Molecular Phylogeny of the Neotropical Knifefishes of the Order Gymnotiformes (Actinopterygii)

by

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Abstract

The order Gymnotiformes, the Neotropical electric knifefishes, is comprised of 200+ species divided into the families Apteronotidae, Gymnotidae, Hypopomidae, Rhamphichthyidae, and Sternopygidae. These species inhabit a variety of freshwater habitats throughout Central and South America. To date, attempts at resolving the internal relationships of Gymnotiformes have yet to produce an unambiguous species-level phylogeny. In order to resolve the phylogeny, I collected molecular data from seven nuclear and two mitochondrial genes for 197 species and performed parsimony and Bayesian phylogenetic analyses. All families were recovered as monophyletic with the exception of Gymnotidae; the electric eel *Electrophorus*, previously considered a member of this family, was instead found to be sister to all other Gymnotiformes. The topologies resulting from this study provide a highly-resolved species phylogeny which will form the basis for future studies of species diversification and ecological and evolutionary patterns.

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List of Abbreviations

AICc – corrected Akaike information criterion

BI – Bayesian inference

BSS – bootstrap support

CI – consistency index

Co1 – cytochrome c oxidase subunit 1

Cyt b – cytochrome b

dNTP – deoxynucleotide

Egr1 – early growth response protein 1

Enc1 – ectoderm-neural cortex protein 1

EO – electric organ

Glyt – glycosyltransferase

ILS – incomplete lineage sorting

LBA – long branch attraction

MCMC – Monte Carlo Markov Chains

MP – maximum-parsimony

mtDNA – mitochondrial DNA

Mya – million years ago

NCB – nucleotide compositional bias

nrDNA – nuclear DNA

PCR – polymerase chain reaction

PP – posterior probability

Rag1 – recombination activating gene 1

Rag2 – recombination activating gene 2

Rh1 – rhodopsin

RI – retention index

Zic1 – zinc finger protein of cerebellum 1

Chapter 1
Introduction

1 Actinopterygii

The class Actinopterygii, or ray-finned fishes, is comprised of approximately 29,000 species, and constitutes about half of all known vertebrate species (Cloutier & Arratia, 2004; Nelson, 2006). These fishes can be found throughout the world in both marine and freshwater environments with almost equivalent diversity (Nelson, 2006). Within Actinopterygii, the superorder Ostariophysi contains five orders of fishes: Gonorynchiformes (milkfishes), Cypriniformes (minnows), Characiformes (characins), Siluriformes (catfishes), and Gymnotiformes (Neotropical knifefishes) (Vari et al., 1998; Nelson, 2006; Betancur-R et al., 2013; Chen et al., 2013). This group contains the majority (~68%) of all freshwater fishes, and more than a quarter (~28%) of all known fish species (Nelson, 2006). Owing to their exceptional diversity, the Ostariophysi present excellent model systems for the study of evolution and diversification (Chen et al., 2013). In this thesis, I focus on the phylogeny and evolution of the Gymnotiformes.

2 Gymnotiformes Biology

The order Gymnotiformes is a group of Neotropical freshwater fishes comprised of 34 genera, and approximately 217+ described species (Nelson, 2006; Brochu, 2011; Tagliacollo et al., 2016). It is hypothesized, however, that up to 100 additional species await formal description (Albert & Crampton, 2005; Nelson, 2006). These fishes are also known as American knifefishes because their bodies resemble the shapes of knives; the anal fin has been greatly elongated, and the dorsal, pelvic, and adipose fins are absent (Mago-Leccia, 1994; Albert, 2001; Brochu, 2011; Tagliacollo et al., 2016). All knifefishes possess electricity-generating organs found along the length of their bodies, which will be described in detail below (Mago-Leccia, 1994; Albert, 2001; Albert & Crampton, 2005; Nelson, 2006; Brochu, 2011; Tagliacollo et al., 2016).

Knifefishes are found throughout Central and South America, as seen in Figure 1. Their geographic range spans from southern Mexico to northern Argentina, and also includes Trinidad in the Caribbean (Albert, 2001; Albert & Crampton, 2005; Nelson, 2006; Brochu 2011; Chen et al., 2013; Tagliacollo, et al., 2016). They are found in river basins east and west of the Andes, with the highest diversity of species occurring within the combined Amazon, Orinoco, and

Guianas river basins (Albert, 2001; Albert & Crampton, 2005; Lovejoy et al., 2010; Brochu, 2011). Within this range, knifefishes occupy a wide diversity of habitats, and waters of different chemistries (Crampton, 2011). These include upland and lowland *terra firme* streams, deep river channels, floodplains, swamps, caves, floating meadows, cataracts, and estuaries (Albert, 2003; Lovejoy, 2010; Crampton, 2011; Tagliacollo et al., 2016). With respect to water chemistry, knifefishes can inhabit blackwaters (acidic, tannin-rich, low mineral content), whitewaters (nutrient-rich, turbid), and clearwaters (moderate nutrient content, transparent) (Ferreira et al., 2010; Crampton, 2011; Yunoki & Velasco, 2016). The relative age of Gymnotiformes has been estimated as early as 100 million years ago (mya), coinciding with the split of South America from Africa (Albert et al. 2001; Mora et al., 2010; Lavoué et al., 2012). Further diversification of the order occurred within the Late Miocene, approximately 11 mya when the modern Amazon River basin was formed (Albert, 2001; Mora et al. 2010; Lavoué et al., 2010). The earliest fossil of the order, *Humboldtichthys kirschbaumi* Gayet & Meunier, also dates back to this period of time (Gayet et al., 1994; Albert & Fink, 2007).

3 Electrogenesis and Electroreception

Owing to the nocturnal lifestyle of knifefishes, and the generally poor visibility of their habitats, these fishes utilize electricity produced by their electric organs (EO) to sense their environments and communicate with one another (Mago-Leccia, 1994; Albert, 2001; Albert & Crampton, 2005; Stoddard & Markham, 2008; Lovejoy et al., 2010; Brochu, 2011; Tagliacollo et al., 2016). Knifefishes are able to produce electricity using special cells called electrocytes found in the EO by way of action potentials produced from trans-membrane sodium channels; the electricity generated is relayed into the external environment (Zupanc & Bullock, 2005; Stoddard & Markham, 2008; Crampton et al., 2013). All gymnotiform fishes are capable of producing weak electric discharges of less than 1V (Zupanc & Bullock, 2005; Tagliacollo et al., 2016). One species, *Electrophorus electricus* Linnaeus, is also capable of producing high-voltage discharges (up to 600V) using specialized organs known as Main and Sachs organs; these high voltage discharges are used for defense and to stun prey (Westby, 1988; Zupanc & Bullock, 2005; Tagliacollo et al., 2016).

The electricity produced by the EO forms a field around the body of the fish, and is used to image the environment (Stoddard, 2002; Stoddard & Markham, 2008; Lovejoy et al., 2010).

When an object enters the electric field, it causes a distortion, as seen in Figure 2. This information is then relayed to the brain where an image of the object is formed, allowing for "visualization" (Hopkins, 1974; Albert & Crampton, 2005; Brochu, 2011; Tagliacollo et al., 2016). An electric field extends up to 5-10 times the fish's body length into the surrounding environment (Albert & Crampton, 2005).

To detect electricity, knifefishes possess specialized electroreceptors (Zupanc & Bullock, 2005; Stoddard & Markham, 2008). These receptors are embedded in the fish's skin, and are distributed over the entire body (Zupanc & Bullock, 2005; Pedraja et al., 2014). The head region in particular possesses a high concentration of receptors (Zupanc & Bullock, 2005 Pedraja et al., 2014). There are two types of electroreceptors: ampullary receptors and tuberous receptors. Ampullary receptors allow for the detection of low-frequency electricity (0-100 hertz), whereas tuberous receptors detect higher frequency electricity (100-3,000 hertz) (Hopkins, 1974; Stoddard, 1999; Stoddard, 2002; Zupanc & Bullock, 2005; Stoddard & Markham, 2008). Ampullary receptors can also be found in catfishes, some of the closest relatives and notable predators of the knifefishes, whereas tuberous receptors can only be found in electricity-producing fishes (Stoddard, 1999; Stoddard & Markham, 2008).

Each species of knifefish produces a unique electric signal that is only shared among conspecifics (Albert & Crampton, 2005; Dunlap et al., 2010; Lovejoy et al., 2010). Two broad categories of electric signal include pulse-type signals and wave-type signals (Hopkins, 1974; Albert, 2001; Stoddard, 2002; Salazar et al., 2013). Pulse-type signals consist of discrete emissions of electricity comprising 1-6 phases followed by varying periods of silence (Albert, 2001; Albert & Crampton, 2005; Brochu, 2011). In contrast, a wave-type signal is a continuous emission of electricity centered on a certain frequency (Albert, 2001; Albert & Crampton, 2005; Brochu, 2011). Signals may also differ between species with respect to phase number, frequency, and amplitude (Albert & Crampton, 2005; Brochu, 2011).

The vast diversity of knifefish signals is a product of several different evolutionary processes, including environmental pressures, the need to distinguish conspecifics and heterospecifics, reproductive pressures, and predation pressures (Westby, 1981; Albert, 2001; Stoddard, 2002; Albert & Crampton, 2005; Stoddard & Markham, 2008; Dunlap et al., 2010; Brochu, 2011; Ho et al., 2013). For example, pulse-type signals provide high sensory resolution of the physical

environment, but typically fall within detection range of electroreceptive predators (Stoddard, 2002; Brochu, 2011). This is contrasted by wave-type signals that occupy a narrow frequency range outside of the sensory capabilities of electroreceptive predators, but require constant emissions to provide sensory input and oxygen-rich environments to offset high energetic costs (Albert, 2001; Stoddard, 2002; Brochu, 2011). Furthermore, it is common for several species of knifefish to occur in sympatry (Albert, 2001; Lovejoy et al., 2010). This necessitates the ability to distinguish between conspecifics and heterospecifics, which has driven a diversification of electric signal as a form of reproductive character displacement (Westby, 1981; Brochu, 2011; Crampton et al., 2011). If accidental mating between heterospecifics produces low-fitness offspring or results in wasted mating opportunities, natural selection will favor those individuals who produce and respond to signals distinguishable from those of heterospecifics. This, in turn, drives a separation of signal characteristics between species (Rice & Pfennig, 2007; Pfennig, 2009; Dyer et al., 2013). Electric signals also carry information between conspecifics relating to their sex, fitness, social hierarchy position, and intentions to breed (Ho et al., 2013). For this to be possible, variations in signals within species are required, and some knifefish species show sexual dimorphism with respect to their signals (Dunlap et al., 2010; Ho et al., 2013). Lastly, predation has also contributed to electric signal diversity. Knifefishes use their electric signals in hunting prey, such as aquatic arthropods and other fishes (Mago-Leccia, 1994; Stoddard, 2002). Hunting behavior, courting, and environmental imaging using electric signals, however, poses a problem for knifefishes in that they advertise their location to electroreceptive predators (Westby, 1988; Stoddard, 1999; Stoddard & Markham, 2008). This creates an evolutionary arms race between knifefishes and predators that has resulted in signal diversity to escape predation. Some examples include knifefishes that have shifted their electric signal frequency outside of the detection range of potential predators, and others that utilize discrete electric discharges followed by a period of silence to avoid detection (Westby, 1988; Stoddard, 1999; Stoddard, 2002; Stoddard & Markham, 2008; Lovejoy et al., 2010).

It is for these reasons that the Gymnotiformes present an excellent model system for studying the evolution of communication (Albert, 2001). This includes, but is not limited to, the influence of physical environment, social recognition requirements, reproductive success, and predator-prey interactions on the nature of communication. For these studies to be possible, however, we must first understand how our units of comparison, the species of Gymnotiformes, are taxonomically

classified and phylogenetically related. Otherwise, it is not possible to understand the evolution of communication and other traits, such as morphology, physiology, other behaviors, etc.

4 Taxonomy and Phylogeny of Gymnotiformes

4.1 Taxonomic Classification

The order Gymnotiformes *sensu* Mago-Leccia (1994) has traditionally been divided into five families, which include: Apteronotidae Jordan (ghost knifefishes), Gymnotidae Regan (banded knifefishes), Hypopomidae Mago-Leccia (bluntnose knifefishes), Rhamphichthyidae Regan (sand knifefishes), and Sternopygidae Mago-Leccia (glass and rat-tail knifefishes). Family classification of each species is based in gross-morphology, particularly head and snout shape, and the presence or absence of teeth, adipose fins, and caudal fins (Mago-Leccia, 1994; Albert, 2001; Albert & Crampton, 2005).

Apteronotidae is characterized by the presence of a caudal fin, and a modified adipose fin known as a dorsal organ (Mago-Leccia, 1994; Albert & Crampton, 2005). It is currently the most species-rich gymnotiform family with ~88 species divided into 15 genera: *Adontosternarchus* Ellis, *Apteronotus* Lacépede, *Compsaraia* Albert, *Magosternarchus* Lundberg, Cox Fernandes, & Albert, *Megadontognathus* Mago-Leccia, *Orthosternarchus* Ellis, *Parapteronotus* Albert, *Pariosternarchus* Albert & Crampton, *Platyurosternarchus* Mago-Leccia, *Porotergus* Ellis, *Sternarchella* Eigenmann, *Sternarchogiton* Eigenmann, *Sternarchorhamphus* Eigenmann, *Sternarchorhynchus* Castelnau, and *Tembeassu* Triques (Mago-Leccia, 1994, Lundberg et al., 1996; Albert 2001, Albert & Crampton, 2005; Campos-da-Paz, 2005; Albert & Crampton, 2006). Representatives of each genus are presented in Plates 1, 2, and 3.

Gymnotidae is characterized by a cylindrical body with a long body cavity, and displays the largest geographical distribution of all Gymnotiformes spanning the entire range of the order (Figure 1) (Albert & Crampton, 2005; Lovejoy et al., 2010). It contains only two genera; *Electrophorus* (the electric eel) and *Gymnotus* Linnaeus (Mago-Leccia, 1994; Albert & Crampton, 2005). *Gymnotus* is the most species-rich genus (~38 species) of all Gymnotiformes (Albert & Crampton, 2005 Lovejoy et al., 2010). It should be noted that *Electrophorus* was once classified separately from *Gymnotus* under the family Electrophoridae Gill, but was reclassified

by Ellis (1913) within Gymnotidae due to shared characters, such as body shape, and phylogenetic placement as sister taxa, which will be discussed in detail below (Albert, 2001). Representatives of each genus are presented in Plate 4.

Hypopomidae is characterized by short snouts with well-separated nares, and an absence of teeth and caudal fins (Sullivan, 1997; Mago-Leccia, 1994; Albert & Crampton, 2005). This family contains ~30 formally described species, with quite a number of new species awaiting description (Albert & Crampton, 2005). There are eight genera within Hypopomidae: *Akawaio* Maldenado-Ocampo, López-Fernández, Taphorn, C. R. Bernard, Crampton, & Lovejoy, *Brachyhypopomus* Mago-Leccia, *Hypopomus* Gill, *Hypopygus* Hoedeman, *Microsternarchus* Fernández Yépez, *Procerusternarchus* Cox Fernandes, Nogueira, & Alves-Gomes, *Racenisia* Mago-Leccia, and *Steatogenys* Boulenger (Mago-Leccia, 1994; Cox Fernandes et al., 2014; Maldonado-Ocampo et al., 2014). Representatives of each genus are presented in Plates 5 and 6.

Rhamphichthyidae is characterized by long, tubular snouts, and the absence of teeth and caudal fins (Mago-Leccia, 1994; Albert & Crampton, 2005). Rhamphichthyids typically bury themselves in the sand during the day (Mago-Leccia, 1994). This is the smallest family within Gymnotiformes containing only ~14 species, and is divided into three genera: *Gymnorhamphichthys* Ellis, *Iracema* Triques, and *Rhamphichthys* Müller & Troschel (Mago-Leccia, 1994; Carvalho & Albert, 2011). Representatives of each genus are presented in Plate 7.

Sternopygidae is characterized by the presence of teeth, large eyes, and the absence of caudal and adipose fins (Mago-Leccia, 1994; Albert & Crampton, 2005). Sternopygids occupy a wide variety of habitats, and one species, *Eigenmannia vicentespelaea* Triques, inhabits caves along tributaries of the Tocantins in Brazil (Albert & Crampton, 2005; Bichuette & Trajano, 2015). This family contains ~35 species, divided into six genera: *Archolaemus* Korringa, *Distocyclus* Mago-Leccia, *Eigenmannia* Jordan & Evermann, *Japigny* Meunier, Jégu & Keith, *Rhabdolichops* Eigenmann & Allen, and *Sternopygus* Müller & Troschel (Mago-Leccia, 1994; Meunier et al., 2011). Representatives of each genus are presented in Plate 8.

4.2 Phylogenetic History of Gymnotiformes

The earliest explicit phylogenetic tree for Gymnotiformes was produced by Ellis (1913) based on gross morphology (Figure 3). In this phylogeny, Ellis (1913) placed *Gymnotus* and

Electrophorus together in subfamily Gymnotinae as sister to all other Gymnotiformes based on the absence of a cranial fontanel. Sternarchinae (now known as Apteronotidae) was designated as a clade based on the presence of caudal fins (Ellis, 1913). Ellis (1913) grouped Hypopomidae, Rhamphichthyidae, and Sternopygidae in the monophyletic subfamily Sternopyginae based on the absence of caudal fins.

No other phylogenetic hypotheses were postulated for Gymnotiformes until Triques (1993) constructed a phylogeny using an increased number of morphological characters, including osteological data. Triques (1993) placed Apteronotidae as sister to all other Gymnotiformes (Figure 4). Shortly after, Gayet et al. (1994) produced a phylogeny using morphology, anatomy, and physiology that included a newly discovered gymnotiform fossil, *Humboldtichthys kirschbaumi* Gayet & Meunier (Figure 5). In agreement with Triques (1993), Gayet et al. (1994) placed Apteronotidae as the sister lineage to remaining Gymnotiformes. With some uncertainty, *Humboldtichthys kirschbaumi* was then proposed to have diverged from remaining Gymnotiformes, followed soon after by Sternopygidae (Gayet et al., 1994). Hypopomidae and Rhamphichthyidae were proposed to constitute the monophyletic superfamily Rhamphichthyoidea, and Gymnotidae and Electrophoridae formed the superfamily Gymnotoidea (Gayet et al., 1994).

In 1995, Alves-Gomes et al. produced the first phylogeny of Gymnotiformes to combine genetic molecular data (12S and 16S mitochondrial rRNA) with morphological characters using a maximum-parsimony (MP) approach (Figure 6). This analysis supported six distinct lineages: Sternopygidae formed solely of the genus *Sternopygus*, Gymnotidae, Electrophoridae, Rhamphichthyoidea, a new Eigenmanniidae (formed of *Rhabdolichops*, *Eigenmannia*, and *Distocyclus*), and Apteronotidae. This study showed that Rhamphichthyidae and Hypopomidae actually formed paraphyletic groups due to the placement of *Brachyhypopomus* and *Microsternarchus* as sister to rhamphichthyids rather than other Hypopomids. It also separated *Sternopygus* from all other sternopygids. These results were supported in a consensus phylogeny compiled by Alves-Gomes (1999) from previously discussed phylogenies using both morphological and molecular data.

Another morphology-based phylogeny was created by Albert (2001) for all Gymnotiformes using a MP approach. This tree placed *Gymnotus* and *Electrophorus* as sister to all other

Gymnotiformes, and in agreement with Ellis (1913), incorporated both within the family Gymnotidae, effectively abolishing Electrophoridae (Albert, 2001). Following this was a divergence of the Rhamphichthyoidea, with a notable internal structure change; *Hypopygus* and *Steatogenys* were placed as sister to other Hypopomidae rather than Rhamphichthyidae. This restored the monophyly of Rhamphichthyidae, but Hypopomidae remained a paraphyletic group. This study found a monophyletic sister pairing of Sternopygidae and Apteronotidae, and also showed that *Sternopygus* and Eigenmanniidae formed a monophyletic clade, reestablishing Sternopygidae *sensu* Mago-Leccia (1994). Albert & Crampton (2005) created a composite tree by compiling morphological and molecular data from multiple sources, which further supported the phylogeny by Albert (2001), as seen in Figure 7.

In addition to creating the order-wide phylogeny of Gymnotiformes, Albert (2001) delineated subclades within each family. Within Gymnotidae, *Gymnotus* was divided into three named species groups based on shared body morphology, named for an eponymous species within each species group: *Gymnotus anguillaris* Hoedeman clade, *Gymnotus cylindricus* La Monte clade, and *Gymnotus carapo* Linnaeus clade (Albert, 2001). In Hypopomidae, Albert (2001) named the following clades: *Hypopomus artedi* Kaup (sister to all other hypopomids), *Brachyhypopomus*, Microsternarchini (comprising *Microsternarchus*, *Racenisia*, and *Procerusternarchus* (described after this phylogeny)), and Steatogini (*Steatogenys* and *Hypopygus*). Sternopygidae was divided as follows: Sternopyginae (*Sternopygus* and *Humboldtichthys*) and Eigenmanninae (*Eigenmannia*, *Archolaemus*, *Distocyclus*, and *Rhabdolichops*) (Albert, 2001). Finally, Albert (2001) divided Apteronotidae as follows: Sternarchorhamphini (*Sternarchorhamphus* and *Orthosternarchus*), Sternarchorhynchini (*Sternarchorhynchus* and *Platyurosternarchus*), *Parapteronotus*, *Apteronotus sensu stricto*, and Navajini (*Apteronotus*, *Sternarchella*, *Magosternarchus*, *Porotergus*, *Sternarchogiton*, *Compsaraia*, and *Adontosternarchus*). Of note, *Apteronotus* showed paraphyly, appearing in both Sternarchorhynchini and Navajini (Albert, 2001).

Albert et al. (2005), Lovejoy et al. (2010), and Brochu (2011) produced phylogenies for Gymnotidae using morphological and molecular data, and MP, maximum-likelihood, and BI approaches. All show agreement on the placement (with strong support) of *Electrophorus electricus* as sister to all other gymnotids. These studies also showed strong support for the monophyletic *Gymnotus carapo* clade, *Gymnotus cylindricus* clade, and *Gymnotus anguillaris*

clade (also known as the G2 clade), and in turn designated three new monophyletic clades: *Gymnotus tigre* J.S. Albert & Crampton clade (also known as the G3 clade), *Gymnotus coatesi* La Monte clade (also known as the G1 clade), and *Gymnotus pantherinus* Steindachner as its own lineage (Albert et al., 2005; Lovejoy et al., 2010; Brochu, 2011). However, the internal arrangement of these clades within Gymnotidae is ambiguous, as each phylogeny shows a different topology (Albert et al., 2005; Lovejoy et al., 2010; Brochu, 2011).

Investigating the effect of gene paralogs on electric signaling, Arnegard et al. (2010) created a phylogeny using a maximum-likelihood approach that included Gymnotiformes. This tree (Figure 8) was created using molecular data from *Scn4aa* and *Scn4ab*, genes responsible for the creation of voltage gated sodium channel subunits (Arnegard et al., 2010). This tree shows an early divergence of wave-type fishes followed by pulse-type fishes, potentially indicating a plesiomorphic wave-state (Arnegard et al., 2010). This tree shows Apteronotidae as sister to all Gymnotiformes. It should be noted that taxon sampling of Gymnotiformes for this study was quite low (Arnegard et al., 2010).

To further investigate the internal structure of Rhamphichthyoidea, Maldonado-Ocampo et al. (2013) created of phylogeny of the group using molecular data (*cyt b*, *co1*, and *rag2*) and a MP approach. This phylogeny (Figure 9) was also the first to include the newly described species *Akawaio penak* Maldenado-Ocampo, López-Fernández, Taphorn, C. R. Bernard, Crampton, & Lovejoy (Maldonado-Ocampo et al., 2013). This tree recovered both Hypopomidae and Rhamphichthyidae with some differences with the original designations of Mago-Leccia (1994). The Steatogini *sensu* Albert (2001) formed a monophyletic group with *Rhamphichthys* and *Gymnorhamphichthys*, whereas all other hypopomids formed a monophyletic group sister to Rhamphichthyidae (Maldonado-Ocampo et al., 2013). It was also found that *Akawaio penak* was sister to all remaining Hypopomidae (Maldonado-Ocampo et al., 2013).

The most recent phylogeny of Gymnotiformes was presented by Tagliacollo et al. (2016), and was created using a combination of morphological and molecular data (6 genes, the largest molecular dataset to date), and used maximum-likelihood and Bayesian inference (BI) approaches (Figure 10). The topology of this phylogeny corroborated the findings of Albert (2001) with respect to the arrangement of Gymnotiform families: Gymnotidae as sister to all other Gymnotiformes; monophyly of Rhamphichthyoidea (Hypopomidae + Rhamphichthyidae);

and a monophyletic Sternopygidae + Apteronotidae (Tagliacollo et al., 2016). Changes were made in the designation of clades within some families. Within Rhamphichthyidae, Steatogini was renamed Steatogenae, and the genera *Rhamphichthys*, *Gymnorhamphichthys*, and *Iracema* were grouped as the Rhamphichthyinae (Tagliacollo et al., 2016). Major revisions were also made to the structuring of Apteronotidae. The sister lineage of Apteronotidae, Sternarchorhamphini, was renamed as the Sternarchorhamphinae, which was followed by a divergence of *Adontosternarchus* as a new lineage separate from Navajini. The next diverging group within Apteronotidae consisted of a new clade designated the Apteronotini and consisting of *Parapteronotus*, *Megadontognathus*, and *Apteronotus sensu stricto* (Tagliacollo et al., 2016). *Apteronotus sensu stricto* was further divided into three clades: *Apteronotus albifrons* Linnaeus clade, *Apteronotus leptorhynchus* Ellis clade, and *Apteronotus magdalenensis* Miles clade (Tagliacollo et al., 2016). The family included a sister pairing of Sternarchorhynchini (*Platyurosternarchus* and *Sternarchorhynchus*) and Navajini (*Pariosternarchus*, *Sternarchella*, *Magosternarchus*, *Sternarchogiton*, *Compsaraia*, *Porotergus*, and *Apteronotus*) (Tagliacollo et al., 2016).

5 Purpose of Study, Hypotheses, and Predictions

As summarized above, there are many contradictions among different phylogenetic hypotheses for Gymnotiformes. This study aims to resolve the ambiguous arrangement of knifefish families and species. To accomplish this task, I construct a species-level molecular phylogeny using the largest molecular character set and taxon sampling to date. I use two approaches to construct trees, including MP and BI. The resulting trees will allow several hypotheses concerning the topology of Gymnotiformes to be tested. First, I will test the hypothesis that Gymnotidae (including *Electrophorus*) constitutes the sister lineage to all remaining Gymnotiformes. This family has been consistently placed as sister to all other Gymnotiformes in most recent phylogenetic hypotheses. Second, I will test the hypothesis that Rhamphichthyoidea forms a monophyletic clade, and that Steatogenae group within Rhamphichthyidae, as opposed to Hypopomidae, as seen in all recent molecular phylogenies. Third, I will test the hypothesis that *Sternopygus* groups within Sternopygidae, even though this genus is morphologically distinct from all other sternopygids. Lastly, I will test the hypothesis that *Apteronotus* forms a

polyphyletic genus distributed in both Apteronotini and Navajini, as proposed in recent phylogenies.

6 Significance of Present Study

Comparative biology requires a clear understanding of the units of comparison (e.g., species) and their relationships. For comparative biology to be possible, we require the use of taxonomy to define units, and phylogeny to understand how these units are related. Without these tools, it is difficult to compare species physiology, biochemistry, behavior, genetics, and ontogeny. Indeed, knifefishes present an excellent study system for understanding the evolution of communication, as well as speciation and diversification in the Neotropics. This study, which is intended to determine how the fishes of Gymnotiformes are evolutionarily related, will provide the basis for further comparative studies of this fascinating clade of fishes.

Chapter 2
Materials and Methods

1 Selection of Taxa, Outgroups, and Genes

To reconstruct an order-wide phylogeny of Gymnotiformes, I included specimens from as many described knifefish taxa as possible. 197 ingroup species were included, representing 31 of the 34 recognized Gymnotiformes genera (*Pariosternarchus*, *Tembeassu*, and *Iracema* were not included due to unavailability of tissue samples). 11 outgroup species were included to root the tree, and include representatives from each of the closest orders to Gymnotiformes from within Ostariophysi. A total of 223 specimens were used in this study, and are listed in Table 1 with voucher information for each specimen.

Genes were selected for this study using one or more of three criteria: (1) to provide a mixture of quickly evolving and slowly evolving genes to provide phylogenetic resolution for both deep-level (~60-100 mya) and shallow-level (present - ~50 mya) relationships (Lovejoy, 2000; Lavoué et al., 2012), (2) availability from other molecular studies of Gymnotiformes to allow for Gymnotiformes-specific primer design when necessary, and (3) genes which provided the largest amount of nucleotide bases from coding sequences per polymerase chain reaction (PCR) amplification. Nine genes were included in this study with a total of 10,603 base pairs used in phylogenetic analyses. Two of these genes were mitochondrial: cytochrome c oxidase subunit 1 (*co1*, 685 base pairs (bp)) and cytochrome b (*cyt b*, 1,282 bp). Seven of these genes were nuclear: early growth response protein 1 (*egr1*, 1,285 bp), ectoderm-neural cortex protein 1 (*enc1*, 1,340 bp), glycosyltransferase (*glyt*, 1,161 bp), recombination activating gene 1 (*rag1*, 1,620 bp), recombination activating gene 2 (*rag2*, 1,324 bp), rhodopsin (*rh1*, 906 bp), and zinc finger protein of cerebellum 1 (*zic1*, 1,000 bp).

Co1 is a gene commonly used in DNA barcoding, and has shown to be useful in providing species-level resolution in fishes (Nicolas et al., 2012). *Cyt b* has been frequently used in fish molecular phylogenetic studies. Both mitochondrial genes show rapid rates of evolution when compared to nuclear genes, and are useful in providing shallow-level (i.e. species-level) resolution in phylogenies (Lovejoy, 2000; Russell et al., 2010; Nicolas et al., 2012).

Like most nuclear genes, *egr1* exhibits a slower rate of evolution than mitochondrial DNA (mtDNA), which allows for resolution at deep-level phylogenetic relationships (Chen et al., 2008). Gene trees constructed using *egr1* show consistently congruent tree topologies with those of nuclear multi-gene species-level phylogenies (Chen et al., 2008). *Enc1*, *glyt*, and *zic1* all show similar patterns of evolution with *rag1* and *rag2*; all of these genes possess long, uninterrupted, fairly-conserved exons useful in providing deep-level phylogenetic resolution, and lack saturation of the third codon position (Lovejoy, 2000; Lovejoy & Collette, 2001; Li et al., 2007; Chiari et al, 2009). *Rh1* has been shown to be useful in providing species-level resolution, and lacks introns within its sequence (Sevilla et al., 2007). All nuclear genes chosen for this study are single-copy genes preventing phylogenetic biases associated with gene paralogs (Lovejoy & Collette, 2001; Li et al., 2007; Chen et al., 2008; Chiari et al., 2009).

Using genes with a diversity of rates of evolution also mitigates the effects of phylogenetic biases (Prychitko & Moore, 2000; Bergsten, 2005; Baum & Smith, 2013). One such bias is long branch attraction (LBA), a problem common to parsimony that can also occur to a lesser extent in maximum-likelihood and BI (Bergsten, 2005). LBA occurs when taxa that exhibit a great amount of evolutionary changes (i.e. long branches of the tree) are grouped together as the most parsimonious tree arrangement rather than shared ancestry (Bergsten, 2005; Baum & Smith, 2013). This may also result in slowly evolving lineages (i.e. short branches of the tree) to be erroneously grouped together (Bergsten, 2005). By including both quickly- and slowly-evolving genes, LBA can be avoided because taxa that exhibit a great amount of change in one gene may not exhibit the same degree of change in a much more slowly-evolving gene (Bergsten, 2005; Baum & Smith, 2013).

Another phylogenetic bias to consider is nucleotide compositional bias (NCB). NCB occurs when taxa are grouped with respect to shared nucleotide content and nucleotide combinations regardless of shared ancestry (Prychitko & Moore, 2000; Praz & Packer, 2014). For example, protein-coding mitochondrial genes tend to show high adenine and cytosine content, specifically in the third codon position (Prychitko & Moore, 2000). This can lead to artificial groupings of species that may contain analogous adenine- and cytosine-rich nucleotide sequences in which only so many combinations are possible (Prychitko & Moore, 2000). Although individual genes may display NCB, the exact nucleotide biases of each gene are unlikely to be shared (Li et al.,

2007). By including a variety of genes, both mitochondrial and nuclear, we can dilute the influence of any specific NCB (Prychitko & Moore, 2000; Li et al., 2007).

2 DNA Extraction, Amplification, and Sequencing

The majority of fish tissues used in this study were obtained from Dr. Nathan Lovejoy's collection of tissues stored in 95% ethanol, and frozen at -20°C. Muscle and fin tissues were collected by Dr. Lovejoy and previous students of his lab on collection trips to Central and South America. His collection, however, did not contain all described Gymnotiformes species, so collection trips were organized to obtain as many missing species as possible. During my program, I travelled to Guyana, Peru, Brazil, and Colombia where I collected both described and currently undescribed species. Tissues loans were also obtained from natural history institutions throughout North and South America.

Muscle and fin tissues were extracted using DNeasy Blood and Tissue Kits (QIAGEN) following a protocol provided with each kit. DNA extractions were then used to amplify genes selected for this study, as described above, using PCR. Primer sequences used to amplify each gene are provided in Table 2. *Rh1* was amplified in two overlapping halves using two external and two internal primers. *Rag2* was amplified for all fishes using the primers *rag2* GYF and *rag2* R6. For fishes that failed to amplify, a second PCR was conducted using the primers *rag2* JF1 and *rag2* JR1. *Egr1*, *enc1*, *glyt*, *rag1*, and *zic1* were amplified using external primers, and then sequenced using internal primers due to the presence of non-specific PCR product after amplification.

For *co1*, each amplification reaction contained 1X mixed *Taq* buffer (buffer made by combing *Taq* buffer with $(NH_4)_2SO_4$ and *Taq* buffer with KCl in a ratio of 6:4 respectively; Thermo Fisher Scientific Inc.), 1.5 mM $MgCl_2$ (Thermo Fisher Scientific Inc.), 0.8 mM of deoxynucleotides (dNTPs) containing equal proportions of dATP, dCTP, dGTP, and dTTP (Thermo Fisher Scientific Inc.), 0.4 μM of each primer, 0.04 U of *Taq* DNA polymerase, recombinant (Thermo Fisher Scientific Inc.), approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water (Sigma-Aldrich Canada) to adjust to the final reaction volume of 25 μL. Using a Mastercycler pro thermocycler (Eppendorf – Mississauga, ON), the PCR reactions were cycled through a series of cyclical heating phases to facilitate amplification. Cycling conditions for COI included an initial heating to 94°C for 2 min, then 40 cycles of DNA denaturation at

94°C for 30s, primer annealing at 52°C for 40s, and DNA extension at 72°C for 1 min. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

The same PCR recipe used for amplifying *co1* was also used for *cyt b*, with the exception of using 0.1 U of *Taq* DNA polymerase, recombinant. Cycling conditions for *cyt b* included an initial heating to 95°C for 30s, then 35 cycles of DNA denaturation at 95°C for 30s, primer annealing at 50°C for 1 min, and DNA extension at 72°C for 1 min and 30s. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

Both *enc1* and *glyt* were amplified using the same PCR reaction recipe: 1X mixed *Taq* buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 μM of each primer, 0.1 U of *Taq* DNA polymerase, recombinant, approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water to adjust to the final reaction volume of 25 μL. Cycling conditions for *enc1* and *glyt* included an initial heating to 95°C for 2 min, then 40 cycles of DNA denaturation at 95°C for 30s, primer annealing at 56°C for 1 min (for *glyt*)/58°C for 1 min (for *enc1*), and DNA extension at 72°C for 1 min and 30s. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

For *egr1*, each amplification reaction contained 1X mixed *Taq* buffer, 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 μM of each primer, 0.1 U of *Taq* DNA polymerase, recombinant, approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water to adjust to the final reaction volume of 25 μL. Cycling conditions for *egr1* included an initial heating to 95°C for 4 min, then 35 cycles of DNA denaturation at 95°C for 40s, primer annealing at 55°C for 40s, and DNA extension at 72°C for 1 min and 30s. A final extension phase at 72°C for 7 min took place at the end of the cycling program.

For *rag1*, each amplification reaction contained 1X mixed *Taq* buffer, 1.67 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 μM of each primer, 0.05 U of *Taq* DNA polymerase, recombinant, approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water to adjust to the final reaction volume of 30 μL. Touchdown PCR cycling conditions for *rag1* included an initial heating to 94°C for 4 min, then 6 cycles of DNA denaturation at 95°C for 30s, primer annealing at 58°C for 30s (decreasing by 1°C each cycle), and DNA extension at 72°C for 1 min. Once completed, a second series of heating cycles took place with 34 cycles of DNA denaturation at

95°C for 30s, primer annealing at 52°C for 30s, and DNA extension at 72°C for 1 min. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

The same PCR reaction recipe used for amplifying *co1* was also used for *rag2*. Touchdown PCR cycling conditions for *rag2* included an initial heating to 95°C for 2 min and 30s, then 8 cycles of DNA denaturation at 95°C for 30s, primer annealing at 58°C for 1 min (decreasing by 2°C each cycle), and DNA extension at 72°C for 1 min and 30s. Once completed, a second series of heating cycles took place with 32 cycles of DNA denaturation at 95°C for 30s, primer annealing at 50°C for 1 min, and DNA extension at 72°C for 1 min and 30s. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

For RH1, each amplification reaction contained 1X mixed *Taq* buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 μM of each primer, 0.05 U of *Taq* DNA polymerase, recombinant, approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water to adjust to the final reaction volume of 30 μL. Touchdown PCR cycling conditions for *rh1* included an initial heating to 94°C for 4 min, then 6 cycles of DNA denaturation at 95°C for 30s, primer annealing at 54°C for 30s (decreasing by 1°C each cycle), and DNA extension at 72°C for 1 min. Once completed, a second series of heating cycles took place with 34 cycles of DNA denaturation at 95°C for 30s, primer annealing at 48°C for 30s, and DNA extension at 72°C for 1 min. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

For *zic1*, each amplification reaction contained 1X mixed *Taq* buffer, 2 mM $MgCl_2$, 1.2 mM dNTPs, 0.4 μM of each primer, 0.1 U of *Taq* DNA polymerase, recombinant, approximately 20-30 ng of template DNA extract, and enough sterile, nuclease-free water to adjust to the final reaction volume of 25 μL. Cycling conditions for *zic1* included an initial heating to 95°C for 2 min and 30s, then 40 cycles of DNA denaturation at 95°C for 30s, primer annealing at 52°C for 1 min, and DNA extension at 72°C for 1 min and 30s. A final extension phase at 72°C for 5 min took place at the end of the cycling program.

PCR products were purified by combining 2 μL of ExoSAP-IT (Affymetrix USB) with 5 μL PCR product, and heated in a thermocycler using a two-step cycling program according to the manufacturer's protocol (USB Corporation, 2006). Samples were first heated to 37°C for 15 min, allowing enzymes in the ExoSAP-IT to degrade primers and unused dNTPs, and then were heated further to 80°C for 15 min to inactivate the enzymes (USB Corporation, 2006; Watanabe,

2010). Once purified, 2 μL of PCR product was combined with 0.7 μL of the appropriate sequencing primer and 5 μL of sterile, nuclease-free water in a sequencing plate. Samples were then sent to TCAG DNA Sequencing and Synthesis Facilities at the Toronto SickKids Hospital for sequencing.

3 Phylogenetic Analyses

Sequences were imported, assembled, and edited using Geneious 9.1.4 (Kearse et al., 2012). Individual alignments were assembled for each gene using the ClustalW plug-in of Geneious (Chenna et al., 2003). A concatenated alignment of all genes consisting of 10,603 characters was made using SequenceMatrix (Vaidya et al., 2011) by combining NEXUS files of each individual gene alignment exported from Geneious. This alignment was then used to conduct a MP analysis and a BI.

The MP analysis was conducted using PAUP* (Swofford, 2003). Owing to alignment ambiguities in the sequence matrix, typically at the beginnings and endings of individual genes due to unequal sequence lengths, the following characters were excluded from analysis: 80, 87, 628-629, 685-732, 747-748, 823-824, 1,770, 1,848, 1,861-2,025, 2,092, 2,127-2,130, 2,283, 2,495, 2,731, 2,789, 3,253-3,265, 3,309-3,312, 4,583, 4,586-4,605, 5,803, 5,815, 5,879, 5,891, 5,908, 6,076, 6,288, 6,340-6,341, 6,427, 6,689, 6,791, 6,849-6,850, 6,968, 7,031, 7,614, 8,072, 8,434, 8,461, 9,694, 9,889, 9,923, 9,988, 10,083, and 10593-10603. *Chanos chanos* was designated as the outgroup taxon, and a heuristic search with 1,000 random addition replicates with the MULTREES option was conducted to find the most parsimonious trees. Once completed, a strict consensus tree was produced for the most parsimonious trees, and the consistency index (CI) and the retention index (RI) were calculated (Farris, 1989). Bootstrap support (BSS) for internal branches of the consensus tree were calculated using a heuristic search with 1,000 replicates using the MULTREES option (Felsenstein, 1985).

Before the concatenated alignment was analyzed using BI, it was partitioned by gene to account for differing rates and patterns of molecular evolution (Baum & Smith, 2013). With nine partitions, the alignment was run through PartitionFinder 1.1.1 (Lanfear et al., 2012) to find the best nucleotide substitution model for each gene selected based on the Corrected Akaike

Information Criterion (AICc). Substitution models that best fit the partitioned data were found for MrBayes 3.2.5 (Ronquist & Huelsenbeck, 2003), as presented in Table 3.

A BI of the concatenated alignment was conducted using MrBayes 3.2.5. Models of nucleotide substitution (Table 3) were applied to each gene partition, and were unlinked. Two independent runs were performed, each composed of four Markov Chains. Monte Carlo Markov Chains (MCMC) were run for 10,000,000 generations, and trees were sampled every 1,000 generations. Of these trees, the first 25% were discarded as burn-in due to convergence having not been achieved in this portion of sampled trees. Convergence of both runs was assessed using Tracer 1.6.0 (Rambaut & Drummond, 2007). Individual gene alignments were also analyzed in MrBayes using the above parameters, but for half as many generations (5,000,000). For all BI trees, posterior probabilities (PP) were recovered for internal branches of the trees.

Chapter 3
Results

1 Overview of Gymnotiformes Phylogeny

This study produced species-level phylogenies for Gymnotiformes using both MP and BI. In the case of MP, 4,662 of the 10,297 characters included in the concatenated dataset were parsimony-informative. A total of 60 most parsimonious trees were found, of 9,748 steps with a CI of 0.24 and a RI of 0.76. The strict consensus tree is shown in Figure 11. In the case of BI, both MrBayes runs achieved for convergence. For the combined runs, I found an effective sample size of 1,650.60, a LnL value of 1,651, a LnPr value of 8,577, and a TL(all) value of 8,573. The final consensus tree produced using a 50% majority rule is shown in Figure 12.

Tree topologies for both MP and BI analyses were highly congruent, and both analyses provided well-resolved species-level relationships with high BSS and PP values for most nodes. In both analyses, the monophyly of Gymnotiformes is well-supported (BSS of 100, PP of 1.00). One of our most notable results is that the electric eel genus, *Electrophorus*, was placed as the sister group to all other Gymnotiformes rather than in Gymnotidae, as hypothesized by most previous studies. The monophyly of other Gymnotiformes, excluding *Electrophorus*, was strongly supported with a BSS of 100, and a PP of 1.00. Within Gymnotiformes excluding *Electrophorus*, the families Apteronotidae, Hypopomidae, Rhamphichthyidae and Sternopygidae were monophyletic, as expected based on previous analyses. In both analyses, the superfamily Rhamphichthyoidea (Hypopomidae + Rhamphichthyidae) was well supported (BBS of 100, PP = 1.00). Both analyses also supported a clade composed of Apteronotidae and Sternopygidae (BBS of 82, PP of 1.00).

The arrangement of Gymnotiformes families, however, shows a discrepancy between the MP analysis and the BI. In the MP phylogeny (Figure 11), Gymnotidae (excluding *Electrophorus*) was found to be the sister group of Hypopomidae + Rhamphichthyidae, and Sternopygidae + Apteronotidae. In the BI phylogeny Gymnotidae (excluding *Electrophorus*) was found to be the sister group of Hypopomidae + Rhamphichthyidae. Both analyses recovered somewhat weak support for these arrangements (85 BSS, 0.76 PP).

2 Gymnotiformes Family Phylogenies

2.1 Gymnotidae

As previously mentioned, *Electrophorus* did not group within Gymnotidae, but was placed as sister to all other Gymnotiformes. With regard to the remaining gymnotids, both the MP analysis and the BI analyses recovered previously proposed major lineages: the *Gymnotus coatesi* (G1) clade, *Gymnotus anguillaris* (G2) clade, *Gymnotus tigre* (G3) clade, *Gymnotus cylindricus* clade, *Gymnotus carapo* clade, and *Gymnotus pantherinus*. These lineages of Gymnotidae were strongly supported as monophyletic groups, with BSS >90 and PP of 1.00. However, the arrangement of the species groups differed between analyses, as seen in Figures 13 and 14.

2.2 Rhamphichthyidae

Relationships within Rhamphichthyidae were identical in the MP and BI phylogenies (Figure 15). All genera were well-supported within this family, and we found a relationship of *Hypopygus* + *Steatogenys* (Steatogenae) sister to *Rhamphichthys* + *Gymnorhamphichthys* (Rhamphichthyinae).

2.3 Hypopomidae

The relationships among Hypopomidae were found to be identical between the MP and BI phylogenies (Figure 16). *Akawaio penak* was sister to all other hypopomids with a strong BSS of 100 and a PP of 1.00. The monophyly of the species-rich genus *Brachyhypopomus* was strongly supported (BSS of 100, PP of 1.00). The phylogenies presented in this study included 19 currently undescribed species of *Brachyhypopomus* in addition to all 13 described species. *Hypopomus artedi* was the sister group of the Microsternarchini (*Microsternarchus* + *Racenisia* + *Procerusternarchus*). Within Microsternarchini, *Racenisia fimbriipinna* was placed as the sister lineage of *Procerusternarchus pixuna* + *Microsternarchus*. There are currently two described *Microsternarchus* species, of which only one (*Microsternarchus bilineatus*) was included in this study. I have also included four currently undescribed species, three of which were collected along the Tapajós River of Brazil. All species of the Tapajós form a monophyletic

group within *Microsternarchus* separate from *Microsternarchus bilineatus* (from Tefé, Brazil) and *Microsternarchus* n. sp. SMYT (from the Amazon River, Bolivia).

2.4 Sternopygidae

Sternopygidae was found to be a monophyletic group which included both *Sternopygus* and the genera of Eigenmanninae. The same topology of Sternopygidae (Figure 17) was recovered for both the MP and BI analysis. Genera were found to be well-supported monophyletic clades, with the exception of *Rhabdolichops* and *Eigenmannia*, as currently defined. Two species of *Rhabdolichops*, *Rhabdolichops lundbergi* and *Rhabdolichops nigrimans*, were found nested as a sister pair within *Eigenmannia*, albeit with somewhat weak support (BSS of 52, PP of 0.74). The placement of these two species caused *Rhabdolichops* and *Eigenmannia* to become polyphyletic and paraphyletic genera, respectively. In addition, the species *Rhabdolichops* cf *stewarti*, was found to be nested within several specimens of *Rhabdolichops caviceps*.

2.5 Apteronotidae

The topology of Apteronotidae was found to be consistent between the MP and BI analyses (Figure 18). All major clades of Apteronotidae were recovered in addition to two new lineages designated in this study. Sternarchorhamphinae (*Sternarchorhamphus muelleri* and *Orthosternarchus tamandua*) was placed as sister to all other apteronotids. Our study recovered monophyletic *Adontosternarchus* and Navajini clades. The Apteronotini and Sternarchorhynchini clades were recovered as non-monophyletic.

Apteronotini did not form a monophyletic clade due to the placement of the *Apteronotus leptorhynchus* clade as sister to the sister-pair of Sternarchorhynchini and Navajini rather than with other Apteronotini clades. Placement of the *Apteronotus leptorhynchus* clade was strongly supported in the BI with a PP of 1.00, but only weakly supported in the MP analysis with a BSS of 66. Apteronotini was strongly supported as monophyletic (BSS of 99, PP of 1.00) when the *Apteronotus leptorhynchus* clade was excluded. In the BI, the *Apteronotus magdalenensis* clade was sister to *Megadontognathus kaitukaensis* + *Parapteronotus hasemani* + the *Apteronotus albifrons* clade. *Apteronotus* was shown to be a polyphyletic genus, occurring in Apteronotini and Navajini.

Platyurosternarchus diverged after the *Apteronotus leptorhynchus* clade, placed as sister to Sternarchorhynchini and Navajini with a PP of 1.00. The Sternarchorhynchini, therefore, displayed paraphyly as neither phylogeny placed *Platyurosternarchus* as sister to *Sternarchorhynchus*. *Sternarchorhynchus* showed strong monophyly with a BSS of 89 and a PP of 1.00.

Navajini was strongly supported as a monophyletic clade with a BSS of 100, and a PP of 1.00. All genera within Navajini with the exception of *Compsaraia*, however, were found to be paraphyletic or polyphyletic. *Sternarchogiton preto* was placed as sister to all other Navajini species, separate from other *Sternarchogiton* species. Its placement was strongly supported with a BSS of 100 and a PP of 1.00. Within Navajini, genera were arranged in three strongly supported (BSS >97, PP of 1.00) monophyletic clades: Magosternarchus + Sternarchella, Compsaraia + Sternarchogiton, and Apteronotus + Porotergus. The two species of *Magosternarchus* were interspersed within *Sternarchella*, causing paraphyly in both genera. *Compsaraia* and *Sternarchogiton* (excluding *Sternarchogiton preto*) also showed paraphyly as *Sternarchogiton* n. sp. LONG was grouped more closely with *Compsaraia compsa* and *Compsaraia samueli* than other *Sternarchogiton* species. Finally, *Apteronotus* and *Porotergus* also displayed paraphyly. The three species of *Porotergus* were interspersed within *Apteronotus*. Also, *Apteronotus sensu stricto* was placed in Apteronotini, separate from Navajini species.

3 Individual Gene Phylogenies

Individual gene matrices were analyzed in MrBayes 3.2.5, resulting in nine gene trees. None of the single gene tree topologies matched the overall topology of the consensus species trees. The diversity of gene tree topologies, however, provided support at various phylogenetic levels of the species tree. For instance, *co1* (Figure 19) presented strong support for most sister-pairings of species also seen in the species tree, and recovered most major clades within each family. *Co1*, however, could not resolve deep-level relationships of the families, and presented weakly-supported polytomies. The other mitochondrial gene, *cyt b* (Figure 20), recovered all families as monophyletic clades, and recovered all clades within each family with strong support. Like *co1*, the deep-level phylogenetic arrangement of the families in *cyt b* did not match that of the species tree.

The gene tree of *egr1* (Figure 21) recovered most major clades within each family with good support. Families, however, were not always recovered as monophyletic. For example, *Sternopygus* formed a clade separate from all remaining sternopygids. Also, arrangement of families in a polytomy presented ambiguity within the order. *enc1*, in contrast, produced a tree (Figure 22) with stronger deep-level support. Although not identical, the arrangement of families using *enc1* most resembled the topology of the species tree. The gene tree produced for *glyt* (Figure 23) recovered all families as monophyletic, and also recovered with strong support all major clades within each family. Arrangement of the families in a polytomy, however, presented ambiguity.

Rag1 produced a gene tree (Figure 24) that recovered all major clades found within each family, but did not recover monophyly for all families. *Rag2*, in contrast, produced a gene tree (Figure 25) that recovered monophyly for all families and all major clades with good support. Arrangement of the families within this phylogeny, however, was ambiguous. *Rh1* (Figure 26) and *zic1* (Figure 27) both showed patterns similar to *rag1*, in that all intra-family clades were recovered as monophyletic groups with strong support. Like *rag1*, not all families displayed monophyly, specifically Sternopygidae and Apteronotidae. Although each gene trees presented differing topologies, the concatenated dataset produced a species tree with strong statistical support at both shallow- and deep-levels of phylogeny.

Regarding the placement of *Electrophorus*, most gene trees (*egr1*, *enc1*, *rag1*, *rag2*, *rh1*, and *zic1*) designated it as a lineage separate from Gymnotidae. Four of these genes (*rag1*, *rag2*, *rh1*, and *zic1*) placed *Electrophorus* as sister to all other Gymnotiformes. In contrast, *co1* nested *Electrophorus* within *Microsternarchus*, and both *cyt b* and *glyt* placed *Electrophorus* within Gymnotidae as sister to *Gymnotus*.

Chapter 4
Discussion

1 Overview of Gymnotiformes Phylogeny

1.1 *Electrophorus*

Topology of the order Gymnotiformes has historically been ambiguous owing to conflicting signal in all existing phylogenies. The purpose of this study was to seek a robust species-level phylogeny of the order. This was accomplished by including the largest number of taxa and the largest amount of molecular characters of any phylogenetic analysis to date. Regarding the sister lineage to all remaining Gymnotiformes, previous studies have presented one of two possibilities: Gymnotidae or Apteronotidae. This study, however, was the first to place *Electrophorus* alone as the sister of all other knifefishes.

Phylogenies by Ellis (1913), Albert (2001), Albert & Crampton (2005), and Tagliacollo et al. (2016) all placed *Electrophorus* and *Gymnotus* (Gymnotidae) as the sister to all other knifefish lineages. *Gymnotus*, however, was not found to be sister to *Electrophorus* in the present study. Owing to the phylogenetic separation of *Gymnotus* and *Electrophorus*, the first hypothesis I proposed stating Gymnotidae was the sister to all other Gymnotiformes was unsupported, and therefore rejected.

This is the first study to date presenting *Electrophorus* and Gymnotidae as separate families that do not form a sister-pair. As with the present study, Triques (1993), Gayet et al. (1994), Alves-Gomes et al. (1995), and Alves-Gomes (1999) all proposed the inclusion of *Electrophorus* in its own family, Electrophoridae. All phylogenies following Alves-Gomes (1999), however, have grouped *Electrophorus* within Gymnotidae (Albert, 2001; Albert et al., 2005; Albert & Crampton, 2005; Lovejoy et al., 2010; Tagliacollo et al., 2016). A possible explanation for this could owe to the inclusion of a greater or equal proportion of mtDNA to nuclear DNA (nrDNA) in their analyses (Arnason et al., 1999). As *Electrophorus* shows a deep-level divergence from Gymnotiformes in the present study, it is possible that mitochondrial genes with fast rates of evolution cannot provide resolution for this deep-level phylogenetic relationship (Arnason et al., 1999). By including 50% or more mtDNA in analyses, previous phylogenies may have recovered the mtDNA grouping of *Electrophorus* within Gymnotidae, as seen in the *cyt b* gene tree (Figure

19). A greater number of nuclear genes would provide more accurate deep-level resolution (Arnason et al., 1999). In fact, this study has included the largest amount of nuclear genes (seven); of these genes, six reject a sister relationship between *Electrophorus* and the remaining Gymnotidae, and four place it as sister to all other Gymnotiformes.

Owing to its position as sister to remaining Gymnotiformes and as a separate lineage from Gymnotidae, I propose *Electrophorus* be reclassified within a resurrected Electrophoridae. This designation would be morphologically supported as *Electrophorus* is unlike any other currently described knifefish. One unique feature of this genus is the presence of an upturned anal fin continuous with the posterior region of the tail, forming a false caudal fin (Ellis, 1913; Mago-Leccia, 1994). A second unique feature of *Electrophorus* is the presence of three well-developed electric organs (Main organ, Hunter's organ, and Sach's organ) capable of producing strong electric discharges used in stunning prey (Ellis, 1913; Mago-Leccia, 1994).

1.2 Arrangement of Families

Phylogenies presented in the current study displayed some ambiguity regarding the arrangement of Gymnotiformes families. The MP analysis placed Gymnotidae as sister of remaining Gymnotiformes (excluding *Electrophorus*), while the BI analysis recovered Gymnotidae as sister to Hypopomidae + Rhamphichthyidae. This ambiguity could be due to differences in the theoretical frameworks underlying phylogenetic reconstruction. MP produces a tree with the fewest number of character state changes to reduce homoplasy, whereas BI produces a tree by relating possible topologies back to a priori assumptions, and selecting the most-probable phylogeny (Merl et al., 2005; Baum & Smith, 2013).

With respect to the arrangement of the Hypopomidae and Rhamphichthyidae, the first part of my second hypothesis states that these families would form the monophyletic superfamily Rhamphichthyoidea. Both of the phylogenies presented in this study found strong statistical support for a monophyletic Rhamphichthyoidea. The sister-pairing of Hypopomidae and Rhamphichthyidae is also supported in all previous Gymnotiformes phylogenies produced after the Ellis (1913) phylogeny (Triques, 1993; Gayet et al., 1994; Alves-Gomes et al., 1995; Alves-Gomes, 1999; Albert, 2001; Albert & Crampton, 2005; Arnegard et al., 2010; Maldonado-Ocampo et al., 2014; Tagliacollo et al., 2016).

2 Gymnotiformes Family Phylogenies

2.1 Gymnotidae

Of all families, Gymnotidae showed the greatest discrepancy between MP and BI topologies. All major clades were recovered in both analyses; BI placed the *Gymnotus anguillaris* clade as sister to all other gymnotid lineages. The MP analysis, however, divided gymnotids into two large clades. The first clade contained the *Gymnotus anguillaris* clade, *Gymnotus coatesi* clade, and *Gymnotus pantherinus* as a polytomy. The second clade contained the *Gymnotus tigre* clade, the *Gymnotus cylindricus* clade, and the *Gymnotus carapo* clade. Both phylogenies agree on the relationships of the *Gymnotus tigre*, *Gymnotus cylindricus*, and *Gymnotus carapo* clades, but disagree on the arrangement of the three other clades.

Arrangement of Gymnotidae clades in previous studies interestingly show similar patterns of ambiguity as those found between the MP and BI phylogenies of this study. Since the designation of the *Gymnotus tigre* clade, all phylogenies have agreed that Gymnotidae contains the *Gymnotus tigre*, *Gymnotus cylindricus*, and *Gymnotus carapo* clades (Brochu, 2011; Tagliacollo et al., 2016). Placement of the other three clades, however, has differed greatly between all studies. Albert et al. (2005) placed the *Gymnotus cylindricus* clade as sister to all other gymnotids, and placed all other clades in a polytomy. Lovejoy et al. (2010) and Brochu (2011) both agreed on placing the *Gymnotus coatesi* clade as sister to all other gymnotids. Tagliacollo et al. (2016) placed *Gymnotus pantherinus* as sister lineage to all other Gymnotidae. The present study is the first to place the *Gymnotus anguillaris* clade as sister to all other gymnotids.

The intra-family relationships of Gymnotidae may be resolved through two strategies. The first is the inclusion of additional genes that provide deep-clade resolution within Gymnotidae. Each gene included in the present study (Figures 19-27) structures Gymnotidae differently; by including more slowly evolving nuclear genes, it may be possible to resolve these ambiguities. The second strategy is to increase taxon sampling within Gymnotidae. The *Gymnotus tigre*, *Gymnotus cylindricus*, and *Gymnotus carapo* clades all show consistent arrangement within Gymnotidae between studies. By including missing species of the ambiguous clades, i.e. the

Gymnotus anguillaris and *Gymnotus coatesi* clades, it is possible that key intermediary species may possess molecular characters that contribute to providing inter-clade resolution.

2.2 Rhamphichthyidae

The genera found within Rhamphichthyidae in this study included *Rhamphichthys*, *Gymnorhamphichthys*, *Hypopygus*, and *Steatogenys*. Both analyses placed *Steatogenys* and *Hypopygus* (as Steatogenae) as the sister lineage of remaining rhamphichthyids (*Rhamphichthys* + *Gymnorhamphichthys*). Together, the monophyletic *Rhamphichthys* and *Gymnorhamphichthys* form the Rhamphichthyinae. The inclusion of *Hypopygus* and *Steatogenys* within Rhamphichthyidae supported the second part of my second hypothesis, that *Hypopygus* and *Steatogenys* would be more closely related to rhamphichthyids than hypopomids.

This result disagrees with the previously held hypothesis that *Hypopygus* and *Steatogenys* nest within Hypopomidae, as presented by Ellis (1913), Triques (1913), Albert (2001), and Albert & Crampton (2005). Interestingly, Alves-Gomes et al. (1995) and Alves-Gomes (1999) found paraphyly in Rhamphichthyidae, placing *Gymnorhamphichthys* as sister to *Microsternarchus* and *Brachyhypopomus*; this result, however, was not supported in the current phylogeny. The results presented here do agree with those of Arnegard et al. (2010), Maldonado-Ocampo et al. (2013), and Tagliacollo et al. (2016).

The difference in placement of Steatogenae between older and more recent studies could be a result of homoplasy. All studies that grouped Steatogenae within Hypopomidae were constructed using a majority of morphological characters. Fishes of *Hypopygus* and *Steatogenys* share more morphological characters with *Akawaio*, *Brachyhypopomus*, *Hypopomus*, *Microsternarchus*, *Procerusternarchus*, and *Racenisia* than fishes of Rhamphichthyidae *sensu* Mago-Leccia (Mago-Leccia, 1994). The first two studies, however, to include a majority of mitochondrial molecular characters in reconstructing Gymnotiformes found paraphyly within Rhamphichthyidae, albeit a relationship unsupported in any subsequent phylogeny (Alves-Gomes et al., 1995; Alves-Gomes, 1999). Finally, when studies began to include nuclear and mitochondrial genes in analyses, a consistent placement of Steatogenae as sister to Rhamphichthyinae was recovered (Arnegard et al., 2010; Maldonado-Ocampo et al., 2013; Tagliacollo et al., 2016).

It is for these reasons that I propose the shared morphology of Steatogenae and Hypopomidae is a result of convergent evolution, and not shared ancestry. The exclusion of morphology from phylogenetic analyses allows molecular data to overcome the problem of morphological homoplasy. Owing to the support of constructed phylogenies in the present study and previous molecular phylogenies, I support the recommendation of Maldonado-Ocampo et al., (2013) that *Hypopygus* and *Steatogenys* be classified in Rhamphichthyidae.

2.3 Hypopomidae

The placement of *Akawaio penak* as sister to all other Hypopomids is supported by the first phylogeny constructed by Maldonado-Ocampo et al. (2014) to include this monotypic genus. This was further supported by Tagliacollo et al. (2016) who recovered the same result. In contrast Albert (2001) and Albert & Crampton (2005) placed *Hypopomus artedi* as sister lineage of Hypopomidae. These studies, however, predated the description of *Akawaio*, and therefore could not include it within their analyses.

The placement of *Brachyhypopomus*, the most speciose genus of Hypopomidae, as the sister to *Hypopomus* + *Racenisia* + *Procerusternarchus* + *Microsternarchus* is supported by Tagliacollo et al. (2016). This result, however, is not observed in any other phylogeny; in fact, the genus has typically been placed in a polytomy with the genera *Hypopomus* + *Racenisia* + *Procerusternarchus* + *Microsternarchus* (Albert, 2001; Albert & Crampton, 2005; Maldonado-Ocampo et al., 2014). This difference in phylogenetic position is possibly due to the inclusion of more nuclear genes in phylogenetic analyses. This study and that of Tagliacollo et al. (2016) included the most nuclear data with slower rates of evolution of any study to date. This may have provided deep-level phylogenetic resolution of the genera within Hypopomidae unavailable in all previous studies. It should be noted that the present study was first to include 19 soon to be described species of *Brachyhypopomus*, effectively more than doubling the amount of taxa previously included in phylogenetic analyses of this genus (Sullivan, 1997; Carvalho, 2013).

The arrangement of the remaining genera of Hypopomidae has historically been within a polytomy (Albert, 2001; Albert & Crampton, 2005; Maldonado-Ocampo et al., 2013). Tagliacollo et al. (2016), however, recovered the same topological arrangement of the genera as

the present study. These most recent studies present a more accurate arrangement of hypopomids.

The present study is the first to include molecular data for *Procerusternarchus pixuna*. This species was described in 2014, and tissues for DNA extraction were not available until recently. The original study describing *Procerusternarchus* used morphological characters to propose its phylogenetic placement as sister to a clade of *Racenisia* + *Microsternarchus* (Cox Fernandes et al., 2014). Also using morphology, Tagliacollo et al. (2016) placed *Procerusternarchus* as sister to only *Microsternarchus*, with this clade being sister to *Racenisia*. With molecular character support, this study agreed with the topology proposed by Tagliacollo et al. (2016), placing *Procerusternarchus* as sister to *Microsternarchus*.

Lastly, this study is the first to include four currently undescribed species of *Microsternarchus*, three of which were collected from the Tapajós River in Brazil. These species (*Microsternarchus* n. sp. AIGA, *Microsternarchus* n. sp. CIGA, and *Microsternarchus* n. sp. TAPA) formed a clade separate from *Microsternarchus bilineatus* and *Microsternarchus* n. sp. SMYT. This could indicate that sympatric speciation has occurred in the Tapajós River area, and suggests that actual diversity of Microsternarchus could be considerably greater.

2.4 Sternopygidae

The topology recovered here for relationships within Sternopygidae has not been recovered in previous studies; in fact, no two phylogenies of Gymnotiformes have ever recovered the same topology for Sternopygidae aside from those produced by the same investigator. Triques (1993) placed *Rhabdolichops* as sister to Sternopygidae, followed by a polytomy of all other genera. Alves-Gomes et al. (1995) and Alves-Gomes (1999) resolved this ambiguity and found *Archolaemus* to be sister to *Rhabdolichops* + Eigenmannia + *Distocyclus*. *Sternopygus* was not found to group within Sternopygidae, which is discussed in greater detail below. Albert (2001) and Albert & Crampton (2005), in contrast placed *Sternopygus* as sister to *Archolaemus* and a polytomy containing *Distocyclus*, *Eigenmannia*, and *Rhabdolichops*.

The topology of Sternopygidae presented in this study is the most robust of any to date owing to two factors. The first is that this study includes the largest amount of nuclear genes useful in providing deep-level phylogenetic support within Sternopygidae. The second factor is the

inclusion of more sternopygid species than any other study. I have included all recognized *Sternopygus* species, and the highest number of *Eigenmannia*, *Rhabdolichops*, and *Archolaemus* species of any study to date. With more species, it is possible I have included intermediary species useful in providing inter-genus phylogenetic resolution.

This study found strong support for the monophyly of Sternopygidae (including *Sternopygus*), in the BI. My third hypothesis, therefore is supported; *Sternopygus* and Eigenmanninae form a monophyletic Sternopygidae. This result agrees with those presented in Ellis (1913), Triques (1993), Gayet et al. (1994), Albert (2001), Albert & Crampton (2005), Arnegard et al. (2010), and Tagliacollo et al. (2016). However, not all phylogenies constructed for Gymnotiformes have found this monophyletic grouping. Alves-Gomes et al. (1995) and Alves-Gomes (1999) both proposed that *Sternopygus* formed a lineage not forming a sister-pair with all other sternopygids, and reclassified *Eigenmannia*, *Rhabdolichops*, *Archolaemus*, and *Distocyclus* into the family Eigenmanniidae (*Japigny* had not yet been described). Both of these studies constructed trees using a small number of mitochondrial genes. In the present study, individual gene trees for *co1* (Figure 19), *egr1* (Figure 21), *rag1* (Figure 24), *rh1* (Figure 26), and *zic1* (Figure 27) do not resolve a sister group relationship between *Sternopygus* and Eigenmanninae. If these genes had been the only ones included in my analyses, a monophyletic Sternopygidae would not have been resolved.

This is the first study to include molecular data for *Japigny kirschbaum*. The only other study to include *Japigny* was Tagliacollo et al. (2016) using morphological characters. They nested *Japigny* within *Eigenmannia*, creating paraphyly within *Eigenmannia*. Using molecular characters, the present study placed *Japigny* as sister group of sternopygids excluding *Sternopygus*. For this reason, I conclude that the inclusion of *Japigny* within *Eigenmannia* was a result of morphological homoplasy or homology.

The present study found a sister-pairing of *Rhabdolichops lundbergi* and *Rhabdolichops nigrimans* to nest within *Eigenmannia*. As this is the first phylogeny to include either species, it is not possible to compare these results with other studies. I suggest that a morphological investigation should be conducted to determine if these species require reclassification as *Eigenmannia*. This would restore the monophyly of *Eigenmannia*. *Rhabdolichops* cf *stewarti* was found nested within several specimens of *Rhabdolichops caviceps* in both the MP analysis

and the BI analysis. This could indicate either the specimen of *Rhabdolichops* cf *stewarti* is misidentified, and is in fact *Rhabdolichops caviceps*, or further investigation is required to determine if *Rhabdolichops stewarti* is a true recognized species.

2.5 Apteronotidae

This present study is the only one to recover the presented topology of Apteronotidae (Figure 18) of any to date; in fact, no two phylogenies of Gymnotiformes have ever recovered the same topology for Apteronotidae aside from those produced by the same investigator. Of all studies to date, the current phylogeny presents the most comprehensive and best resolved phylogeny of Apteronotidae as I have included more nuclear genes used in resolving deep-level phylogenies, and more taxa of each genus than any other previous study. Furthermore, this is the first study to include molecular data for *Megadontognathus* and *Magosternarchus*.

This study recovered the Apteronotini, Sternarchorhynchini, and the Navajini designated in Tagliacollo et al. (2016), but did not find all groups to be monophyletic. Regarding Apteronotini, all major clades (*Parapteronotus*, *Megadontognathus*, the *Apteronotus albifrons* clade, the *Apteronotus leptorhynchus* clade, and the *Apteronotus magdalenensis* clade) were recovered, but did not arrange them as done by Tagliacollo et al. (2016) (Figure 10). With increased taxon sampling and a larger molecular character set, this study placed the *Apteronotus magdalenensis* clade as sister to *Megadontognathus* + *Parapteronotus hasemani* + the *Apteronotus albifrons* clade. The *Apteronotus leptorhynchus* clade was not found to be monophyletic with Apteronotini, but constituted the sister lineage of Sternarchorhynchini + Navajini. Therefore, I propose that the *Apteronotus leptorhynchus* clade be designated as a distinct Leptorhynchini lineage separate from Apteronotini.

Sternarchorhynchini was also found to be paraphyletic in the present study. According to Tagliacollo et al. (2016), Sternarchorhynchini should consist of *Platyurosternarchus* as sister to all *Sternarchorhynchus* species. The present study, however, found *Platyurosternarchus* to be sister to both *Sternarchorhynchus* and Navajini, causing paraphyly within Sternarchorhynchini. The results presented in this study are more robust owing to the inclusion of more nuclear genes providing inter-clade resolution within Apteronotidae. To restore monophyly to this group, I propose the two species of *Platyurosternarchus* be designated as their own distinct

Platyurosternarchini lineage, sister to Sternarchorhynchini (consisting of only *Sternarchorhynchus*) and Navajini.

Unlike Apteronotini and Sternarchorhynchini, Navajini was found to be monophyletic in both this study and that of Tagliacollo et al. (2016). Internal structuring of this clade, however, showed some differences between studies. The present study found *Sternarchogiton preto* to be the sister lineage of Navajini, separate from other *Sternarchogiton* species. Navajini genera were arranged in three clades: *Sternarchella* + *Magosternarchus*, *Sternarchogiton* + *Compsaraia*, and *Porotergus* + *Apteronotus*. Tagliacollo et al. (2016) proposed a clade containing *Pariosternarchus* (using only morphological data) + *Sternarchella* + *Magosternarchus* as sister to all Navajini. They recovered monophyletic *Sternarchogiton*, *Compsaraia*, *Apteronotus*, and *Porotergus*. This studies results are less robust than the present study because taxa sampling of all genera was higher in the present study, and more nuclear genes were incorporated, providing higher inter-clade resolution of Navajini.

Unlike Taglicollo et al. (2016), almost all genera of Navajini were found to be paraphyletic. First and foremost was *Sternarchogiton preto* being separated as the sister lineage of Navajini. Owing to its unique phylogenetic position, *Sternarchogiton preto* should be reclassified into a new genus to restore the monophyly of *Sternarchogiton*. *Sternarchogiton*, however, requires another reclassification to restore monophyly. The *Sternarchogiton* + *Compsaraia* clade found *Compsaraia* nested within *Sternarchogiton*, causing paraphyly in *Sternarchogiton*. A simple reclassification of the undescribed *Sternarchogiton* n. sp. LONG to *Compsaraia* n. sp. LONG would restore monophyly to *Sternarchogiton*. The *Magosternarchus* + *Sternarchella* clade also display paraphyly as the two species of *Magosternarchus* are interspersed within *Sternarchella*. *Magosternarchus* does not constitute a valid genus designation; *Magosternarchus duccis* and *Magosternarchus raptor* should be reclassified as *Sternarchella* species.

As with the other clades of Navajini, *Apteronotus* and *Porotergus* also display polyphyly and paraphyly, respectively, owing to the three species of *Porotergus* being interspersed with *Apteronotus*. *Apteronotus*, in fact, displayed a great degree of polyphyly, being found in Apteronotini, the new Leptorhynchini *sensu* this study, and Navajini. To restore monophyly to this genus, reclassifications are required. *Apteronotus sensu stricto* of the Apteronotini should remain taxonomically unchanged as these species were used in the description of the genus.

Apteronotus of Leptorhynchini should be reclassified into a new genus as they form a monophyletic clade. Finally, *Apteronotus* of Navajini should be reclassified as *Porotergus* to restore monophyly to both *Apteronotus* and *Porotergus*. The present study found strong support for my fourth hypothesis, that *Apteronotus* was a polyphyletic genus found in several clades within Apteronotidae.

3 Future Directions

Although this study presents the most robust phylogeny of Gymnotiformes to date, some clades still demonstrate ambiguity. The most apparent example of this is the differing internal structuring of Gymnotidae between the MP analysis and the Bayesian inference. Future studies that construct Gymnotiformes phylogenies should include as many missing species as possible. Certain key intermediary species may possess molecular characters that contribute to providing higher resolution of the clades within each family. Of particular interest would be the inclusion of *Pariosternarchus, Iracema,* and *Tembeassu* as no study has ever included molecular data for these monotypic genera. Future phylogenies of Gymnotiformes should also focus on resolving the ambiguous placement of the families by including additional slowly evolving nuclear genes to provide deep-level phylogenetic resolution of the order.

In this study, all phylogenies were produced using a concatenated molecular character matrix. A criticism of phylogenetic analyses using concatenated data matrices involves discrepancies in tree topologies between the individual gene trees, and when compared to the resulting species tree (Drummond et al., 2012; Tonini et al., 2015; Edwards et al., 2016). One explanation for the observed discrepancies is incomplete lineage sorting (ILS; Maddison, 1997; Rogers & Gibbs, 2014). When an ancestral species possesses multiple alleles for a given character, it may pass on all possible alleles to both resulting descendants following a speciation event (Maddison, 1997; Rogers & Gibbs, 2014). All alleles may again be passed on in subsequent speciation events (Maddison, 1997; Rogers & Gibbs, 2014). ILS occurs when some, but not all, descendant species lose alleles at a given locus, as sometimes occurs with recombination of nrDNA (Maddison, 1997; Rogers & Gibbs, 2014). This results in species sharing allele-loss to be phylogenetically grouped, regardless of evolutionary history (Maddison, 1997; Rogers & Gibbs, 2014). Furthermore, occurrences of ILS will not be uniform amongst all gene trees, leading to discrepancies (Maddison, 1997; Rogers & Gibbs, 2014). Theoretical frameworks used to build

species-level phylogenies using concatenated data do not take ILS into account, which reduces the topological accuracy of the final phylogeny (Kubatko & Degnan, 2007; Thiergart et al., 2014; Tonini et al., 2015). Specifically, using a concatenated matrix may result in what is known as an anomaly zone, which occurs when a gene tree shows greater topological probability than a discrepant amalgamated species tree (Kubatko & Degnan, 2007; Edwards et al., 2016). The better supported gene tree is then erroneously favored and incorporated into the species tree topology, compromising its accuracy (Kubatko & Degnan, 2007; Edwards et al., 2016). The solution to this bias is the use of multispecies coalescent models, in which gene trees and the species tree are simultaneously analyzed using models that accommodate for ILS. This arguably produces phylogenies with greater topological accuracy by avoiding anomaly zones (Drummond et al., 2012; Tonini et al., 2015; Edwards et al., 2016). Multispecies coalescent models can also accommodate for introgression and lateral gene transfer (Kubatko & Degnan, 2007; Edwards et al., 2016).

Finally, future studies can make use of the Gymnotiformes phylogeny in understanding the evolution of character traits pertaining to physiology, biochemistry, behavior, genetics, and ontogeny. As previously stated, knifefishes present an excellent study system for understanding Neotropical speciation and diversification. With a phylogeny, scientists can investigate the factors which result in differing levels of diversity between genera and families, and between species occurring in different habitat types.

4 Conclusions

This study presented the most robust phylogeny of Gymnotiformes of any study to date. It included molecular data for 31 of the 34 recognized knifefish genera, and more species than any other study. Although most phylogenetic relationships within the order were strongly supported, there remained some ambiguity across analyses in the arrangement of families, and phylogenetic structure within Gymnotidae. A major unambiguous result, however, was the new placement of *Electrophorus* as the sister lineage of all other Gymnotiformes. *Electrophorus* was also found to group separately from Gymnotidae, supporting its reclassification within the family Electrophoridae.

Strong support was also found for the monophyletic superfamily Rhamphichthyoidea, consisting of Hypopomidae and Rhamphichthyidae. Within Rhamphichthyoidea, *Hypopygus* and *Steatogenys* (traditionally grouped within Hypopomidae) were placed within Rhamphichthyidae. *Sternopygus* was found to group within Sternopygidae, despite being morphologically distinct from other sternopygids. In this family, all genera were monophyletic with the exception of *Rhabdolichops lundbergi* and *Rhabdolichops nigrimans* nesting within *Eigenmannia*. Further investigation is required to evaluate the taxonomic status of these species. Within Apteronotidae, two new clades were recovered in addition to those designated in Tagliacollo et al. (2016). These clades were given the names Leptorhynchini (previously the *Apteronotus leptorhynchus* clade of Apteronotini), and Platyurosternarchini (previously grouped within Sternarchorhynchini). Finally, the genera of Navajini were found to be paraphyletic. To restore monophyly, I proposed *Magosternarchus* be subsumed within *Sternarchella*, *Compsaraia* be subsumed within *Sternarchogiton*, *Apteronotus* of Navajini be subsumed within *Porotergus*, and *Sternarchogiton preto* be given a new genus designation owing to its unique placement within Navajini.

References

Albert, J. S. 2001. Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei). Miscellaneous Publications Museum of Zoology, University of Michigan 190:1-127

Albert, J.S. 2003. Family Apterontoidae. In: [Reis, R.E., Kullander, S.O., & Ferraris, Jr., C.J. (eds)] Checklist of the Freshwater Fishes of South and Central America, pp. 503-508. Edipucrs, Porto Alegre, Brazil

Albert, J.S., & Crampton, G.R. 2005. Diversity and phylogeny of Neotropical electric fishes (Gymnotiformes). In: [Bullock, T.H., Hopkins, C.D., Popper, A.N., & Fay, R.R. (eds.)] Electroreception, Vol. 21, pp. 360-409. Springer Science, New York

Albert, J.S. & Crampton, G.R. 2006. *Pariosternarchus amazonensis*: A new genus and species of Neotropical electric fish (Gymnotiformes: Apteronotidae) from the Amazon River. Ichthyological Exploration of Freshwaters 17(3): 267-274

Albert, J.S., Crampton, W.G.R., Thorsen, D.H., & Lovejoy, N.R. 2005. Phylogenetic systematics and historical biogeography of the Neotropical electric fish *Gymnotus* (Teleostei: Gymnotidae). Systematics and Biodiversity 2(4): 375-417

Albert, J.S. & Fink, W.L. 2007. Phylogenetic relationships of fossil Neotropical electric fishes (Osteichthys: Gymnotiformes) from the Upper Miocene of Bolivia. Journal of Vertebrate Paleontology 27(1): 17-25

Alves-Gomes, J.A. 1999. Systematic biology of Gymnotiform and Mormyriform electric fishes: Phylogenetic relationships, molecular clocks and rates of evolution in the mitochondrial rRNA genes. The Journal of Experimental Biology 202: 1167-1183

Alves-Gomes, J.A. Ortí, G., Haygood, M., Heiligenberg, W., & Meyer, A. Phylogenetic analysis of the South American electric fishes (Order Gymnotiformes) and the evolution of their electrogenic system: A synthesis based on morphology, electrophysiology, and mitochondrial sequence data. Molecular Biology and Evolution 12(2): 298-318

Arnason, U., Gullberg, A., & Janke, A. 1999. The mitochondrial DNA molecule of the aardvark, *Orycteropus afer*, and the position of the Tubulidentata in the eutherian tree. Proceedings of the Royal Society of London 266: 339-345

Arnegard, M.E., Zwickl, D.J., Lu, Y., & Zakon, H.H. 2010. Old gene duplication facilitates origin and diversification of an innovative communication system – twice. Proceedings of the National Academy of Science doi: 10.1073/pnas.1011803107

Baum, D.A. & Smith, S.D. 2013. Tree thinking: An introduction to phylogenetic biology. Roberts & Company, Colorado, USA

Bergsten, J. 2005. A review of long-branch attraction. Cladistics 21: 163–193

Betancur-R. R., Broughton, R.E., Wiley, E.O., Carpenter, K., López, J.A., Li, C., Holcroft, N.I., Arcila, D., Sanciangco, M., Cureton, II, J.C., Zhang, F., Buser, T., Campbell, M.A., Ballesteros, J.A., Roa-Varon, A., Willis, S., Borden, W.C., Rowley, T., Reneau, P.C., Hough, D.J., Lu, G., Grande, T., Arratia, G., & Ortí, G. 2013. The tree of life and a new classification of bony fishes. PLOS Currents Tree of Life Edition 1. doi: 10.1371/currents.tol.53ba26640df0ccaee75bb

Bichuette, M.E. & Trajano, E. 2015. Population density and habitat of an endangered cave fish *Eigenmannia vicentespelaea* Triques, 1996 (Ostariophysi: Gymnotiformes) from a karst area in central Brazil. Neotropical Ichthyology 13(1): 113-122

Birky, C.W. 2008. Uniparental inheritance of organelle genes. Current Biology 18: R692-R695

Brochu, K. 2011. Molecular phylogenetics of the Neotropical electric knifefish genus *Gymnotus* (Gymnotidae, Teleostei): Biogeography and signal evolution of the trans-Andean species. Masters thesis. University of Toronto, Canada

Campos-da-Paz, R. 2005. Previously undescribed dental arrangement among electric knifefishes, with comments on the taxonomic and conservation status of Tembeassu marauna Triques (Otophysi: Gymnotiformes: Apteronotidae). Neotropical Ichthyology 3(3): 395-400

Carvalho, T.P. 2013. Systematics and evolution of the toothless knifefishes Rhamphichthyoidea Mago-Leccia (Actinopterygii: Gymnotiformes): Diversification in South American freshwaters. Doctoral Thesis. University of Louisiana at Lafayette, USA

Carvalho, T.P. & Albert, J.S. 2011. Redescription and phylogenetic position of the enigmatic Neotropical electric fish *Iracema caiana* Triques (Gymnotiformes: Rhamphichthyidae) using x-ray computed tomography. Neotropical Ichthyology 9(3): 457-469

Chen, W-J., Bonillo, C., & Lecointre, G. 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Molecular Phylogenetics and Evolution 26: 262-288

Chen, W-J., Lavoué, S., & Mayden, R.L. 2013. Evolutionary origin and early biogeography of Otophysan fishes (Ostariophysi: Teleostei). Evolution 67(8): 2218-2239

Chen, W-J., Miya, M., Saitoh, K., & Mayden, R.L. 2008. Phylogenetic utility of two existing and four novel nuclear gene loci in reconstructing Tree of Life of ray-finned fishes: The order Cypriniformes (Ostariophysi) as a case study. Gene 423: 125-134

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgens, D.G., & Thompson, J.D. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31(13): 3497-3500

Chiari, Y., van der Meijden, A., Madsen, O., Vences, M., Meyer, A. 2009. Base composition, selection, and phylogenetic significance of indels in the recombination activating gene-1 in vertebrates. Frontiers in Zoology 6: 32-46

Cloutier, R. & Arratia, G. 2004. Early diversification of actinopterygians. In: [Arratia, G., Wilson, M.V.H., & Cloutier, R. (eds.)] Recent advances in the origin and early radiation of vertebrates, pp. 217-270. Verlag Dr. Friedrich Pfeil, München

Cox Fernandes, C., Nogueira, A., & Alves-Gomes, J.A. 2014. *Procerusternarchus pixuna*, a new genus and species of electric knifefish (Gymnotiformes: Hypopomidae, Microsternarchini) from the Rio Negro, South America. Proceedings of the Academy of Natural Sciences of Philadelphia 163: 95-118

Crampton, W.G.R. 2011. An ecological perspective on diversity and distributions. In: [Albert, J.S. & Reis, R.E. (eds.)] Historical biogeography of Neotropical freshwater fishes, pp. 165-189. University of California Press, Berkeley & Los Angeles

Crampton, W.G.R., Lovejoy, N.R., & Waddell, J.C. 2011. Reproductive character displacement and signal ontogeny in a sympatric assemblage of electric fishes. Evolution 65(6): 1650-1666

Crampton, W.G.R., Rodríguez-Cattáneo, A., Lovejoy, N.R., & Caputi, A.A. 2013. Proximate and ultimate causes of signal diversity in the electric fish *Gymnotus*. Journal of Experimental Biology 216(13): 2523–2541

Drummond, A.J., Suchard, M.A., Xie, D., & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29(8): 1969-1973

Dunlap, K.D., DiBenedictis, B.T., and Banever, S.R. 2010. Chirping response of weakly electric knife fish (*Apteronotus* leptorhynchus) to low-frequency electric signals and to heterospecific electric fish. The Journal of Experimental Biology 213: 2234-2242

Dyer, K.A., White, B.E., Sztepanacz, J.L., Bewick, E.R., & Rundle, H.D. 2013. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. Evolution 68(4): 1163-1175

Edwards, S.V., Xi, Z., Janke, A., Faircloth, B.C., McCormack, J.E., Glenn, T.C., Zhong, B., Wu, S., Moriarty Lemmon, E., Lemmon, A.R., Leaché, A.D., Liu, L., & Davis, C.C. 2016. Implementing and testing the multispecies coalescent model: A valuable paradigm for phylogenomics. Molecular Phylogenetics and Evolution 94: 447-462

Ellis, M.M. 1913. The gymnotid eels of tropical America. In: Memoires of the Carnegie Museum, pp. 112-113, 197. Carnegie Museum, Pittsburgh

Farris, J.S. 1989. The retention index and the rescaled consistency index. Cladistics 5: 417-419

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist 125: 1-15

Ferreira, L.V., Soares de Almeida, S., & Parolin, P. 2010. Amazonian white- and blackwater floodplain forests in Brazil: large differences on a small scale. Ecotropica 16: 31-41

Gayet, M., Meunier, F.J. & Kirschbaum F. 1994. *Ellisella kirschbaum* Gayet & Manuel-, 1991, gymnotiformes fossile de Bolivie et ses relations phylogenetiques au sein des formes actuelles. Cybium 18(3): 273-306

Ho, W.W., Turner, C.R., Formby, K.J., & Smith, G.T. 2013. Sex differences in the electrocommunication signals of *Sternarchogiton nattereri* (Gymnotiformes: Apteronotidae). Japan Ethological Society 31: 335-340

Hopkins, C. D. 1974. Electric communication: Functions in the social behaviour of *Eigenmannia virescens*. Behaviour 50:270-305

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647-1649

Kubatko, L.S. & Degnan, J.H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology 56(1): 17-24

Kuo, L-Y., F-W. Li, W-L. Chiou, & C-N. Wang. 2011. First insights into fern matK phylogeny. Molecular Phylogenetics and Evolution 59: 556-566

Lanfear, R., Calcott, B., Ho, S.Y.W., & Guindon, S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701

Lavoué, S., Miya, M., Arnegard, M.E., Sullivan, J.P., Hopkins, C.D., & Nishida, M. 2012. Comparable ages for the independent origins of electrogenesis in African and South American weakly electric fishes. Public Library of Science ONE 7(5): e36287 doi: 10.1371/journal.pone.0036287

Li, C., Ortí, G., Zhang, G., & Lu, G. 2007. A practical approach to phylogenomics: The phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evolutionary Biology 7: 44-54

López, J.A., Chen W.-J., & Ortí G. 2004. Esociform phylogeny. Copeia 3: 449–464

Lovejoy, N.R. 2000. Reinterpreting recapitulation: Systematics of needlefishes and their allies (Teleostei: Beloniformes). Evolution 54(4): 1349-1362

Lovejoy, N.R. & Collette, B.B. 2001. Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. Copeia 2: 324-338

Lovejoy, N.R., Lester, K., Crampton, W.G.R., Marques, F.P.L., & Albert, J.S. 2010. Phylogeny, biogeography, and electric signal evolution of Neotropical knifefishes of the genus Gymnotus (Osteichthyes: Gymnotidae). Molecular Phylogenetics and Evolution 54: 278-290

Lundberg, J.G., Cox Fernandes, C., Albert, J.S., & Garcia, M. 1996. *Magosternarchus*, a new genus with two new species of electric fishes (Gymnotiformes: Apteronotidae) from the Amazon River basin, South America. Copeia 3: 657-670

Maddison, W.P. 1997. Gene trees in species trees. Systematic Biology 46(3): 523-536

Mago-Leccia, F. 1994. Electric fishes of the continental waters of America. FUDECI, Caracas

Maldonado-Ocampo, J.A., López-Fernández, H., Taphorn, D.C., Bernard, C.R., Crampton, W.G.R., & Lovejoy, N.R. 2014. *Akawaio penak*, a new genus and species of Neotropical electric fish (Gymnotiformes, Hypopomidae) endemic to the upper Mazaruni River in the Guiana Shield. The Norwegian Academy of Science and Letters 43(1): 24-33

Merl, D., Escalante, A., & Prado, R. 2005. Comparison of Bayesian, maximum likelihood and parsimony methods for detecting positive selection. Report No. AMS2005-03

Meunier, F.J., Jégu, M., & Keith, P. 2011. A new genus and species of Neotropical electric fish, *Japigny kirschbaum* (Gymnotiformes: Sternopygidae), from French Guiana. Cybium 35(1): 47-53

Mora A., Baby, P., Roddaz, M., Parra, M., Brusset, S., Hermoza, W., & Espurt, N. 2010. Tectonic history of the Andes zones: Implications for the development of the Amazon drainage basin. In: [Hoorn, C. & Wesselingh, F.P. (eds.)] Amazonia: Landscape and species evolution, edition 1: pp. 38-57. Wiley-Blackwell, West Sussex

Nelson, J.S. 2006. Fishes of the world, 4th edition. John Wiley & Sons, New Jersey

Nicolas, V., Schaeffer, B., Missoup, A.D., Kennis, J., Colyn, M., Denys, C., Tatard, C., Cruaud, C., & Laredo, C. 2012. Assessment of Three Mitochondrial Genes (16S, Cytb, CO1) for Identifying Species in the Praomyini Tribe (Rodentia: Muridae). Public Library of Science ONE 7(5): e36586. doi:10.1371/journal.pone.0036586

Palumbi, S. R. 1996. Nucleic acids II: The polymerase chain reaction. In: [Hillis, D. M., Moritz, C., & Mable, B. K. (eds.)] Molecular systematics second edition, pp. 205-247. Sinauer Associates, Inc., Sunderland

Palumbi, S. R., Martin, A., Romano, S., McMillan, W.O., Stice, L., & Grabowski, G. 1991. The Simple Fool's Guide to PCR. University of Hawaii Press, Honolulu

Pedraja, F., Aguilera, P., Caputi, A.A., & Budelli, R. 2014. Electric imaging through evolution, a modeling study of commonalities and differences. Public Library of Science ONE 10(7): e1003722. doi:10.1371/journal.pcbi.1003722

Pfennig, K.S. & Pfennig, D.W. 2009. Character displacement: Ecological and reproductive responses to a common evolutionary problem. The Quarterly Review of Biology 84(3): 253-276

Praz, C.J. & Packer, L. 2014. Phylogenetic position of the bee genera *Ancyla* and *Tarsalia* (Hymenoptera: Apidae): A remarkable base compositional bias and an early Paleogene geodispersal from North America to the Old World. Molecular Phylogenetics and Evolution 81: 258-270

Prychitko, T.M. & Moore, W.S. 2000. Comparative evolution of the mitochondrial cytochrome *b* gene and nuclear b-Fibrinogen intron 7 in woodpeckers. Molecular Biology and Evolution 17(7): 1101-1111

Rambaut, A. & Drummond, A.J. 2007. Tracer v1. 4. Retrieved from http://beast.bio.ed.ac.uk/Tracer

Rice, A.M. & Pfennig, D.W. 2007. Character displacement: *in situ* evolution of novel phenotypes or sorting of pre-existing variation? Journal of Evolutionary Biology 20: 448-459

Rogers, J. & Gibbs, R.A. 2014. Comparative primate genomics: Emerging patterns of genome content and dynamics. Nature Reviews Genetics 15: 347-359

Ronquist, F., & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574

Russell, P.J., S.L. Wolfe, P.E. Hertz, C. Starr, M.B. Fenton, H. Addy, D. Maxwell, T. Haffie, & K. Davey. 2010. In: Biology, Exploring the Diversity of Life, pp. 349-352, 429-431. Nelson Education Ltd., USA.

Salazar, V.L., Krahe, R., & Lewis, J.E. 2013. The energetics of electric organ discharge generation in gymnotiform weakly electric fish. The Journal of Experimental Biology 216: 2459-2468

Sevilla, R.G., Diez, A., Norén, M., Mouchel, O., Jérôme, M., Verrez-Bagnis, V., Van Pelt, H., Favre-Krey, L., Krey, G., & Bautista, J.M. 2007. Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome *b* and nuclear rhodopsin genes. Molecular Ecology Notes 7: 730-734

Stoddard, P.K. 1999. Predation enhances complexity in the evolution of electric fish signals. Nature 400: 254-256

Stoddard, P.K. 2002. Electric signals: Predation, sex, and environmental constraints. Advances in the Study of Behavior 31: 201-242

Stoddard, P.K., & Markham, M.R. 2008. Signal cloaking by electric fish. BioScience 58 (5): 415-425

Sullivan, J.P. 1997. A phylogenetic study of the Neotropical Hypopomid electric fishes (Gymnotiforme: Rhamphichthyoidea). Doctoral Thesis. Duke University, USA

Swofford, D.L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tagliacollo, V.A., Bernt, M.J., Craig, J.M., Oliveira, C., & Albert, J.S. 2016. Model-based total evidence phylogeny of Neotropical electric knifefishes (Teleostei, Gymnotiformes). Molecular Phylogenetics and Evolution 95: 20-33

Thiergart, T., Landan, G., & Martin, W.F. 2014. Concatenated alignments and the case of the disappearing tree. BMC Evolutionary Biology 14: 266-277

Tonini, J., Moore, A., Stern, D., Shcheglovitova, M., & Ortí, G. 2015. Concatenation and species tree methods exhibit statistically indistinguishable accuracy under a range of simulated conditions. PLOS Currents Tree of Life Edition 1. doi: 10.1371/currents.tol.34260cc27551a527b124ec5f6334b6be

Triques, M.L., 1993. Filogenia dos gêneros de Gymnotiformes (Actinopterygii, Ostariophysi), com base em caracteres esqueléticos. Comunicações do Museu de Ciências da PUCRS, Série Zoologia 6: 85-130

USB Corporation. 2006. ExoSAP-IT – PCR Clean-Up Reagent [Internet]. https://www.biolynx.ca/pdf/admin/ub_exosapitoverview.pdf . Date accessed: Aug 03, 2016.

Vaidya, G., Lohman, D.J., & Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27: 171-180

Vari, R.P., Malabarba, L.R., Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S. & Lucena, C.A.S. 1998. Phylogeny and classification of Neotropical fishes. Phylogeny and classification of Neotropical fishes. Edipucrs, Porto Alegre, Brazil

Ward, R.D., Hanner, R., & Hebert, P.D.N. 2009. The campaign to DNA barcode all fishes, FISH-BOL. Journal of fish Biology 74: 329-356

Watanabe, K., N. Emoto, M. Sunohara, M. Kawakami, H. Kage, T. Nagase, N. Ohishi, & D. Takai. 2010. Treatment of PCR products with exonuclease I and heat-labile alkaline phosphatase improves the visibility of combined bisulfite restriction analysis. Biochemical and Biophysical Research Communications 399: 422-424

Westby, G.W.M. 1981. Communication and jamming avoidance in electric fish. Trends in Neurosciences 4: 205-210

Westby, G.W.M. 1988. The ecology, discharge diversity and predatory behaviour of gymnotiforme electric fish in the coastal streams of French Guiana. Behavioural Ecology and Sociobiology 22: 341-354

Yunoki, T. & Velasco, L.T. 2016. Fish metacommunity dynamics in the patchy heterogeneous habitats of varzea lakes, turbid river channels and transparent clear and black water bodies in the Amazonian Lowlands of Bolivia. Environmental of Fishes 99: 391-408

Zupanc, G.K.H. & Bullock, T.H. 2005. From electrogenesis to electroreception: An overview. In: [Bullock, T.H., Hopkins, C.D., Popper, A.N., & Fay, R.R. (eds.)] Electroreception, Vol. 21, pp. 5-46. Springer Science, New York.

Figure 1: Geographical distribution of Gymnotiformes. Range of the order extends from southern Mexico to northern Argentina, as depicted in green. Modified from Chen et al. (2013).

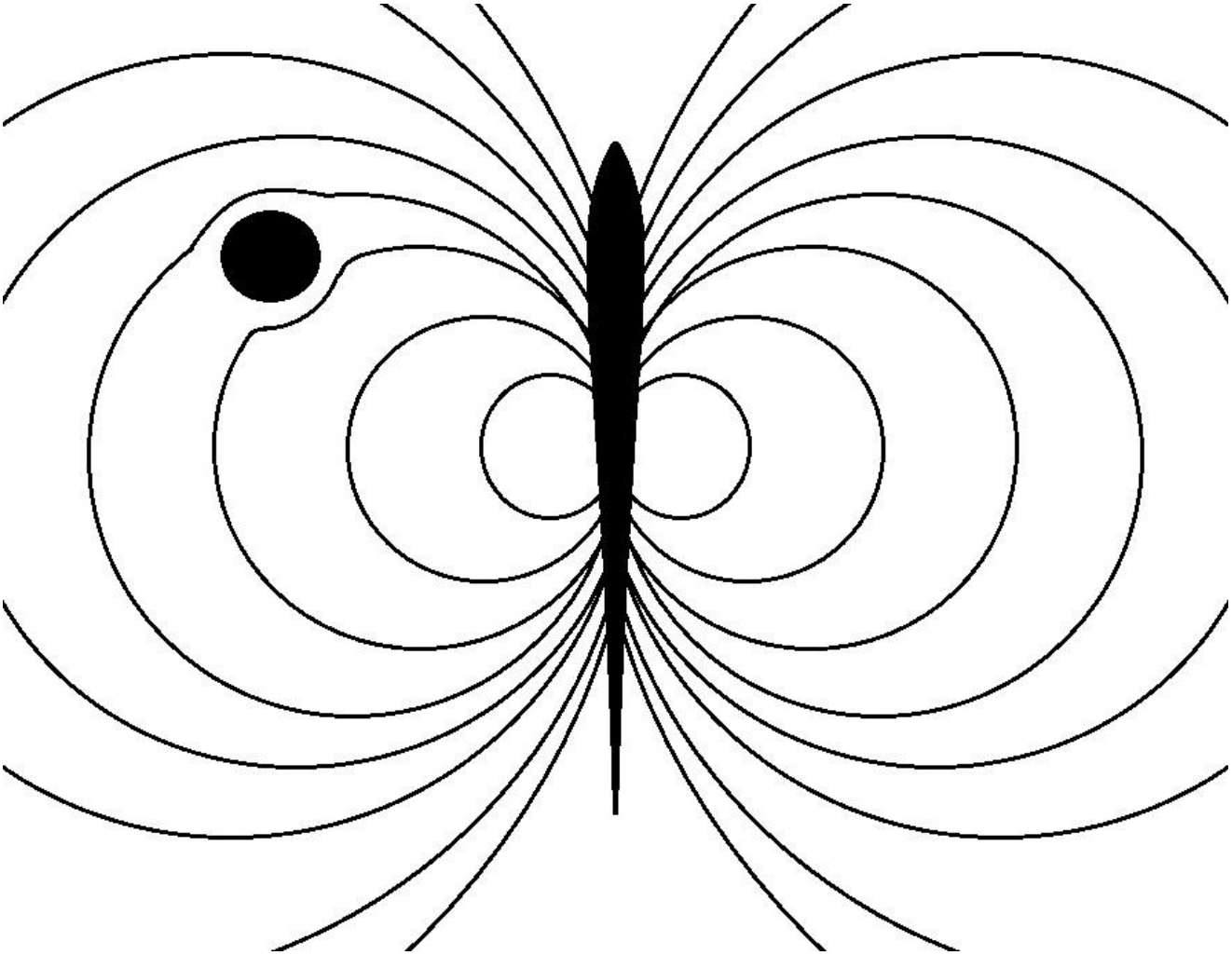


Figure 2: Visual depiction of the electric field produced by Gymnotiformes fishes. As an object enters the field, the path of electricity becomes distorted. The distortion is detected by the fish's electroreceptors, allowing it to sense the object. Modified from Stoddard (2002).

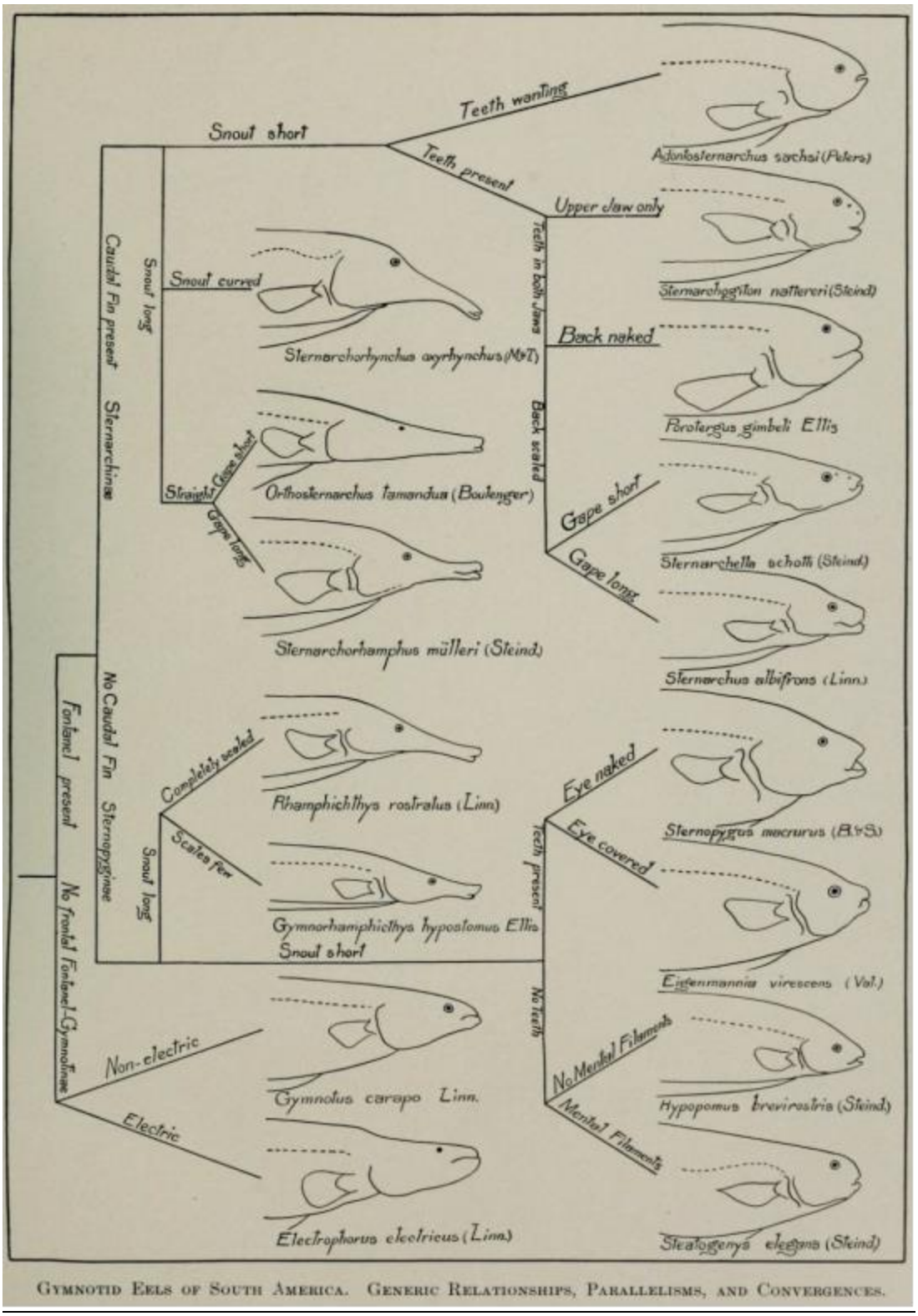


Figure 3: First explicit phylogeny of Gymnotiformes based on gross morphology. Gymnotidae including *Electrophorus* and *Gymnotus* constitutes the sister lineage of all other knifefishes. Taken from Ellis (1913).

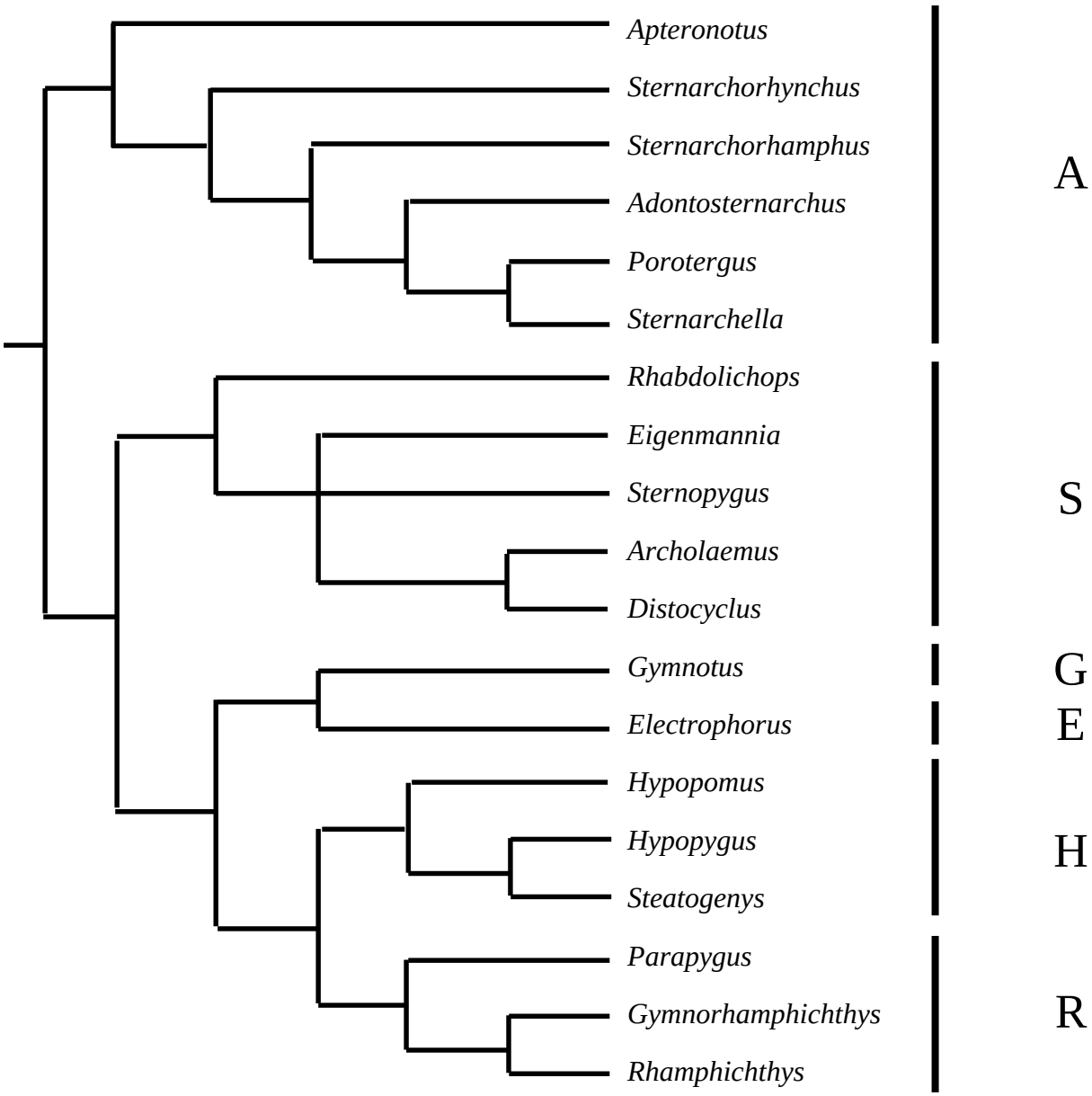


Figure 4: Phylogenetic tree of the Gymnotiformes constructed using gross morphology character data. Letters represent families as follows: A = Apteronotidae, S = Sternopygidae, G = Gymnotidae, E = Electrophoridae, H = Hypopomidae, R = Rhamphichthyidae. Modified from Triques (1993).

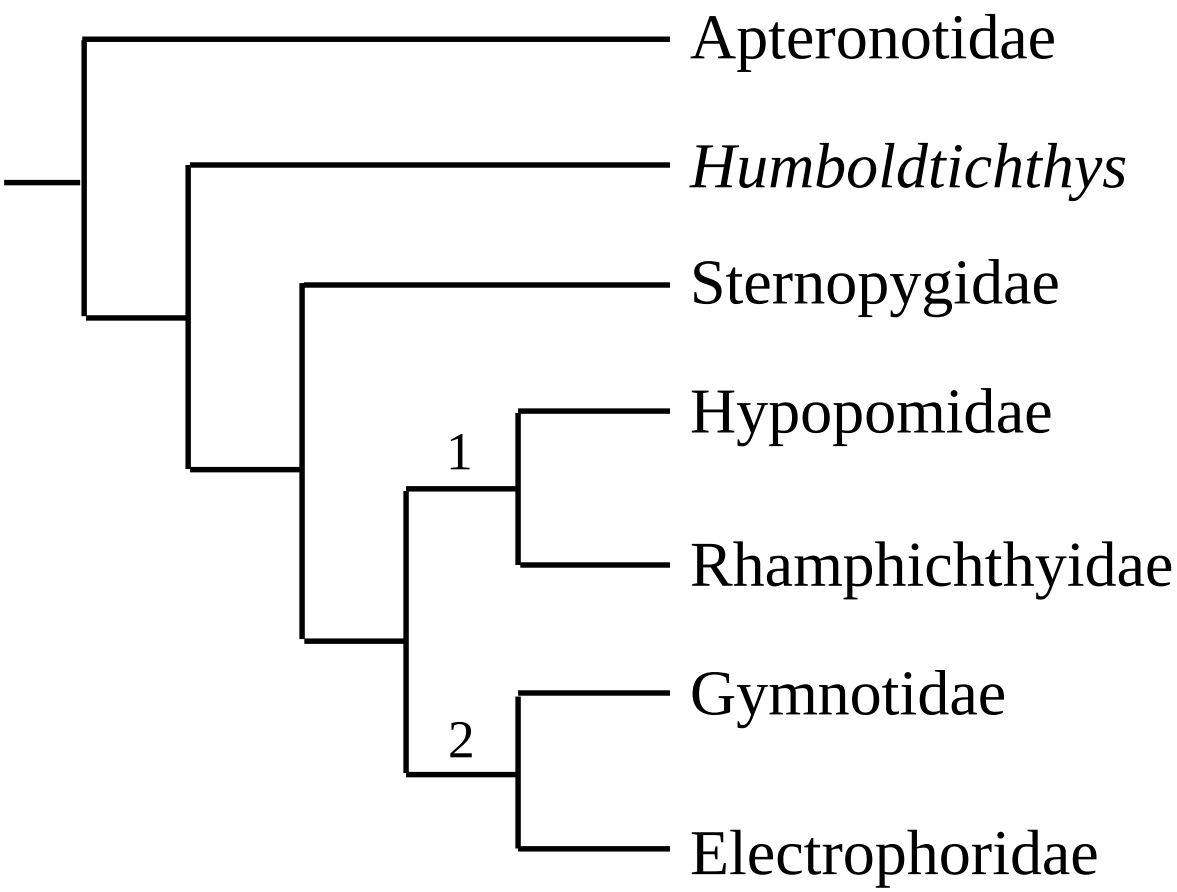


Figure 5: Phylogenetic tree of the Gymnotiformes constructed using morphological, anatomical, and physiological characters, and including the fossil genus *Humboldtichthys*. Hypopomidae and Rhamphichthyidae form the super family Rhamphichthyoidea (1), and Gymnotidae and Electrophoridae form the super family Gymnotoidea (2). Modified from Gayet et al. (1994).

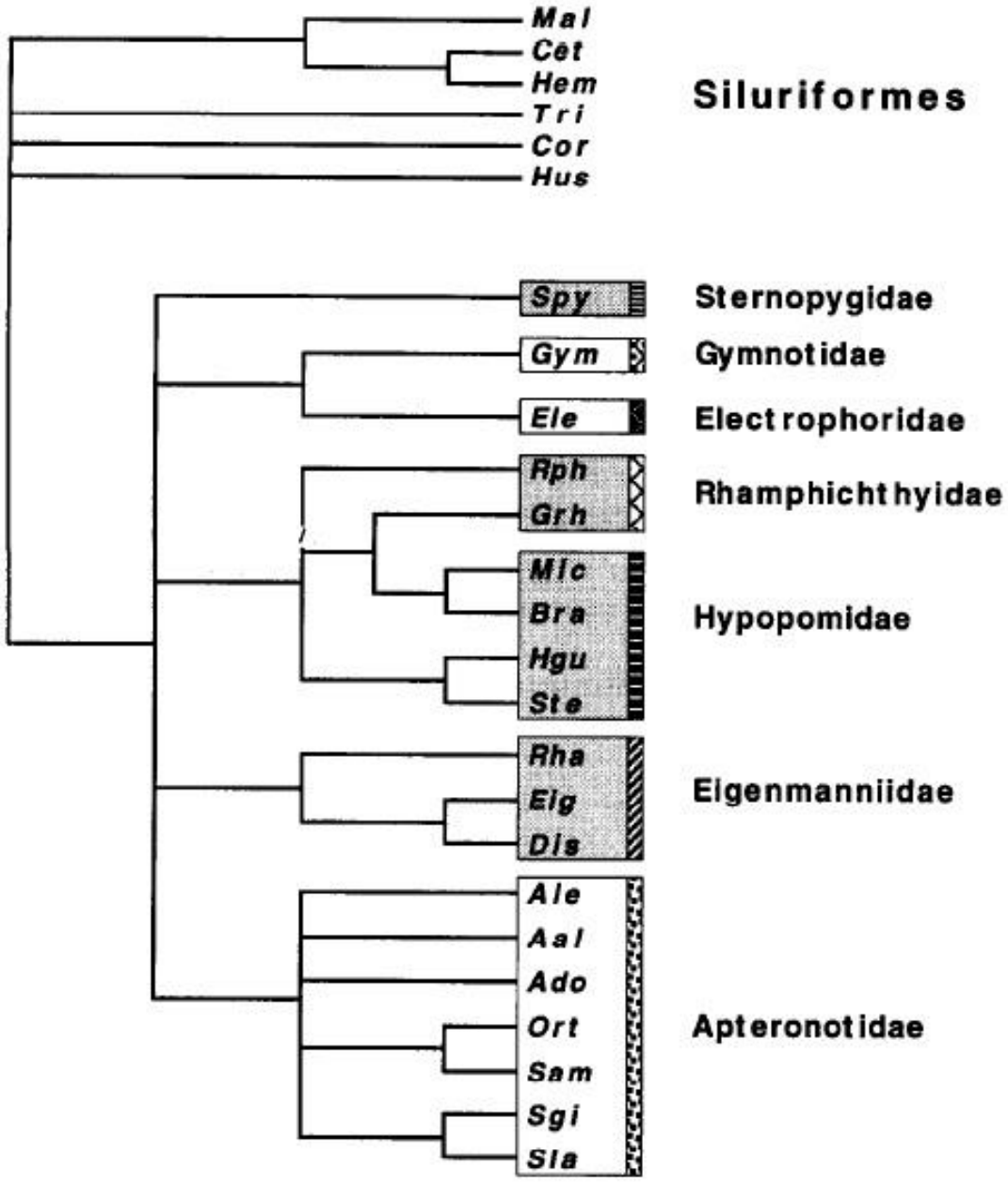


Figure 6: Maximum-parsimony phylogeny of Gymnotiformes constructed using mitochondrial molecular data of 12S and 16S. Three-letter codes used to represent genera are as follows: Mal = *Malapterurus*, Cet = *Cetopsis*, Hem = *Hemicetopsis*, Tri = *Trichomycterus*, Cor = *Corydoras*, Hus = *Hypostomus*, Spy = *Sternopygus*, Gym = *Gymnotus*, Ele = *Electrophorus*, Rph = *Rhamphichthys*, Grh = *Gymnorhamphichthys*, Mic = *Microsternarchus*, Bra = *Brachyhypopomus*, Hgu = *Hypopygus*, Ste = *Steatogenys*, Rha = *Rhabdolichops*, Eig = *Eigenmannia*, Dis = *Distocyclus*, Ale = *Apteronotus leptorhynchus*, Aal = *Apteronotus albifrons*, Ado = *Adontosternarchus*, Ort = *Orthosternarchus*, Sam = *Sternarchorhamphus*, Sgi = *Sternarchogiton*, Sla = *Sternarchella*. Taken with permission from Alves-Gomes et al. (1995).

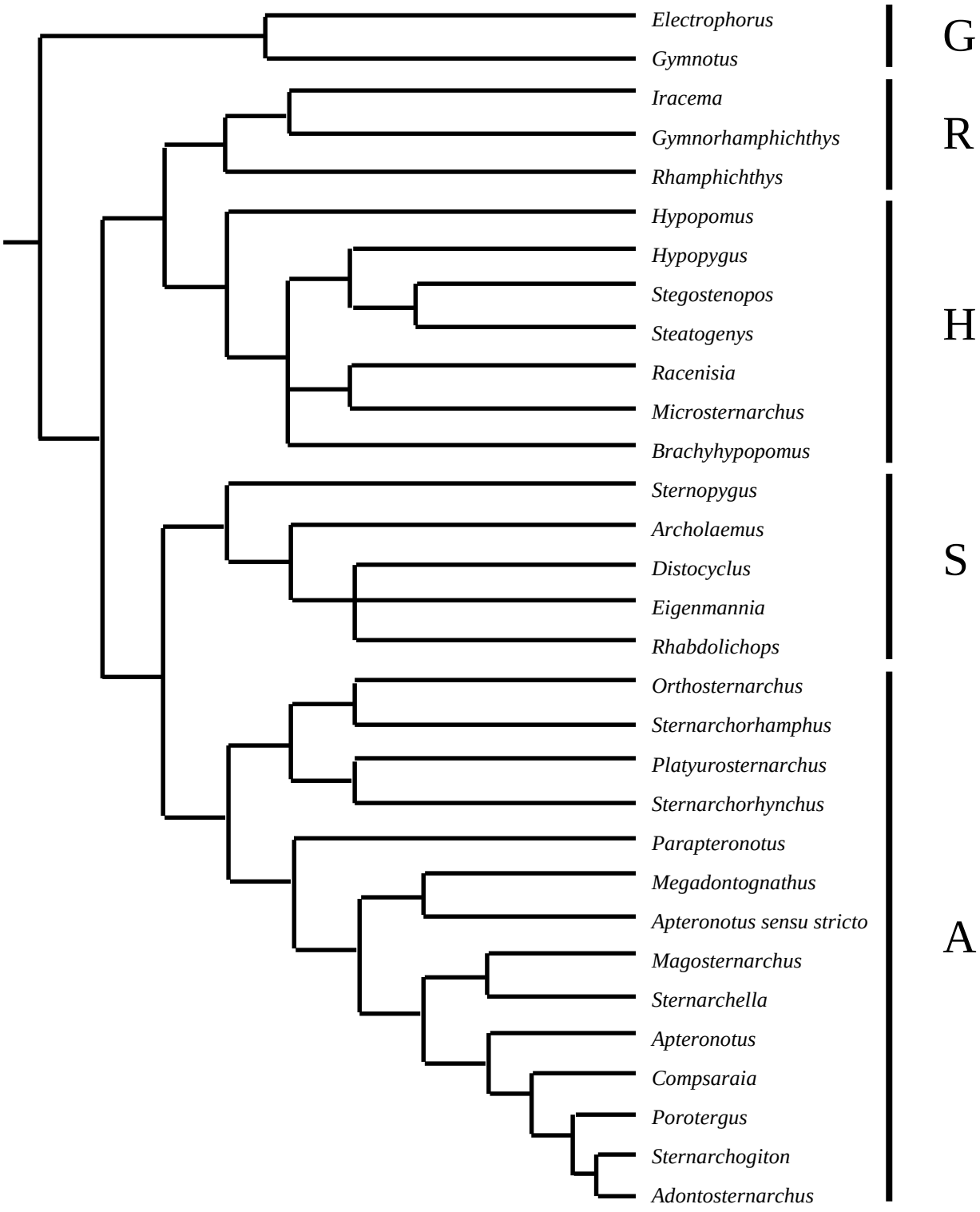


Figure 7: Gymnotiformes phylogeny with composite topology from several previous studies. Letters represent families as follows: G = Gymnotidae, R = Rhamphichthyidae, H = Hypopomidae, S = Sternopygidae, A = Apteronotidae. Modified from Albert & Crampton (2005).

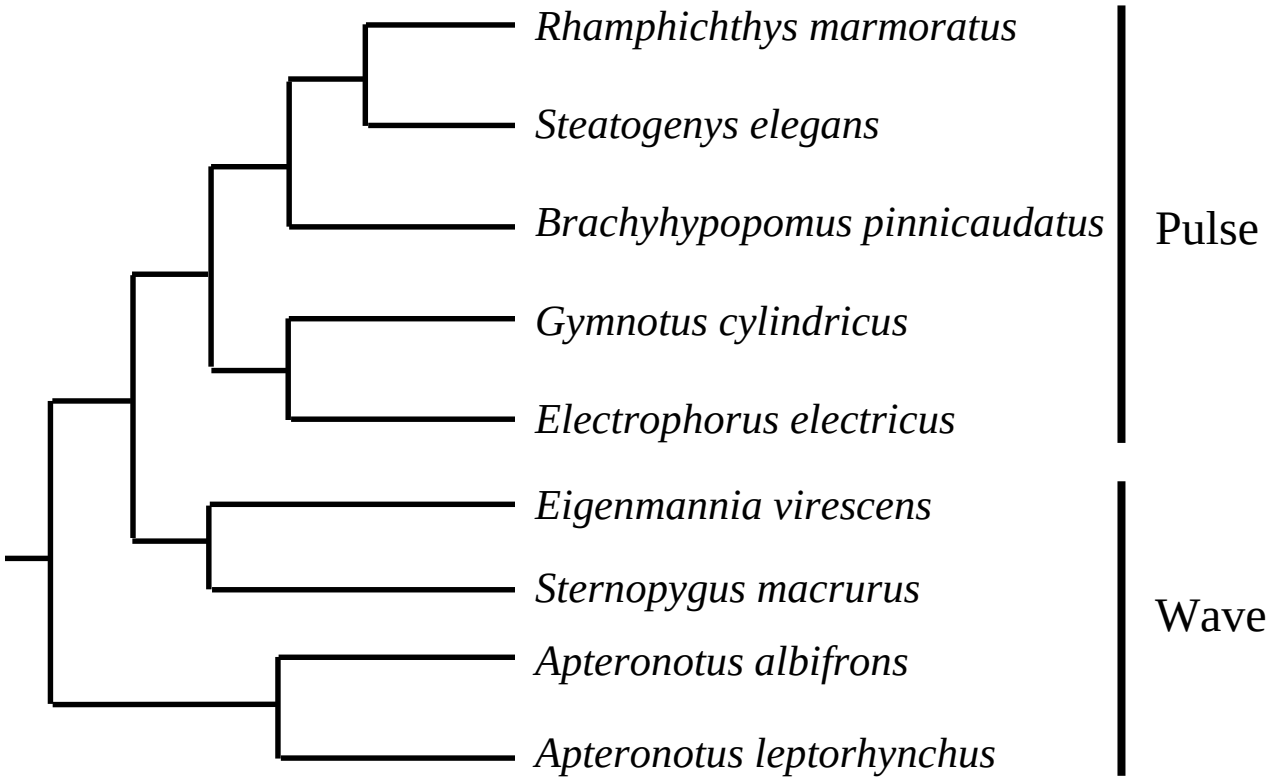


Figure 8: Gymnotiformes phylogeny constructed using molecular characters from *Scn4aa* and *Scn4ab*. Apteronotidae represents the sister lineage of all other knifefishes. Modified from Arnegard et al. (2010).

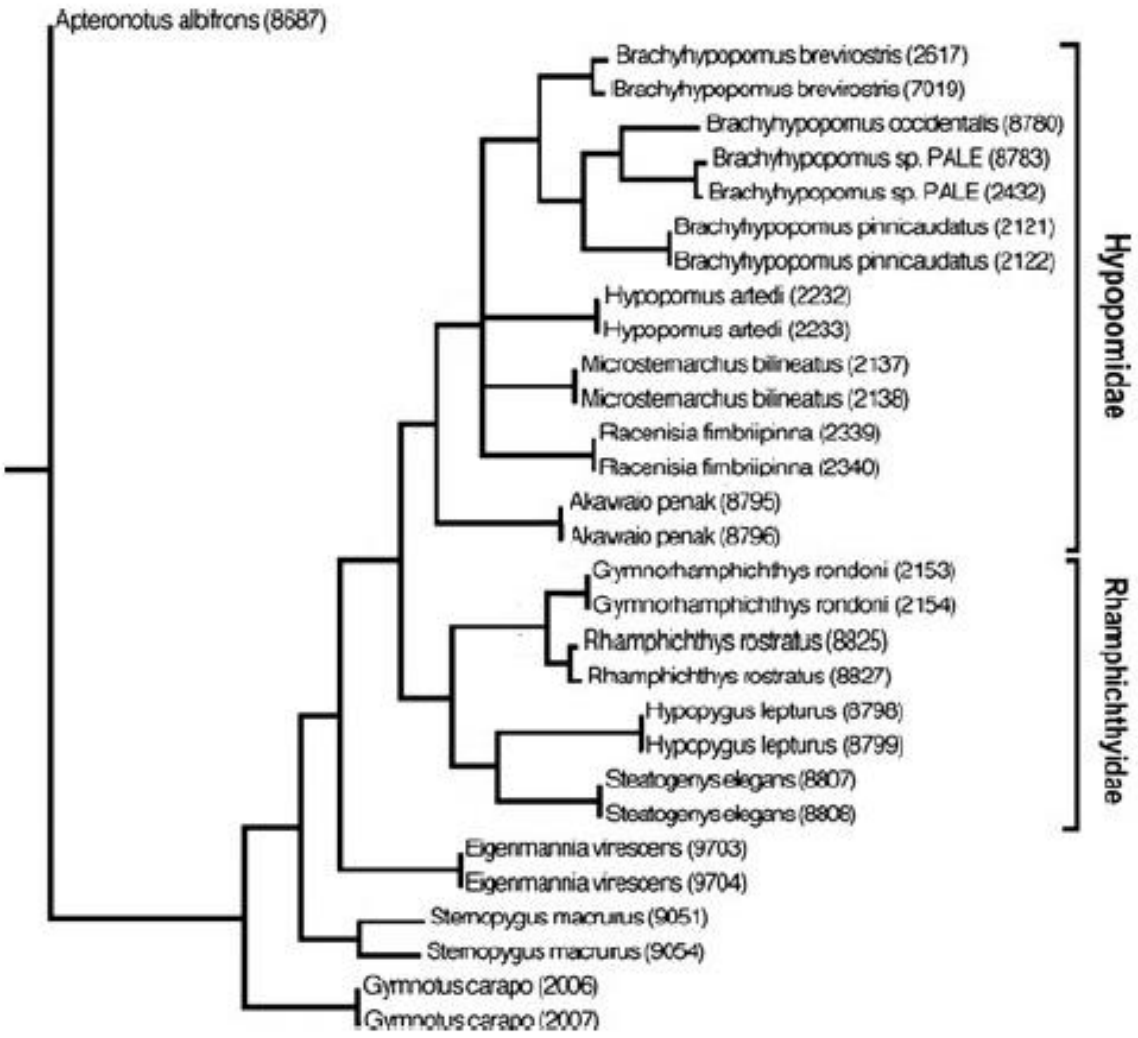


Figure 9: Maximum-parsimony phylogeny of Gymnotiformes constructed using mitochondrial molecular data of *cyt b*, *co1*, and nuclear molecular data of *rag2*. *Hypopygus* and *Steatogenys* (traditionally grouped within Hypopomidae) are reclassified as Rhamphichthyidae. Taken with permission from Maldonado-Ocampo et al. (2014).

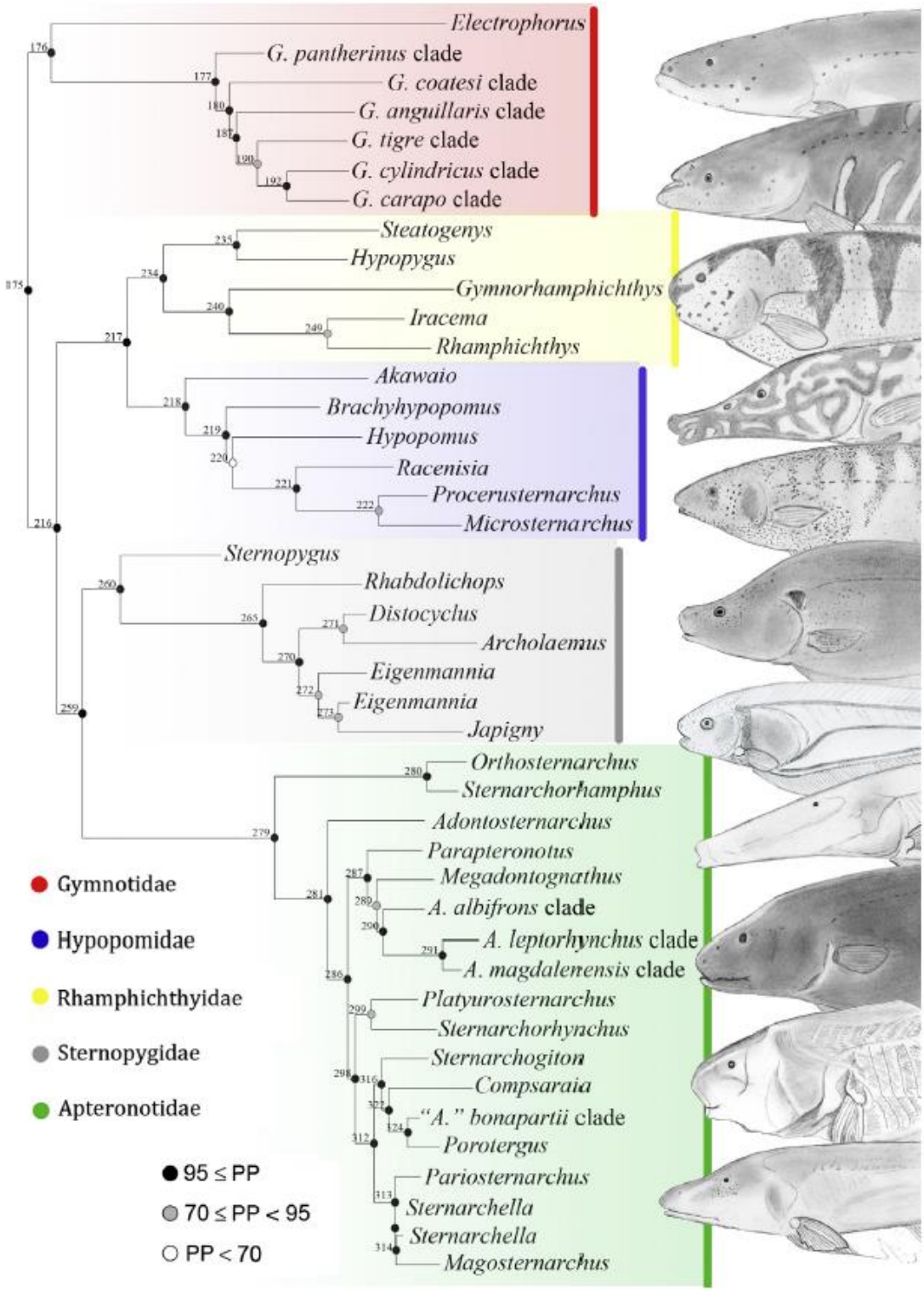


Figure 10: Combined maximum-likelihood and Bayesian inference phylogeny of Gymnotiformes constructed using molecular and morphological characters. Taken with permission from Tagliacollo et al. (2016).

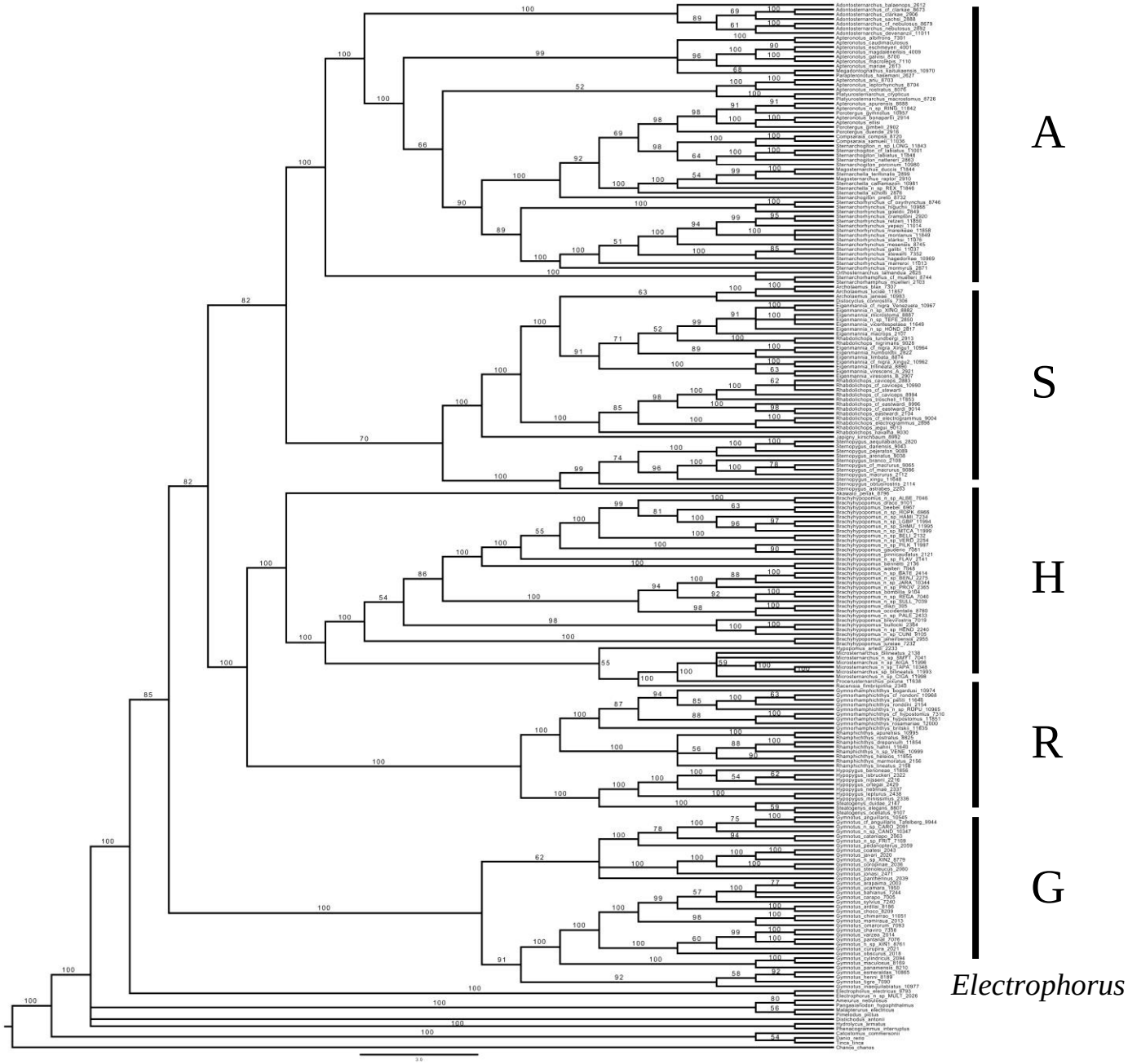


Figure 11: Strict consensus Gymnotiformes phylogeny of the 60 most parsimonious trees recovered from maximum-parsimony analysis of a nine gene concatenated data matrix. Letters represent families as follows: G = Gymnotidae, R = Rhamphichthyidae, H = Hypopomidae, S = Sternopygidae, A = Apteronotidae. Bootstrap supports are shown above each branch.

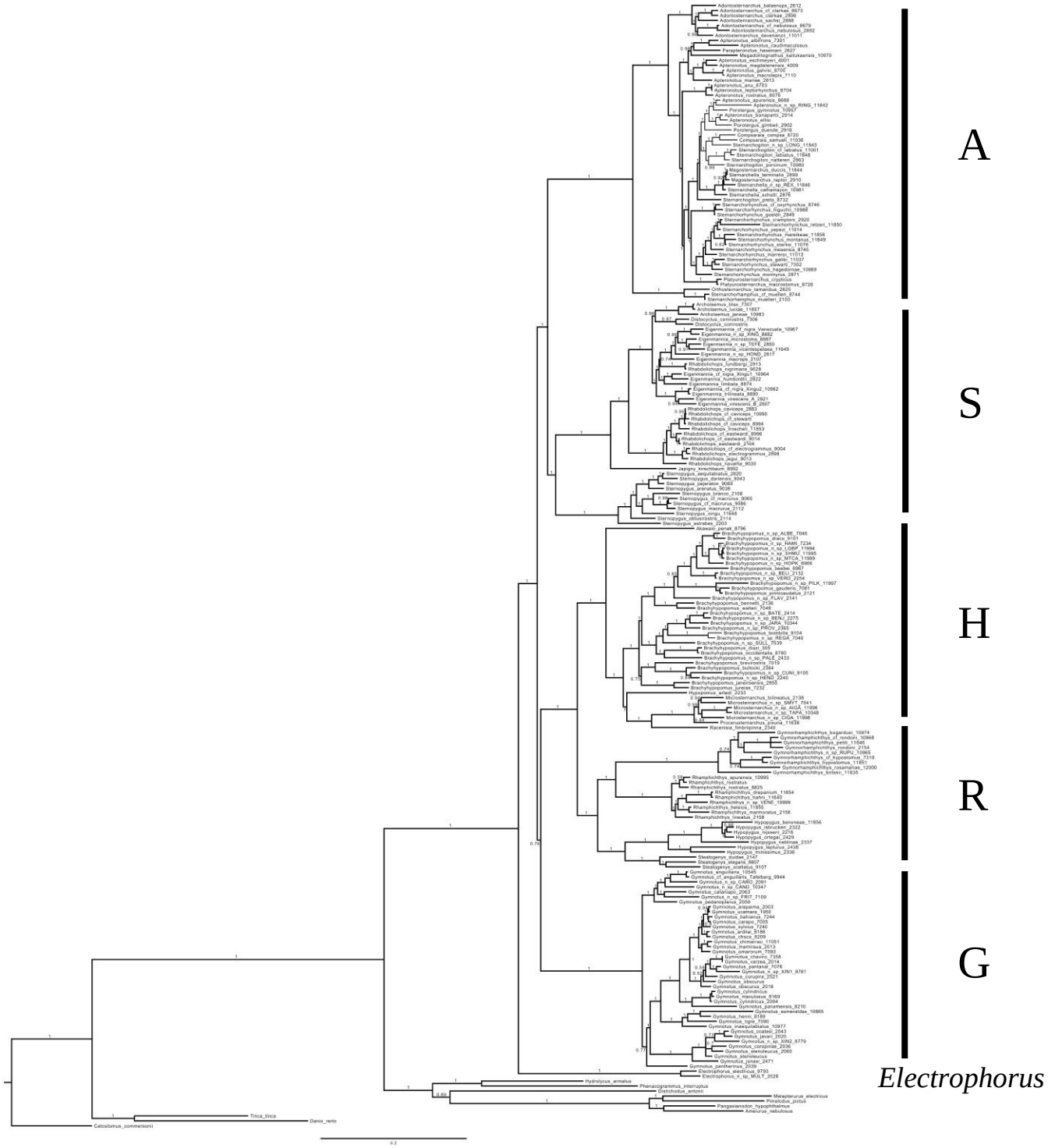


Figure 12: Bayesian inference Gymnotiformes phylogeny produced using a nine gene concatenated data matrix. Letters represent families as follows: G = Gymnotidae, R = Rhamphichthyidae, H = Hypopomidae, S = Sternopygidae, A = Apteronotidae. Posterior probabilities are shown above each branch.

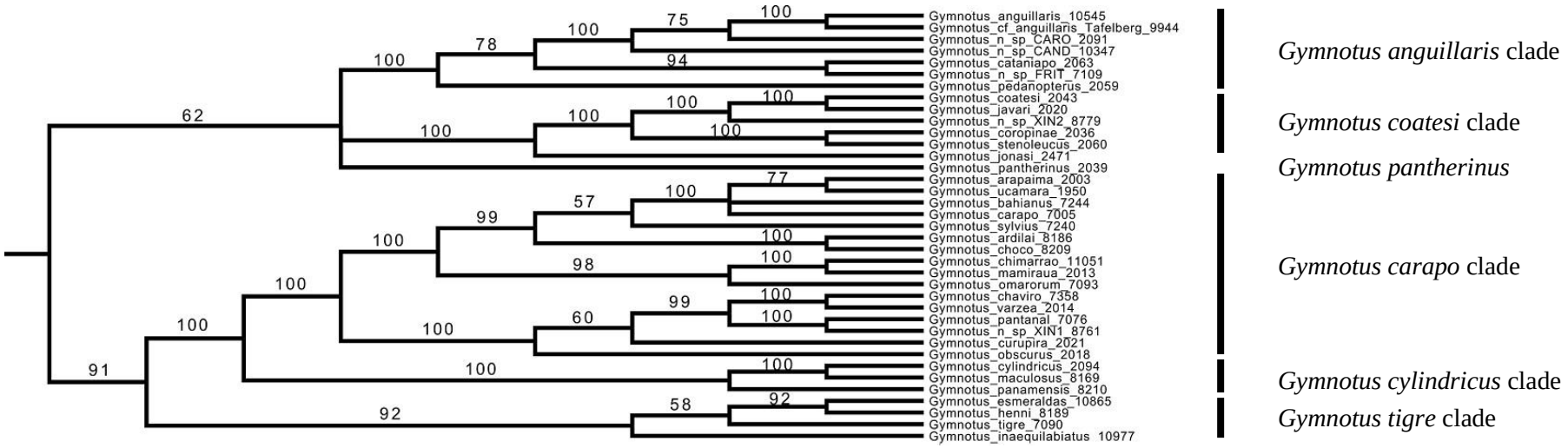


Figure 13: Family Gymnotidae from the strict consensus of the 60 most parsimonious trees recovered from maximum-parsimony analysis of a nine gene concatenated data matrix. Designations right of the specific epithets denote major recognized clades of Gymnotidae. Bootstrap supports are found above each branch.

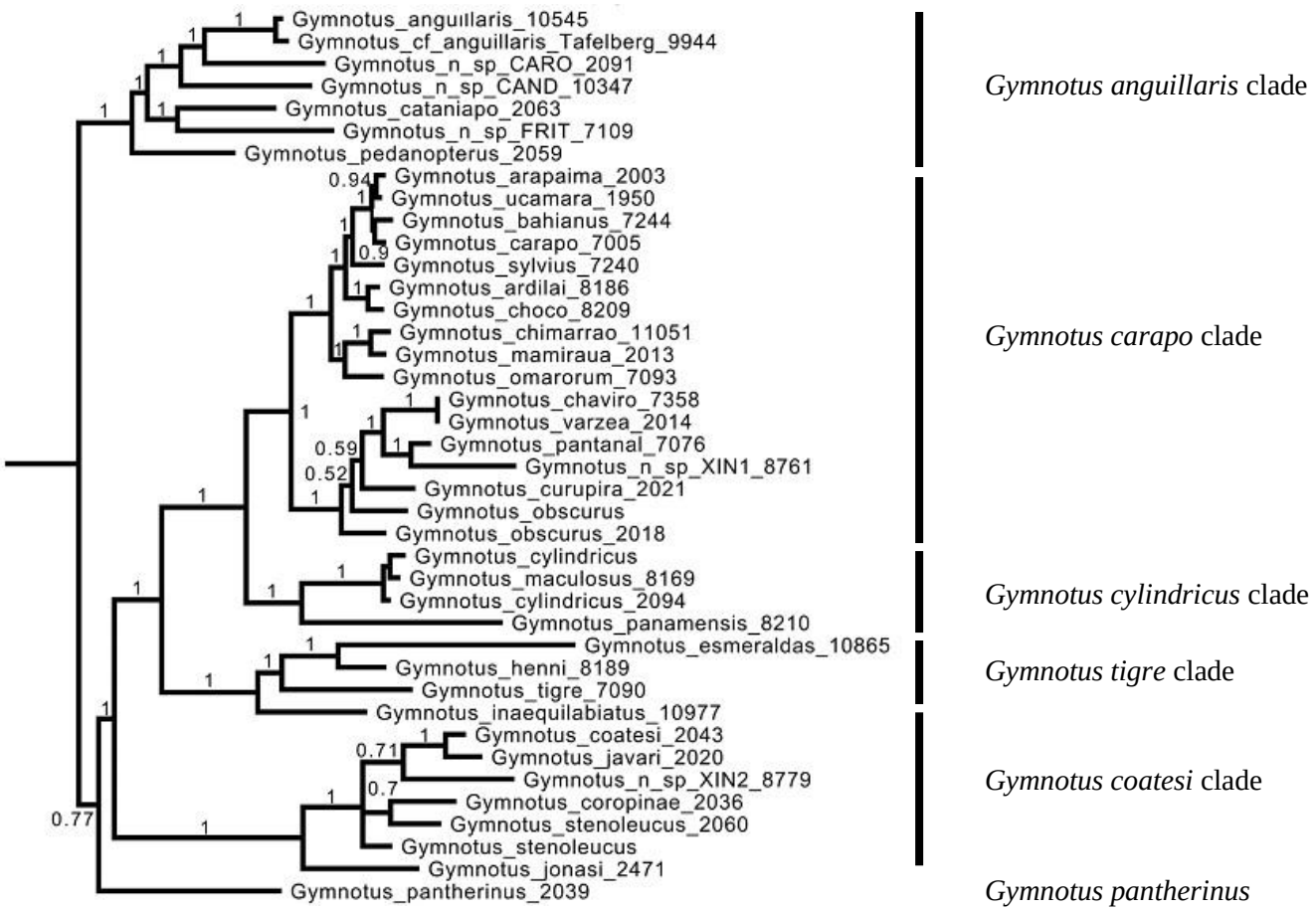


Figure 14: Family Gymnotidae from the Bayesian inference phylogeny produced using a nine gene concatenated data matrix. Designations right of the specific epithets denote major recognized clades of Gymnotidae. Posterior probabilities are found above each branch.

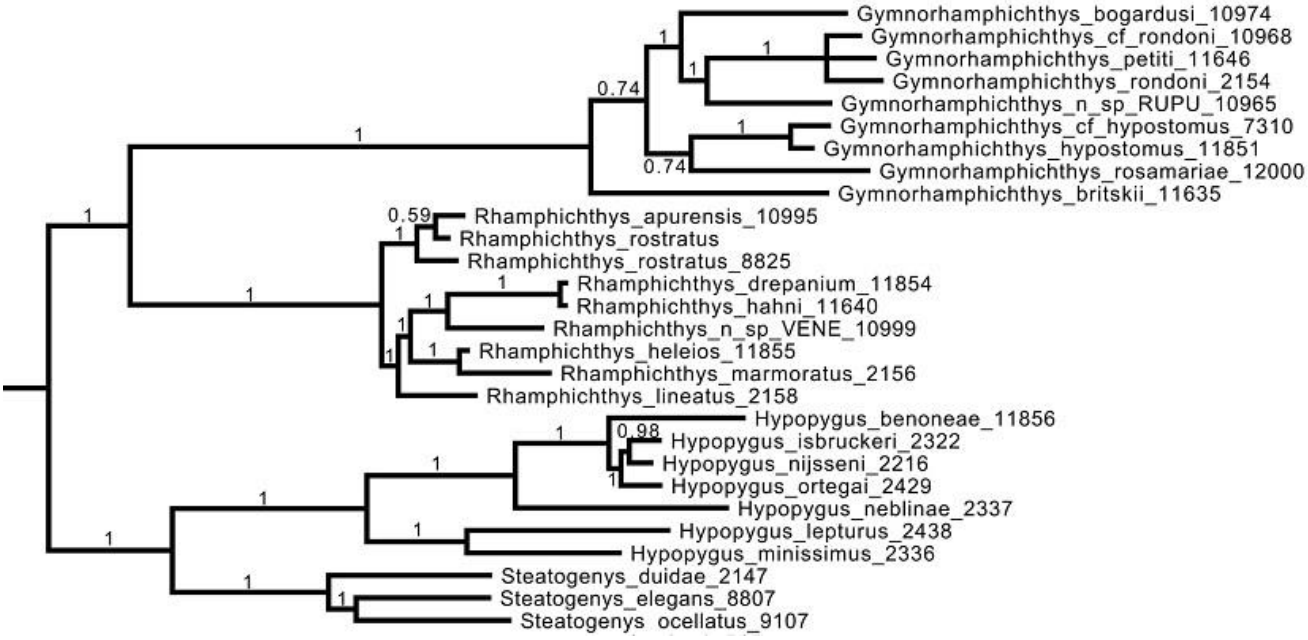


Figure 15: Family Rhamphichthyidae from the Bayesian inference phylogeny produced using a nine gene concatenated data matrix. *Rhamphichthys* and *Gymnorhamphichthys* form the Rhamphichthyinae, and *Hypopygus* and *Steatogenys* form the Steatogenae. Posterior probabilities are found above each branch.

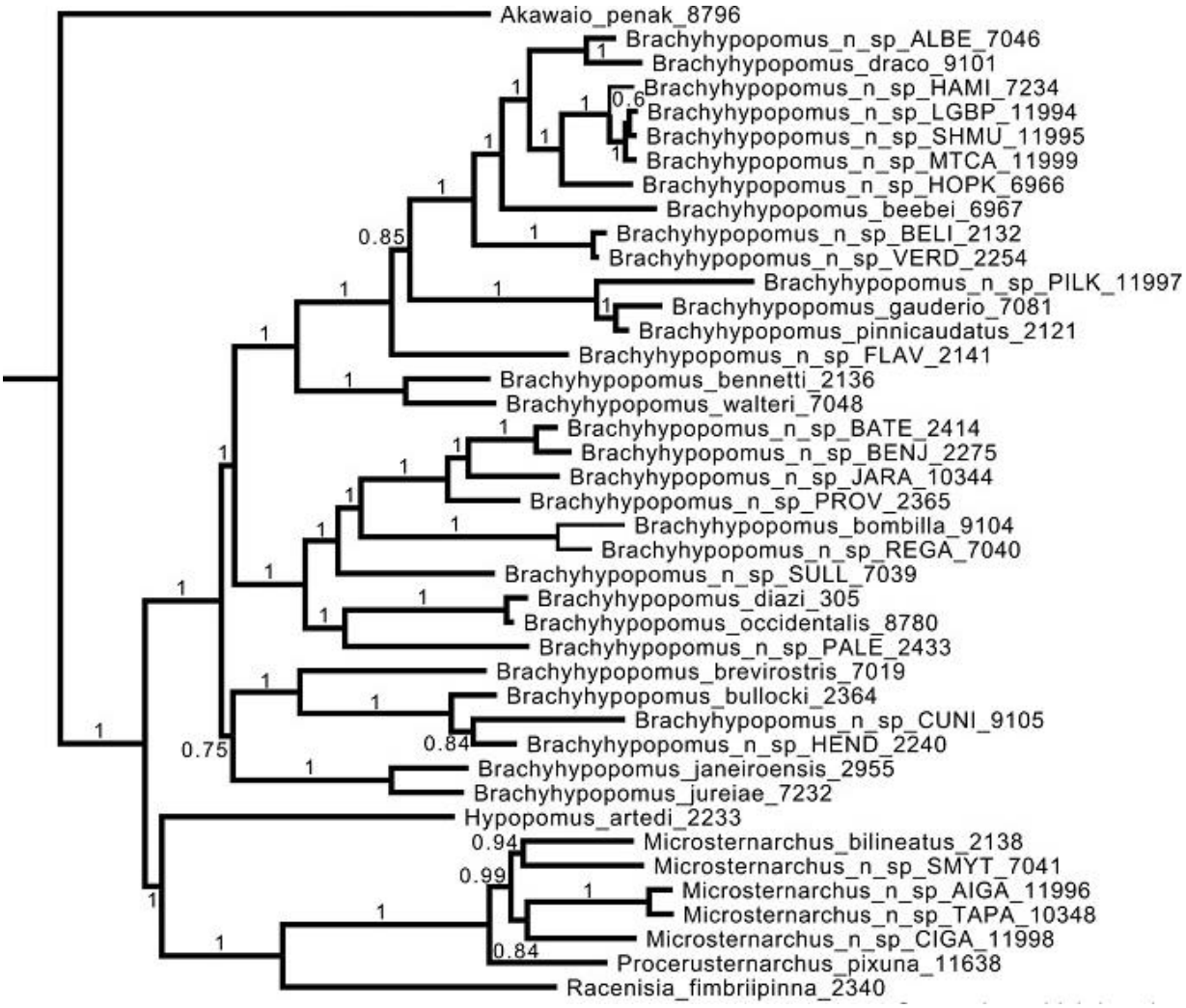


Figure 16: Family Hypopomidae from the Bayesian inference phylogeny produced using a nine gene concatenated data matrix. *Microsternarchus*, *Procerusternarchus*, and *Racenisia* form the Microsternarchini. Posterior probabilities are found above each branch.

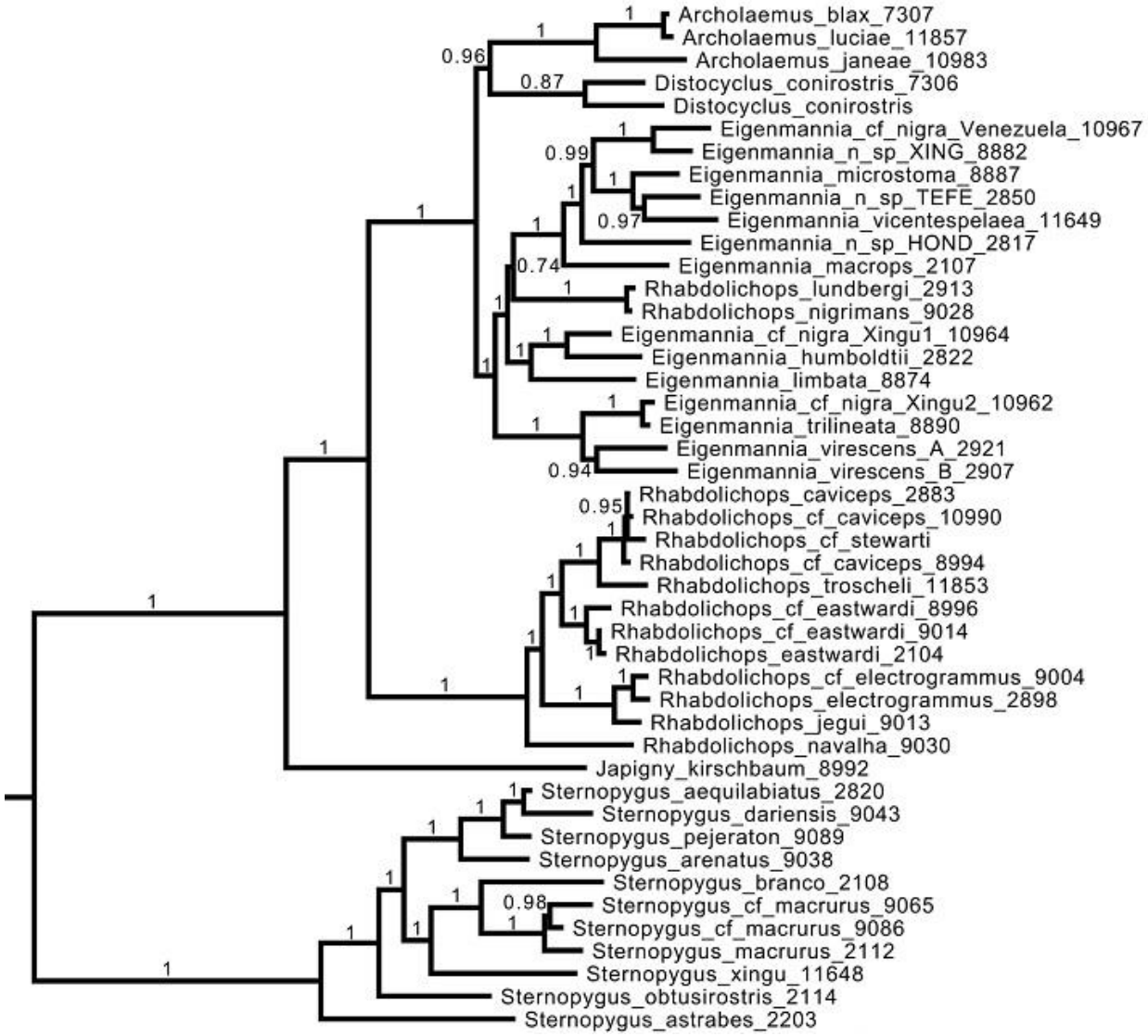


Figure 17: Family Sternopygidae from the Bayesian inference phylogeny produced using a nine gene concatenated data matrix. Posterior probabilities are found above each branch.

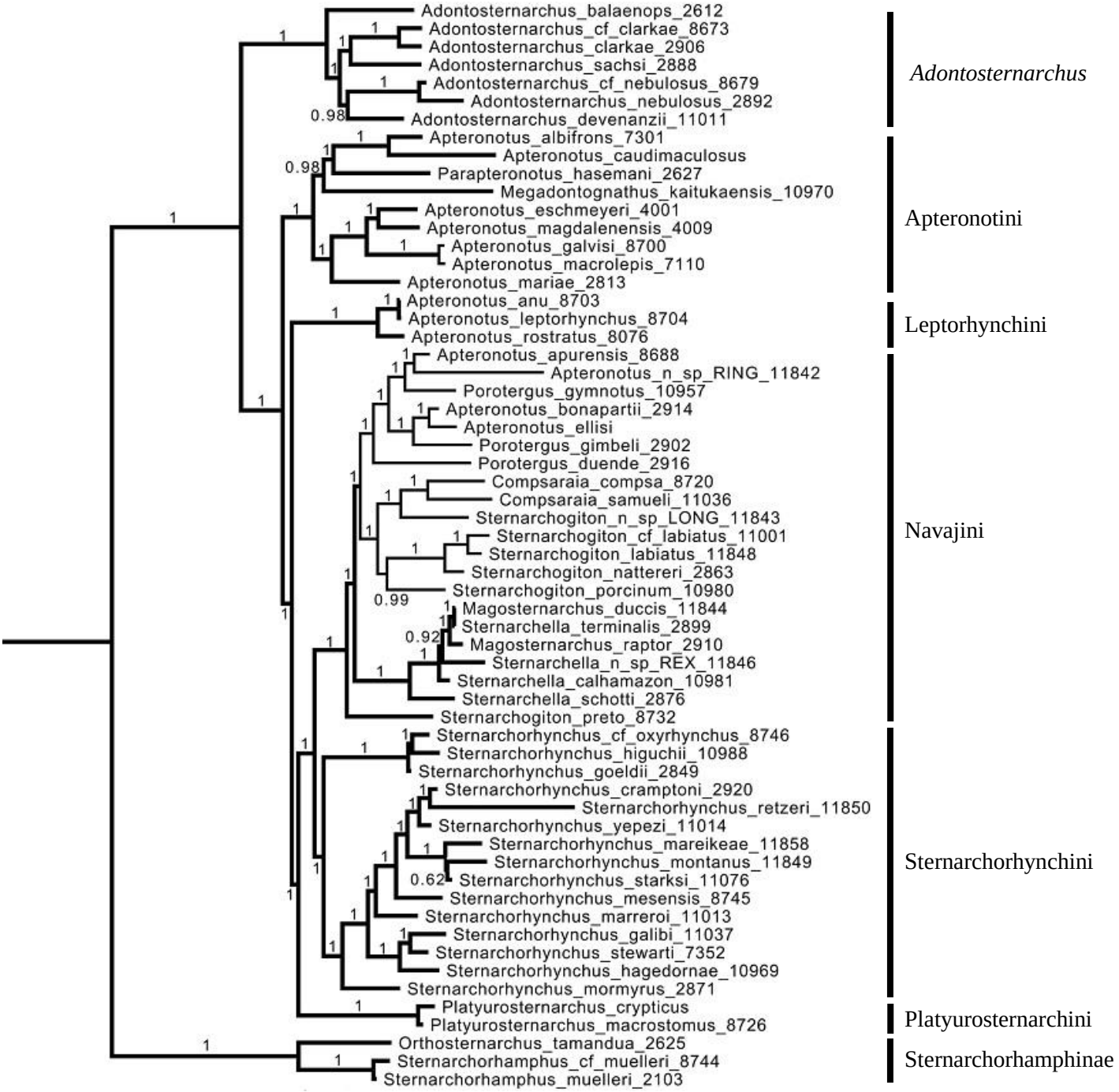


Figure 18: Family Apteronotidae from the Bayesian inference phylogeny produced using a nine gene concatenated data matrix. Designations right of the specific epithets denote both major recognized clades and new clades found in the present study. Posterior probabilities are found above each branch.

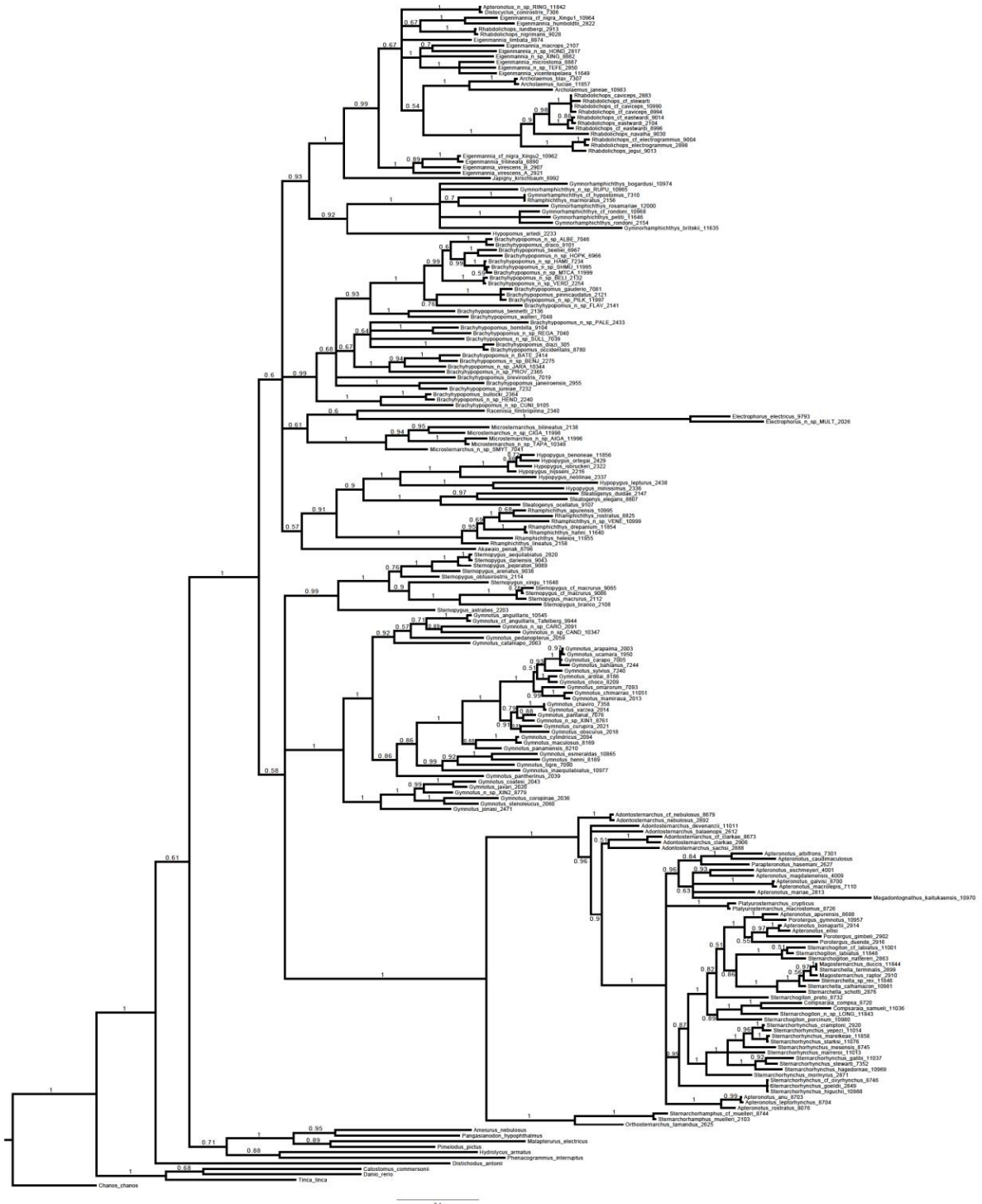


Figure 19: *Co1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

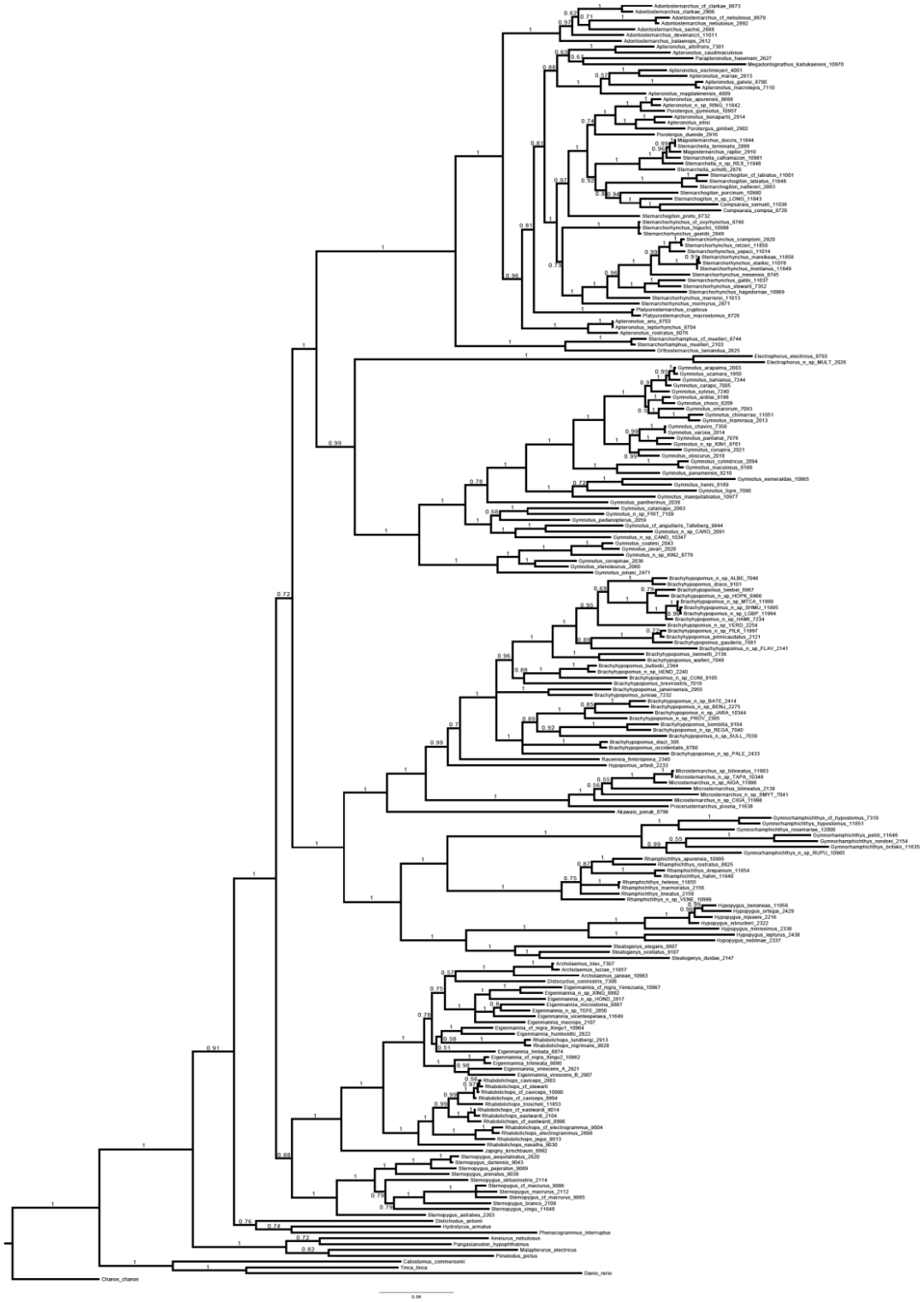


Figure 20: *Cyt b* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

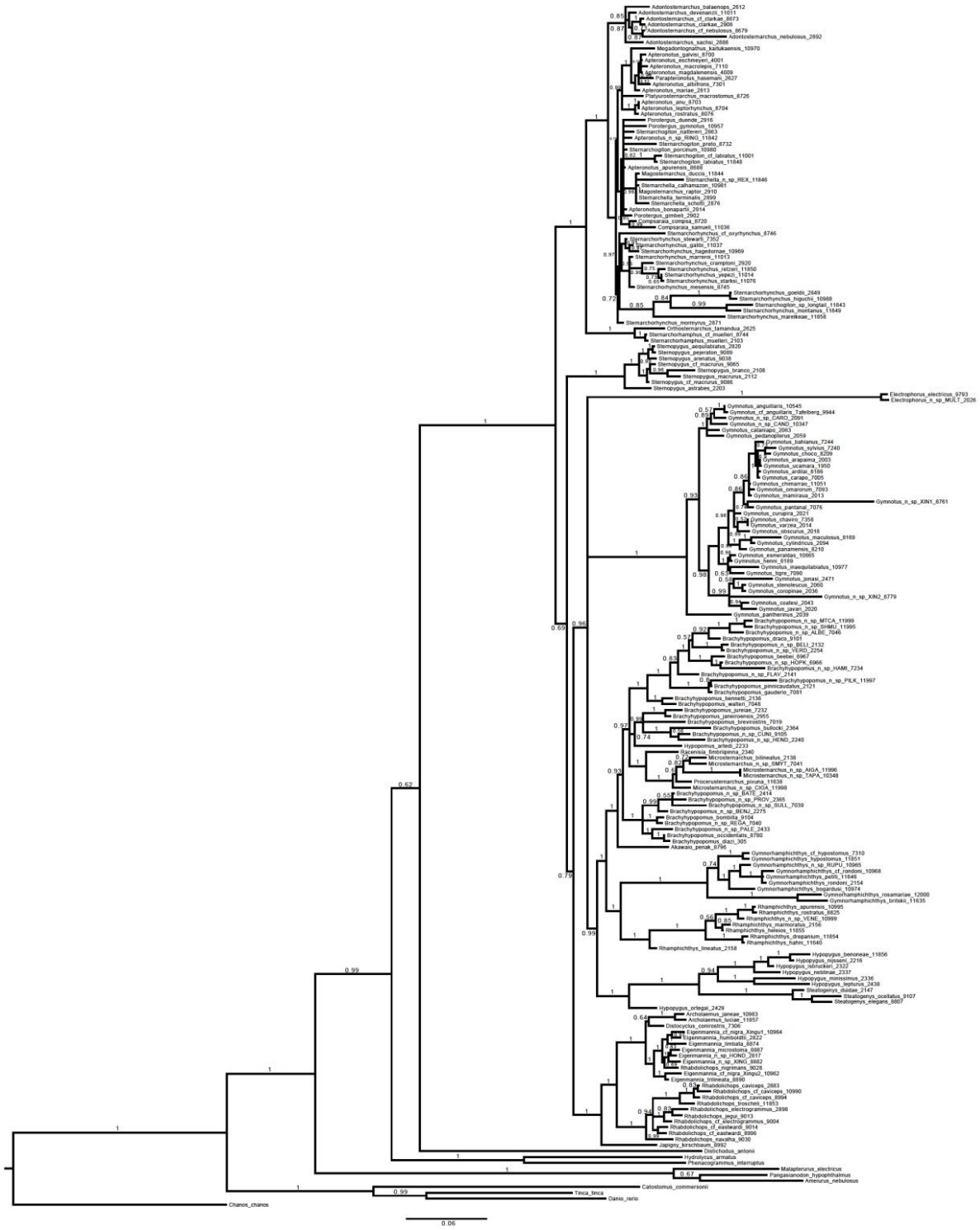


Figure 21: *Egr1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

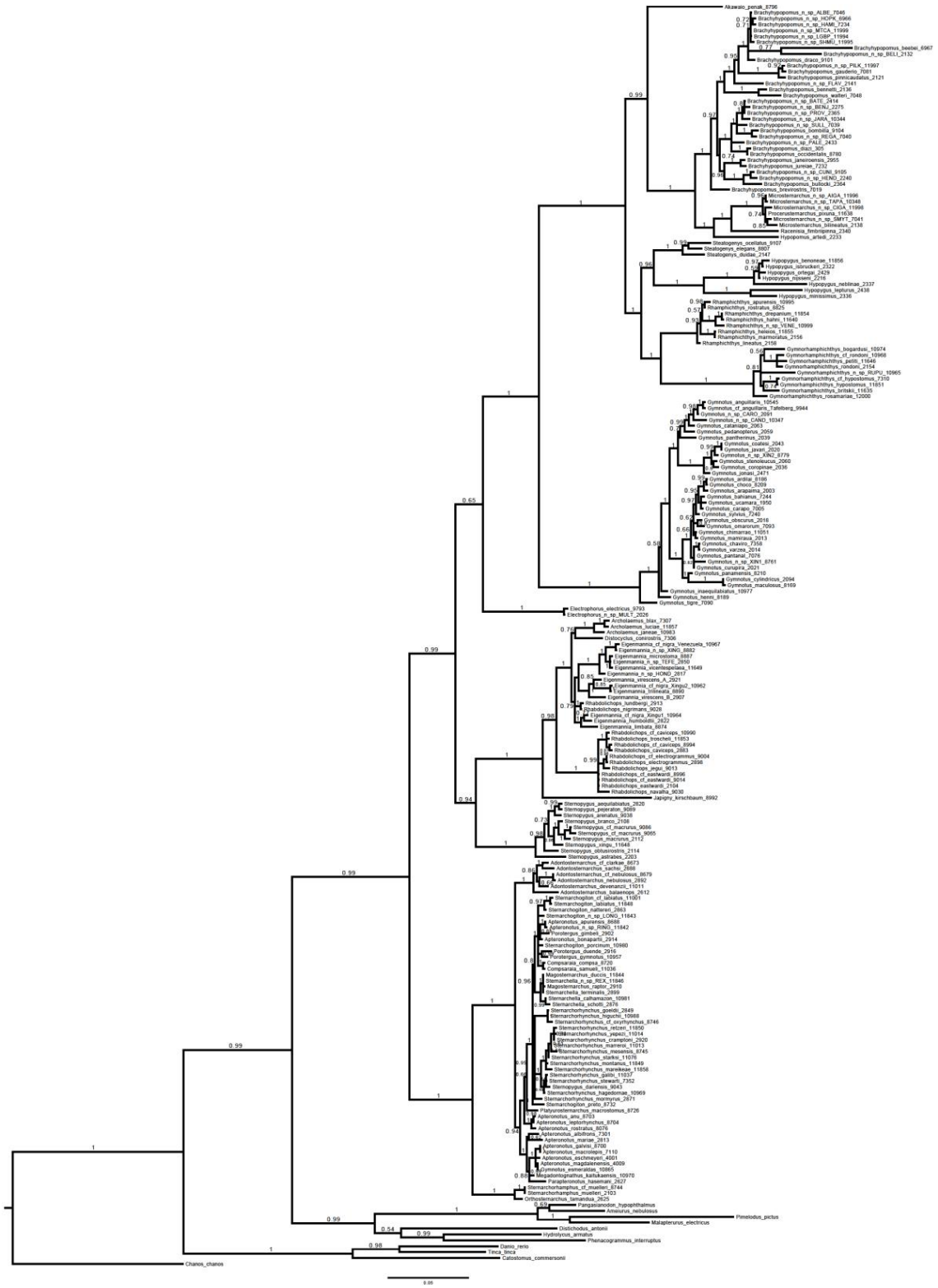


Figure 22: *Enc1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

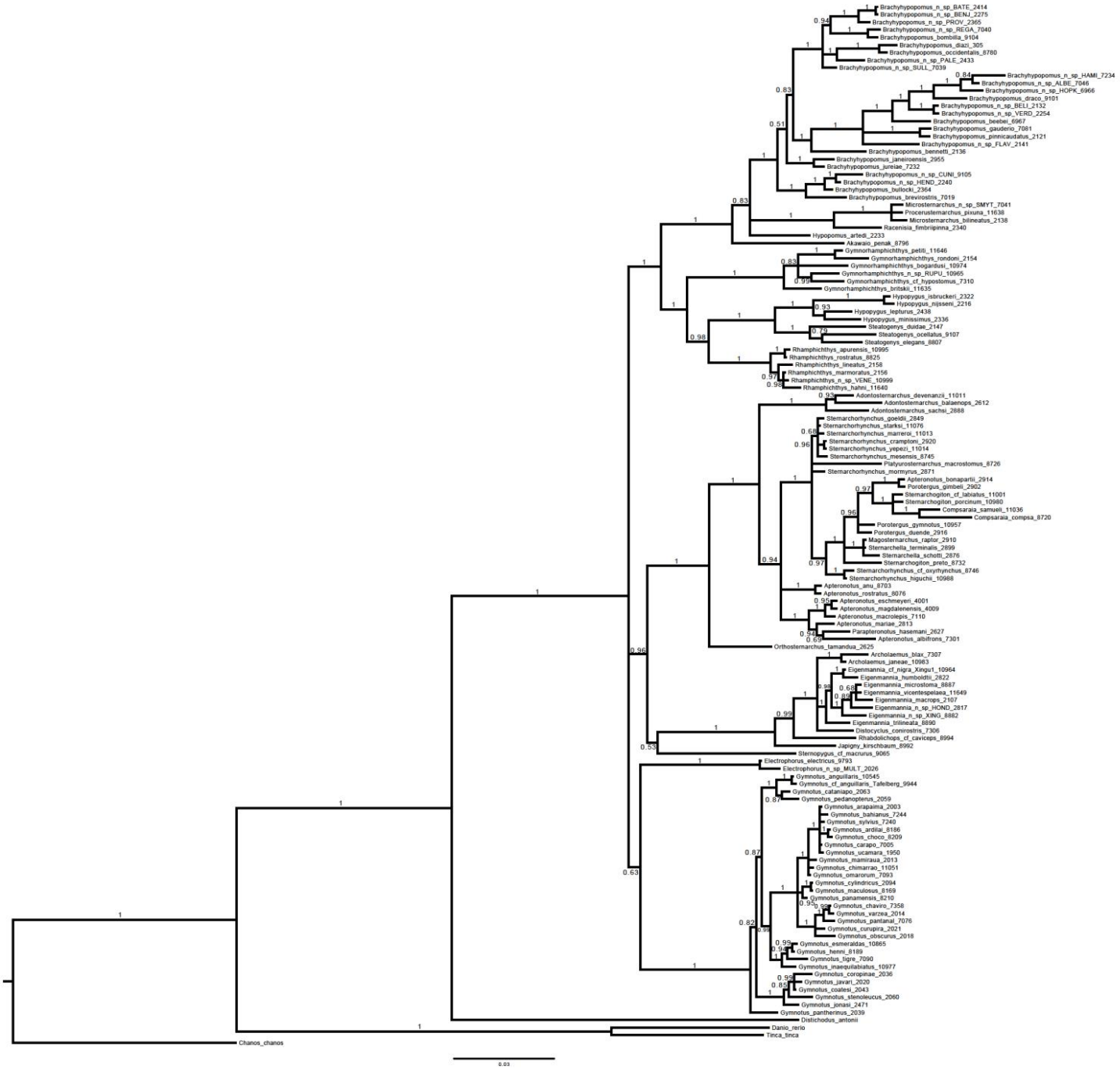


Figure 23: *Glyt* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

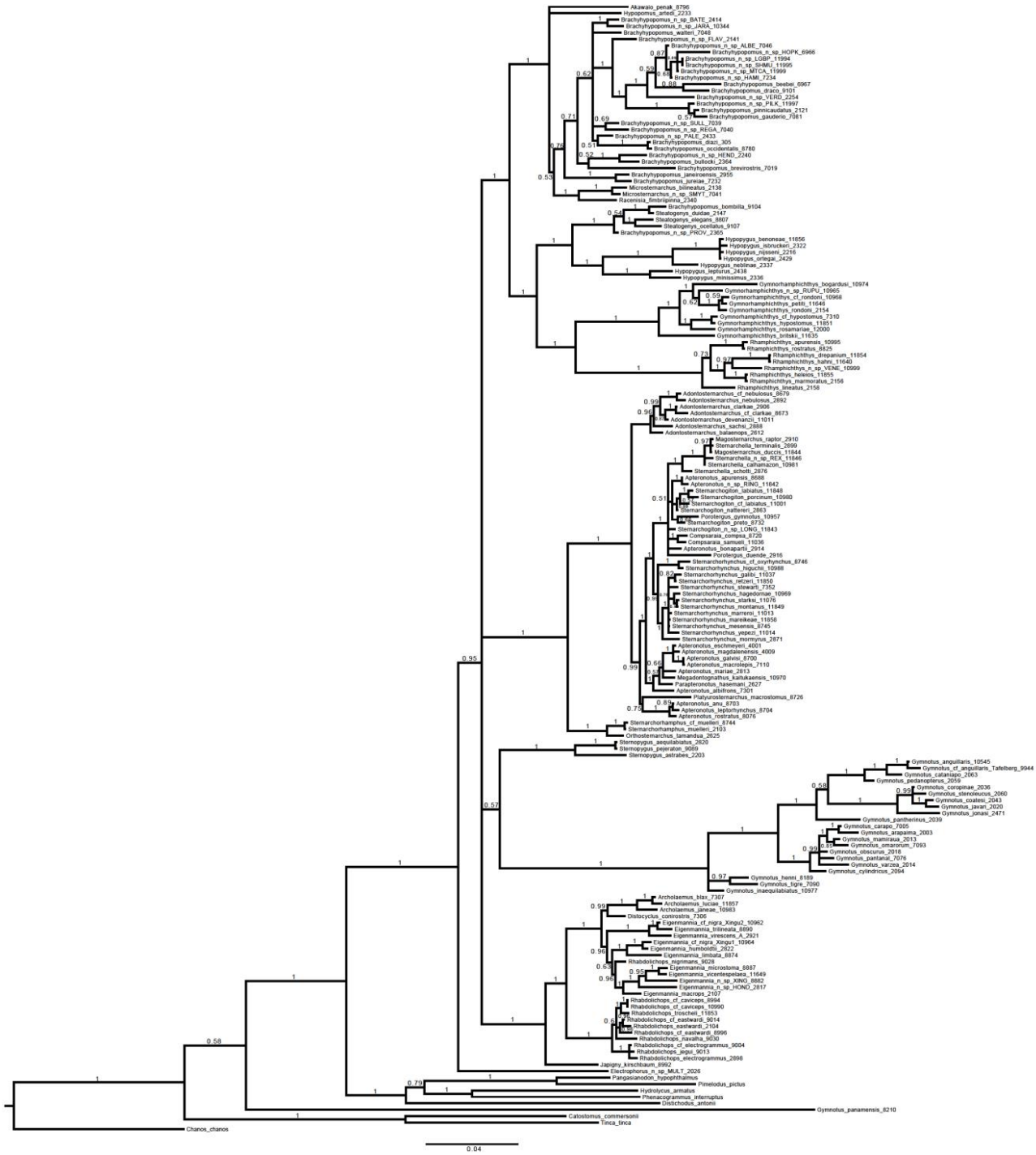


Figure 24: *Rag1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

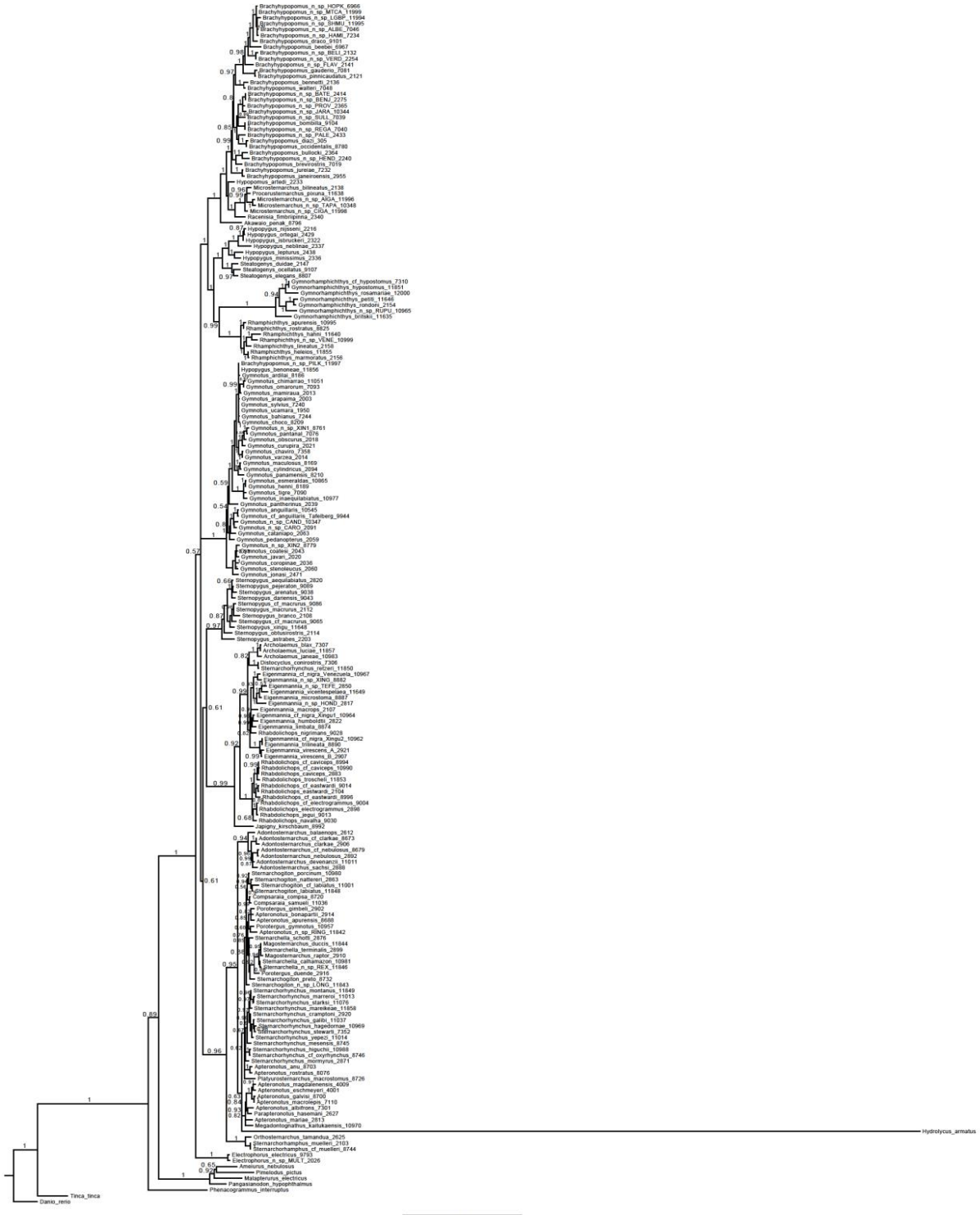


Figure 25: *Rag2* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

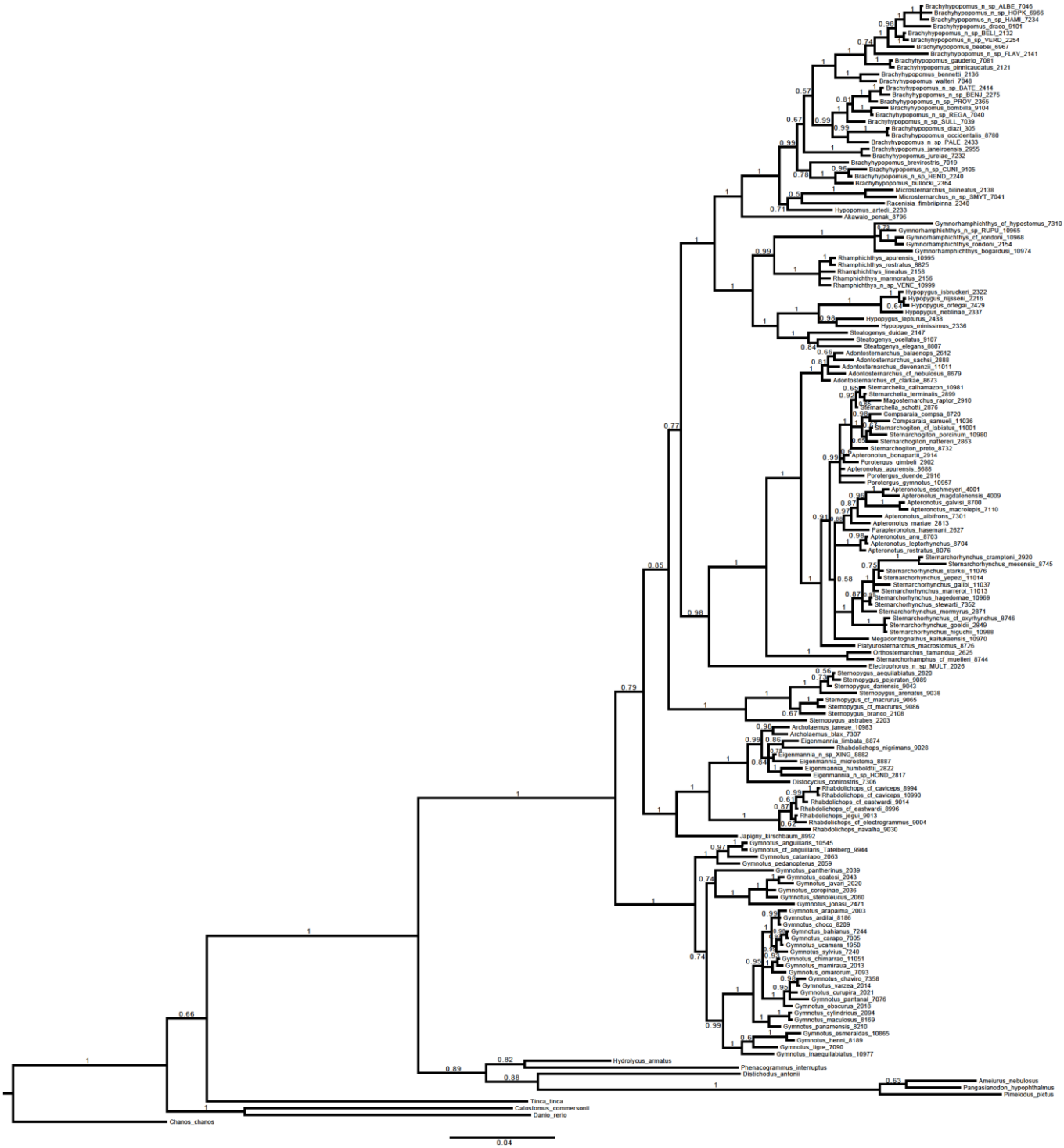


Figure 26: *Rh1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

Figure 27: *Zic1* gene tree of Gymnotiformes using Bayesian inference. Posterior probabilities are found above each branch.

Table 1: Classification *sensu* Tagliacollo et al. (2016) and voucher information for each taxon used in the present study.

Family	Species	Specimen	Voucher	Locality
Apteronotidae	*Adontosternarchus balaenops* Cope	2612	UF 116559	Iquitos, Peru (Aquarium)
	Adontosternarchus clarkae Mago-Leccia, Lundberg, & Baskin	2906	MCP 39341	Alvaraes, Brazil
	Adontosternarchus cf *clarkae*	8673	INPA 28867	Rio Negro, Brazil
	Adontosternarchus devenanzii Mago-Leccia, Lundberg, & Baskin	11011	ANSP 198405	Rio Portuguesa, Venezuela
	Adontosternarchus nebulosus Lundberg & Cox-Fernandes	2892	MCP 39313	Alvaraes, Brazil
	Adontosternarchus cf *nebulosus*	8679	INPA 26	Rio Negro, Brazil
	Adontosternarchus sachsi Peters	2888	MCP 39354	Alvaraes, Brazil
	Apteronotus albifrons Linnaeus	7301	MNRJ 33616	Xingú-Tapajós, Brazil
	Apteronotus anu de Santana & Vari	8703	UNELLEZ 35	Maracaibo, Venezuela
	Apteronotus apurensis Fernández-Yépez	8688	UNELLEZ 41	Rio Apure, Venezuela
	Apteronotus bonapartii Castelnau	2914	UCF Uncat.	Alvaraes, Brazil
	Apteronotus caudimaculosus de Sanatana	GenBank	LBP 43246	Data Unavailable
	Apteronotus ellisi Alonso de Arámburu	GenBank	LBP 24040	Data Unavailable
	Apteronotus eschmeyeri de Santana, Maldenado-Ocampo, Severi, & Mendes	4001	Data Missing	Honda, Colombia
	Apteronotus galvisi de Santana, Maldenado-Ocampo, & Crampton	8700	IAvH-BT 7611	Rio Meta, Colombia
	Apteronotus leptorhynchus Ellis	8704	UNELLEZ 36	Maracaibo, Venezuela
	Apteronotus macrolepis Steindachner	7110	Data Missing	Rio Meta, Colombia
	Apteronotus magdalenensis Miles	4009	Data Missing	Colombia
	Apteronotus mariae C.H. Eigenmann & Fisher	2813	Data Missing	Honda, Colombia
	Apteronotus rostratus Meek & Hildebrand	8076	ROM 89761	Piriati River, Panama
	Apteronotus n. sp. RING	11842	UCF Uncat.	Rio Nanay, Peru
	Compsaraia compsa Mago-Leccia	8720	INPA 28876	Rio Negro, Brazil
	Compsaraia samueli Albert & Crampton	11036	ANSP 182209	Rio Amazonas, Peru
	Magosternarchus duccis Lundberg, Cox Fernandes, & Albert	11844	UCF Uncat.	Rio Amazonas, Peru
	Magosternarchus raptor Lundberg, Cox Fernandes, & Albert	2910	UCF Uncat.	Alvaraes, Brazil
	Megadontognathus kaitukaensis Campos-da-Paz	10970	ANSP 195961	Rio Xingú, Brazil
	Orthosternarchus tamandua Boulenger	2625	UF 116562	Rio Amazonas, Peru
	Parapteronotus hasemani Ellis	2627	Data Missing	Iquitos, Peru (Aquarium)
	Platyurosternarchus crypticus de Santana & Vari	GenBank	ANSP 179153	Rupununi, Guyana
	Platyurosternarchus macrostomus Günther	8726	MNRJ 33614	Xingú-Tapajós, Brazil

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Porotergus duende de Santana & Crampton	2916	MCP 37359	Rio Solimões, Brazil
	Porotergus gimbeli Ellis	2902	MCP 37529	Alvaraes, Brazil
	Porotergus gymnotus Ellis	10957	ROM	Marowijne River, Suriname
	Sternarchella calhamazon Lundberg, Cox Fernandes, Campos-da-Paz, & Sullivan	10981	ANSP 193103	Rio Amazonas, Peru
	Sternarchella schotti Steindachner	2876	MCP 49429	Alvaraes, Brazil
	Sternarchella terminalis Eigenmann & Allen	2899	MCP 49436	Alvaraes, Brazil
	Sternarchella n. sp. REX	11846	MUSM 54500	Rio Amazonas, Peru
	Sternarchogiton labiatus de Santana & Crampton	11848	UCF Uncat.	Iquitos, Peru
	Sternarchogiton cf *labiatus*	11001	ANSP 198348	Rio Apure, Venezuela
	Sternarchogiton nattereri Steindachner	2863	MCP 38306	Alvaraes, Brazil
	Sternarchogiton porcinum Eigenmann & Allen	10980	ANSP 182319	Rio Amazonas, Peru
	Sternarchogiton preto de Santana & Crampton	8732	INPA 28880	Rio Negro, Brazil
	Sternarchogiton n. sp. LONG	11843	UCF Uncat.	Rio Nanay, Peru
	Sternarchorhamphus muelleri Steindachner	2103	MCP 41658	Alvaraes, Brazil
	Sternarchorhamphus cf *muelleri*	8744	INPA 26	Rio Negro, Brazil
	Sternarchorhynchus cramptoni de Santana & Vari	2920	MCP 41638	Alvaraes, Brazil
	Sternarchorhynchus galibi de Santana & Vari	11037	ANSP 187155	Lawa River, Suriname
	Sternarchorhynchus goeldii de Santana & Vari	2849	MCP 41643	Mamiraua Lake, Brazil
	Sternarchorhynchus hagedornae de Santana & Vari	10969	ANSP 180637	Rio Inambari, Peru
	Sternarchorhynchus higuchii de Santana & Vari	10988	INPA 40463	Rio Xingú, Brazil
	Sternarchorhynchus mareikeae de Santana & Vari	11858	UCF Uncat.	Rio Tapajós, Brazil
	Sternarchorhynchus marreroi de Santana & Vari	11013	ANSP 198345	Rio Apure, Venezuela
	Sternarchorhynchus mesensis Campos-da-Paz	8745	MNRJ 33617	Rio Xingú, Brazil
	Sternarchorhynchus montanus de Santana & Vari	11849	UCF Uncat.	Rio Amazonas, Peru
	Sternarchorhynchus mormyrus Steindachner	2871	MCP 41640	Alvaraes, Brazil
	Sternarchorhynchus cf *oxyrhynchus* Müller & Troschel	8746	INPA 47	Rio Negro, Brazil
	Sternarchorhynchus retzeri de Santana & Vari	11850	UCF Uncat.	Rio Nanay, Peru
	Sternarchorhynchus starksi de Santana & Vari	11076	MCP 47080	Manaus, Brazil
	Sternarchorhynchus stewarti de Santana & Vari	7352	MUSM 33822	Data Missing
	Sternarchorhynchus yepezi de Santana & Vari	11014	ANSP 198401	Rio Portuguesa, Venezuela

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
Gymnotidae	*Electrophorus electricus* Linnaeus	9793	Uncatalogued	Raleigh Vallen, Suriname
	Electrophorus n. sp. MULT	2026	MZUSP 103218	Lago Secretaria, Brazil
	Gymnotus anguillaris Hoedeman	10545	Data Missing	Marowijne River, Suriname
	Gymnotus cf *anguillaris*	9944	KU 41321	Tafelberg, Suriname
	Gymnotus arapaima Albert & Crampton	2003	MZUSP 103219	Tefé, Brazil
	Gymnotus ardilai Maldonado-Ocampo & Albert	8186	IAvHP 11510	Rio de Oro, Colombia
	Gymnotus bahianus Campos-da-Paz & Costa	7244	MZUSP 102898	Rio Almada, Brazil
	Gymnotus carapo Linnaeus	7005	UF 180169	Suriname River, Suriname
	Gymnotus cataniapo Mago-Leccia	2063	UF 174332	Rio Cataniapo, Venezuela
	Gymnotus chaviro Maxime & Albert	7358	MUSM 33729	Rio Yurua, Peru
	Gymnotus chimarrao Cognato, Richer-de-Forges, Albert, & Crampton	11051	MCP 28583	Porto Alegre, Brazil
	Gymnotus choco Albert, Crampton, & Maldonado-Ocampo	8209	IAvHP 10646	Rio Atrato, Colombia
	Gymnotus coatesi La Monte	2043	MCP 34472	Tefé, Brazil
	Gymnotus coropinae Hoedeman	2036	AUM 35848	Rupununi, Guyana
	Gymnotus curupira Crampton, Thorsen, & Albert	2021	MZUSP 75146	Tefé, Brazil
	Gymnotus cylindricus La Monte	2094	ROM 84772	Rio Tortuguera, Costa Rica
	Gymnotus esmeraldas Albert & Crampton	10865	ZOO.A.V.Pe0310	Ecuador
	Gymnotus henni Albert, Crampton, & Maldonado-Ocampo	8189	IMCN 4521	Rio Dagua, Colombia
	Gymnotus inaequilabiatus Valenciennes	10977	ANSP 192991	Rio Guayquiraro, Argentina
	Gymnotus javari Albert, Crampton, & Hagedorn	2020	UF 122824	Iquitos, Peru (Aquarium)
	Gymnotus jonasi Albert & Crampton	2471	UF 131410	Rio Ucayali, Peru
	Gymnotus maculosus Albert & Miller	8169	ROM 89784	Nicoya, Costa Rica
	Gymnotus mamiraua Albert & Crampton	2013	MCP 29805	Tefé, Brazil
	Gymnotus obscurus Crampton, Thorsen, & Albert	2018	MZUSP 75157	Tefé, Brazil
	Gymnotus omarorum Richer-de-Forges, Crampton, & Albert	7093	AMNH 239656	Laguna del Cisne, Uruguay
	Gymnotus panamensis Albert & Crampton	8210	STRI-01579	Rio Cricamola, Panama
	Gymnotus pantanal Fernandes-Matioli, Albert, Daniel-Silva, Lopes, Crampton, & Almeida-Toledo	7076	Data Missing	Corrientes, Argentina
	Gymnotus pantherinus Steindachner	2039	No Voucher	Rio Perequê-Açu, Brazil
	Gymnotus pedanopterus Mago-Leccia	2059	UF 174328	Caño Viejita, Venezuela

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Gymnotus stenoleucus Mago-Leccia	2060	UF 174329	Caño Viejita, Venezuela
	Gymnotus sylvius Albert & Fernandes-Matioli	7240	MZUSP 100267	Rio Ribeira, Brazil
	Gymnotus tigre Albert & Crampton	7090	Uncatalogued	Aquarium
	Gymnotus ucamara Crampton, Lovejoy, & Albert	1950	UF 126184	Pacaya Samiria Reserve, Peru
	Gymnotus varzea Crampton, Thorsen, & Albert	2014	MZUSP 75163	Tefé, Brazil
	Gymnotus n. sp. CAND	10347	UCF Uncat.	Rio Tapajós, Brazil
	Gymnotus n. sp. CARO	2091	AUM 36616	Guyana
	Gymnotus n. sp. FRIT	7109	Uncatalogued	Tefé, Brazil
	Gymnotus n. sp. XIN1	8761	MNRJ 33642	Xingú-Tapajós, Brazil
	Gymnotus n. sp. XIN2	8779	MNRJ 33630	Xingú-Tapajós, Brazil
Hypopomidae	*Akawaio penak* Maldenado-Ocampo, López-Fernández, Taphorn, Bernard, Crampton, & Lovejoy	8796	ROM 83884	Mazaruni, Guyana
	Brachyhypopomus beebei Schultz	6967	UF 177358	Commewijne River, Suriname
	Brachyhypopomus bennetti Sullivan, Zuanon, & Cox Fernandes	2136	MCP 45255	Tefé, Brazil
	Brachyhypopomus bombilla Loureiro & Ana Silva	9104	UFRGS 10561	Rio Grande do Sul, Brazil
	Brachyhypopomus brevirostris Steindachner	7019	UF 177359	Commewijne River, Suriname
	Brachyhypopomus bullocki Sullivan & Hopkins	2364	UF 177348	Guyana Region, Venezuela
	Brachyhypopomus diazi Fernández-Yépez	305	UF 174334	Rio Las Marias, Venezuela
	Brachyhypopomus draco Giora, Malabarba, & Crampton	9101	UFRGS 14562	Rio Grande do Sul, Brazil
	Brachyhypopomus gauderio Giora & Malabarba	7081	UF 177364	Corrientes, Argentina
	Brachyhypopomus janeiroensis Costa & Campos-da-Paz	2955	UF 183780	Rio de São João, Brazil
	Brachyhypopomus jureiae Triques & Khamis	7232	MZUSP 100268	Rio Ribeira, Brazil
	Brachyhypopomus occidentalis Regan	8780	UNELLEZ Uncat.	Maracaibo, Venezuela
	Brachyhypopomus pinnicaudatus Hopkins	2121	MCP 45281	Tefé, Brazil
	Brachyhypopomus walteri Sullivan, Zuanon, & Cox Fernandes	7048	CBF 10257	Rio Amazonas, Bolivia
	Brachyhypopomus n. sp. ALBE	7046	CBF 10278	Rio Amazonas, Bolivia
	Brachyhypopomus n. sp. BATE	2414	MCP 45312	Tefé, Brazil
	Brachyhypopomus n. sp. BELI	2132	MCP 45431	Tefé, Brazil
	Brachyhypopomus n. sp. BENJ	2275	UF 148512	Quebrada Fierro Caño, Peru
	Brachyhypopomus n. sp. CUNI	9105	MCP 46937	Rio Amazonas, Brazil

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Brachyhypopomus n. sp. FLAV	2141	MCP 45265	Tefé, Brazil
	Brachyhypopomus n. sp. HAMI	7234	MCP 45681	Tefé, Brazil
	Brachyhypopomus n. sp. HEND	2240	MCP 45397	Tefé, Brazil
	Brachyhypopomus n. sp. HOPK	6966	UF 177365	Commewijne River, Suriname
	Brachyhypopomus n. sp. JARA	10344	UCF Uncat.	Rio Tapajós, Brazil
	Brachyhypopomus n. sp. LGBP	11994	UCF Uncat.	Rio Tapajós, Brazil
	Brachyhypopomus n. sp. MTCA	11999	UCF Uncat.	Rio Tapajós, Brazil
	Brachyhypopomus n. sp. PALE	2433	UF 148572	Rio Palenque, Ecuador
	Brachyhypopomus n. sp. PILK	11997	UCF Uncat.	Rio Tapajós, Brazil
	Brachyhypopomus n. sp. PROV	2365	UF 177347	Guyana Region, Venezuela
	Brachyhypopomus n. sp. REGA	7040	UMSS 7038	Rio Amazonas, Bolivia
	Brachyhypopomus n. sp. SHMU	11995	UCF Uncat.	Rio Tapajós, Brazil
	Brachyhypopomus n. sp. SULL	7039	UF 177341	Rio Amazonas, Bolivia
	Brachyhypopomus n. sp. VERD	2254	UF 148520	Quebrada, Peru
	Hypopomus artedi Kaup	2233	AUM 35574	Cuyuni-Mazaruni, Guyana
	Microsternarchus bilineatus Fernández-Yépez	2138	MCP 45480	Tefé, Brazil
	Microsternarchus n. sp. AIGA	11996	UCF Uncat.	Rio Tapajós, Brazil
	Microsternarchus n. sp. CIGA	11998	UCF Uncat.	Rio Tapajós, Brazil
	Microsternarchus n. sp. SMYT	7041	CBF 10270	Rio Amazonas, Bolivia
	Microsternarchus n. sp. TAPA	10348	UCF Uncat.	Rio Tapajós, Brazil
	Procerusternarchus pixuna Cox Fernandes, Nogueira, & Alves-Gomes	11638	LBP 7006	Rio Negro, Brazil
	Racenisia fimbriipinna Mago-Leccia	2340	UF 177352	Rio Atabapo, Venezuela
Rhamphichthyidae	*Gymnorhamphichthys bogardusi* Lundberg	10974	ANSP 199558	Rio Xingú, Brazil
	Gymnorhamphichthys britskii Carvalho, Ramos, & Albert	11635	LBP 3813	Data Missing
	Gymnorhamphichthys hypostomus Ellis	11851	UCF Uncat.	Iquitos, Peru
	Gymnorhamphichthys cf *hypostomus*	7310	MOU 027	Brazil
	Gymnorhamphichthys petiti Géry & Vu	11646	ROM 97536	Cuyuni-Mazaruni, Guyana
	Gymnorhamphichthys rondoni Miranda-Ribeiro	2154	MCP 46936	Tefé, Brazil
	Gymnorhamphichthys cf *rondoni*	10968	Data Missing	Data Missing
	Gymnorhamphichthys rosamariae Schwassmann	12000	Data Missing	Data Missing

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Gymnorhamphichthys n. sp. RUPU	10965	AUM 48205	Rupununi River, Guyana
	Hypopygus benoneae Peixoto, Dutra, de Santana, & Wosiacki	11856	UCF Uncat.	Rio Tapajós, Brazil
	Hypopygus isbruckeri de Santana & Crampton	2322	UF 148537	Rio Atabapo, Venezuela
	Hypopygus lepturus Hoedeman	2438	Uncatalogued	Rio Nanay, Peru
	Hypopygus minissimus de Santana & Crampton	2336	UF 175389	Rio Atabapo, Venezuela
	Hypopygus neblinae Mago-Leccia	2337	UF 148540	Rio Atabapo, Venezuela
	Hypopygus nijsseni de Santana & Crampton	2216	MCP 44651	Tefé, Brazil
	Hypopygus ortegai de Santana & Crampton	2429	UF 176879	Rio Nanay, Peru
	Rhamphichthys apurensis Fernández-Yépez	10995	ANSP 198380	Rio Apure, Venezuela
	Rhamphichthys drepanium Triques	11854	UCF 1456	Santarém, Brazil
	Rhamphichthys hahni Meinken	11640	LBP 3096	Rio Baia, Brazil
	Rhamphichthys heleios Carvalho & Albert	11855	UCF Uncat.	Rio Tapajós, Brazil
	Rhamphichthys lineatus Castelnau	2158	UCF Uncat.	Tefé, Brazil
	Rhamphichthys marmoratus Castelnau	2156	MCP 46932	Tefé, Brazil
	Rhamphichthys rostratus Linnaeus	8825	INPA 46	Rio Negro, Brazil
	Rhamphichthys n. sp. VENE	10999	ANSP 198379	Rio Apure, Venezuela
	Steatogenys duidae La Monte	2147	MCP 31958	Tefé, Brazil
	Steatogenys elegans Steindachner	8807	INPA 28860	Rio Negro, Brazil
	Steatogenys ocellatus Crampton, Thorsen, & Albert	9107	MUSM 44772	Rio Amazonas, Peru
Sternopygidae	*Archolaemus blax* Korringa	7307	MNRJ 33663	Rio Xingú, Brazil
	Archolaemus janeae Vari, de Santana, & Wosiacki	10983	INPA 39971	Rio Iriri, Brazil
	Archolaemus luciae Vari, de Santana, & Wosiacki	11857	UCF Uncat.	Rio Tapajós, Brazil
	Distocyclus conirostris Eigenmann & Allen	7306	CAR 022	Rio Negro, Brazil
	Eigenmannia humboldtii Steindachner	2822	Data Missing	Honda, Colombia
	Eigenmannia limbata Schreiner & Miranda-Ribeiro	8874	ANSP 182586	Rio Itaya, Peru
	Eigenmannia macrops Boulenger	2107	UCF Uncat.	Alvaraes, Brazil
	Eigenmannia microstoma Reinhardt	8887	MNRJ 31524	Jaboticatubas, Brazil
	Eigenmannia cf *nigra* Venezuela Mago-Leccia	10967	AUM 53750	Rio Ventuari, Venezuela
	Eigenmannia cf *nigra* Xingu1	10964	ANSP 194529	Rio Xingú, Brazil
	Eigenmannia cf *nigra* Xingu2	10962	INPA 40674	Rio Xingú, Brazil

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Eigenmannia trilineata López & Castello	8890	Data Missing	Rio Riachuelo, Argentina
	Eigenmannia vicentespelaea Triques	11649	LBP 2012072201	Rio Tocantins, Brazil
	Eigenmannia virescens A Valenciennes	2921	UCF Uncat.	Alvaraes, Brazil
	Eigenmannia virescens B	2907	UCF Uncat.	Tefé, Brazil
	Eigenmannia n. sp. HOND	2817	Data Missing	Honda, Colombia
	Eigenmannia n. sp. TEFE	2850	UCF Uncat.	Tefé, Brazil
	Eigenmannia n. sp. XING	8882	MNRJ 33658	Rio Xingú, Brazil
	Japigny kirschbaum Meunier, Jégu, & Keith	8992	MHNG 2682.031	Oyapock, French Guiana
	Rhabdolichops caviceps Fernández-Yépez	2883	MCP 36007	Alvaraes, Brazil
	Rhabdolichops cf *caviceps*	10990	INPA Uncat.	Rio Xingú, Brazil
	Rhabdolichops cf *caviceps*	8994	Data Missing	Rio Apure, Venezuela
	Rhabdolichops eastwardi Lundberg & Mago-Leccia	2104	MCP 36025	Alvaraes, Brazil
	Rhabdolichops cf *eastwardi*	9014	Data Missing	Altoorinoco, Venezuela
	Rhabdolichops cf *eastwardi*	8996	INPA 28911	Rio Negro, Brazil
	Rhabdolichops electrogrammus Lundberg & Mago-Leccia	2898	MCP 36029	Alvaraes, Brazil
	Rhabdolichops cf *electrogrammus*	9004	INPA 28863	Rio Negro, Brazil
	Rhabdolichops jegui Keith & Meunier	9013	ANSP 189021	Marowijne River, Surniame
	Rhabdolichops lundbergi Correa, Crampton, & Albert	2913	MCP 36044	Alvaraes, Brazil
	Rhabdolichops navalha Correa, Crampton, & Albert	9030	Data Missing	Altoorinoco, Venezuela
	Rhabdolichops nigrimans Correa, Crampton, & Albert	9028	ANSP 182578	Rio Itaya, Peru
	Rhabdolichops cf *stewarti* Lundberg & Mago-Leccia	GenBank	LBP	Data Unavailable
	Rhabdolichops troscheli Kaup	11853	UCF Uncat.	Iquitos, Peru
	Sternopygus aequilabiatus Humboldt	2820	Data Missing	Honda, Colombia
	Sternopygus arenatus Eydoux & Souleyet	9038	MNRJ 734	Guayas, Ecuador
	Sternopygus astrabes Mago-Leccia	2203	Data Missing	Tefé, Brazil
	Sternopygus branco Crampton, Hulen, & Albert	2108	MCP 32246	Tefé, Brazil
	Sternopygus dariensis Meek and Hildebrand	9043	IAvHP-8477	Rio Atrato, Colombia
	Sternopygus macrurus Bloch & Schneider	2112	UF 122829	Maynas, Peru
	Sternopygus cf *macrurus*	9086	Data Missing	Altoorinoco, Venezuela
	Sternopygus cf *macrurus*	9065	MNRJ 33649	Rio Xingú, Brazil

Table 1: Continued.

Family	Species	Specimen	Voucher	Locality
	Sternopygus obtusirostris Steindachner	2114	MCP 32261	Tefé, Brazil
	Sternopygus pejeraton Schultz	9089	UNELLEZ 40	Maracaibo, Venezuela
	Sternopygus xingu Albert & Fink	11648	Data Missing	Rio Araguaia, Brazil
Chanidae	*Chanos chanos* Forsskål	GenBank	Data Unavailable	Jawa Tengah, Indonesia
Cyprinidae	*Danio rerio* Hamilton	GenBank	Data Unavailable	Wayanad, India
	Tinca tinca Linnaeus	GenBank	Data Unavailable	River Alcantara, Italy
Catostomidae	*Catostomus commersonii* Lacépède	GenBank	Data Unavailable	Data Unavailable
Alestidae	*Phenacogrammus interruptus* Boulenger	GenBank	AMNH 233444	Data Unavailable
Cynodontidae	*Hydrolycus armatus* Jardine	GenBank	Data Unavailable	Data Unavailable
Distichodontidae	*Distichodus antonii* Schilthuis	GenBank	AMNH 246450	Data Unavailable
Ictaluridae	*Ameirurus nebulosus* Lesueur	GenBank	Data Unavailable	Saratoga, New York
Malapteruridae	*Malapterurus electricus* Gmelin	GenBank	AMNH 250725	Kolenté River, Guinea
Pangasiidae	*Pangasianodon hypophthalmus* Sauvage	GenBank	Data Unavailable	Mekong River, Thailand
Pimelodidae	*Pimelodus pictus* Steindachner	GenBank	Data Unavailable	Aquarium Trade

Table 2: Primer Sequences used for DNA amplification and sequencing. An asterisk (*) is used to identify primers used only for DNA sequencing, and not amplification.

Gene	Primer Name	Primer Sequence (5'-3')	Primer Source
co1	*co1*fishF1	TCAACYAATCAYAAAGATATYGGCAC	Ward et al. 2009
	*co1*fishR1	ACTTCYGGGTGRCCRAARAATCA	Ward et al. 2009
cyt b	GLUDGL	CGAAGCTTGACTTGAARAACCAYCGTTG	Palumbi et al. 1991
	*cyt b*R	CTCCGATCTTCGGATTACAAG	Palumbi et al. 1991
egr1	*egr1* FJf	CACCCAGCGCCTGCCGCCCA	This study
	egr1 FJr	TTCTTGTCCTTCTGCCGCA	This study
	egr1 FJfb*	CTGAACTGTGAGAAGTCCCTGGCTGA	This study
	egr1 FJrb*	GCCTGCATGTCGGCCAGCG	This study
enc1	*enc1* FJf	CAGGCTAAGGAGGTGGACTTCAGAGA	This study
	enc1 FJr	ACCATGCTCCACTTGTTGGC	This study
	enc1 intF*	TCCTGGAGTATGTGCCTCAG	This study
	enc1 intR*	GGAGACATTCTCCATGAGGAA	This study
glyt	*glyt* FJf	ATGCCGAAGCCTGTGTTTGT	This study
	glyt FJr	GCCTGCACTGATGTCTGRCA	This study
	glyt FJfb*	TATCAGTATGGCTTTGTACAGCC	This study
	glyt intR*	GTGTCTTGATAAATCCGGAAAAG	This study
rag1	*rag1* F2	CTGAGCTGCAGTCAGTACCATAAGATGT	López et al. 2004
	rag1 R1	GTGTAGAGCCAGTGGTGYTT	López et al. 2004
	rag1 intF*	TGGAGGAGGACATCATAGA	This study
	rag1 intR*	ATGTCACAGTGCAGGGCATC	This study
rag2	*rag2* GYF	ACAGGCATCTTTGGKATTCG	Lovejoy et al. 2010
	rag2 R6	TGRTCCARGCAGAAGTACTTG	Lovejoy & Collette, 2001
	rag2 JF1	TGCTATCTTCCACCACTGCGVTGCC	This Study
	rag2 JR1	TCATCYTCCTCATCKTCCTCATTGTA	This Study
rh1	*rh1* 93F	CNTATGAATAYCCTCAGTACTACC	Chen et al. 2003
	rh1 GR2int	GCCRTAGCAGAAGCAGATGGTGAA	This Study
	rh1 GF2int	GAGAACCAYGCCATCATGGGYGTG	This Study
	rh1 073R	CCRCAGCACARCGTGGTGATCATG	Chen et al. 2003
zic1	*zic1* F9	TCCTCGAACGTGGTGAACAG	Li et al. 2007
	zic1 R967	CTGTGTGTGTCCTTTTGTGRATYTT	Li et al. 2007
	zic1 intF*	TCCTCGAACGTGGTGAACAG	Brochu, 2011
	zic1 intR*	TTCGGGTTAGTTAGTTGCTCCGG	Brochu, 2011

Table 3: Best nucleotide substitution model found for each gene using PartitionFinder 1.1.1 and the Corrected Akaike Information Criterion. Best-fit models were found for MrBayes.

Gene Partition	Best Model for MrBayes
co1	GTR + I + G
cyt *b*	GTR + I + G
egr1	GTR + I + G
enc1	SYM + I + G
glyt	SYM + G
rag1	SYM + I + G
rag2	SYM + G
rh1	GTR + I + G
zic1	GTR + I + G

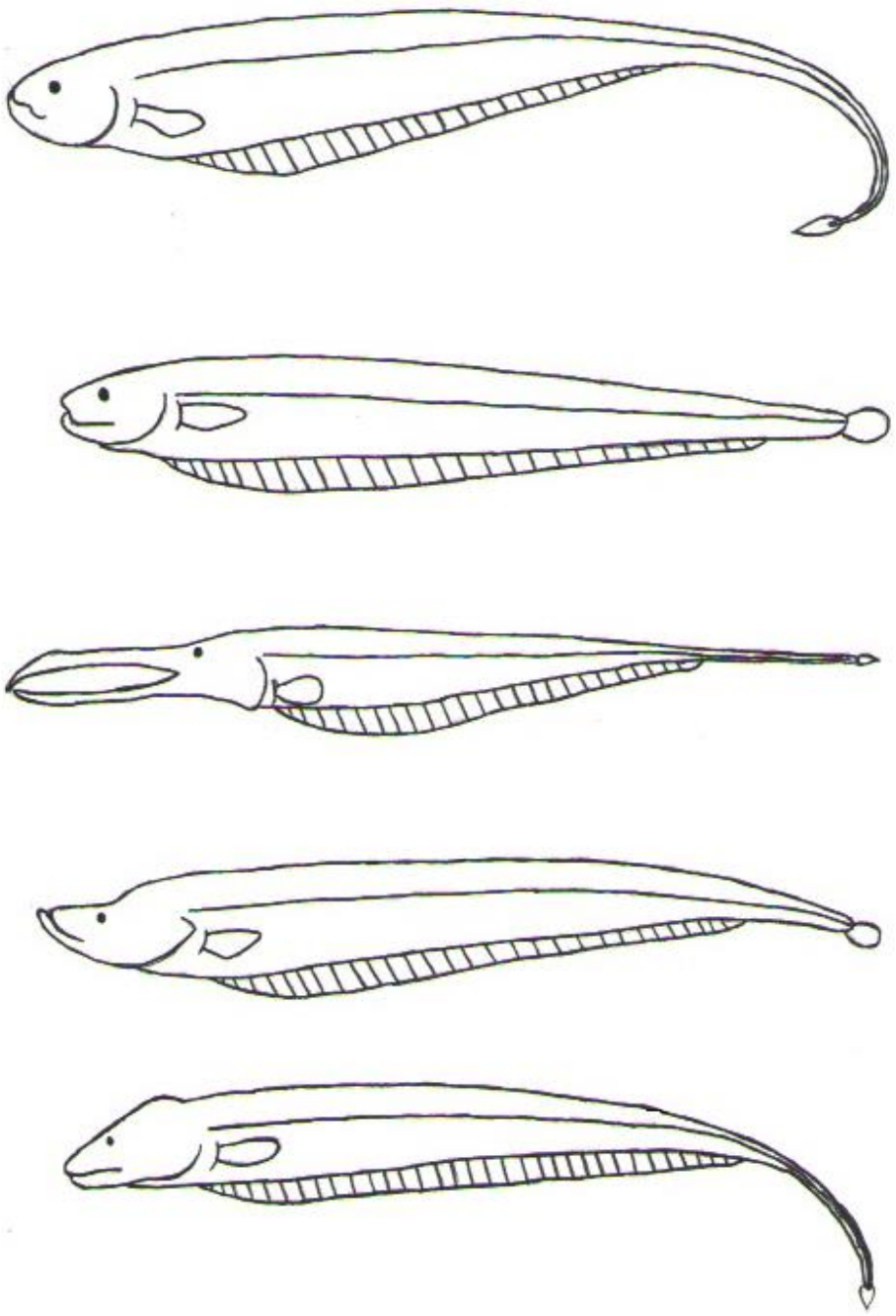


Plate 1: Line drawings of apteronotid genera representatives. Labels correspond to the following species: A – *Adontosternarchus sachsi*, B – *Apteronotus albifrons*, C – *Compsaraia compsa*, D – *Magosternarchus duccis*, E – *Megadontognathus cuyuniense*.

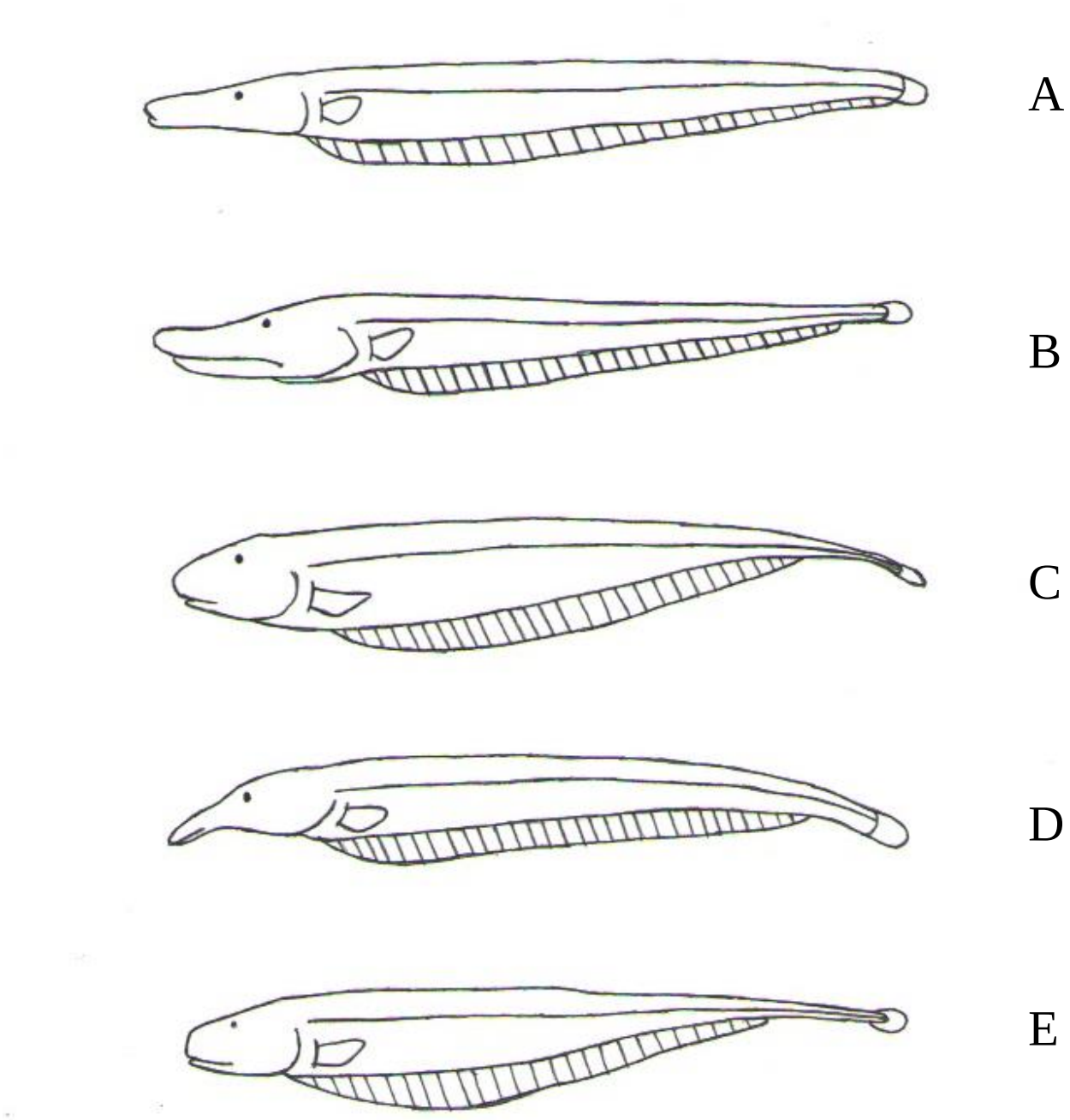


Plate 2: Line drawings of apteronotid genera representatives. Labels correspond to the following species: A – *Orthosternarchus tamandua*, B – *Parapteronotus hasemani*, C – *Pariosternarchus amazonensis*, D – *Platyurosternarchus macrostomus*, E – *Porotergus gymnotus*.

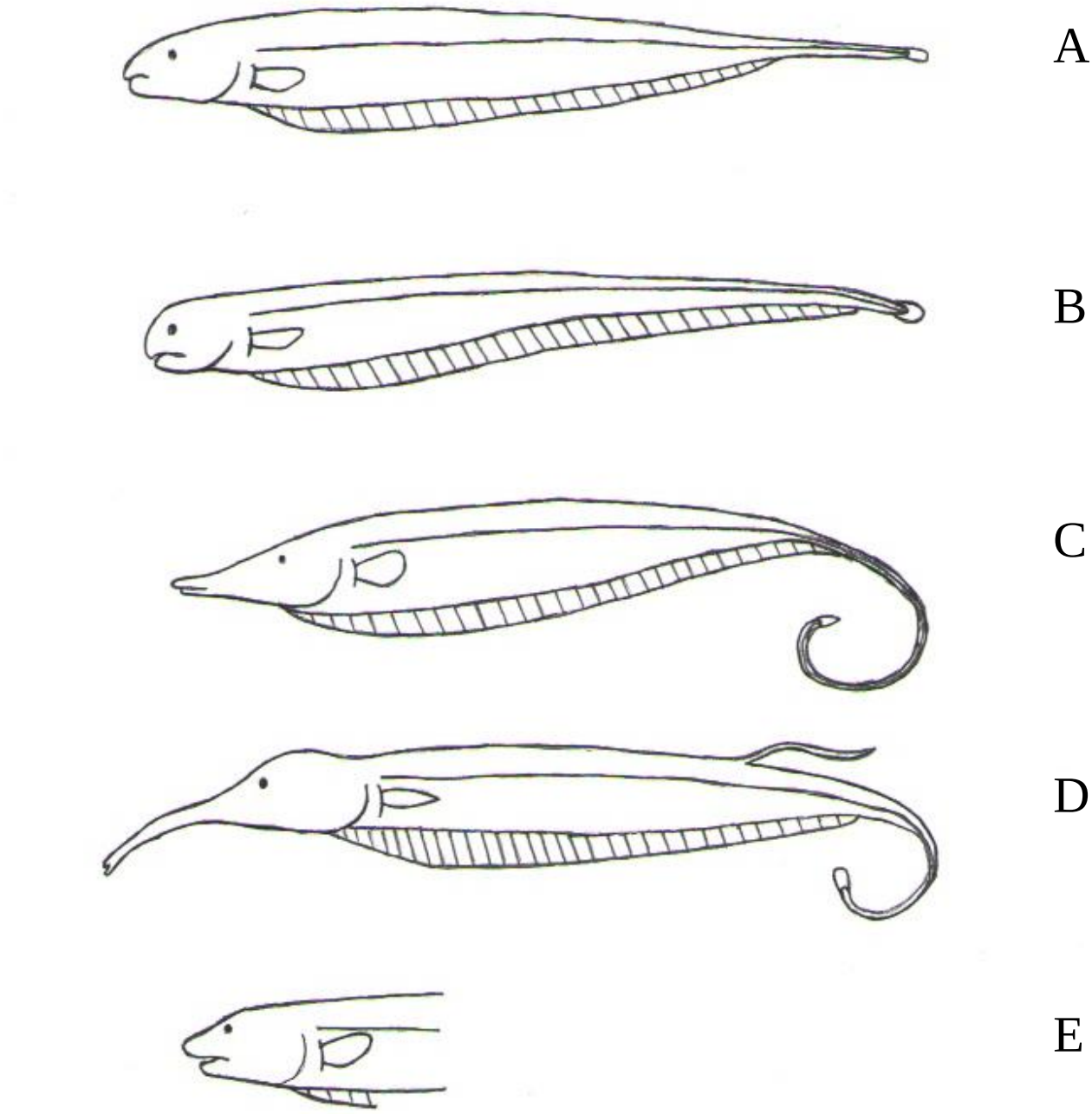
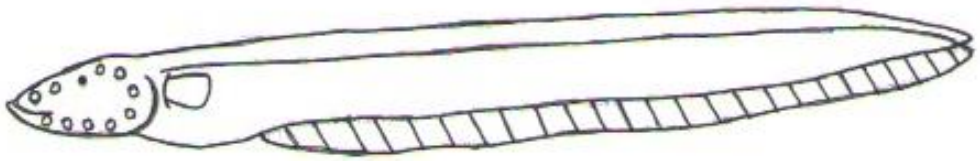
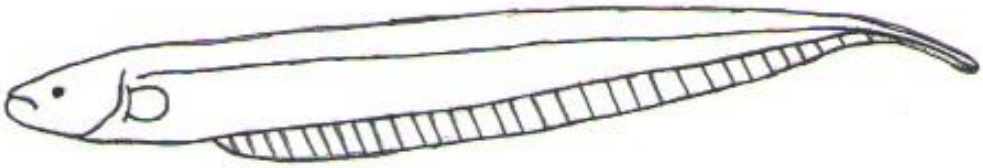


Plate 3: Line drawings of apteronotid genera representatives. Labels correspond to the following species: A – *Sternarchella schotti*, B – *Sternarchogiton nattereri*, C – *Sternarchorhamphus muelleri*, D – *Sternarchorhynchus oxyrhynchus*, E – *Tembeassu marauna*.



A



B

Plate 4: Line drawings of gymnotid genera representatives. Labels correspond to the following species: A – *Electrophorus electricus*, B – *Gymnotus carapo*.

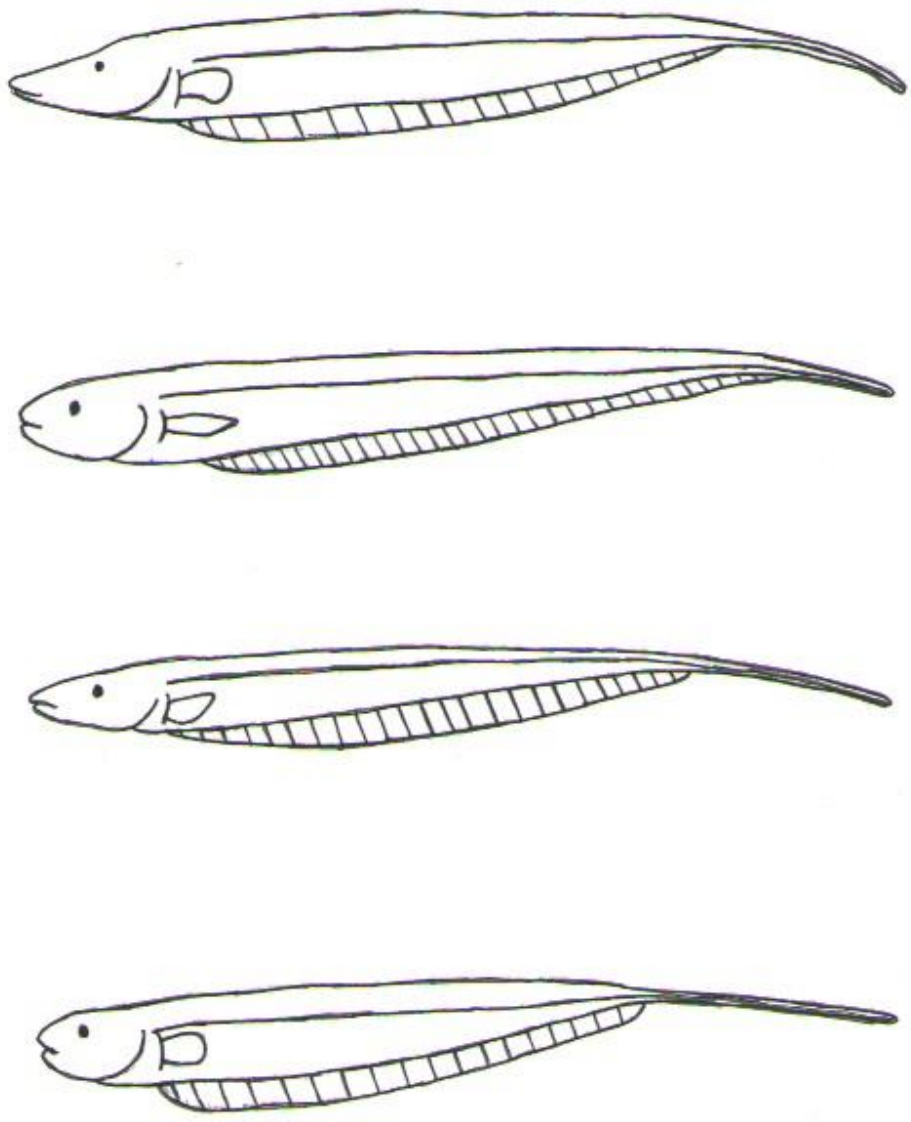


Plate 5: Line drawings of hypopomid genera representatives *sensu* Mago-Leccia (1994). Labels correspond to the following species: A – *Akawaio penak*, B – *Brachyhypopomus pinnicaudatus*, C – *Hypopomus artedi*, D – *Hypopygus lepturus*.

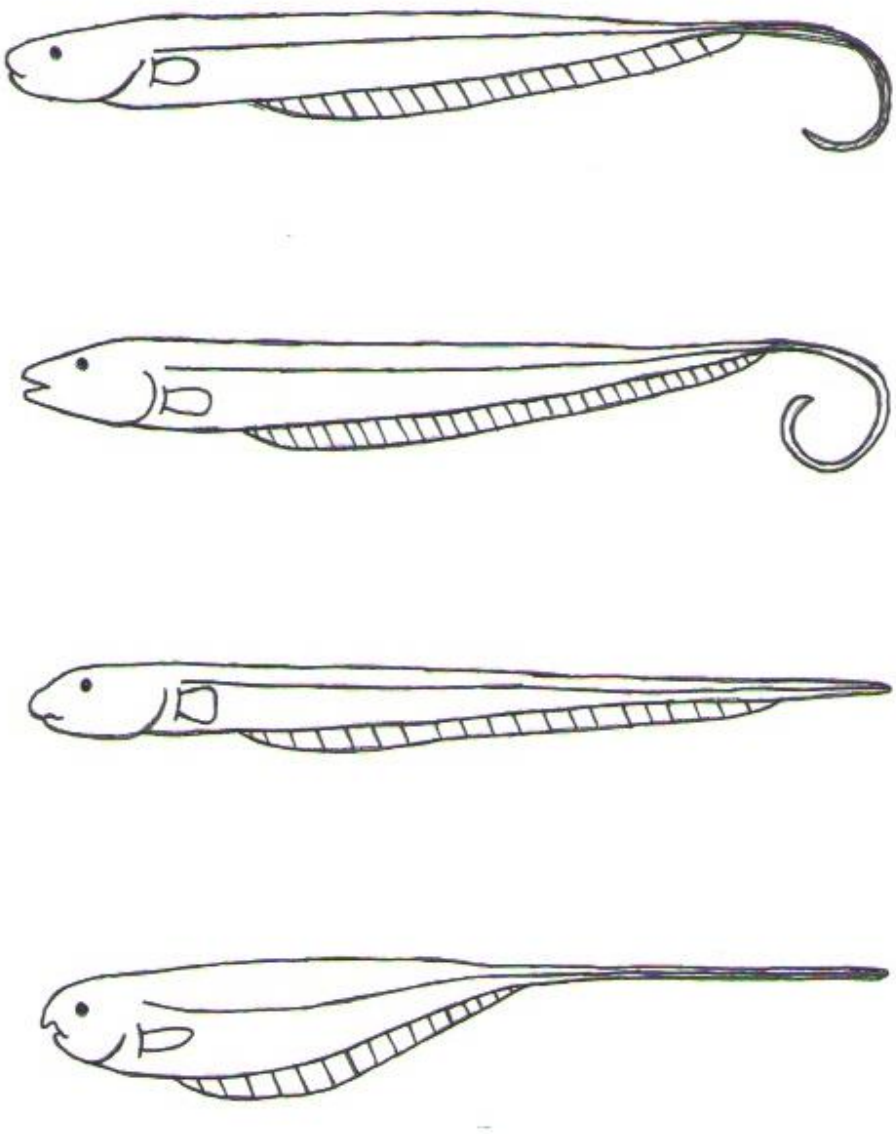


Plate 6: Line drawings of hypopomid genera representatives *sensu* Mago-Leccia (1994). Labels correspond to the following species: A – *Microsternarchus bilineatus*, B – *Procerusternarchus pixuna*, C – *Racenisia fimbriipinna*, D – *Steatogenys duidae*.

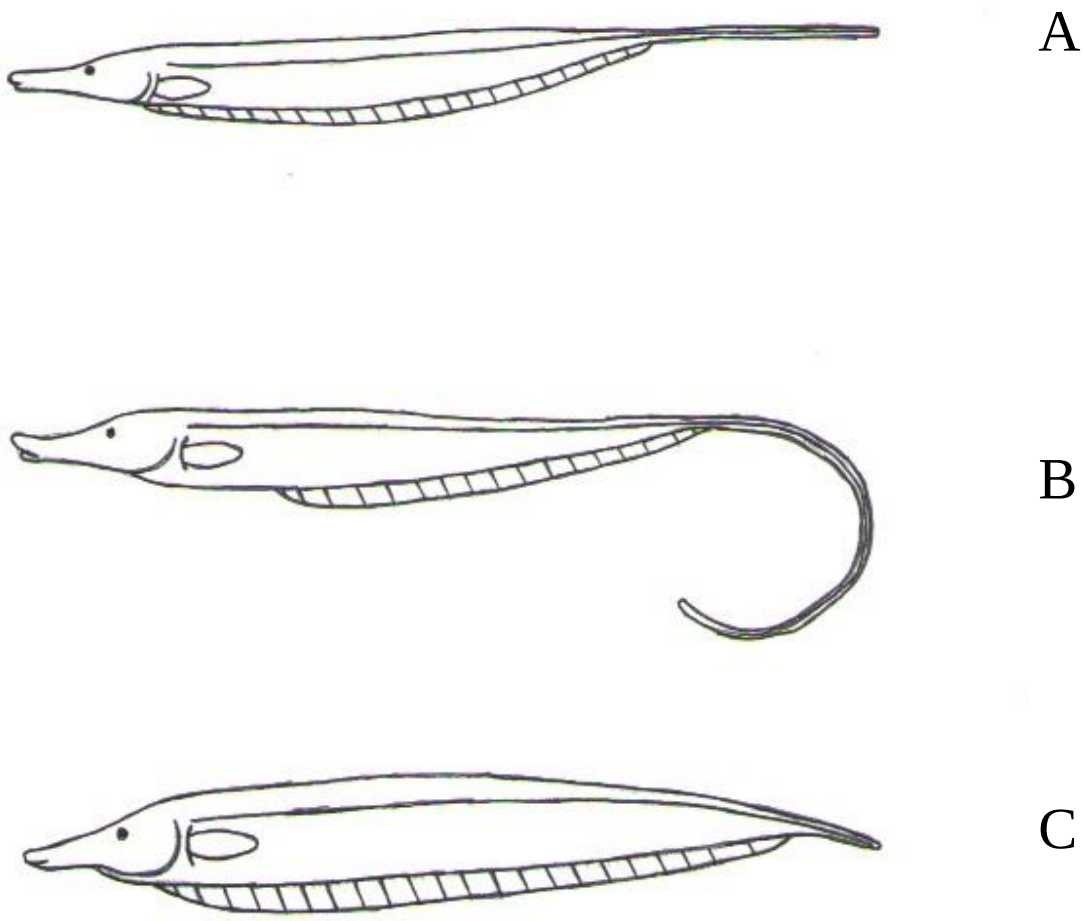


Plate 7: Line drawings of rhamphichthyid genera representatives *sensu* Mago-Leccia (1994). Labels correspond to the following species: A – *Gymnorhamphichthys rondoni*, B – *Iracema caiana*, C – *Rhamphichthys marmoratus*.

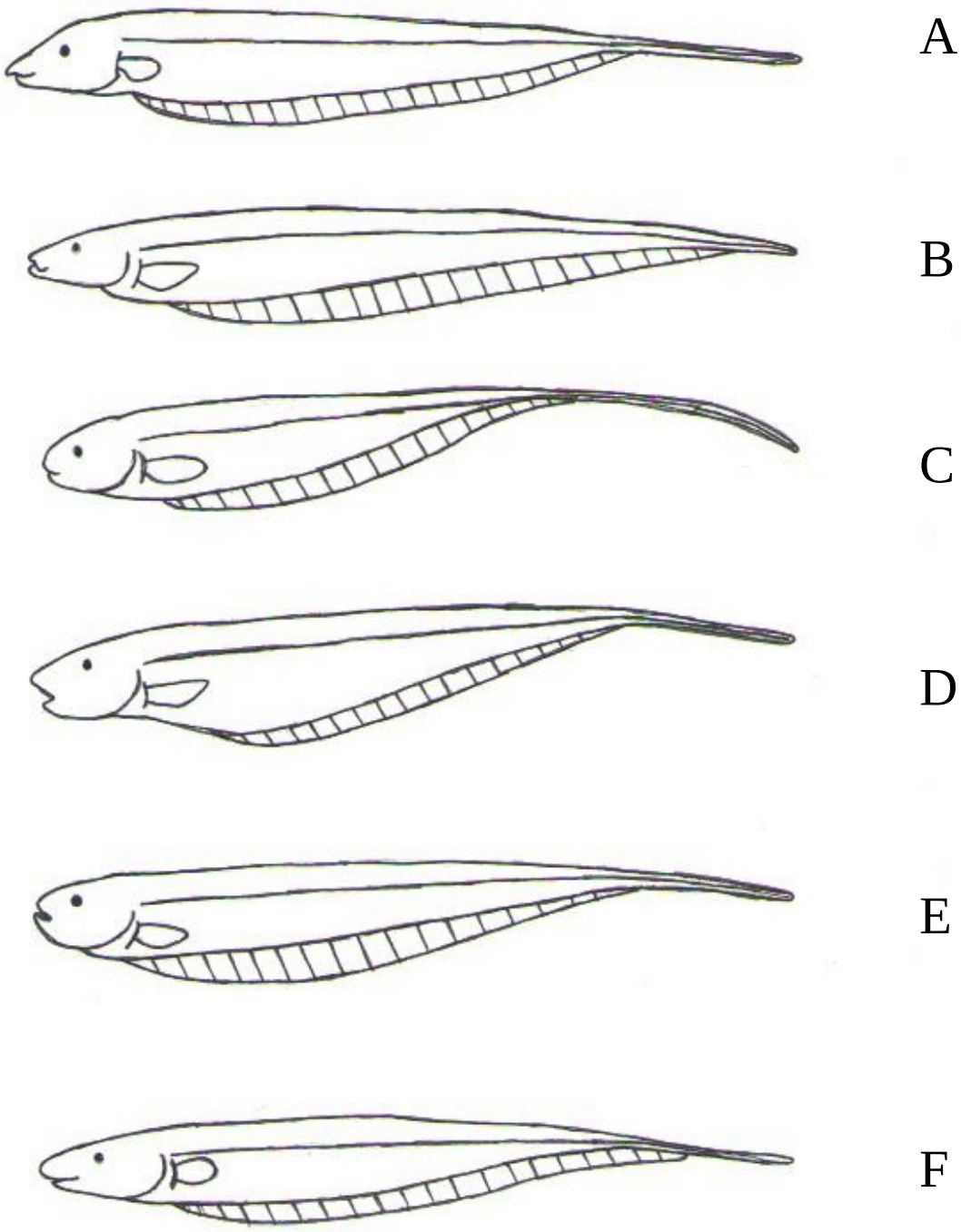


Plate 8: Line drawings of sternopygid genera representatives. Labels correspond to the following species: A – *Archolaemus blax*, B – *Distocyclus conirostris*, C – *Eigenmannia virescens*, D – *Japigny kirschbaum*, E – *Rhabdolichops stewarti*, F – *Sternopygus macrurus*.

Copyright Acknowledgements