840) (AF370675), *Uaru amphiacanthoides* Heckel, 1840 (AF370678 and AY050622) and *Heros* sp. Heckel, 1840 and *H. appendiculatus* (Castelnau, 1855)—now *Heros efasciatus* Heckel, 1840 (AF370672 and AF009951). Also included was the cytochrome *b* sequence submitted for *Symphysodon aequifasciatus* (AF370677).

MODELTEST software (Posada & Crandall, 1998) was used to determine the best model of molecular evolution for the cytochrome *b* sequences. The general time reversible model with proportion of invariant sites = 0·5491 and gamma shape of 1·4368 (GTR + I + G) was chosen under both heirarchical likelihood test criteria and Akaike Information Criteria (AIC) and used for subsequent analysis. Phylogenetic analysis using parsimony (PAUP*) (Swofford, 1998) was used to construct trees using the sequence of *Acaronia* as an outgroup. Sequences were then analysed with 1000 bootstrap replicates under parsimony (options: ACCTRAN, TBR, MULTREES, Gaps = missing, random addition, transition to transversion (ti/tv) weighting as estimated in MODELTEST), 100 heuristic bootstrap replicates using the maximum likelihood setting described under MODELTEST and 1000 bootstrap replicates for neighbour joining (weighted least squares and parameters described by MODELTEST). Bayesian support was obtained using MrModeltest software (Nylander, 2002) to determine the model (also GTR + I + G) and MrBayes (Huelsenbeck & Ronquist, 2001). The support was obtained using 2 000 000 MCMC iterations, sampling every 100 iterations with a burn in where the first 10% of trees sampled were discarded (likelihood values were stable after this point). A consensus of the remaining trees was then obtained in PAUP* (Swofford, 1998).

Six colour pattern characters and two body shape characters were classed for each photograph using the characters and character states outlined in Table I. The eight characters were each classed into at most five states, with the first state being the most common in the outgroup (Table I).

Morphological distance measurements were made using digital callipers accurate to 0·01 mm. Methods for taking meristic counts follow Kullander (1983; 1986). Measurements and counts were made on the left side of the specimen when possible. Specimen lengths are standard length (SL). Vertebrae, pterygiophore, fin spine and soft ray counts and the measurement of SL were taken from radiographs made on Kodak X-Omat V plates, with a Philips MGC 30 low voltage X-ray unit. Distances between landmark points on specimens were determined from radiographs using a double rhomboid system (Fig. 2).

Statistical analyses and graphs were made using the SYSTAT package (Wilkinson *et al.*, 1992). Log transformations of body distances relative to SL, square root transformed meristic counts and raw data for classed characters were used in correlation and principal component analyses (PCA).

RESULTS

The aligned mitochondrial cytochome *b* gene sequence was 1134 bp long, and the nuclear rhodopsin partial sequence obtained was 514 bp long. Rhodopsin sequences showed no variation within *Symphysodon*. A blast search of sequences

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TABLE I. Coding of characters used for analysis of shape and colour in *Symphysodon*. State 1 is most common character states in photographs of related taxa (*Heros, Mesonauta, Uaru*)

Character	State 1	State 2	State 3	State 4	State 5
1 Anal fin red colour	Absent	Little	Medium	Much	
2 Continuation of colour from anal fin to body	Absent	Slight	Extended		
3 Anal fin pattern	None	Red spots or small streaks on blue base	Thin red lines on blue base (lines thinner than base)	Thick red lines on blue base (lines and base colour equal width)	Thick red lines reticulating over blue base
4 Forehead shape	Flat slope	Slightly rounded	Mainly rounded	Completely rounded	
5 Body base colour	Light brown	Brown	Green/brown	Grey green	
6 Forehead marking	Absent	Few blue stripes	Moderate number of blue stripes	Many blue stripes continuing to dorsal fin	
7 Body shape	Elongate	Intermediate	Discoid		
8 Red spots on body	Absent	Present			

in GenBank indicated that *Symphysodon* sequences were most similar to rhodopsin sequences of African cichlids. No Neotropical cichlid rhodopsin sequences were found in GenBank. Unambiguous alignment of the deduced amino acid sequence in *Symphysodon* with sequences from GenBank confirmed the single open reading frame.

Analysis of mitochondrial DNA (mt DNA) (cytochrome *b* gene) indicates distinct lineages of *Symphysodon* that do not conform to previous taxonomic classifications (Fig. 3).

One lineage comprises western populations that under the existing classification would have been identified as *S. aequifasciatus*. However, a second large lineage included not only sequences from central and eastern populations conforming to *S. aequifasciatus* but also sequences of *S. discus* from Manaus and the Rio Abacaxis. There is one notable exception. An individual from the Madeira River, which is central in the geographical distribution of samples, possesses both the phenotype and the mtDNA haplotype typical of western populations. Additionally, a far eastern genetic lineage may also exist from which only two samples were collected, Boím samples 2 and 3. However, their divergence from the central and the eastern lineage of *Symphysodon aequifasciatus* is small.

When compared with those of published Maximum Likelihood-based trees of mitochondrial sequences including African rift lake cichlids (Farias *et al.*, 2001;

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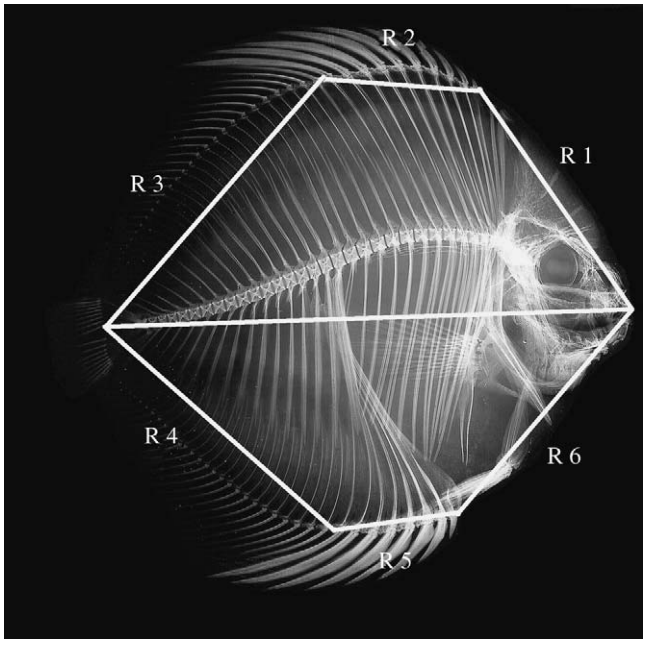

FIG. 2. Rhomboid measurements of shape in *Symphysodon*. R 1–R 6 and SL.

Klett & Meyer, 2002) the branch lengths between *Symphysodon* species (0·02 substitutions/site, Fig. 3) are comparable in some cases to branch lengths between genera of rift lake cichlids.

The resolution of the position of *Symphysodon* within the Heroini is weak. This is not surprising as many of the sequences used are those obtained from previous investigations (Farias *et al.*, 2001).

Principal Component Analysis (PCA) of colour pattern and body shape places specimens from the same population in the same cluster, but with many populations the overall pattern becomes ambiguous. Groups of specimens defined by environmental differences between sites, or by sex, did not lead to any distinct clusters in the PCA. However, when specimens were classified by region (western *v*. central/eastern groups), two weakly overlapping groups appeared in the PCA (Fig. 4). A re-examination of photographs of four individuals from the central/eastern group that lie within or close to the western group show that these individual fish possess the red spots typical for fish in the western group, even though these four fish were collected from a central population dominated by fish lacking red spots. The main western and central/eastern groupings in Fig. 4 were largely defined by Factor 1 that had high loadings for the presence or the absence of red spots and for anal fin red colouration. Even under very close inspection, darker bodied fish from central and eastern populations always lack red-pigmented spots on the anal fin and body and remains distinct from western fish. Two sub-groups appear to be present in the central/eastern cluster, though the current data do not support the recognition of a far eastern Boím genetic lineage.

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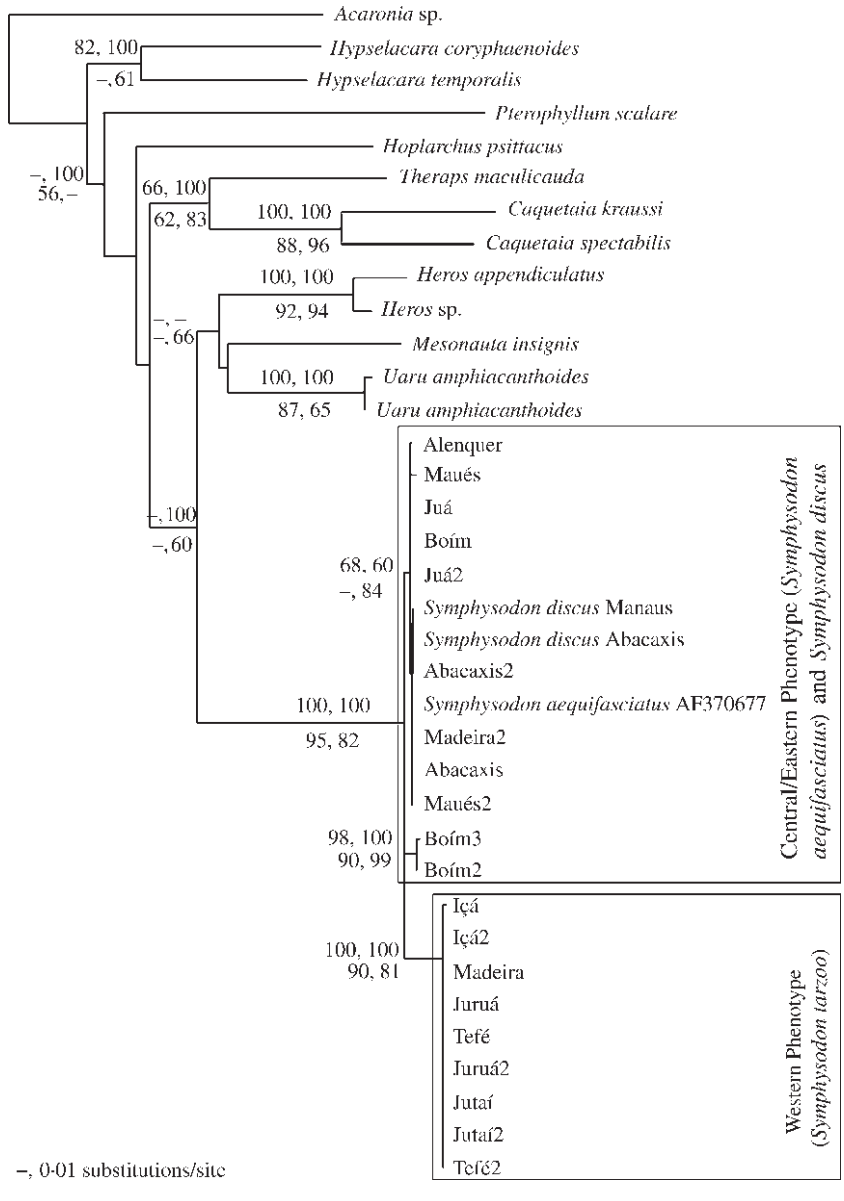

FIG. 3. Maximum Likelihood phylogram of *Symphysodon* sequences (including GenBank sequence of *S. aequifasctiatus*) and outgroup taxa. Support values: Above line left indicates per cent support from 1000 parsimony bootstrap replicates; above line right indicates Bayesian posterior probability as per cent; below line left indicates per cent support from 1000 Neighbour Joining bootstrap replicates; below line right indicates per cent support from 100 Maximum Likelihood bootstrap replicates. Consistency Index (CI), 0·4637; Retention Index (RI), 0·5363 and Rescaled Consistency Index (RC), 0·34 for consensus parsimony tree.

PCA of morphology (meristic counts and rhomboid measurements) on all populations indicated no clear population groupings. Within-population variation is greater than between-population variation.

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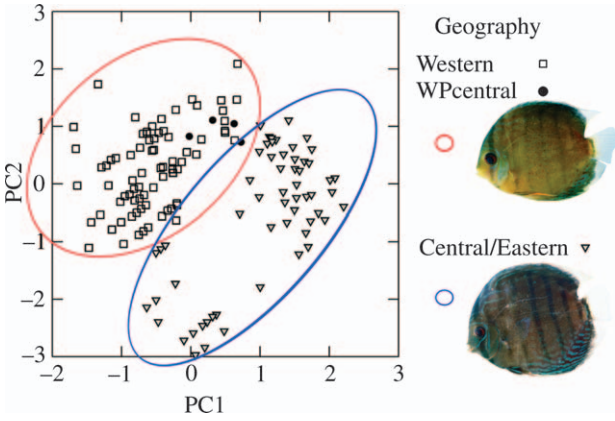

FIG. 4. PCA for geographic variation in the colour pattern and body shape of *Symphysodon*. Factor 1 is determined by a combination of characters 'Anal fin pattern' 'Presence of red spots' and 'Anal fin red colour' while Factor 2 is determined by the combination of characters 'Forehead markings' 'Body colour' and 'Continuation of anal fin pattern onto the body'. Factors 1 and 2 account for 57·3% of all variance in the analysis (32·3% and 24·0%, respectively). Specimens have been assigned to western phenotype (indicates *S. tarzoo*) and central/eastern phenotype (indicates *S. aequifasciatus*). WP central indicates fish with western phenotype from the Madeira River (filled circles).

DISCUSSION

The analysis of mitochondrial DNA haplotypes, colour pattern and morphology indicates that the western Amazonian *Symphysodon* are a distinct species. This is supported by the distinct mitochondrial lineage and by the diagnostic phenotypic character of red spots on the sides of the body.

Schultz (1960) described several sub-species of *S. aequifasciatus* based on the colour slides of individuals obtained through the ornamental fish industry. Among those, *S. a. haraldi*, is described from Benjamin Constant in the Western Amazon. However, this locality is unlikely to be correct as the specimen illustrated and described has a colour pattern conforming to eastern populations.

The type series of *S. aequifasciatus* includes specimens from Tefé (MNHN 1902–134 and 1902–135) and Santarém (MNHN 1902–130) that would represent the distribution of the western and eastern species, respectively. No attempts were made to extract DNA from these specimens, and the live colour patterns of preserved specimens could not be determined. Therefore, MNHN 1902–130 from Santarém is selected as lectotype of *S. aequifasciatus*, thus restricting the name to the central-eastern species. The specimen is 107 mm SL and has the following meristic data: Dorsal fin, IX.31; anal fin, XIII.30; vertebrae, 13 abdominal, 18 caudal; scales, 56 on row E1, 20 in anterior lateral line, 14 in posterior lateral line. *Symphysodon aequifasciatus* is distinguished from *S. discus* by having a melanic colour pattern of lateral bars of equal intensity and width. *Symphysodon aequifasciatus* does not possess distinct red spots on the anal fin and body, although it may have red reticulated pigmentation primarily on the anal fin. *Symphysodon haraldi* and *S. axelrodi* are synonyms of this species.

The western species is *S. tarzoo* Lyons, 1959, that was described as having distinct red spots on its anal fin and body. The description was based on live

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aquarium specimens from Leticia, Colombia, and no type specimens were apparently preserved (Kullander, 1996; 2003). The availability of the name *tarzoo* was rejected by Schultz (1960) based on the supposed absence of a diagnosis in Lyons (1959). However, several statements in the latter study clearly describe the new species. Kullander (1996) discusses the availability of names for *Symphysodon* species and recognized that the epithet *tarzoo* was available under the International Code of Zoological Nomeclature. Because of the nomenclatural and taxonomic problems in *Symphysodon*, a neotype for *S. tarzoo* was selected. INPA 25960 from Brazil, Est. Amazonas, Rio Jutaí. 1998, S.O. Kullander and E.F.J.G. Ferreira. It is an adult male of 132·4 mm SL and has the following meristic data: Dorsal fin, X.30; anal fin, XIII.31; vertebrae, 14 abdominal, 17 caudal; scales, 58 on row E1, 20 in anterior lateral line, 14 in posterior lateral line. The red spots on the anal fin and in the body distinguishes *S. tarzoo* from all other *Symphysodon* species that have reticulations.

Symphysodon aequifasciatus and *S. tarzoo* overlap in distribution slightly as shown by individuals from the Madeira River that are phenotypically and genetically *S. tarzoo*.

Traditional morphometric characters do not easily distinguish species, although average lateral line scale counts and melanic pigmentation still distinguish *S. discus* and *S. aequifasciatus* (Kullander, 1996). However, the mtDNA sequence shared between *S. discus* and *S. aequifasciatus* from the eastern and the central part of the distribution of the genus must be explained.

This appears to be the only case of a Neotropical cichlid genus with a distribution that, although marked and extensive, is limited to lowland Amazonian habitats and that includes a geographic boundary thought to restrict an endemic species to the western lowland region.

The Amazon historically flowed in the opposite direction from east to west. Evidence documenting this change is summarized by Lundberg *et al.* (1998). The current distribution of *Symphysodon* probably developed due to the changes in drainage pattern caused by tectonic processes. Species of *Symphysodon* are restricted to areas experiencing floods, and it is likely that their unusual body shape is an adaptation to this biotope. Under the proposed history of Lundberg *et al.* (1998), large-scale flood habitats [*e.g.* Lago Santa Lucía (60–42 MYA), Lago Pozo (43–30 MYA) and Lago Pebas (20–11·8 MYA)] occurred first in western regions of present day Amazonia, caused by the initial uplift of the Andes and the diversion of drainages to the north. Such habitats would have extended eastward when the northern drainage route was cut off and when the Amazon began to drain eastwards into the Atlantic as it does now. Given the sample locations, the ranges of *S. aequifasciatus* and *S. tarzoo* are roughly divided by the Purus arch (located between Manaus and Rio Bauana in Fig. 1) with only a few *S. tarzoo* specimens from the Madeira river downstream of the arch, and no *S. aequifasciatus* specimens from locations upstream of the arch. This arch was formed during one of the last phases of Andean uplift (*C.* 3 MYA) and was probably breached when the Amazon changed direction (Lundberg *et al.*, 1998). Arguably, these subterranean arches may have affected organisms in the Amazonian floodplain (Lougheed *et al.*, 1999), although the extent of its influence on surface topology at that time cannot be known. Two scenarios have been suggested: 1) the arch may have forced

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the overlying sediments to form a temporary barrier that isolated ancestral populations of *S. tarzoo* from other *Symphysodon* that subsequently have come into secondary contact, 2) alternatively, arch dynamics may have led to a narrowing of the floodplain that reduced gene flow enough to allow divergence between upstream and downstream forms. This latter scenario is embodied in a parapatric model of divergence between *S. tarzoo* and other *Symphysodon* species. The latter case is supported by geological analyses of the Amazon River today, which showed that where the river flows over arches, such as the Purus arch, water flow becomes slow and entrenched. The valley narrows to <20 km compared to an average breadth of nearly 45 km, the water-surface gradient decreases, sediment is deposited and water flow through the channel is negligible (Mertes *et al.*, 1996).

The shared mtDNA sequences of *S. discus* and *S. aequifasciatus* and the lack of any variation among rhodpsin sequences supports at least two possible explanations for the observed phenotypic variation. 1) Branch lengths in the phylogram (Fig. 3) are consistent with an ancient divergence between upper (above Purus arch) and lower (below Purus arch) Amazonian fishes, and a divergence between the two species in the lower Amazon that is so recent that mtDNA differences between these species could not be found. 2) The genomes of all three species have diverged significantly, but the similarity of the mtDNA of *S. aequifasciatus* and *S. discus* results from introgressive hybridization. Under this scenario, the observed mtDNA sequence probably originally belonged to *S. aequifasciatus* because of the smaller number of sequences obtained for *S. discus*. However, it also possible that an mtDNA sweep may have occurred if there is any bias in the direction of crosses, *e.g.*, due to Haldane's rule. As such, the possibility of the sequences having orginated from *S. discus* should not be ruled out.

The distribution of *Symphysodon* is unusual in consisting of a very large transect across equatorial lowland South America. The habitat consists of major river channels, tributaries and the associated flooded forest, across which continuity should be sufficiently high to maintain gene flow between populations. This adaptation to a narrow ecological niche and the absence of competition from sympatric species may explain why morphology is so relatively constant over such a large range. The different colour forms that have evolved may indicate that mate choice plays a role in the evolution of *Symphysodon*.

The importance of colour vision and the light spectrum in water have been used to explain differentiation through, and subsequent breakdown of, the reproductive isolation between colour forms of African lake cichlids (Seehausen *et al.*, 1997), and colour pattern has been shown to be important for species identity in South American cichlids (Ready *et al.*, 2006). Discus fishes breed in flooded forests, therefore, the different light conditions produced by blackwater, whitewater and clearwater flooded forest habitats (Furch & Junk, 1997) could provide a mechanism for differentiation of populations. *Symphysodon tarzoo* and *S. aequifasciatus* distributions are generally consistent with the distribution of whitewater and clearwater tributaries of the Amazon, while the distribution of *S. discus* is generally consistent with the distribution of blackwater tributaries. Water colour is influenced by the geographical origins of the water, with whitewater originating from the Andes, clearwater from the south bank tributaries (Brazilian shield) and blackwater from the north bank tributaries

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(Guyanan shield and eastern Andes). The latter two sources are believed to have remained unchanged for many millions of years (Lundberg *et al.*, 1998).

Therefore, the current distributions of the species may reflect ancient speciation if flood habitats were isolated prior to the change in direction of flow of the Amazon (possibly with hybridization after secondary contact), or may represent parapatric speciation within the modern Amazon drainage pattern (possibly with a hybrid zone).

There are reports that the bones of discus fishes from whitewater (five *Symphysodon aequifasciata*) and clearwater (four *Symphysodon discus*) habitats differ in chemical composition as a result of the different water chemistry (Geisler & Schneider, 1976). However, the current water conditions in the Amazon are very variable on small spatial and temporal scales. All species of discus fishes can be found in all water types, and this result may merely indicate a plastic ability of these fishes to utilise different chemicals depending on those available in the environment.

In summary, it is likely that the limited species number in the genus *Symphysodon* is a result of the adaptation to the floodwater habitats of the Amazon. Such adaptation has ensured that populations across a large geographical area share a continous habitat resulting in high enough gene flow to prevent much differentiation. Apparently, even major differences between the water types and the effect of large-scale tectonic processes have only provided enough selection pressure for three species to have evolved in this genus.

Further investigation of the genus, with larger sample sizes for molecular analyses, faster evolving nuclear markers and samples from elsewhere in the distribution of the genus, may further improve our understanding of these fishes and the evolutionary and the geohistorical processes that have led to their unusual distribution. The challenging result of the present study is the absence of genetic differentiation between two morphologically distinct species. It confirms the need for complementing molecular analyses with morphological analyses to distinguish discovery of morphologically and genetically distinct, but very similar, species that are geographically separated across an apparently homogeneous habitat without obvious physical present day barriers.

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