LETTER

Apparent signal of competition limiting diversification after ecological transitions from marine to freshwater habitats

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Abstract

Adaptive radiations are typically triggered when a lineage encounters a significant range of open niche space (ecological opportunity), stemming from colonisation of new areas, extinction of competitors or key innovations. The most well-known of these is the colonisation of new areas, through either dispersal into new regions or the invasion of novel ecological regimes. One aspect of ecological opportunity that has rarely been studied, however, is the extent to which pre-existent competitors act to limit diversification in newly colonised adaptive zones. Herein, we show that in multiple geographically independent invasions of freshwaters by marine Sea Catfishes (Ariidae), rates of both morphological disparification and lineage diversification are inversely related to the presence and diversity of other freshwater fish lineages. Only in one region (Australia-New Guinea) with an otherwise depauperate freshwater fauna, has an ariid invasion gained any substantial traction. This is true at both regional and community scales, suggesting that competitive constraints may be an important factor regulating adaptive radiation.

Keywords

Adaptive radiation, Ariidae, disparification, diversification, ecological opportunity, freshwaters, habitat transitions, marine, morphological evolution, phylogeny.

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INTRODUCTION

A longstanding question in evolutionary ecology concerns the extent to which colonisation of novel, underutilised habitats promotes diversification (Schluter 2000). Classical theory predicts that shifting between adaptive zones promotes speciation, morphological variation and resource partitioning, the ultimate outcome of which is adaptive radiation (Simpson 1953). These adaptive radiations are dependent on ecological opportunity (EO) (Schluter 2000; Losos 2010) typically stemming from (1) colonisation of new areas, (2) extinction of competitors or (3) key innovations, leading to ecological release and the evolution a variety of ecomorphologies to maximise resource utilisation along the niche spectrum (Yoder *et al.* 2010). As the main driver of adaptive radiation, EO is a direct function of resource abundance and an inverse function of competition (Simpson 1953; Schluter 2000; Losos 2010; Yoder *et al.* 2010). However, these sources of EO may be interdependent. For instance, colonisation of a new region or habitat may not result in adaptive radiation if it is already filled with competitors, as incumbent lineages may limit invasions of the niches they hold (Harvey & Pagel 1991).

Competition among species within rapidly diversifying lineages may promote diversifying selection along the disputed resource axes (Schluter 2000). However, the ecological theory of adaptive radiation also implies that the prior existence of competitors will affect the ability of newly colonising lineages to diversify. This is supported by laboratory experiments showing a strong impact of immigration history (Fukami *et al.* 2007), competitive interactions (Meyer & Kassen 2007) and niche occupancy (Brockhurst *et al.* 2007) on the structure of adaptive radiations in experimental populations.

Because directly testing the effects of past competition in natural systems is more difficult, little is known about the extent to which EO presented by colonisation of new areas can be limited by the effects of extra-clade competition.

For most groups of organisms with a history of recent and repeated colonisation of new habitats we would thus predict that given similar resource availability among adaptive zones, the existence of competing lineages would limit diversification after ecological transitions. The relative contributions of resource availability and competitive interactions in determining EO are rarely evaluated (but see Bolnick 2001; Parent & Crespi 2009), and studies tend to focus instead on the presence or absence of competitors. Many indirect tests of interspecific competition have focused on intra-clade competition during diversification (Moen & Wiens 2009). Although the effects of competition are difficult to address in a phylogenetic context (Moen & Wiens 2009), the present-day signal of historical competition limiting adaptive radiation for the invasive lineage should be reduced species richness and lowered rates of morphological disparity and lineage diversification in adaptive zones with many pre-existing competitors. Thus, we predict the signal of competitive limitation of adaptive radiation to be a negative correlation between richness in competing lineages in novel adaptive zones, and the response variables of diversity and rates of diversification in invasive lineages.

A major category of adaptive zone shifts results from incursion into freshwater habitats by marine organisms. Ecological transitions from marine to freshwater habitats have occurred repeatedly in several animal groups, including annelids, molluscs, crustaceans, lampreys, elasmobranchs and teleost fishes (McDowall 1997; Lee & Bell 1999; Vega & Wiens 2012). These transitions typically involve

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euryhaline groups that are able to overcome the physiological stress imposed by the new osmotic environment (Lee *et al.* 2012). Among fishes, many major lineages have euryhaline species but not all have produced species radiations in freshwater environments (Evans *et al.* 2005; Lovejoy *et al.* 2006). Given the hyperdiversity of freshwater fishes (~40% of all fish species) relative to the tiny fraction (< 0.01%) of the aquatic realm represented by freshwater (Berra 2001; Vega & Wiens 2012), it seems clear that freshwater habitats provide a wealth of ecological resources and opportunities for diversification.

So, what factors determine successful freshwater radiations? Biome conservatism or the tendency to remain in certain types of habitats, will restrict frequent marine to freshwater transitions, and may be the result of competitive advantage of incumbent lineages already established in freshwaters (Bloom & Lovejoy 2012). Thus, a system with repeated, geographically isolated invasions of freshwater by marine lineages with a documented phylogenetic history would provide a prime opportunity to gain insights into the competitive limitation of EO-induced adaptive radiation. Given the presence of existing competitive lineages in freshwater environments (incumbents), we predict that: (1) species diversity in freshwater after invasion will be negatively correlated with the number of incumbent species (at local and regional levels), (2) rates of disparification (i.e. morphological diversification; sensu Evans *et al.* 2009) and lineage diversification will be lower in regions with high levels of pre-existing competition, and (3) lineages colonising freshwater regions with lower incumbent diversity may undergo accelerated adaptive radiation.

Sea Catfishes (Ariidae) offer a promising system to test these hypotheses, given their global distribution in warm-temperate and tropical regions and the varying habitat preferences among its members, ranging from marine, brackish and freshwater. Extant ariid diversity comprises ~170 species and their fossil record dates back 70 million years (my; Betancur-R. 2009). Recent data show an initial transition from fresh to marine waters before the onset of ariid diversification (i.e. most catfish families live in freshwaters but the origin of extant ariids is marine), followed by multiple events of freshwater recolonisation in all major geographical regions where they occur (Betancur-R. 2010). A great proportion of the ariid freshwater diversity (~62 purely freshwater or euryhaline species) is concentrated within a clade endemic to the Australia-New Guinea (AU-NG) region, comprising 34 endemic species, 28 of which live in freshwaters. In contrast, freshwater ariid clades in other regions are considerably more species-poor, usually including less than five species each (Betancur-R. 2010). Remarkably, the high proportion of freshwater ariids in rivers of AU-NG seems to be associated with the region's depauperate freshwater fish community, including < 30 species (vs. 600–6000 in other regions) of primary freshwater fish.

We test the hypothesis that competition limits ariid diversification after marine to freshwater transitions in regions with high pre-existing freshwater fish diversity. We find overall support for the above-mentioned predictions, suggesting that ariid radiations in novel ecological habitats are limited by other freshwater lineages as competition imposes constrains on diversification. If this pattern holds true for other groups across regions, this would indicate that interspecific competition might be as or more important than resource availability for determining the success and magnitude of adaptive radiations via EO.

MATERIALS AND METHODS

Taxon sampling and molecular data

The molecular data set used to infer phylogeny is primarily based on previous studies of ariids (Betancur-R. *et al.* 2007; Betancur-R. 2009), using sequences from five mitochondrial and one nuclear gene fragments (3844 sites) from 123 species. The taxonomic sampling comprises representatives of 28 ariid genera from the two subfamilies (Galeichthyinae and Ariinae), and species from all major biogeographical provinces. One ariid genus (out of ~29 valid genera) and an estimated 43 species (including undescribed taxa) were not included due to unavailability of material. In terms of habitat utilisation, our sampling represents 14 of 17 euryhaline species (82.4%), 82 of 104 marine species (78.8%), and 27 of 45 freshwater species (60.0%).

In terms of geographical distribution, our sampling represents 70.0% of the African species, 84.2% of the Australia-New Guinean species (including four marine species outside the AU-NG clade), 57.1% of the India-SE Asia and Madagascar species and 81.2% of the New World species. Twenty-one out-group taxa spanning a broad phylogenetic diversity of fishes were selected for calibrations of the molecular clock outside the Ariidae. We generated additional sequences for three nuclear markers (encompassing five gene regions) and sequences from one additional marker were also obtained from GenBank. The final alignment included 7190 sites and 11 gene fragments (five mitochondrial and six nuclear). The new sequences are available from GenBank (accession numbers JN672685–JN672954). Details on marker selection and laboratory protocols are given in Appendix S1A; the material examined is listed in Appendix S1B.

Phylogeny, divergence times and habitats

To assess tempo and mode of ariid diversification, we inferred phylogeny and divergence times under maximum likelihood (ML; phylogeny only) and Bayesian inference (BI; phylogeny and divergence times) criteria. Divergence times were estimated in BEAST v.1.6.1 using the uncorrelated log-normal (UCLN) clock-model (Drummond *et al.* 2006). Details on phylogenetic reconstructions, calibrations points and priors are given in Appendix S1C and Table S1; the XML Beast file is available from the Dryad repository doi:10.5061/dryad.35d85c0p.

To reconstruct the evolution of habitat utilisation we implemented the dispersal-extinction-cladogenesis (DEC) model in the package Lagrange (Ree & Smith 2008), which incorporates probabilities of discrete range transitions as a function of time. Unlike typical methods of character optimisation (such as those based on parsimony or ML) that assume instantaneous transitions between character states, the DEC model interprets the character in an explicitly biogeographical context, allowing taxa or nodes to occur in two areas or habitats simultaneously (e.g. in marine and freshwater simultaneously, to accommodate euryhaline species). We coded marine, freshwater and euryhaline species as 1, 2 and 1–2 respectively. Euryhaline species are those commonly found in both marine and freshwaters (e.g. diadromous); coastal species approaching or entering river mouths were counted as marine. Ancestral ranges were assumed to include no more than two areas and ancestral dispersal rates were unconstrained.

As an explicit phylogenetic test for differences in diversification rates and dispersal between freshwater and marine environments, we used the Geographic-state Speciation and Extinction (GeoSSE)

model introduced by (Goldberg *et al.* 2011) and implemented in the R package 'diversitree.' The GeoSSE model is an extension of the BiSSE (Binary-State Speciation and Extinction), which tests whether or not evolutionary rates vary as a function of a binary character. Unlike BiSSE and like the DEC model, GeoSSE allows lineages to occur simultaneously in two areas. We tested ten distinct GeoSSE models using our time-calibrated tree. Details are given in Appendix S1D.

Disparity

We quantified morphological diversity using data for traditional morphometrics (body shape), maximum total length (MTL), and meristics (counts of anal-fin rays and gill rakers). For the morphometrics, we included 666 ariid specimens in the analyses (of 912 examined specimens of which 246 were discarded; see Appendix S1E) from over 20 museums, representing 118 (of 123) species in the molecular phylogeny (1–33 specimens per species, mean = 5.6, mode = 2). MTL and meristic data were obtained from the museum specimens or compiled from the literature. Preliminary disparity analyses (see below) conducted on the morphometric, MTL, and meristic data separately vs. all data combined yielded similar results; for simplicity and to capture as much variation as possible, we report analyses on the combined data set only.

Log-transformed MTL data and square-root-transformed meristic data were appended to the morphometric data matrix after estimating mean values for each species. The final morphological matrix included 118 species and 30 variables (file available from the Dryad repository doi: 10.5061/dryad.35d85c0p). A phylogenetically corrected principal component analysis (PCA) was performed on the transformed data to reduce dimensionality in the matrix, while accounting for evolutionary dependence (Revell 2009). The first PC axis captures mostly size variation; the remaining components explain mostly size-free disparity in shape, for which we used PC2 and PC3. Details on morphological data are given in Appendix S1E.

Maximum-likelihood estimates of rates of morphological evolution were calculated using a modification of O'Meara *et al.*'s (2006) censored-rates test (Burbrink *et al.* 2011). This test compares a model in which rates vary among regional assemblages in the phylogeny against a single-rate model. We first determined the optimal model of trait evolution for each PC by fitting a series models using the Akaike information criterion corrected for small sample size (AICc). We tested Brownian motion (BM), Ornstein–Uhlenbeck (OU) and early burst/late burst (EB/LB; see Harmon

et al. 2010; Burbrink *et al.* 2011). We also tested the non-phylogenetic white noise (WN) model, as a control model for comparison (Sheets & Mitchell 2001). Values of ΔAICc > 4 are interpreted as having substantial statistical support. In cases where OU was favoured over BM as the best-fit model, we repeated the tests after transforming the tree branches using the OU α parameter. Transformations were conducted in the R package Geiger (Harmon *et al.* 2008).

Testing hypotheses of competition and diversification

To determine if competition has limited diversification in freshwater ariids, we tested three explicit predictions for the present-day signal of historical competition derived under the ecological theory of adaptive radiation.

Does competition influence species richness after ecological transitions?
We compared the total number of freshwater ariid species per colonisation event (including species not represented in the phylogeny) against the remaining freshwater fish species per region (Table 1), using linear regression on log-transformed data. This test assumes that the total number of fish species per region is a rough estimator of past competition, as diversity would likely have been similar in the relatively recent time periods represented by the freshwater ariid colonisations. We realise that the present-day competitive environment does not necessarily reflect that in which newly arrived freshwater-ariid colonisers evolved. However, this is likely a proportionally similar metric given the early evolutionary diversification of ostariophysan fishes – the world's dominant group of freshwater fishes, including two thirds of the species. Diversification of major ostariophysan orders (including catfishes) occurred during the Jurassic through early Cretaceous, with most families (including ariids) having diversified in multiple continents by the late Cretaceous-early Cenozoic (Nakatani *et al.* 2011). Thus, by the time of first freshwater ariid colonisation (16 mya; see below), rivers in major freshwater regions were already filled with ostariophysan competitors (except for AU-NG, which lacks primary freshwater ostariophysans; Table 1).

However, it is still possible that the two faunas (i.e. ariids and non-ariids) in a region are occupying separate habitats. To determine if this pattern holds true on the scale actually encountered by species (i.e. the local community), we repeated this test by regressing the number of freshwater ariids from 12 rivers against the remaining fish species in each river (see details on river selection in Table S4).

Table 1 Regional composition of freshwater ariids in relation to freshwater fish (FF) families and species (total and primary freshwater). Family level estimates modified from (Berra 2001); species numbers follow conservative estimations by (Lundberg *et al.* 2000), with an arbitrary addition of 5% for the well-characterised North America and 20% for other regions. Euryhaline ariid species included in estimates. Values in bold highlight outstanding proportions of freshwater ariid species in the Australia-New Guinea region.

Region	Total FF families	Primary FF families	Total FF species	Primary FF species	Freshwater ariid spp.	Ariids/total FF species	Ariids/primary FF species
North America and northern Mesoamerica	32	16	1100	60–90%*	6	5.5×10^{-3}	$6.1–9.1 \times 10^{-3}$
South and Central America	51	35	6600	60–90%*	11	1.7×10^{-3}	$1.9–2.8 \times 10^{-3}$
Africa	37	29	3600	60–90%*	2	5.5×10^{-4}	$6.2–9.3 \times 10^{-4}$
India-SE Asia and Madagascar	42	28	3600	60–90%*	15	4.2×10^{-3}	$4.6–6.9 \times 10^{-3}$
Australia-New Guinea	21	2–5	600	3–26	28	**4.7×10^{-2}**	**1.1–9.3**

*The number of primary freshwater fish species is conservatively estimated as 60–90% of the total number of freshwater species per region, except for Australia-New Guinea that is known to host 3–26 such species.

Do ariid colonisations in freshwater regions with low incumbent diversity have higher rates of disparification and lineage diversification?

We compared rates of disparification (see Disparity above) between regional freshwater assemblages and marine assemblages (freshwater species in AU-NG, freshwater species in other regions and marine species) and estimated ratios for all possible pairwise comparisons (i. e. relative rates of disparification). To account for ambiguous habitat states, euryhaline species were either coded as marine (criterion 1) or freshwater (criterion 2). Preliminary analyses using the two criteria gave similar results and only results from criterion 2 are discussed (see also Table S3). We also compared relative rates of morphological evolution against the proportion of freshwater ariids relative to the total number of freshwater fish by region; we did not perform correlations for disparification rates due to small sample sizes.

To quantify net diversification rates for various clades, we used the method-of-moments estimator (Magallon & Sanderson 2001; Wiens *et al.* 2011), implemented in the R package Geiger. Given extant diversity and age, we obtained net diversification rates for all freshwater colonisation events that included at least three species (i. e. have non-zero rates; see Table S2). We selected various values for the extinction parameter (ε = 0.0, 0.5 and 0.9), all of which yielded similar results; thus, only results using ε = 0.5 are reported. To make all calculations comparable, we used the stem rather than the crown equations given uncertainty in the placement of missing species in specific nodes. We assessed the phylogenetic placement for missing species based on our taxonomic expertise while taking geography into consideration; in addition, missing freshwater species were assigned into clades in the most conservative manner by minimising the number of transition events. For comparisons, net diversification rates for each freshwater clade were standardised against background rates in the Ariidae.

Finally, even if competition is limiting diversification, freshwater lineages may still accumulate over time (Stephens & Wiens 2003). We tested the time-for-speciation hypothesis by regressing log-transformed species richness in freshwater clades (Table S2) against the stem age of those lineages.

Is adaptive radiation via EO facilitated by the relative lack of competitors?

Given similar levels of resource wealth, we expect that regions with a relative lack of competitors would facilitate adaptive radiations of greater magnitude than regions with high incumbent diversity. Determining whether or not the evolutionary radiations match the criteria for an ecological adaptive radiation (sensu Schluter 2000) is often difficult, as it requires demonstrating phenotype-environment correlations and a selective advantage of trait variation (but see Olson & Arroyo-Santos 2009). We thus test whether or not the AU-NG clade, including mostly freshwater representatives, shows the signal of adaptive radiation in the classic Simpsonian sense. Unlike the AU-NG clade, no other freshwater invasions have diversified to the point where these hypotheses can even be tested (see Fig. 1a).

We assessed whether or not patterns of cladogenesis and disparity in this clade are accelerated, showing an early burst of diversification. We generated lineage-through-time (LTT) plots and estimated the lineage diversification index (LDI) statistic to determine if cladogenesis fits pure-birth (LDI $\approx$ 0), early burst (positive LDIs) or recent speciation (negative LDIs) models. Significance in LDI values was assessed using 95% CIs from a null (Yule) model of speciation (Harmon *et al.* 2003). As an alternative metrics of lineage accumulation, we estimated the γ statistic (Pybus & Harvey 2000), for which

values significantly different from zero indicate strong departures from the constant-rate null model. To assess the potential impact of missing taxa, we used the procedure of Burbrink *et al.* (2011), which modifies the Monte Carlo Constant Rates test (Pybus & Harvey 2000) of the γ statistic to account for non-random phylogenetic sampling (see Brock *et al.* 2011). Given that our phylogeny lacks 43 species, of which six belong in the AU-NG clade, we were able to randomly assign the missing taxa within their putative clades as explained above, with placement weighted by the length of the internal branches. This creates a distribution of fully sampled trees with phylogenetically randomised placement of the missing species, consistent with the expectation of a null (Yule) model of speciation. We created 1000 replicates, and repeated the LDI and γ analyses on these trees.

We also compared the relative net diversification and disparification rates (see above) of the AU-NG clade by standardising both by the respective overall (background) ariid rates. Finally, we assessed whether or not morphological diversity in this clade is indirectly associated with niche width expansion, using available information on trophic ecology (Allen & Coates 1990; Allen 1991).

RESULTS

Phylogeny, divergence times and habitats

Details of the phylogeny and divergence time estimates are presented in Appendix S2 and Fig. S1. Our reconstruction of the evolution of habitat utilisation (Fig. 1a) confirms findings from the previous study (Betancur-R. 2010), indicating multiple independent events of freshwater colonisation by marine ariids. With the implementation of the DEC model here, we detected 13 single-origin events of ecological transitions (including euryhaline taxa). One lineage in the AU-NG clade that comprises two species (one represented in the phylogeny and one missing) appears to be the result of a secondary freshwater invasion; whenever necessary, these two species are excluded from diversity estimations of the AU-NG freshwater assemblage in downstream analyses. In addition, we hypothesise that missing taxa in the tree may account for five additional events (Table S2). Our optimisation indicates that the oldest freshwater transition took place ~16 my (Fig. 1a). The GeoSSE analysis favoured a model in which speciation and extinction are invariant between marine and freshwaters, suggesting no relationship between diversification and habitat occupancy overall.

Disparity

In the phylogenetic PCA, the first three axes explained 86.6% of the variation (PC1 = 76.21%; PC2 = 5.82%, and PC3 = 4.51%), while PC4–PC30 in conjunction explained only 13.4% of the variation (2.7% in PC4 to 3.6 $\times$ 10^{-6} in PC30); thus, only the first three PCs were retained for subsequent analyses. The highest loadings from PC1 and PC3 were size components [e.g. standard length (0.99) and preadipose-fin length (0.99)] and meristic variables [number of gill rakers (−0.84) and anal-fin rays (0.45)], respectively. The second axis was dominated by mostly shape variables [e.g. upper lip width (0.50) and barbels length (−0.46 to −0.48)], but a meristic variable [anal-fin rays (−0.67)] had a strong weight on this component (see details in Table S5). Although variation in size (standard and preadipose-fin lengths) may be associated with niche partition-
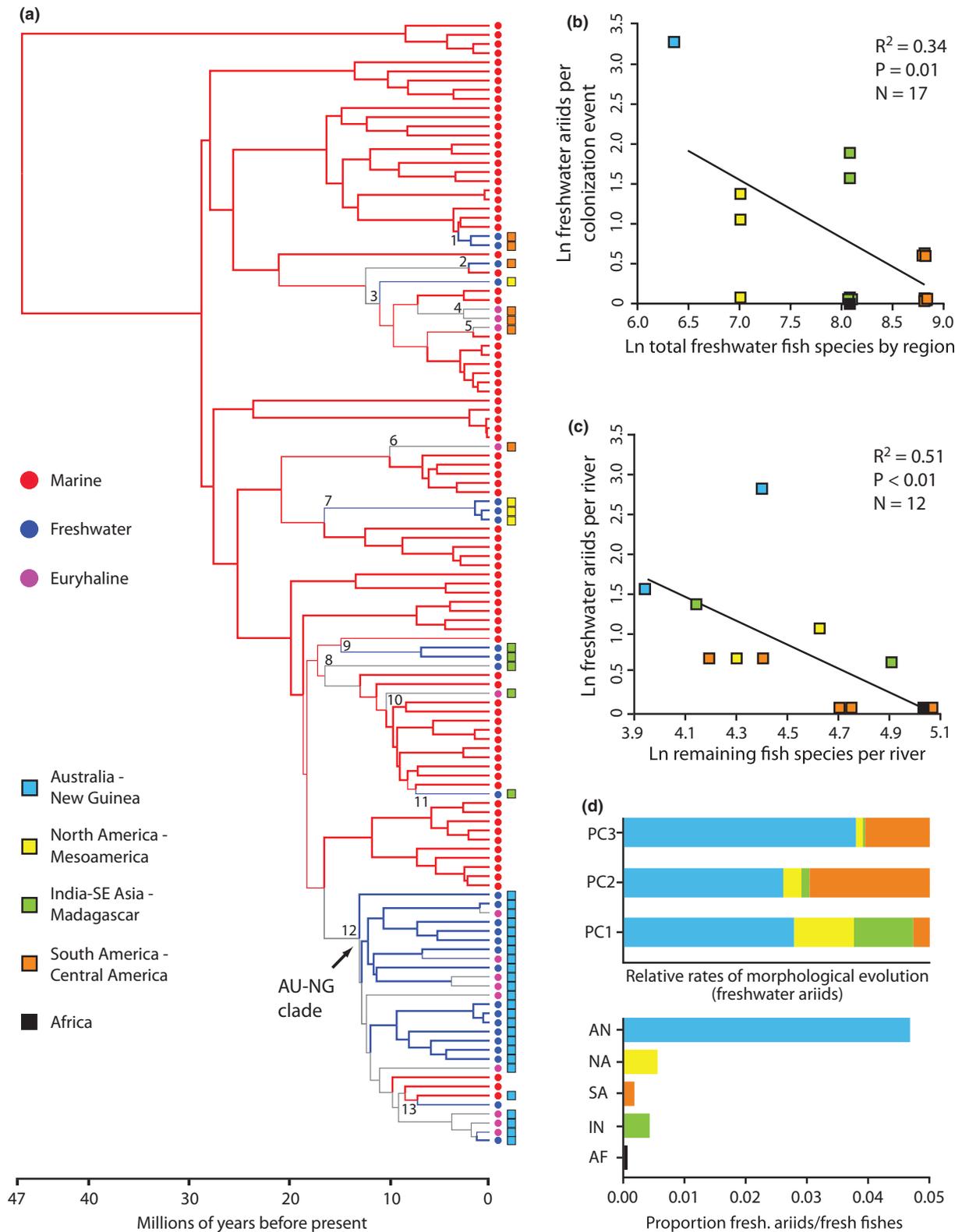
Figure 1 (a) Time-calibrated phylogeny for 123 ariid species estimated from combined mitochondrial and nuclear data sets (7190 sites). Branch colours indicate ancestral habitat reconstructions, with thick and thin colour lines denoting unambiguous and most likely (relative probability ≥ 0.67) reconstructions respectively; thin grey lines indicate ambiguous optimisations (probability < 0.67); numbered nodes are inferred marine-to-freshwater transitions (including euryhaline lineages; Table S2). (b–c) Scatterplots of (b) freshwater ariids per colonisation event against the number of freshwater fishes by region and (c) number of riverine ariids against total number of fish species per river. (d) Relative rates of morphological evolution [principal components (PC) 1–3] in regional assemblages of freshwater ariids compared to the proportion of freshwater ariids/freshwater fishes in each region.

ing, the number of anal-fin rays likely vary as a function of locomotion performance and the remaining variables as a function of feeding behaviour.

The best-fit models of trait evolution for the ariid tree and for the AU-NG clade were WN (PC1; ariids ΔAICc = 71.5, AU-NG clade ΔAICc = 18.1) and OU (PC2; ariids ΔAICc = 18.3, AU-NG clade ΔAICc = 2.4; PC3, ariids ΔAICc = 56.4, AU-NG clade ΔAICc = 18.7). The WN is difficult to interpret biologically, as it provides a phylogeny-free null model that is similar to OU but with infinitely high selection (Kozak & Wiens 2010; Burbrink *et al.* 2011). Nonetheless, the fit of PC1 to OU is not significantly different from that to WN (ΔAICc = 0.92); thus, we conducted disparifi-

cation analyses using untransformed (UB) and transformed branches under the OU model (OUTB; all OUTB trees subsequently fitted a BM model). Although the analyses from UB and OUTB trees revealed differences in rates of evolution, the results of the hypothesis tests are robust to the transformations. Analyses based on OUB trees are summarised in Table S3.

Competition and diversification

Is competition influencing species richness after ecological transitions?
Scatterplots of freshwater ariid species per colonisation event against the total freshwater fish species per region (Table 1) and
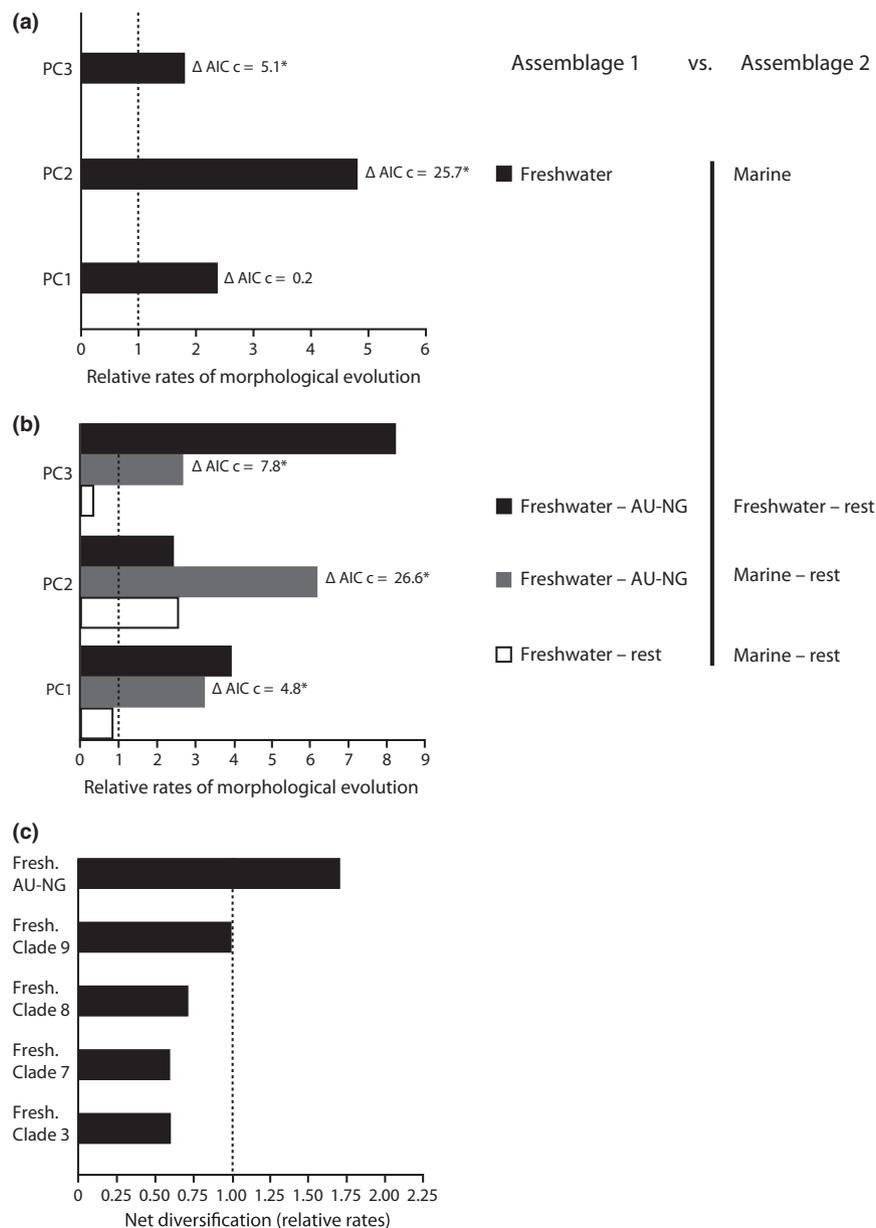

Figure 2 Relative rates of morphological evolution (a–b) and lineage diversification (c) for multiple pairwise comparisons. (a) Rates of disparification in marine vs. freshwater taxa (euryhaline species coded as freshwater; see Table S3). (b) Rates of disparification in freshwater ariids in AU-NG vs. freshwater ariids in other regions vs. marine ariids. The dashed line denotes equal rates of morphological evolution, with values > 1 illustrating higher rates in assemblage 1 and < 1 higher rates in assemblage 2. (c) Net diversification rates estimated for multiple clades (see Fig. 1; details in Table S2) using the method-of-moments and standardised for each clade against background rates in ariids; dashed line denotes equal rates.

number of freshwater ariids from 12 rivers against the remaining fish species both show a strong negative correlation ($R^2 = 0.34$, $P = 0.01$, Fig. 1b; $R^2 = 0.51$, $P < 0.01$, Fig. 1c respectively), suggesting that freshwater ariid diversification is limited by competition from incumbent, non-ariid freshwater fish lineages.

Do ariid colonisations in freshwater regions with low incumbent diversity have higher rates of disparification and lineage diversification?

Disparification rates in freshwater ariids are 2–5× faster than in marine taxa, with strong support for a two-rate (i.e. freshwater vs. marine) model ($\Delta AICc = 5$–26 for PC2 and PC3; PC1 was not supported; Fig. 2a). Rates in AU-NG freshwater species are 3–6× and 2–8× faster relative to marine and non-AU-NG freshwater taxa respectively ($\Delta AICc = 5$–27). In contrast, compared to marine taxa, relative rates in freshwater species outside AU-NG are slower for PC1 and PC3 (0.8× and 0.3× respectively) but faster for PC2 (2.4×; Figs 1d, 2b; Table S3). Net diversification rates, on the other hand, are 1.7× faster in AU-NG freshwater taxa relative to ariids as a whole, whereas in the remaining four freshwater clades that include at least three species, net diversification rates are slower than background (ariid) rates (0.60–0.96×; Fig. 2c). These results overall support the prediction that higher rates of morphological evolution and lineage diversification in freshwater ariids are driven by the AU-NG taxa, and that the rates in freshwater taxa elsewhere tend to be constrained. In addition, relative rates of diversification in freshwater ariids conform to the richness of freshwater fish competitors in the respective regions (Figs 1d, 2c).

Plots of log-transformed species richness against stem age for 13 freshwater clades/lineages in the phylogeny (Table S4) show a significant positive correlation ($R^2 = 0.46$; $P = 0.01$), indicating that older freshwater colonisations have accumulated more species over time.

Is adaptive radiation via EO facilitated by the relative lack of competitors?

As noted above, we find no support for an early burst of disparification in the AU-NG clade (see Discussion). However, the LTT plots using either our empirical tree or the thousand trees with random placement of missing species, show a clear pattern of rapid initial accumulation of lineages falling outside the 95% CI (Fig. 3b) with positive LDIs (empirical = 0.70; random placement mean = 0.74; Fig. 3b) and significantly negative values of γ (empirical value = -3.1, $P = 0.001$; random placement mean = -3.7, P mean = 0.000). In contrast to the pattern observed in the AU-NG clade, the ariid tree shows a more constant pattern of LTT accumulation, with negative LDIs values (empirical = -0.48; random placement mean = -0.56; Fig. 3a) but significantly negative values of γ (empirical = -1.93, $P = 0.026$; random placement mean = -1.56, P mean = 0.036; Fig. 3a).

Rates of disparification and lineage diversification are ~2× faster (PC1 = 2.0×, PC2 = 2.4×, PC3 = 1.9×; net diversification rates = 2.1×) for the AU-NG clade relative to background rates in ariids. Finally, high levels of morphological diversity in AU-NG (Fig. S2) are coupled with a broad range of trophic adaptations, including filter feeding, plant feeding, frugivory, piscivory, mollusc and prawn feeding, lepidophagy, omnivory, detritivory and insectivory (Allen & Coates 1990; Allen 1991); this is indicative of wide resource exploitation along ecological gradients.

DISCUSSION

Although it is difficult to rigorously test hypotheses of past competition, under the ecological theory of adaptive radiation via EO we would expect the present-day signal of historical competition limiting diversification to manifest itself in three ways. First, freshwater species richness should be inversely related to incumbent diversity in both regions and communities, due to the earlier arrival and radiation of those lineages. Second, freshwater colonisation into regions with high pre-existing diversity should exhibit lowered rates of lineage diversification and reduced rates of disparification, due to a reduced volume of occupied niche space. Third, lineages colonising habitats lacking competing lineages (e.g. AU-NG) may undergo accelerated adaptive radiation relative to regions with abundant EO but competing lineages.

We find support for all these predictions in ariids, with strong evidence that freshwater radiations are limited outside the AU-NG
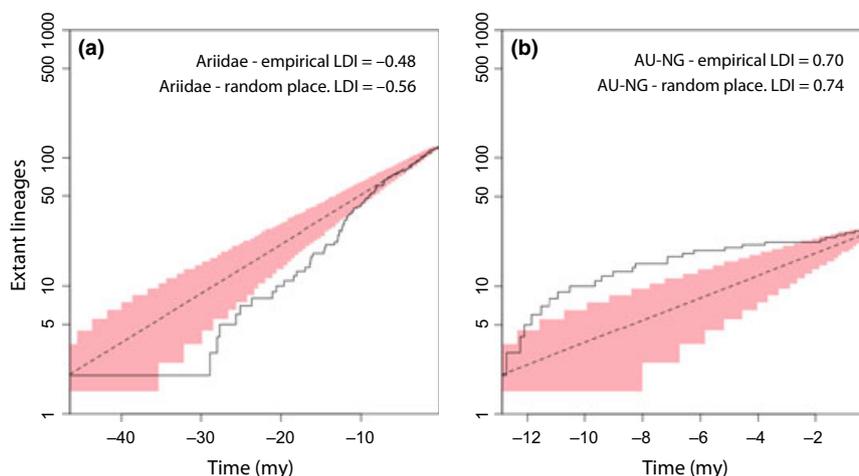

Figure 3 Lineage diversification index (LDI) and lineage through time (LTT) plots for the ariid tree (a) and the Australia-New Guinea clade (AU-NG; (b). The dashed line indicates the null distribution under a Yule process; the shaded area denotes the 95% confidence intervals. The LTT plots were estimated from the empirical (incomplete) tree; LDI values are shown for both the empirical and the randomised trees (mean value).

clade and that the EO presented by the colonisation of new habitats can be blunted by the effects of competition from incumbent lineages. We show that the diversity of freshwater ariid taxa is negatively correlated with the regional abundance of freshwater fish competitors at both regional and community scales: areas and rivers hosting fewer incumbent species tend to support either more diverse clades of freshwater ariids, regardless of the co-occurrence of other ariid clades (Fig. 1b), or larger assemblages of freshwater ariids, regardless of their phylogenetic affinity (Fig. 1c). Although the multiple freshwater invasions have accumulated species over time, as would be expected under the time-for-speciation hypothesis (Stephens & Wiens 2003), this has apparently occurred at a greatly reduced rate (particularly outside AU-NG) compared to ariids (Fig. 2C) and to what would be expected under a typical adaptive radiation (Simpson 1953; Schluter 2000; Brockhurst et al. 2007).

Freshwater ariids overall have higher rates of diversification than marine taxa (Fig. 2a), in agreement with the expectation that ecological habitat transitions promote radiations. However, this pattern is mostly driven by freshwater species in AU-NG (Fig. 2b,c). In fact, relative to marine ariids, rates of disparification and diversification tend to be slower in other freshwater lineages (Fig. 2b–d). Accordingly, these rates are strongly tied to the number of competitor lineages in each region (see below; Table 1; Fig. 1d). On the other hand, rates of disparification and cladogenetic diversification in the AU-NG clade (including mostly freshwater taxa) are approximately twice as fast as the background rates, and species exhibit high phenotypic diversity (Figure S2) related to resource utilisation. Lineages also accumulate rapidly during the early history of the clade followed by a steady decline (Fig. 3b), which is the typical signature of Simpsonian adaptive radiation (Simpson 1953). Although we find no support for an early burst of disparification in the AU-NG clade, as predicted by classic adaptive radiation models, empirical studies have shown that early bursts in comparative data are rare, even for recognised adaptive radiations (Harmon et al. 2010).

Tests of the EO hypothesis have usually focused on the colonisation of empty habitats (Schluter 2000; Harmon et al. 2003; Burbrink & Pyron 2010), such as islands by terrestrial animals (e.g. finches and Anolis lizards; Schluter 1988; Harmon et al. 2003) or lakes by fishes (e.g. cichlids and sticklebacks; e.g. Bolnick 2004), but the colonisation of habitats with varying degrees of incumbent diversity is rarely examined in this context.

Phylogenetic studies on marine-derived fish groups (e.g. anchovies and pufferfishes; Betancur-R. 2010; Yamanoue et al. 2011; Bloom & Lovejoy 2012) have suggested that competition from incumbent lineages may be a major force regulating the frequency of freshwater transitions. However, until now, no explicit tests were conducted to assess the relationship between pre-existing freshwater diversity and diversification of the invading marine group. Herein, we show that the prior existence of competitors limits radiations during ecological transitions in Sea Catfishes. Despite repeated independent reinvasions of freshwaters in multiple geographic areas, only the clade in AU-NG, an area with an otherwise depauperate freshwater fish fauna, has achieved a successful radiation.

Thus, the advantage of EO presented by new areas and ecological regimes has apparently been limited in freshwater ariids by the effects of competition from non-ariid freshwater lineages present prior to colonisation. Potential constraints on adaptive radiation are an understudied, but important avenue of research for studies of evolutionary diversification (Wiens et al. 2011). Despite the intrinsic

difficulties in testing historical competition in a phylogenetic context (Moen & Wiens 2009), our results demonstrate the importance of assessing the impact of competing lineages when analysing adaptive radiation (Kassen et al. 2004; Brockhurst et al. 2007). Additional questions include decoupling the relative contribution between competition and wealth of resources in determining the extent of EO, and whether or not the phylogenetic similarity of existing lineages affects the constraints imposed by competition (Ricklefs 2004; Wiens et al. 2011). Ultimately, adaptive radiation via EO is unlikely to take place in a vacuum of competitors, so assessing the relative impact of EO and incumbent diversity will be important to explain variation in the speed and magnitude of evolutionary radiations.

AUTHORSHIP

RBR, RAP and GO conceived the study. RBR and AMS generated molecular data; RBR, APM and AMS examined museum specimens. RBR and RAP performed the analyses and wrote the manuscript. All authors contributed to the writing and approved the final version of the paper.

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