Abstract

Phylogenetic relationships of populations and species within *Potamorrhaphis*, a genus of freshwater South American needlefishes, were assessed using mitochondrial cytochrome \( b \) sequences. Samples were obtained from eight widely distributed localities in the Amazon and Orinoco rivers, and represented all three currently recognized species of *Potamorrhaphis*. The phylogeny of haplotypes corresponded imperfectly to current morphological species identities: haplotypes from *P. guianensis*, the most widespread species, did not make up a monophyletic clade. Geography played a strong role in structuring genetic variation: no haplotypes were shared between any localities, indicating restricted gene flow. Possible causes of this pattern include limited dispersal and the effects of current and past geographical barriers. The haplotype phylogeny also showed a complex relationship between fishes from different river basins. Based on the geographical distribution of clades, we hypothesize a connection between the middle Orinoco and Amazon via rivers of the Guianas. More ancient divergence events may have resulted from Miocene alterations of river drainage patterns. We also present limited data for two other Neotropical freshwater needlefish genera: *Belonion* and *Pseudotylosurus*. *Pseudotylosurus* showed evidence of substantial gene flow between distant localities, indicating ecological differences from *Potamorrhaphis*.

Keywords: Amazon, Belonidae, Orinoco, phylogeny, South America

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Introduction

South American rivers contain the most diverse, but also one of the more poorly known, freshwater fish faunas of the world. Significant progress is being made on the taxonomy and systematics of many Neotropical fish groups (Malabarba et al. 1998). However, compared to Palearctic and Neartic fishes (Bernatchez & Wilson 1998), very little is known about the genetics of Neotropical fish populations (the Central American isthmian fauna excepted; Bermingham & Martin 1998). Intraspacific gene genealogies and their biogeographical distributions provide a basis for testing alternative models of speciation and diversification (e.g. Harrison 1991; Templeton 1994; McCune & Lovejoy 1998; Patton & da Silva 1998). Also, intraspacific biogeographical patterns can be used in concert with interspecific and geological data in historical biogeographical analysis (Rosen 1978; Nelson & Platnick 1981; Chernoff 1982; Avise et al. 1987). Thus, biogeographical and gene genealogical data should provide insight into the origins and distribution of the remarkable diversity of Neotropical fishes.

At present, the South American fauna presents unique challenges for these studies. Many taxa remain poorly defined taxonomically, phylogenetically and geographically (Vani & Weitzman 1990); ranges of species may be very large, and sympatric distributions of closely related species or species-complexes complicate interpretation. To develop data useful for analyses of biogeography and speciation, we have investigated Neotropical freshwater needlefishes of the genus *Potamorrhaphis*, a group of small...
insectivorous and piscivorous fishes common in rivers and lakes of South America (Goulding & Carvalho 1984). In contrast to many Neotropical fish genera, Potamorrhaphis includes only three species, each of which is clearly diagnosed morphologically and has been closely studied and revised (Collette 1974a; Collette 1982). The species are allopatrically distributed (Fig. 1) and their total range encompasses many of the major South American drainages (Amazonas, Orinoco, Paraná and rivers of the Guianas). These characteristics make Potamorrhaphis a tractable and potentially informative candidate for biogeographical analysis.

For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). For this study on a representative South American fish taxon, we focused on two main issues. First, we examined the distribution of genetic variation in relation to river drainages and morphologically defined species. P. guianensis (like a number of other Neotropical fish species) occurs in both the Amazon and Orinoco basins, but it is unknown to what extent the populations from these two basins are genetically distinct. It is also not known whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA). Second, we considered whether currently recognized Potamorrhaphis species are monophyletic in terms of their mitochondrial DNA (mtDNA).

Materials and methods

Needlefish specimens were collected in the field by ourselves or colleagues (see Table 1 and Fig. 1 for the list of vouchers and localities – more detailed collection data are available from the authors). Specimens were usually collected at night using a dipnet and flashlight. Whenever possible, multiple individuals were collected from each site. Gill tissue was preserved in buffer comprising 20% dimethylsulphoxide (DMSO) and 0.25 m EDTA, pH 8.0, saturated with NaCl (Seutin et al. 1991). Tissue preserved in this buffer and stored at room temperature has always yielded amplifiable DNA (even after storage for up to 4 years). Voucher specimens were preserved in 10% buffered formalin, transferred to 70% ethanol or 50–55% isopropanol and deposited in museum collections (institutional abbreviations are those provided by Leviton et al. 1985).

For each sample, ~25 mg of tissue was rinsed briefly in water, then DNA purified using QiaGen’s spin-column tissue kit. Briefly, cells were lysed at 55 °C in 20 μL of proteinase K (20 mg/mL) for 3–6 h. Lysate was bound to the spin column membrane and washed twice by centrifugation. DNA was then eluted by centrifugation twice with 200 μL of low-salt buffer. Template for sequencing was amplified by the polymerase chain reaction (PCR) using the cytochrome b primers GLUDG-5', L14725 (CGAAAGCTTCCATCCAACATCTCAARACCGTGGT) and CB3-3', H15580 (GGCAAAATGGAARTATCATTC), where L and H correspond to the light or heavy strand and numbers represent the position of the 3' end of the primer in the human mtDNA sequence (see Palumbi 1996). We used 50 μL reaction mixtures containing 1 μL of DNA, 3 mM MgCl2, 20 mM Tris HCl, pH 8.4, 50 mM KCl, 200 μM of dNTPs, 0.4 μM of each primer and 1 U of Gibco Taq polymerase. Amplifications were usually performed under the following conditions: an initial 30-s denaturation at 95 °C; 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 60 s, and extension at 72 °C for 90 s; and a final 5-min extension at 72 °C. For Belonion, primer CB1-5', L14841 (AAAAAACGCTTCCATCCAAACGATCTCAGCATGAAA) of Kocher et al. (1989) was used instead of GLUDG-5', and the annealing temperature was reduced.
TreeRot (Sorenson 1996) and the branch-and-bound search algorithm. Bootstrap values were calculated using paup* (100 replications using the heuristic search option with 50 replicates of random taxon addition), and TreeRot (Sorensen 1996) and paup* were used to calculate decay indices. Consensus trees, branch lengths and reconstructions of character state changes were calculated and analysed using PAUP* and MacClade (Maddison & Maddison 1992). Sequence divergence was measured in two ways. Uncorrected divergence was calculated using PAUP*, and tree-based divergences were calculated by dividing the patristic distance between taxa (estimated from the most-parsimonious tree(s), or a consensus thereof) by the total number of compared base pairs.

To evaluate comparative levels of support for nodes, each matrix using the branch-and-bound search algorithm. Bootstrap values (Felsenstein 1985) and decay indices (Bremer 1994) were calculated. Bootstrap values were measured in two ways. Uncorrected divergence was calculated using PAUP*, and tree-based divergences were calculated by dividing the patristic distance between taxa (estimated from the most-parsimonious tree(s), or a consensus thereof) by the total number of compared base pairs.

Results

For Potamorrhaphis and Belonion, our aligned matrix for 48 individuals yielded 23 different haplotypes defined by 228 variable sites, 158 of which were parsimony informative. Sequences have been deposited in GenBank (accession nos: AF185070–AF185092 and AF186098–AF186106). All haplotypes were unique to particular localities and were thus named after the localities (or the drainages) where they were collected, and further identified by lower case letters (a, b, c, etc.). Within each locality, the number of different haplotypes ranged from 1 to 5, and uncorrected sequence divergence ranged from 0 to 1% (Table 2); these numbers are not directly comparable because the number of individuals sequenced from different localities varied (Table 1). The two Belonion haplotypes from Rio Atabapo are identical.

Phylogenetic analysis of the 23 Potamorrhaphis and Belonion haplotypes produced 20 equally parsimonious trees of length 373 and a consistency index (CI) of 0.65, excluding uninformative characters. Figure 2 shows a strict consensus of these trees for Potamorrhaphis (the divergence between Belonion haplotypes is shown separately in Fig. 3). We have chosen to represent the relationships between haplotypes as trees rather than networks because branches between localities are relatively long; within localities, some haplotypes could clearly be placed at nodes. From character state optimizations on the most-parsimonious trees, an approximate transition/transversion ratio of 3.5 was observed and 42 amino acid changes were identified.

There was an imperfect relationship between species
identity and haplotype monophyly in *Potamorrhaphis*. Haplotypes from the widespread species *P. guianensis* were not monophyletic because their clade also included the *P. eigenmanni* (*Apere*) haplotypes (Fig. 2). As haplotypes from the same localities are monophyletic and minimally diverged, we presented average sequence divergences between haplotypes from each locality (Table 2). Within *Potamorrhaphis*, the *P. petersi* haplotype (Atabapo) was basal and highly diverged from the other two species (11.2–14.1% tree-based). Within the *P. guianensis* and *P. eigenmanni* clade, divergences ranged from 3.6 to 9.6% (tree-based). Tree-based divergences are usually higher than uncorrected estimates, particularly as distances between taxa increase, because homoplastic changes are included in the calculations. The divergences discussed below are all tree-based.

![Figure 2 Phylogeny and distribution of *Potamorrhaphis* haplotypes. Consensus tree was rooted with *Belonion* (shown separately). Numbers above nodes are decay indices; numbers below nodes are bootstrap proportions; branch lengths correspond to number of changes. Localities: 1, Apure; 2, Santa Rita; 3, Atabapo; 4, Yasuni; 5, Barcelos; 6, Santarém; 7, Belém; 8, Apere.](image-url)
the clade involved the upper Orinoco (Atabapo and Santa Rita) and upper Amazon (Yasuni). Haplotypes from the middle and lower Amazon and Madeira clustered in a large clade that, surprisingly, also included the haplotypes from the Apure (Orinoco). Thus, neither the Orinoco nor the Amazon possess monophyletic clades of haplotypes.

For *Pseudotylosurus*, four distinct haplotypes were determined (the two specimens from Napo had identical sequences). Figure 3 and Table 3 show, respectively, the relationships and divergences between these sequences.

**Table 3** Sequence divergence between localities for *Pseudotylosurus* haplotypes

<table>
<thead>
<tr>
<th></th>
<th>Santarém</th>
<th>Napo</th>
<th>Paraná</th>
<th>Manu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santarém</td>
<td>0.001</td>
<td>0.067</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>Napo</td>
<td>0.067</td>
<td>0.065</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>Paraná</td>
<td>0.061</td>
<td>0.059</td>
<td>0.022</td>
<td></td>
</tr>
</tbody>
</table>

Numbers above the diagonal represent tree-based divergences. Numbers below the diagonal represent uncorrected divergences.

Phylogenetic analysis of *Pseudotylosurus* and its marine sister group produced two trees of 229 steps and a CI of 0.90, excluding uninformative characters (the two trees differed only in the relationships among outgroups). Only a single change distinguished the Napo and Santarém haplotypes, which together form a clade that is 6.1–6.7% diverged from its sister group, Paraná and Manu.

**Discussion**

Most of our data concern *Potamorrhaphis*; thus, we concentrated on this group in the following discussion. Comparisons were then made with *Belonion* and *Pseudotylosurus*.

**Species and genes**

Within each genus, species of Neotropical freshwater needlefishes are conservative in morphology, and hence the number of taxa that have been described is low. *Potamorrhaphis* is widely distributed in the major river systems of South America but includes only three species; one of these, *P. guianensis*, occurs in the Orinoco, Amazon and drainages of the Guianas. Genetic markers, however, reveal that underlying this morphological uniformity is
a considerable amount of DNA diversity. Moreover, this diversity is extremely structured by geography — no haplotypes were shared among any localities.

The phylogenetic relationships of *Potamorrhaphis* mtDNA haplotypes corresponded somewhat imperfectly with current species definitions within the genus. The single *P. petersi* haplotype (Atabapo) is basal and rather distantly related to the other samples, thus genetic data confirmed the evolutionary distinctiveness of this species. On the other hand, *P. eigenmanni* haplotypes (Apere) were nested within *P. guianensis*. There are at least two interpretations of the latter pattern. One possibility is that speciation is recent and lineage sorting is incomplete. Various modes of speciation produce haplotype phylogenies that are nonreciprocally monophyletic until the passage of sufficient time (Neigel & Avise 1986; Harrison 1991). Thus, given a sufficiently large interval, new collections from Santa Rita, Yasuni, Belém and the other *P. guianensis* localities might be hypothesized to yield a monophyletic group of haplotypes, to the exclusion of Apere. However, the expectation of eventual reciprocal monophony between sister species depends on continuing gene flow between *P. guianensis* sites. Both the substantial divergence between haplotypes from distant localities and the current presence of barriers between some populations (see below), argues against this scenario, and suggests an alternative interpretation: that many of the localities represent independently evolving units. From this perspective, *P. eigenmanni* may merit species status for pragmatic reasons (by possessing morphological features that permit its identification), but is no more an independent evolutionary entity than are several of the other diagnosable haplotype clades. In this regard, the separate lineages within *P. guianensis* might be considered cryptic species.

Untangling the relationship among morphology, genetics and geography will require denser sampling. Considerable divergence exists between haplotypes from different localities; however, these localities are widely distributed across the South American continent. We need collections from intervening habitats to determine which localities are simply isolated by distance and form components of larger populations, and which are independent units, isolated by particular landscape features.

Gene flow and biogeography

The biogeography of the *Potamorrhaphis* phylogeny shows two interesting and related patterns. First, each locality has a monophyletic clade of haplotypes that is deeply divergent from other localities in the same river basin. This is despite the apparent absence of barriers to gene flow in at least some cases (for example, between Belém and Santaréam). Second, relationships between haplotypes may cut across river basins, such that the closest relative of an Orinoco haplotype may be from the Amazon, rather than the Orinoco.

Why are localities within the same river system divergent? Some Amazonian fishes undertake long-distance migrations along major South American rivers and tributaries (Goulding 1980) and might be expected to show a lack of association between clades of genes and particular geographical areas. For example, G. Orti & E. Bermingham (manuscript in preparation) have found little geographical structure among haplotype clades in the highly migratory fish *Prochilodus lineatus* in spite of its > 1500-km distribution in the Paraná–Paraguay basin. The geography of the *Potamorrhaphis* genealogy shows a different pattern. No sharing of haplotypes was observed, even between localities that are situated along uninterrupted stretches of open river, such as Belém and Santaréam. This pattern is characteristic of a species with limited dispersal, at least on the scale of localities in the study. *Potamorrhaphis* is observed more frequently in backwater lakes and streams than in major rivers (N. R. Lovejoy & M. L. J. de Araújo, personal observations), thus the open water of larger rivers may represent a significant barrier to gene flow. A related explanation for the level of divergence between some localities is isolation-by-distance. For example, Yasuni, from the upper Amazon, is more than 2000 km distant from the other Amazon basin localities (Barcelos, Santaréam and Belém).

Past and present geographical barriers probably also influence the genetic structure of *Potamorrhaphis*. In some cases, barriers along rivers may be important. A series of rapids (Atures) separate the Atabapo (upper Orinoco) from downstream Santa Rita, and may represent a formidable obstacle to needlefish gene flow. Similarly, the Apere locality in the Rio Mamoré is separated from the rest of the Amazon by rapids of the upper Rio Madeira. These overt geological structures may play an important role in defining borders for fish populations. Patton & da Silva (1998) have also demonstrated the potential importance of more cryptic landscape features. Their studies of small-bodied rodents and marsupials along the Rio Juruá in western Brazil identified a geographical break in mitochondrial haplotype distributions that corresponds to the location of an underlying structural arch separating two Amazonian sub-basins. Such tectonic features might also organize genetic variation in fishes — more sampling will be required to discover whether this is the case. A further important factor affecting patterns of haplotype phylogeny and distribution is the history of connections between river basins. The evolution of fishes is intimately tied to the geological changes that have affected river drainage patterns. Recent work confirms the dynamic nature of South American rivers and basins at both regional (e.g. Räsänen et al. 1987) and continental
During the Miocene, in particular, ancient drainages that had persisted for many millions of years shifted to the modern arrangement seen today (Hoorn et al. 1995; Diaz de Gamero 1996). A null hypothesis for taxa distributed in rivers is that their genetic relationships should reflect current hydrological connections (Meffe & Vrijenhoek 1988; Hurwood & Hughes 1998). Thus, fishes from the same river basin are expected to be most closely related, and, within basins, populations from adjacent localities should be most similar. For *Potamorrhaphis*, this simple model is not upheld. The haplotype phylogeny shows that individuals from the same large river system are not each other’s closest relatives. Notably, Apure individuals from the Orinoco drainage are closely related to individuals from the Amazon (Santa Rém), and Rio Negro (Barcelos), rather than to individuals from Santa Rita, a much closer Orinoco locality (see Fig. 2). To further examine these patterns, the *Potamorrhaphis* phylogeny can be compared with current and previous connections between drainages, and to biogeographical patterns from other aquatic organisms.

Current connections between the Amazon basin and the Orinoco and Guianas occur via: (i) the Rio Casiquiare, which connects the upper Rio Negro (Amazon) to the upper Orinoco; and (ii) the inundated savannah of the Rupununi, which connects the Rio Branco (Amazon) to the Guianan Rio Essequibo (Lowe-McConnell 1964). The basal position of *P. petersi* (Fig. 2), combined with its allopatric distribution (Fig. 1), suggests that the Rio Casiquiare route (and also the Guaviare/Uaupés route — see Roberts 1972) was closed early in the history of *Potamorrhaphis* phylogeny. However, the relationship between the Apure (Orinoco), Santa Rém (Amazon) and Barcelos (Rio Negro) haplotypes suggests a continued connection between the Amazon and Orinoco after this period. We therefore hypothesize that this link took place through the Guianas drainages and the Rupununi or Mapuera (Fig. 4). A similar pattern has been observed in freshwater crabs of the genus *Fredius* (Rodriguez & Pereira 1992; Rodriguez & Campos 1998). In this taxon, a reconstructed area cladogram shows the Orinoco to be basal to a clade including the rivers of the Guianas (Essequibo, Cuyuni and Atlantic drainages of Guyana, Surinam and French Guyana) and the Amazon. Clearly, in order to test the pattern in *Potamorrhaphis*, we need samples from the lower Orinoco, the Guianas and the Rio Branco; haplotypes from these localities should fall within clade B of Fig. 4.

To determine the extent to which geology and changes in river drainage patterns have produced the patterns outlined above, a greater number of unrelated groups with similar distributions need to be considered. Also, increased sampling of individuals and the use of appropriate...
molecular markers might allow tests of alternative modes of speciation and population histories: e.g., recent range expansions vs. allopatric divergence (Harrison 1991; Templeton 1998). An advantage of the molecular approach to biogeography is the potential to estimate the approximate age of nodes. Unfortunately, we cannot at present calibrate a rate of cytochrome *b* evolution specifically for *Potamorrhaphis* because no clearly dated geological event can be associated with a particular divergence. However, even with a rapid rate of molecular evolution, the large divergences between some haplotypes are suggestive of a comparatively ancient age. Using a “teleost” rate of 1.2% per million years (Bermingham et al. 1997), the origin of *P. petersi* dates to the Late Miocene, and might therefore have been associated with changes in river drainage patterns initiated by the orogeny of the Caribbean Andes (Hoorn et al. 1998).

**Pseudotylosurus and Belonion**

During the course of this study, we collected data for two other genera of endemic Neotropical freshwater needlefishes, *Pseudotylosurus* and *Belonion*. *Belonion* includes two species of very small (maximum body length = 42 mm), possibly paedomorphic, needlefishes that are infrequently collected (Collette 1986). Our samples from the Atalaia and Rio Negro (Barcelos) showed significant haplotype divergence (> 10%), indicative of a history of isolation whose duration parallels that of *Potamorrhaphis*.

*Pseudotylosurus*, on the other hand, is a genus of larger fishes (maximum body length = 275 mm, Collette 1974b), compared with *Potamorrhaphis* (maximum body length = 259 mm; Collette 1982). Unlike *Potamorrhaphis* species, which appear to be most common in smaller rivers and lakes (however, see Goulding & Carvalho 1984), *Pseudotylosurus* species are often found in open-water habitats of large rivers (N. R. Lovejoy & M. L. J. de Araújo, personal observations). This ecological difference may be reflected in the haplotype phylogeny for the group. Like *Potamorrhaphis*, there is considerable geographical differentiation of *Pseudotylosurus* haplotypes: Manu, Napo and Paraná are diverged from one another by 2.2–6.7%. Surprisingly, however, the Santarém haplotype differs by only a 1-bp change from Napo (divergence < 0.2%). This indicates that *Pseudotylosurus* individuals from Napo and Santarém, although separated by more than 2000 km, may have shared a common ancestor quite recently. We therefore hypothesize that *Pseudotylosurus* is more mobile than *Potamorrhaphis* and has correspondingly more expansive geographical boundaries that define populations. Although based on minimal data, this hypothesis will be easy to test: haplotypes from *Pseudotylosurus* individuals from intermediate locations along the Amazon should fall within the Napo/Santarém clade and show minimal geographical structure.

Our hypothesis of extensive gene flow within *Pseudotylosurus* is not falsified by the clear divergence of the Manu/Paraná clade. The Rio Manu drains into the upper Madeira, which has been isolated from the rest of the Amazon by a series of rapids, while the Paraná represents a different basin from the Amazon. The close relationship between the Manu and Paraná haplotypes reflects a historical relationship between the upper Madeira and Paraná that is recorded by the distribution of *P. eigenmanni* and several other fish groups (see Schaefer 1997).

**Conclusion**

Large differences in cytochrome *b* sequences characterize *Potamorrhaphis* specimens from different river localities. Surprisingly, individuals from the upper, middle and lower reaches of the Amazon are not each other’s closest relatives, but are, in some cases, more closely related to populations from other drainages. These patterns suggest that *Potamorrhaphis* may be a useful indicator of historical connections between rivers since the late Miocene. A hypothesis proposed here is that the connection between the Orinoco and Amazon through the Guianas was closed more recently than the connection through the Rio Casiquiare. Such events have structured genetic variation in *Potamorrhaphis*, and may have contributed to speciation in other taxa. Thus, ongoing investigations should improve our understanding of the recent diversification of Neotropical fishes.

South American rivers may represent highways to some migratory fishes, allowing widespread gene flow over thousands of km. For *Potamorrhaphis*, this is not the case, but in the related taxon, *Pseudotylosurus*, we have a glimpse of a dramatically different pattern. Clearly, the ecology of fishes can have profound affects on the geographical distribution of genetic variation (see, for example, Avise et al. 1987). Understanding this variation is important for both conservation (Moriya 1995; da Silva & Patton 1998) and management. Because Neotropical fishes are an important natural resource, collection of more data for different taxa is a clear objective. However, the vast size of South American drainage systems, the remote-ness of many biogeographically important localities and the extraordinary richness of the fish fauna, present a considerable challenge.

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