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TESTING FOR UNEQUAL RATES OF MORPHOLOGICAL DIVERSIFICATION IN THE ABSENCE OF A DETAILED PHYLOGENY: A CASE STUDY FROM CHARACIFORM FISHES

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This study develops the random phylogenies rate test (RAPRATE), a likelihood method that simulates morphological evolution along randomly generated phylogenies, and uses it to determine whether a considerable difference in morphological diversity between two sister clades of South American fishes should be taken as evidence of differing rates of morphological change or lineage turnover. Despite identical ages of origin, similar species richness, and sympatric geographic distributions, the morphological and ecological diversity of the superfamily Anostomoidea exceeds that of the Curimatoidea. The test shows with 90% confidence (using variance among species as the measure of morphological diversity) or 99% confidence (using volume of occupied morphospace) that the rate of morphological change per unit time in the Anostomoidea likely exceeded that of the Curimatoidea. Variation in the rate of lineage turnover (speciation and extinction rates) is not found to affect greatly the morphological diversity of simulated clades and is not a likely explanation of the observed difference in morphological diversity in this case study. Though a 17% or greater delay in the onset of diversification in the Curimatoidea remains a possible alternative explanation of unequal morphological diversification, further simulations suggest that two clades drawn from the possible treespace of the Anostomoidea and Curimatoidea will rarely differ so greatly in the onset of diversification. Several uniquely derived morphological and ecological features of the Anostomoidea and Curimatoidea may have accelerated or decelerated their rate of morphological change, including a marked lengthening of the quadrate that may have relaxed structural constraints on the evolution of the anostomoid jaw.

KEY WORDS: **Adaptive radiation, Brownian evolution, disparity, evolutionary rates, freshwater fishes, South America, tempo and mode.**

Why is morphological diversity distributed unevenly across the tree of life? Many groups of organisms, including the Lake Tanganyika cichlids (Fryer and Iles 1972; Chakrabarty 2005), the Hawaiian silversword plants (Robichaux et al. 1990), the *Anolis* lizards (Warheit et al. 1999), and the domed-nest building tanagers (Burns et al. 2002), the lineage that includes Darwin's celebrated finches (Lack 1947), have evolved a range of morphologies that dwarfs that of their close relatives. Shifts in rates of speciation, extinction and morphological change within evolving lineages

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can increase or decrease morphological diversity in descendant clades (Simpson 1944; Raup and Gould 1974; Foote 1996; Foote 1997; Roopnarine 2003) and may help explain the unequal morphological diversification of closely related clades. However, the stochastic nature of evolution implies that evolutionary scenarios with unvarying rates of cladogenesis and morphological evolution can produce clades with widely varying morphological diversities (Gould et al. 1977; Raup 1977, 1985; Foote 1993). It can therefore prove difficult to determine whether observed differences in morphological diversity (or any other evolutionary endpoint) are best explained as outcomes of the same or different evolutionary scenarios.

Most extant tests identifying clades with evolutionary rates differing from their close relatives require extensive phylogenetic information (Garland 1992; Purvis et al. 1995; Wagner 1997; Barraclough et al. 1998a; Collar et al. 2005; O'Meara et al. 2006). Lacking a full phylogenetic reconstruction, one can use combined simulations of cladogenesis and morphological diversification to test for rate differences provided that estimates of clade age, species richness, and morphological diversity are available (Ackerly and Nyffeler 2004). Though the power of tests that simulate phylogenies remains low compared to the power of methods that use explicit phylogenetic reconstructions, simulation methods can be applied to the large number of case studies in which the phylogeny is unknown, incomplete, or poorly supported (Martins 1996).

The central goals of this study are (1) to develop a simulation-based likelihood method, the random phylogenies rate test (RAPRATE), that tests for heterogeneity in evolutionary rates in the absence of a detailed phylogeny and (2) apply that method to the Anostomoidea and Curimatoidea, sister clades of South American fishes that differ considerably in morphological diversity but that lack a comprehensive phylogeny. These fishes represent a natural experiment and an ideal case study because monophyly, equal species richness, and sympatry rule out different phylogenetic backgrounds, dissimilar clade ages, unequal net lineage diversification rates, and different environmental or geographic histories as primary agents of unequal morphological diversification. The large number of similarities between the clades reduces the number of parameters that must be considered when modeling their evolution and simplifies the analysis of their case study. The method can be generalized to clades that are not sisters as long as relative ages can be estimated.

At least three scenarios can explain the unequal morphological diversification of clades. First, the rate of morphological evolution (average morphological change per lineage per unit time) could have differed. Felsenstein (1985) demonstrated how the value of a Brownian rate parameter affects the subsequent morphological diversification of simulated clades, and many papers have subsequently used a Brownian model of morphological change when testing for rate heterogeneity (Garland 1992; Mooers et al. 1999; Collar et al. 2005; O'Meara et al. 2006). In this first scenario, more often than not the clade with a higher morphological diversity experienced a higher rate of morphological evolution.

Second, variation in the historical rate of lineage turnover (combined speciation and extinction rates) can produce unequal morphological diversities. An increase in turnover rates concentrates nodes connecting surviving species near the modern time horizon (O'Meara et al. 2006) and thereby decreases average crown clade age, divergence time among lineages (Raup 1983), and node age. Ricklefs (2006) demonstrated that a decrease in the average age of nodes within simulated clades decreases average morphological variance, with average node age better predicting morphological variance than does clade age. In this second scenario, the expectation is that clades with higher morphological diversities experienced lower turnover rates.

Finally, it is well known that a clade's morphological diversity (variance) correlates with the time elapsed during its evolution (Felsenstein 1985; Valentine et al. 1994; Gavrilets 1999; Collar et al. 2005). On average, older clades have a higher morphological diversity. Even in the case of sister clades, a delay in the onset of diversification (defined as the timing of the first bifurcation within a clade after separation from its sister) in either clade will give that clade less time to accumulate variance. The clade with a delayed onset of diversification will more often than not have a younger crown group and a lower morphological diversity (Collar et al. 2005). In this third scenario, explanation of unequal diversification may not require heterogeneity in the rates of morphological change or lineage turnover.

In any comparison, the clade with a larger morphological diversity most likely experienced a higher rate of morphological change, a lower rate of lineage turnover, an earlier onset of diversification, or possibly more than one of these, but the converses are still possible because evolution is highly stochastic (Raup and Gould 1974; Raup 1977; Slowinski and Guyer 1989). The underlying rates of lineage turnover or morphological change or the onsets of diversification could have been identical or the more diverse clade could have had a higher turnover rate, a later onset of diversification, or a lower rate of morphological change. At the same time, evolution is not infinitely stochastic, and some possible rate scenarios are less likely than others.

This analysis determines whether some rate scenarios are significantly better supported than others by (1) quantifying the morphological diversity of the Anostomoidea and Curimatoidea, (2) calculating probabilities that each clade's observed morphological diversity evolved under various rates of morphological change and lineage turnover by simulating evolution in an empirically determined morphospace on many possible phylogenies, (3) determining via likelihood tests, which have been employed in many similar studies (Sanderson and Donoghue 1994; Wagner 1997;

Sims and McConway 2003) whether hypotheses of higher rates of morphological change (scenario 1) or lower rates of turnover (scenario 2) in the Anostomoidea fit the data significantly better than their converses, and (4) determining the extent to which simulating a delayed onset of diversification in the Curimatoidea erodes confidence in a conclusion of a higher rate of morphological change in the Anostomoidea (scenario 3).

Materials and Methods

STUDY SYSTEM

The families Anostomidae, Chilodontidae, Curimatidae, and Prochilodontidae form a monophyletic lineage of South American freshwater fishes within the Characiformes, the order which also contains the tetras and piranhas (Vari 1983). Members of all four families inhabit most river systems throughout tropical and subtropical South America and southern Central America (Reis et al. 2003). The Anostomidae and Chilodontidae (hereafter, superfamily Anostomoidea) and the Curimatidae and Prochilodontidae (hereafter, Curimatoidea) are sister clades (Fig. 1; Vari 1983, 1989; Castro and Vari 2004). Consequently, they share a common phylogenetic background and have evolved independently for the same length of time (for discussion of the equivalent ages of sister clades, see Mayden 1986; Brooks and McLennan 1991; Barraclough et al. 1998b). The usage of the term Anostomoidea herein differs from that of Buckup (1998) and Calcagnotto et al. (2005), who equated the term with the entire assemblage of the four families referenced above.

COLOUR FIG.

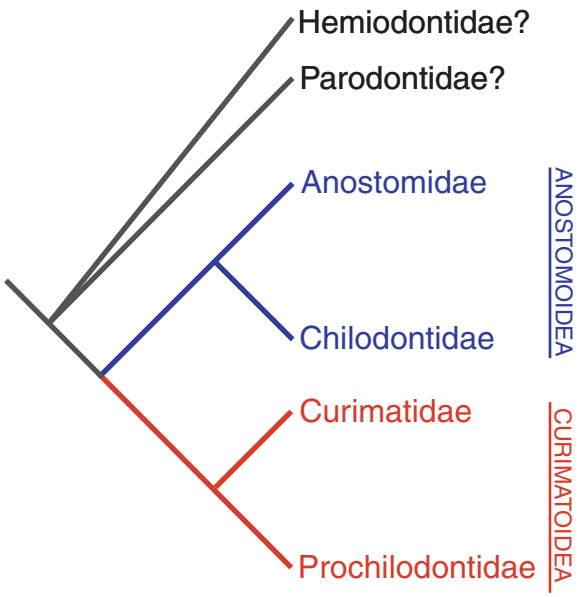

Figure 1. Phylogenetic relationships among six characiform families. Relationships among the Anostomoidea (Anostomidae and Chilodontidae, in blue) and Curimatoidea (Curimatidae and Prochilodontidae, in red) are based on parsimony analyses of more than 100 morphological characters (Vari 1983, 1989; Castro and Vari 2004). A close relationship of the Hemiodontidae and/or Parodontidae to the Anostomoidea, Curimatoidea and/or to each other has been suggested on morphological (Roberts 1974; Buckup 1998) and molecular grounds (Calcagnotto et al. 2005).

Though both clades possess similar species richness, the 138 species in the Anostomoidea have significantly more morphological diversity than the 120 species in the Curimatoidea. Species richnesses were compiled from Reis et al. (2003). Species in the Anostomoidea have highly differentiated teeth and jaws, including mouths which face forwards, downwards, upwards, and even backwards in *Sartor* (Fig. 2D; Myers 1950; Myers and Carvalho 1959; Géry 1977). The Anostomoidea includes generalist feeders, herbivores, insectivores, and species that consume fish scales and freshwater sponges (Géry 1977; Goulding 1980; Taphorn 1992; Santos and Rosa 1998; Balassa et al. 2004). Conversely, all members of the Curimatoidea feed on detritus and organic debris, lack teeth on their jawbones as adults, and possess one of only two distinctive jaw morphologies (Fig. 3; Géry 1977; Vari 1989; Castro and Vari 2004).

Despite the equality of overall ages, the timing of the initial split between the superfamilies and subsequent branching events within each is difficult to determine. Phylogenetic information is very incomplete, particularly for the Anostomoidea, which has never been the subject of a full phylogenetic study. The curimatoid fossil *Cyphocharax mosesi* from the late Oligocene and early Miocene places a minimum age for the most recent common ancestor of the two lineages at approximately 22.5 million years (Reis 1998). Because *Cyphocharax mosesi* is a member of an extant genus, the minimum date of the crown clade of the Curimatoidea is also 22.5 million years. The derived position of *Cyphocharax* within the curimatoid phylogeny (Vari 1989) and the fact that South American characiform fossils are minimally of Maastrichtian age (~70 million years, Lundberg 1998) suggest that the basal split between the Anostomoidea and Curimatoidea and much of the diversification within the Curimatoidea occurred well before the preservation of *C. mosesi*.

Two recent phylogenies for the Characiformes place the Anostomoidea and Curimatoidea among the most basal members of that order (Buckup 1998; Hubert et al. 2005), whereas two others place them in a somewhat more derived position (Orti and Meyer 1997; Calcagnotto et al. 2005). None of these phylogenies contradicts a most recent common anostomoid–curimatoid ancestor that is significantly older than the minimum estimate provided by *Cyphocharax mosesi*. A major group of African characiform fishes, the Alestidae, is nested deeply within a clade of South American characins (Calcagnotto et al. 2005; Zanata and Vari 2005) and has a minimum age of 90–112 million years (Zanata and Vari 2005). The derived position of the African Alestidae taken together with other fossil and phylogenetic evidence indicates that

COLOUR FIG.

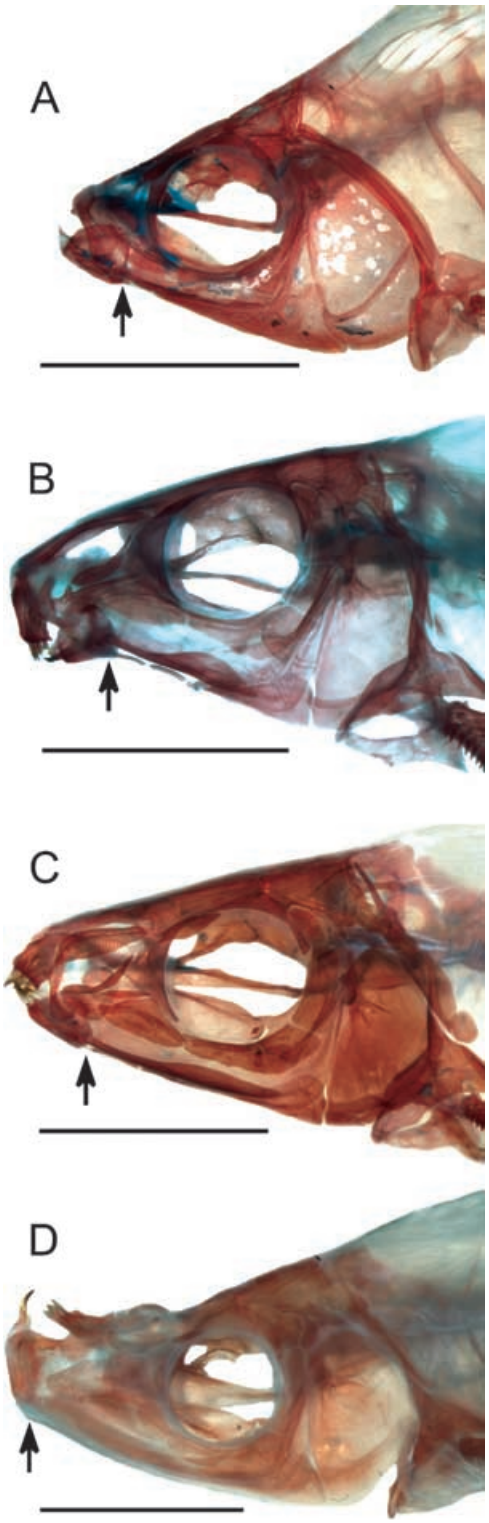

Figure 2. Cleared and stained skulls illustrating the considerable range of morphological variation within the Anostomoidea. Gill arches, eye, and hyoid series removed. Scale bar indicates 1 cm. Arrows indicate positions of lower jaw (anguloarticular/quadrate) joints. (A) FMNH 102061 *Chilodus punctatus*, Chilodontidae, (B) INPA 6706 *Leporinus pachycheilus*, Anostomidae, (C) INPA 15371, *Leporinus aripuanaensis*, Anostomidae *aripuanaensis*, (D) INPA 1168, paratype of *Sartor elongatus*, Anostomidae.

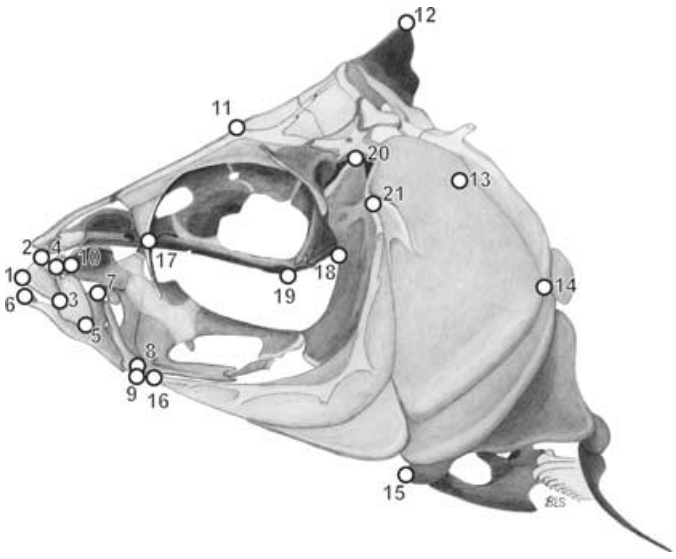

Figure 3. Left lateral view of neurocranium and suspensorium of FMNH 101529, *Curimatella alburna*, Curimatidae, with gill arches, eye, infraorbitals, and hyoid series removed. Numbered dots indicate the following landmarks used in morphometric analysis: (1) anterior limit of premaxilla, (2) tip of ascending process of premaxilla, (3) posteroventral corner of premaxilla, (4) dorsal tip of maxilla, (5) ventral tip of maxilla, (6) anterior limit of dentary, (7) posterodorsal corner of dentary, (8) anguloarticular/quadrate joint, (9) retroarticular, (10) anterior of palatine, (11) epiphyseal bar, (12) tip of supraoccipital crest, (13) joint of basioccipital with first vertebra (obscured by opercle), (14) posterior point on opercle, (15) anterior limit of cleithrum, (16) anterior limit of interopercle (covered by preopercle), (17) anterior of bony orbit, (18) posterior of bony orbit, (19) bend of parasphenoid (attachment point of pharyngeal jaws), (20) dorsal limit of hyomandibular, and (21) joint of hyomandibular and opercle.

significant cladogenesis occurred within the Characiformes prior to or contemporaneous with separation of the South American and African continents at approximately 90 million years ago (Fink and Fink 1981; Lundberg et al. 1998; Calcagnotto et al. 2005). Overall the fossil, biogeographic and phylogenetic evidence implies a Cretaceous radiation of the major clades in the Characiformes and a basal split between the Anostomoidea and Curimatoidea that took place well before the Eocene, and possibly as early as the Cretaceous. Dating the radiation more precisely is currently impossible.

QUANTIFICATION OF MORPHOLOGICAL DIVERSITY

Morphological diversity was measured in a morphospace derived from the configuration of 21 skull landmarks (Fig. 3) located on radiographs in TpsDig version 1.40 (Rohlf 2004a). Though the functional and adaptive implications of the morphological variation quantified in this study have not been examined in detail, skull bones support a diversity of functions including food acquisition and processing, respiration, sight, hearing, olfaction, and

protection of the brain. The wealth of biological functions dependent on skull morphology indicates that the morphological variation of skulls often has adaptive significance and is frequently of biological interest (Schluter 2000; Losos and Miles 2002). Many of the landmarks were assumed to be functionally significant, marking joints such as the hinge of the lower jaw on the quadrate (landmark 8) or the pivot around which the opercle rotates (landmark 21) which have been used in biomechanical models of jaw function (Hulsey and Wainwright 2002; Westneat 2003, 2006). Others (such as landmarks 11, 12, 14, 15) outlined the general shape of the head. The results obtained herein describe only variation in the head and oral regions; future investigation of other systems, such as coloration, behavior, body shape, and gill arch osteology may reveal different or similar patterns of diversity.

Analysis included 83 anostomoid and 68 curimatoid species representing about 60% of the species diversity and every nominal genus and subgenus in each superfamily. The selected species represented all externally obvious morphological variants and were assumed to capture nearly the entire range of morphological variation. In total, 1263 specimens were included (see online Table S1). Another 363 specimens were imaged but excluded from analysis because they were tilted during the x-ray procedure and produced a skewed image.

A generalized least-squares Procrustes consensus configuration (Rohlf and Slice 1990) of up to 20 specimens represented the characteristic skull shape of each species. Although most points in morphospace represented a consensus of several conspecific individuals (mean of eight specimens per species), 20 of the 151 points represented only a single individual. Each species' consensus skull configuration was aligned to a second among-species Procrustes consensus to eliminate variation among species due to scale, rotation, translation, and shearing. The remaining shape variation among species was described with relative warp analysis (Rohlf 1993) in the program TpsRelw version 1.39 (Rohlf 2004b), yielding a series of independent eigenvectors, or relative warps (RWs). A regression of distance among species in the relative warp tangent plane onto Procrustes distance in the program TpsSmall version 1.20 (Rohlf 2003) confirmed that the relative warp procedure did not introduce a significant shape distortion. The relative warps that described a greater percentage of variance than would be expected via a broken stick model (Frontier 1976; Jackson 1993) were identified with the "Brokestk" MATLAB script (Strauss 1999) and formed the morphospace axes.

Because no single measure of morphological diversity can capture all of the information contained in a morphospace distribution, it is important to use multiple diversity measures (Wills et al. 1994; Foote 1997; Ciampaglio et al. 2001) and to compare estimates of the total range of morphospace occupied as well as measurements of the dispersion and packing of species within that space (Foote 1994; Neige 2003; McClain et al. 2004). Diversity in the empirical morphospace was measured with multidimensional variance (Van Valen 1974; Foote 1993) which estimates the average dispersion of a group of species from the center of the group's distribution, and occupied morphospace volume (Briggs et al. 1992; Wills et al. 1994), which estimates the range of morphospace spanned by a group of species without regard to the number of species occupying that space. A hypercubic approximation of volume equal to the product of the ranges of values on each morphospace axis was used. Because both observed clades have reasonably large ranges on all four morphospace axes (see Results), the problem of hypervolumes collapsing to zero when the range on any axis approaches zero (Van Valen 1974) was avoided. The standard error (SE) of the estimates of volume and variance was evaluated as the standard deviation of those values in 1000 bootstrap iterations (Efron and Tibshirani 1993).

In addition to the simulation-based tests described below, the hypothesis that the anostomoid sample variance equaled the curimatoid sample variance was tested with Levene's test, which is preferred over the F-test when the measured distributions cannot be assumed to be normal (Van Valen 1978; Garland et al. 1993; Hutcheon and Garland 2004), at a 95% critical value. Neither Levene's test nor the F-test, however, accounts for the dependence of datapoints (species) on the underlying phylogenetic structure. Garland et al. (1993) developed an alternative to the F-test which accounts for the dependence of morphology on phylogeny, but their method requires much more phylogenetic resolution than is currently available for the Anostomoidea and Curimatoidea. Ackerly and Nyffeler (2004) developed a phylogenetically corrected F-test based on simulated phylogenies, but their test was designed for a univariate case and does not account for the underlying morphospace structure in particular case studies. The simulation method outlined below accounts for the dependence of morphology on phylogeny, explicitly models multivariate evolution in an empirical morphospace, and is most appropriate in this application.

SIMULATIONS

The RAPRATE method described below is an example of inverse modeling using likelihood, in which one determines which of several models and the associated parameter values is most likely to have produced the observed data (reviewed in Oakley 2003). The data in this case are the measured variance, volume, and species richness of the Anostomoidea and Curimatoidea, and the tested models are variations of a linked Markovian simulation of cladogenesis (e.g., Gould et al. 1977; Raup 1977, 1985; Slowinski and Guyer 1989) and Brownian simulation of morphological diversification (in the spirit of Raup and Gould 1974; Felsenstein 1985; Foote 1993; Harmon et al. 2003; Pie and Weitz 2005; and many others). There is no known mathematical distribution (such as the Poisson) that can predict the probabilities of generating clades

with specific morphological diversities; thus, a simulation-based approach is necessary.

Simulations were accomplished using the Matlab script "morphtreegen" (available upon request, see Ricklefs 2006 for another application of morphtreegen), which takes the following parameters as inputs: number of timesteps (t), speciation rate (p), extinction rate (q), rate of morphological change (r), upper and lower bounds on species richness (*maxsize* and *minsize*), and morphospace eigenvalues (λ). Values for the model's parameters were chosen to match the available data on species richness, clade age, and morphological diversity as closely as possible. Rationales for the chosen values are discussed in a separate section below.

A single run of morphtreegen begins with a single modeled lineage (species) positioned at zero on all morphospace axes. In each timestep, each lineage present in the analysis has probability of branching p, going extinct q, or persisting, $1-(p+q)$. If a lineage branches, the simulation adds a morphological copy of that lineage, and both the original and the new lineage will evolve independently thereafter. For each lineage, random numbers corresponding to each morphospace axis are drawn from a normal distribution centered on 0 with variance 1 and standard deviation 1. Those numbers are multiplied by $(r\lambda)^{0.5}$, where r is a Brownian rate parameter controlling variance in morphological step size and λ is the eigenvalue of each morphospace axis. Because r is scaled by λ, morphological change will, on average, be higher on the axes with larger eigenvalues. The multiplication results in the morphological change experienced by each lineage in a single timestep, and these values are added to the values representing the current position of the lineage in morphospace. The direction of change on each axis is independent of change on all other axes. The expected (mean) morphological change in a lineage is equal in the originating and subsequent timesteps, so this is an anagenetic rather than punctuational model of evolution.

The simulation iterates for $t-1$ additional timesteps. If all lineages go extinct before t timesteps are simulated, the simulation begins again. After the last timestep, the simulation counts the number of living lineages and checks whether the number of living lineages equals or falls between the parameters *minsize* and *maxsize*. If too many or too few lineages are present the simulation discards the current clade and repeats until a clade of the desired size is produced. Once a clade with the desired species richness is obtained, the simulation outputs a matrix containing the final morphologies (positions in morphospace) of all living and extinct lineages and calculates the morphological diversity (variance and volume) of the surviving lineages.

PARAMETER CHOICES

The number of timesteps (t) was set to 90 in all simulations because 90 million years is a rough upper bound on the age of the Curimatoidea and Anostomoidea. Whether or not each of the 90 timesteps can be interpreted accurately as one million years is irrelevant; it is crucial only that clades evolve for the same number of timesteps in all simulations because the Anostomoidea and Curimatoidea are sister clades and have evolved independently for the same length of time. The equivalence of t in all simulations does not mandate that all simulated clades have equal onsets of diversification or crown clade ages.

The mean number of extant lineages in simulated clades after t timesteps (assuming a single starting lineage and conditioned on the clades' survival to the end of the simulation) depends on the values of p (speciation) and q (extinction) and can be calculated via Raup's (1985) equation (A25). For any given value of p only a single value of q will yield any particular desired mean number of lineages (Raup 1985). Because of the one-to-one mapping of p to q their combination can be treated as a single parameter in this application, the turnover rate pq.

Given that the Anostomoidea and Curimatoidea both have a modern diversity of approximately 130 lineages and the number of timesteps was chosen to be 90, Raup's (1985) equation (A25) was used to identify five pq that yielded mean 130 lineages in 90 timesteps (Table 1). By choosing among these specific values of pq, turnover rate was allowed to vary while ensuring that most of the simulated phylogenies would have a number of surviving taxa similar to the species richness of the Anostomoidea and Curimatoidea. Because of the clades' similar species richnesses, it was possible to maintain the same set of potential values of p and q across this whole analysis. The five rate sets ranged from a pure branching or Yule model (Yule 1924) with zero turnover ($p = 0.0541$, $q = 0$) to a rate set that generated relatively high lineage turnover with over 80% of the lineages ever existing within the clade having gone extinct in a typical simulation ($p = 0.2500$, $q = 0.2183$). Even higher turnover rates could have been simulated, but extremely high turnover rates send Markovian simulations into chaotic episodes of explosive speciation and rapid extinction that have been suggested to be biologically unrealistic (Magallón and Sanderson 2001).

Table 1. Values of *p* (speciation rate), *q* (extinction rate), and *r* (rate of morphological change) used in this analysis. The parameters *p* and *q* varied dependently; five values of *pq* (turnover rate) were considered, each yielding a mean 130 surviving lineages in 90 timesteps (Raup 1985). The parameter *r* varied independently of *pq*.

p	q	$r\times10^{-6}$			
0.0541	0.0000	5	35	65	95
0.1000	0.0546	10	40	70	100
0.1500	0.1105	15	45	75	105
0.2000	0.1648	20	50	80	110
0.2500	0.2183	25	55	85	115
		30	60	90	120

Only simulation runs yielding between 110 and 150 extant lineages at the end of the simulation (the *minsize* and *maxsize* parameters in morphtreegen) were analyzed. All other runs were discarded. This conditioning tailored the analysis to consider only the portion of treespace that could contain the true phylogenies for the Anostomoidea and Curimatoidea. Alfaro et al. (2004) recently used similar, if more stringent, conditioning to ensure that simulated clades match real clades in species richness.

The rate *r* represents the overall rate of morphological change in the Brownian simulation. It is analogous to Felsenstein's (1985) Brownian parameter in that higher values of *r* will generate clades with larger mean variances and volumes. However, *r* is scaled among the morphospace axes in accordance with their eigenvalues (see the section on simulations above). The eigenvalues input into morphtreegen were empirically determined from the relative warp analysis, so the simulated clades have eigenstructures similar to that of the empirically determined morphospace. Mathematically, *r* equals the ratio of the variance in simulated step size on each morphospace axis in each timestep to λ, the eigenvalue of the corresponding eigenvector.

In the case of the Anostomoidea and Curimatoidea, preliminary simulations revealed that values of *r* between 5×10^{-6} to 120×10^{-6} yielded morphological variances and volumes similar to those observed in the real clades and would include the maximum likelihood solutions. That range was sampled every 5×10^{-6} rate units to obtain the potential values of *r* listed in Table 1. The small magnitude of the potential values for *r* is not surprising given that they were integrated over 90 timesteps and an increasingly large number of lineages to produce a final diversity. If the chosen values for *r* were much larger or smaller, the simulated variances and volumes would all have been much larger or smaller than the values calculated from the real clades.

TESTING SCENARIO 1: WAS THE RATE OF MORPHOLOGICAL CHANGE HIGHER IN THE ANOSTOMOIDEA?

Scenario 1 tested the alternate hypotheses that the Anostomoidea experienced a higher rate of morphological evolution than did the Curimatoidea (denoted $r_A > r_C$) and that the rate of morphological evolution in the Anostomoidea was equal to, or less than, the rate of morphological evolution in the Curimatoidea ($r_A \leq r_C$). First, 1000 clades with between 110 and 150 terminal taxa for all 120 possible combinations of the chosen values for *pq* and *r* (120,000 clades total) were simulated, each was rarefied to 60% of living species to mimic the sampling intensity of real species and the variances and volumes of the rarefied clades were calculated. Because clade age is identical and species richness is similar between the Anostomoidea and Curimatoidea, a single set of simulations was used to investigate the evolution of both clades.

The simulated variances and volumes were used to estimate the probability of each of the 120 rate combinations simulating the morphological diversity of the Anostomoidea and of the Curimatoidea. For each clade, one set of estimates was based on variance and one on volume. Each probability estimate equaled the proportion of the 1000 simulations using a specific rate combination that generated a variance or volume falling within one SE of the clade's true value. The total probability of each *r* for each clade equaled the proportion of the 5000 simulations using that *r* (1000 for each of the five potential values of *pq*) that matched the empirical variance or volume of that clade. The maximum likelihood estimates were the values of *r* with the highest total probabilities of generating the morphological diversities of the Anostomoidea or the Curimatoidea. The tests outlined below were performed twice, once with variance-based probability estimates and once with volume-based probability estimates.

Next, a 24×24 probability matrix was assembled with columns representing the potential values of *r* in the Curimatoidea (r_C) and rows representing the potential values of *r* in the Anostomoidea (r_A). Each box in the matrix represented the probability of a particular combination of r_A and r_C generating the morphological diversity of both real world clades, denoted $P(A|\, r_A, C|\, r_C)$. That probability is obtained by multiplying the total probability that the Anostomoidea evolved according to rate r_A, denoted $P(A|\, r_A)$, and the total probability that the Curimatoidea evolved according to rate r_C, denoted $P(C|\, r_C)$.

$$P(A|r_A, C|r_C) = P(A|r_A) * P(C|r_C).$$

For a similar example, see Wagner (1997). The sum of values in the upper triangular portion of the probability matrix exclusive of the diagonal was proportional to the likelihood of the true rate set falling into $r_A > r_C$, whereas the sum of values in the lower triangular matrix, including the diagonal, was proportional to the likelihood of the true rate set falling into $r_A \leq r_C$ (for discussion of the relationship between probability and likelihood, see Edwards 1992, chapter 2). The natural logarithm of the sum of probabilities in each class was the total support for the corresponding hypothesis, and the hypothesis with the largest support was preferred (the most likely, given the data).

The significance of the difference in support was evaluated by the X^2 approximation to the *G* statistic (Sokal and Rohlf 1995). *G* was compared to the X^2 distribution for a single degree of freedom because the sorting of rate sets into the two hypothesis classes was not independent (Sokal and Rohlf 1995); if rate r_A was not greater than r_C, it must have been equal to or less than r_C. Because the same data (A, C) was used to determine all probabilities, the arbitrary constant of proportionality was equivalent for all sets of rates and could be ignored in the support tests (Edwards 1992).

The inclusion of the probability matrix's diagonal in $r_A \leq r_C$ biased the test slightly. If all sets of rates were equally likely,

the likelihood of the two hypotheses would be determined only by the number of rate sets in each category, and more sets of rates fall into $r_A \leq r_C$ than into $r_A > r_C$. By setting the probabilities of all rate sets to the same arbitrary value and calculating the *G* statistic, it was determined that the bias was approximately 0.1 support units in favor of $r_A \leq r_C$ given 24 potential values of *r*. To compensate, a correction factor of 0.2 (twice the support bias) was added to the calculated *G* statistics.

TESTING SCENARIO 2: WAS THE TURNOVER RATE LOWER IN THE ANOSTOMOIDEA?

Next, the alternate hypotheses that the Anostomoidea experienced a lower rate of lineage turnover than the Curimatoidea (denoted $pq_A < pq_C$) and that the rate of lineage turnover was equal in the two clades or greater in the Anostomoidea ($pq_A \geq pq_C$) were tested. The test used in this scenario parallels the method used in scenario 1. The same set of simulated clades was employed, but in this case the total probability of generating the morphological diversity of each clade for each of the five values of *pq* equaled the proportion of the 24,000 clades simulated under that value of *pq* (1000 for each of the 24 values of *r*) that matched the empirical variance or volume.

The probability matrix in this case was 5×5, with columns representing potential *pq* values for the Curimatoidea (pq_C) and rows representing potential *pq* values for the Anostomoidea (pq_A). Fewer potential values were examined for *pq* than for *r*, and the bias caused by the inclusion of the matrix diagonal in one hypothesis class was stronger in this scenario than in scenario 1. The bias in favor of $pq_A \geq pq_C$ was calculated as 0.4 support units, and the *G* values were corrected accordingly by 0.8.

TESTING SCENARIO 3: WAS THE ONSET OF DIVERSIFICATION LATER IN THE CURIMATOIDEA?

Because morphological variance does not begin to accumulate within a clade until its first internal bifurcation (Collar et al. 2005), a delayed onset of diversification in the Curimatoidea could explain its lower morphological diversity. To determine the magnitude of the delay in the onset of diversification in the Curimatoidea that would erode any significant result returned by the analysis of scenarios 1 and 2, those analyses were repeated five times with the anostomoid phylogeny constrained to branch in the first timestep and the first bifurcation in the phylogeny of the Curimatoidea delayed by 0, 10, 20, 30, or 40 timesteps. The minimum delay resulting in *G* values below the 90%, 95%, and 99% confidence levels was determined via interpolation. In the repeated trials the morphological diversity of the Anostomoidea had the potential to accumulate through the full 90 timesteps, whereas the Curimatoidea accumulated no morphological diversity during the delay before the onset of bifurcation. Treatment of *pq*, *r*, *minsize* and *maxsize* was the same as in the original trials without a constraint on the onset of diversification.

Ten thousand random draws of the anostomoid and curimatoid phylogenies were then taken from a treespace containing 10,000 clades of between 110 and 150 surviving taxa (2,000 from each of the five turnover rates), and the percentage of draws in which the age of first bifurcation in the Curimatoidea was delayed sufficiently to erode confidence in a conclusion of higher *r* or lower *pq* in the Anostomoidea was calculated. If a sufficient delay in the Curimatoidea was statistically common (depending on desired confidence, occurring in more than 1%, 5%, or 10% of random draws), then scenario 3 would be maintained as a candidate explanation for unequal morphological diversification.

Results

MORPHOMETRICS AND MEASURED DIVERSITIES

The eigenvalues for four eigenvectors exceeded the percentage of variance predicted by the broken stick model (Jackson 1993) and were distinguishable from measurement error. The corresponding four morphospace axes summarized 49.6%, 17.2%, 9.4%, and 6.2% of the non-uniform shape variation (exclusive of uniform scaling, rotation, translation, and shearing) among species, respectively, together representing 82.4% of the non-uniform shape variation present in the original dataset. RW1 partitioned the superfamilies into distinct regions of morphospace whereas the ranges of morphology exhibited by the two superfamilies overlapped considerably on the other three axes (Fig. 4). The Anostomoidea spanned a greater range of values and had a higher variance than did the Curimatoidea on RW1, RW2, and RW4; only on RW3 were the Curimatoidea more morphologically diverse (Fig. 4). When all four warps were considered together, the Anostomoidea exhibited 1.6 times the variance of the Curimatoidea and occupied approximately six times their volume (Table 2). Confidence intervals extending one SE away from the observed variances and volumes for each clade did not overlap. Levene's test for the Anostomoidea and Curimatoidea yielded a T-statistic of 21.4, with a critical value of 1.98 at 149 degrees of freedom, indicating that the chance that the true variance of the Anostomoidea equaled that of the Curimatoidea did not exceed 5%. Based on the non-overlap of the bootstrap confidence intervals and the significance of Levene's test, the morphological diversities of the two clades were significantly different.

Much of the variation along the first morphospace axis (RW1) involved the landmarks on the quadrate and lower jaw, and this warp primarily described the lower jaw's angle and length, the location of the quadrate–anguloarticular joint relative to the orbit and the length of the interopercular–mandibular ligament, which runs along the ventral surface of the quadrate. Negative values of RW1 corresponded to anteriorly positioned lower jaw joints,

COLOUR FIG.

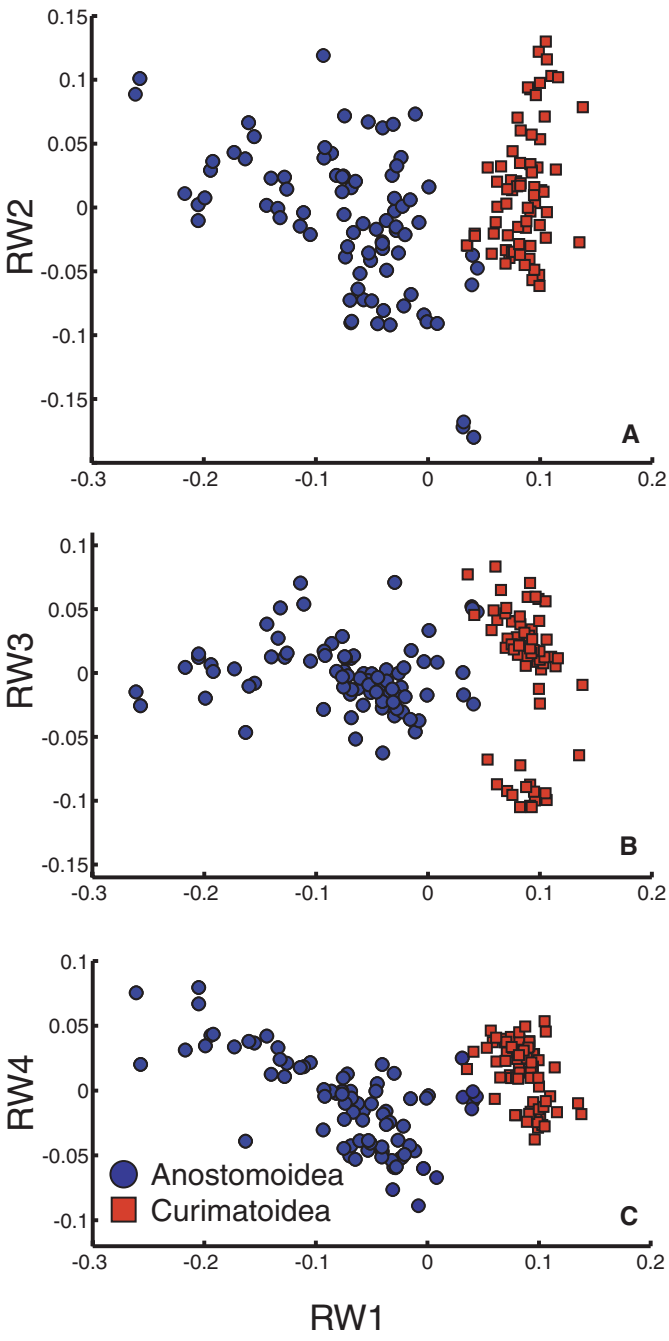

Figure 4. Scatterplots of the Anostomoidea (blue circles) and Curimatoidea (red squares) of RW2 (A), RW3 (B), and RW4 (C) against RW1. The disjunction in the curimatoid scatter on RW3 corresponds to the taxonomic division of these fishes into the families Prochilodontidae and Curimatidae.

long interopercular–mandibular ligaments, and terminal or upturned mouths, whereas positive values of RW1 corresponded to posteriorly positioned lower jaw joints and short interopercular–mandibular ligaments, and terminal or subterminal mouths. The species with the most negative scores on RW1 (Fig. 4) were members of *Sartor* (Fig. 2D) that possessed the most strongly upturned mouths of any examined species. RW1 also correlated with the length of the supraoccipital crest, the orientation of the premaxilla and maxilla, the anteroposterior positioning of the orbit, the attachment of the pharyngeal jaws to the neurocranium relative to the posterior margin of the orbit, and the dorsal extent of the hyomandibular. Many of the differences summarized by RW1 were diagnostic of the two superfamilies, and as such the distributions of the Anostomoidea and Curimatoidea abutted on RW1 but scarcely overlapped (Fig. 4).

Table 2. Observed variances and volumes, with standard errors (SE) of the Anostomoidea and Curimatoidea. Variances and volumes are calculated from distributions of species on the four morphospace axes, with SEs estimated via the bootstrap (Efron and Tibshirani 1993).

Group	Variance	Volume
Anostomoidea	$1.0\times10^{-2}\pm1.1\times10^{-3}$	$2.0\times10^{-3}\pm2.4\times10^{-4}$
Curimatoidea	$6.2\times10^{-3}\pm5.8\times10^{-4}$	$3.4\times10^{-4}\pm3.8\times10^{-5}$

RW2 primarily described the overall aspect ratio of the skull, the rotation of the premaxilla, and the dorsoventral positioning of the orbit. Species with negative values of RW2 had fairly elongate, dorsoventrally compressed skulls with the orbit positioned dorsally and the premaxilla rotated counterclockwise relative to the condition in species with positive values of RW2. Species with positive values of RW2 tended to have more ventrally positioned eyes and abbreviated skulls in which height about equaled length. The cluster of species in the Anostomoidea with extremely negative scores on RW2 (Fig. 4A) are members of the subgenus *Hypomasticus* within *Leporinus* that possess the most strongly downturned mouths of any examined species (Fig. 2B). Species with positive or less negative values of RW2 may have subterminal, terminal, or superterminal mouths.

RW3 described further aspects of jaw morphology, particularly the shape of the dentary and the premaxilla. Species with negative values of RW3 tended to have relatively long premaxillas and short dentaries with a reduced distance between the anterior margin of the dentary and the anguloarticular-quadrate joint. Species with positive values of RW3 had more elongate dentaries and shorter premaxillas. RW3 also described variation in the position of the cleithra, the length of the opercular series, and the diameter of the orbit. The morphological differences described by RW3 separated the Curimatoidea into the two recognized families, the Prochilodontidae and the Curimatidae (Fig. 4B). RW4 described almost exclusively the size of the premaxilla relative to the remainder of the head.

PROBABILITIES

The ridged topology of the probability surfaces in Figure 5 indicated that variation in the rate of morphological change r had

COLOUR FIG.

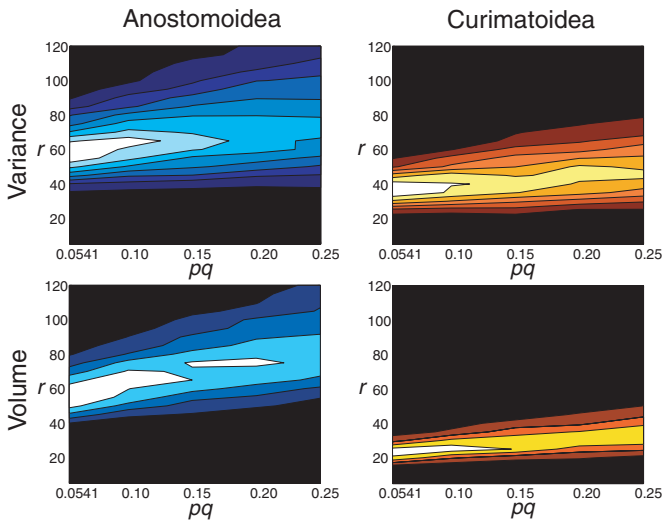

Figure 5. Probability plots for the Anostomoidea (left, in blue) and Curimatoidea (right, in red) showing the probability of simulating the observed variance (top) or volume (bottom) of each modern clade for all 120 combinations of turnover rates (*pq*, the x-axis) with rates of morphological change (*r*, the y-axis). White or light regions indicate rate sets with high probabilities of simulating the observed morphological diversities and include the maximum likelihood solutions; darker blue or red regions indicate lower probabilities of simulating the observed morphological diversities for the Anostomoidea and Curimatoidea respectively. Black indicates zero probability.

a much stronger effect on morphological diversification in the simulations than did variation in the turnover rate *pq*. For any *r*, variation in *pq* only slightly altered the probabilities of producing the variance or volume of either real clade, but for any given *pq*, variation in *r* affected the probabilities greatly. The slight slant to the ridges of high probability in Figure 5 suggests that *pq* did have a minor effect on morphological diversification in the simulations.

SCENARIO 1: VARYING RATES OF MORPHOLOGICAL CHANGE

The variance-based test for a higher rate of morphological change in the Anostomoidea supported $r_A > r_C$ over $r_A \leq r_C$ with 90% confidence and very close to 95% confidence, particularly when *G* was corrected for the support bias in favor of $r_A \leq r_C$ (Table 3). The volume-based test was significant at 99% confidence. The variance-based test indicated that the hypothesis of elevated rates of morphological change in the Anostomoidea was about six times more likely than the alternative, whereas the volume-based test indicated that the elevated rates hypothesis for the Anostomoidea was about 75 times more likely than the alternative. A visualization of the likelihood tests as probability surfaces appears in Figure 6, in which the gridlines represent the probability matrices assembled during the likelihood tests and the height of the surface at each gridline intersection represents the probability of a particular rate combination. The thick white lines divide the probability surfaces into the alternate hypothesis classes, and the summed height of the surface in each hypothesis class is proportional to the likelihood of that hypothesis. The position of the peak in the probability surfaces in the left panels of Figure 6 indicates the maximum likelihood estimates of *r*, which based on variance are 1.6 times greater in the Anostomoidea than in the Curimatoidea, and based on volume are 2.6 times greater in the Anostomoidea than in the Curimatoidea (Table 4).

SCENARIO 2: VARYING RATES OF LINEAGE TURNOVER

The flatness of the probability surfaces in Figure 6 (right panels) shows that all turnover rates *pq* were roughly equiprobable for both clades, and that neither $pq_A < pq_C$ nor $pq_A \geq pq_C$ was strongly preferred. After factoring in the 0.4 support unit bias in favor of $pq_A \geq pq_C$ there was a very slightly higher likelihood

Table 3. Likelihood tests of the hypotheses $r_A > r_C$ versus $r_A \leq r_C$ (scenario 1) and $pq_A < pq_C$ versus $pq_A \geq pq_C$ (scenario 2). The test statistic *G* was evaluated at critical values of 2.71, 3.84, and 6.64 corresponding to 90%, 95%, and 99% confidence in a higher rate of morphological evolution (*r*) or lower rate of turnover (*pq*) in the Anostomoidea. If the 0.1 support-unit bias in favor of $r_A \leq r_C$ (see methods) is taken into account (corrected *G*), the variance-based test of differences in *r* returned results within 0.11 support units of significance at the 95% confidence level (noted as marginal). The negative uncorrected values of *G* in the tests of turnover rate are explained by the 0.4 support unit bias in favor of $pq_A \geq pq_C$ in that test (equivalent to a 0.8 unit bias in *G*, see Methods) and all values of *pq* were found to be essentially equiprobable.

r	Data	Support $r_A > r_C$	Support $r_A \leq r_C$	*G*	Corrected *G*	Significant at 90%?	Significant at 95%?	Significant at 99%?
	Variance	18.441	16.727	3.429	3.629	yes	marginal	no
	Volume	17.243	12.893	8.701	8.901	yes	yes	yes
pq	Data	Support $pq_A < pq_C$	Support $pq_A \geq pq_C$	*G*	Corrected *G*	Significant at 90%?	Significant at 95%?	Significant at 99%?
	Variance	16.045	16.364	−0.638	0.162	no	no	no
	Volume	16.250	16.611	−0.722	0.078	no	no	no

COLOUR FIG.

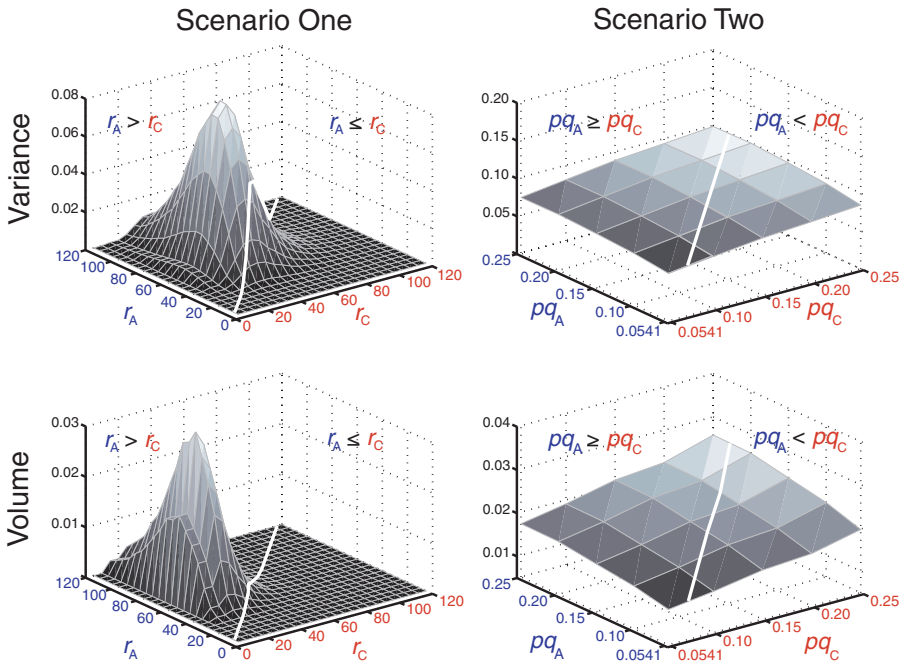

Figure 6. Surfaces illustrating the probabilities of producing the morphological diversities of the Anostomoidea and Curimatoidea for all examined rate combinations. Height on the z-axis represents the probability of each rate set identified by the x- and y-axes, with light areas representing rate sets with the highest probability. The thick white line drawn through each probability surface divides that surface into alternate hypothesis classes, with the summed height of the surface in each hypothesis class proportional to the likelihood of that hypothesis. The two left panels illustrate the variance- and volume-based tests for variation in rate of morphological change (scenario 1), whereas the two right panels illustrate the tests for variation in turnover rates (scenario 2). Whether variance or volume is used as the measure of morphological diversity, the peak of high probability in the leftmost panels is well displaced from the diagonal corresponding to $r_A = r_C$, indicating maximal likelihood of $r_A > r_C$. The flatness of the surfaces in the rightmost panels illustrates that neither $pq_A < pq_C$ nor $pq_A \geq pq_C$ is preferred.

that the turnover rates in the Anostomoidea were lower than in the Curimatoidea (Table 3). This slight preference for $pq_A < pq_C$ over $pq_A \geq pq_C$ agreed with the original prediction, but the small difference in support indicated that very little confidence could be placed in the preference. Calculations of the total probability of each potential value of pq identified high turnover rates as most likely for both clades (Table 4). Volume-based estimates identified the highest turnover rate as most likely for both the Anostomoidea and Curimatoidea, whereas variance-based estimates identified a slightly lower turnover rate in the Anostomoidea than in the Curimatoidea (Table 4). No major difference in the most likely turnover rate between the clades was indicated.

SCENARIO 3: VARYING ONSETS OF DIVERSIFICATION

Though the support tests (Table 3) favored an elevated rate of morphological evolution in the Anostomoidea when the ages of first bifurcation in the two superfamilies were allowed to vary freely (scenario 1), simulations of delayed onset of diversification in the Curimatoidea revealed that a 20 timestep delay in the first bifurcation of the curimatoid phylogeny brought the significance of the support test based on variances below the 90% confidence level (Fig. 7). The volume-based support test remained significant at 95% confidence even with a 40 timestep delay in the first bifurcation of the curimatoid phylogeny. Interpolation between points estimated that approximately a 15 timestep (17%) delay in the first bifurcation of the Curimatoidea would be sufficient to edge the variance-based G statistic below the 90% confidence level. Random draws of the anostomoid and curimatoid phylogenies from the possible treespace indicated that in 5.6% of trials, two clades were selected that differed by at least 15 timesteps in the age of first bifurcation (Fig. 8). In only half of these trials (2.8% of all random draws) was the Curimatoidea the clade with the later onset of diversification and a lower expected morphological diversity.

Table 4. Maximum likelihood solutions for rates of turnover (speciation, p and extinction, q) and morphological change (r) in the Anostomoidea and Curimatoidea.

Group	Measure	p	q	$r \times 10^{-6}$
Anostomoidea	variance	0.2000	0.1648	65
Anostomoidea	volume	0.2500	0.2183	65
Curimatoidea	variance	0.2500	0.2183	40
Curimatoidea	volume	0.2500	0.2183	25

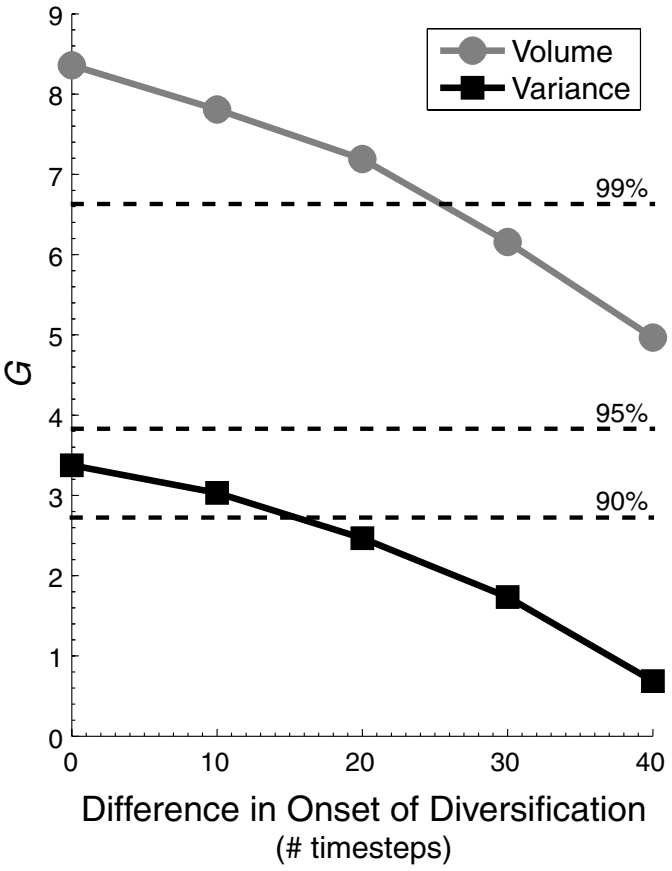

Figure 7. Likelihood test statistics (*G*) based on variance and volume for the comparison of alternate hypotheses ($r_A > r_C$) and ($r_A \leq r_C$) conditioned on varying delays in the age of first bifurcation in the Curimatoidea, relative to the Anostomoidea. Dashed horizontal lines represent critical values of *G* for 90%, 95%, and 99% confidence. By interpolation, the variance-based *G* statistic drops below the 90% confidence limit at approximately a 15 timestep difference in the ages of first bifurcation between the sister clades (17% difference in total diversification time).

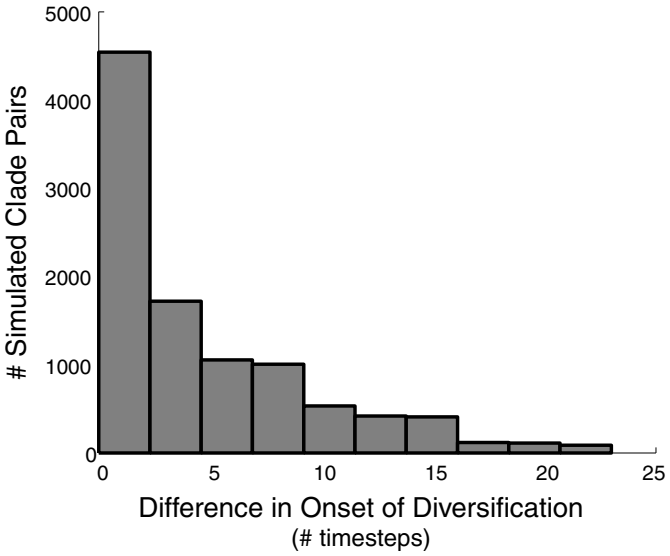

Figure 8. Histogram showing the distribution of differences in the onset of diversification (age of first bifurcation) in sister clade comparisons drawn randomly from all possible phylogenies with 110 to 150 modern lineages assembled under all five simulated rates of lineage turnover. About 5.6% of comparisons differ by 15 timesteps or more, but in only half of these is the Curimatoidea the clade with a later onset of diversification.

Discussion

GENERAL IMPLICATIONS

The identification of a probable higher rate of morphological change in the Anostomoidea relative to the Curimatoidea suggests that the RAPRATE method can test effectively for heterogeneity of rates of morphological evolution when a detailed phylogeny is lacking. As the first step in a comparative analysis, this method could be used to identify pairs of clades likely to exhibit variation in the underlying rate of morphological change. Such clades could then be targeted for phylogenetic reconstruction and comparative analysis at a finer scale.

One limitation of an approach using simulated phylogenies is low power compared to tests for rate heterogeneity that use an explicit phylogenetic reconstruction (e.g., Purvis et al. 1995; Collar et al. 2005; O'Meara et al. 2006). As a result, this method is fairly conservative. Given that the fairly large differences in morphological diversity between the Anostomoidea and Curimatoidea did not yield 95% confidence in rate divergence in all tests based on simulated phylogenies, the differences in variance necessary to accept a hypothesis of unequal rates with 95% or 99% confidence on the basis of simulated phylogenies may be uncommon in nature. In a similar simulation-based analysis, Foote (1993) found that even in comparisons involving the most morphologically diverse groups of trilobites in the analysis, the null model of equal rates could only be rejected with 90% confidence. To avoid the inappropriate conclusion that further investigation of the divergence of evolutionary processes is rarely justified, I suggest that future tests use a critical *G* value based on 90% confidence, particularly when variance is the chosen diversity metric. If a significant result can be obtained at a 95% confidence level that result should be regarded as particularly strong support for rate heterogeneity, but use of a 95% confidence level in this test may be too conservative as a general rule of thumb.

Because variance-based tests return lower *G* values than those based on hypervolumes (Table 4) the variance-based tests appear to be more conservative. This effect presumably results from a stronger correlation between *r* and simulated hypervolume than between *r* and clade variance. Note that the volume-based probability peak in Figure 6 is narrower than the variance-based peak, indicating that fewer examined values of *r* were able to generate

a given volume than were able to generate a given variance. The increased conservatism of the variance-based tests suggests that a determination of a significant difference in rates of morphological change from a comparison of variances is stronger evidence than a similar conclusion drawn from a comparison of hypervolumes.

Although the case study of the Anostomoidea and Curimatoidea included many similarities that eased analysis, including a sister group relationship and similar species richness, these simplifications are not mandatory. By modifying the number of timesteps simulated for each clade, the method could test the equivalence of rates of morphological evolution in any pair of clades for which the species richnesses and relative ages of origin are known (from fossils or a molecular phylogeny). The exploration of the potential effect of a delayed onset of diversification in the Curimatoidea on expected morphological diversification (scenario 3) provided an example of one possible modification.

The most important result from the case study of the Anostomoidea and Curimatoidea demonstrates that, irrespective of the measure of morphological disparity used and without constraining the timing of the onset of diversification, there is at least 90% confidence that the rate of morphological evolution was higher in the Anostomoidea than in the Curimatoidea (scenario 1, Table 4). Conversely, variation in the lineage turnover rate *pq* in scenario 2 exerted only a weak effect on simulated morphological diversity. The value of *pq* may or may not have varied between the clades, but even if it did, scenario 2 is not a likely explanation of unequal morphological diversification in this case study.

If one assumes that the Curimatoidea experienced at least a 17% delay in the onset of diversification relative to the Anostomoidea (scenario 3), confidence in a higher rate of morphological change in the Anostomoidea erodes below 90% (Fig. 7). Because random draws of pairs of phylogenies from anostomoid/curimatoid treespace rarely differ by 17% (15 timesteps) (Fig. 8), it is unlikely that the ages of onset of diversification in the two real clades differed by that extent. It remains unknown, however, whether the true timing of the onset of diversification in the Anostomoidea and Curimatoidea falls near the statistical expectation. In the final analysis a large delay in the onset of diversification in the Curimatoidea cannot be ruled out as the cause of unequal morphological diversification, but based on the statistical improbability of such a delay scenario 3 (delayed onset of diversification in the Curimatoidea) is less likely than scenario 1 (higher *r* in the Anostomoidea). If it is eventually shown through fossils or a well-sampled molecular phylogeny that the Anostomoidea began to diversify before the Curimatoidea, the conclusion that unequal rates of morphological diversification best explain unequal morphological diversity in these two clades should be revisited. Meanwhile, further research to determine why the rate of morphological change may have increased in the Anostomoidea or decreased in the Curimatoidea is appropriate and ongoing. Several possible underlying processes that may have influenced the rate of morphological evolution in these fishes are discussed in a separate section below.

Unlike the tests of rates of morphological evolution, the tests of speciation and extinction rates (Table 3) failed to support heterogeneity in turnover rate in the Anostomoidea and Curimatoidea and suggested instead that all simulated turnover rates were roughly equiprobable (Figs. 5 and 6). The ability of every examined turnover rate to produce clades with identical species richness and morphological diversity (Fig. 5) suggested that variation in turnover rate (scenario 2) had a comparatively minor effect on morphological diversity in these simulations. The very small, but non-zero, support differences in the tests for variation in *pq* (Table 3) suggested that a negligible effect of turnover rate on morphological diversity existed in these simulations, but was unlikely to be a major source of the observed differences in morphological diversity.

Why did variation in turnover rate have such a limited effect on simulated variance and volume? The expectation that turnover rate would affect morphological diversification was based on the assumption that lower turnover rates would generate clades with older average node ages, corresponding to an increase in mean morphological diversity. Conditioning the analysis to examine only clades with similar species richness may have factored some of the variation in morphological diversity due to differing average node ages out of the analysis. Ordinarily, variance is independent of sampling intensity (e.g., species richness), but Ricklefs (2006) recently demonstrated that in clades simulated under time-dependent morphological diversification (such as Brownian evolution), the logarithm of species richness predicts average node age, crown clade age, and morphological variance better than does clade age. The positive relationship between species richness and variance was confirmed and a similar result for volume was obtained by simulating 1000 clades under a constant *pq* and *r* and plotting morphological diversity against species richness (Fig. 9). The full relationship between turnover rate, species richness, morphological variance, average node age, crown clade age, and the onset of diversification has not been thoroughly explored and could be a fruitful avenue for further analysis.

Removing the constraint on species richness in the simulations would probably increase the influence of turnover rate on morphological diversity, but would also introduce error by simulating morphological diversification on phylogenies that could not be true for the real clades. Furthermore, it would eliminate a control on the difference in morphological diversity that is attributable to the observed difference in species richness. I conclude that phylogenetic comparative methods that simulate phylogenies must condition the simulations on observed species richness to account for the dependence of morphological diversity on average node age. The necessity of conditioning on species richness

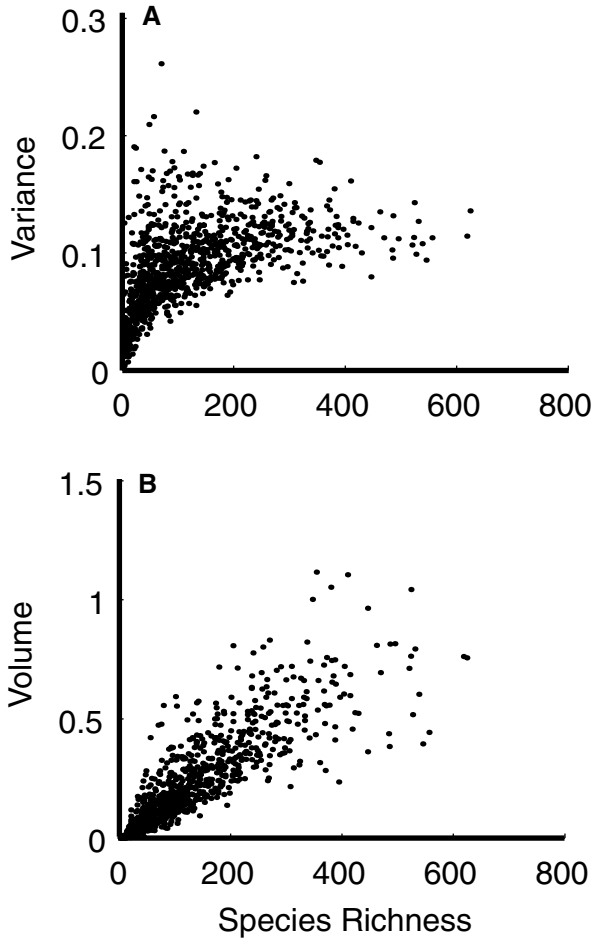

Figure 9. Species richness varies positively with variance and volume in 1000 simulated clades. Number of timesteps (t) = 90, speciation rate (p) = 0.10, extinction rate (q) = 0.0546, rate of morphological change (r) = 60×10^{-6}. Simulations were conditioned on survival of at least one lineage to the end of the simulation.

may limit the ability of the simulations to test for variation in turnover rate. These results support Nee's (1994) statement that simulation-based analyses cannot identify speciation and extinction rates based solely on data from recent organisms. On a more encouraging note, the equiprobability of all examined *pq* implies that prior knowledge of the rates of speciation and extinction is not required to test for heterogeneity in the rate of morphological change. In cases where rates of extinction and speciation can be estimated from the fossil record (Sepkoski 1998) or molecular phylogenies (Nee et al. 1994; Nee 2001), said rates may be used to set the internal branching parameters in morphtreegen and refine the results.

Though the maximum likelihood solutions for *pq* (Table 4) are not well constrained, high turnover rates were still identified as most likely. This result most likely stems from the increased variance of outcomes in simulations with a high turnover. At high turnover rates, the surviving species at the end of the simulation frequently diverge late from just a few ancestors (Raup 1983); these clades tend to have unusually low morphological diversities and their inclusion in the distribution increases the range and variance of morphological diversities that can be produced given a particular value of r. Summation across distributions with higher variances results in higher probability scores, and because the highest values of *pq* result in the greatest variance in simulation output, high values of *pq* also have maximum likelihood.

The match of simulation outputs to measured morphological diversities does not imply that the organisms in question diversified according to the Brownian model of evolution (Foote 1993; O'Meara et al. 2006). These simulations are clearly simplified, particularly in their assumptions of undirected and unconstrained morphological change at each timestep and constancy of rates. Nevertheless, the Brownian model is the standard starting point in comparative rate studies and has been shown many times to be capable of identifying evolutionary discontinuities that can then be targeted for closer investigation (Felsenstein 1985; Garland et al. 1993; Ackerly and Nyffeler 2004). The true model of evolution in the Anostomoidea and Curimatoidea was surely more complex, and may have involved factors such as rate heterogeneity within as well as between clades, forbidden regions of morphospace, and ecological interactions among species. Recent, very interesting work by Harmon et al. (2003) offers one potential way to investigate some of these more complex models by testing for the specific types of rate heterogeneity that are the hallmarks of adaptive radiation, such as high rates of cladogenesis early in the diversification of a clade and the partitioning of the majority of morphological diversity among, rather than within subclades. Unfortunately those tests require a well-resolved phylogeny and cannot currently be applied to the Anostomoidea and Curimatoidea. Still, this current work reveals one probable feature of the true model of evolution for these fishes; it will involve a rate of morphological evolution for the Anostomoidea that exceeds the rate in the Curimatoidea.

POSSIBLE UNDERLYING PROCESSES

The shared phylogenetic and environmental histories of the Anostomoidea and Curimatoidea imply that a biological or ecological feature arising after their divergence may have accelerated the rate of morphological evolution in the Anostomoidea or decelerated that rate in the Curimatoidea (see discussion of clade-specific factors in Lovette et al. 2002). Determination between these alternatives will require comparison with the currently unknown outgroup (Sanderson and Donoghue 1994). A test of whether any of the synapomorphies of the Anostomoidea or Curimatoidea promoted unequal morphological diversification would examine other sister clades in which one clade has evolved similar characteristics to the Anostomoidea or Curimatoidea independently

(Jensen 1990), but such a test is beyond the scope of this current work. Nevertheless, some aspects of the morphology and ecology of both sister clades stand out as excellent candidate promoters of unequal morphological diversification.

If the rate of morphological evolution was accelerated in the Anostomoidea, restructuring of the lower jaw early in the evolution of this lineage may have promoted that increase in rate. All examined anostomoids have a longer quadrate than do curimatoids or other examined characiforms, with many species in the Anostomidae (which contains more than 90% of the species in the Anostomoidea) possessing extraordinarily long morphologies of this bone (e.g. *Sartor*, Fig. 2D). The elongation of the quadrate found in anostomoids relocates the joint of the lower jaw well in front of the eye (indicated by arrows in Fig. 2) and away from a large ventral flange of the lateral ethmoid, a bone which forms part of the neurocranium. The repositioning of the lower jaw joint may have relaxed a structural constraint on jaw orientation. Specifically, the upwards- or backwards-facing morphologies possessed by some anostomoids would not be biomechanically possible without relocation of the lower jaw joint because the neurocranium lies directly dorsal to the joint in the primitive condition possessed by the Curimatoidea (Fig. 3).

Phylogenetic work in progress suggests that the elongation of the anostomoid quadrate evolved early in the history of this group, prior to the evolution of the upturned and backwards-facing jaw morphologies found in the most morphologically extreme anostomoids. This sequence of evolutionary events supports the hypothesis that the early lengthening of the quadrate facilitated the evolution of novel jaw morphologies by releasing structural constraints. If so, the elongation of the anostomoid quadrate could be viewed as a parallel to the restructuring of the pharyngeal jaws implicated in the explosive evolution of cichlids (Liem 1974) and the decoupling of the oral jaws from the neurocranium and opercular series that may help explain the remarkable diversity of loricarioid catfishes (Schaefer and Lauder 1996).

Release of a structural constraint by elongation of the quadrate appears to have been a prerequisite for the evolution of the modern diversity of anostomoid skulls and jaws, but the mere possibility of change does not necessitate change, and an evolutionary force such as directional selection, character displacement or genetic drift is needed to produce morphological change over time (Simpson 1944). Given the highly modified nature of some anostomoid jaws (Fig. 2), morphological diversification may have been driven by a complex interplay between morphology, jaw function, and trophic ecology. It is known that in addition to their diversity of jaw forms, anostomoids exploit a variety of food resources including macrophytes, seeds, fungus, aquatic invertebrates, fish scales, and freshwater sponges (Géry 1977; Goulding 1980; Taphorn 1992). Even though many members of the Anostomoidea are generalists in the sense that they will consume a variety of foods, preference for various food items varies among taxa (Knöeppel 1972; Santos and Rosa 1998; Albrecht and Pellegrini-Caramaschi 2003; Mendonça et al. 2004), and co-occurring species of *Leporinus* consume very different proportions of the available resources (Balassa et al. 2004). Future research in the Anostomoidea should synthesize this scattered ecological literature and map it to a phylogeny to determine whether trophic preference correlates with jaw diversity. A final step would add a biomechanical component to investigate whether quantifiable changes in jaw kinematics closely track changes in feeding ecology and morphology along phylogeny, as has been done for the diverse cichlid (Hulsey and Léon 2005) and labrid (Westneat 1995; Wainwright et al. 2004) fishes.

It may also be that the rate of morphological evolution has decreased in the Curimatoidea, similar to the decrease in the rate of morphological change that Wagner identified in a clade of conocardioid gastropods (Wagner 1997). Several features of the Curimatoidea may help explain their failure to diversify morphologically despite an abundance of speciation events. First, all curimatoids lack well-developed teeth attached to their jaws as adults (adult prochilodontids have numerous spatulate teeth attached to their lips, and adult curimatids lack teeth entirely) and have experienced major body plan shifts to accommodate a detritivorous lifestyle. The body plan shifts include modifications to hard and soft anatomy of the gill arches and the evolution of a large epibranchial organ which concentrates food out of the water column (Vari 1983, 1989; Castro and Vari 2004). Loss of attached teeth, modification of the gill arches, and the need to maintain the epibranchial organ may have closed some trophic strategies such as herbivory or durophagy to the curimatoids and may help explain their lack of diversity. Second, curimatoids are specialists on very abundant resources in South American rivers: detritus and the aufwuchs or organic slime that coats river bottoms and other subaquatic hard surfaces. This resource abundance appears to help explain why curimatoids achieve some of the highest population densities of any South American fishes, can account for up to 70% of commercial freshwater fish harvests (Géry 1977; Goulding 1980; Vari 1989; Castro and Vari 2004), and form critical links in the carbon cycle (Taylor et al. 2006). The abundance of food may imply that populations of curimatoids are not trophically limited but, like many other Neotropical fishes, are limited by competition for preferred habitats or by predation (Arrington et al. 2005; Layman and Winemiller 2005). If competition among curimatoids for habitat or shelter from predators is stronger than competition for food, there may be little evolutionary pressure on curimatoids to diversify in diet or in jaw shape.

Any attempt to determine which, if any, of these potential explanations best explains the unequal rates of morphological

diversification of the Anostomoidea and Curimatoidea will require several additional datasets: more detailed phylogenetic information, a list of the morphological and ecological synapomorphies of the various clades within both superfamilies, measurements of morphological diversity, and estimates of rates of morphological diversification in characiform families such as the Hemiodontidae and Parodontidae in which the sister group to the Anostomoidea and Curimatoidea can probably be found (Orti and Meyer 1997; Buckup 1998; Calcagnotto et al. 2005). The phylogeny of the Curimatoidea has been largely resolved (Vari 1989, 1992; Castro and Vari 2004) and research into the phylogeny, morphology, and ecology of the Anostomoidea is ongoing by the author of this paper in collaboration with Vari. Several other studies have demonstrated the use of phylogenies to test key innovation hypotheses in a probabilistic framework (Ree 2005) or to localize changes in rates of cladogenesis (Purvis et al. 1995), morphological diversification (Wagner 1997), or both (Harmon et al. 2003) at or near particular nodes. When such tests can be used to identify phylogenetic nodes at which the rates of morphological change began to diverge most markedly in the Anostomoidea and Curimatoidea, anatomical and ecological changes associated with those nodes will then offer greater insight into the evolutionary events that launched the ancestors of these modern fishes onto very different trajectories of morphological diversification.

SYNOPSIS

An inverse model of cladogenesis and morphological diversification was combined with an empirically determined morphospace to identify likely heterogeneity in the historical rate of morphological change in two sister clades of fishes. Variation in historical rates of lineage turnover was not found to be a sufficient explanation of observed differences in morphological diversity, though inequality in the timing of onset of diversification remains a potential explanation. The method employed did not require phylogenetic resolution within each clade and can be applied to a wide variety of questions in evolutionary biology. Morphological and ecological factors that may have been instrumental in accelerating morphological change in the Anostomoidea and/or decelerating morphological change in the Curimatoidea were identified and discussed.

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