SYSTEMATICS AND EVOLUTIONARY HISTORY OF SEA CATFISHES

(SILURIFORMES: ARIIDAE)

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Ricardo Betancur-R.

Certificate of Approval:

_____      _____
Kenneth M. Halanych                      Jonathan W. Armbruster, Chair
Associate Professor                        Associate Professor
Biological Sciences                        Biological Sciences

_____      _____
Scott R. Santos                              Mark H. Sabaj
Associate Professor                        Collection Manager of Fishes
Biological Sciences                        Academy of Natural Sciences,
                                          Philadelphia

_____
George T. Flowers
Interim Dean
Graduate School

SYSTEMATICS AND EVOLUTIONARY HISTORY OF SEA CATFISHES

(SILURIFORMES: ARIIDAE)

Ricardo Betancur-R.

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SYSTEMATICS AND EVOLUTIONARY HISTORY OF SEA CATFISHES

(SILURIFORMES: ARIIDAE)

Ricardo Betancur-R.

_____
Signature of Author

_____
Date of Graduation

VITA


Ricardo Betancur Rodríguez, son of Christian Betancur Botero and Maria Cristina Rodríguez Vélez, was born in Medellín, Colombia on December 15, 1976. He received a Bachelor of Science in Marine Biology in 2000 from Universidad Jorge Tadeo Lozano (Bogotá, Colombia). Ricardo pursed his degree for Magister in Sciences in Marine Biology from 2001 through 2004 at Universidad Nacional de Colombia (Bogotá) and received *Cum Laude* honors. He entered Graduate School, Auburn University, in August, 2004. Ricardo finished his degree requirements for a Doctor of Philosophy in Biological Sciences in May, 2009.

DISSERTATION ABSTRACT

SYSTEMATICS AND EVOLUTIONARY HISTORY OF SEA CATFISHES

(SILURIFORMES: ARIIDAE)

Ricardo Betancur-R.

Doctor of Philosophy, May 9, 2009
(M.Sc. Universidad Nacional de Colombia, 2004)
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Ariids or sea catfishes are one of the two otophysan fish families (out of 67 families in four orders) that inhabit primarily marine and brackish waters, although some ariid species occur strictly in fresh waters. The Ariidae includes over a 150 species and many are of primary importance for tropical fisheries. Their classification has remained in disarray and recent studies that have intended to elucidate relationships among ariids have mostly focused on taxa from restricted geographic areas and comprehensive phylogenies are lacking. Furthermore, few efforts have been made to hypothesize biogeographic scenarios and evolutionary trends among ariids. This study inferred molecular phylogenies (up to ~4 kb) for ariids based on the most inclusive taxon sampling to date (123 species/entities). The results support the monophyly of the Ariidae (four anatomical and three molecular synapomorphies) and the sister-taxa relationship between ariids and the Malagasy family Anchariidae. The Ariidae is divided into two

basal lineages. The Galeichthyinae, new subfamily, includes one genus and four species from southern Africa (three species) and southwestern South America (one species), representing a remarkable case of transoceanic disjunction. Molecular data provided fully-resolved and well-supported phylogenies for galeichthyines, indicating that the South American species is nested within the African clade. An earlier study attributed galeichthyines' disjunct distribution to vicariance promoted by the final separation of Africa and South America (~105 mya). However, chronological estimations via molecular clocks show that the timing of intercontinental divergence was 15.4–2.5 mya, implying transoceanic dispersal or recent vicariance. The subfamily Ariinae includes the remaining taxa (~97% of ariid diversity). The topologies support the monophyly of ariines but up to ten genera previously validated are incongruent with the molecular phylogenies. New World ariines were paraphyletic and Old World ariines were grouped into a well-supported clade further divided into subclades mostly restricted to major Gondwanan landmasses. The general area cladogram derived from the area cladograms of ariines and other fish groups is largely congruent with the geological area cladogram of Gondwana. Nonetheless, molecular clock analyses provided highly variable estimations on the timing of ariine diversification (~28–105 mya). Habitat distributions (freshwater vs. marine) were optimized onto the phylogeny to test whether freshwater ariids are primitive (i.e., retain the ancestral freshwater otophysan condition) or derived (i.e., involve marine-to-freshwater transitions). The reconstructions support the latter scenario, suggesting a single invasion of marine waters at the root of the ariid tree followed by multiple events of freshwater recolonization in the Ariinae. Ariids provide an extraordinary example of bidirectional habitat transitions in fishes.

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Style manual of journal used: *Molecular Phylogenetics and Evolution*

Computer software used: Microsoft Word 2003 (text), Microsoft Excel 2003 (tables), Corel Draw X1 (figures), Corel Photopaint X1 (figures), Sequencher 4.8 (contig assemblage), Clustal X (sequence alignment), PAUP* 4.0b10 (maximum parsimony reconstructions, genetic distance calculations, hypothesis testing), ModelTest 3.7 (model testing), Garli 0.96 (maximum likelihood reconstructions), RAxML 7.04 (maximum likelihood reconstructions), MrBayes 3.1.2 (Bayesian inference), MacClade 4.06 (dataset handling, ancestral character reconstructions), Mesquite 2.5 (ancestral character reconstructions), DIVA 1.2 (dispersal-vicariance analyses), Component 2.0 (Component analyses), r8s 1.71 (divergence time estimations), MULTIDIVTIME (divergence time estimations), and others.

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# CHAPTER I – MONOPHYLY, AFFINITIES, AND SUBFAMILIAL CLADES OF SEA CATFISHES

## ABSTRACT

The sea catfish family Ariidae is a natural group defined by four anatomical synapomorphies: lapillus otolith extraordinarily developed, bones of the otic capsules (prootic, pterotic, exoccipital, and epioccipital) profoundly inflated, presence of a well-developed ventral process of basioccipital, and male mouthbrooding of eggs and embryos. Recent authors agree that the sister group to ariids is the freshwater family Anchariidae from Madagascar; however, there is no consensus about the phylogenetic relationships of the Ariidae + Anchariidae clade among other siluroid families. The family Ariidae can be divided into two monophyletic lineages. Galeichthyinae, new subfamily, including one genus and four species, is defined by one derived morphological state: postcleithral process fused to the posterior dorsal process of cleithrum, forming a fan-shaped lamina. The subfamily Ariinae, including all remaining ariids, is characterized by four anatomical synapomorphies: posterior process of epioccipital produced and connected to the sustentaculum of Weberian apparatus, ventral process of basioccipital and ventral ossification of complex vertebra forming an aortic tunnel, presence of anterodorsal bony block of the orbitosphenoids, and absence of anterior nuchal plate.

Additionally, mitochondrial and nuclear evidence strongly support the monophyly of the Ariidae (three amino acid synapomorphies) and its subfamilies (Galeichthyinae, six amino acid autapomorphies of *Galeichthys peruvianus*; Ariinae, four amino acid synapomorphies).

## 1. Introduction

Otophysan fishes include four orders and at least 67 families of teleosts that are widely distributed and diverse in the world's freshwaters (Ng and Sparks, 2005; Rodiles-Hernández et al., 2005; Nelson, 2006). Only two families of the catfish order Siluriformes are well represented in marine waters (Baras and Laleye, 2003): the Plotosidae, restricted to the Indian and Western Pacific oceans, and the Ariidae. Ariids, also known as sea catfishes, are widespread in the tropical and subtropical continental shelves of the Atlantic, Indian and Pacific oceans. The species inhabit mainly brackish estuaries and lagoons, but some are confined to freshwaters and others occur only in totally marine environments.

The number of valid ariid species is uncertain and recent estimates range from 120 to 200 (Acero P., 2003; Teugels, 2003; Kailola, 2004). In the last decades several authors have attempted to solve the intrafamiliar relationships of ariids on a regional or global basis (Kailola, 1991, 2004; Betancur-R., 2003; Acero P., 2004; Betancur-R. et al., 2004); however, a clear and consistent view of the suprageneric clades of ariids is still lacking. The aim of this paper is to discuss the monophyly of the Ariidae and its interfamiliar relationships, and provide a suprageneric classification within the family,

including the description of a new subfamily.

Names for skeletal structures follow Arratia (2003a, b). Institutional abbreviations for material illustrated are as listed in http://www.asih.org/codons.pdf, with the modification of INVEMAR to INVEMAR-PEC. Molecular evidence is based on ariid sequences from Betancur-R. (2003) [cytochrome *b* (cyt *b*), 49 species; recombination activation gene 2 (*rag2*), 25 species] and other catfish sequences (one per family or major lineage) from Hardman (2005) (cyt *b*, 31 species) and Sullivan et al. (in press) (*rag2*, 37 species). Positions for amino acid synapomorphies are referenced from the start codon as compared to *Danio rerio* for *rag2* (GenBank accession no. NM 131385) and to *Ictalurus punctatus* for cyt *b* (GenBank accession no. AF482987). Unambiguous amino acid transformations are given in bold.

## 2. Results and Discussion

### 2.1 Monophyly of the Ariidae

Ariidae, originally described by Bleeker (1862) as Arii, was hypothesized to be primitive by Regan (1911), in his pioneering systematic study of catfishes. Further studies, such as Bhimachar (1933), Merriman (1940), Tilak (1965), Higuchi (1982), and Rao and Lakshmi (1984), made important contributions on the comparative anatomy of the group. Kailola (1991), Mo (1991), and de Pinna (1993) were the first authors that claimed the monophyly of ariids based mainly on osteological evidence. Oliveira et al.

(2002) reported two myological peculiarities of sea catfishes that might be

synapomorphies. Kailola (2004) proposed several synapomorphies of the family and

discussed other diagnostic features. Diogo (2005) presented additional corroboration of

the monophyly of ariids based on osteological and myological characters. Hardman

(2002, 2005), Betancur-R. (2003), and Sullivan et al. (2006) provided support of ariid

monophyly using molecular data.


*2.1.1 Corroborated synapomorphies*


(1) Lapillus (=utricular) otolith extraordinarily developed (Fig. I-1). In catfishes

the three pairs of otoliths are minute, whereas the lapillus is confined to the central area

of the prootic and is usually larger than the other two (the saccular otolith or sagitta and

the lagenar otolith or asteriscus) (Chardon, 1968; Higuchi, 1982; Mo, 1991; de Pinna,

1993; Oliveira et al., 2001). In three unrelated catfish lineages, ariids, plotosids, and

*Horabagrus* incertae sedis, the lapillus otolith is enlarged and occupies an area

corresponding to several bones of the otic region (Oliveira et al., 2001). The lapillus of

sea catfishes is larger than the lapillus of plotosids and *Horabagrus* (Oliveira et al., 2001;

Diogo, 2005). In the Malagasy anchariids, considered sister to the Ariidae (Diogo, 2005;

Ng and Sparks, 2005; Sullivan et al., 2006), the lapillus is reduced (Kailola, 2004; Diogo,

2005). The lapillus otolith of ariids is oval, conchoidal, and biconvex (Higuchi, 1982;

Nolf, 1985; Betancur-R. et al., 2004).

The lapilli of sea catfishes are so distinctive that they have been widely noted in

the fossil record (Frizzell, 1965; Nolf, 1976; Nolf and Aguilera, 1998). At least 23 fossil

sea catfish species have been described based on their lapillus otolith (Weiler, 1968; Nolf, 1985). Apparently, the best known siluriform fossil described from lapillus material is *Vorhisia vulpes*, quoted as "genus Siluriformorum" *vulpes* by Nolf (1985) and as "genus Ariidarum" *vulpes* by Nolf and Stringer (1996). This species, from the Upper Cretaceous of South Dakota and Maryland, is among the oldest catfish fossils recorded (see Hardman, 2005: fig. 3). *Vorhisia vulpes* has been reported as living in an estuarine-deltaic environment, not entering freshwater (Frizzell and Koening, 1973; Nolf and Stringer, 1996). The shape of *Vorhisia*'s lapillus is relatively similar to that of *Galeichthys* (see Hecht and Hecht, 1981) and other neotropical sea catfishes (Acero P., 2004; Betancur-R. et al., 2004). Based on the shape and size of the lapillus and the environment that *V. vulpes* inhabited, it seems possible that it is a fossil ariid, as was hypothesized by Nolf and Stringer (1996).

(2) Otic capsules or bulla acoustico utricularis swollen: prootic, pterotic, exoccipital, and epioccipital profoundly inflated (Fig. I-2) (Higuchi, 1982; Betancur-R. et al., 2004; Diogo, 2005). The extraordinary development of otic capsules in ariids is likely related to the size of lapillus. In other catfish groups with enlarged lapillus (i.e., plotosids and *Horabagrus*), the otic capsules are also inflated, but only the prootic and the pterotic are involved (Oliveira et al., 2001; Diogo, 2005), probably because their lapilli are smaller than in sea catfishes.

(3) Presence of well-developed ventral process of basioccipital, forming a cone-shaped projection (Fig. I-3). The union between the basioccipital and the first vertebra is expressed externally by a toothed suture and by the subvertebral process, which is absent in other catfish families (Tilak, 1965; Higuchi, 1982; Rao and Lakshmi, 1984; Kailola,

5

1991, 2004).

(4) Male mouthbrooding of eggs and embryos (Rimmer and Merrick, 1983; Betancur-R. et al., 2004; Kailola, 2004). This trait has been found in all ariid species with known life histories, including *Galeichthys feliceps* (Tilney and Hecht, 1993). Mouthbrooding has not been reported in other catfish families, with the exception of males and females of the claroteid *Phyllonemus typus* (Ochi et al., 2000). The multiple occurrence of this feature in non-closely related teleost lineages will be discussed elsewhere.

The monophyly of sea catfishes is also strongly supported by evidence derived from mitochondrial (cyt *b,* ATP synthase 8/6, ribosomal 12S and 16S) and nuclear (*rag*1 and *rag*2) sequences (Hardman, 2002, 2005; Betancur-R., 2003; Sullivan et al., 2006). Amino acid synapomorphies of the Ariidae are: *rag2*, **116: cysteine→serine**, 118: arginine→lysine; cyt *b*, 305: leucine→methionine.

*2.1.2 Other putative synapomorphies*

Oliveira et al. (2002) and Diogo (2005) reported two myological peculiarities of ariids: the adductor arcus palatini muscle inserting on the mesial margin of the suspensorium and on a significant part of the lateral surface of this complex structure and the adductor mandibulae Aw or A3'' obliquely orienting with its posterodorsal fibers significantly dorsal to the upper edge of the coronoid process. Kailola (2004) proposed that the possession of strong pelvic musculature, unique epidermal mucous secretions, and the maximal consolidation of the anterior vertebrae may also support the monophyly

of the family. Sea catfishes are also characterized by lacking larvae, males

mouthbrooding eggs and embryos, releasing batches as juveniles much longer than one

centimeter (Tilney and Hecht, 1993); this feature was named precocial larvae by Kailola

(2004). She also commented that high DNA complement per cell (LeGrande, 1980) and

chromosome number and arm length (Fitzsimmons et al., 1988) might be unique features

of ariids. We also hypothesize that sexually dimorphic pelvic fins (i.e., larger in females

than in males) might be a derived condition of the Ariidae (Fig. I-4), but this feature

needs corroboration in several taxa.

*2.1.3 Other diagnostic characters*

Fossa between dorsomedial limb of posttemporo-supracleithrum, extrascapular,

and pterotic remarkably developed in most ariids (Fig. I-5b). The fossa is absent in most

catfish families, such as doradids and auchenipterids (Fig. I-5a), or present but less

developed in claroteids, schilbeids, pangasiids, ictalurids, and *Ancharius* (Diogo, 2005).

Mesocoracoid arch reduced or absent from the pectoral girdle (condition shared with

anchariids, akysids, aspredinids, bagrids, bunocephalins, and doradoids) (Kailola, 2004;

Diogo, 2005). Lateral ethmoid and frontal bones usually articulated through two facets

(vs. single facet in the remaining siluriforms except pangasiids) (Kailola, 2004).

*2.2 Interfamilial relationships*

The phylogenetic position of sea catfishes within the order Siluriformes is under

debate. Mo (1991) first proposed that the Ariidae is the sister family of a clade including the African Mochokidae and the neotropical doradoids (Doradidae and Auchenipteridae). Arratia (1992), in her analysis of the suspensorium of catfishes, suggested that ariids are closely related to pimelodids and more distantly to heptapterids. Lundberg's (1993) hypothesis is similar to that of Mo (1991), with the addition of the undescribed "titanoglanis", a coastal Eocene fossil from Arkansas. Lundberg (1993) provisionally placed ariids, mochokids, doradoids, and "titanoglanis" in the arioid group, which was supported by six skeletal synapomorphies. Such relationship was not supported by de Pinna (1993), who considered the Ariidae (including *Ancharius*) the sister group to the African Claroteidae, and that clade the sister group to the Schilbeidae plus Pangasiidae, both from the Old World. In a later work, de Pinna (1998) adopted an eclectic point of view, accepting a doradoid clade that includes Mochokidae, Doradidae, and Auchenipteridae, plus Ariidae, the African Malapteruridae, the Asian Pangasiidae, and the African and Asian Schilbeidae. De Pinna's (1998) doradoid clade is diagnosed by the presence of an elastic spring mechanism associated to the Weberian complex; however, the condition may not be homologous in all those families (de Pinna, 1998; Diogo, 2005).

Diogo et al. (2002) discussed four derived states from the cephalic region muscles shared by ariids, claroteids, and cranoglanidids. Diogo (2005) did a fairly complete analysis of the phylogeny of siluriforms based on morphological information. He concluded that ariids (including *Ancharius*) are the sister group to claroteids, and that clade plus the African Austroglanididae are sister to a clade including Cranoglanididae and Ictaluridae. According to Diogo (2005), those five families plus schilbeids and pangasiids form a monophyletic group.

Recent molecular evidence also reveals incongruence regarding the affinities of ariids to other siluriform groups. Hardman (2002) hypothesized the relationships of 22 catfish families using different reconstruction criteria on cyt *b* and *rag*2 sequences. Ariids were often recovered close to ictalurids and sometimes close to the neotropical doradoids, or to schilbeids and pangasiids, or to the Asiatic chacids. In most topologies, however, the nodes relating ariids to those families were weakly supported. Hardman (2005) expanded his catfish sampling to 29 families (anchariids not included) using only cyt *b* sequences. He found a poorly supported relationship between ariids and schilbeids, ictalurids, cranoglanidids, mochokids, claroteids, and pangasiids. Sullivan et al. (2006), based on *rag*1 and *rag*2 sequences of 35 catfish families, revaluated the phylogenetic relationships of siluriforms. They recovered the anchariid genus *Gogo* sister to five ariid genera and placed both lineages in the superfamily Arioidea, but failed to resolve the phylogenetic position of the arioid clade among other siluroid groups. Our comparison of the amino acid data reveals two arioid synapomorphies for *rag2*, **346: phenylananine→leucine** and 214: aspartic acid→asparagine.

The familial status of anchariids, a freshwater lineage endemic to Madagascar, has been controversial. The genus *Ancharius* has been traditionally treated as an ariid, but also placed within its own family (Stiassny and Raminosa, 1994) or in the family Mochokidae (Mo, 1991; Ng and Sparks in Sparks and Stiassny, 2003; Kailola, 2004; Nelson, 2006). Diogo (2005) supported de Pinna's (1993) hypothesis that *Ancharius* is the sister taxa of the remaining ariids. Diogo (2005) provided five ambiguous synapomorphies placing *Ancharius* as the sister taxa to *Carlarius heudelotii* plus *Genidens genidens*. He also gave reasons to reject a close relationship between *Ancharius*

and mochokids and stated that *Ancharius* should not be placed in its own family, but could be recognized as the ariid subfamily Anchariinae. The recent revision of anchariids by Ng and Sparks (2005) brought a new perspective to this controversy. Ng and Sparks (2005) described a new anchariid genus (*Gogo*) and diagnosed the group based on two putative apomorphic states, providing support for its familial status. We concur that anchariids are not sea catfishes. Despite the fact that some ariids are confined to freshwaters (e.g. *Potamarius* spp.), the family is originally a marine group and the freshwater restriction condition in some species implies secondarily acquisition and reversion to the primitive state in Otophysi (Betancur-R., 2003). Sullivan et al. (2006) argued that there is insufficient information to hypothesize the habitat preferences (i.e., coastal marine or freshwater) of the arioid ancestor. The position of the Anchariidae in their siluriform tree would parsimoniously imply that it retains the plesiomorphic Otophysi condition (i.e., freshwater confinement).

## 2.3 Suprageneric systematics of the Ariidae

The results obtained by Betancur-R. (2003), Betancur-R. et al. (2004), and Acero P. (2004), based on mitochondrial, nuclear, and morphological evidence, suggest that the Ariidae can be divided into two clades. These two clades are diagnosed below.

*2.3.1 Subfamily Galeichthyinae New Subfamily*

Type genus. *Galeichthys* Valenciennes.

Diagnosis. The subfamily Galeichthyinae is distinguished from the other catfishes by a uniquely derived state: postcleithral (=humero-cubital *sensu* Arratia, 2003b) process fused to posterior dorsal process of cleithrum, forming a fan-shaped lamina (Fig. I-6a). Primitively in other catfishes, including ariines (Fig. I-6b) and anchariids, the postcleithral process is produced and distinct from the posterior dorsal process. Additionally, the new subfamily is characterized by having a long and narrow supraoccipital process, usually grooved along its entire extension (Fig. I-7b). The polarization of this feature is, however, equivocal because the supraoccipital process of ariines (Fig. I-7c) and other catfishes, if present, is widely variable in shape and size (Arratia, 2003a). Amino acid autapomorphies of *Galeichthys peruvianus* (only galeichthyine species sequenced) are: *rag2*, **214: asparagine→serine**; cyt *b*, **37: leucine→methionine**, 171: asparagine→serine, **232: isoleucine→alanine, 245: serine→tryptophan**, 355: isoleucine→threonine.

Genus and species included. *Galeichthys*: *G. feliceps* Valenciennes (type species), *G. ater* Castelnau, *G. peruvianus* Lütken. The genus also includes an undescribed species from South Africa (Kulongowski, 2001).

Distribution and habitat. The species of *Galeichthys* occur in subtropical and temperate

waters, with three species in southern Africa and one in the Eastern Pacific in Perú. The

biogeographic implications of the disjunct distribution of galeichthyines will be discussed

elsewhere (see also Betancur-R., 2003; Acero P., 2004). The species inhabit coastal

marine and estuarine waters, suggesting that the invasion of marine waters by ariids

predated subfamilial cladogenesis.


*2.3.2 Subfamily Ariinae Bleeker*


Type genus. *Arius* Valenciennes.


Diagnosis. The subfamily Ariinae is diagnosed by four synapomorphies. (1) Posterior

process of epioccipital (=epiotic *sensu* Higuchi, 1982; Rao and Lakshmi, 1984; Arratia,

2003a) produced and connected to the sustentaculum of Weberian apparatus (Fig. I-7c).

In galeichthyines the posterior process of epioccipital does not contact the sustentaculum

(Fig. I-7b). Primitively in other catfish families, including anchariids, the posterior

extension of the epioccipital is absent, except for auchenipterids, doradids, pangasiids,

and schilbeids, in which the extension is not considered homologous to that of ariids (Fig.

I-7a) (Kailola, 2004; Diogo, 2005). (2) Ventral process of basioccipital and ventral

ossification of complex vertebra mesially closed forming an aortic tunnel (Fig. I-8b). The

basioccipital and the complex vertebra in galeichthyines are mesially opened, producing

an incomplete aortic tunnel (Fig. I-8a). The basioccipital and the ventral ossification of

the complex centrum do not form an aortic tunnel in anchariids or in other catfish

families (Diogo, 2005). (3) Presence of antero-dorsal bony block of the orbitosphenoids (Fig. I-9b). This block is present homoplasically in some pimelodoids (J .G. Lundberg, pers. comm.), but it is absent in galeichthyines (Fig. I-9a) and anchariids (Ng and Sparks, 2005: fig. 14b). (4) Anterior nuchal plate absent (Fig. I-7c). In galeichthyines the plate is reduced but distinct from the middle nuchal plate (=predorsal plate) (Fig. I-7b). In many catfish groups (Fig. I-7a), including anchariids (Ng and Sparks, 2005), both plates are well developed. Amino acid ariine synapomorphies are: *rag2*, **147: serine→asparagine**, 108: isoleucine→valine;cyt *b*, 45: leucine→methionine, 344: histidine→aspartic acid.

Genera included. All non-*Galeichthys* sea catfishes; genera currently recognized are listed in Table I-1. There are several undescribed and incertae sedis taxa not included in Table I-1, such as the ariid fauna of the eastern Atlantic (at least four species, Acero P. and Betancur-R., in press), which generic affinities are unclear.

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R.*, 2007. Monophyly, affinities, and subfamilial clades of the sea catfishes

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http://www.pfeil-verlag.de/04biol/pdf/ief18_2_06.pdf. *Both authors contributed equally

to the work, authorship in alphabetical order.

**Table I-1.** List of ariine genera. Classification of New World genera is based on Betancur-R. (2003) and Acero P. (2004) except for *Genidens* which follows Marceniuk and Ferraris (2003). Classification of Old World genera is based on Kailola (2004), excluding Old World species from *Ariopsis* (otherwise treated as '*Ariopsis*') and New World species from *Hemiarius*. EI, Eastern Indian; EP, Eastern Pacific; M, Madagascar; SA, Sahul Shelf; SU, Sunda Shelf; WA, Western Atlantic; WI, Western Indian; B, brackish waters; F, freshwaters; M, marine waters.

| Genera | Approx. number of spp. | Distribution | Habitat |
|---|---|---|---|
| **New World** | | | |
| *Ariopsis* Gill | 5 | EP, WA | B, F, M |
| *Bagre* Cloquet | 4 | EP, WA | B, M |
| *Cathorops* Jordan and Gilbert | 15 | EP, WA | B, F, M |
| *Genidens* Castelnau | 4 | WA | B, F, M |
| *Notarius* Gill | 15 | EP, WA | B, F, M |
| *Potamarius* Hubbs and Miller | 3 | WA | F |
| *Sciades* Müller and Troschel | 6 | EP, WA | B, F, M |
| **Old World** | | | |
| *Amissidens* Kailola | 1 | SA | B, M |
| '*Ariopsis*' | 11–14 | SA, M? | B, F, M |
| *Arius* Valenciennes | 16 | EI, SU | B, F, M |
| *Batrachocephalus* Bleeker | 1 | EI, SU | B, F, M |
| *Brustiarius* Herre | 2 | SA | F |
| *Cephalocassis* Bleeker | 2 | SU | F |
| *Cinetodus* Ogilby | 3–4 | SA | B, F, M |
| *Cochlefelis* Whitley | 3 | EI, SA, SU | B, F |
| *Cryptarius* Kailola | 2 | EI, SU | B, F, M |
| *Hemiarius* Bleeker | 5 | EI, SA, SU | B, F, M |
| *Hexanematichthys* Bleeker | 2 | EI, SA, SU | B, M |
| *Ketengus* Bleeker | 1 | EI, SU | B, M |
| *Nedystoma* Ogilby | 2 | SA | B, F |
| *Nemapteryx* Ogilby | 6 | EI, SA, SU | B, M |
| *Netuma* Bleeker | 3 | WI, EI, SA, SU | B, M |
| *Osteogeneiosus* Bleeker | 1 | EI, SU | B, F?, M |
| *Plicofollis* Kailola | 7 | WI, EI, SA, SU | B, F, M |

**FIGURES**

**Figure I-1.** *Potamarius nelsoni*, UMMZ 198713-S, 480 mm SL, lapillus (=utricular) otolith.

**Figure I-2.** Ventral view of posterior portion of cranium: (a) *Trachelyopterus insignis* (Auchenipteridae), INVEMAR PEC 6819, 171 mm SL; and (b) *Galeichthys ater*, INVEMAR PEC 6799, 239 mm SL. EO, exoccipital; PR, prootic; PT, pterotic.

**Figure I-3**. *Notarius kessleri*, INVEMAR PEC 6785, 281 mm SL; lateral view of cranium. **VPB,** ventral process of basioccipital.

**Figure I-4.** Pelvic fins of (a) males and (b) females of Bagre marinus (after Merriman, 1940).

**Figure I-5.** Dorsal view of posterior portion of cranium: (a) *Trachelyopterus insignis* (Auchenipteridae), INVEMAR PEC 6819, 171 mm SL; and (b) *Notarius kessleri*, INVEMAR PEC 6785, 328 mm SL. ES, extrascapular; F, fossa; PS, posttemporo-supracleithrum; PT, pterotic.

**Figure I-6.** Cleithrum of: (a) *Galeichthys ater*, INVEMAR PEC 6799, 239 mm SL; and (b) *Occidentarius platypogon*, INVEMAR PEC 6802, 261 mm SL. FL, fan-shaped lamina; PCP, postcleithral process; PDP, posterior dorsal process.

**Figure I-7.** Dorsal view of posterior portion of cranium: (a) *Trachelyopterus insignis* (Auchenipteridae), INVEMAR PEC 6819, 171 mm SL; (b) *Galeichthys ater*, INVEMAR PEC 6799, 239 mm SL; and (c) *Ariopsis* sp., INVEMAR PEC 6781, 274 mm SL. ANP, anterior nuchal plate; EP, epioccipital; MNP, middle nuchal plate; SPP, supraoccipital process; SW, sustentaculum of the Weberian apparatus.

**Figure I-8.** Ventral view of posterior portion of cranium: (a) *Galeichthys ater*, INVEMAR PEC 6799, 239 mm SL; and (b) *Sciades dowii*, INVEMAR PEC 6803, 296 mm SL. AT, aortic tunnel; BO, basioccipital; CV, complex vertebra.

**Figure I-9.** Dorsal view of anterior portion of cranium: (a) *Galeichthys ater*, INVEMAR PEC 6799, 239 mm SL; and (b) *Sciades proops*, INVEMAR PEC 6809, 377 mm SL. BL, block; ORB, orbitosphenoids.

**Fig. I-1**



1 cm

**Fig. I-2**



**Fig. I-3**

**Fig. I-4**



a

b

**Fig. I-5**



a

b
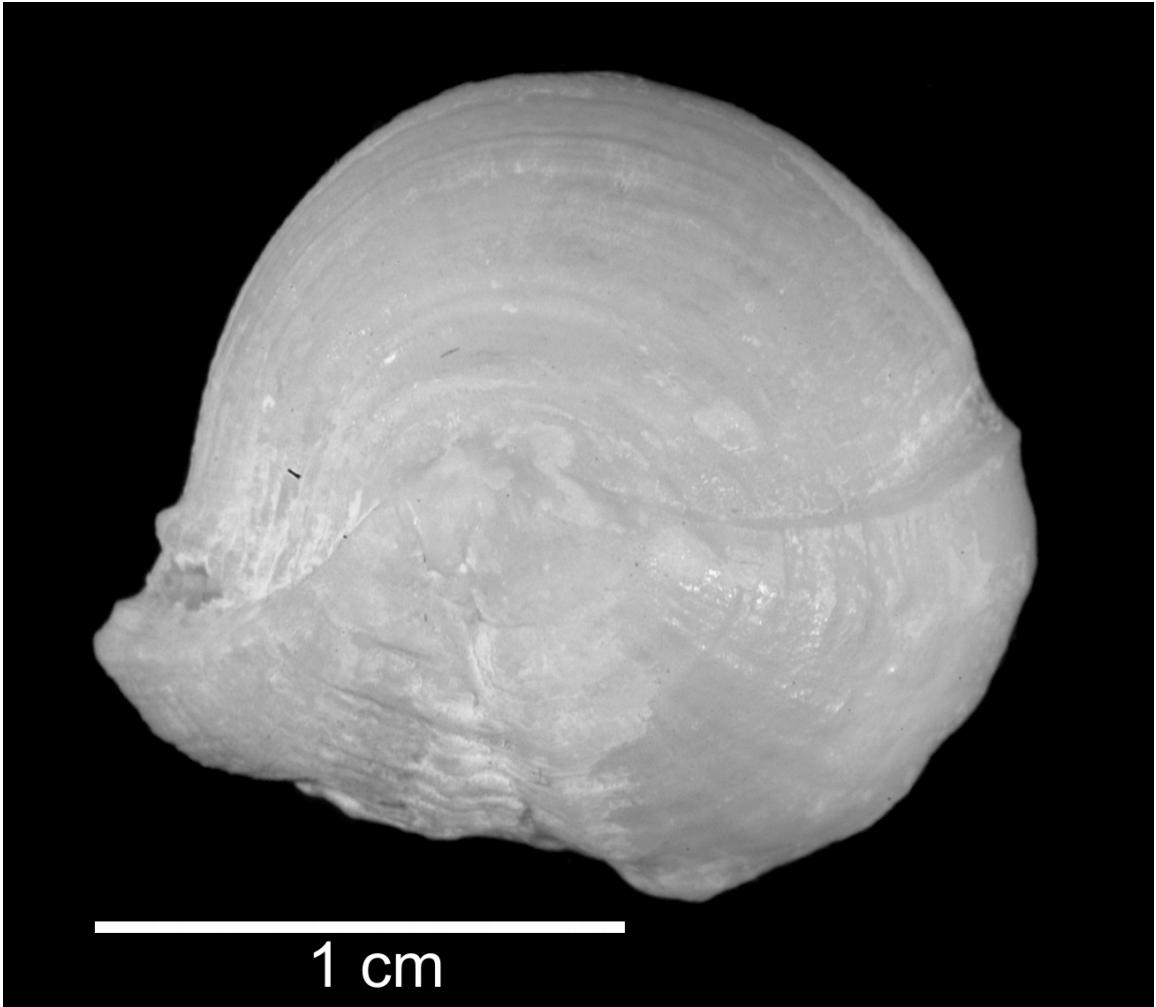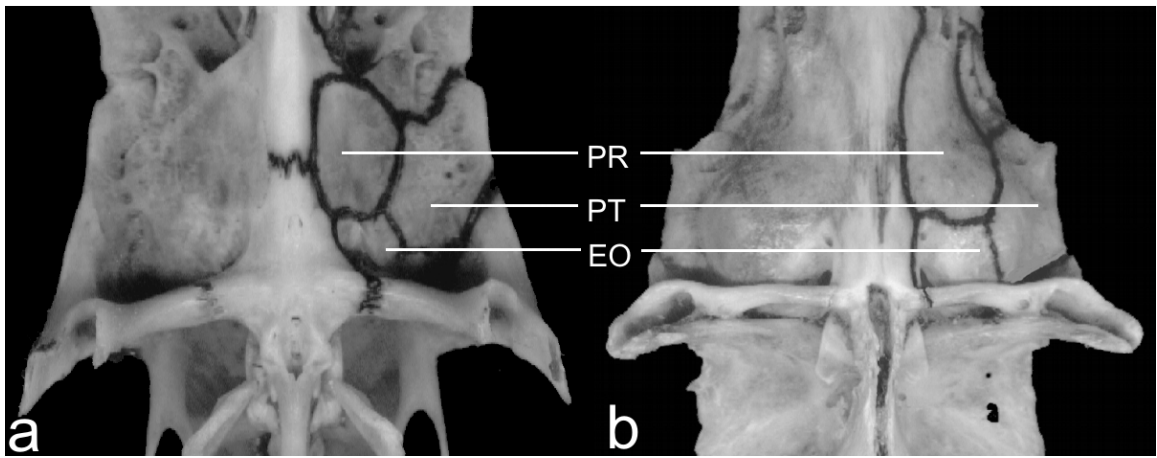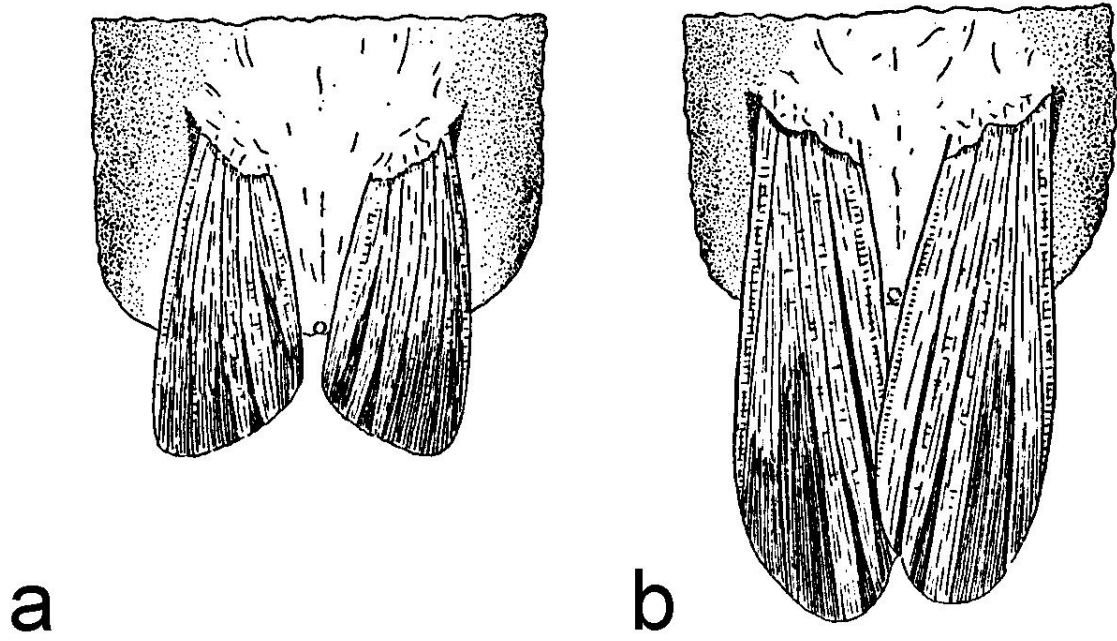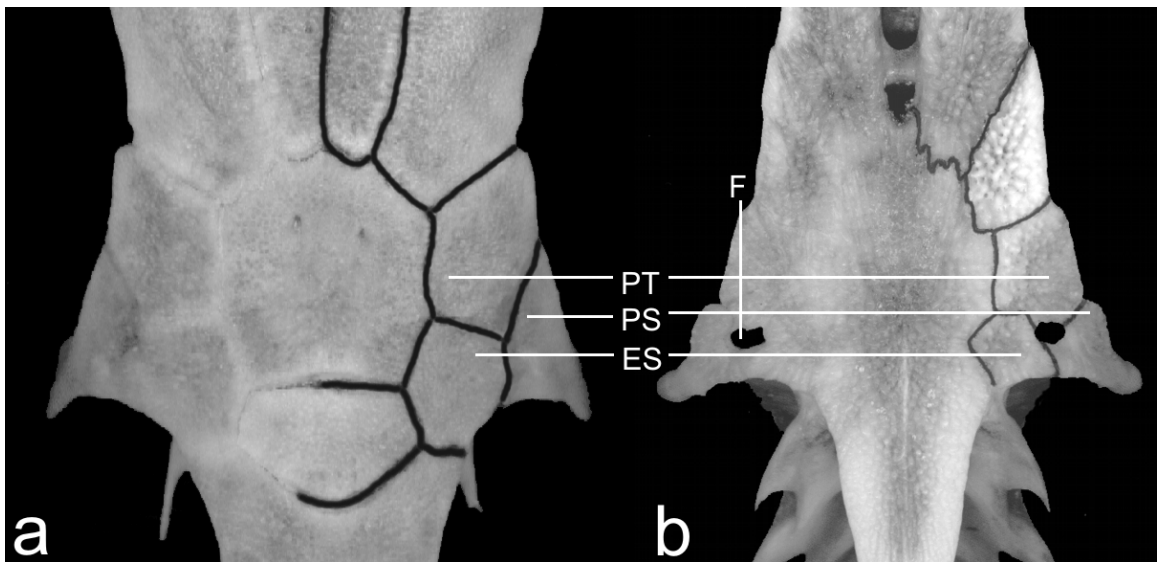
**Fig. I-6**
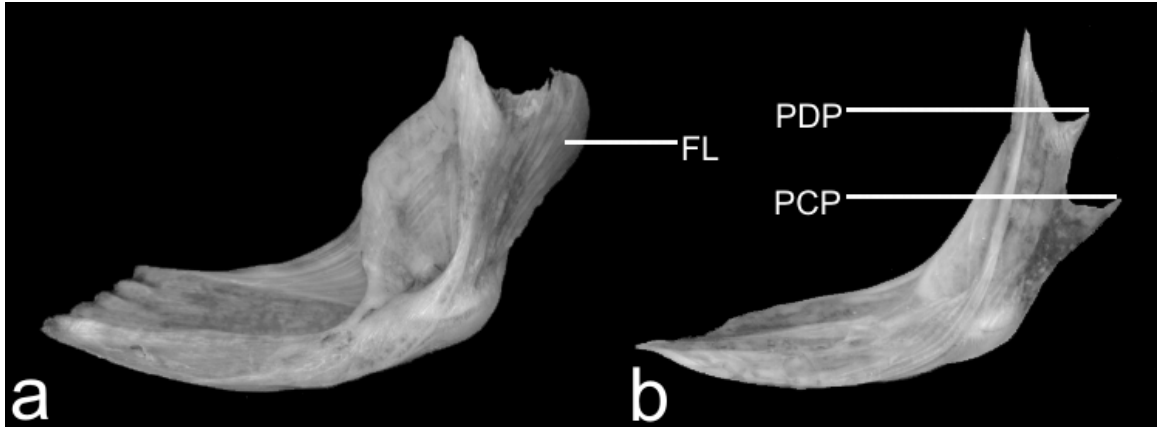


**Fig. I-7**
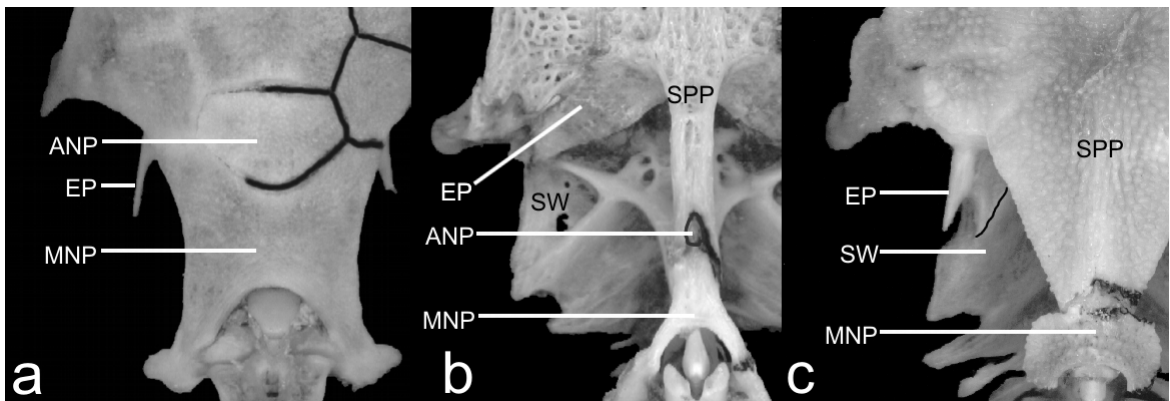
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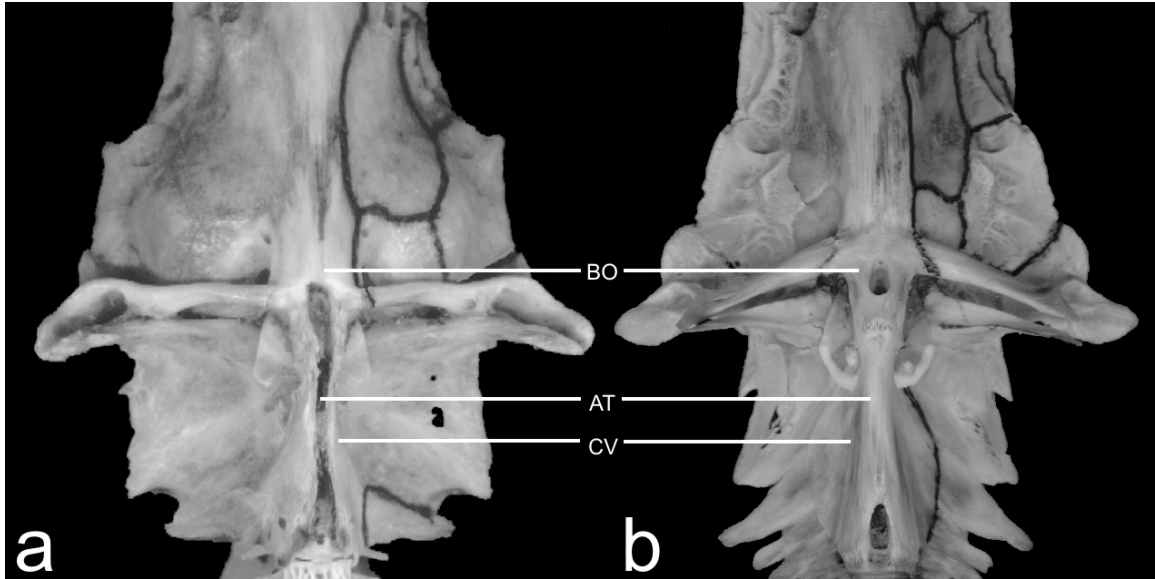


**Fig. I-9**
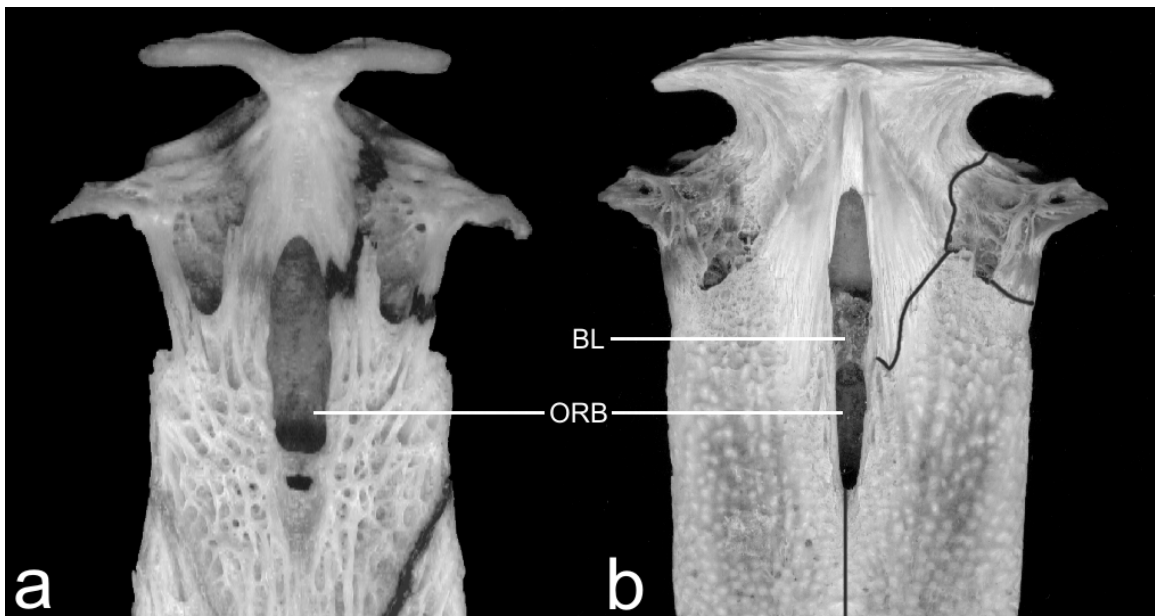
# CHAPTER II – SUBFAMILY GALEICTHYINAE: PHYLOGENETIC RELATIONSHIPS AND EVOLUTIONARY HISTORY

## ABSTRACT

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.

## 1. Introduction

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 landmasses in the southern hemisphere were the result of Gondwanan vicariance or transoceanic dispersal (McGlone, 2005; de Queiroz, 2005). The latter explanation was the paradigm held in classic biogeography. It was not until the 1960's 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 world-wide Ariinae, including 97% of ariid diversity, and the

Galeichthyinae (Acero P. and Betancur-R., 2007). With three extant species in southern

Africa (*Galeichthys* sp., *G. ater*, *G. feliceps*) and one in the Peruvian Pacific (*G.*

*peruvianus*), the Galeichthyinae represents a striking example of biogeographic

disjunction (Fig. II-1D; Kulongowski, 2001; Acero P. and 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 an

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. II-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. II-1B). The subsequent cooling of Antarctica 37 mya (Veevers and Ettriem, 1988)

and southern South America, probably caused a progressive migration towards lower latitudes, finally reaching today's subtropical waters of Peru (Fig. II-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 *Galeichthys 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. In order to test Gondwanan vicariance vs. 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.

## 2. Materials and Methods

### 2.1 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 *Galeichthys peruvianus* and nine ariine species in seven genera examined in Betancur-R. et al. (2007: Appendix II-1), 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 out 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 II-1. 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 convergence of likelihood scores. Ancestral distributions were reconstructed under

parsimony.


## 2.2 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 out 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.

Penalized likelihood is a semiparametric approach that combines a parametric

model for branches with different substitution rates and a non-parametric 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.

Bayesian relaxed clock 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 rttmsd 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. (2006).

The rtrate and rtratesd 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 rttm (=0.0043). The

brownmean and the brownsd 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. In order

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.

II-2): (†[1]) 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 II-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 maximum age constraint of 3.1 my and a minimum

age constraint of 2.8 my were applied to this node.  (†[2]) The oldest fossil of *Cathorops* is

an unidentified skull from the Cantaure formation (Early Miocene), Venezuela (Aguilera

and Rodríguez de Aguilera, 2004). A minimum age of 23–16 my was applied to the root

of *Cathorops*. (†[3]) 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

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.

## 3. Results

### *3.1 Dataset attributes and phylogenetic inference*

All sequences obtained during this study are available in GenBank. Accession

numbers of sequences are listed in Appendix II-1. The final alignment of the

mitochondrial protein coding genes included 1937 sites: 1095 bp for partial cyt *b* and 842

bp for ATPase 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 -23001.75, Fig. II-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 ~135k post-burn-in trees (mean lnL –23020). 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. II-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 II-1). The African

galeichthyines were rendered 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 II-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.

## 3.2 Divergence time estimations

Node D in Figure II-3 indicates the most recent disjunct clade (*Galeichthys peruvianus* (*G. ater*, *G. feliceps*)), i.e. 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 II-2 and Figure II-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 II-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).

# 4. Discussion

## 4.1 Molecular clocks and the divergence time of node D

Molecular data offer new perspectives into the evolutionary history of galeichthyines and re-evaluates the previous Gondwanan hypothesis. Even though different methods provide variable results on the divergence time of the disjunct clade (15.4–2.5 my, Table II-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 vs. corrected distances for all positions (not shown) provide evidence of saturation above 10% of genetic divergence. The genetic distances between *Galeichthys 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 Maastrichthian; 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 at the root of the tree. Due to the fact that the calibration of node †[1] 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 †[1] 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 †[1] calibration is accurate. Of course, this method would not allow us to conclude an age older than 73 my for node D, so it can not 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 (Zardoya and Doadrio, 1999; Bermingham et al., 1997). 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 $\dagger^2$ and $\dagger^3$) are shown in Table II-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 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 Arioidea (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).

## 4.2 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 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 (Tilney and Hecht, 1990, 1993; Taylor, 1986). 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 world-wide 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 (see Chapter III). 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 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 to 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 Lombari, 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. II-4).

Finally, phylogeny can provide a potential mode of dispersal or vicariance. The hypothesized phylogenies within the Galeichthyinae are robust and the ancestral distribution reconstructed from the topology suggests a southern African origin (Fig. II-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 though subtropical waters. Also, given that *Galeichthys 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. II-4).  The pros

and cons of each of these dispersal patterns are listed in Table II-4 and the areas where

fossils would need to be found to support each dispersal route are given in Table II-5.

Dispersal routes are named and lettered as in Figure II-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. II-4).  *Genidens barbus*, another subtropical ariid, has been found in

the Magellan strait (Sielfeld, 1979), suggesting that this route could 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*,

i.e. 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

towards Benguela in Africa (Fig. II-4, Table II-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.

*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 Lundburg, 1998 for review). The Marañon Portal may have drained the western Amazon into the Pacific until about 15 mya (although it is uncertain whether or not 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; Kulongowsky, 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 northwards, 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 vs. 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 *Genidens barbus*. 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 II-5).

## ACKNOWLEDGMENTS

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**Table II-1.** Support values for the *Galeichthys* tree (see Fig. II-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 |
|---|---|---|---|
| *Galeichthys ater*/*G. feliceps* | 100/100 | 97/69 | 100/1.0/100 |
| D | 96/100 | 85/87 | 99/1.0/100 |
| *Galeichthys* root | 100/100 | 89/99 | 100/1.0/100 |

**Table II-2**. Age estimations for node D (see Fig. II-3).

| Method | Time (mya) | SD | 95% credibility interval lower | upper |
|---|---|---|---|---|
| Penalized likelihood, additive penalty YFA (S= 10000) | 6.4 | – | – | – |
| Penalized likelihood, additive penalty OFA (S= 10000) | 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 *b* +ATPase 8/6, 0.7%/my/lineage) | 2.5-2.7 | – | – | – |

YFA, youngest fossil ages.

OFA, oldest fossil ages.

S, smoothing parameter.

**Table II-3.** Molecular age estimations for nodes constrained by fossils.  Values within brackets indicate fossil ages.

| Method | Node †2 (23-16 my) | Node †3 (73-68 my) |
|---|---|---|
| Penalized likelihood, additive penalty YFA (S= 10000) | 26.4 | 74.2 |
| Penalized likelihood, additive penalty OFA (S= 10000) | 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 |

YFA, youngest fossil ages.

OFA, oldest fossil ages.

S, smoothing parameter.

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

**Table II-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 Angola, Namibia, Congo, D. R. of Congo, and Gabon). Central South America (SA) would be currently freshwater regions of Argentina, Bolivia, Brazil, Ecuador, Peru, and 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 |

# FIGURES

**Figure II-1**. A–C, Betancur-R. et al.'s (2007) hypothesis of galeichthyine 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.odsn.de/).

**Figure II-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.

**Figure II-3.** Maximum likelihood phylogram (lnL -23001.75) 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 II-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.

**Figure II-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; 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 II-4. Gray arrows indicate key paleocurrents (modified from Haq, 1981; Kennett, 1985). Map based on 20-11 mya map of Golonka (2000).

**Figure II-1**



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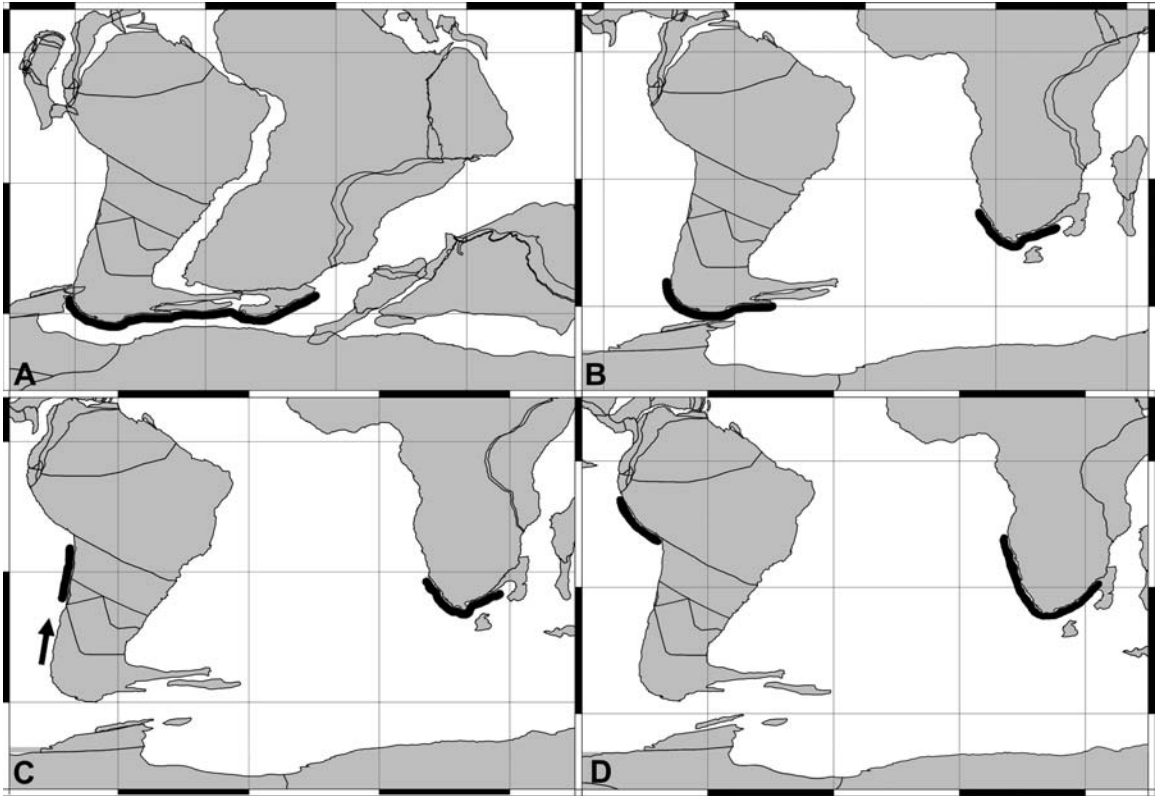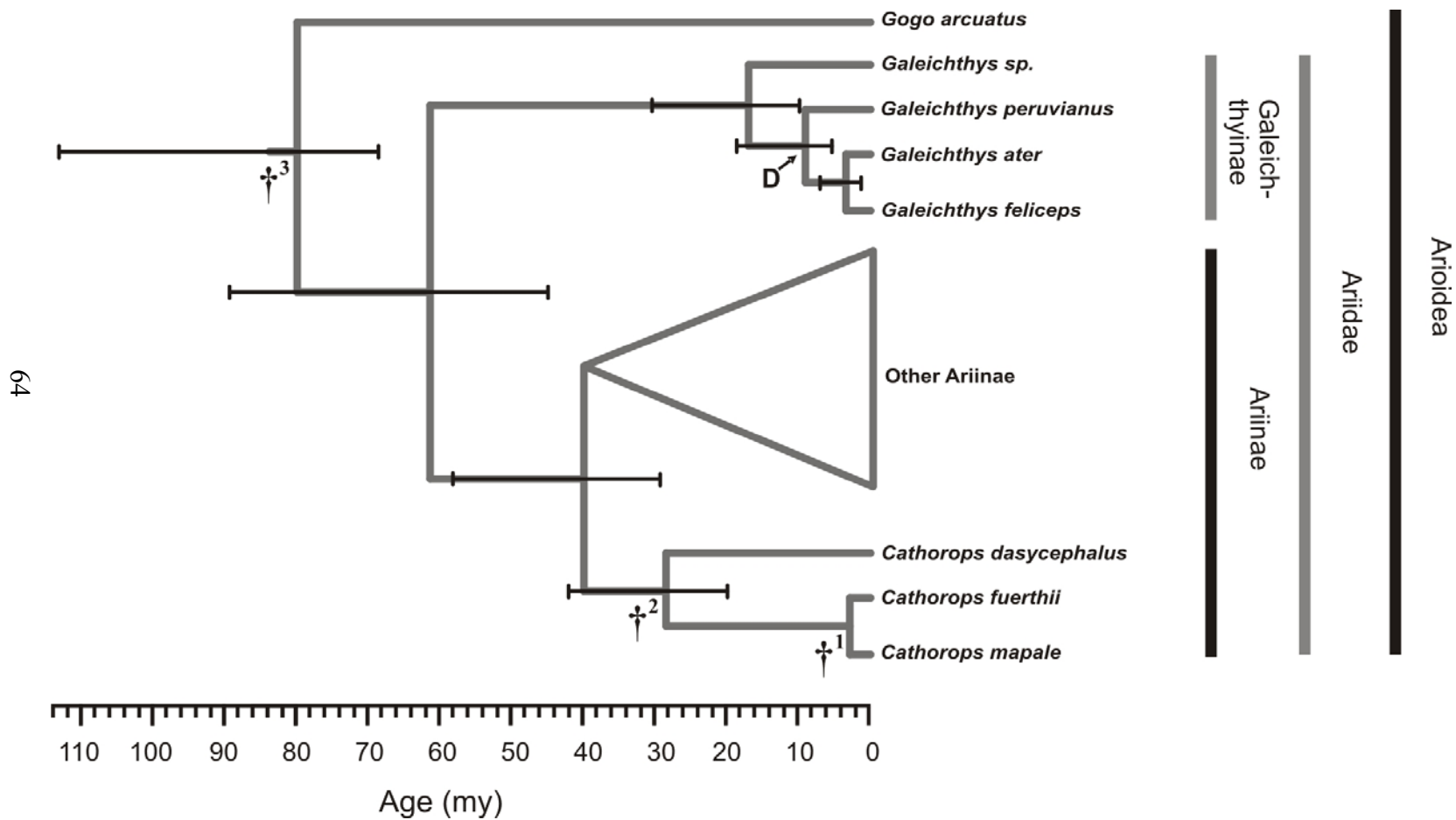
**Figure II-2**

**Figure II-3**
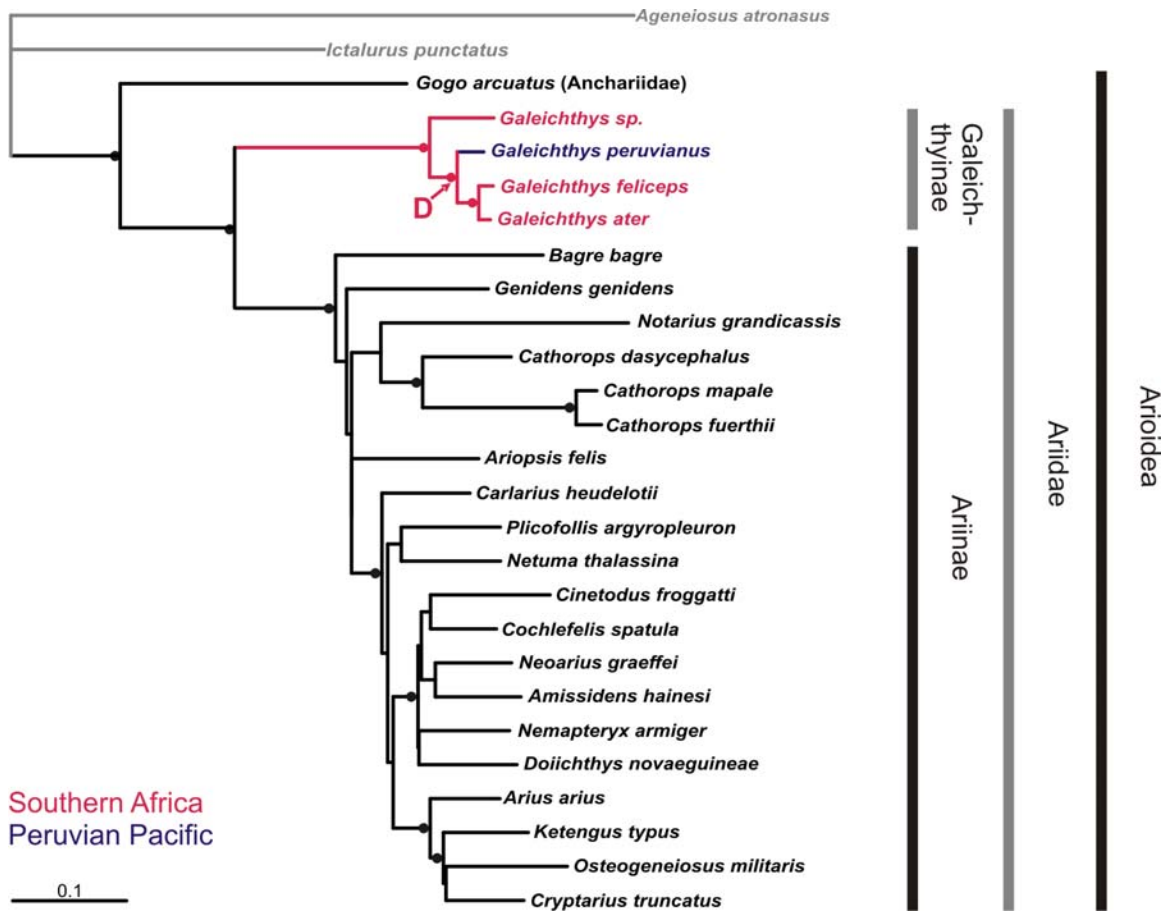
**Figure II-4**
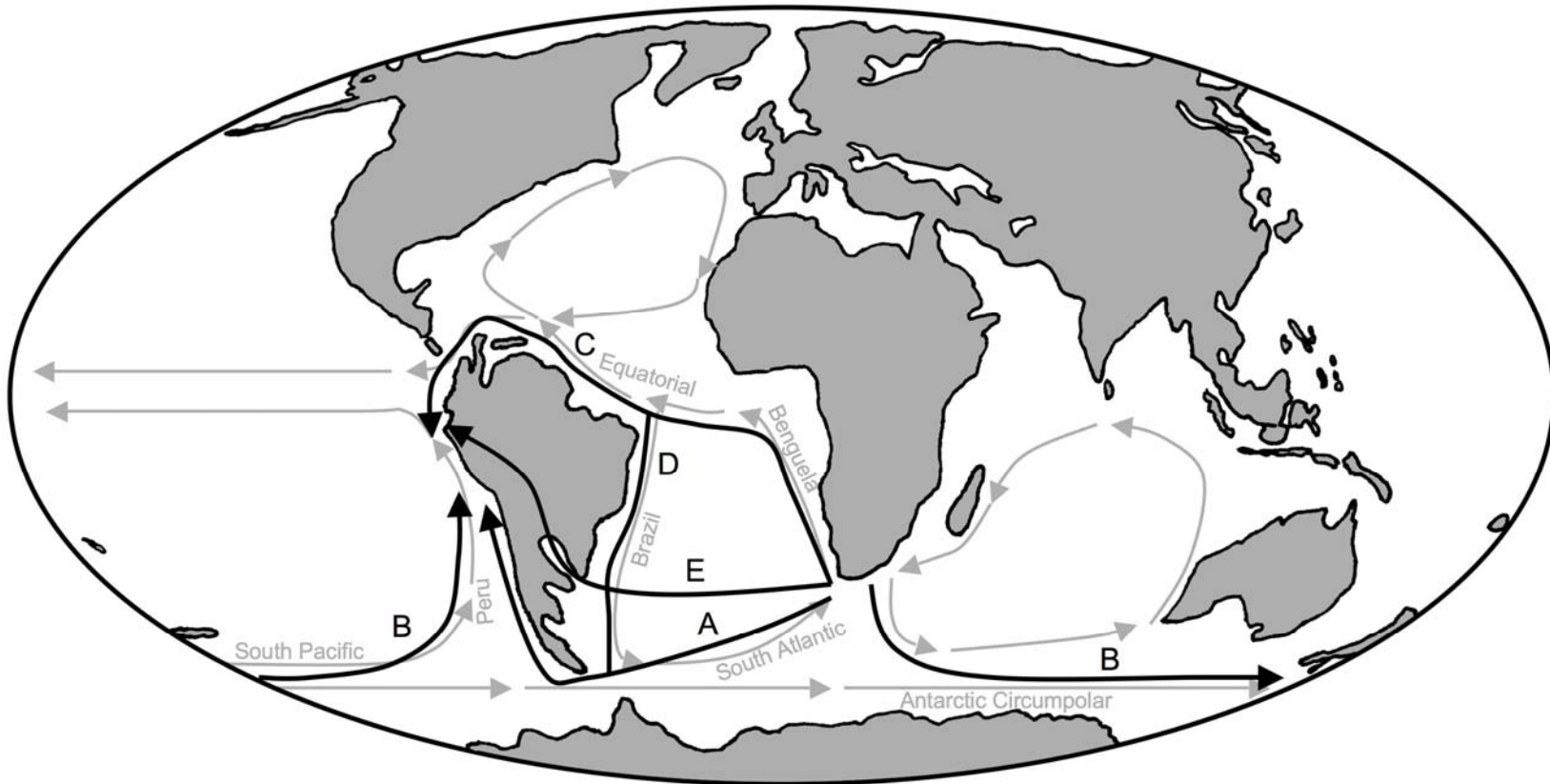
**Appendix II-1.** List of new material examined and GenBank Accession numbers. Additional material is listed in Betancur-R. et al. (2007).

| Species | Tissue No. | Catalog No. | GenBank No. |
|---|---|---|---|
| Anchariidae | | | |
| *Gogo arcuatus* | _ | UMMZ 238042 | **FJ013144, FJ013160, FJ013176, FJ013191** |
| Ariidae: Galeichthyinae | | | |
| *Galeichthys* sp. | T593, T592 | SAIAB 78771 | **FJ013147, FJ013163, FJ013179, FJ013194, FJ013208** |
| *G. ater* | 59.2 # 1, 59.2 # 2 | NV | **FJ013145, FJ013159, FJ013161, FJ013177, FJ013192, FJ013206** |
| *G. feliceps* | AC112, AC114 | NV | **FJ013146, FJ013162, FJ013175, FJ013178, FJ013193, FJ013207** |
| Ariidae: Ariinae | | | |
| *Amissidens hainesi* | HL0106 | NTM S.15533-001 | **FJ013153, FJ013169, FJ013185, FJ013200, FJ013216** |
| *Carlarius heudelotii* | 1710 | NV | **FJ013149, FJ013165, FJ013181, FJ013196, FJ013212** |
| *Cathorops mapale* | stri-x3600 | INVEMAR-PEC 5333 | **FJ013210** |
| *Cathorops fuerthii* | stri-17563 | STRI-5720 | **FJ013209** |
| *Cinetodus froggatti* | P4151 | ANSP 180336 | **FJ013154, FJ013170, FJ013186, FJ013201, FJ013217** |
| *Cochlefelis spatula* | PNG116 | AUM 47553 | **FJ013155, FJ013171, FJ013187, FJ013202, FJ013218** |
| *Doiichthys novaeguineae* | PNG097 | AUM 47711 | **FJ013156, FJ013172, FJ013188, FJ013203, FJ013219** |
| *Genidens genidens* | ID141 | MZUSP 99721 | **FJ013148, FJ013164, FJ013180, FJ013195, FJ013211** |
| *Nemapteryx armiger* | HL0116 | NV | **FJ013158, FJ013174, FJ013190, FJ013205, FJ013221** |
| *Neoarius graeffei* | RW0140 | NTM S.15362-002 | **FJ013157, FJ013173, FJ013189, FJ013204, FJ013220** |
| *Netuma thalassina* | ID103 | AUM 46282 | **FJ013150, FJ013166, FJ013182, FJ013197, FJ013213** |
| *Osteogeneiosus militaris* | ID007 | AUM 44874 | **FJ013152, FJ013168, FJ013184, FJ013199, FJ013215** |
| *Plicofollis argyropleuron* | ID002 | AUM 44750 | **FJ013151, FJ013167, FJ013183, FJ013198, FJ013214** |

NV, not vouchered.

# CHAPTER III – SUBFAMILY ARIINAE: PHYLOGENETIC RELATIONSHIPS AND EVOLUTIONARY HISTORY

## ABSTRACT

Ariids or sea catfishes are one of the two otophysan fish families (out of about 67 families in four orders) that inhabit mainly marine and brackish waters (although some species occur strictly in fresh waters). The group includes over 150 species placed in ~29 genera and two subfamilies (Galeichthyinae and Ariinae). Despite their global distribution, ariids are largely restricted to the continental shelves due in part to their specialized reproductive behaviour (i.e., oral incubation). Thus, among marine fishes, ariids offer an excellent opportunity for inferring historical biogeographic scenarios. Phylogenetic hypotheses available for ariids have focused on restricted geographic areas and comprehensive phylogenies are still missing. This study inferred phylogenetic hypotheses for 123 ariid species in 28 genera from different biogeographic provinces using both mitochondrial and nuclear sequences (up to ~4kb). While the topologies obtained support the monophyly of basal groups, up to ten genera validated in previous morphological studies were incongruent with the molecular topologies. New World ariines were recovered as paraphyletic and Old World ariines were grouped into a well-

supported clade that was further divided into subclades mainly restricted to major

Gondwanan landmasses. A general area cladogram derived from the area cladograms of

ariines and three other fish groups was largely congruent with the geological area

cladogram of Gondwana. Nonetheless, molecular clock estimations provided highly

variable results on the timing of ariine diversification (~28–105 mya). This study

provides the most comprehensive phylogeny of sea catfishes to date and stress the need

for re-assessment of their classification.


## 1. Introduction


The catfish order Siluriformes is a very diverse natural group that occurs

primarily in freshwater. Catfishes are widespread and their distribution encompasses all

continents even Antarctica, as evidenced by Eocene-Oligocene fossils (Grande and

Eastman, 1986). The order includes 36 extant families and over 3000 valid species plus

an estimated ~1500 undescribed species (Ferraris, 2007; Sabaj et al., 2004). Several

morphological and molecular studies have addressed the relationships among catfish

families (e.g., de Pinna, 1998; Mo, 1991; Sullivan et al., 2006) and recent evidence

indicates that large basal clades are restricted to particular continental masses, suggesting

a long history of intercontinental isolation (Sullivan et al., 2006). Thus, catfishes offer an

exceptional opportunity for studying evolutionary and biogeographic trends. The fossil

record of Siluriformes is relatively well represented and includes material from every

continent (Gayet and Meunier, 2003). Although the earliest fossils date back to the Late

Campanian–Early Maastrichtian (ca. 68–73 mya), molecular clocks predict a much older

origin for Siluriformes (i.e., 130–175 mya [Briggs, 2005; Lundberg et al., 2007; Peng et al., 2006]).

While most catfishes inhabit freshwater, only two families are well represented in marine environments: the Plotosidae from the Indo-West Pacific and the Ariidae. The Ariidae, or sea catfishes, is the only siluriform group with a global distribution that includes over 150 species occurring in warm-temperate to tropical regions. Although most members of the family live in brackish and marine waters, several species occur in freshwater as well. Sea catfish distributions include the continental margins of the Eastern Pacific and the Western Atlantic (New World), the Eastern Atlantic (Western Africa), and the Indo-West Pacific (Eastern Africa, Madagascar, India-SE Asia, and Australia-New Guinea; Fig. 1). Ariids play a significant role in tropical fisheries, with many species having high economic value due to their large size, local abundance, and flesh quality. Some species have been recently listed as vulnerable on the IUCN red list (http://www.sci.odu.edu/gmsa/).

Members of the Ariidae exhibit a specialized reproductive mode: male mouthbrooding of eggs and embryos. This condition is absent in nearly all other catfishes, the sole exception being the biparental mouthbrooding claroteid *Phyllonemus typus* (Ochi et al., 2000). Oral incubation means ariids have limited dispersal capabilities and subsequent high level of species endemism, which has ultimately resulted in continentally restricted distributions. In fact, ariids are absent from the Pacific plate (Springer, 1982), the nonmarginal portion of the Antilles, and other oceanic islands of recent volcanic origin (Betancur-R. and Armbruster, in press). Restriction of most species to the continental shelves make ariids an exceptional marine fish group to infer historical

biogeography scenarios (although see Betancur-R. and Armbruster [in press] for evidence

of recent transoceanic dispersal in *Galeichthys*).  Such studies require a conceptual

framework derived from robust phylogenetic hypotheses. Recent interfamilial

phylogenies based on morphological (Diogo, 2004; Kailola, 2004) and molecular

(Sullivan et al., 2006) data have placed the Malagasy family Anchariidae as sister to the

Ariidae and both families are ascribed in the superfamily Arioidea within the suborder

Siluroidei (Sullivan et al., 2006). The monophyly of the Ariidae has not been seriously

questioned and is strongly supported on both molecular and morphological grounds

(Acero P. and Betancur-R., 2007; Kailola, 2004; Sullivan et al., 2006). The group is

divided into two subfamilies, the monogeneric Galeichthyinae (four species) and the

Ariinae (remaining taxa) (Acero P. and Betancur-R., 2007). Although the basal arioid

clades are well defined, much controversy has arisen regarding the phylogeny and

classification of ariid taxa, particularly within the diverse Ariinae.

Recent studies that have attempted to elucidate relationships among ariids have

mostly focused on taxa from restricted geographic areas and comprehensive phylogenies

are still missing. Using anatomical data, Kailola (2004) inferred relationships for 45 Old

World and eight New World species (Fig. 2A) and provided a revised classification

accepting 23 genera. Based on combined evidence from mitochondrial and nuclear

sequence data in addition to morphological characters (modified from Betancur-R. et al.,

2004), Betancur-R. et al. (2007) hypothesized relationships for 46 New World and three

Old World species (Fig. 2B) and provided a revised classification for New World taxa

only, validating eight genera. Although the studies by Kailola and Betancur-R. et al. dealt

with different taxon-sampling schemes, their resulting topologies are highly incongruent

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regarding the position of *Galeichthys* and the Indo-Pacific *Ketengus typus* and *Cryptarius truncatus* (Fig. 2). In his unpublished doctoral dissertation, A. P. Marceniuk inferred phylogenetic relationships for 80 ariid species from different biogeographic provinces using morphological characters (Marceniuk, 2003). Following the results derived from this work, Marceniuk and Menezes (2007) presented the most inclusive taxonomy for the Ariidae to date, recognizing 26 genera. More recently, Betancur-R. and Armbruster (in press) inferred molecular phylogenies for the four species of galeichthyines showing that the Eastern Pacific species (*G. peruvianus*) is nested within a clade comprising the remaining three African species, and that the timing of intercontinental divergence occurred from Mid to Late Miocene, likely implying transoceanic dispersal. As will be discussed below, not only the molecular and the anatomical hypotheses differ markedly, but also the two morphological classifications show considerable disagreement.

This study expanded previous molecular phylogenies on New World ariids and galeichthyines using mitochondrial sequences (*cytochrome b*, *ATP synthase 8/6*, *12S*, and *16S*; ~3 kb) and a nuclear marker (*rag2*, ~1 kb) to cover a wide spectrum of taxa from different biogeographic provinces. In addition to the 63 ariid species examined previously (Betancur-R. et al., 2007; Betancur-R. and Armbruster, in press), new molecular data was obtained for 60 additional species. These data were utilized for three main purposes: (1) hypothesize ariid relationships via maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) reconstruction criteria; (2) discuss morphological phylogenies and current classifications in the light of molecular phylogenies; and (3) infer historical biogeography scenarios for the Ariinae using cladistic and chronological

methods. This study provides the most inclusive phylogeny of ariid taxa to date and a resource for future classifications and other comparative studies in the family.

## 2. Materials and Methods

### 2.1 Taxon sampling

Generic nomenclature for New World and Old World ariids follows Betancur-R. et al. (2007) and Marceniuk and Menezes (2007), respectively. The datasets included a total of 123 ariid species/entities (see below), representing 28 genera and the two subfamilies (Galeichthyinae and Ariinae) (~230 total individuals sequenced). Based on current check lists and classifications (Betancur-R. et al., 2007; Marceniuk, 2007), all but one (*Batrachocephalus*) ariid genus was examined and at least 37 species in 16 genera were not examined due to unavailability of tissue samples. The phylogenetic sampling includes ariid species from 26 countries and all major biogeographic provinces. One species of the sister family Anchariidae (*Gogo arcuatus*) was also included in the ingroup (Arioidea sensu Sullivan et al. [2006]); three distantly-related catfish families were used as outgroups (Heptapteridae, Ictaluridae, and Auchenipteridae, listed in Betancur-R. et al. [2007]). New material examined is listed in Appendix III-1. Institutional abbreviations are as listed at http://www.asih.org/codons.pdf.

For several taxa, two or more individuals were sequenced for at least one gene region (mostly *ATP synthase 8/6*). As many as 15 species revealed significant morphological and/or molecular (>1.5 % genetic divergence) differentiation among localities, suggesting that several potential undescribed/unrecognized species exist. In such cases, different entities were analyzed as separate terminals, referring the individuals collected close to the type locality (if known) as the nominal species and the allopatric entities as *affinis* (aff.). Undescribed or unidentified species were listed to the genus level (sp.), whereas dubious identifications were treated as *confer* (cf.). Old World species placed in *Sciades* by Marceniuk and Menezes (2007) were referred as '*Sciades*' (see Discussion).

## 2.2 DNA data and phylogenetic reconstructions

Targeted mitochondrial regions included 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 included partial *recombination activating gene 2* (*rag2*). Laboratory protocols, PCR conditions, utilized primers, and sequence alignment procedures are as described in Betancur-R. et al. (2007). Two additional *rag2* primers were designed for several ariid taxa that failed to amplify using the combination MHRAG2-F1 and MHRAG2-R1 (Betancur-R. et al., 2007; Hardman and Page, 2003): rag2.Ari.F, 5'-GAGCCTCACAGTGAAAACCCYGAG-3'; rag2.Ari.R, 5'-CTCCCTCTCCATCACTGCTGTAC-3'. Different combinations of these four primers yielded successful amplifications in all cases.

Phylogenetic reconstructions were performed under MP, ML, and BI criteria. The initial conditions used for different analyses are summarized in Table III-1. The MP reconstructions were conducted in PAUP* v.4.0b10 (Swofford, 2002) via heuristic searches with random addition (RA) of sequences and tree-bisection-reconnection (TBR); clade support was evaluated using non-parametric bootstrapping with RA and TBR. For ML and BI, the best-fit models of sequence evolution were estimated using the Akaike information criterion (AIC) in ModelTest version 3.7 (Posada and Crandall, 1998). All analyses were run unpartitioned. The ML analyses were performed in the programs Garli v. 0.951/0.96 (Zwickl, 2006) and RAxML v.7.04 (Stamatakis, 2006). Garli searches were conducted using automatic termination (genthreshfortopoterm command). RAxML searches were run in the CIPRES portal version 1.13 (http://www.phylo.org/) under default configurations. ML nodal support was evaluated in RAxML using the rapid bootstrapping algorithm with automatic estimation of runs. For both Garli and RAxML searches, several runs from random-starting seeds were performed to check convergence of likelihood scores. Model parameters were estimated simultaneously (i.e., unfixed). Remaining settings were left at their default values.

The BI analyses were performed in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) via Markov chain Monte Carlo (MCMC) iterations. The MCMC analyses were conducted in triplicate using four chains and sampling trees every 100 generations. Conservatively, 25% of the first trees sampled in each MCMC run were discarded as burn-in. Marginal probabilities of summary parameters, consensus phylograms, and posterior probabilities of nodes were estimated from the post-burn-in samples of the three independent runs combined (Table III-1). To confirm that post-burn-

in trees were sampled from the actual MCMC posterior distribution, marginal parameters (MrBayes log file) were analyzed using the Effective Sample Size (ESS) statistic in the program Tracer (Drummond et al., 2007); ESS greater than 200 suggests that MCMC searches were run long enough to accurately represent the posterior distribution (Drummond et al., 2007).

## 2.3 Hypothesis testing

Alternative hypotheses were compared to the molecular topologies using the parsimony-based nonparametric Templeton test and the likelihood-based Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) as implemented in PAUP*. Three major hypotheses were tested: (1) Kailola's (2004) morphological phylogeny (Fig. III-2A), (2) recent generic classifications derived from anatomical data (Kailola, 2004; Marceniuk and Menezes, 2007), and (3) a Gondwanan vicariance model for ariines. A MP tree that represented a particular hypothesis was estimated using constrained tree searches in PAUP*. The constrained trees were compared to the unconstrained MP topologies using the Templeton test. Likewise, constrained and unconstrained topologies were estimated under ML in Garli and compared via SH tests (1000 replicates and RELL sampling). For ML comparisons, the best-fit model and parameters were selected using the AIC.

## 2.4 Biogeographic inferences

Ancestral areas were reconstructed via dispersal-vicariance analyses as implemented in the program DIVA version 1.2 (Ronquist, 1997). DIVA analyses were performed for inferring: (1) 'center of origin' for New World ariines (unit areas coded as South America/southern Central America, Mesoamerica/North America, Old World); (2) ancestral distribution of basal ariine lineages and widespread Indo-Pacific taxa (unit areas coded as New World, Africa, Madagascar, India-SE Asia, Australia-New Guinea). The number of ancestral areas was restricted to two using the maxareas command. All DIVA analyses were run on MP and ML topologies.

An area cladogram was constructed by replacing the names of terminal taxa with their distributions. The area cladograms for the Ariinae were compared to those inferred by Sparks and Smith (2004) for cichlids, aplocheiloid killifishes, and rainbowfishes (Melanotaenioidei). A general area cladogram based on the four fish groups was then derived and compared to the general area cladogram hypothesized by Sparks and Smith using the latter three groups only. To handle widespread taxa, redundant distributions, and missing areas, a component analysis (Morrone and Crisci, 1995; Nelson and Platnick, 1981; Page, 1988) was performed in the program Component v. 2.0 (Page, 1993, 1994). Component analyses were run using the nearest-neighbor interchanges algorithm by minimizing the number of leaves added. Host without associates (= missing areas) were treated as missing information. Widespread associates were dealt under assumption 2 (i.e., areas including widespread taxa have monophyletic, paraphyletic, or polyphyletic relationships [Morrone and Crisci, 1995; Nelson and Platnick, 1981; Page, 1988; van

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Veller et al., 1999]) by arbitrarily removing all but one area from the distribution of each

widespread taxon (Page, 1993, 1994). The general fish area cladograms were further

compared to the geological area cladogram of Gondwanan breakup (summarized by

Sparks and Smith [2004] based on Smith et al. [1994] and Storey [1995]).


## 2.5 Divergence time estimations


Relative rates tests based on likelihood were performed on eight clades with

different nesting hierarchies using the software r8s version 1.71 (Sanderson, 2003;

Sanderson, 2004). Four out of the eight comparisons suggested significant departures

from a clock-like behaviour ($p<0.05$). Thus, two different methods that do not assume a

strict molecular clock were used for chronological estimations, penalized likelihood (PL)

as implemented in r8s and Bayesian relaxed clock (BRC) as implemented in

MULTIDIVTIME (Thorne and Kishino, 2002). Detailed procedures are as outlined in

Betancur-R. and Armbruster (in press), with the following modifications: BRC analyses

were run unpartitioned and PL analyses were performed under additive penalty. For both

PL and BRC analyses, the initial tree was estimated using ML (Garli) on combined

protein-coding sequences (mitochondrial *cyt b* and *ATPase 8/6*, and nuclear *rag2*). In

addition to the combined dataset, based on the recommendations outlined in Hurley et al.

(2007), divergence times were also estimated excluding the entire *ATPase 8* fragment as

well as third codon positions of *cyt b* and *ATPase 6* (see also Kumazawa et al., 2004).

Eighteen calibration points on thirteen nodes were set as maximum (six points only) and

minimum age constraints based on median fossil ages (Table III-2). Priors for the BRC

analyses were calibrated as follows: rttm, rttmsd, and bigtime parameters were both to

45.0 (= 450 my; estimated divergence time between sarcopterygians and actinopterygians

[Azuma et al., 2008; Kumar and Hedges, 1998]); rtrate and rtratesd 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 rttm (rtrate and rtratesd = 0.0468, combined dataset; rtrate and rtratesd =

0.0603, reduced dataset). Other priors were set to their default values and/or as specified

in Betancur-R. and Armbruster (in press).


## 3. Results and Discussion


### 3.1 Dataset attributes


All sequences obtained during this study are available from GenBank. Accession

numbers of sequences are listed in Appendix III-1. The final alignment of the

mitochondrial protein coding genes included 1095 bp for partial *cyt b* and 842 bp for

*ATPase 8/6* (see details in Betancur-R. et al., 2007). Indels were only observed in *ATPase

8*, where *Cinetodus carinatus*, ten species of *Notarius* (including *N. lentiginosus*), and

'*Sciades*' *sagor* lacked a codon 108, 111, and 141 bases downstream of the start codon,

respectively. For *ATPase 6*, three initiation codons were observed: UUG in *S. sagor* and

the species of the subgenus *Cathorops*, GUG in *Notarius planiceps*, *Notarius aff.

planiceps*, and *N. lentiginosus*, and the typical AUG in the remaining taxa. Likewise, *N.

lentiginosus* presented UUG instead of AUG in *ATPase 8*. The start codons GUG and

UUG have been shown to be less efficient variants of AUG in some genes (Stenstrom et al., 2001). In addition to the differences observed in *Notarius lentiginosus* and *S. sagor* at the amino acid level, both species revealed the highest variation in substitution rates of mtDNA among ariid taxa (Fig. III-3B). It is noteworthy that no ambiguous chromatogram readings, stop codons or frameshifts were observed in these two species, suggesting that these sequences are likely not pseudogenes.

Partial ribosomal 12S and 16S sequences ranged in size from 385 to 394 bp and 556 to 571 bp, with final alignment lengths of 406 and 595 bp, respectively. In the final alignments, 18 sites of 12S and 54 sites of 16S were excluded due to ambiguous positional homology. The mitochondrial dataset assembled included 129 terminal taxa (124 arioids species including one anchariid, four galeichthyines, and 119 ariines) and 2866 sites. The dataset had missing data for *Notarius luniscutis* in *ATPase 8/6* (due to amplification failure) and for *Cathorops manglarensis* (65 bp at 3' end) and *N. lentiginosus* (35 bp at 3' end) in *16S* (due to polymerase slippage producing noisy sequences). Many other taxa also had missing data on sequence ends due to noisy chromatogram reads. Considering only arioid taxa, the mitochondrial alignment contained 1273 (44.4%) variable sites and 1097 (38.3%) parsimony-informative sites, respectively.

The nuclear *rag2* gene has been shown to have low variability within the Ariidae (Betancur-R. et al., 2007), thus nuclear sequences were obtained only from major lineages (as indicated by the mitochondrial topologies) representing all biogeographic provinces and sampled genera (73 species). In the nuclear *rag2* alignment not all ingroup taxa had the same sequence length, which varied from 837 to 978 bp; outgroups had 720

bp. The numbers of variable and parsimony-informative sites for the ingroup in the *rag2* dataset were 174 (17.8%) and 67 (6.9%), respectively. In addition to the mitochondrial and nuclear partitions, a combined dataset including 73 common taxa was assembled (73 species, 3844 sites, 1525 variable sites, 1157 parsimony-informative sites).

## 3.2 Phylogenetic inference

Optimality results obtained under different analyses and model testing on the three data partitions are summarized in Table III-1. Among the four reconstruction methods conducted (MP, ML-Garli, ML-RAxML, BI) on the mitochondrial dataset, BI analyses resulted in the least resolved tree. The BI consensus is shown in Figure III-3; congruent nodes recovered under other methods are indicated by thicker lines. As found in previous molecular studies (Betancur-R. et al., 2007; Sullivan et al., 2006), the superfamily Arioidea sensu Sullivan et al. was recovered as monophyletic, with the Anchariidae sister to the Ariidae (all nodes strongly supported). The subfamilial divisions within the Ariidae (Galeichthyinae and Ariinae) were fully congruent among different methods and well supported. The relationships within the Galeichthyinae and among New World ariine genera are basically identical to those reported in previous molecular studies (Betancur-R. et al., 2007; Betancur-R. and Armbruster, in press) (Fig. III-2B). The new finding is that the genus *Genidens* from the Western Atlantic (previously not examined) was monophyletic and sister to the monotypic *Occidentarius* from the Eastern Pacific (node F, Fig. III-3).

Delimitations of New World genera (sensu Betancur-R. et al., 2007) were congruent with the mitochondrial topologies; however, seven Old Word genera (sensu Marceniuk and Menezes, 2007) were found to be non-monophyletic (Fig. III-3, colored taxa; see below). In general, the relationships among basal ariine taxa were poorly resolved or supported, with short internodes (Fig. III-3B). While New World taxa were not monophyletic in any of the analyses, Old World ariines were grouped into a well-supported clade (node I, Fig. III-3). The basal nodes linking some New World genera (e.g., *Occidentarius*, *Genidens*, *Potamarius*, *Ariopsis*, and *Sciades*) to the Old World clade were often incongruent among different reconstruction criteria and poorly supported (node E, Fig. III-3). Within the Old World clade, taxa were generally grouped into well-defined biogeographic assemblages (Fig. III-3, colored bars). Overall inshore species conform to major Gondwanan provinces (Africa, Madagascar, India-SE Asia, and Australia-New Guinea or Sahul). Delimitations and nodal supports for such groups were high, except for the India-SE Asia assemblage that was often recovered as non-monophyletic with the Madagascar species (*Arius madagascariensis*) nested within. Offshore taxa found widely distributed along Indo-West Pacific shelves fell into three categories: the genera *Netuma* (Indo-West Pacific 1) and *Plicofollis* (Indo-West Pacific 2), and *Brustiarius proximus* (Indo-West Pacific 3, nested within the Australia-New Guinea clade; Fig. III-3). Resolution among Old World biogeographic clades was poor, except for the sister-relationship between Australia-New Guinea and *Plicofollis* (node N, Fig. III-3), which was fully congruent and well supported.

The India-SE group comprised two well-supported basal clades, with one including 'Sciades' sagor, *Cephalocassis melanochir*, and *Hemiarius stormii* (node K,

Fig. III-3) and other grouping the remaining taxa (node L, Fig. III-3). The latter clade was largely unresolved. The relationships within *Netuma*, *Carlarius*, and *Plicofollis* were entirely resolved with high nodal support. There was poor resolution within the Australia-New Guinea assemblage (node O, Fig. III-3), except for the following well-supported clades: *Brustiarius proximus* + *Amissidens hainesi* (node P, Fig. III-3); *Neoarius* (in part; node Q, Fig. III-3); node R (Fig. III-3): node S (*Potamosilurus*, in part) + node T (*Cinetodus carinatus*, ('*Sciades*' *utarus*, (*B. nox*, *B. solidus*))); *Pachyula crassilabris* + *Doiichthys novaeguineae* + *Nedystoma dayi* (node V, Fig. III-3); and *Cochlefelis* (in part; node W, Fig. III-3).

The congruences between methods and data partitions (mitochondrial, nuclear and combined) are summarized in Table III-3. There was strong phylogenetic signal in the mitochondrial dataset, often yielding well-resolved and well-supported nodes. The weak signal and the small number of characters in the nuclear *rag2* dataset (67 parsimony-informative sites) resulted in the greatest number of incongruent nodes (Table III-3). Although using a more reduced taxon-sampling scheme, the combined partition yielded highly congruent topologies as compared to the mitochondrial topologies. The major difference is that the combined scheme recovered the India-SE Asia group as monophyletic in all topologies (only MP and ML-Garli in the mitochondrial partition), but failed to place the Madagascar species sister to the India-SE clade. The lack of resolution and short internodes evidenced among basal ariine lineages is consistent with the reconstructions conducted on the mitochondrial, nuclear, and combined partitions. Given that mitochondrial and nuclear markers contain different levels of phylogenetic signal (Betancur-R. et al., 2007), this pattern may be the result of rapid ariine radiations;

however, further analyses are required to test this hypothesis (see Whitfield and Lockhart, 2007).

*3.3 Morphological hypotheses, classifications, and alpha taxonomy*

The morphological phylogeny hypothesized by Kailola (Fig. III-2A) differs substantially from molecular topologies (Fig. III-3; Table III-3). Of the 34 possible nodes common to both datasets (mitochondrial partition), only eight clades are congruent (Fig. III-2A, asterisks). Further MP and ML reconstructions constraining Kailola's topology into the mitochondrial dataset yielded scores significantly worse than those obtained under unconstrained searches (Templeton and SH tests; Table III-4). Some of the most striking differences between the two hypotheses are the nested position of *Galeichthys*, the basal position and non-monophyly of Old World taxa, and the polyphyly of *Genidens* in Kailola's cladogram. Thus, Kailola's topology does not support the Galeichthyinae and Ariinae as basal subfamilial divisions. Remarkably, unlike the molecular topologies, there are no distinguishable biogeographic patterns in the morphological hypothesis as ariine taxa from different regions appear randomly scattered across the tree. Based on Hennig's (1966) principle of reciprocal illumination, consilience between biogeography and phylogeny provides a logical framework to favor the molecular over the morphological hypothesis.

Conflicts in phylogenetic hypotheses also have profound implications on the classification. Despite their economic importance, the taxonomy of the Ariidae has for a

long time remained in a chaotic state and is probably the most unresolved among catfish families (Ferraris, 2007). This has resulted in instability of the taxonomic nomenclature, with species commonly jumping from one genus to another. An example of this is the three recent studies that have addressed the classification of ariids, which reveal considerable disagreement (Betancur-R. et al., 2007; Kailola, 2004; Marceniuk and Menezes, 2007).

The results presented here also differ from previous classifications in several aspects. Despite the more restricted taxon sampling, the previous classification of New World taxa based on combined molecular and morphological characters (Betancur-R. et al., 2007) is fully congruent with current topologies. This study also validates the status and confirms monophyly of the New World genus *Genidens* (not included in Betancur-R. et al., 2007). The major conflicts concern the morphology-based taxonomies as ten genera recognized by Kailola (2004) and nine by Marceniuk and Menezes (2007) (Fig. III-3A) were not monophyletic in any of the reconstructions conducted on the mitochondrial partition (broadest taxon sampling). Furthermore, Templeton and SH tests constraining each non-monophyletic genus into the mitochondrial dataset identified strong departures from congruence in 16 (nine in Kailola [2004]; seven in Marceniuk and Menezes [2007]) out of the 19 comparisons (Table III-4).

The definition and delimitation of most conflicting genera not only differ markedly among the two anatomical studies, but also have no logical biogeographical circumscriptions. For instance, the genera *Ariopsis*, *Aspistor*, and *Hemiarius* sensu Kailola and *Sciades* sensu Marceniuk and Menezes are ubiquitous and comprise numerous unrelated species/clades (>16 species in *Ariopsis* and *Sciades*). Likewise,

*Hexanematichthys* and *Nemapteryx* sensu Kailola both include species that belong either in the India-SE Asia or the Australia-New Guinea clades. Interestingly, *Hexanematichthys* sensu Kailola provides a remarkable example of morphological convergence (Fig. III-4). As defined by Kailola, *Hexanematichthys* includes two species ('*Sciades*' *sagor* from India-SE Asia and *S. mastersi* from Australia-New Guinea) and is diagnosed by the presence of a broad and depressed head, a short and broad supraoccipital process, a large butterfly-shaped nuchal plate, and a dark peritoneum, among other features (Kailola, 2004) (some of these characteristics also present in the Neotropical *Sciades* and some *Notarius* [Betancur-R. et al., 2008; Marceniuk and Menezes, 2007]). While there is an extraordinary similarity between the neurocrania (and other features) of *S. sagor* and *S. mastersi* (Fig. III-4) and the morphological phylogeny places the two species as sister taxa (Fig. III-2A), the monophyly of *Hexanematichthys* is not supported by the molecular data (Fig. III-3; Table III-4). *Cephalocassis* (from SE Asia) and *Cinetodus* (from Australia-New Guinea) are additional examples of genera that are reasonably well established morphologically (although their delimitations vary in Kailola [2004] and Marceniuk [2007]), but incongruent on a molecular basis (Table III-4). Interestingly, *Cephalocassis melanochir* and *C. borneensis* are two of the eight purely freshwater species found in SE Asia. Both species share several features, including a fenestra between the supraoccipital, the pterotic, and the sphenotic, unique among ariids (Kailola, 2004; Marceniuk and Menezes, 2007: fig. 37). Their morphological similarity may be the result of convergence associated with the transitions between marine and freshwaters.

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The genus *Arius* has been one of the major problems concerning ariid systematics. As traditionally recognized, *Arius* is the largest and most widespread genus in the family (e.g., Acero P., 2002; Kailola, 1999). All recent studies (Betancur-R. et al., 2007; Kailola, 2004; Marceniuk and Menezes, 2007) concur that the genus includes considerably fewer species than was previously presumed (< 25) and, unlike prior classifications, no New World species are currently placed in *Arius*. Although *Arius* sensu Kailola was not recovered as monophyletic, it is restricted to the India-SE Asia province and the topology with enforced monophyly was not rejected by the mitochondrial data. Similarly, the monophyly of *Arius* sensu Marceniuk and Menezes is not rejected by the topological tests, but only if the Malagasy species (*A. madagascariensis*) is excluded (Table III-4). From a phylogenetic standpoint, the present results also suggest that the genera *Netuma* and *Sciades* sensu Kailola and *Brustiarius*, *Cochlefelis*, *Neoarius*, and *Potamosilurus* sensu Marceniuk and Menezes each include only a few species incorrectly assigned to them.

In the light of the classification adopted for this study, *Notarius* (sensu Betancur-R. et al., 2007), *Cathorops*, *Arius*, and *Plicofollis* are among the most species-rich genera in the family. Delimitation of the Neotropical *Notarius* sensu Marceniuk and Menezes is similar to that of Betancur-R. et al., except that Marceniuk and Menezes placed *N. rugispinis* and *N. phrygiatus* in their new genus *Amphiarius*, and *N. luniscutis* and *N. quadriscutis* in *Aspistor*. Validating *Amphiarius* and *Aspistor* would render *Notarius* paraphyletic (although in some analyses *Aspistor* was recovered as sister to all other *Notarius*; see Table III-3). *Aspistor* was also recognized by Kailola but with a different circumscription, including seven species of *Notarius* sensu Betancur-R. plus *Hemiarius*

*hardenbergi* (from Australia-New Guinea) and *Occidentarius platypogon*. The

monophyly of *Notarius* sensu Marceniuk and *Aspistor* sensu Kailola are not supported by

the molecular evidence (Table III-4). A consensus solution for *Notarius* would be

providing subgeneric assignments.

Molecular evidence also brings new perspectives at the alpha taxonomy level.

While recent taxonomic check lists estimate that the number of valid ariid species range

from 125 to 136 (Kailola, 2004; Marceniuk and Menezes, 2007; plus five more recent

additions: Betancur-R. and Willink, 2007; Marceniuk, 2007; Marceniuk and Betancur-R.,

2008), there are 53 species designated as inquirendae in the family (i.e., uncertain

validity), the greatest number within the order Siluriformes (Betancur-R. et al., 2008;

Ferraris, 2007). The major difficulties in undertaking a comprehensive taxonomic study

of the Ariidae are the overall similarity in external morphology, the widespread

distribution of the group coupled with the high degree of species endemism, and the poor

representation of species diversity in museums (see also Marceniuk and Menezes, 2007).

Although estimating the total number of valid species is a challenging task, examination

of wide variety of taxa on both mitochondrial and/or morphological grounds enabled

identification of 20 putative undescribed/unrecognized species (referred as *affinis* or sp.;

see Materials and Methods). The alpha taxonomy of the Ariidae is in need of revision.

Despite the recent efforts to clarify ariid taxonomy (Betancur-R. et al., 2007;

Kailola, 2004; Marceniuk and Menezes, 2007), these results show that it is still far from

settled. This instability prevents an adequate management of the fisheries, making ariids a

difficult group for conservational purposes. A total evidence approach based on

combined morphological (including A.P. Marceniuk data matrix upon publication) and

molecular data would provide a suitable framework to redefine genera and to reassess the classification of the Ariidae. This issue will be addressed elsewhere.

## 3.4 Historical biogeography of the Ariinae

### 3.4.1 The evolutionary history of ariines from a topological perspective

Presently, ariid distribution encompasses the New World, Africa, Madagascar, India-SE Asia, and the Sahul continent (Fig. III-1); however, as evidenced by the fossil record, the group also ranged into Europe until the Late Miocene. The current poor representation of catfishes in Europe has been attributed to extinctions caused by Pleistocene glaciations (Teugels, 1996). While galeichthyines are restricted to subtropical waters in southern Africa and southwestern South America, Sea catfishes owe their broad distribution to ariines. The disjunct biogeography of galeichthyines was recently addressed (Betancur-R. and Armbruster, in press), thus I will focus on ariines only for this section.

The presence of ariines on major landmasses of the southern hemisphere suggests (*a priori*) a Gondwanan vicariance (but see de Queiroz, 2005; McGlone, 2005). To further test this hypothesis, the biogeography of the Ariinae was approached under topological and chronological frameworks. Area cladograms for Gondwana (based on geological data) and ariine taxa (based on combined evidence trees), and general area cladograms derived from various fish groups are shown in Figure III-5. Different reconstruction methods yielded different topologies (Table III-3), thus ariine area

cladograms were derived from MP (Fig. III-5B) and ML (Fig. III-5C) trees (BI and ML topologies are congruent/combinable). The reconstructions indicate that New World ariines are basal and paraphyletic while Old World taxa form a nested clade further subdivided into groups endemic to major areas (i.e., Africa, Madagascar, India-SE Asia and Australia-New Guinea).

In addition to the marked biogeographic associations, the Old World clade includes two subclades with widespread Indo-West Pacific distributions (*Netuma* and *Plicofollis*). Likewise, although *Brustiarius proximus* is nested within the Australia-New Guinea clade, its range extends into SE Asia (reported here for the first time). DIVA analyses indicate that the presence of *B. proximus* in SE Asia is most parsimoniously the result of dispersal from Australia-New Guinea. Thus, for component analyses *B. proximus* is treated as a Sahul taxon. Inferring the biogeographic history of the widespread *Netuma* and *Plicofollis* is less straightforward because of ambiguous relationships among major Old World clades (Figs. 5B, 5C). According to DIVA optimizations on the mitochondrial dataset (broadest taxon sampling), the distribution of the common *Plicofollis* ancestor was either India-SE Asia or the composite India-SE Asia/Australia-New Guinea, whereas the ancestral distribution of *Netuma* was India-SE Asia/Australia-New Guinea.

Overall, the major differences between ariine (Figs. 5B, 5C) and geological area cladograms (Fig. III-5A) are the absence of ariine endemics from Sulawesi and the Seychelles and the lack of a close relationship between New World and African taxa. However, further analyses constraining the monophyly of the New World node G (*Potamarius + Ariopsis + Sciades*; see Fig. III-3) plus the African genus *Carlarius*,

yielded scores similar to those obtained under unconstrained searches (SH test *p* value= 0.35; Templeton test *p* value= 0. 06–0.29). Similarly, while the placement of Australia-New Guinea, India-SE Asia, and Madagascar is variable among different reconstructions (Figs. 5B, 5C) and the predicted area relationship based on the Gondwanan model was not recovered (but see Garli ML reconstructions on the mitochondrial dataset; Table III-3), additional analyses enforcing the Australia-New Guinea + (India-SE Asia + Madagascar) topology are not statistically rejected by the data (SH test *p* value= 0.472; Templeton test *p* value= 0.35–0.54; note that *Netuma* and *Plicofollis* were excluded from the analysis because their widespread distributions).

Component analyses on both MP and ML topologies derived identical general area cladograms (Fig. III-5E). The general fish area cladogram based on ariines, cichlids, aplocheiloid killifishes, and rainbowfishes (Fig. III-5E) is largely congruent with Sparks and Smith's (2004) general area cladogram derived from the latter three groups only (Fig. III-5D) and the geological area cladogram (Fig. III-5A). The main difference is the truncated placement of Australia-New Guinea and India-SE Asia (Fig. III-5).

Although ariines are predominantly marine and therefore potentially capable of dispersing along contiguous intercontinental shelves (e.g., *Netuma, Plicofollis, Brustiarius proximus*), the fact that most taxa with limited dispersal capabilities were grouped into major Gondwanan clades and that relationships among these clades largely coincide (or at least are not significantly incongruent) with the geological history of the super continent, suggest vicariance via continental drift. While restricted distributions are the generalized condition within the Ariinae, widespread Indo-West Pacific taxa are derived from three independent acquisitions (Fig. III-3). If dispersal is historically the

major force driving ariine biogeography, no provincial associations would be observed. As pointed out by Sparks and Smith (2005) for cichlids, a close relationship between African and Malagasy taxa across the Mozambique canal is expected under a dispersalist model. However, the reconstructions presented here offer no evidence for Afro-Malagasy clades (although the two ariine species from Eastern Africa were not examined). Similarly, the sharp faunal division across Wallace's line (dividing SE-Asia and Australia-New Guinea plates) of most (i.e., non-widespread) ariine taxa disfavors dispersal acting as a homogenizing force. The collision of the India subcontinent into Asia 43 mya (Lee and Lawver, 1995; McLoughlin, 2001) permitted faunal dispersal to SE Asia but not to the Australia-New Guinea plate due to deep oceanic barriers preventing faunal exchange across Wallace's line (except for the three Indo-Pacific taxa found in SE Asia and Australia-New Guinea). Remarkably, with the exception being the basal node linking New and Old World ariines (e.g., node E, Fig. III-3), the topologies do not show any transpacific or transatlantic nested clades within the Ariinae, supporting the idea that sea catfishes have limited transoceanic dispersal capability (but see Betancur-R. and Armbruster [in press] for evidence of recent dispersal from Africa to South America in the Galeichthyinae). As Sparks and Smith (2005) argued, not only freshwater fishes in the primary division but also continentally restricted marine groups may be of great value for inferring ancient land connections.

The presence of ariines in non-Gondwanan areas such as Mesoamerica/North America and Europe (until Late Miocene) was probably the result of contiguous intercontinental marine dispersal from South America and perhaps Africa, respectively. In support for this scenario, DIVA analyses derived from the mitochondrial dataset

(broadest taxon sampling) indicates South America as center of origin for New World taxa. Given that extant ariids are absent from Europe (but see records of the African *Carlarius parkii* in the Mediterranean [Golani and Sonin, 1996]), further morphological studies including European fossils are required to test dispersal into this continent.

*3.4.2 The timing of ariine diversification in the context of Gondwanan vicariance*

Recent studies have shown a variety of floral and faunal taxa whose disjunct distributions in the southern hemisphere, previously associated with Gondwanan vicariance, likely represent instances of transoceanic dispersal. Particularly, molecular dating techniques have revealed divergence times that are too young to be explained by continental-drift vicariance. Remarkable examples of recent transoceanic dispersal include geckos, monkeys, lemurs, carnivores, chameleons, frogs, insects, and several angiosperm families, among others (reviewed in de Queiroz, 2005). Contemporary advocates of dispersal even argue that vicariance should be revaluated as the most likely a priori assumption in biogeography (e.g., \de Queiroz, 2005; McGlone, 2005). Accordingly, it seems appropriate to test the Gondwanan hypothesis of ariine diversification in a chronological framework.

The final separation of South America and Africa is dated to be as recent as 105 mya (Maisey, 2000; Pitman et al., 1993), Madagascar and India separated at about 95–84 mya (McLoughlin, 2001; Smith et al., 1994; Storey, 1995), and Australia and Antarctica initiated rifting 95 mya but remained connected until at least 35 mya (Li and Powell, 2001). While the temporal context for the separation of India-Madagascar from the major

Gondwanan landmass is more controversial (Hay et al., 1999; Storey, 1995), it has been

suggested that the Kerguelen Plateau served as a corridor allowing exchange of terrestrial

and freshwater faunas between India-Madagascar and Australia via Antarctica until about

80 mya (Hay et al., 1999; McLoughlin, 2001; Sparks and Smith, 2004). Before the

Eocene, Antarctica had a temperate climate and included fish fauna found at lower

latitudes today (Bellwood and Wainwright, 2002), such as catfishes reported from the

Eocene/Oligocene boundary (Grande and Eastman, 1986). Likewise, after the

fragmentation of Gondwana, shallow marine connections between major landmasses

might have remained open until ~80 mya (e.g., Africa and South America at 84 mya

[Pitman et al., 1993]) or later, permitting exchange of continentally-restricted marine taxa

such as ariines.

Under the Gondwanan model a Mesozoic origin of major ariine clades is

predicted. Previous studies addressing the timing of catfish diversification provide no

support for this scenario. Lundberg et al. (2007) inferred nodal ages for siluriform

families using nuclear *rag1* and *rag2* sequences and seven fossil calibration points via

BRC and PL analyses. Their divergence time estimation for arioids and ariines is ~73 my

and ~20 my, respectively (Lundberg et al., 2007: fig. 2). It is remarkable, however, that

Lundberg et al. arbitrarily selected 144 my (informed by the fossil record of

actinopterygians) as the maximum age constraint for the split of gymnotiforms and

siluriforms. While addressing the timing of galeichthyine intercontinental disjunction,

Betancur-R. and Armbruster (in press) inferred 94–74 my (95% credibility interval 104–

69 my) for the arioid split and 40 my (95% credibility interval 59–29 my) for the ariine

split based on mitochondrial sequences and three calibration points. Instead of fixing the

root of the tree to an arbitrary age, they used minimum and maximum age constraints on a terminal node based on the rising of the Panama isthmus 3.1–2.8 mya. In order to provide more accurate estimates on the divergence time of basal nodes in the arioid tree, this study selected 18 calibration points on two deep osteichthyan nodes, six non-arioid catfish nodes, and five arioid nodes (Table III-2).

Divergence times for selected nodes derived from PL and BRC analyses are shown in Table III-5. Both methods provided significantly divergent estimations on the temporal context of ariine diversification. The BRC analyses estimated much older (twofold to threefold greater) divergence times for most nodes than PL analyses. For instance, according to BRC analyses, the Ariinae stem group originated 104.8–63.9 mya (95% credibility intervals 162.2–50.0 my) and the split between New World and Old World ariines occurred 80.6–49.4 mya (95% credibility intervals 136.4–45.7 my); PL inferred 38.0–28.5 my and 35.1–21.1 my, respectively (Table III-5). The dates obtained with PL, although most similar to those reported in previous studies (Betancur-R. and Armbruster, in press; Lundberg et al., 2007), offer no support for a Gondwanan origin of ariines. Conversely, the divergence times inferred using BRC are within the range of those predicted under the vicariant model (Table III-5). Thus, the present results on the timing of ariine diversification do not permit accepting or rejecting Gondwanan vicariance on a chronological framework with confidence. Similar discrepancies on the timing of cichlid divergence in the context of Gondwanan vicariance are reported in recent molecular studies. Vences et al. (2001) inferred a Cenozoic origin for cichlids (consistent with the fossil record) suggesting recent transatlantic dispersal from Africa to South America.  In contrast, Azuma et al. (2008) inferred divergence times for cichlids

that are consistent with a Gondwanan origin during the Cretaceous (see also Genner et al., 2007). While the latter hypothesis is more widely accepted (but See Briggs, 2003; Genner et al., 2007; e.g., Sparks and Smith, 2005), interestingly, different calibrations and methods have inferred ages for cichlids that are two- to three-fold different.

An alternative solution for improving accuracy on divergence time estimations of basal arioid nodes could come from assigning additional ariid fossils to particular subclades. However, such work requires an exhaustive morphological examination of both fossil and recent taxa. Among catfish families, ariids include the oldest and most abundant elements in the fossil record. Ariid fossils have been identified from bony (48 localities) and otolith (68 localities) remains (Gayet and Meunier, 2003) and the fragments are widespread, including records from North and South America, Europe, Asia, and Africa. The oldest ariid fossils date from the Late Campanian–Early Maastrichtian in North and South America. Old World fossil remains also include Maastrichtian otoliths assigned to the Ariidae, but the oldest bony elements are from the lowermost Eocene (Europe, Africa and Asia) (Cione and Prasad, 2002; Gayet and Meunier, 2003). Considering that the stratigraphic record indicates that by the Early Maastrichtian (~70 mya) ariids were already widely distributed (see Gayet and Meunier, 2003: fig. 17.2), the origin of the group might be older, implying a failure of preservation and/or detection of ancient fossils. If that is the case, a precise assignment of the oldest known specimens to particular ariid subclades might provide more accurate molecular clock estimations in support of the vicariant scenario.

# 4. Conclusions

This study utilized mitochondrial, nuclear, and combined (up to ~4 kb) sequence data to infer phylogenies for arioids based on the most comprehensive taxon sampling to date (124 species/entities).  While the reconstructions support the monophyly of basal groups (Arioidea, Ariidae, Galeichthyinae, and Ariinae), up to ten ariine genera (out of 25–30) validated by previous morphological studies are incongruent with the molecular phylogenies. These results stress the need for re-assessment of ariid classification.

The topologies recovered New World ariines as paraphyletic and Old World species were grouped into a well-supported clade. In further disagreement with morphological hypotheses that follow no biogeographic patterns, the molecular phylogenies group inshore ariine species into well-defined clades restricted to particular Gondwanan provinces (New World [three to five basal clades], Africa, Madagascar, India-SE Asia, and Australia-New Guinea). The general area cladogram derived from the area cladograms of cichlids, aplocheiloid killifishes, rainbowfishes, and ariines is largely congruent with the temporal sequence of events during the fragmentation of Gondwana. Nonetheless, the results obtained on the timing of ariine diversification using PL and BRC methods are too variable to accept or reject Gondwanan vicariance on a chronological context. Further examination of additional ariid fossils might provide better calibration points for more accurate molecular clock estimations.

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**Table III-1.** Summary of initial conditions and results obtained in phylogenetic reconstructions and model testing.

| Analysis | Mitochondrial 129 taxa, 2866 bp | Nuclear 73 taxa, 978 bp | Combined 73 taxa, 3844 bp |
|---|---|---|---|
| **MP** | | | |
| RA replicates | 100 | 5 | 100 |
| Bootstrap replicates | 1000 (10 RA rep.) | _ | 1000 (10 RA rep.) |
| Optimal trees retained | 8 | $>5 \times 10^4$ | 36 |
| Optimal tree score (steps) | 11208 | 387 | 9002 |
| Consistency index | 0.188 | 0.749 | 0.25 |
| Consensus type | strict | 50% majority rule | 90% majority rule |
| **Initial model (ML and BI)** | | | |
| Akaike information criterion | GTR+I+Γ | HKY+I+Γ | GTR+I+Γ |
| Number of substitution rate parameters | 6 | 2 | 6 |
| **ML - Garli** | | | |
| Search replicates | 10 | 5 | 10 |
| Automatic termination* (generations) | $1 \times 10^4$ | $1 \times 10^4$ | $1 \times 10^4$ |
| Optimal tree score (lnL) | -52003.50 | -3791.51 | -43666.78 |
| Bootstrap | _ | _ | _ |
| **ML - RAxML** | | | |
| Search replicates | 10 | 5 | 5 |
| Partitions | none | none | none |
| Optimal tree score (lnL) | -52145.37 | -3790.72 | -43726.10 |
| Bootstrap replicates** | 150 | 400 | 200 |
| **BI** | | | |
| Search replicates | 3 | 3 | 3 |
| Partitions | none | none | none |
| Generations | $1 \times 10^7$ | $6 \times 10^6$ | $6 \times 10^6$ |
| Burn-in | $2.5 \times 10^6$ | $1.5 \times 10^6$ | $1.5 \times 10^6$ |
| Post-burn-in trees (combined searches) | $\sim 2.15 \times 10^5$ | $\sim 1.35 \times 10^5$ | $\sim 1.35 \times 10^5$ |
| Mean lnL | -52160 | -4119.14 | -43760 |
| Efective Sample Size (all parameters) | >200 | >200 | >200 |
| Consensus type | 50% majority rule | 50% majority rule | 50% majority rule |

RA, random addition

*genthreshfortopoterm command

**rapid bootstrapping algorithm via automatic termination

**Table III-2.** Minimum and maximum ages constraints used as calibration points for divergence time estimations. MRCA, most recent common ancestor (continued on next page).

| Taxon | MRCA | Description |
|---|---|---|
| Osteicthyes | *Latimeria, Arioidea* | Split between sarcopterygians and actinopterygians based on fossils and molecules |
| Osteicthyes | *Latimeria, Arioidea* | *Psarolepis, Lophosteus,* and *Andreolepis* fossil (Ludlow, Silurian) |
| Actinopterans | *Polypterus, Arioidea* | Stem-actinopterans from the Givetian/Eifelian boundary |
| Doradoidea + Aspredinidae | *Ageneiosus, Pterobunocephalus* | Doradoid fossil of Maastrichtian |
| Ictaluridae | *Ameiurus, Ictalurus* | *Ameiurus pectinatus* of Late Eocene |
| Ictaluridae + Cranoglanidae | *Ictalurus, Cranoglanis* | Ictalurid stem fossil Astephus sp. of Early Paleocene |
| Callichthyidae | *Callichthys, Corydoras* | *Corydoras revelatus* fossil dated as Late Paleocene |
| *Leiarius + Perrunichthys* | *Leiarius, Perrunichthys* | Uplift of the Mérida Andes and isolation of the Maracaibo basin (10–8 my) |
| *Leiarius + Perrunichthys* | *Leiarius, Perrunichthys* | Uplift of the Mérida Andes and isolation of the Maracaibo basin (10–8 my) |
| Pimelodidae | *Steindachneridion, Leiarius* | *Steindachneridion* fossil (30–20 my) |
| Arioidea | *Gogo, Arius* | Ariid fossils of Late Campanian–Early Maastrichtian |
| *Cathorops* | *C. dasycephalus, C. mapale* | *Cathorops* fossil of Early Miocene |
| *Cathorops mapale + C. fuerthii* | *C. mapale, C. fuerthii* | Last rising of Panama isthmus (3.1–2.8 my) |
| *Cathorops mapale + C. fuerthii* | *C. mapale, C. fuerthii* | Last rising of Panama isthmus (3.1–2.8 my) |
| *Notarius kesleri + N. neogranatensis* | *Notarius kesleri, N. neogranatensis* | Last rising of Panama isthmus (3.1–2.8 my) |
| *Notarius kesleri + N. neogranatensis* | *Notarius kesleri, N. neogranatensis* | Last rising of Panama isthmus (3.1–2.8 my) |
| *Ariopsis seemanni + Ariopsis sp.* | *Ariopsis seemanni, Ariopsis sp.* | Last rising of Panama isthmus (3.1–2.8 my) |
| *Ariopsis seemanni + Ariopsis sp.* | *Ariopsis seemanni, Ariopsis sp.* | Last rising of Panama isthmus (3.1–2.8 my) |

**Table III-2.** (Continued).

| Taxon | Age constraint (my) | Reference |
|---|---|---|
| Osteicthyes | 450 (max) | (Azuma et al., 2008; Kumar and Hedges, 1998) |
| Osteicthyes | 416 (min) | (Azuma et al., 2008; Botella et al., 2007; Hurley et al., 2007) |
| Actinopterans | 392 (min) | (Azuma et al., 2008; Hurley et al., 2007) |
| Doradoidea + Aspredinidae | 68 (min) | (Gayet and Meunier, 2003; Lundberg et al., 2007) |
| Ictaluridae | 36 (min) | (Lundberg et al., 2007) |
| Ictaluridae + Cranoglanidae | 64 (min) | (Lundberg et al., 2007) |
| Callichthyidae | 58.35 (min) | (Lundberg et al., 2007) |
| *Leiarius + Perrunichthys* | 10 (max) | (Hardman and Lundberg, 2006; Lundberg et al., 1998) |
| *Leiarius + Perrunichthys* | 8 (min) | (Hardman and Lundberg, 2006; Lundberg et al., 1998) |
| Pimelodidae | 25 (min) | (Hardman and Lundberg, 2006; Lundberg et al., 1998) |
| Arioidea | 70.5 (min) | (Betancur-R. and Armbruster, in press; Gayet and Meunier, 2003; Lundberg et al., 2007) |
| *Cathorops* | 19.5 (min) | (Aguilera and De Aguilera, 2004; Betancur-R. and Armbruster, in press) |
| *Cathorops mapale + C. fuerthii* | 3.1 (max) | (Bermingham et al., 1997; Betancur-R. and Armbruster, in press; Coates and Obando, 1996) |
| *Cathorops mapale + C. fuerthii* | 2.8 (min) | (Bermingham et al., 1997; Betancur-R. and Armbruster, in press; Coates and Obando, 1996) |
| *Notarius kesleri + N. neogranatensis* | 3.1 (max) | (this study, Bermingham et al., 1997; Coates and Obando, 1996) |
| *Notarius kesleri + N. neogranatensis* | 2.8 (min) | (this study, Bermingham et al., 1997; Coates and Obando, 1996) |
| *Ariopsis seemanni + Ariopsis sp.* | 3.1 (max) | (this study, Bermingham et al., 1997; Coates and Obando, 1996) |
| *Ariopsis seemanni + Ariopsis sp.* | 2.8 (min) | (this study, Bermingham et al., 1997; Coates and Obando, 1996) |

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**Table III-3.** Congruence among reconstruction methods and data partitions. For each analysis filled and open cells indicate presence or absence of a particular clade, respectively. Numbers in cells indicate nodal support (whenever available, see Table III-1), with bolded values for MP or ML bootstrap ≥ 75% and BI posterior probabilities ≥ 0.95. Nodes refer to clades in Figure III-3 (continued on next page).

| Taxon/Clade | Node | Mitochondrial 124 ingroup taxa 2866 bp 1097 informative sites | | | | Nuclear 70 ingroup taxa 978 bp 67 informative sites | | | | Combined 70 ingroup taxa 3844 bp 1157 informative sites | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MP | ML - Garli | ML - RAxML | BI | MP | ML - Garli | ML - RAxML | BI | MP | ML - Garli | ML - RAxML | BI |
| Arioidea | | **94** | | **100** | **1.0** | | | **99** | **1.0** | **100** | | **100** | **1.0** |
| Ariidae | | **100** | | **100** | **1.0** | | | **99** | **1.0** | **100** | | **100** | **1.0** |
| Galeichthyinae | | **100** | | **100** | **1.0** | | | **96** | **1.0** | **100** | | **100** | **1.0** |
| Ariinae | A | **100** | | **100** | **1.0** | | | **88** | **0.99** | **100** | | **100** | **1.0** |
| *Bagre* | | **100** | | **100** | **1.0** | | | **91** | **1.0** | **100** | | **100** | **1.0** |
| *Notarius + Cathorops* | B | | | 70 | **1.0** | | | | | | | 81 | **1.0** |
| *Notarius* | | **100** | | **100** | **1.0** | | | **85** | **1.0** | **100** | | **100** | **1.0** |
| *Aspistor* sensu Marceniuk and Menezes | C | **100** | | **100** | **1.0** | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* |
| *Notarius* minus *Aspistor* | | 50 | | | | | | <50 | 0.61 | | | | |
| *Cathorops* | | **91** | | **99** | **1.0** | | | | | **88** | | **100** | **1.0** |
| Subgenus *Cathorops* | D | **100** | | **100** | **1.0** | | | **98** | **1.0** | **100** | | **100** | **1.0** |
| Node G + Node F + Old World Ariinae (Node I) | E | <50 | | <50 | 0.59 | | | | | | | | |
| *Occidentarius + Genidens* | F | 52 | | **91** | **0.98** | | | | | 59 | | **95** | **0.99** |
| *Genidens* | | **100** | | **100** | **1.0** | | | **87** | **0.97** | **100** | | **100** | **1.0** |
| *Sciades + Potamarius + Ariopsis* | G | 72 | | **99** | **1.0** | | | | 0.59 | **90** | | **100** | **1.0** |
| *Potamarius + Ariopsis* | H | **90** | | **100** | **1.0** | | | | | **95** | | **100** | **1.0** |

# Table III-3. (Continued).

| Taxon | Node | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sciades* | | 100 | 100 | 1.0 | | | 100 | 1.0 | 100 | | 100 | 1.0 |
| *Potamarius* | | 100 | 100 | 1.0 | | | 76 | 1.0 | 100 | | 100 | 1.0 |
| *Ariopsis* | | 100 | 99 | 1.0 | | | | | 100 | | 100 | 1.0 |
| Old World Ariinae | I | 91 | 100 | 1.0 | | | 58 | 0.88 | 94 | | 100 | 1.0 |
| Indo-Pacific Ariinae (Old World minus W. Africa) | | | | | | | | | | | 59 | 0.92 |
| *Netuma* (Indo-West Pacific 1) | | 100 | 100 | 1.0 | | | 98 | 1.0 | 100 | | 100 | 1.0 |
| *Carlarius* (W. Africa) | | 99 | 100 | 1.0 | | | 65 | 0.93 | 99 | | 100 | 1.0 |
| Madagascar + India-Southeast Asia | J | | | 0.78 | | | | | | | | |
| India-Southeast Asia | | 67 | | | | | | | 71 | | <50 | 0.68 |
| *S. sagor* + *C. melanochir* + *H. stormii* | K | 91 | 85 | 0.95 | | | | | 94 | | 90 | 0.98 |
| India-Southeast Asia minus node K | L | 96 | 99 | 1.0 | | | 83 | 1.0 | 98 | | 100 | 1.0 |
| *Ketengus* + *Osteogeneiosus* | M | <50 | <50 | 0.53 | | | | | | | | |
| *Plicofollis* + Australia-N. Guinea | N | 60 | 94 | 1.0 | | | | | | | 87 | 1.0 |
| *Plicofollis* (Indo-West Pacific 2) | | 99 | 100 | 1.0 | | | 70 | 0.98 | 100 | | 100 | 1.0 |
| Australia-N. Guinea | O | 96 | 99 | 1.0 | | | | | 96 | | 100 | 1.0 |
| *Amissidens* + *B. proximus* | P | 74 | 70 | 0.95 | | | | | 75 | | 89 | 0.99 |
| *Neoarius* (in part) | Q | 100 | 100 | 1.0 | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* |
| Node S + node T | R | 97 | 97 | 1.0 | | | | | 91** | ** | 95** | 1.0** |
| *Potamosilurus* (in part) | S | 99 | 100 | 1.0 | | | 67 | 0.94 | 95 | | 100 | 1.0 |
| *C. carinatus* + *S. utarus* + *Brustiarius* (in part) | T | 75 | 89 | 0.99 | | | | | 69** | ** | 70** | 0.91 |
| *Doiichthys* + *Nedystoma* | U | 90 | 67 | 0.79 | | | 66 | 0.98 | 94 | | 84 | 1.0 |
| *Pachyula* + *Doiichthys* + *Nedystoma* | V | 100 | 100 | 1.0 | | | | 0.92 | 100 | | 100 | 1.0 |
| *Cochlefelis* (in part) | W | 97 | 100 | 1.0 | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* | N/A* |

*Only one taxon included in dataset

**S. utarus* not included in dataset

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**Table III-4.** Incongruence between phylogeny and classifications derived from morphological studies and the molecular evidence. Results obtained with Templeton and Shimodaira–Hasegawa (SH) tests of topology congruence between trees constrained under the morphological hypotheses and unconstrained trees (significant *p* values in bold). Hypothesis testing was performed on Kailola's phylogeny (2004 Fig. III-2A) and those genera defined by Kailola (2004) and Marceniuk and Menezes (2007) that were recovered as non-monophyletic (see also Fig. III-3). For generic comparisons, only one node was constrained. Taxa in constrained clade include common species only; however, for some comparisons the number of taxa with enforced monophyly is greater than the number of taxa assigned to a particular genus in previous studies due to the greater number of species recognized here (e.g., *affinis* entities).

| | # of taxa in constrained clade | SH (*p* value) | Templeton (*p* value) |
|---|---|---|---|
| **Kailola (2004)** | | | |
| Phylogeny | 48 (34 constrained nodes) | **0.000** | **<0.0001** |
| *Ariopsis* | 20 | **0.000** | **<0.0001** |
| *Arius* | 7 | 0.411 | 0.72–0.88 |
| *Aspistor* | 11 | **0.000** | **<0.0001** |
| *Cephalocassis* | 2 | **0.000** | **<0.0001** |
| *Cinetodus* | 3 | **0.000** | **<0.0001** |
| *Hemiarius* | 4 | **0.000** | **<0.0001** |
| *Hexanematichthys* | 2 | **0.027** | **<0.01** |
| *Nemapteryx* | 5 | **0.000** | **<0.001** |
| *Netuma* | 5 | **0.018** | **<0.01** |
| *Sciades* | 8 | **0.000** | **<0.0001** |
| **Marceniuk and Menezes (2007)** | | | |
| *Arius* | 10 | **0.004** | **<0.04** |
| *Arius* excluding *A. madagascariensis* | 9 | 0.341 | 0.59–0.77 |
| *Brustiarius* | 3 | **0.000** | **<0.0001** |
| *Cephalocassis* | 2 | **0.000** | **<0.0001** |
| *Cinetodus* | 2 | 0.153 | **<0.08\*** |
| *Cochlefelis* | 3 | 0.355 | 0.31–0.41 |
| *Neoarius* | 6 | 0.132 | 0.18–0.25 |
| *Notarius* | 13 | **0.013** | **<0.03** |
| *Potamosilurus* | 4 | **0.014** | 0.09–0.13 |
| *Sciades* | 18 | **0.000** | **<0.0001** |

*In 52 out of 56 comparisons *p* value < 0.05

**Table III-5.** Divergence time estimations for selected nodes. Values in parenthesis indicate 95% credibility interval. Complete dataset: *cyt b*, *ATPase 8/6*, and *rag2* (2934 bp). Reduced dataset: excluding *ATPase 8* and third codon positions of *cyt b* and *ATPase 6* (2173 bp). Nodes refer to clades in Figure III-3. BRC, Bayesian relaxed clocks; PL, penalized likelihood; S, smoothing parameter.

| Taxon/Clade | Node | BRC (my) | | PL (my) | |
| --- | --- | --- | --- | --- | --- |
| | | Complete | Reduced | Complete (S= 10) | Reduced (S= 100) |
| Osteicthyes | | 432.8 (448.8–417.1) | 431.8 (448.8–416.8) | 450.0 | 450.0 |
| Actinopterans | | 405.6 (431.2–392.5) | 417.1 (442.5–394.1) | 392.0 | 392.0 |
| Gymnotiformes-Siluriformes | | 229.9 (274.8–186.9) | 313.2 (401.5–219.0) | 153.3 | 138.6 |
| Arioidea | | 111.8 (140.9–86.9) | 156.4 (228.5–100.6) | 70.5 | 70.5 |
| Ariidae | | 88.4 (111.9–68.9) | 130.7 (196.4–81.3) | 52.4 | 42.9 |
| Galeichthyinae | | 33.5 (51.9–19.9) | 53.2 (102.7–21.0) | 13.4 | 12.2 |
| *Galeichthys peruvianus* + *G. ater/G. feliceps* | | 18.8 (32.5–9.6) | 23.7 (56.3–6.13) | 6.65 | 4.7 |
| Ariinae | A | 63.9 (80.9–50.0) | 104.8 (162.2–63.0) | 38.0 | 28.5 |
| Some New World + Old World | E | 58.8 (75.3–45.7) | 86.7 (136.4–50.8) | 35.1 | 21.1 |
| Old World Ariinae | I | 49.4 (64.1–37.7) | 80.6 (127.8–46.5) | 29.6 | 18.6 |
| Indo-Pacific Ariinae (split India, Madagascar, and Asutralia-N. Guinea) | J | 47.0 (61.2–35.6) | 76.3 (121.1–44.1) | 28.5 | 17.8 |
| India-Southeast Asia | | 42.5 (56.1–31.8) | 71.9 (115.3–40.9) | 26.7 | 17.6 |
| Australia-N. Guinea + Madagascar + *Netuma* + *Plicofollis* | | 46.1 (60.2–34.9) | 70.2 (113.4–39.3) | 28.4 | 17.8 |
| Australia-N. Guinea | O | 36.9 (49.2–27.4) | 59.4 (98.2–32.2) | 22.4 | 15.5 |

# FIGURES

**Figure III-1.** Approximate distribution of ariids. Some shaded areas represent extrapolated localities (after Betancur-R. et al., 2007).

**Figure III-2.** Alternative hypotheses of relationships among ariid taxa. (A) Kailola's (2004) phylogeny on 45 Old World and eight New World ariid species based on 57 morphological characters. Taxa examined during this study are in bold; asterisks (*) indicate clades that are congruent with the topologies recovered (see Fig. III-3). (B) Betancur-R. et al.'s (2007) phylogeny on 46 New World and three Old World ariid species. The summarized phylogeny is derived from trees obtained from mitochondrial (2842 bp), nuclear (978 bp), and morphological (55 characters) datasets. Both studies deal with different taxon-sampling schemes, and both topologies are highly incongruent regarding the position of *Galeichthys*, *Ketengus typus* and *Cryptarius truncatus*.

**Figure III-3.** BI phylogeny of 124 arioid species derived from the mitochondrial dataset (2866 bp). Fifty percent majority rule consensus on ~2.15x105 post-burn-in trees (mean lnL -52160). (A) cladogram; thicker branches indicate clades that are congruent with MP and ML (Garli and RAxML) analyses. Asterisks (*) designate clade support (see also Table III-3); capital letters indicate nodes referred in text and Table III-3 (symbols and letters always on left of nodes); vertical bars indicate subfamilial divisions and distribution of major ariine groups. Generic placement for New World and Old World ariines follows Betancur-R. et al. (2007) and Marceniuk and Menezes (2007),

respectively. Colored taxa indicate non-monophyletic genera validated by Marceniuk and

Menezes (yellow and red taxa correspond to *Notarius* and *Sciades* sensu Marceniuk and

Menezes [2007], respectively). Two letter country codes follow ISO-3166. (B)

phylogram (Ariidae only) elucidating the short internodes at the base of the Ariinae and

the rate variation across lineages (taxon arrangement follows the same order in both

figures). Gray dots indicate long branches in *N. lentiginosus* (left) and *H. sagor* (right).

**Figure III-4.** A remarkable example of morphological convergence. The genus

*Hexanematichthys* sensu Kailola (2004) includes two species (*'Sciades' sagor* and *S.

mastersi*) and is defined by the presence of a broad and depressed head, a short and broad

supraoccipital process (SP) and a large butterfly-shaped nuchal plate (NP), among other

features. Although the neurocrania of *S. sagor* and *S. mastersi* are most similar in this

sample, the molecular evidence suggests that they are more closely related to *Arius nenga*

(India-SE Asia clade) and *Cochlefelis dioctes* (Australia-New Guinea clade), respectively

(see Fig. III-3). Also, Templeton and SH tests reject monophyly of *Hexanematichthys*

(see Table III-4). (A), AUM 46280, 87 mm cranial length (CL); (B), AUM 50242, 131

mm CL; (C), AUM 47562, 117 mm CL; (D), AUM 47507, 170 mm CL.

**Figure III-5.** Area cladograms. (A) Geological area cladogram of Gondwanan

progression (summarized by Sparks and Smith [2004] based on Smith et al. [1994] and

Storey [1995]). Ariine area cladograms based on MP (B) and ML (C) topologies

estimated on the combined dataset (see details in Tables III-1, III-3). (D) Sparks and

Smith (2004) general area cladogram derived from the area cladograms of cichlids,

aplocheiloid killifishes, and rainbowfishes (for particular area cladograms see Sparks and

Smith [2004: fig. 4]). (E) General area cladogram derived from the component analysis

of the four fish groups using either MP (strict consensus of three optimal trees, minimal

value= 51) or ML (strict consensus of three optimal trees, minimal value= 32) topologies.

Widespread *Netuma* and *Plicofollis* were handled under assumption 2 (Morrone and

Crisci, 1995; Nelson and Platnick, 1981; Page, 1988; van Veller et al., 1999) by

arbitrarily removing all but one area from their distributions (areas used: India-SE Asia

for *Netuma*, Australia-New Guinea for *Plicofollis* [Page, 1993, 1994]). Letters in

parentheses refer to nodes in Figure III-3 and Table III-3.

**Figure III-1**



Legend:
- Galeichthyinae
- Ariinae
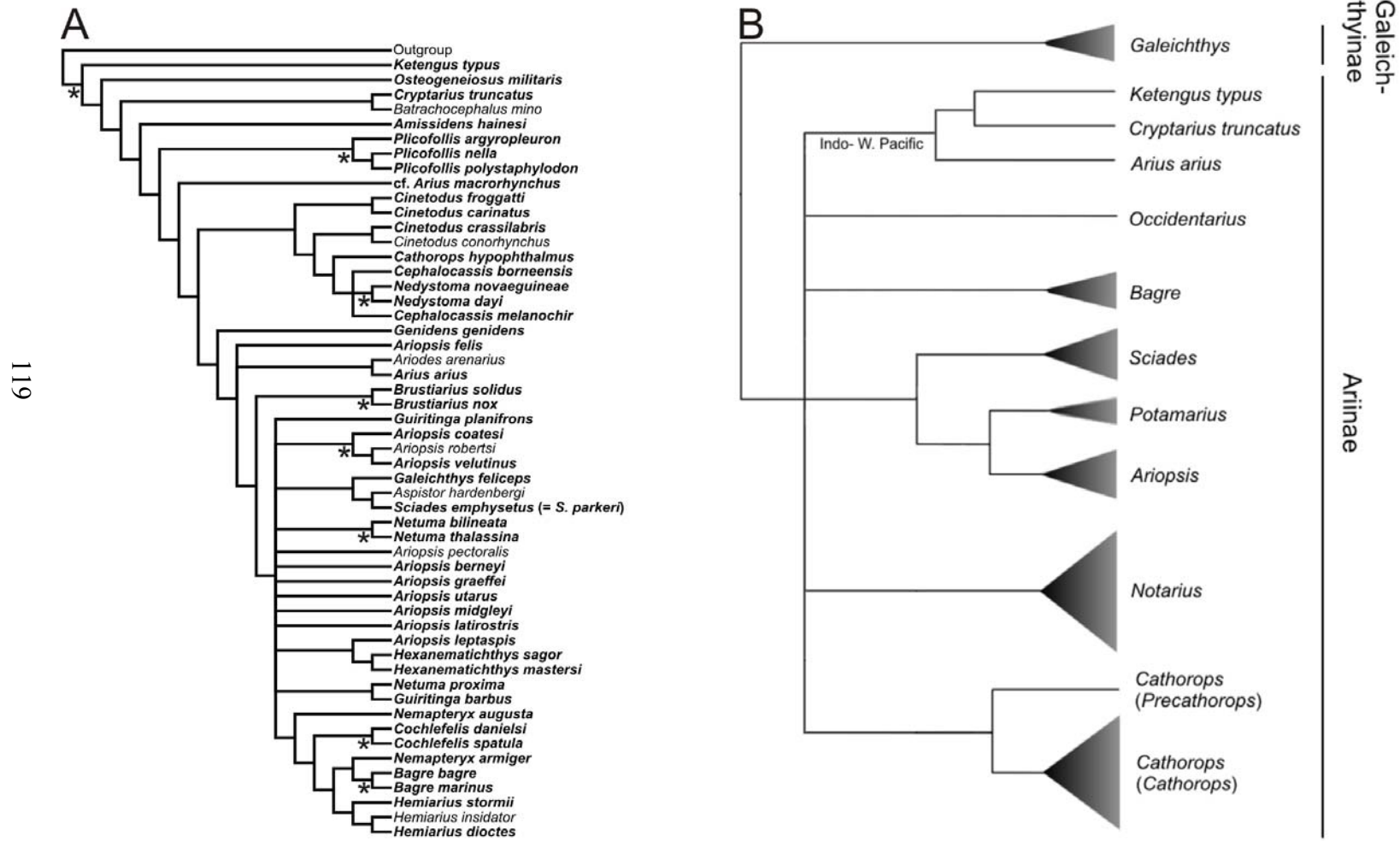
**Figure III-2**
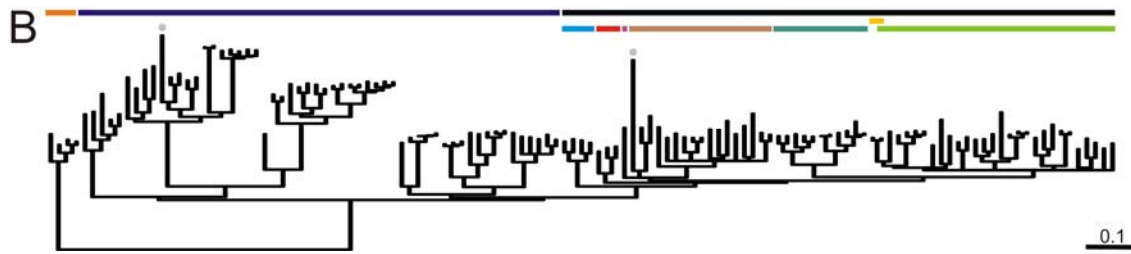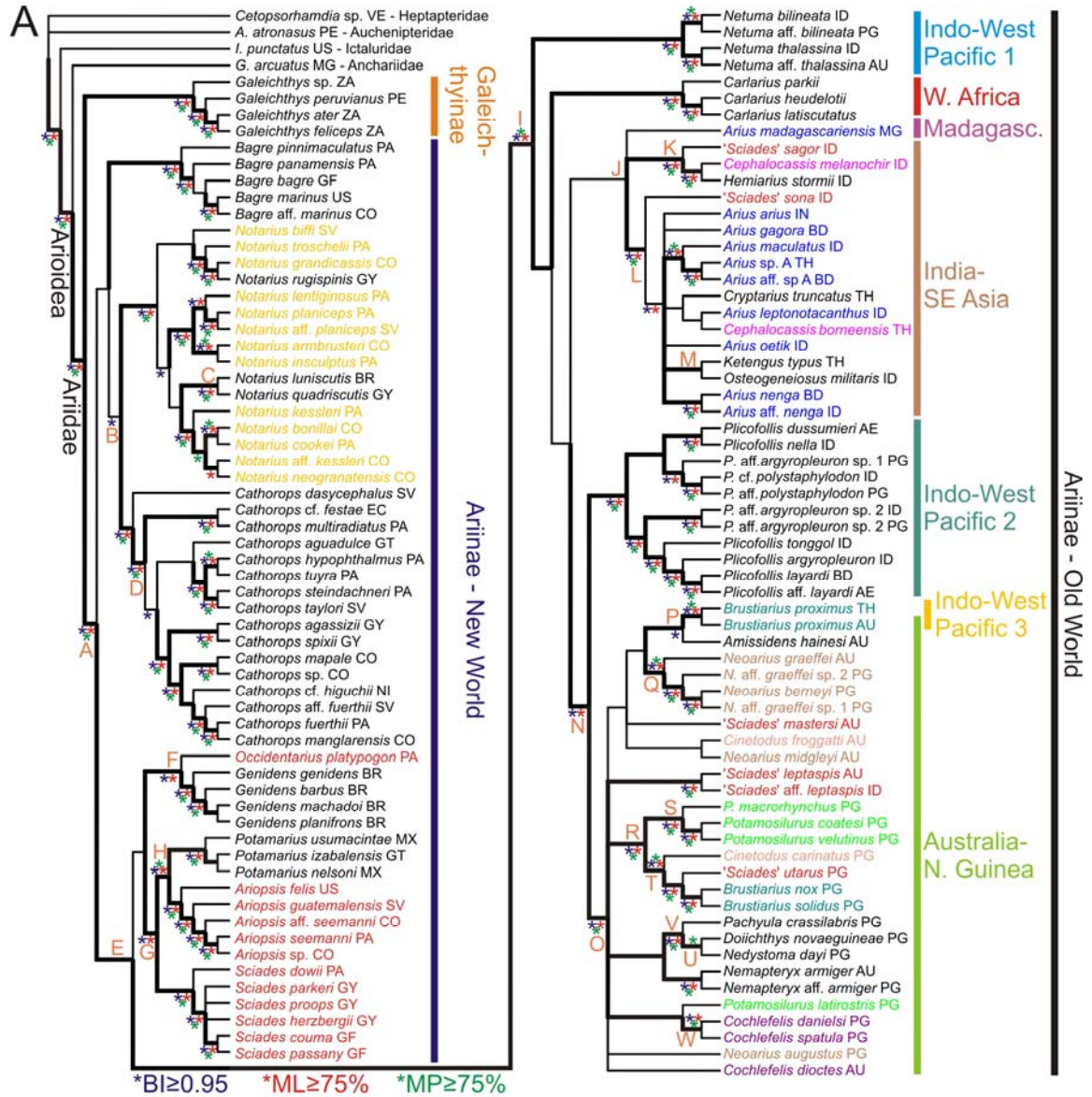
**Figure III-3**

**Figure III-4**
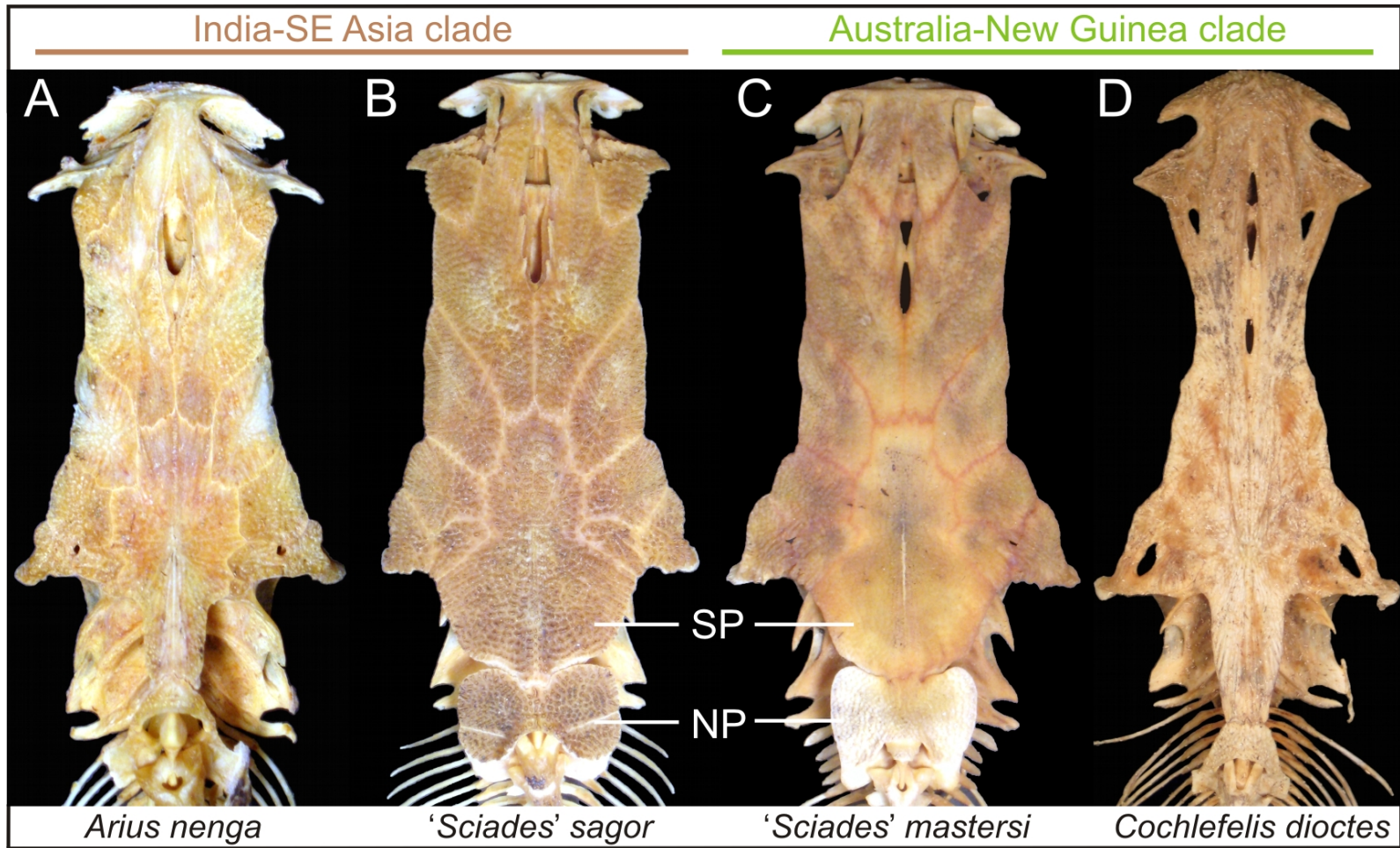
**Figure III- 5**
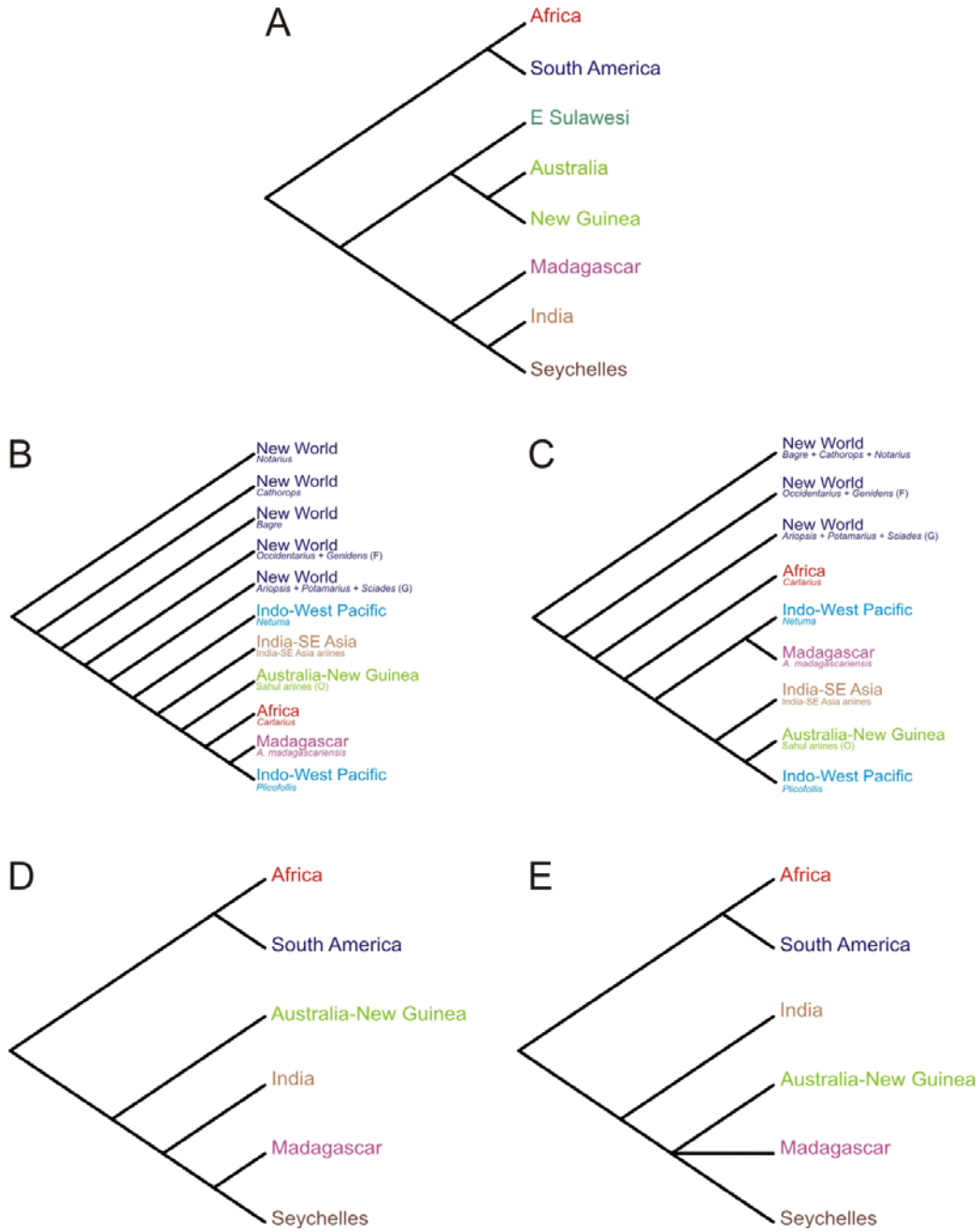
**Appendix III-1.** List of new material examined and GenBank accession numbers. Additional material is listed in Betancur-R. et al. (2007) and Betancur-R. and Armbruster (in press). Two letter country codes follow ISO-3166. NV, not vouchered; NCA, not catalogued (Continued on next page).

| Species/entity | Country | Tissue No. | Catalog No. |
|---|---|---|---|
| *Ariopsis* sp. (Eastern Pacific) | CO/PE | 509/ID182 | INVEMAR-PEC (NCA)/AUM 50249 |
| *Ariopsis* sp. (Caribbean) | CO | CO0604 | INVEMAR-PEC (NCA) |
| *Bagre bagre* | BR | ID125 | MZUSP 99706 |
| *Cathorops spixii/arenatus* | BR | ID140 | MZUSP 99717 |
| *Cathorops* cf. *festae* | EC | JM 744 | MNRJ (NCA) |
| *Cathorops* cf. *higuchii* | NI | PB20/oto4 | MNHN (NCA) |
| *Cathorops manglarensis* | CO | 526/535 | INVEMAR-PEC (NCA) |
| *Cathorops multiradiatus* | CO | 506 | INVEMAR-PEC (NCA) |
| *Cathorops spixii* | BR | ID135 | MZUSP 99715 |
| *Genidens barbus* | BR/UR | 19018/ID129/AA2/NC191 | LBP 3007/MZUSP 99709/NV/UFRGS (NCA) |
| *Genidens genidens* | BR | ID130 | MZUSP 99710 |
| *Genidens machadoi* | BR | ID128/ID124 | MZUSP 99708/99705 |
| *Genidens planifrons* | BR | NC 196/NC 195 | UFRGS (NCA) |
| *Notarius* aff. *kessleri* | CO | 514/524/522/523 | INVEMAR-PEC (NCA) |
| *Notarius lentiginosus* | PA | stri-21651 | STRI 07120 |
| *Notarius luniscutis* | BR | ID132/ID134 | MZUSP 99712/99714 |
| *Sciades herzbergii* | CO | 490 | INVEMAR-PEC (NCA) |
| *Sciades passany* | VE | VEN0529 | AUM 44220 |
| *Carlarius latiscutatus* | W. Africa | 1717 | NV |
| *Carlarius parkii* | W. Africa | 1709/174/176 | NV |
| *Arius madagascariensis* | MG | stri-x3536/stri-x3534/T.003.0293-0295 | MHNG 2623.055/AMNH 241670-241672 |
| *Netuma bilineata* | ID | ID029 | AUM 46279 |
| *Netuma* aff. *bilineata* | PG | PNG026 | AUM 47559 |
| *Netuma* aff. *thalassina* | AU | RW 0421 | NV |
| *Plicofollis argyropleuron* | ID | ID105 | AUM 46298 |

**Appendix III-1.** (Continued).

| Species/entity | GenBank Accession No. |
|---|---|
| *Ariopsis* sp. (Eastern Pacific) | FJ625863, FJ625864, FJ626160, FJ626097, FJ626034 |
| *Ariopsis* sp. (Caribbean) | FJ625865 |
| *Bagre bagre* | FJ625866 |
| *Cathorops spixii/arenatus* | FJ625867 |
| *Cathorops* cf. *festae* | FJ625868, FJ626161, FJ626098, FJ626035 |
| *Cathorops* cf. *higuchii* | FJ625869, FJ625870, FJ626162, FJ626163, FJ626099, FJ626036 |
| *Cathorops manglarensis* | FJ625871, FJ625872, FJ626164, FJ626165, FJ626100, FJ626037 |
| *Cathorops multiradiatus* | FJ625873, FJ626166 |
| *Cathorops spixii* | FJ625874 |
| *Genidens barbus* | FJ625875–FJ625878, FJ626167, FJ626101, FJ626038, FJ626006 |
| *Genidens genidens* | FJ625879 |
| *Genidens machadoi* | FJ625880, FJ625881, FJ626168, FJ626102, FJ626039 |
| *Genidens planifrons* | FJ625882, FJ625883, FJ626169, FJ626103, FJ626040 |
| *Notarius* aff. *kessleri* | FJ625884–FJ625887, FJ626170, FJ626104, FJ626041 |
| *Notarius lentiginosus* | FJ625888, FJ626171, FJ626105, FJ626042 |
| *Notarius luniscutis* | FJ626172, FJ626106, FJ626107, FJ626043, FJ626044 |
| *Sciades herzbergii* | FJ625889 |
| *Sciades passany* | FJ625890 |
| *Carlarius latiscutatus* | FJ625891, FJ626173, FJ626108, FJ626045 |
| *Carlarius parkii* | FJ625892, FJ625893, FJ626175, FJ626174, FJ626109, FJ626046, FJ626007 |
| *Arius madagascariensis* | FJ625894–FJ625898, FJ626176, FJ626110, FJ626047, FJ626008 |
| *Netuma bilineata* | FJ625899, FJ626177, FJ626111, FJ626048 |
| *Netuma* aff. *bilineata* | FJ625900, FJ626178, FJ626112, FJ626049, FJ626009 |
| *Netuma* aff. *thalassina* | FJ625901, FJ626179, FJ626113, FJ626050 |
| *Plicofollis argyropleuron* | FJ625902 |

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| | | | |
|---|---|---|---|
| *Plicofollis* aff. *argyropleuron* sp.1 | PG | PNG054/PNG064 | AUM 47484/47505 |
| *Plicofollis* aff. *argyropleuron* sp.2 | AU/PG/ID | HL0116/ID055/PNG033 | NV/AUM 44854/AUM 47485 |
| *Plicofollis dussumieri* | AE/BD | PNG147/PNG143/PNG528 | NV/AUM (NCA) |
| *Plicofollis layardi* | BD | PNG523 | AUM (NCA) |
| *Plicofollis* aff. *layard* | AE | PNG149 | NV |
| *Plicofollis nella* | ID, SG | ID039/HH004/ID106/ID104 | AUM 44864/ZRC ?51223/AUM 46283 |
| *Plicofollis* cf. *polystaphylodon* | ID | ID054 | AUM 44856 |
| *Plicofollis* aff. *polystaphylodon* | PG | PNG197/PNG196 | AUM 47560 |
| *Plicofollis tonggol* | ID/BD | ID095/ID100/PNG529 | AUM 44862/(NCA) |
| *Brustiarius proximus* | AU/PG/TH | RW0429/I.33460-019/PNG030/stri-x3644 | NV/AMS I.33460-019/AUM 47486/INHS 93671 |
| *Arius gagora* | BD | BAN07-244 | AUM (NCA) |
| *Arius leptonotacanthus* | ID | ID040/HH002 | AUM 44872/ZRC ?51225 |
| *Arius maculatus* | ID/TH | ID031/stri-x3643/T1013/T1016/T1017/T1022 | AUM 46293/INHS 93668/ANSP 178833/INHS 93668, 93704 |
| *Arius oetik* | ID/SG | ID099/HH001/HH003 | AUM 46295/ZRC 51225/ZRC 51224 |
| *Arius* sp.A | TH | stri-x3646/T1032/T1034 | INHS 93702/ANSP 178837 |
| *Arius* aff. sp.A | BD | BAN07-264/BAN07-261 | AUM (NCA) |
| *Cephalocassis melanochir* | ID | – | UMMZ 243391 |
| *Cryptarius truncatus* | ID/SG | ID087/T016-1514 | AUM 50243/AMNH 240581 |
| *Hemiarius stormii* | SG | HH007 | ZRC 51843 |
| *Cephalocassis borneensis* | TH | T1104/T1074/T1135 | INHS 93567, 93582, 93728 |
| 'Sciades' sagor | ID/SG/TH | ID023/ID045/stri-x3648/T1237/T.016.1540 | AUM 44868/AUM 50242/INHS 93709/AMNH 240582 |
| Osteogeneiosus militaris | BD | PNG522 | AUM (NCA) |
| *Arius nenga* | BD | PNG516/PNG527 | AUM (NCA) |
| *Arius* aff. *nenga* | ID/TH | ID081/stri-x3645/T1025/T1027-T1030 | AUM 46280/INHS 93701/ANSP 178836/INHS 93703, 93701 |
| 'Sciades' sona | ID/BD | ID098/PNG544 | AUM 44861/AUM (NCA) |
| *Amissidens hainesi* | AU/PG | P4150/PNG039 | ANSP 180335/AUM 47563 |

| | |
|---|---|
| *Plicofollis* aff. *argyropleuron* sp.1 | FJ625903, FJ625904, FJ626180, FJ626114, FJ626051 |
| *Plicofollis* aff. *argyropleuron* sp.2 | FJ625905–FJ625907, FJ626181, FJ626182, FJ626115, FJ626116, FJ626052, FJ626053, FJ626010 |
| *Plicofollis dussumieri* | FJ625908–FJ625910, FJ626183, FJ626117, FJ626054 |
| *Plicofollis layardi* | FJ625911, FJ626184, FJ626118, FJ626055 |
| *Plicofollis* aff. *layard* | FJ625912, FJ626185, FJ626119, FJ626056 |
| *Plicofollis nella* | FJ625913–FJ625916, FJ626186, FJ626120, FJ626057, FJ626011 |
| *Plicofollis* cf. *polystaphylodon* | FJ625917, FJ626187, FJ626121, FJ626058 |
| *Plicofollis* aff. *polystaphylodon* | FJ625918, FJ625919, FJ626188, FJ626122, FJ626059, FJ626012 |
| *Plicofollis tonggol* | FJ625920–FJ625922, FJ626189, FJ626123, FJ626060 |
| *Brustiarius proximus* | FJ625923–FJ625926, FJ626190, FJ626191, FJ626124, FJ626125, FJ626061, FJ626062, FJ626013 |
| *Arius gagora* | FJ625927, FJ626192, FJ626126, FJ626063, FJ626014 |
| *Arius leptonotacanthus* | FJ625928, FJ625929, FJ626193, FJ626127, FJ626064 |
| *Arius maculatus* | FJ625930–FJ625935, FJ626194, FJ626128, FJ626065, FJ626015 |
| *Arius oetik* | FJ625936–FJ625938, FJ626195, FJ626129, FJ626066 |
| *Arius* sp.A | FJ625939–FJ625941, FJ626196, FJ626130, FJ626067 |
| *Arius* aff. sp.A | FJ625942, FJ625943, FJ626197, FJ626131, FJ626068 |
| *Cephalocassis melanochir* | FJ625944, FJ626198, FJ626132, FJ626069, FJ626016 |
| *Cryptarius truncatus* | FJ625945, FJ625946 |
| *Hemiarius stormii* | FJ625947, FJ626199, FJ626133, FJ626070, FJ626017 |
| *Cephalocassis borneensis* | FJ625948–FJ625950, FJ626200, FJ626134, FJ626071, FJ626018 |
| 'Sciades' sagor | FJ625951–FJ625954, FJ626201–FJ626203, FJ626135, FJ626072, FJ626019 |
| Osteogeneiosus militaris | FJ625955 |
| *Arius nenga* | FJ625956, FJ625957, FJ626204, FJ626136, FJ626073 |
| *Arius* aff. *nenga* | FJ625958–FJ625964, FJ626205, FJ626137, FJ626074, FJ626020 |
| 'Sciades' sona | FJ625965, FJ625966, FJ626206, FJ626138, FJ626075, FJ626021 |
| *Amissidens hainesi* | FJ625967, FJ625968 |

**Appendix III-1.** (Continued).

| | | | |
|---|---|---|---|
| *Brustiarius nox* | PG | PNG011/PNG024 | AUM 47488 |
| *Brustiarius solidus* | PG | PNG022/PNG010 | AUM 47487/47635 |
| *'Sciades' utarus* | PG | PNG019/PNG018 | AUM 47491 |
| *Cinetodus carinatus* | PG | PNG083/PNG109 | AUM 47550/47508 |
| *Cinetodus froggatti* | PG | PNG107 | AUM 47494 |
| *Cochlefelis danielsi* | PG | PNG080 | AUM 47492 |
| *Cochlefelis spatula* | PG | PNG095 | AUM 47506 |
| *Doiichthys novaeguineae* | PG | PNG132 | AUM 47499 |
| *Nedystoma dayi* | PG | PNG125 | AUM 47500 |
| *Nemapteryx armiger* | AU/PG | P4152/PNG038 | ANSP 180337/AUM 50247 |
| *Nemapteryx* aff. a*rmiger* | PG | PNG115 | AUM 47552 |
| *Neoarius berneyi* | PG | GA3 | NV |
| *Neoarius* aff. *graeffei* sp.1 | PG | PNG002 | AUM 47481 |
| *Neoarius* aff. *graeffei* sp.2 | PG | PNG036 | AUM 47561 |
| *Pachyula crassilabris* | PG | PNG072 | AUM 47509 |
| *Potamosilurus coatesi* | PG | PNG017 | AUM 47490 |
| *Potamosilurus macrorhynchus* | PG | GA5 | NV |
| *Potamosilurus velutinus* | PG | PNG014/PNG007 | AUM 47489 |
| *Neoarius augustus* | PG | PNG133/PNG134 | AUM 47501/NV |
| *Cochlefelis dioctes* | AU/PG | HL0117/t640/PNG121 | NV/ANSP 182837/AUM 47507 |
| *Potamosilurus latirostris* | PG | PNG001/GA4 | AUM 47482/NV |
| *'Sciades' leptaspis* | AU/PG | HL0119/PNG049 | NV/AUM 47565 |
| *'Sciades' aff. leptaspis* | ID* | PNG151 | AUM  50248 |
| *'Sciades' mastersi* | AU/PG | HL0107/PNG140 | NV/AUM 47497 |
| *Neoarius midgleyi* | AU | RW0140 | NTM S.15362-003 |

*Irian Jaya (New Guinea)

**Appendix III-1.** (Continued).

| | |
|---|---|
| *Brustiarius nox* | FJ625969, FJ625970, FJ626207, FJ626139, FJ626076, FJ626022 |
| *Brustiarius solidus* | FJ625971, FJ625972, FJ626208, FJ626140, FJ626077 |
| *'Sciades' utarus* | FJ625973, FJ625974, FJ626209, FJ626141, FJ626078 |
| *Cinetodus carinatus* | FJ625975, FJ625976, FJ626210, FJ626142, FJ626079, FJ626023 |
| *Cinetodus froggatti* | FJ625977 |
| *Cochlefelis danielsi* | FJ625978, FJ626211, FJ626143, FJ626080 |
| *Cochlefelis spatula* | FJ625979 |
| *Doiichthys novaeguineae* | FJ625980 |
| *Nedystoma dayi* | FJ625981, FJ626212, FJ626144, FJ626081, FJ626024 |
| *Nemapteryx armiger* | FJ625982, FJ625983 |
| *Nemapteryx* aff. a*rmiger* | FJ625984, FJ626213, FJ626145, FJ626082 |
| *Neoarius berneyi* | FJ625985, FJ626214, FJ626146, FJ626083 |
| *Neoarius* aff. *graeffei* sp.1 | FJ625986, FJ626215, FJ626147, FJ626084 |
| *Neoarius* aff. *graeffei* sp.2 | FJ625987, FJ626216, FJ626148, FJ626085 |
| *Pachyula crassilabris* | FJ625988, FJ626217, FJ626149, FJ626086, FJ626025 |
| *Potamosilurus coatesi* | FJ625989, FJ626218, FJ626150, FJ626087, FJ626026 |
| *Potamosilurus macrorhynchus* | FJ625990, FJ626219, FJ626151, FJ626088, FJ626027 |
| *Potamosilurus velutinus* | FJ625991, FJ625992, FJ626220, FJ626152, FJ626089 |
| *Neoarius augustus* | FJ625993, FJ625994, FJ626221, FJ626153, FJ626090, FJ626028 |
| *Cochlefelis dioctes* | FJ625995, FJ625996, FJ625997, FJ626222, FJ626154, FJ626091, FJ626029 |
| *Potamosilurus latirostris* | FJ625998, FJ625999, FJ626223, FJ626155, FJ626092, FJ626030 |
| *'Sciades' leptaspis* | FJ626000, FJ626001, FJ626224, FJ626156, FJ626093, FJ626031 |
| *'Sciades'* aff. *leptaspis* | FJ626002, FJ626225, FJ626157, FJ626094 |
| *'Sciades' mastersi* | FJ626003, FJ626004, FJ626226, FJ626158, FJ626095, FJ626032 |
| *Neoarius midgleyi* | FJ626005, FJ626227, FJ626159, FJ626096, FJ626033 |

*Irian Jaya (New Guinea)

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# CHAPTER IV – HABITAT TRANSITIONS AND EVOLUTIONARY IMPLICATIONS

## ABSTRACT

Transitions between the marine and freshwater environments represent an extraordinary ecological shift that has promoted diversification in many groups of aquatic organisms. Here, molecular phylogenetics is utilized to investigate habitat transitions in a group of catfishes (Ariidae) that includes species inhabiting marine and brackish waters (>110 species) as well as freshwater environments (~40 species). The Ariidae is nested within the Otophysi, the largest clade of primary freshwater fishes with four orders and 67 families. Optimizations of habitat transitions (freshwater vs. marine) onto an inferred phylogeny suggest a single invasion of marine waters at the root of the ariid tree followed by 10 to 15 events of freshwater colonization, implying reversion to the primitive otophysan condition. Thus, ariids provide an extraordinary example of bidirectional habitat transitions in fishes. Freshwater recolonization has occurred in all major provinces where ariids are present, such as the New World (Mesoamerica and South America), Madagascar, Africa, Southeast Asia, and the Sahul continent (Australia and Papua New Guinea). Freshwater ariids are remarkably diverse in Australia-New Guinea and Mesoamerica, facilitated by the originally depauperate freshwater ichthyofauna in these

regions, as well as the low diversity (or absence in Australia and New Guinea) of otophysan families in the primary division. Sahul ariids form a well-supported endemic clade that includes nearly half of the freshwater species in the family. Sahul ariids form a well-supported endemic clade that includes nearly half of the freshwater species in the family. The lack of phylogenetic resolution among basal Sahul lineages coupled with their extraordinary level of morphological divergence and trophic diversity suggests an ancient rapid radiation promoted by freshwater colonization. For this reason, Sahul ariids represent an excellent system for studying diversification associated with habitat transitions.

# 1. Introduction

For aquatic organisms, the boundary between marine and freshwater habitats is a significant physicochemical barrier that imposes physiological and ecological constrains. As a result, 12 out of approximately 30 animal phyla have failed to colonize freshwaters (e.g., Ctenophora, Echinodermata, and Chaetognata; Little, 1990; Lee and Bell, 1999). A wide range of taxa has achieved physiological adaptations to overcome the osmotic barriers (Lee and Bell, 1999; McDowall, 1988). For instance, among invertebrates, transitions from marine to freshwater have occurred in several groups within annelids, mollusks, and crustaceans (Lee and Bell, 1999). Among fishes, distantly related taxa such as lampreys, elasmobranches, and teleosts have also colonized freshwaters multiple times (McDowall, 1988; Little, 1990). These include species that migrate between marine and freshwater habitats (i.e., diadromous life histories; McDowall, 1997) or simply tolerate a

wide range of salinities (i.e., eurhyhaline; Lee and Bell, 1999). A fraction of such groups

have become landlocked (e.g., Bell and Foster, 1994; Lovejoy et al., 2006; Lovejoy et al.,

1998; McDowall, 1997). On a macroevolutionary scale, environmental factors facilitating

freshwater colonization and subsequent landlocking of marine-derived fishes include

historical marine incursions (Lovejoy et al., 2006; Lovejoy et al., 1998), rivers with high

ionic concentrations that buffer osmotic gradients (Lee and Bell, 1999; Lovejoy and

Collette, 2001), habitats with depauperate fish communities (Lee and Bell, 1999), and

postglacial events (Bell and Foster, 1994; Schluter, 1996), among others.

While marine-to-freshwater transitions are relatively common in fishes and

invertebrates (Little, 1990; Lee and Bell, 1999; Lovejoy and Collette, 2001), colonization

of marine waters by freshwater organisms is far less frequent (McDowall, 1997). Such is

the case of a few otophysan fish groups. The Otophysi is the largest clade of fishes

confined to freshwater (primary freshwater sensu Myers, 1938) and includes four orders

(Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes) and 67 families

(Ferraris, 2007; Nelson, 2006), accounting for about 27% and 64% of the total fish

species and freshwater fish species, respectively (Briggs, 2005). Although phylogenetic

relationships among otophysan orders are controversial (e.g., Dimmick and Larson, 1996;

Fink and Fink, 1996; Peng et al., 2006), all hypotheses coincide in placing the order

Siluriformes (catfishes) in a rather terminal position. Thus, the Siluriformes retains the

ancestral otophysan condition of freshwater confinement; however, two families of

catfishes are predominantly marine (Ariidae and Plotosidae) and several others include

species approaching river mouths (e.g., Auchenipteridae, Loricariidae and Pimelodidae)

or even inhabiting coastal-marine waters (Aspredinidae; Ferraris, 2002). Although

mapping the condition of saltwater tolerance in the siluriform tree imply multiple origins (i.e., the aforementioned families form a polyphyletic assemblage; see Sullivan et al., 2006), catfishes appear to have a phylogenetic propensity for invading brackish and marine waters.

Ariids or sea catfishes are the only siluriform family widely distributed on the world's tropical and subtropical marine continental shelves. While specific life histories and migratory behaviors for most ariid species are poorly understood, there is a great diversity of habitat distribution within the family, including offshore marine taxa (e.g., *Bagre, Netuma, Plicofollis, Brustiarius proximus*, and most *Galeichthys* species), inshore coastal and brackish taxa (e.g., *Notarius, Cathorops dasycephalus,* '*Sciades*' *mastersi, S. sagor*), and euryhaline taxa in the biogeographic sense, i.e. with broad habitat distributions (Lee and Bell, 1999). Additionally, as many as 43 ariid species in 19 genera (among over 150 species in ~29 genera) from different regions around the world inhabit predominantly freshwater (Table IV-1).

Phylogenetic hypotheses provide a framework to investigate the evolution of habitat distributions. Although there is a wide spectrum of habitat states within the Ariidae, which behave more as continuous rather than categorical variables, assuming a binary condition (i.e., freshwater vs. marine, estuarine, or euryhaline) provides a simple method toward approaching questions of habitat transitions. In the light of the ancestral otophysan condition, the most parsimonious a priori explanation is that freshwater ariids are basal, implying a single transition from fresh to marine waters (i.e., marine taxa form a monophyletic group). Alternatively, freshwater species may be derived, which would imply secondary invasion of rivers (i.e., reversed to the primitive otophysan state). To

address this question, this study optimized habitat transitions onto a comprehensive phylogeny inferred for ariids (123 taxa) using mitochondrial sequences (~3 kb; see Chapter III). Additionally, noteworthy instances of freshwater confinement on different regions are discussed and examined as a possible case for an ancient rapid radiation associated with habitat transitions.

## 2. Materials and Methods

Generic nomenclature for New World and Old World ariids follows Betancur-R. et al. (2007) and Marceniuk and Menezes (2007), respectively. Datasets include mitochondrial (*cytochrome b* [*cyt b*], *ATP synthase* subunits 8 and 6 [*ATPase 8/6*], and ribosomal *12S* and *16S*; total 2866 bp and 123 ariid taxa) and nuclear (*rag2*; 978 bp and 70 ariid taxa) sequences. Methods of sequence acquisition and phylogenetic reconstructions are detailed in Chapter III with additional analyses described below.

Despite the wide variety of habitat distributions among ariid taxa, the primary focus of this study is tracing transitions between freshwaters and waters with some degree of salinity. Thus, habitat type was coded as a binary character (0, freshwater; 1, marine, estuarine or euryhaline) (compiled from unpublished data, H.H. Ng, pers. comm., M. Kottelat, pers. comm., G. Allen, pers. comm., Acero and Betancur-R., 2006; Allen, 1991; Allen and Coates, 1990; Allen et al., 2002; Betancur-R. and Willink, 2007; Ferraris, 2007; Jayaram, 1983; Kailola, 2000; Kottelat, 2001; Marceniuk and Betancur-R., 2008; Marceniuk and Menezes, 2007; Ng and Sparks, 2003; Roberts, 1989; Swales et al., 2000; Taylor, 1986). While the binary coding hinders inferences on evolutionary

transitions among estuarine, marine, and euryhaline taxa, it allows large-scale

interpretations of habitat transitions within the Ariidae in the context of the freshwater

otophysan condition. Two taxa with uncertain habitat condition (*Neoarius* aff. *graeffei* sp.

1 and '*Sciades*' aff. *leptaspis*) were coded as missing data ("?"). These discrete character

data were optimized onto the mitochondrial phylogeny (inferred under Bayesian

Inference [BI], maximum parsimony [MP], and maximum likelihood [ML]; see Chapter

III) under both likelihood (Pagel, 1999) and parsimony criteria in Mesquite v. 2.5

(Maddison and Maddison, 2008). For likelihood and parsimony reconstructions, the

evolution models "mk1" (Pagel, 1999) and Wagner optimization, respectively, were

selected, under which any particular change ($0 \rightarrow 1$ or $1 \rightarrow 0$) is equally likely.

The parsimony-based nonparametric Templeton test and the likelihood-based

Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) as implemented in

PAUP* v.4.0b10 (Swofford, 2002), were performed to evaluate alternative hypotheses on

the evolution of the habitat transitions in the Ariidae. To test whether freshwater ariids

are basal (i.e., single transition from fresh to marine waters) or derived (i.e., multiple

independent recolonization of freshwaters), MP and ML searches (see procedures in

Chapter III) constraining the monophyly of marine taxa were conducted and the optimal

scores with those obtained under unconstrained searches were compared.

Testing for ancient rapid radiations involved additional ML and MP

reconstructions on separate (nuclear *rag2*, mitochondrial ribosomal *12S + 16S*,

mitochondrial protein-coding *cyt b + ATPase 8/6*) and combined (mitochondrial,

mitochondrial + nuclear) partitions (see also Chapter III: table III-1). Additionally,

congruence among data partitions was assessed using 100 replicates of the partition

homogeneity test in PAUP* (= incongruence length difference test of Farris et al., 1994).

Genetic distances were calculated under the Kimura two-parameter model.

## 3. Results and Discussion

### 3.1 Tracing habitat transitions

Mapping of the habitat condition via parsimony and likelihood analyses onto the mitochondrial phylogeny suggests multiple marine-to-freshwater transitions in ariid catfishes (Fig. IV-1). Further MP and ML analyses constraining marine ariids to a monophyletic group yielded scores significantly worse than those obtained under unconstrained searches (SH: $p$ value= 0.000; Templeton test: $p$ value< 0.0001). The optimizations suggest a single invasion of marine waters at the root of the ariid tree, followed by 10 to 15 events of freshwater transition and confinement in the subfamily Ariinae. It is noteworthy that at least 16 freshwater ariid species in 10 genera from different regions were not examined (Table IV-1); thus, the number of habitat transitions is likely underestimated. Optimizations of marine-to-freshwater transitions in the New World (four transitions), Madagascar (one transition), and India-SE Asia (two transitions) were straightforward; however, the number of (three to eight transitions) and, in some cases, direction of habitat transitions in the Australia-New Guinea clade (node O, Fig. IV-1) were equivocal. Four major factors account for these ambiguous optimizations: (1) poor phylogenetic resolution (i.e., large basal polytomy; see below), (2) many instances

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of interleaved marine and freshwater taxa, (3) binary coding (combines states), and (4) missing taxa.

Lee and Bell (1999) suggested that the ability for freshwater invasion by saltwater animals requires an evolutionary innovation, and once the innovation arises the transition may occur repeatedly. Remarkable examples of invertebrate taxa that are frequent colonizers of freshwater include heterodont mollusks (e.g., zebra mussels) and amphipod, decapod, and copepod crustaceans (Lee and Bell, 1999). Among marine fishes, needlefishes, drums, anchovies, toadfishes, and others, also appear to have entered freshwaters multiple times (Roberts, 1989; Lovejoy and Collette, 2001; Lovejoy et al., 2006). The pattern of multiple recolonization of rivers in ariids may be the result of a phylogenetic bias facilitating transitions into freshwater and is probably a primitive signal in the Otophysi. Plotosids, the other catfish group that inhabit chiefly marine waters, also include many species that are freshwater inhabitants (Allen, 1991; Allen et al., 2002). As in the Ariidae, freshwater plotosids are probably derived (P. Unmack pers. comm.), but this issue remains to be tested in a phylogenetic framework. Among fishes, ariids (and possibly plotosids) provide an extraordinary example of bidirectional habitat transitions (i.e., original invasion of salt waters and subsequent recolonization of rivers).

## 3.2 Biogeography of the habitat transitions

Marine to freshwater transitions occurred in all major regions where ariids are present, including the New World, Madagascar, Africa, SE Asia, and Australia-New Guinea. Not surprising, freshwater ariid diversity is highest in regions that host a rather

depauperate ichthyofauna in the primary division, such as Australia-New Guinea and Mesoamerica (Table IV-1). This section discusses remarkable instances of ariid habitat transitions on major provinces. Freshwater species and their distribution are summarized in Table IV-1 and Fig. IV-2.

*3.2.1. New World*

The Neotropics host the most diverse freshwater fish fauna in the world, which is estimated to include up to 8000 species (Lundberg et al., 2000). The freshwater ichthyofauna in South and Central America is dominated by siluriforms, characiforms, gymnotiforms, cyprinodontiforms, and cichlids (Lundberg et al., 2000), but many families of marine origin have invaded Neotropical rivers as well (Lovejoy et al., 2006; Lovejoy et al., 1998; Lovejoy and Collette, 2001). Within the Ariidae, three species of *Potamarius* (Betancur-R. and Willink, 2007) and two species in the *Cathorops aguadulce* species group (*C. kailolae* and *C. aguadulce*; Marceniuk and Betancur-R., 2008) provide evidence of two independent transitions into the Río Usumacinta and Lago Izabal basins in Mesoamerica.  The high percentage of secondary freshwater or peripheral species (sensu Myers, 1938) and micro-endemism in the Río Usumacinta basin is a reflection of the region's originally depauperate freshwater fish fauna that was subsequently invaded by North American, South American, and marine species (Myers, 1966; Rosen, 1979). Remarkable examples of marine-derived fishes that co-occur with *Potamarius* and *Cathorops* in the Río Usumacinta basin include needlefishes (*Strongylura*), toadfishes (*Batrachoides*), and halfbeaks (*Hyporhamphus*) (Lovejoy and Collette, 2001). Lovejoy

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and Collette (2001) suggested that the coincidental origin and distribution of such taxa may either be indicative of shared biogeographic histories (i.e., an ancient vicariant event that isolated marine taxa in the region) or particular ecological conditions of the Usumacinta that may facilitate transitions from adjacent marine waters into the system (e.g., high ionic concentrations).

Additional instances of Neotropical freshwater ariids include the transisthmian pair *Notarius cookei*, from rivers in the Pacific Panama and potentially Colombia and Costa Rica (Acero and Betancur-R., 2002), and *N. bonillai*, from the Río Atrato and Río Magdalena basins draining into the Colombian Caribbean (Acero and Betancur-R., 2006). Such distributions are paralleled by other marine-derived fishes, such as *Daector quadrizonatus* (Batrachoididae) and *Strongylura fluviatilis* (Belonidae), suggesting an ancient connection between the Río Atrato and some Pacific-slope drainages (Lovejoy and Collette, 2001). While there are numerous marine-derived fishes that have extensively colonized the Amazon River, including stingrays, anchovies, needlefishes, drums, flatfishes, and pufferfishes (Lovejoy et al., 2006; Lovejoy et al., 1998), surprisingly no extant freshwater ariids occur in the Amazon or other rivers in northeastern South America. However, this may not always have been the case. La Venta paleontological site (Middle Miocene) in the Magdalena valley in Colombia includes many fossil freshwater fish species presently endemic to the Amazon or the Orinoco basins (but absent from the Magdalena basin) (Lundberg, 1997). Thus, fossil records of aff. *Sciades parkeri* from La Venta (Lundberg, 1997, 1998) suggest ancient ariid transitions into the proto-Amazon/Orinoco basins.

*3.2.2. Madagascar*


The freshwater ichthyofauna of Madagascar mainly includes marine families that frequently colonize rivers (i.e., peripheral species), such as gobiids, mugilids, and anguillids, as well as three representative groups of the secondary division (aplocheilids, cichlids, and poeciliids; Sparks and Stiassny, 2003). As reported in other faunal and floral groups, many Madagascar freshwater fish taxa are phylogenetically basal (but highly derived) as a result of the long period of isolation of the island from the continental mass (Sparks and Stiassny, 2003). Sparks and Stiassny (2003) pointed out that no primary fishes occur in Madagascar and suggested that the endemic Bedotiidae and Anchariidae are in the secondary division as well. Although from a historical biogeography perspective there are inherent limitations and contradictions of Myer's primary, secondary and peripheral divisions (see Rosen [1974] and Sparks and Smith [2005] for discussion), the concept is still of great value in descriptive zoogeographical and ecological contexts. Optimizations of the habitat transitions suggest that the Anchariidae retains the plesiomorphic otophysan freshwater condition, and hence it should be included in the primary division. However, the position of the Arioidea (Anchariidae + Ariidae) among other catfish families remains unresolved (Sullivan et al., 2006), which hinders further inferences on the biogeography of Anchariidae as related to continental drift.

Ariids in Madagascar are represented by four species, with one (*Plicofollis dussumieri*) being marine and widely distributed along the continental Indian Ocean; the remaining three (*Arius madagascariensis*, *A. uncintatus*, and *A. festinus*) inhabit rivers in

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eastern Madagascar (Ng and Sparks, 2003). Although the only Malagasy species examined in this study is *A. madagascariensis*, preliminary morphological evidence suggests that the three freshwater species may be related (Ng and Sparks, 2003) implying a single habitat transition.

## 3.2.3. Africa

The ariid diversity endemic to Africa is impoverished, including only three species in *Galeichthys* (southern Africa), two in *Arius* (eastern coast), and four in *Carlarius* (western coast). Of these, *Arius brunellii* (Juba River) and *Carlarius gigas* (Volta and Niger Rivers) are freshwater inhabitants (Marceniuk and Menezes, 2007), likely involving independent habitat transitions.

## 3.2.4. SE Asia

The freshwater biota of SE Asia hosts a high diversity of fish species (Lundberg et al., 2000). While most species in the region are cypriniforms and siluriforms in the primary division, there are several marine-derived groups as well, including clupeids, ariids, and tetraodontids (Kottelat, 2001; Roberts, 1989). Based on the affinities revealed by the freshwater fish assemblages in SE Asia, biogeographic studies show that the region comprises five to nineteen provinces (Kottelat, 1989; Yap, 2002; Zakaria-Ismail, 1994). According to Zakaria-Ismail (1994), five major subregions compose SE Asia: (1) the Salween basin in Burma, (2) the Indochina Peninsula (ICP; Mekong, Chao Phraya,

and Mae Khlong drainages), (3) the Malay Peninsula (MPN), (4) the Indo-Malayan

Archipelago (IMA; Borneo, Sumatra, and Java), and (5) the Philippines. Freshwater

ariids are widely distributed in SE Asia, occupying all these provinces (Table IV-1, Fig.

IV-2). Interestingly, the distribution of *Cephalocassis borneensis* extends to different

locations on the Sunda shelf (Kottelat, 2001; Kottelat and Widjanarti, 2005; Roberts,

1989), a broad subregion that encompasses the ICP, MPN, and IMA provinces. During

the Pleistocene glacial maxima, the sea level reached 120 m below present and the Sunda

Shelf was exposed, connecting major river systems of the islands and the mainland

(Dodson et al., 1995; McConnell, 2004; Pelejero et al., 1999). Population genetic studies

on the bagrid *Hemibagrus nemurus* (Dodson et al., 1995) and the cyprinid *Barbodes

gonionotus* (McConnell, 2004) from disjunct locations on the Sunda Shelf suggests their

present distribution reflects faunal exchanges during the Pleistocene. The distribution of

*H. nemurus*, *B. gonionotus*, and *C. borneensis* implies a similar and shared biogeographic

history. Alternatively, multiple marine transitions onto different landmasses on the Sunda

Shelf might explain the disjunct distribution of *C. borneensis*. Considering the freshwater

restriction of *C. borneensis*, a vicariant rather than a dispersalist scenario is favored;

however, phylogeographic studies are required for accurate biogeographic inferences to

be made. Similarly, the disjunct distribution of *C. melanochir* and *Hemiarius stormii* in

Sumatra and Borneo (IMA province) merits further investigation.

*3.2.5. Australia-New Guinea (= Sahul continent)*

Relative to the diverse inland ichthyofauna of tropical regions such as South

America, Africa and Asia, the freshwater fish biota in Australia and New Guinea is rather

impoverished (Allen, 1991; Allen et al., 2002; Berra, 1998; but see Lundberg et al.,

2000). The faunal composition in Australia and New Guinea also differs markedly from

other regions as they lack otophysan families from the primary division. Except for the

lungfish (Ceratodontidae) and bony tongues (Osteoglossidae), most freshwater fish

representatives in the region are of marine origin, including the otophysan ariids and

plotosids (Allen, 1991; Allen et al., 2002). Despite the present proximity of the Sunda

Shelf and the Sahul, the striking differences in faunal composition of the latter province

are a reflection of its long-term isolation after the separation from Antarctica 95–35 mya

(Li and Powell, 2001). Sahul hosts the most diverse ariid freshwater fauna, accounting for

nearly half of the purely freshwater ariids (Table IV-1), but also including many

euryhaline species that inhabit both inshore coastal waters and lower and middle reaches

of rivers (e.g., *Neoarius graeffei*, *Cochlefelis danielsi*, 'Sciades' *leptaspis*).

The Sahul Shelf proper lies beneath the Torres Strait and the Arafaura Sea

separating Northern Australia (NA) and SNG. As with the Sunda Shelf, the Sahul Shelf

was exposed during Pleistocene glaciations until 8000–6000 years ago and major river

drainages in SNG and NA were connected facilitating faunal exchange (Allen, 1991;

Filewood, 1984; Lundberg et al., 2000; Voris, 2000). Although many brackish and

marine ariid species are common to both the SNG and NA provinces, surprisingly only

one strictly freshwater ariid (*Neoarius berneyi*) occurs in both New Guinea and Australia

(Allen, 1991; Allen et al., 2002). The natural history of New Guinea has been both

complex and catastrophic. The island includes two major biogeographic provinces

separated by the central mountain range (Allen, 1991; van Ufford and Cloos, 2005;

Whitley, 1938). The Northern New Guinea (NNG) province, formed ~10 mya as a result

of collision between the Pacific and Australian plates, includes a rather impoverished

freshwater ichthyofauna (~55 purely freshwater species; Allen, 1991; Allen and Coates,

1990). The Southern Province (= SNG), on the other hand, hosts a richer biota of

freshwater fishes including over 160 species (Allen and Coates, 1990). The highland

drainages of both provinces near the mountain range are largely depleted of fish faunas

and no species occur above elevations of 1800 m, reflecting the catastrophic geological

history of the region (e.g., volcanism and glaciations; Allen, 1987).

In a zoogeographical analysis of the freshwater ichthyofaunas in the Sahul, Allen

and Coates (1990) found 34 species shared between the NA and SNG, thus reflecting

their recent land connection. On the other hand, only three species were common to both

northern and southern provinces in New Guinea. This pattern led Allen and Coates to

infer the area cladogram shown in Figure IV-3A (NNG, (SNG, NA)). As mentioned

previously, the presence of the freshwater *N. berneyi* in the NA and SNG match the

expected sister-area relationship between these two provinces. Additionally, the

topologies place the five ariids from the NNG province into two sister clades (node R:

node S + node T; Fig. IV-1), each of which also includes one species from the SNG

province, rendering a duplicate NNG/SNG pattern (Fig. IV-3B). All nodes within clade R

are congruent among different methods and well supported, suggesting robust

reconstructions. Furthermore, optimizations of the habitat transition indicate the

evolutionary history of node R took place entirely in freshwaters (Fig. IV-1), and thus marine dispersal is an unlikely scenario. The biogeographic pattern of node R differs from Allen and Coates' area cladogram in that the NA province is absent from both node S and node T. Nonetheless, the duplicate NNG/SNG pattern is compatible with the idea that New Guinean ichthyofauna from the north evolved from ancestral southern species due to cladogenesis induced by orogenesis of the central range (Allen, 1991; van Ufford and Cloos, 2005).

*3.3 The Australia-New Guinea clade: an ancient rapid radiation associated with freshwater colonization?*

Species penetrating into new habitats or geographical areas may diversify to occupy a wide range of niches via ecological speciation (Hendry et al., 2000; Schluter, 2001). Specialization for different niches may promote rapid radiations (Hendry et al., 2000) and enormous phenotypic diversification (Bell and Foster, 1994; Schluter, 1996). While some of classical examples of rapid radiations associated with habitat specialization involve terrestrial animals (e.g., Darwin's Galapagos finches Schluter, 1988), freshwater colonization by marine organisms provides a major case study including well-studied fish systems such as North American sticklebacks (Bell and Foster, 1994; Schluter, 1996) and New Zealand galaxiids (Waters and Wallis, 2001).

Australia-New Guinea is among the most diverse provinces for ariids in terms of both species richness and morphological divergence. New Guinea, the world's second largest island ($7.86 \times 10^5$ km$^2$), hosts the greatest diversity per area of ariids in the world,

including over 38 species in 11 genera (including potential new taxa), of which 21 species and five genera are endemic. New Guinean ariids exhibit extraordinary levels of morphological divergence, which is most pronounced among freshwater taxa (e.g., *Doiichthys novaeguineae*, *Cochlefelis spatula*, *Brustiarius nox*, *Pachyula crassilabris*; Fig. IV-4). There also is a wide range of trophic specialization, including filter feeding (e.g., *Brustiarius nox*), fruit and plant feeding (e.g., *Potamosilurus taylori*, *P. macrorhynchus*), carnivory (e.g., *Cochlefelis* spp.), omnivory (*'Sciades' leptaspis*), and insectivory (e.g., *Pachyula crassilabris*; Allen, 1991; Allen and Coates, 1990; Kailola, 1999).

Excluding the genera *Netuma* and *Plicofollis* (Indo-West Pacific clades 1 and 2, Fig. IV-1), which encompass wide Indo-West Pacific distributions, ariids from Australia and New Guinea form a well supported and mostly endemic clade (but see comments on Fig. IV-1 caption and Chapter III regarding the distribution of *Brustiarius proximus*; node O, Fig. IV-1). Genetic differentiation among lineages is high, with intergeneric divergence values calculated from mitochondrial and nuclear (*rag 2*) protein-coding genes varying between 6.1%–13.8% and 0.33%–3.0%, respectively. Despite the large genetic distances, reconstructions on the mitochondrial dataset (2866 bp) provide little phylogenetic resolution among basal lineages, as evidenced by a large polytomy containing six major branches showing interleaved habitat patterns (Fig. IV-1, node O). Further analyses conducted under MP and ML on different partitions (including nuclear *rag2*) using a reduced taxon sampling all resolved the relationships among basal Sahul lineages with short internodes and/or low support values (Fig. IV-5).

Clades lacking phylogenetic resolution in trees may result from inadequate sampling (soft polytomies) or may signal ancient radiations that cannot be resolved with increased sampling effort (hard or near-hard polytomies; Fishbein et al., 2001; Whitfield and Lockhart, 2007). Deciphering whether unresolved clades are the result of inadequate character sampling or a reflection of the true evolutionary history of the group requires analytical examination of the dataset and the reconstruction methods. Whitfield and Lockhart (2007) outlined four situations where poorly supported or short internodes reflect problems inherent to the data or the method rather than rapid radiations: (1) inappropriate marker choice (e.g., low variability), (2) conflicting signal among data partitions, (3) use of incorrect reconstruction method or substitution model, (4) insufficient data. Here, the first and third factors are likely not the case considering the high genetic distances and the similar patterns obtained under different reconstruction criteria. Furthermore, at least three circumstances make the second scenario unlikely: (a) partition homogeneity tests performed among separate partitions (i.e., mitochondrial protein-coding genes, mitochondrial ribosomal genes, nuclear *rag2*) reveal no conflicting phylogenetic signal (*p* value= 0.85); (b) clades strongly supported at either shallower (e.g., nodes Q, R, W) or deeper levels (e.g., Ariinae, node I) in the ariid tree, suggest that the large polytomy is not the result of phylogenetic signal loss due to substitutional saturation; (c) plots of third codon position transitions vs. Kimura two-parameter distances for mitochondrial protein-coding genes (fastest evolving markers; Betancur-R. et al., 2007) reveal no asymptotic accumulation of third codon transitions among Australia-New Guinea ariids (results not shown).

Unfortunately, ruling out the fourth factor is more difficult as it would involve obtaining additional DNA data (e.g., Rokas et al., 2003). However, the different markers utilized in this study contain differing levels of phylogenetic signal (Betancur-R. et al., 2007) and combined vs. partitioned analyses show no improvement in resolution among basal Sahul lineages (although more terminal nodes do reveal better resolution and/or support in combined analyses; Fig. IV-5). Thus, the basal polytomy observed here likely reflects a true hard or near hard polytomy resulting from an ancient rapid radiation. Divergence time estimations derived from molecular clock analyzes indicate that the timing of the putative radiation is 59–15 my (see Chapter III for discussion on variable molecular clock estimations).

As mentioned previously, nearly half of the freshwater ariid species occur in the rivers of Australia and New Guinea, which are otherwise impoverished of fish species and lack otophysan groups in the primary division (Allen, 1991; Allen et al., 2002; Berra, 1998). The high degree of morphological divergence and trophic specialization among freshwater ariid forms in this geographic region raises the possibility that, upon colonization of rivers, availability of new and variable habitats promoted an ancient radiation. While further research is required to test correspondence between the varying phenotypes and the divergent environments as well as functionality of traits (Schluter, 2000), Sahul ariids might provide a valuable opportunity for studying the underlying mechanisms driving diversification in relation to habitat transitions.

# ACKNOWLEDGEMENTS

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**Table IV-1.** Freshwater ariids and their distribution. Compiled from (unpublished data, H.H. Ng, pers. comm., M. Kottelat, pers. comm., G. Allen, pers. comm., Acero and Betancur-R., 2006; Allen, 1991; Allen and Coates, 1990; Allen et al., 2002; Betancur-R. and Willink, 2007; Ferraris, 2007; Jayaram, 1983; Kailola, 2000; Kottelat, 2001; Marceniuk and Betancur-R., 2008; Marceniuk and Menezes, 2007; Ng and Sparks, 2003; Roberts, 1989; Swales et al., 2000; Taylor, 1986). Two letter country codes follow ISO-3166. ICP, Indochina Peninsula; MPN, Malay Peninsula; IMA, Indo-Malayan Archipelago (continued on next page).

| Species | Distribution | Major region/province |
|---|---|---|
| **New World** | | |
| *'Arius' labiatus\** | Río Peripa, EC | Tropical Eastern Pacific slope |
| *Cathorops aguadulce* | Río Usumacinta, GT and MX | Mesoamerica (Atlantic slope) |
| *Cathorops kailolae\** | Lago Izabal, GT | Mesoamerica (Atlantic slope) |
| *Cathorops melanopus\** | Río Motagua, GT possibly HN | Mesoamerica (Atlantic slope) |
| *Cathorops festae* | Río Guayas basin, EC | Tropical Eastern Pacific slope |
| *Notarius bonillai* | Río Magdalena and Río Atrato basins, CO | Southern Caribbean slope |
| *Notarius cookei* | Pacific-slope rivers, PA and possibly CO | Tropical Eastern Pacific slope |
| *Potamarius grandoculis\** | Rio Doce and Rio Parabaiba do Sul, Southern BR | Southern Brazil |
| *Potamarius izabalensis* | Lago Izabal, GT | Mesoamerica (Atlantic slope) |
| *Potamarius nelsoni* | Río Usumacinta, GT and MX | Mesoamerica (Atlantic slope) |
| *Potamarius usumacintae* | Río Usumacinta, GT and MX | Mesoamerica (Atlantic slope) |
| **Africa** | | |
| *Arius brunellii\** | Juba River, SO | Eastern Africa |
| *Carlarius gigas\** | Volta and Niger Rivers, GH and NG | Gulf of Guinea, Western Africa |
| **Madagascar** | | |
| *Arius festinus\** | Amboaboa River | Northwestern Madagascar |
| *Arius madagascariensis* | Western Madagascar | Western Madagascar |
| *Arius uncinatus\** | Ankofia River basin | Northwestern Madagascar |
| **SE Asia** | | |
| *Arius acutirostris\** | Salween River, MM | Salween basin, Burma |
| *Arius borneensis\** | Bandjarmasin, Borneo | Borneo |
| *Arius burmanicus\** | Irrawaddy River, MM | Salween basin, Burma |
| *Cephalocasis borneensis* | IMA, MPN, and ICP (ID, MY, TH, KH, and VN) | Sunda Shelf |
| *Cephalocasis melanochir* | Sumatra and Borneo | IMA |
| *Hemiarius stormii* | Sumatra and Borneo | IMA |

**Table IV-1.** (Continued).

| Species | Distribution | Major region/province |
|---|---|---|
| *Hemiarius verrucosus** | Mekong River | ICP |
| *Plicofollis magatensis** | Magat River, PH | Philippines |
| **Australia-N. Guinea** | | |
| *Brustiarius nox* | Sepik River basin, PG | Northern New Guinea |
| *Brustiarius solidus* | Sepik River basin, PG | Northern New Guinea |
| *Cinetodus carinatus* | Southern PG and Papua, ID | Southern New Guinea |
| *Cochlefelis spatula* | Southern PG and Papua, ID | Southern New Guinea |
| *Doiichthys novaeguineae* | Southern PG and Papua, ID | Southern New Guinea |
| *Nedystoma dayi* | Southern PG and Papua, ID | Southern New Guinea |
| *Neoarius augustus* | Fly River system in PG and Papua, ID | Southern New Guinea |
| *Neoarius berneyi* | Fly River system in PG and Papua, ID; northern Australia | Sahul Shelf |
| *Neoarius midgleyi* | Victoria, Katherine, Daly and other rivers sytems in Northern AU | Northern Australia |
| *Nemapteryx* aff. *armiger* | Fly River system in PG | Southern New Guinea |
| *Pachyula conorhynchus** | Lorentz River system, Southern Papua, ID | Southern New Guinea |
| *Pachyula crassilabris* | Southern PG and Papua, ID | Southern New Guinea |
| *Potamosilurus coatesi* | Sepik River basin, PG | Northern New Guinea |
| *Potamosilurus latirostris* | Southern PG and Papua, ID | Southern New Guinea |
| *Potamosilurus macrorhynchus* | Southern PG and Papua, ID | Southern New Guinea |
| *Potamosilurus taylori** | Fly River system in PG and Papua, ID | Southern New Guinea |
| *Potamosilurus velutinus* | Sepik River basin, PG | Northern New Guinea |
| *'Sciades' paucus** | Roper and Flinder river systems in Northern AU | Northern Australia |
| *'Sciades' utarus* | Sepik River basin, PG | Northern New Guinea |

*Not examined

# FIGURES

**Figure IV-1.** BI phylogeny of 124 arioid species derived from the mitochondrial dataset (2866 bp; Chapter III). (A) cladogram; thicker branches indicate clades that are congruent with MP and ML analyses (se details in Chapter III). Asterisks (*) designate clade support; capital letters indicate nodes referred in text and Chapter III (symbols and letters always on left of nodes); vertical bars indicate subfamilial divisions and distribution of major ariine groups. Pie charts and color branches represent ancestral habitat reconstructions based on likelihood (mk1 model) and parsimony (Wagner optimization), respectively; gray question mark "?" indicates uncertain states or ambiguous optimizations. Two letter country codes follow ISO-3166. (B) phylogram (Ariidae only) elucidating rate variation across lineages (taxon arrangement follows the same order in both figures); gray arrow indicates basal polytomy in the Australia-New Guinea clade (= node O).  Although *Brustiarius proximus* occurs in Australia-New Guinea as well as in SE Asia, for the purpose of this study it is treated as a Sahul taxon (see additional comments in Chapter III).

**Figure IV-2.** Approximate distribution of freshwater ariids. Blue circles indicate regions wherein purely freshwater ariids occur (some symbols represent more than on locality; see also Table IV-1). Euryhaline species found in other freshwater locations not plotted (e.g., *Neoarius graeffei*; see Discussion).

**Figure IV-3.** Area relationships among Australia and New Guinea provinces based on freshwater fish affinities. (A) Allen and Coates' (1990) area cladogram reconstructed from the number of freshwater fish species shared among the two provinces (32 freshwater common to Southern New Guinea and Northern Australia; three species common to Northern New Guinea and Southern New Guinea). (B) Relationships among Northern and Southern New Guinea freshwater ariids based on mitochondrial data. Nodal letters are as in Figure 2. All nodes within clade R are well supported (see details in Fig. IV-1) and the habitat optimizations suggest that their evolutionary history occurred in freshwaters only. Although Northern Australia is missing from node R, another freshwater ariid, *Neoarius berneyi*, is common to both Northern Australia and Southern New Guinea provinces, supporting their sister-area relationship.


**Figure IV-4.** Remarkable examples of morphological divergence among New Guinean freshwater ariids. (A) *Doiichthys novaeguineae*; (B) *Cochlefelis spatula*; (C) *Brustiarius nox*; (D) *Pachyula crassilabris*.


**Figure IV-5.** MP and ML analyses using a reduced taxon sampling on different partitions, showing poor phylogenetic resolution among basal Australia-New Guinea ariid lineages (black branches; = node O, Fig. IV-1). Nodal dots indicate ML and MP bootstrap values ≥ 75% (MP clades with < 50% bootstrap support were collapsed). Gray branches are outgroups (*Galeichthys ater* [most basal] and *Cathorops dasycephalus*).
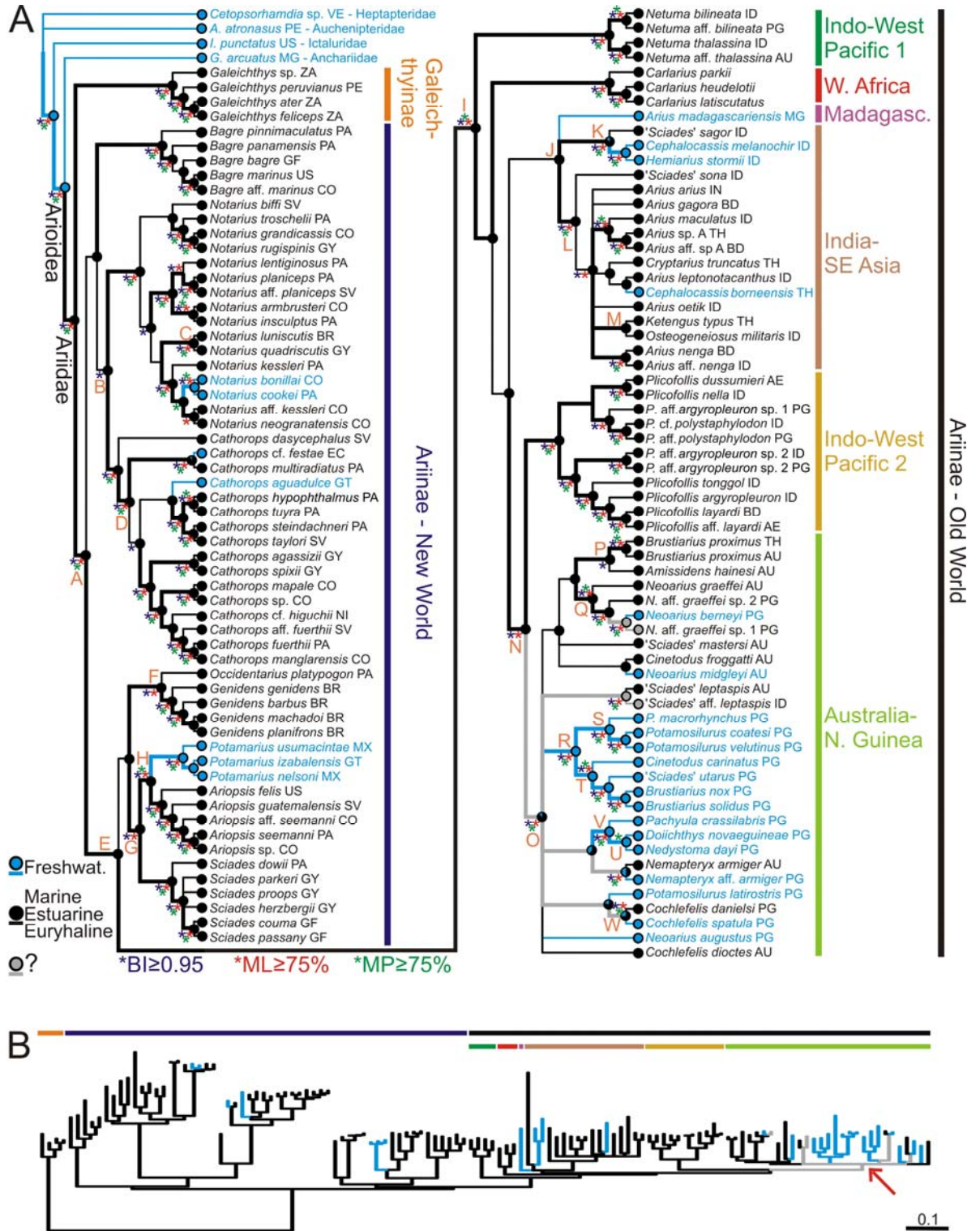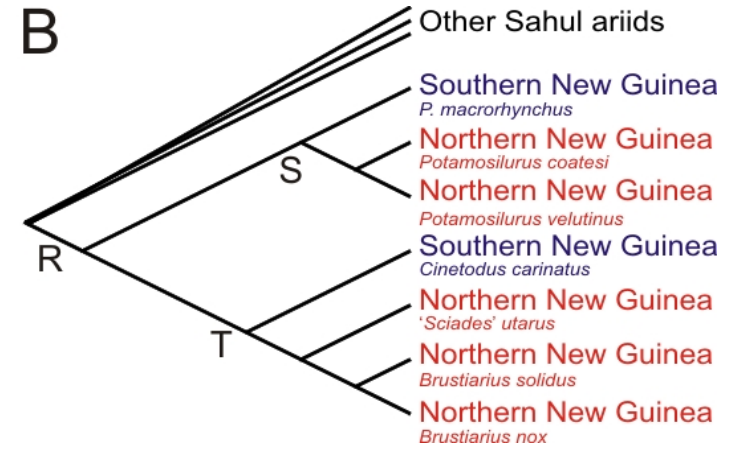
**Figure IV-1**
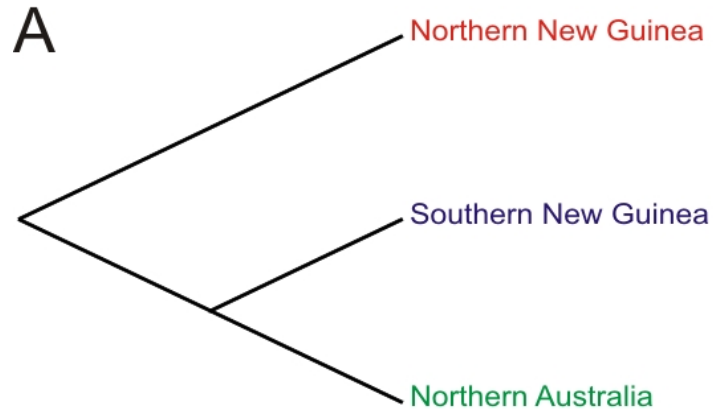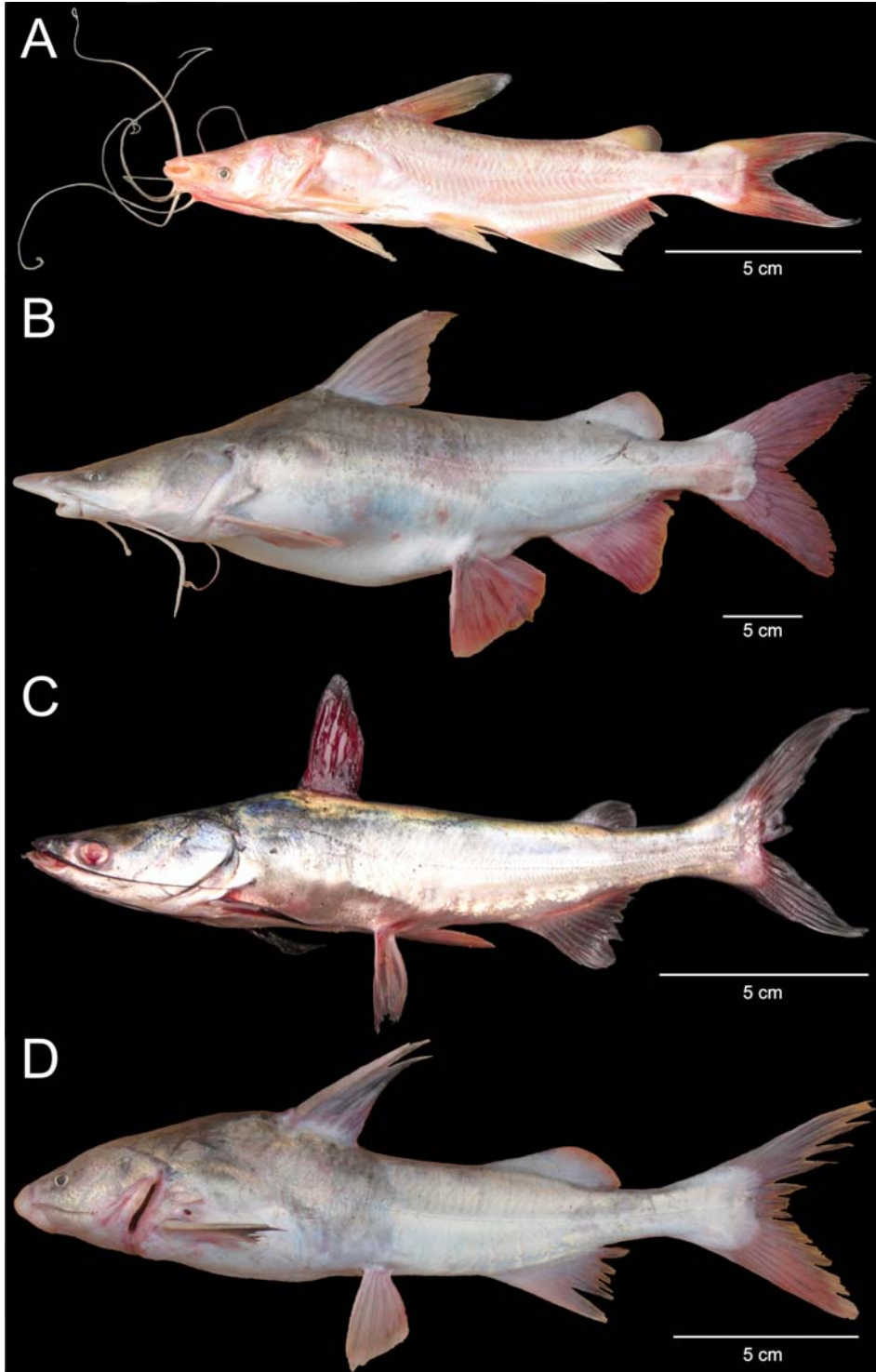
**Figure IV-2**

**Figure IV-1**

**Figure IV-2**

**Figure IV-3**
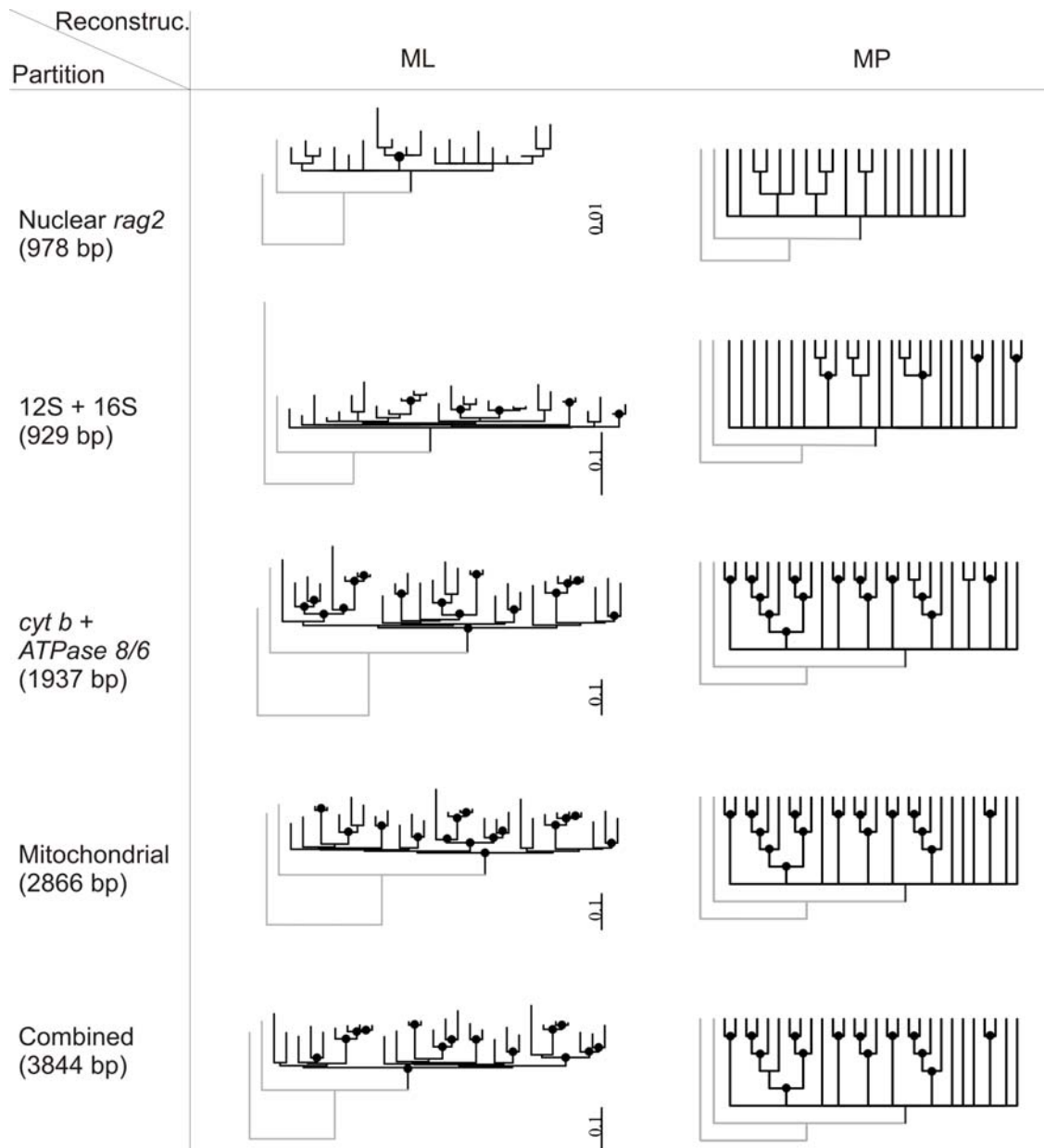
# CHAPTER V – CONCLUSIONS AND FUTURE DIRECTIONS

The Otophysi is the largest clade of primary freshwater fishes, including four orders (Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes) and about 67 families. Among the 36 catfish families in the order Siluriformes, the Ariidae or sea catfishes are the most widespread members, distributed along world's tropical and subtropical continental shelves. Unlike most catfishes and other otophysan groups, ariids occur primarily in marine and brackish waters; however, several species inhabit freshwaters as well. Sea catfishes include over 150 valid species, with many others in need of formal description. Members of the Ariidae practice male oral incubation, which has ultimately resulted in limited dispersal capabilities and high rates of species endemism. Thus, ariids provide an exceptional opportunity among marine fish groups to infer historical biogeography scenarios. The group is also of primary importance for tropical fisheries and numerous species have high economic value. Despite their importance on both biogeographic and economic aspects, few attempts have been made to elucidate phylogenetic relationships and to unravel their evolutionary history. Furthermore, ariid classification has traditionally remained in disarray and even recent studies provide substantially different taxonomies.

The chapters compiled in this dissertation address the systematics and evolution of sea catfishes from different angles. The first chapter revises major ariid clades and summarizes the morphological and molecular evidence supporting their monophyly and relationships to other siluriform families. Additionally, a subfamilial classification is proposed. The monophyly of the Ariidae has not been disputed and is strongly supported on both morphological and molecular grounds. The group is defined by four anatomical/life history and two amino acid (based on nuclear *rag2* and mitochondrial *cytochrome b*) synapomorphies. While morphology-based phylogenetic hypotheses from last decade place ariids in radically different positions in the catfish tree, recent studies based on molecular and morphological evidence concur that the sister group of ariids is the freshwater family Anchariidae from Madagascar. Ariidae and Anchariidae are placed in the superfamily Arioidea within the suborder Siluroidei; however, there is no consensus regarding the phylogenetic position of arioids among other siluroid families. The Ariidae is divided into two well-defined subfamilies. The Galeichthyinae, new subfamily, includes one genus and four species from southern Africa and southwestern South America, and is diagnosed by one or two anatomical synapomorphies and six molecular autapomorphies of *Galeichthys peruvianus*. The subfamily Ariinae, including ~97% of sea catfish diversity, is defined by four anatomical and four amino acid synapomorphies.

The second chapter addresses the phylogeny and historical biogeography of the subfamily Galeichthyinae in a chronological framework. A previous study suggested that the disjunct biogeography of galeichthyines in South America (Peruvian Pacific) and Africa was the result of Gondwanan vicariance. The vicariant model predicts an Early

Cretaceous origin for galeichthyines based on the final breakup of the supercontinent (~105 my). However, neither the monophyly nor the timing of galeichthyine cladogenesis was tested before by means of molecular data. The mitochondrial and nuclear sequence data obtained here (~ 4kb) provided fully dichotomous and well-supported phylogenies for the four species of *Galeichthys*, indicating that the Peruvian species is nested within the African taxa. Molecular clock estimations suggest that the timing of intercontinental divergence occurred from Mid to Late Miocene, which by far postdates the final separation of Africa and South America. The inferred chronologies reject the Gondwanan hypothesis, and thus the current distribution of galeichthyines must be explained by transoceanic dispersal from Africa to Peru (five likely scenarios) or recent vicariance.

The third chapter considers the evolutionary history of the subfamily Ariinae. Mitochondrial and nuclear (~4 kb) phylogenies were inferred for arioids based on the most comprehensive taxon sampling to date (124 species/entities). As with previous chapters, while the reconstructions support the monophyly of basal groups (Arioidea, Ariidae, Galeichthyinae, and Ariinae), up to ten ariine genera (out of 25–30) validated by previous morphological studies are incongruent with the molecular phylogenies. These results stress the need for re-assessment of ariid classification. The topologies recovered New World ariines as paraphyletic and Old World species were grouped into a well-supported clade. In further disagreement with morphological hypotheses that evidenced no logical biogeographic circumscriptions, the molecular phylogenies group inshore ariine species into well-defined clades restricted to particular Gondwanan provinces (New World [three to five basal clades], Africa, Madagascar, India-SE Asia, and Australia-New Guinea). The general area cladogram derived from the area cladograms of

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cichlids, aplocheiloid killifishes, rainbowfishes, and ariines is largely congruent with the temporal sequence of events during the fragmentation of Gondwana. Nonetheless, the results obtained on the timing of ariine diversification using PL and BRC methods are too variable to accept or reject Gondwanan vicariance on a chronological context. Further examination of additional ariid fossils might provide better calibration points for more accurate molecular clock estimations.

The last chapter investigates the evolution of habitat distributions (marine vs. freshwater) within the Ariidae.  Because ariids are nested within the Otophysi (the largest clade of freshwater fishes) a parsimonious explanation for the existence of freshwater species is that they are basal, implying a single transition from fresh to marine waters. Alternatively, freshwater species may be derived which would imply secondary colonization of rivers (i.e., reversion to the primitive otophysan condition). Optimizations of the habitat transitions onto the inferred phylogeny support the latter hypothesis, indicating a single invasion of marine waters in the stem ariid node followed by multiple events of freshwater colonization. Among fishes, ariids provide an extraordinary example of bidirectional habitat transitions. Freshwater recolonization has taken place in all major provinces where ariids occur (New World, Madagascar, Africa, Southeast Asia, and Australia-Papua New Guinea). Remarkably, ariids from the Australia-New Guinea (=Sahul) province form a well-supported endemic clade that includes nearly half of the freshwater ariid species. The lack of phylogenetic resolution among basal Sahul lineages coupled with their extraordinary level of morphological divergence and trophic diversity suggests an ancient rapid radiation promoted by freshwater colonization. Sahul ariids

represent an excellent model for studying diversification associated with habitat transitions.

The taxonomy of the Ariidae is a major issue that remains to be addressed. Despite their economic importance, ariid classification is probably the most controversial among catfish families. This has resulted in vast nomenclatural instability with species jumping from one genus to another. Even recent phylogenetic studies derived from anatomical and molecular data have provided markedly divergent classifications for the Ariidae. This instability precludes an adequate management of the resource, making ariids a difficult group for conservational purposes. A suitable approach for arriving at a stable taxonomy of the family is to combine all phylogenetic matrixes available into a supermatrix and derive a total evidence phylogeny. The consensus hypothesis would be useful for redefining genus limits as well as for addressing additional evolutionary questions.

The problems on ariid systematics at the genus level also extend to the alpha taxonomy level. While recent checklists estimate that the number of valid species ranges from 125 to 140, the Ariidae includes the greatest number of nominal species with uncertain status (i.e., species inquirendae) within the order Siluriformes (~50). Furthermore, examination of wide variety of taxa based on mitochondrial and morphological data in this study enabled identification of 20 putative undescribed/unrecognized species. In summary, despite the recent efforts to describe and redescribe ariid species (e.g., Appendixes), more collections and revisions are still required to complete the alpha taxonomy of the family.

# APPENDIXES – OTHER PUBLICATIONS DERIVED FROM THE DOCTORATE RESEARCH

**APPENDIX A-1.** Description of *Notarius biffi* n. sp. and redescription of *N. insculptus* (Jordan and Gilbert) (Siluriformes: Ariidae) from the eastern Pacific, with evidence of monophyly and limits of *Notarius*.

**Abstract**

A new species of ariid catfish, *Notarius biffi* n. sp., from the eastern Pacific, is described. The species is distinguished from other eastern Pacific species of *Notarius* by the following features: mouth small (width 34.2–39.3% head length); eye large (diameter 3.8–4.5% standard length); mandibulary barbels short (length 10.2–13.1% standard length); distance between anterior nostrils short (17.9–20.8% head length); caudal peduncle relatively slender (depth 6.1–6.7% SL); gill rakers on the first arch 11–12. *N. insculptus* (Jordan and Gilbert, 1883) new combination is redescribed herein. This species is distinguished from other eastern Pacific *Notarius* as follows: highly developed, sculptured epioccipital bones, forming with the supraoccipital a basally wide complex process which tapers drastically posteriorly; supraoccipital process length 0.7–0.9 in the base of the complex process; predorsal plate narrow and crescent-shaped; mouth large

(width 45.8–54.8% head length); maxillary barbels long (length 26.7–30.3% standard length). Based on mitochondrial phylogenetic evidence (combined cytochrome *b* and ATP synthase 8/6 data set with 1937 base pairs) and general morphology, the amphiamerican genus *Notarius* is expanded to include a total of 14 species, eight of which are presented as new combinations (*N. cookei*, *N. insculptus*, *N. kessleri*, *N. luniscutis*, *N. neogranatensis*, *N. phrygiatus*, *N. quadriscutis*, and *N. rugispinis*). The nomenclatural status of the eastern Pacific ariids *Hexanematichthys henni*, *Arius hassleriana*, *A. festae*, *A. labiatus*, *A. planiceps*, and *A. osculus* is discussed. Finally, a key to identify seven described species of *Notarius* from the eastern Pacific is presented.

**Citation**

Betancur-R., R., Acero., A., 2004. Description of *Notarius biffi* n. sp. and redescription of *N. insculptus* (Jordan and Gilbert) (Siluriformes: Ariidae) from the eastern Pacific, with evidence of monophyly and limits of *Notarius*. Zootaxa 703, 1-20.

**APPENDIX A-2.** Description of *Cathorops mapale*, a new species of sea catfish (Siluriformes : Ariidae) from the Colombian Caribbean, based on morphological and mitochondrial evidence.

**Abstract**

A new species of sea catfish (Ariidae), *Cathorops mapale*, is described from the central and southwestern Colombian Caribbean coast. The species is distinguished from other *Cathorops* species from the western Atlantic by the following combination of features: 20–24 anterior gill rakers on first gill arch, 18–21 anterior gill rakers on second gill arch; maxillary barbels 27.8–39.3% standard length; medial head groove long and deep, extending posteriorly almost to supraoccipital keel. This species has been widely misidentified as *C. spixii* (Agassiz), a species known from Brazil to the Guianas. Based on mitochondrial evidence (cytochrome *b* and ATP synthase 8/6), *C. mapale* is more closely related to the eastern Pacific *C. fuerthii* (Steindachner) species group (2.2–2.8% sequence divergence) than to the *C. spixii* clade (5.9–6.2% sequence divergence). *C. mapale* is distinguished from the *C. fuerthii* group by having higher anterior gill raker counts on the first (14–15 in the *C. fuerthii* group) and second (15–17 in the *C. fuerthii* group) gill arches, and by having a smaller mouth (8.3–10.6% standard length in *C. mapale* and 11.0–11.6% standard length in the C. *fuerthii* group)

**Citation**

Betancur-R., R., Acero, A., 2005. Description of *Cathorops mapale*, a new species of sea catfish (Siluriformes : Ariidae) from the Colombian Caribbean, based on morphological and mitochondrial evidence. Zootaxa, 45-60.

**APPENDIX A-3.** A new species of *Notarius* (Siluriformes: Ariidae) from the Colombian Pacific.

**Abstract**

*Notarius armbrusteri* n. sp. is described from specimens purchased in the fish market of Buenaventura, Valle del Cauca, Colombia. The species is distinguished from other eastern Pacific species of *Notarius* by the following combination of features: mouth small, width 11.1–11.8% HL; eye large, diameter 4.3–4.9% SL; distance between anterior nostrils 6.1–6.9% SL, distance between posterior nostrils 5.9–6.9% SL; short maxillary barbels, length 20.5–22.2% SL; and gill rakers on first arch 3–4+8–9 (total 11–13). Based on mitochondrial evidence (cytochrome *b* and ATP synthase 8/6, total 1937 base pairs), the new species is closely related to *N. insculptus*, from the Pacific Panama. An updated key to identify the eight described species of *Notarius* from the eastern Pacific is provided.

**APPENDIX A-4.** Real identity of the northern Colombian endemic sea catfish

*Galeichthys bonillai* Miles, 1945 (Siluriformes: Ariidae).

**Abstract**

*Notarius bonillai* (Miles, 1945) new combination is redescribed as a large sized freshwater ariid species endemic to northern Colombia. *N. bonillai* is the sister species of the eastern tropical Pacific *N. cookei*, differing from it in body depth (20.0-20.5% SL in N. bonillai vs 17.3-17.9% SL in N. cookei), in having a less exposed and rugose head shield and 1.7% of mitochondrial sequence divergence (combined cytochrome b and ATP synthase 8/6 data set, with 1937 bp). It can be distinguished from other tropical western Atlantic ariid species by the presence of three pairs of barbels, one maxillary and two mandibular; parieto-supraoccipital process broader at base than distally, 1.6 to 2.0 times longer than the width of its base; predorsal plate narrow and crescent-shaped; teeth on palate villiform, forming a U-shaped pattern of four closely adjacent patches, the lateral pair largest and subtriangular; and by the lack of a fleshy furrow between the nostrils, a longitudinal fleshy groove in the median depression of the head, and gill rakers on rear surfaces of the first two gill arches. The species seems to be restricted to freshwaters, living in the large Colombian rivers that run to the southern Caribbean. The name Ariopsis bonillai has been wrongly used for the New Granada sea catfish, a medium sized species common in brackish water estuaries and muddy shallow marine bottoms along the Colombian Caribbean.

**Citation**

Acero, A., Betancur-R., R., 2006. Real identity of the northern Colombian endemic sea catfish *Galeichthys bonillai* Miles, 1945 (Siluriformes: Ariidae). Cybium 30, 215–219.

**APPENDIX A-5.** Systematics and biogeography of New World sea catfishes (Siluriformes: Ariidae) as inferred from mitochondrial, nuclear, and morphological evidence.

**Abstract**

Ariid or sea catfishes include around 150 species that inhabit marine, brackish, and freshwater environments along world's tropical and subtropical continental shelves. Phylogenetic relationships for 46 New World and three Old World species of ariids were hypothesized using maximum parsimony and Bayesian inference reconstruction criteria on 2842 mitochondrial (cytochrome *b*, ATP synthase 8 and 6, ribosomal 12S and 16S) and 978 nuclear (*rag2*) nucleotide sites. The molecular topologies were compared to a previously compiled morphological dataset that was expanded herein to a total of 25 ariid species and 55 characters. Mitochondrial data yielded clades highly resolved at subfamilial, generic, and intrageneric levels. Nuclear *rag2* reconstructions showed poor resolution at supra- and intrageneric levels, but provided support for the monophyly of most genera (except *Ariopsis* and *Cathorops*) as well as for the subfamilial clades. The hypothesized phylogeny derived from the morphological data was congruent with the molecular topologies at infrafamilial and generic levels. As indicated by the statistical tests of topological congruence, Kailola's phylogenetic hypothesis of ariids based on anatomical data is significantly different from our molecular trees. All reconstructions agree in the division of the Ariidae into two subfamilies, the Ariinae and the monogeneric Galeichthyinae. Basal ariine resolution was negligible suggesting that early diversification events occurred rapidly. The three Indo-Pacific taxa were grouped into a

clade, but New World ariines were never recovered as monophyletic. We provide a revised classification for New World ariines examined, which is consistent with the molecular and the morphological evidence. Our classification scheme includes the genera *Ariopsis*, *Bagre*, *Cathorops*, *Notarius*, *Potamarius*, and *Sciades*, and the description of two new genus-level taxa (*Occidentarius* n. gen and *Precathorops* n. subgen.). We also hypothesize plausible biogeographic scenarios that explain distributional patterns of major ariid lineages. Diversification of the predominantly circumtropical ariines likely occurred throughout the Tethys Sea, whereas speciation events in the subtropical galeichthyines were probably tied to the southern coast of Gondwana.

**Citation**

Betancur-R., R., Acero P., A., Bermingham, E., Cooke, R., 2007. Systematics and biogeography of New World sea catfishes (Siluriformes: Ariidae) as inferred from mitochondrial, nuclear, and morphological evidence. Mol. Phylogenet. Evol. 45, 339-357.

**APPENDIX A-6.** A new freshwater ariid (Otophysi: Siluriformes) from the Río Usumacinta basin. Copeia 2007, 818–828.

## Abstract

*Potamarius usumacintae*, new species, is described based on specimens collected in the Río Usumacinta basin in Guatemala and México. The species is diagnosed from other freshwater ariids distributed in Mesoamerica by the following combination of features: lateral gill rakers on first gill arch 17–20 (mode 19); mesial gill rakers on first two gill arches well developed; accessory tooth patches on palate present; premaxillary tooth band length 2.4–2.9 in its width; snout length 1.4–1.8 in postorbital length; maxillary barbel not extending behind gill membrane; dorsomedian fleshy groove on head absent. A key to identify nine Atlantic slope ariid species from North and Central America is provided.

## Citation

Betancur-R., R., Willink, P.W., 2007. A new freshwater ariid (Otophysi: Siluriformes) from the Río Usumacinta basin. Copeia 2007, 818–828.

**APPENDIX A-7.** Revision of the species of the genus *Cathorops* (Siluriformes; Ariidae) from Mesoamerica and the Central American Caribbean, with description of three new species.

**Abstract**

The ariid genus *Cathorops* includes species that occur mainly in estuarine and freshwater habitats of the eastern and western coasts of southern Mexico, Central and South America. The species of *Cathorops* from the Mesoamerica (Atlantic slope) and Caribbean Central America are revised, and three new species are described: *C. belizensis* from mangrove areas in Belize; *C. higuchii* from shallow coastal areas and coastal rivers in the Central American Caribbean, from Honduras to Panama; and *C. kailolae* from río Usumacinta and lago Izabal basins in Mexico and Guatemala. Additionally, *C. aguadulce*, from the río Papaloapan basin in Mexico, and *C. melanopus* from the río Motagua basin in Guatemala and Honduras, are redescribed and their geographic distributions are revised.

**Citation**

**APPENDIX A-8.** Taxonomic status and redescription of the Gillbacker Sea Catfish (Siluriformes: Ariidae: *Sciades parkeri*).

**Abstract**

The Gillbacker Sea Catfish is a valid species of ariid catfish from the northeastern coast of South America. There are many synonyms in the literature for the Gillbacker Sea Catfish and even recent classifications have used different scientific names. Examination of a wide range of sizes of individuals from different localities and examination of types and original species descriptions of *Silurus parkeri*, *Bagrus flavescens*, *B. emphysetus*, *Arius physacanthus*, *A. bonneti*, *A. clavispinosus*, and *A. despaxi*, has lead us to the conclusion that all these names refer to the Gillbacker Sea Catfish and the valid name for the species is *Sciades parkeri*. The species is distinguished from all other ariid species by the following combination of features: body coloration yellow; swim bladder divided into three chambers, posterior chamber moderately sized; nuchal plate shield-shaped, usually larger than supraocciptal process; anterior notch of nuchal plate absent; head shield exposed and granulated in orbital and postorbital regions; lateral edge of accessory patches not emarginated or shallowly notched; fleshy furrow connecting posterior nares absent; and mesial gill rakers absent from first two gill arches. Striking intraspecific and/or ontogenetic variation in eye size, maxillary-barbel length, supraoccipital-process size, nuchal-plate size and shape, and dorsal-spine thickness, contributed to the numerous synonyms and misidentifications for *Sciades parkeri*. *Bagrus albicans*, described from French Guiana, has at times been listed as a synonym of *Sciades parkeri*. Our

examination of the holotype of *B. albicans,* however*,* led us to conclude that it is a synonym of *Sciades proops.*

**Citation**

Betancur-R., R., Marceniuk, A.P., Béarez, P., 2008. Taxonomic status and redescription of the Gillbacker Sea Catfish (Siluriformes: Ariidae: *Sciades parkeri*). Copeia 2008, 827–835.