

# MOLECULAR CLOCKS PROVIDE NEW INSIGHTS INTO THE EVOLUTIONARY HISTORY OF GALEICHTHYINE SEA CATFISHES

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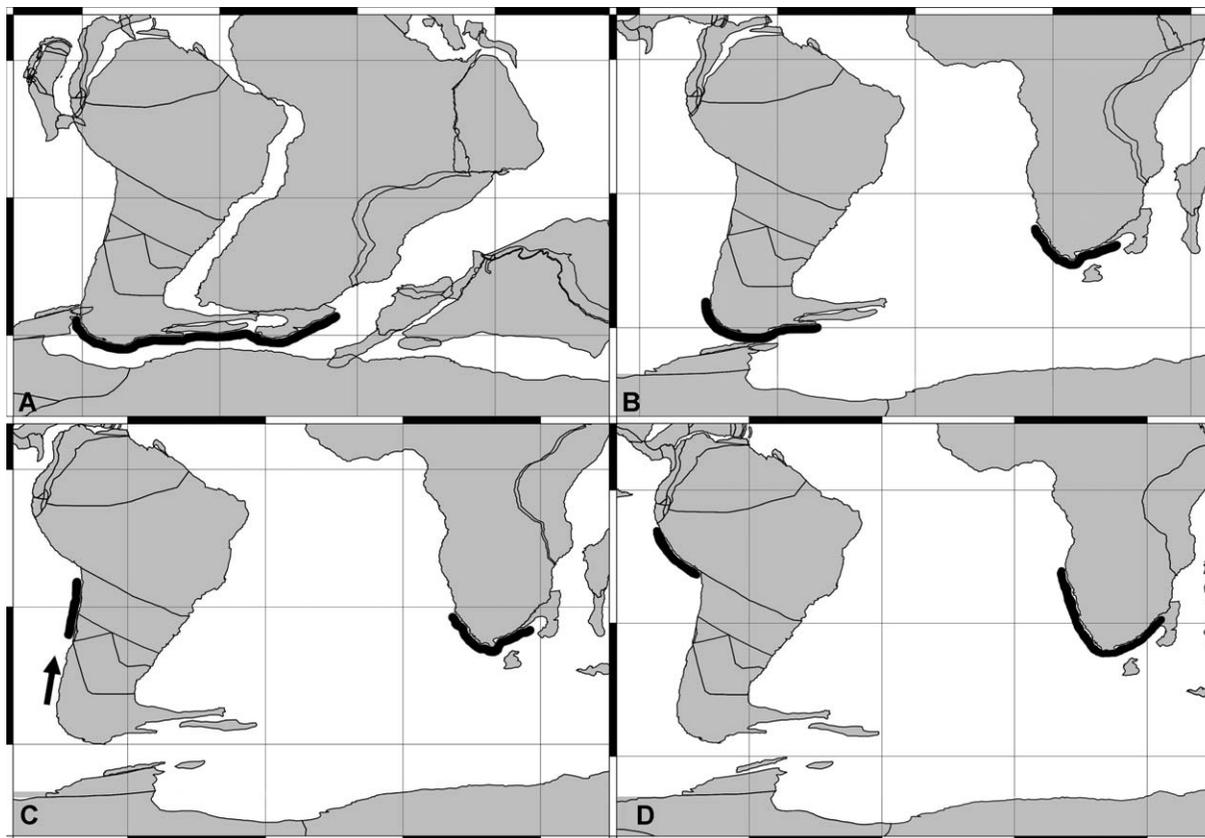
Intercontinental distributions in the southern hemisphere can either be the result of Gondwanan vicariance or more recent transoceanic dispersal. Transoceanic dispersal has come into vogue for explaining many intercontinental distributions; however, it has been used mainly for organisms that can float or raft between the continents. Despite their name, the Sea Catfishes (Ariidae) have limited dispersal ability, and there are no examples of nearshore ariid genera with a transoceanic distribution except for *Galeichthys* where three species occur in southern Africa and one in the Peruvian coast. A previous study suggested that the group originated in Gondwana, and that the species arrived at their current range after the breakup of the supercontinent in the Early Cretaceous. To test this hypothesis, we infer molecular phylogenies (mitochondrial cytochrome *b*, ATP synthase 8/6, 12S, and 16S; nuclear *rag2*; total ~4 kb) and estimate intercontinental divergence via molecular clocks (penalized-likelihood, Bayesian relaxed clock, and universal clock rates in fishes). Age ranges for cladogenesis of African and South American lineages are 15.4–2.5 my, far more recent than would be suggested by Gondwanan vicariance; thus, the distribution of galeichthyines must be explained by dispersal or more recent vicariant events. The nested position of the Peruvian species (*Galeichthys peruvianus*) within the African taxa is robust, suggesting that the direction of the dispersal was from Africa to South America. The progenitor of the Peruvian species likely arrived at its current distribution with the aid of ocean currents, and several scenarios are discussed.

**KEY WORDS:** Ariidae, Gondwana, relaxed-clock methods, transoceanic dispersal, vicariance.

A key challenge in evolutionary biology is hypothesizing historical events that explain disjunct distributional patterns. There has been recent debate on whether disjunct biogeographies on different land masses in the southern hemisphere were the result of Gondwanan vicariance or transoceanic dispersal (de Queiroz 2005; McGlone 2005). The latter explanation was the paradigm held in classic biogeography. It was not until the 1960s that Gondwanan vicariance became a mainstream concept, induced by the development of the continental-drift hypothesis and the boom of cladistics. Nevertheless, recent studies using modern molecular dating techniques are now causing a shift in this debate by revealing younger divergence times and thus ruling out Gondwanan vicariance as an explanation (e.g., Baum et al. 1998; Raxworthy et al. 2002; Schrago and Russo 2003). Striking examples of

oceanic dispersal include a variety of faunal and floral groups, such as geckos, monkeys, lemurs, carnivores, chameleons, frogs, insects, and several angiosperm families, among others (reviewed in de Queiroz 2005).

The ariids, or Sea Catfishes, comprise over 150 species that inhabit warm temperate to tropical marine or estuarine regions around the world (Betancur-R. et al. 2007; Marceniuk and Menezes 2007). Two subfamilies of Sea Catfishes are currently recognized, the worldwide Ariinae, including 97% of ariid diversity, and the Galeichthyinae (Acero and Betancur-R. 2007). With three extant species in southern Africa (*Galeichthys* sp., *G. ater*, *G. feliceps*) and one in the Peruvian Pacific (*Galeichthys peruvianus*), the Galeichthyinae represents a striking example of biogeographic disjunction (Fig. 1D; Kulonowski 2001; Acero P. and



**Figure 1.** (A–C) Betancur-R. et al.'s (2007) hypothesis of Galeichthyinae distribution during the plate tectonic progression of Gondwana (indicated by solid black line). Reconstructions are based on the following events: (A) Gondwana after the separation of Australia (before Early Cretaceous), (B) final separation of the supercontinent before the opening of Drake Passage (between Early Cretaceous to Late Eocene), (C) opening of Drake Passage and cooling of Antarctica (after Late Eocene), and (D) present configuration (reconstructions from: <http://www.ods.de/>).

Betancur-R. 2007; Betancur-R. et al. 2007). Several aspects of the biology and habitat preferences of this group are critical for understanding their evolutionary history. Like other ariids, galeichthyines are mouthbrooders of eggs and alevins (Tilney and Hecht 1993). These fishes inhabit shallow subtropical waters and exhibit bottom-dwelling habits as juveniles or adults with maximum depths of up to 60 m (Tilney and Hecht 1990). Therefore, pelagic or deep-sea dispersal and long-distance dispersal via planktonic larvae stages is disfavored as explanation for the disjunct distribution of galeichthyines. The aforementioned examples of intercontinental dispersal include taxa that may cross oceanic barriers via rafting or wind; however, the habitat preferences and basic life-history strategies of galeichthyines suggest that these are unlikely dispersal mechanisms. Their apparent continental restriction disfavors a priori transoceanic dispersalist scenario.

Gondwanan vicariance seems a plausible scenario for explaining galeichthyines' disjunct distribution. Betancur-R. et al. (2007) hypothesized that the group originated in the southern coast of the supercontinent after the separation of Australia

(Fig. 1A). Following the final breakup of Gondwana in the mid Cretaceous (Barron et al. 1981), the lineages may have remained in the southern portions of both South America and Africa (Fig. 1B). The subsequent cooling of Antarctica 37 million years ago [mya] (Veevers and Ettriem 1988) and southern South America, probably caused a progressive migration toward lower latitudes, finally reaching today's subtropical waters of Peru (Fig. 1C). A Gondwanan galeichthyine origin implies that African and South American lineages diverged at least 105 mya (Late Albian). A major con for the Gondwanan hypothesis is that the fossil record of ariids only dates back to Late Campanian–Early Maastrichtian (~73–68 mya; Gayet and Meunier 2003; Betancur-R. et al. 2007).

The monophyly of the Galeichthyinae has been supported from morphological evidence (Acero P. and Betancur-R. 2007; Marceniuk and Menezes 2007), but previous molecular phylogenies included only *G. peruvianus* (Betancur-R. et al. 2007); hence, both the monophyly and the temporal context of diversification remain to be tested with molecular data. In this study, we infer molecular phylogenies (2.88 kb mitochondrial + 0.98 kb

nuclear) for the four species of galeichthyines among representative ariid and anchariid taxa. To test Gondwanan vicariance versus transoceanic dispersal in a chronological context, we provide divergence time estimations for the disjunct group based on a number of molecular-clock methods via geological and paleontological calibrations.

## Materials and Methods

### TAXON SAMPLING, DNA DATA, AND PHYLOGENETIC RECONSTRUCTIONS

The ingroup (Arioidea) includes sequence data from the two subfamilies of the Ariidae (Galeichthyinae and Ariinae) plus one species of the sister family Anchariidae (*Gogo arcuatus*). In addition to *G. peruvianus* and nine ariine species in seven genera examined in Betancur-R. et al. (2007: Appendix A), molecular data were obtained in this study for *G. arcuatus*, the three galeichthyine species from southern Africa (*Galeichthys* sp., *G. ater*, and *G. feliceps*), and 11 ariine species representing different genera (total 25 ingroup taxa). Our taxon-sampling scheme includes ariine representatives of 18 of the 25 genera currently validated (according to Marceniuk and Menezes 2007) and major biogeographic provinces (New World, Africa, India-SE Asia, Australia-New Guinea). Two distantly related catfish families were used as outgroups (Ictaluridae and Auchenipteridae; listed in Betancur-R. et al. 2007). New material examined is listed in Appendix A. Institutional abbreviations are as listed at <http://www.asih.org/codons.pdf>.

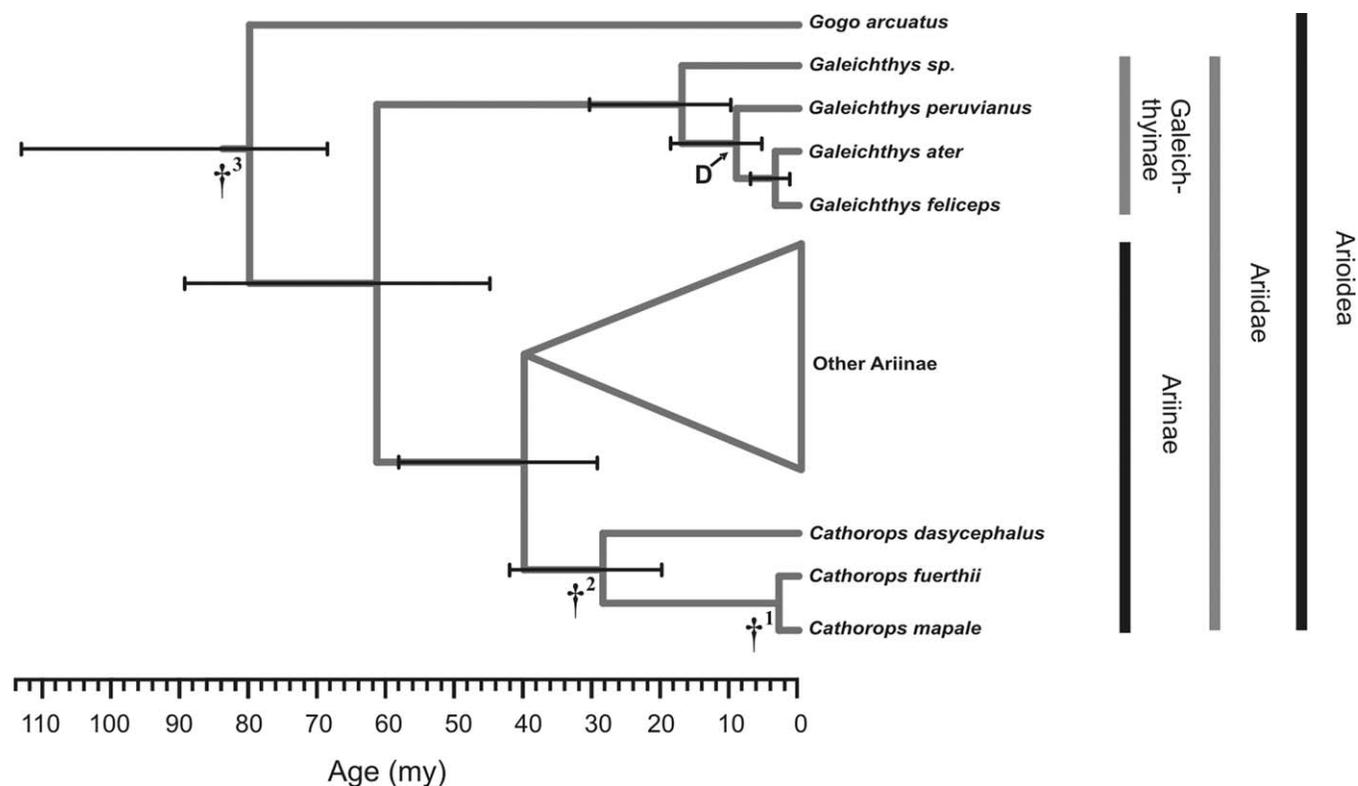
Target mitochondrial regions include partial cytochrome *b* (*cyt b*) and complete ATP synthase subunits 8 and 6 (ATPase 8/6) protein-coding genes, and partial 12S and 16S ribosomal genes. Nuclear evidence includes partial recombination activating gene 2 (*rag2*). Laboratory protocols, primers used for obtaining molecular data, sequence alignments, model testing, and phylogenetic inferences using maximum parsimony (MP) and Bayesian inference (BI) criteria are as described in Betancur-R. et al. (2007), with two modifications: (1) MP nodal support was estimated using 1000 bootstrap pseudoreplicates, (2) BI analyses were run unpartitioned. Maximum-likelihood (ML) reconstructions were performed in the program RAxML version 7.04 (Stamatakis 2006) as implemented in the CIPRES portal version 1.13 (<http://www.phylo.org/>). The initial model was estimated using the Akaike information criterion (AIC) in ModelTest version 3.7 (Posada and Crandall 1998); model parameters were estimated simultaneously. ML nodal support was evaluated using the rapid bootstrapping algorithm with automatic estimation of runs. At least five runs from random-starting seeds were performed to check the convergence of likelihood scores. Ancestral distributions were reconstructed under parsimony.

### DIVERGENCE TIME ESTIMATIONS

Only mitochondrial protein-coding genes (*cyt b* and ATPase 8/6) were used for chronological estimations. Relative rates tests based on likelihood were performed on five clades with different nesting hierarchies using the software r8s version 1.71 (Sanderson 2003, 2004). Four of the five comparisons suggested significant departures from a clock-like behavior. Thus, two different methods that do not assume a strict molecular clock were used for inferring nodal ages: penalized likelihood (PL) as implemented in r8s and Bayesian relaxed clock (BRC) as implemented in MULTIDIVTIME (Thorne and Kishino 2002). For both PL and BRC analyses, the tree topology (and branch lengths in PL) was estimated using ML.

PL is a semiparametric approach that combines a parametric model for branches with different substitution rates and a nonparametric roughness penalty. The relative contribution of the two components is determined by the smoothing parameter. As recommended by Sanderson (2004), PL analyses were performed using the truncated Newton algorithm with optimal values of the smoothing parameter being estimated via cross-validation procedures (four orders of magnitude). The software r8s includes two types of penalty functions. The additive penalty function penalizes the squared difference in rates on neighboring branches. The log penalty penalizes the squared difference in the log of the rates across neighboring branches. As Sanderson (2004) pointed out, it is not always clear which of these functions should be selected a priori. Thus, all analyses were carried out using either additive or log penalties. The correctness of the solutions found was checked using the checkgradient command.

BRC uses a probabilistic model to approximate the posterior distribution of rates and divergence times, with their associated standard deviations and 95% credibility intervals, via Markov chain Monte Carlo (MCMC) iterations. For BRC analyses, we followed the instructions compiled by Rutschmann (2004) and the program manuals. Estimation of the F84 + G model parameters was done in the BASEML program implemented in PAML (Yang 1997). Other programs used are part of the MULTIDISTRIBUTE package (available from J. Thorne): BASEML output files were converted into ESTBRANCHES input files using PAML2MODELINF; branch lengths on the tree topology and the variance-covariance matrix were estimated under maximum likelihood in ESTBRANCHES; MCMC analyses were conducted in MULTIDIVTIME. The priors for the Bayesian approach were calibrated as follows (ingroup only): the *rttm* and *rtmsd* parameters were both set to 73 my, based on Lundberg et al.'s (2007: Fig. 2) estimation of the Anchariidae/Ariidae split. Because assuming a Gondwanan divergence of the disjunct group requires a minimum age of 105 my, the *Bigtime* parameter was set to 157 my. This value equals the origin of Siluriformes inferred by Peng et al.



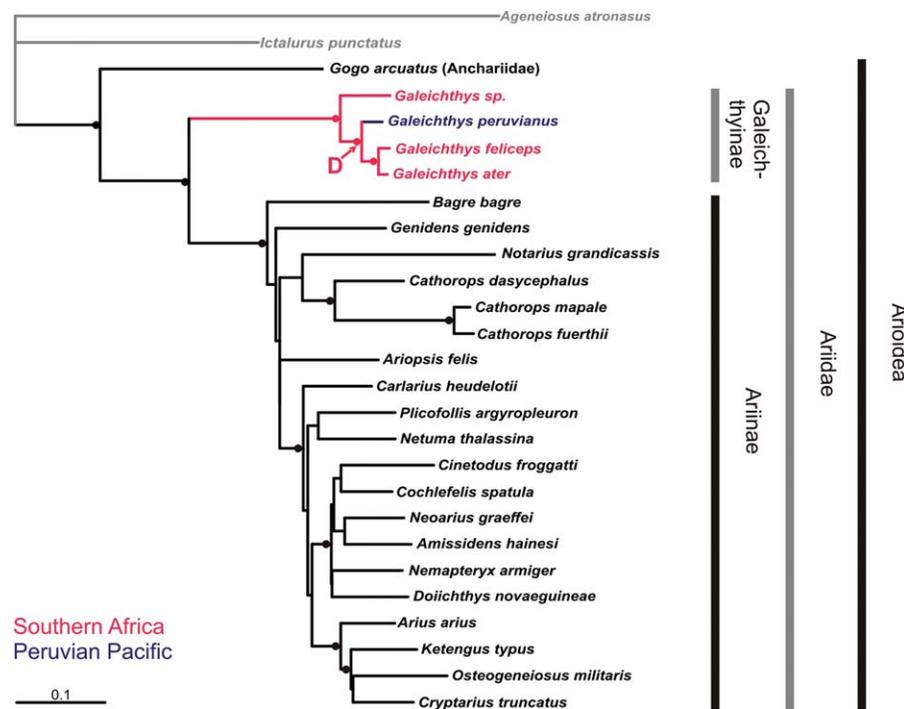
**Figure 2.** Bayesian relaxed-clock chronogram based on two partitions (*cyt b* and ATPase 8/6; 1937 sites) and youngest fossil ages. The initial topology was estimated under maximum likelihood. Daggers (†) indicate calibration points and constraints based on fossils or geological data (see details on Materials and Methods). Gray bars indicate 95% credibility intervals. D, disjunct clade.

(2006). The  $r_{rate}$  and  $r_{ratesd}$  parameters were both set to the mean value for the total evolution of all branches from the root to the tip of the tree divided by  $rtm$  ( $= 0.0043$ ). The  $brnmean$  and the  $brnsd$  parameters were both set to 1.0. Other priors were set to their default values. The MCMC was run for  $1.0 \times 10^6$  generations, sampling trees every 100 generations. Ten percent of the first trees sampled were discarded as burn-in. To check convergence of the Markov chain, all analyses were run in duplicate from random starting seeds. BRC analyses were carried out using either one partition or two partitions (ATPase 8/6 and *cyt b*). For analyses conducted under two partitions, the commonbrown parameter was set to 1 (i.e., genes having similar tendency to change rate).

Three sets of dates were used as calibration points for PL and BRC analyses (Fig. 2): (†<sup>1</sup>) the final rising of the Panama isthmus (3.1–2.8 mya; Coates and Obando 1996) is hypothesized as the vicariant event leading to divergence into the modern geminate pair *Cathorops mapale* (Caribbean) and *C. fuerthii* group (Eastern Pacific). The mitochondrial distances using protein-coding genes between these two taxa (2.2–2.8%; Betancur-R. and Acero P. 2005: Table 3) are similar to those reported for other transisthmian fish pairs assumed to have diverged during the final rising of the isthmus (Bermingham et al. 1997). Both a maxi-

imum age constraint of 3.1 my and a minimum age constraint of 2.8 my were applied to this node. (†<sup>2</sup>) The oldest fossil of *Cathorops* is an unidentified skull from the Cantaura formation (Early Miocene), Venezuela (Aguilera and Rodríguez de Aguilera 2004). A minimum age of 23–16 my was applied to the root of *Cathorops*. (†<sup>3</sup>) The oldest ariid is known from otolith fossil remains of Late Campanian to Early Maastrichtian from South and North America (Gayet and Meunier 2003). A minimum age constraint of 73–68 my was applied to the root of the ingroup (Ariidae/Anchariidae split; see also Lundberg et al. 2007). For both PL and BRC analyses, we explored the effect of using either the youngest or the oldest fossil ages on nodal time estimations.

A third more general approach for estimating divergence ages was conducted by a direct translation of genetic distances into time based on reported molecular clock rates in fishes using protein-coding genes. For *cyt b*, it has been estimated 0.75%/my/lineage in cyprinid fishes (Zardoya and Doadrio 1999); for ATPase 6, it has been estimated 0.65%/my/lineage in percomorph fishes (Bermingham et al. 1997). A rough rate of 0.70%/my/lineage was applied to the combined *cyt b* + ATPase 8/6 dataset. Genetic distances were estimated under the Kimura two-parameter model.



**Figure 3.** Maximum-likelihood phylogram (lnL  $-23,001.75$ ) based on the combined dataset with 3860 bp (2880 bp mitochondrial+978 bp nuclear). Nodal circles represent well-supported clades that are congruent with BI and MP topologies (posterior probability=1.0; ML and MP bootstrap > 85%; see Table 1 for support values within *Galeichthys*). Other clades are incongruent and/or poorly supported. Blue and red branches represent ancestral distribution reconstructions assuming dispersal (under parsimony). D, disjunct clade.

## Results

### DATASET ATTRIBUTES AND PHYLOGENETIC INFERENCE

All sequences obtained during this study are available in GenBank. Accession numbers of sequences are listed in Appendix A. The final alignment of the mitochondrial protein-coding genes included 1937 sites: 1095 bp for partial *cyt b* and 842 bp for AT-Pase 8/6 (see details in Betancur-R. et al. 2007). Partial ribosomal 12S and 16S sequences ranged in size from 388 to 392 bp and 556 to 569 bp, with final alignment lengths of 398 and 573 bp, respectively. In the final alignments nine sites of 12S and 17 sites of 16S were excluded due to ambiguous positional homology. In the nuclear *rag2* dataset not all ingroup taxa had the same sequence length, which varied from 837 to 978 bp; both outgroups had 720 bp.

The combined molecular dataset included 3860 aligned sites. Both ML and BI analyses were performed unpartitioned with initial model GTR + I + G as estimated by the AIC. Five independent analyses using ML each yielded one tree with similar optimal scores (best lnL  $-23,001.75$ , Fig. 3); in all cases, rapid bootstrap analyses were terminated automatically after 250 runs. A majority rule (50%) consensus phylogram was obtained from the BI analysis of  $\sim 135k$  post-burnin trees (mean lnL  $-23,020$ ). The MP search resulted in one optimal tree of 4137 steps and consistency index of 0.41 (BI and MP trees not shown, but see Fig. 3 legend). All topologies are identical at basal ingroup nodes (i.e., Arioidea: Ariidae + Anchariidae). All analyses supported the monophyly of the Galeichthyinae and yielded a fully resolved (*Galeichthys* sp. (*G. peruvianus* (*G. ater*, *G. feliceps*))) and well-supported topology (Table 1). The African galeichthyines were rendered

**Table 1.** Support values for the *Galeichthys* tree (see Fig. 3); all reconstructions recovered identical topologies. ML, maximum likelihood bootstrap; MP, maximum parsimony bootstrap; BI, Bayesian posterior probability.

Node (see Fig. 3)	Mitochondrial (2.88 kb) ML/MP	Nuclear rag2 (0.98 kb) ML/MP	Combined (3.86 kb) ML/BI/MP
<i>Galeichthys ater</i> / <i>G. feliceps</i>	100/100	97/69	100/1.0/100
D	96/100	85/87	99/1.0/100
<i>Galeichthys</i> root	100/100	89/99	100/1.0/100

paraphyletic due to the nested position of the Peruvian species (*G. peruvianus*). Identical topologies within the Galeichthyinae were recovered in separate MP and ML analyses on mitochondrial and nuclear partitions (see summary in Table 1; BI analyses were not performed on separate partitions).

The Ariinae was recovered as monophyletic with high nodal support, but several low-supported clades within the group are incongruent among different reconstruction criteria. Despite the fact that this study and the previous molecular study (Betancur-R. et al. 2007) include rather different taxon-sampling schemes, the resolution patterns identified across the tree are similar, having well-resolved and highly supported family-level clades but low-supported and often incongruent intergeneric clades within the Ariinae. Describing the relationships among ariine genera will be addressed comprehensively elsewhere.

### DIVERGENCE TIME ESTIMATIONS

Node D in Figure 3 indicates the most recent disjunct clade (*G. peruvianus* (*G. ater*, *G. feliceps*)), that is cladogenesis between African and South American lineages. The results obtained on the chronology of node D using different methods of molecular dating are summarized in Table 2 and Figure 2. The PL analyses estimated a divergence time of 9.3–6.4 my. The greatest variation detected is associated with the penalty functions employed (i.e., additive vs. log), whereas calibrations using either youngest or oldest fossil ages had little or no influence on the results. BRC estimations are variable, depending on whether one partition (15.4–14.8 my) or two partitions (9.9–9.2 my) are used. Also, the 95% credibility interval is considerably narrower using two partitions (18.7–4.7 my) vs. one (30.2–6.2 my). The BRC analyses also revealed minor differences associated with youngest and oldest fossil calibrations (Table 2). The universal fish mitochondrial clock inferred a much younger divergence time of node D (2.7–2.5 my) than relaxed-clock methods (15.4–6.4 my).

## Discussion

### MOLECULAR CLOCKS AND THE DIVERGENCE TIME OF NODE D

Molecular data offer new perspectives into the evolutionary history of galeichthyines and reevaluates the Gondwanan hypothesis. Even though different methods provide variable results on the divergence time of the disjunct clade (15.4–2.5 my, Table 2), the oldest estimate is far too recent to be explained by Gondwanan vicariance (i.e., at least 105 my). It could be argued that the age of node D is being underestimated due to the high potential for saturation of mitochondrial protein-coding genes. At least three circumstances disfavor this possibility: (1) plots of third-codon position transitions versus corrected distances for all positions (not shown) provide evidence of saturation above 10% of genetic

divergence. The genetic distances between *G. peruvianus* and *G. ater*/*G. feliceps* are only 3.5–3.8%. (2) Further PL and BRC analyses constraining node D to a minimum age of 105 my (assuming Gondwanan vicariance) estimate the age of the Arioidea being 532–251 my. This is at the minimum 94 my older than the oldest estimate of siluriform origin based on molecular clocks (i.e., 157 my; Peng et al. 2006) and 180 my older than predicted by the fossil record of Siluriformes (i.e., Late Campanian to Early Maastrichtian; Gayet and Meunier 2003). (3) It has been shown in vertebrates (tetrapods) that many mitochondrial genes, including *cyt b* and ATPase 8/6, are good dating estimators even for old divergence times (>100 my; Kumazawa et al. 2004).

Relaxed-clock methods, such as PL, require at least one node in the tree fixed or having a maximum age constraint. Such constraints are often arbitrarily placed in the root of the tree. Due to the fact that the calibration of node †<sup>1</sup> is based on a geological event, it was constrained using both minimum and maximum ages. This conveys the advantage that no other nodes in the tree need an arbitrary constraint (i.e., require fewer assumptions). A possible shortcoming is that inferring the age of deeper nodes using a maximum age constraint on a terminal node might be inaccurate. It is noteworthy, however, that node †<sup>1</sup> has a similar nesting hierarchy to that of node D. Furthermore, supplementary analyses fixing the root of the ingroup to 73 my, based on Lundberg et al.'s (2007) estimation of the Ariidae/Anchariidae split, provide similar results on the divergence time of node D (12.1–7.2 my; PL and BRC using median fossil ages). This suggests that node †<sup>1</sup> calibration is accurate. Of course, this method would not allow us to conclude an age older than 73 my for node D, so it cannot test Gondwanan vicariance.

The universal fish mitochondrial clock and the relaxed-clock methods provide divergent results on the origin of the disjunct clade (2.7–2.5 my vs. 15.4–6.4 my, respectively). Assuming that the latter methods are more accurate (e.g., do not assume a clock-like fashion), the disparity suggests that galeichthyines have slower mitochondrial rates than other fishes whose clock rate has been roughly estimated to 0.65%/my/lineage using *cyt b* and ATPase 8/6 (Bermingham et al. 1997; Zardoya and Doadrio 1999). The BRC and PL analyses inferred substitution rates of 0.2–0.3%/my/lineage for Galeichthyines. In catfishes, typical mitochondrial rates have been reported in pimelodids (0.5–0.8%/my/lineage using *cyt b*; Hardman and Lundberg 2006), whereas slower rates are also known in East Asian bagrids (0.2–0.3%/my/lineage using *cyt b*; Peng et al. 2002) and some ictalurids (0.3%/my/lineage using *cyt b*; Hardman and Page 2003).

Molecular dates for nodes constrained by fossils (nodes †<sup>2</sup> and †<sup>3</sup>) are shown in Table 3. For both clades, relaxed-clock methods estimated older divergence times than the ages of the fossils assigned to them. For instance, the inferred origin for the split of the Anchariidae and Ariidae is 94.4–74.2 mya but the age

**Table 2.** Age estimations for node D (see Fig. 3). YFA, youngest fossil ages; OFA, oldest fossil ages; S, smoothing parameter.

Method	Time (mya)	95% credibility interval		
		SD	Lower	Upper
Penalized likelihood, additive penalty YFA (S=10,000)	6.4	–	–	–
Penalized likelihood, additive penalty OFA (S=10,000)	6.4	–	–	–
Penalized likelihood, log penalty YFA (S=100)	9.1	–	–	–
Penalized likelihood, log penalty OFA (S=100)	9.3	–	–	–
Bayesian relaxed-clock 1P YFA	14.8	6.2	6.2	30.2
Bayesian relaxed-clock 1P OFA	15.4	6.3	6.6	30.1
Bayesian relaxed-clock 2P YFA	9.2	3.4	4.7	18.0
Bayesian relaxed-clock 2P OFA	9.9	3.5	5.2	18.7
Universal fish clock (cyt <i>b</i> +ATPase 8/6, 0.7%/my/lineage)	2.5–2.7	–	–	–

of the fossil is 73–68 my. Lundberg et al. (2007) hypothesized the chronology of major catfish groups via BRC and PL analyses on nuclear phylogenies. Interestingly, their estimated age for the origin of the Ariioidea (73 my) is similar to that of the fossil (73–68 my). The mismatch observed between constrained and estimated ages in this study either suggests that fossils used are younger than nodes or that molecular dates are overestimated (e.g., extrapolating back in time using a terminal calibration; see above).

### BIOGEOGRAPHIC IMPLICATIONS

The separation of Africa and South America (Early Cretaceous) by far predates the timing of intercontinental cladogenesis (relaxed-clock methods: Mid to Late Miocene), thus any scenario that intends to explain current distributional patterns must invoke dispersal or recent vicariance. Putative transoceanic scenarios for the distributions of galeichthyines must be examined in light of

**Table 3.** Molecular age estimations for nodes constrained by fossils. Values within brackets indicate fossil ages. YFA, youngest fossil ages; OFA, oldest fossil ages; S, smoothing parameter.

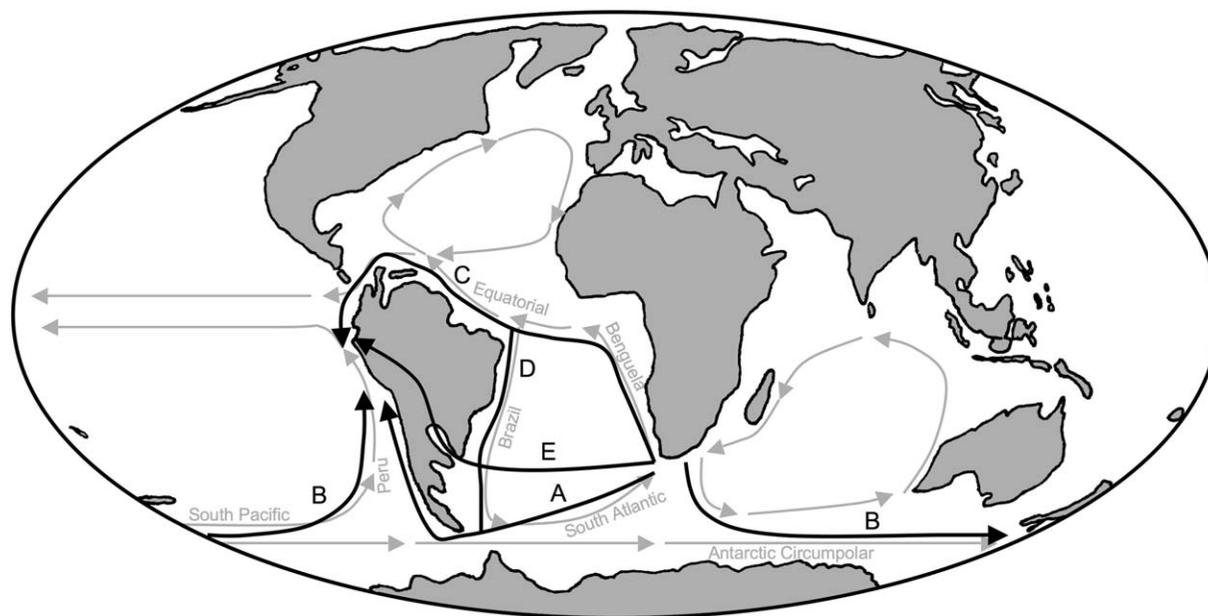
Method	Node †2 (23–16 my)	Node †3 (73–68 my)
Penalized likelihood, additive penalty YFA (S=10,000)	26.4	74.2
Penalized likelihood, additive penalty OFA (S=10,000)	26.4	74.2
Penalized likelihood, log penalty YFA (S=100)	26.0	86.3
Penalized likelihood, log penalty OFA (S=100)	26.5	87.8
Bayesian relaxed-clock 1P YFA	33.8	90.7
Bayesian relaxed-clock 1P OFA	34.9	94.4
Bayesian relaxed-clock 2P YFA	28.8	80.8
Bayesian relaxed-clock 2P OFA	31.0	86.2

three things: (1) the biology of galeichthyines, (2) climate and ocean current changes, and (3) phylogeny.

Galeichthyines are not likely to move across oceans. The fishes are exclusive to continental shelf and are demersal species. The fishes do not have a planktonic stage as males brood the very large eggs in their mouths (Taylor 1986; Tilney and Hecht 1990, 1993). Any transoceanic scenario would have to involve these bottom-dwelling, continental shelf species moving across vast, deep oceans.

A common generalization in marine biogeography is that continental fishes, particularly salt tolerant species, may disperse through oceanic waters (e.g., Lundberg 1993; Vences et al. 2001; Briggs 2003). Sparks and Smith (2005) argued in favor of a Gondwanan vicariance model, instead of oceanic dispersal, for explaining the distributions of cichlids and aplocheiloid killifishes. As stressed by Sparks and Smith (2005), it is continental restriction and not salt intolerance that plays a major role in modeling continental-drift vicariance. For instance, despite the worldwide distribution of ariids, no species occur in the Pacific plate (Springer 1982), the Antilles (except for continental records off Cuba and Trinidad), or any oceanic islands of recent volcanic origin. Moreover, although different morphological phylogenies available for the Ariinae reveal incongruent biogeographic patterns (e.g., Marceniuk 2003; Kailola 2004), molecular evidence offers no support for the existence of any transpacific or transatlantic clade within the group (R. Betancur-R., unpubl. data). As Sparks and Smith proposed for cichlids, if transoceanic dispersal was common in galeichthyines, it would be expected that the South African taxa would have crossed the Mozambique canal to reach the southern subtropical portion of Madagascar; however, no living or fossil galeichthyines have been reported from Madagascar.

The distribution must also be understood by taking into account climate and ocean current changes. The molecular clock suggests a maximum age of cladogenesis of 15.4 mya. This age



**Figure 4.** Possible out-of-Africa dispersal pathways for *Galeichthys* between Middle and Late Miocene (black arrows): (A) South Atlantic—via Drake Passage and Peru current; (B) Antarctic Circumpolar—transpacific route via Antarctic Circumpolar, South Pacific, and Peru currents; (C) Benguela-Equatorial Currents—transatlantic dispersal via Benguela and equatorial currents, and south to Peru; (D) Benguela–Brazil Currents—transatlantic dispersal via Benguela, Brazil and Peru currents through Drake passage; (E) South American Passage—across the south Atlantic and through marine incursions of South America. See pros and cons for each scenario in text and Table 4. Gray arrows indicate key paleocurrents (modified from Haq 1981; Kennett 1985). Map based on 20–11 mya map of Golonka (2000).

correlates with the approximate end of the Miocene Climate Optimum (~16 mya), where temperatures were at their highest in the Neogene (Flower and Kennett 1994). Major growth of the East Antarctic Ice Sheet occurred 14.8–14.1 mya and represented a permanent cooling of the planet. Global water temperatures were highest early in the Miocene and cooled with the end of the Miocene Climate optimum (Savin et al. 1985), although temperatures might have remained higher in South Africa until at least 8 mya based on oxygen isotope data (Savin et al. 1985), and in Patagonia until at least 9 mya based on the presence of fossil sharks, teleosts, mammals, and mollusks (Cione et al. 1996, 2005).

The fragmentation of the circumtropical Tethys seaway, the formation of the Circum-Antarctic current, the closure of the Indo-Pacific passage, and the restriction of water exchange between the Atlantic and the Pacific due to the rising of the Panama isthmus, were the most important events affecting ocean circulation configurations since the Miocene (Romine and Lombardi 1985; Berger and Wefer 1996). Other major surface currents in the southern hemisphere have remained fairly constant since then (Haq 1981; Kennett 1985; Berger and Wefer 1996; Fig. 4).

Finally, phylogeny can provide a potential mode of dispersal or vicariance. The hypothesized phylogenies within the Gale-

ichthyinae are robust and the ancestral distribution reconstructed from the topology suggests a southern African origin (Fig. 3), implying that the South American lineage dispersed out of Africa or that a recent vicariant event split a once much more widespread taxon.

Given that galeichthyines inhabit subtropical latitudes, it is expected that dispersal would have occurred through subtropical waters. Also, given that *G. peruvianus* is nested within the African species, dispersal must have been from Africa to South America. Alternate dispersal scenarios need to be analyzed in the light of water temperatures and ocean circulation patterns. We present several possible scenarios and recognize that none of them possess ideal habitats for galeichthyines (Fig. 4). The pros and cons of each of these dispersal patterns are listed in Table 4 and the areas in which fossils would need to be found to support each dispersal route are given in Table 5. Dispersal routes are named and lettered as in Figure 4. In addition, we present one more recent vicariance scenario.

(A) South Atlantic—the most direct, all marine route is straight from the southern tip of Africa to southern South America, through the Drake Passage, and then up the Pacific coast (Fig. 4). *Genidens barbatus*, another subtropical ariid, has been found in the Magellan strait (Sielfeld 1979), suggesting that this route could

**Table 4.** Possible out-of-Africa dispersal routes and their pros and cons (see also Fig. 4).

Code	Method	Pros	Cons
A	South Atlantic	1. Shortest marine distance 2. Coastal plain extends east from South America west of Falkland Islands, some relatively shallow areas near coast of South Africa	1. Against current 2. Possibly too cold at southern tip of South America
B	Antarctic Circumpolar	Follows currents	1. Longest distance 2. Greatest depths 3. Cold water
C	Benguela-Equatorial Currents	Follows currents	1. Possibly too warm 2. Long distance
D	Benguela – Brazil Currents	1. Follows currents 2. Favorable climate over part of route	1. Long and circuitous route 2. Possibly too cold at southern tip of South America
E	South American Passage	1. Shortest direct route 2. Possibly favorable climate	1. Unknown if the marine incursions were ever connected 2. Species are predominantly marine, not estuarine, and have no tolerance for freshwater

possibly be used by galeichthyines even today. A similar route was hypothesized by Eschmeyer and Hureau (1971) to explain the distributional patterns of the closely related scorpionfishes *Sebastes capensis*, from Tristan da Cunha and South Africa, and *S. ocelata*, from Peru and Chile. It is noteworthy that the inferred direction for *Sebastes* is opposite to that of *Galeichthys*, that is out of Peru via the Drake Passage and the South Atlantic current. Although this route is the most likely, a major con is that the South Atlantic current flows from Patagonia toward Benguela in Africa (Fig. 4, Table 4).

(B) Antarctic Circumpolar—the Atlantic circumpolar current flows between Africa, Australia, and South America and Antarctica. Galeichthyines could have dispersed using this current; however, this long route (~21,000 km) involves crossing the Eastern Pacific barrier (~5000 km), and low temperatures were probably unfavorable for *Galeichthys*.

(C) Benguela-Equatorial Currents—The Benguela current flows north along the African coast and joins the Equatorial current that flows northwest. In the Miocene, the Equatorial Current flowed through what is now the Panamanian isthmus. A galeichthyine could have used these favorable, warm currents. This scenario implies extinction in the western Atlantic. Despite the fact that fossil ariids have been widely recorded from northern and southern South America to up to the Early Miocene, no fossils of galeichthyines have been found (Arratia and Cione 1996; Nolf and Aguilera 1998; Aguilera and Rodríguez de Aguilera 2004).

(D) Benguela–Brazil Currents—Instead of traveling northwards via the Equatorial Current as in (C) above, galeichthyines could have traveled south via the Brazil current, moved through the Drake passage and then north via the Peru Current. This path would generally have favorable, warm currents; however, as above, no fossils are known from eastern South America.

**Table 5.** Possible routes from Africa and fossil locations needed to provide evidence for use of the routes. Two letter country codes given for continental shelf localities (follows ISO-3166). W. Africa indicates countries of western Africa adjacent to the Benguela Current (particularly AO, Angola; NA, Namibia; CD, Congo; CG, D. R. of Congo; and GA, Gabon). Central South America (SA) would be currently freshwater regions of AR, Argentina; BO, Bolivia; BR, Brazil; EC, Ecuador; PE, Peru; and PY, Paraguay. Brazil is broken into north (N, roughly north of Natal) and south (S, roughly south of Natal).

Code	Method	W. Africa	AR	AU/NZ	BR	CL	CO/VE	GF/GY/SR	Central SA
A	South Atlantic		X			X			
B	Antarctic Circumpolar			X		X			
C	Benguela-Equatorial Currents	X			N		X	X	
D	Benguela–Brazil Currents	X	X		NS	X			
E	South American Passage		X						X

(E) South American Passage—South America has had routine marine incursions through the current Paraná River as well as from the Caribbean through the proto-Orinoco (see Lundberg 1998 for review). The Marañon Portal may have drained the western Amazon into the Pacific until about 15 mya (although it is uncertain whether the Marañon Portal ever existed). The Marañon Portal may also have had marine incursions. Although it had been suggested that the Caribbean, Paraná, and Marañon incursions were never connected (Lundberg 1998), recent evidence suggests that the three incursions could have been connected (Hovikoski et al. 2007). Hovikoski et al. (2007) suggest that this combined incursion could have been a dispersal route for freshwater or euryhaline species. There are fossils described from Bolivia originally assigned to the Ariidae (Gayet 1991) but subsequently moved from the family (Arratia and Cione 1996; Gayet and Meunier 2003). Moreover, these fossils are from the Late Cretaceous and thus provide no evidence for this route during the Miocene. An additional con for this scenario is that galeichthyines are not particularly euryhaline. *Galeichthys* sp. and *G. ater* are not found in estuaries or freshwater, *G. feliceps* can be found in estuaries (Tilney and Hetch 1990; Kulongowski 2001), but do not tolerate salinities below 8 ppt (Whitfield et al. 1981), and *G. peruvianus* may only be found in estuaries as juveniles (Béarez et al. 2003). Although this route would be the shortest, it is not certain whether the marine incursions were ever connected, and it is unlikely that the water would have been salty enough for this predominantly marine group.

(F) Recent Vicariance—As mentioned above, ariids have limited capability of dispersal. A vicariance scenario would have to posit either a circumantarctic species of *Galeichthys* or at least presence in southern South America and South Africa. Given that it is unlikely that dispersal would be common across deep oceans to establish gene flow, recent vicariance is not a likely scenario to explain the distribution of galeichthyines. In possible support for recent vicariance, the lowering of sea temperatures after the Miocene Climate Optimum could have decreased the ability of a transoceanic galeichthyine to disperse across now colder water. South American populations may then have been forced to move northward, and the chance of gene flow halted with the decreasing temperatures. Support for recent vicariance would require finding an identical fossil species in southern South America and South Africa that is sister or possible ancestor to the clade of *G. ater*, *G. feliceps*, and *G. peruvianus*.

Testing whether the range of *Galeichthys* can be explained via dispersal (scenarios A–E) or vicariance (F) might prove difficult, and it would involve obtaining many more individuals. Long-range dispersal would likely be accomplished by few individuals resulting in a founder event, suggesting that the Peruvian population would have relatively low genetic diversity and that the coalescence of current genetic diversity would provide the

same date as the origin of the species found in this study. Under vicariance, gene flow might have maintained genetic diversity in South America, coalescence of current diversity would predate the origin of the species found here, and alleles in Peru might not be monophyletic due to incomplete lineage sorting. Coalescence of Peruvian alleles after the origin of the species or evidence of a population bottleneck in African species of around the same age of the origin of the Peruvian species would mean that it is not possible to use this test to determine if the range is a result of dispersal versus vicariance.

Support for any of these scenarios will come from fossils; however, no fossils assignable to the Galeichthyinae have been reported in the literature. Cione et al. (1996) described a fossil ariid from the Middle Miocene in eastern Patagonia based on a dorsal-fin spine. The fossil spine was identified only to family level, as they were unable to assign it to any of the seven examined ariids from the Atlantic South America, including the Patagonian *G. barbatus*. Considering the transatlantic routes discussed and the divergence times of the disjunct clade, the undetermined fossil might enlighten galeichthyine's dispersal path. A morphological examination of the fossil spine (Cione et al. 1996: fig. 2) suggests that it is not *Galeichthys*. The spine is much thicker at the base than distally (vs. uniformly thin in *Galeichthys*) and is markedly ornamented along the proximal half of the anterior surface (vs. proximally smooth in *Galeichthys*). A better understanding of the disjunct biogeography of galeichthyines would require the discovery of fossils or additional extant species from other areas that provide new evidence about the dispersal route (Table 5).

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