

Phylogeny and divergence times of suckers (Cypriniformes: Catostomidae) inferred from Bayesian total-evidence analyses of molecules, morphology, and fossils

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ABSTRACT

Catostomidae (“suckers”) is a diverse (76 species) and broadly distributed family of Holarctic freshwater fishes with a rich fossil record and a considerable number (~35%) of threatened and imperiled species. We integrate DNA sequences (three mitochondrial genes, three nuclear genes), morphological data, and fossil information to infer sucker phylogenetic relationships and divergence times using Bayesian “total-evidence” methods, and then test hypotheses about the temporal diversification of the group. Our analyses resolved many nodes within subfamilies and clarified Catostominae relationships to be of the form ((Thoburniini, Moxostomatini), (Erimyzonini, Catostomini)). Patterns of subfamily relationships were incongruent, but mainly supported two placements of the Myxocyprininae; distinguishing these using Bayes factors lent strongest support to a model with Myxocyprininae sister to all remaining sucker lineages. We improved our Bayesian total-evidence dating analysis by excluding problematic characters, using a clock-partitioning scheme identified by Bayesian model selection, and employing a fossilized birth-death tree prior accommodating morphological data and fossils. The resulting chronogram showed that suckers evolved since the Late Cretaceous–Eocene, and that the Catostomini and Moxostomatini clades have accumulated species diversity since the early to mid-Miocene. These results agree with the fossil record and confirm previous hypotheses about dates for the origins of Catostomidae and catostomine diversification, but reject previous molecular hypotheses about the timing of divergence of ictiobines, and between Asian–North American lineages. Overall, our findings from a synthesis of multiple data types enhance understanding of the phylogenetic relationships, taxonomic classification, and temporal diversification of suckers, while also highlighting practical methods for improving Bayesian divergence dating models by coupling phylogenetic informativeness profiling with relaxed-clock partitioning.

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page 29

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INTRODUCTION

“Suckers” in the family Catostomidae (Cypriniformes) form a large family of Holarctic freshwater fishes with 76 extant species in 14 genera native to North America and Asia (Harris, Hubbard & Sandel, 2014). Seventy-five species from 13 genera occur in North America (Harris, Hubbard & Sandel, 2014; Nelson et al., 2004; Unmack et al., 2014), where they constitute the third largest freshwater fish clade, after darters (Etheostominae) and minnows (Cyprinidae), and ~8% of the continental ichthyofauna (Warren et al., 2000). *Myxocyprinus asiaticus* is endemic to eastern China and *Catostomus catostomus* is the only extant trans-Pacific species (Harris, Hubbard & Sandel, 2014). Suckers are an ancient group whose fossil record spans the Cenozoic, from the early Eocene onwards (Cavender, 1986; Smith, 1992; Smith et al., 2002; Appendix S1). Around 35% of the taxa (26 to >35 species or genetic lineages) are endangered, threatened, or of special conservation concern (Harris, Hubbard & Sandel, 2014; Nelson et al., 2004; Warren et al., 2000).

Geographical distributions of suckers and their taxonomy and relationships have attracted the interest of systematists and biogeographers for over 150 years (Agassiz, 1854). Pre-1900 systematics and taxonomy studies dealt with species descriptions and higher-level classifications of the group (reviewed by Harris, Hubbard & Sandel, 2014; Smith, 1992). Subsequently, key papers on sucker classification designated genera, subgenera, and tribes (Hubbs, 1930; Robins & Raney, 1956) and contributed a pre-cladistics phylogeny (Miller, 1959), as well as the first phylogeny for species in the tribe Moxostomatini (Jenkins, 1970). The findings of post-systematics studies of sucker interrelationships (last 40 years), which were based on a variety of different data types, are summarized in Table 1 and Fig. 1. These studies supported the recognition of four subfamilies (Myxocyprininae, Cycleptinae, Ictiobinae, and Catostominae) and tribes (Catostomini, Erimyzonini, Moxostomatini, Thoburniini) plus the modern allocation of genera to these groups (Harris & Mayden, 2001; Harris et al., 2002). Most phylogenetic analyses of suckers to date have analyzed DNA sequence data from increasing numbers of mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) genes. Recent advances include Clements, Bart & Hurley's (2012) study of phylogenetic relationships of Moxostomatini using the first dataset based on two loci—mtDNA *cytb* and nuclear growth hormone intron (GHI) sequences. Also, Unmack et al. (2014) studied the phylogeny and biogeography of *Pantosteus*, a former subgenus of *Catostomus* (Harris & Mayden, 2001; Harris et al., 2002; Smith, 1966) using independent morphological and mtDNA genetic analyses. Overall, while the inclusion of additional taxa and sequence data in recent analyses has yielded novel insights on sucker relationships, a consensus among hypotheses regarding relationships of higher-level species groups, i.e., subfamilies, has yet to emerge (Table 1; Fig. 1). Moreover, although time-calibrated phylogenies based on comprehensive tip sampling are necessary to understand the tempo and mode of diversification of groups of freshwater fishes (Near et al., 2011, 2012), such a tool has yet to be inferred for suckers. This leaves workers at a distinct disadvantage in considering the macroevolutionary, ecological, or conservation trends of suckers in a phylogenetic context.

Table 1 Summary of post-systematic phylogenetic and taxonomic studies of suckers in the family Catostomidae.

Study	<i>n</i>	Data type	Analysis	Supported clades/relationships
<i>Ferris & Whitt (1978)</i>	30	20 Isozyme loci (loss of duplicate gene expression)	Wagner	Ictiobinae + (Cycleptinae + Catostominae), three catostomin tribes
<i>Smith (1992)</i>	64	157 Morphological, biochemical and early life-history transformation series	MP	Ictiobinae + (Cycleptinae + Catostominae), <i>Moxostoma</i> in paraphyletic grade with “ <i>Scartomyzon</i> ” within Moxostomatini; also trichotomy of <i>M. ariommum</i> + <i>Thoburnia</i> + <i>Hypentelium</i>
<i>Harris & Mayden (2001)</i>	16	mtDNA SSU and LSU rDNA sequences	MP	Four monophyletic subfamilies, Thoburniini resurrected and includes <i>Hypentelium</i> ; Catostomini + Erimyzonini and Moxostomatini + Thoburniini
<i>Harris et al. (2002)</i>	50	mtDNA cytochrome <i>b</i> (<i>cytb</i>) gene	MP and ML	Four monophyletic subfamilies, provisional Thoburniini; “ <i>Scartomyzon</i> ” as a junior synonym of <i>Moxostoma</i>
<i>Sun et al. (2007)</i>	17	mtDNA <i>cytb</i> gene	UPGMA	Monophyletic Ictiobinae and Catostominae; Moxostomatini + Catostomini
<i>Doosey et al. (2010)</i>	60	mtDNA NADH subunit 4 and 5 (ND4/5) sequences	ML	((Cycleptinae, (Myxocyprininae, Ictiobinae)), Catostominae); also among catostomins: Erimyzonini + (Moxostomatini + Thoburniini)
<i>Chen & Mayden (2012)</i>	67	Interphotoreceptor retinoid-binding protein (IRBP) sequences	ML	Supported previous relationships including monophyly of subfamilies and tribes except Thoburniini
<i>Clements, Bart & Hurley (2012)</i>	45	Growth hormone intron (GHI)	ML	Para-/polyphyletic <i>Moxostoma</i> and <i>Scartomyzon</i>
<i>Unmack et al. (2014)</i>	24	Morphology, plus <i>cytb</i> and other mtDNA gene sequences	MP, ML, and Bayesian	Monophyletic <i>Pantosteus</i> within Catostomini, except for <i>Catostomus</i> (<i>P.</i>) <i>columbianus</i> (potential hybrid origin)

Note:

ML, maximum likelihood; MP, maximum parsimony; *n*, sample size, including ingroup and outgroup taxa or lineages; UPGMA, unweighted pair-group method with arithmetic mean; Wagner, Wagner method for inferring “most parsimonious” tree.

In this study, we use “total-evidence” analyses of multilocus sequence data from three mtDNA genes and three nuclear genes representing the most comprehensive sampling of suckers to date, combined with available morphological and fossil data, to infer the phylogenetic relationships and divergence times of suckers in a Bayesian framework. We discuss the phylogenetic and taxonomic implications of our results in light of previous phylogenetic studies of suckers. We apply Bayesian divergence time methods to the data (*Drummond et al., 2012*) and take advantage of rich information on the age and distributions of sucker fossils (*Cavender, 1986; Smith et al., 2002; Appendix S1*) to improve divergence time estimation by incorporating extant and fossil sampling in a “fossilized birth-death process” tree prior (*Stadler, 2010; Gavryushkina et al., 2014; Heath, Huelsenbeck & Stadler, 2014*). Moreover, by coupling assessments of the phylogenetic signal of different data subsets with evaluation of clock-partitioning strategies, we were able to avoid potentially confounding effects of problematic characters on our divergence time inferences. The resulting time-calibrated phylogeny is then used to test several hypotheses about the temporal diversification of suckers. Namely, we test the

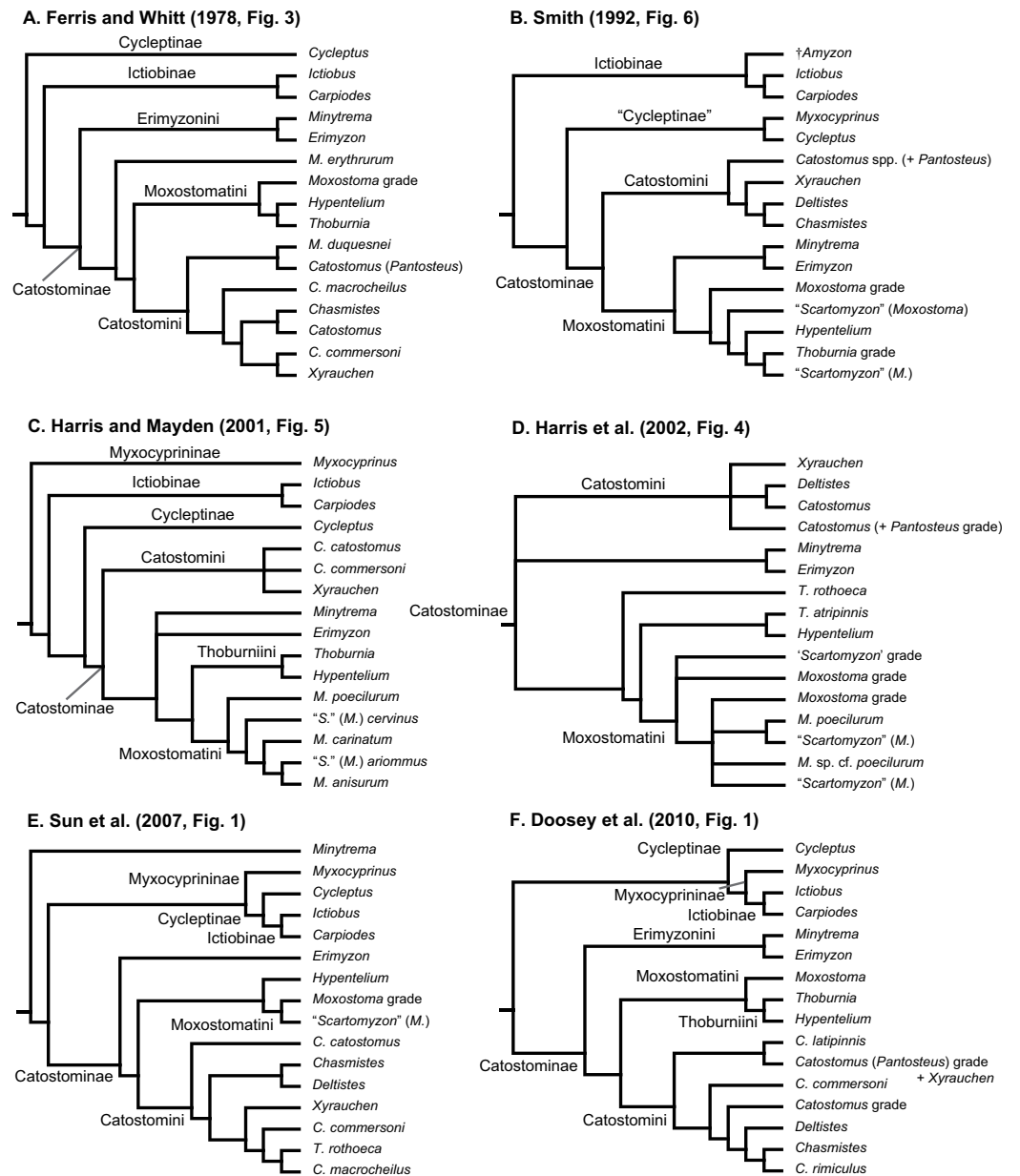


Figure 1 Six alternative hypotheses of phylogenetic relationships among Catostomidae subfamilies and tribes. These results were based on previous studies of electrophoretic data (A); morphological, behavioral, and developmental characters (B); and DNA sequence data (C–F).

Full-size  DOI: [10.7717/peerj.5168/fig-1](https://doi.org/10.7717/peerj.5168/fig-1)

hypotheses (H_1) that Asian Myxocyprinae and North American suckers (Cycleptinae) diverged since ~14 million years ago (Ma) in the mid-Miocene, and (H_2) that the initial divergence of Ictiobinae lineages occurred since ~10 Ma in the late Miocene, as indicated by mtDNA *cytb* gene divergences (Sun et al., 2007). We also tested the hypotheses (H_3) that the diversification of lineages within Catostominae followed ~20 Ma in the Miocene (Smith, 1992; Sun et al., 2007), and (H_4) that Catostomidae species have diversified from a tetraploid ancestor since ~50 Ma in the early Eocene (Uyeno & Smith, 1972).

Overall, by yielding a new phylogeny of suckers and divergence dates for their most recent common ancestors (MRCAs), our study sheds light on the interrelationships, taxonomic classification, and tempo of speciation in a diverse and threatened clade of Holarctic freshwater fishes.

MATERIALS AND METHODS

Molecular taxon sampling, laboratory methods, and sequence alignment

We obtained and sequenced mtDNA and nDNA genes from tissue samples of 121 sucker specimens from throughout the geographical range of the family. Samples were provided by ichthyological collections or colleagues (see Acknowledgements), or were already in-hand at the beginning of the study. We sequenced taxa representing all sucker genera and all Catostominae species except for †*Moxostoma lacerum*, an historically extinct taxon last sampled from the Mississippi River Basin in 1893 ([NatureServe, 2013](#)), and *Chasmistes cujus*, an endangered species ([NatureServe, 2014](#)) for which it was difficult to obtain samples ([Table 2](#)). To complement our sampling and add data from more unlinked loci to our datasets, we obtained additional sequences from GenBank as described below. Overall, we obtained genetic sequences for the most comprehensive taxonomic sampling of the family to date, including 78 species/lineages representing all 14 extant genera, including four “candidate species” within Moxostomatini ([Table 2](#)). Based on recent hypotheses of relationships of suckers and cypriniform fishes ([Mayden et al., 2008](#); [Saitoh et al., 2006](#); [Smith, 1992](#)), we included DNA sequences from five outgroup taxa in our datasets: *Cyprinus carpio* (Cyprinidae); *Gyrinocheilus aymonieri* (Gyrinocheilidae); and *Cobitis striata*, *Chromobotia macracanthus*, and *Leptobotia mantschurica* (Cobitidae). We follow the taxonomy of subfamilies listed in [Harris & Mayden \(2001\)](#) and [Harris et al. \(2002\)](#).

All molecular laboratory work for this project conducted in the R. L. Mayden laboratory was approved under Saint Louis University Institutional Animal Care and Use Committee (IACUC) protocol #2467. We extracted whole genomic DNA using QIAGEN DNeasy Tissue kits (Catalog No. 69506; QIAGEN, Valencia, CA, USA), or the CTAB method of [Saghai-Marooif et al. \(1984\)](#). We amplified and sequenced the mtDNA *cytb* gene using polymerase chain reaction (PCR) primers and parameters in [Harris et al. \(2002\)](#). We amplified and sequenced the mtDNA NADH subunit 2 (ND2) gene with primers 562 (5'-TAA GCT ATC GGG CCC ATA CC-3') and 449 (5'-TGC TTA GGG CTT TGA AGG CTC-3') from LGL Genetics, Bryan, TX, using the same PCR amplification parameters used for *cytb*. We also sequenced the first part of the nuclear interphotoreceptor retinoid binding protein (IRBP) gene using PCR primers and methods in [Chen et al. \(2008\)](#), and we sequenced nuclear ribosomal protein S7 intron 1 (*RPS7*) using PCR primers and parameters in [Chow & Takeyama \(1998\)](#). We purified double-stranded PCR products using columns or gel extraction kits (QIAGEN, Valencia, CA, USA). Given that catostomids are tetraploids ([Uyeno & Smith, 1972](#)), we cloned nuclear PCR products to ensure orthologous sequences were used in subsequent

Table 2 List of sequences used in the present study including museum/field numbers and GenBank accession numbers.

Taxon ^a	Museum/field numbers ^b	<i>cytb</i>	ND2	<i>cox1</i>	IRBP	RPS7	GHI
Order Cypriniformes							
Family Cyprinidae							
Subfamily Cyprininae							
<i>Cyprinus carpio</i>	CToL 1751	AP009047	AP009047	EU524006	FJ197101	DQ163924	FJ265047
Family Gyrinocheilidae							
<i>Gyrinocheilus aymonieri</i>	CToL 612	NC008672	NC008672	JF915620	JX470019	–	FJ265031
Family Cobitidae						–	–
Subfamily Cobitinae							
<i>Cobitis striata</i>	CToL 230	NC004695	NC004695	–	Mayden et al. (2009; K. Saitoh, unpublished data)	–	–
Subfamily Botinae							
<i>Chromobotia macracanthus</i>	CToL 217	AC024175	AC024175	KF738207	FJ197086	–	–
<i>Leptobotia mantschurica</i>	–	AB242170	AB242170	AB242170	FJ197087	–	FJ265035
Family Catostomidae							
Subfamily Myxocyprininae							
<i>Myxocyprinus asiaticus 1</i>	–	NC006401	NC006401	AY526869	–	–	–
<i>M. asiaticus 2</i>	–	AY526869	AY526869	–	–	–	–
<i>M. asiaticus 3</i>	UAIC 11698.01	JX488760	JX488826	–	JX488937	This study	FJ265052
Subfamily Ictiobinae							
<i>Carpiodes carpio 1</i>	–	AY366087	AY366087	–	–	–	–
<i>Car. carpio 2</i>	UAIC 11219.08	AF454867	JX488827	JN024866	–	–	JF837387
<i>Car. cyprinus</i>	TU 157.01	JX488761	JX488828	EU523924	JX488938	–	GU937849
<i>Car. Velifer</i>	TU 108.16	JX488762	JX488829	JN024878	JX488939	–	JF837435
<i>Ictiobus bubalus 1</i>	TU 124.07	JX488763	JX488830	KF929996	JX488940	–	JF799533
<i>I. bubalus 2</i>	TU 120.01	JX488764	JX488831	–	JX488941	–	–
<i>I. bubalus 3</i>	TU 244.03	JX488765	JX488832	–	JX488942	–	–
<i>I. cyprinellus 1</i>	TU 101.05	JX488766	JX488833	EU524687	–	–	–
<i>I. cyprinellus 2</i>	TU 107.15	JX488767	JX488834	–	JX488943	–	GU937840
<i>I. niger 1</i>	TU 121.01	JX488768	JX488835	EU524107	JX488944	–	FJ226251
<i>I. niger 2</i>	TU 124.06	JX488769	JX488836	–	JX488945	–	–
Subfamily Cycleptinae							
<i>Cycleptus elongatus 1</i>	–	NC008645	NC008645	–	–	–	–
<i>Cyc. elongatus 2</i>	UAIC 11371.01	AF454868	JX488837	KF929801	JX488946	–	FJ265028
<i>Cyc. meridionalis</i>	UNL 1-30	JX488770	JX488838	–	JX488947	–	–

(Continued)

Table 2 (continued).

Taxon ^a	Museum/field numbers ^b	cytb	ND2	cox1	IRBP	RPS7	GHI
Subfamily Catostominae							
Tribe Catostomini							
<i>Catostomus ardens</i> 1	USU-UBL	JX488771	JX488839	JN024886	JX488948	–	–
<i>C. ardens</i> 2	USU-USD	JX488772	JX488840	–	JX488949	–	–
<i>C. bernardini</i> Río Papogochic	BRK0507-2	JX488773	JX488841	EU668462	JX488950	–	–
<i>C. bernardini</i> Río Batopilas 1	JEB0504-2	JX488774	JX488842	–	JX488951	–	–
<i>C. bernardini</i> Río Batopilas 2	JEB0505-7	JX488775	JX488843	–	JX488952	–	–
<i>C. bernardini</i> Río Yecora	JEB0510-1	JX488776	JX488844	–	JX488953	–	–
<i>C. cahita</i> 1	BRK0511-6	JX488777	JX488845	EU668375	JX488954	This study	–
<i>C. cahita</i> 2	BRK0511-8	JX488778	JX488846	–	JX488955	This study	–
<i>C. catostomus</i>	–	–	–	W.J. Chen	W.J. Chen, unpublished data	–	–
<i>C. catostomus</i>	UAIC 11237.04	AF454871	JX488847	EU523925	–	–	GU937824
<i>C. columbianus</i>	OS 17548	JX488780	JX488849	–	JX488957	–	–
<i>C. commersoni</i> 1	–	NC008647	NC008647	–	–	This study	–
<i>C. commersoni</i> 2	UAIC 11156.03	JX488781	JX488850	EU523931	JX488958	This study	JF799535
<i>C. fumeiventris</i> 1	–	JX488784	JX488853	–	JX488961	–	–
<i>C. fumeiventris</i> 2	–	JX488785	JX488854	–	JX488962	–	–
<i>C. insignis</i>	MSB 49603-2	JX488786	JX488855	–	JX488963	–	–
<i>C. insignis</i>	MSB 49603-4	JX488787	JX488856	HQ556974	JX488964	This study	–
<i>C. latipinnis</i> 1	MSB 49601	JX488788	JX488857	–	JX488965	This study	–
<i>C. latipinnis</i> 2	MSB 49602	JX488789	JX488858	–	JX488966	–	–
<i>C. leopoldi</i>	JEB15-1	JX488790	JX488859	EU668394	JX488967	–	–
<i>C. macrocheilus</i>	OS 15886	JX488791	JX488860	EU523932	JX488968	–	–
<i>C. microps</i>	OS 17571-1	JX488792	JX488861	HQ557219	JX488969	This study	–
<i>C. microps</i>	OS 17571-2	JX488793	JX488862	–	JX488970	–	–
<i>C. occidentalis humboldtianus</i> 1	OS 15623-1	JX488795	JX488864	–	JX488972	–	–
<i>C. occidentalis humboldtianus</i> 2	OS 15623-2	JX488796	JX488865	–	JX488973	–	–
<i>C. occidentalis lacusanserinus</i>	OS 1943BB	JX488797	JX488866	–	JX488974	–	–
<i>C. occidentalis mniotiltus</i> 1	UAIC 13448.01-2	JX488798	JX488867	–	JX488975	–	–
<i>C. occidentalis mniotiltus</i> 2	UAIC 13448.01-4	JX488799	JX488868	–	JX488976	–	–
<i>C. occidentalis occidentalis</i>	UAIC 11546.02-1	JX488800	JX488869	KF558280	JX488977	This study	–
<i>C. rimiculus</i>	OS 15908	AF454875	JX488874	–	JX488982	–	–

(Continued)

Table 2 (continued).

Taxon ^a	Museum/field numbers ^b	cytb	ND2	cox1	IRBP	RPS7	GHI
<i>C. snyderi</i>	OS 15900	JX488806	JX488876	–	JX488984	–	–
<i>C. tahoensis</i> 1	BuffaloCrk4	JX488807	JX488877	JN024954	JX488985	This study	–
<i>C. tahoensis</i> 2	SmokeCrk1	JX488808	JX488878	–	JX488986	–	–
<i>C. tsiltcoosensis</i> 1	OS X113	JX488809	JX488879	–	JX488987	–	–
<i>C. tsiltcoosensis</i> 2	OS X114	JX488810	JX488880	–	JX488988	–	–
<i>C. warnerensis</i> 1	OS 14251-2	JX488814	JX488884	–	JX488992	This study	–
<i>C. warnerensis</i> 2	OS 14251-3	JX488815	JX488885	–	JX488993	–	–
<i>C. wigginsi</i>	JEB0511-1	JX488816	JX488886	EU668440	JX488994	–	–
<i>Catostomus</i> sp. Coquille River	OS X130	JX488811	JX488881	–	JX488989	–	–
<i>Catostomus</i> sp. Wall Canyon 1	OS X61	JX488812	JX488882	–	JX488990	–	–
<i>Catostomus</i> sp. Wall Canyon 2	OS X67	JX488813	JX488883	–	JX488991	–	–
<i>Chasmistes breviostris</i>	OS 15963	JX488817	JX488887	–	JX488995	–	GU937825
<i>Ch. liorus mictus</i> 1	BYU 56945	JX488818	JX488888	–	JX488996	–	–
<i>Ch. liorus mictus</i> 2	BYU 56946	JX488819	JX488889	–	JX488997	–	–
<i>Deltistes luxatus</i>	OS 15922	AF454870	JX488890	–	JX488998	–	GU937831
<i>Pantosteus clarkii</i>	MSB 49600	JX488779	JX488848	HQ556940	JX488956	–	–
<i>P. discobolus discobolus</i>	BYU 57986	JX488782	JX488851	–	JX488959	–	GU937830
<i>P. discobolus yarrowi</i>	UAIC 12776.01-2	JX488783	JX488852	–	JX488960	–	–
<i>P. nebuliferus</i>	HLB1484	JX488794	JX488863	EU668538	JX488971	–	–
<i>P. platyrhynchus</i> 1	BYU 58618	JX488801	JX488870	EU523934	JX488978	–	–
<i>P. platyrhynchus</i> 2	BYU 58621	JX488802	JX488871	–	JX488979	–	–
<i>P. plebeius</i> 1	DAN0521-10	JX488803	JX488872	EU668409	JX488980	–	GU937833
<i>P. plebeius</i> 2	DAN0526-01	JX488804	JX488873	–	JX488981	–	–
<i>P. santaanae</i>	UAIC 12384.01	JX488805	JX488875	JN024948	JX488983	–	–
<i>Xyrauchen texanus</i> 1	OS X97	JX488824	JX488935	HQ556969	JX489042	This study	–
<i>X. texanus</i> 2	OS X98	JX488825	JX488936	–	JX489043	–	–
Tribe Erimyzonini						–	–
<i>Erimyzon oblongus</i> 1	UAIC 11109.09	AF454876	JX488891	HQ579034	–	This study	–
<i>Erimyzon oblongus</i> 2	BRK05-52	–	–	–	JX488999	–	GU937837
<i>E. sucetta</i> 1	UAIC 12286.01	AF45478	JX488892	EU524567	–	–	–
<i>E. sucetta</i> 2	C306	–	–	–	JX489000	–	–
<i>E. tenuis</i>	UAIC 12370.04	AF454877	JX488893	JN025452	JX489001	This study	GU937838
<i>Minytrema melanops</i> 1	–	AB242116	AB242116	EU524839	–	–	FJ265050
<i>Minytrema melanops</i> 2	–	DQ536432	DQ536432	–	–	–	–
<i>Minytrema melanops</i> 3	UAIC 11141.01	AF454879	JX488897	–	–	–	–

(Continued)

Table 2 (continued).

Taxon ^a	Museum/field numbers ^b	cytb	ND2	cox1	IRBP	RPS7	GHI
<i>Minytrema melanops</i> 4	BRK056	–	–	–	JX489005	–	–
Tribe Moxostomatini						–	–
<i>Moxostoma albidum</i> 1	UAIC 12365.01	AF454901	JX488898	EU751969	JX489006	This study	JF799563
<i>M. albidum</i> 2	UAIC 13446.01	AF454902	JX488899	–	JX489007	This study	–
<i>M. anisurum</i>	UAIC 11606.02	AF454880	JX488900	EU524146	JX489008	This study	JF799537
<i>M. ariommum</i>	UAIC 12071.01	AF454903	JX488901	JN027267	JX489009	This study	JF799557
<i>M. austrinum</i>	UAIC 12375.01	AF454898	JX488902	–	JX489010	This study	JF799565
<i>M. sp. cf. austrinum</i>	UAIC 12373.01	AF454904	JX488903	–	JX489011	This study	JF799564
<i>M. breviceps</i>	UAIC 11314.08	AF454888	JX488904	JN027271	JX489012	This study	JF799542
<i>M. carinatum</i> 1	UAIC 11005.03	AF454883	JX488905	EU524147	JX489013	This study	JF799547
<i>M. carinatum</i> 2	PBB0023	JX488820	JX488906	–	JX489014	This study	–
<i>M. cervinum</i>	UAIC 11004.01	AF454906	JX488907	JN027274	JX489015	This study	JF799556
<i>M. collapsum</i> 1	UAIC 11007.03	AF454882	JX488908	JN027276	JX489016	This study	–
<i>M. collapsum</i> 2	UAIC 12376.05	AF454881	JX488909	–	JX489017	This study	–
<i>M. congestum</i> 1	UAIC 13506.05	AF522290	JX488910	JN027282	JX489018	This study	JF799560
<i>M. congestum</i> 2	UAIC 13508.05	AF522291	JX488911	–	JX489019	This study	–
<i>M. congestum</i> 3	UAIC 13512.05	AF522292	JX488912	–	JX489020	This study	–
<i>M. duquesnei</i> 1	UAIC 11310.09	AF454894	JX488913	EU524861	–	–	–
<i>M. duquesnei</i> 2	JFBM38581	AF454895	JX488914	–	JX489021	This study	JF799554
<i>M. erythrurum</i> 1	UAIC 12237.03	AF454886	JX488915	EU524867	JX489022	This study	JF799551
<i>M. erythrurum</i> 2	JFBM37043	AF454887	JX488916	–	JX489023	This study	–
<i>M. hubbsi</i> 1	NCSM 36440	AF522289	JX488917	EU524877	JX489024	This study	JF799544
<i>M. hubbsi</i> 2	06-19	JX488821	JX488918	–	JX489025	This study	–
<i>M. lachneri</i>	UAIC 12370.02	AF454900	JX488919	JN027292	JX489026	This study	JF799559
<i>M. sp. cf. lachneri</i>	UAIC 12462.03	AF454907	JX488920	–	JX489027	This study	–
<i>M. macrolepidotum</i>	UAIC 11221.10	AF454890	JX488921	EU524149	JX489028	This study	JF799539
<i>M. sp. cf. macrolepidotum</i>	UAIC 11643.01	AF454885	JX488922	–	JX489029	This study	F799541
<i>M. mascotae</i>	UAIC 12374.01	AF454899	JX488923	–	JX489030	This study	JF799566
<i>M. pappillosum</i>	UAIC 13462.01	AF454883	JX488924	JN027303	JX489031	This study	JF799538
<i>M. poecilurum</i> 1	–	NC008674	NC008674	–	–	–	–
<i>M. poecilurum</i> 2	UAIC 11442.01	AF454896	JX488925	HQ579038	JX489032	This study	JF799552
<i>M. sp. cf. poecilurum</i>	UAIC 12746.13	AF454897	JX488926	–	JX489033	This study	–
<i>M. pisolabrum</i>	UAIC 11154.05	AF454889	JX488927	HQ557206	JX489034	This study	–
<i>M. robustum</i>	UAIC 11916.01	AF454891	JX488928	JN027313	JX489035	This study	JF799546
<i>M. rupiscartes</i>	UAIC 12376.06	AF454905	JX488929	JN027315	JX489036	This study	JF799558
<i>M. valenciennesi</i>	JFBM36305	AF454893	JX488930	EU524150	JX489037	This study	JF799543
Tribe Thoburniini							
<i>Hypentelium etowanum</i>	UAIC 12523.08	AF454908	JX488894	JN026830	JX489002	–	GU937836
<i>H. nigricans</i> 1	–	NC008676	NC008676	–	–	–	–

(Continued)

Table 2 (continued).

Taxon ^a	Museum/field numbers ^b	cytb	ND2	cox1	IRBP	RPS7	GHI
<i>H. nigricans</i> 2	UAIC 11138.02	AF454909	JX488895	EU524667	JX489003	This study	JF799571
<i>H. roanokense</i>	UAIC 13449.02	AF454910	JX488896	JN026848	JX489004	This study	JF799570
<i>Thoburnia atripinnis</i>	UAIC 13463.01	AF454911	JX488931	HQ937020	JX489038	This study	JF799569
<i>T. hamiltoni</i> 1	NCSM 45840-1	JX488822	JX488932	–	JX489039	–	JF799567
<i>T. hamiltoni</i> 2	NCSM 45840-3	JX488823	JX488933	–	JX489040	This study	–
<i>T. rhothoeca</i>	UAIC 11009.05	AF454912	JX488934	JN028432	JX489041	This study	JF799568

Notes:

Sequences generated during this study are shown in bold. Dash symbol “–” indicates no sequence was available.

^a Taxonomic classification of suckers used here is based on *Harris & Mayden (2001)* and *Harris et al. (2002)*.

^b Institutional, project, and individual abbreviations: BRK, Bernard R. Kuhajda; CTOL, Cypriniformes Tree of Life Project; DAN, David A. Neely; JEB, James E. Brooks; JFBM, (James Ford) Bell Museum of Natural History, University of Minnesota; HLB, Henry L. Bart, Jr.; MSB, Museum of Southwest Biology; PBB, Peter B. Berendzen; UAIC, University of Alabama Ichthyological Collection; USU, Utah State University; UNL, University of Nebraska Lincoln; OS, Oregon State University; BYU, Monte L. Bean Life Science Museum, Brigham Young University; Louis Bernatchez, Université Laval; NCSM, North Carolina State Museum of Natural Sciences; TU, Tulane University Biodiversity Research Institute (formerly Tulane University Museum of Natural History).

phylogenetic reconstructions. Purified PCR products were cloned using the TOPO TA Cloning[®] Kit (Invitrogen Corp., Carlsbad, CA, USA) with TOP10 chemically competent or electrocompetent cells. Positive colonies were chosen randomly and cultured, and then plasmids preps were purified using the QIAprep Spin Miniprep Kit (QIAGEN, Valencia, CA, USA) and sequenced in both directions using universal M13 primers. We sequenced all genes in both directions on an Applied Biosystems 3100 Genetic Analyzer using ABI PRISM BigDye Terminator v2.0 or v3.0 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA).

We edited sequence chromatographs, assembled sequence contigs, and created final DNA alignments using Geneious v5.4 (*Kearse et al., 2012*). We translated all gene sequences into amino acid sequences to check alignments for stop codons or elevated nonsynonymous substitution numbers, because these signatures can indicate the presence of nuclear mtDNA gene copies, or “NUMTs.” mtDNA and IRBP sequences aligned straightforwardly “by-eye” in Geneious. However, we aligned nuclear *RPS7* sequences, and nuclear genes from other studies listed below, in MAFFT v6.850 (*Katoh & Toh, 2008*) using the local-pair FFTS algorithm with MAXITERATE = 50.

Dataset construction, model selection, and Bayesian phylogenetic analyses

We collated seven datasets for our analyses that we describe here, and which correspond sequentially to datasets listed in [Table 3](#) and trees shown in [Figs. 2A–2G](#) (see Results).

(1) The “concatenated mtDNA” dataset consisted of mtDNA *cytb* and ND2 sequences for 126 tips (121 sucker samples and five outgroup samples) plus 58 mtDNA cytochrome oxidase subunit 1 (*cox1*) gene sequences from GenBank. (2) A “four-locus” DNA dataset with the same 126 tips but adding the following nuclear data to the concatenated mtDNA dataset: 44 GHI sequences from *Clements, Bart & Hurley (2012)* and GenBank, plus 113 IRBP sequences and 52 *RPS7* sequences from our study. (3) We reanalyzed a morphological dataset first presented by *Smith (1992)*, containing 123 morphological

Table 3 Characteristics of each of the seven morphological and molecular datasets analyzed in this study.

Dataset	n (No. ingroup tips)	No. characters (missing data % of total)	No. parsimony informative characters (% total)	Data subsets							
				1	2	3	4	5	6	7	†M
Concatenated mtDNA	126 (121)	2,836 bp (11.9%)	1,216 (42.9%)	+	+	+	–	–	–	–	–
Four-locus	85 (80)	5,925 bp (23.1%)	1,893 (31.9%)	+	+	+	+	+	+	+	–
Morphology	64 (62)	123 (11.7)	120 (97.6%)	–	–	–	–	–	–	–	+
mtDNA + morphology	85 (80)	2,959 (11.9%)	1,336 (45.2%)	+	+	+	–	–	–	–	+
Total-evidence	85 (80)	6,048 (23.3%)	2,013 (33.3%)	+	+	+	+	+	+	+	+
Nuclear IRBP	113 (108)	839 bp (0.3%)	210 (25.0%)	–	–	–	+	+	+	–	–
Concatenated nDNA	113 (108)	3,089 bp (36.4%)	677 (21.9%)	–	–	–	+	+	+	+	–

Notes:

Data subsets: 1 = mtDNA 1st codon sites; 2 = mtDNA 2nd codon sites; 3 = mtDNA 3rd codon sites; 4 = IRBP 1st codon sites; 5 = IRBP 2nd codon sites; 6 = IRBP 3rd codon sites; 7 = GHI + *RPS7* sites, combined; M = morphological characters; “+” symbols indicate that a given subset was included in the dataset while “–” symbols indicate it was not.

† During BEAST analyses, but not MrBayes analyses, morphological characters were subdivided into various subsets described in the text and in the Mendeley-deposited input files (DOI: [10.17632/trw6sb4v7w.2](https://doi.org/10.17632/trw6sb4v7w.2)).

characters for 64 taxa including 62 sucker taxa and two outgroup taxa. This matrix contained two extinct taxa: the historically extinct †*Moxostoma lacerum*, and †*Amyzon*, an Eocene–Oligocene genus of fossil suckers composed of five valid species known from British Columbia, Washington, Nevada, and Wyoming (four species), and Jilin province, China ([Bruner, 1991](#); [Chang et al., 2001](#); [Smith, 1992](#); [Appendix S1](#)). (4) The fourth “mtDNA + morphology” dataset combined datasets 1 and 3. (5) Our “total-evidence” dataset contained all morphological and molecular characters analyzed in this study, for 85 taxa. Two final nDNA datasets were: (6) a “nuclear IRBP” alignment for the only nuclear gene with complete sampling, and (7) a “concatenated nDNA” alignment containing all three nuclear loci. Overall, the morphology dataset was analyzed alone (dataset 3; see Results [Fig. 2C](#)), in concert with mtDNA genes (dataset 4; see Results [Fig. 2D](#)), and combined with the full DNA sequence dataset in total-evidence analyses of dataset 5 (e.g. see Results [Fig. 2E](#)).

We selected the most appropriate partitioning schemes and models of sequence evolution for each of the DNA data “subsets” or “blocks” used in our phylogenetic analyses in PartitionFinder v1.1.1 ([Lanfear et al., 2012](#)), which included codon positions of the mtDNA and IRBP alignments, plus the GHI and *RPS7* genes. We ran PartitionFinder simultaneously on all initial DNA subsets using the greedy heuristic search algorithm, which we set to conduct model comparisons to determine the “best-fit” partitioning scheme based on the Bayesian information criterion (BIC). PartitionFinder relies heavily on PhyML, which we set to link branch lengths, search 56 substitution models, and estimate the base frequencies, proportion of invariant sites (*I*), and the gamma shape distribution (Γ) of each model using maximum-likelihood.

We performed partitioned Bayesian phylogenetic analyses on all seven datasets in MrBayes 3.2.2 ([Ronquist et al., 2012](#)). Analyzing different taxon and character combinations allowed us to evaluate the effect of different data types, and of including morphological data and extinct taxa, on phylogenetic analyses of suckers. During molecular analyses, we specified partitioning schemes and best-fit models of sequence

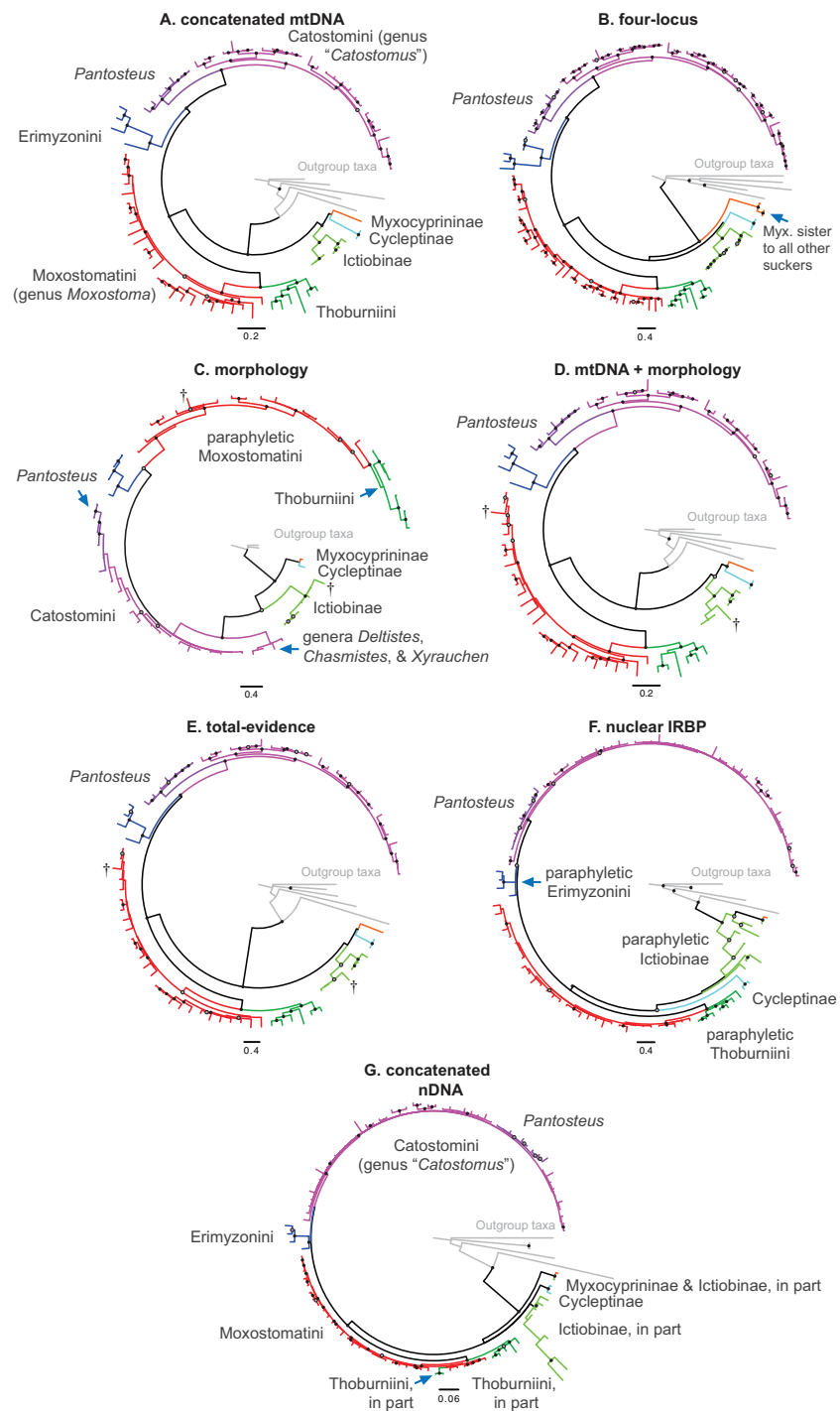


Figure 2 Consensus topologies from MrBayes (Ronquist et al., 2012) analyses of the concatenated mtDNA (A), four-locus (B), morphology (C), mtDNA + morphology (D), total-evidence (E), concatenated nDNA (F), and nuclear IRBP (G) datasets. Subfamily and tribe names and colors shown in panel (A) are followed throughout; in panels (B–G), text labels highlight positions of different taxa, including subfamilies (Myx., Myxocyprinae), tribe Catostomini, and *Pantosteus*, and dagger symbols indicate extinct taxa. Filled black circles indicate nodes with significant Bayesian posterior probabilities (BPP ≥ 0.95), and gray circles indicate near-significant nodes (BPP = 0.80–0.94). Scale bars are given in units of substitutions/site. [Full-size !\[\]\(5f471a71b78d7676bc356df190b88ab4_img.jpg\) DOI: 10.7717/peerj.5168/fig-2](https://doi.org/10.7717/peerj.5168/fig-2)

evolution selected in PartitionFinder, except where the selected model was not implemented in MrBayes we used the next most closely related model in the general time-reversible (GTR) family of models. Given that rate variation among morphological characters can confound phylogenetic branch lengths, applying Γ -distributed rates can greatly improve models of morphological evolution (*Clarke & Middleton, 2008*). Thus, for the morphological analysis, we specified *Lewis's (2001)* Markov variable (Mkv) model with Γ -distributed rate heterogeneity and left characters unordered in state polarity (default). For each dataset, we conducted three independent MrBayes runs of eight chains, each with a Markov chain Monte Carlo (MCMC) chain length of 50 million generations. We diagnosed run convergence using the potential scale reduction factor, which should approach values of 1 when stationarity has been reached (*Ronquist et al., 2012*).

Bayesian total-evidence dating and relaxed-clock partitioning

We estimated divergence times as times to the most recent common ancestor (t_{MRCAS}) through Bayesian relaxed molecular clock analyses of the total-evidence dataset in BEAST v2.4.5 (*Bouckaert et al., 2014*). For our tree prior, we employed the fossilized birth-death (FBD) process model (*Gavryushkina et al., 2014; Heath, Huelsenbeck & Stadler, 2014*) as modified by *Gavryushkina et al. (2017)*, because this model accommodates total-evidence datasets, avoids the need for arbitrary calibration densities, and accommodates all fossils available for a group, and not merely ad hoc selections. In addition to 83 extant ingroup and outgroup samples, 19 extinct sucker taxa and their available minimum ages from the fossil record were included in the BEAST analyses based on evidence provided in [Appendix S1](#). We calculated the approximate sampling proportion for extant lineages (ρ) as 0.97 and set the FBD time of origin prior (t_{or}) to the Cypriniformes “Root” node calibration discussed in [Appendix S1](#). Morphological characters were partitioned into groups having the same number of states, and each partition was assigned an Mkv model (*Lewis, 2001*; conditioning on the use of only variable characters) with Γ -distributed rate variation (“partitioned mode” in *Gavryushkina et al., 2017*). We partitioned the DNA data and set site models according to the best scheme identified in PartitionFinder. Other authors have employed FBD models on a fixed topology (*Heath, Huelsenbeck & Stadler, 2014*) or simultaneously estimated topology and FBD parameters (*Gavryushkina et al., 2017*). We allowed most nodes, including subfamily relationships, to change freely. However, we constrained the Catostomini and Moxostomatini crown groups to be monophyletic, consistent with our MrBayes results and hypotheses of previous studies (see Results and Discussion sections). Posterior nodal support can be markedly weakened when fossils have unscored characters (*Gavryushkina et al., 2017*) or lack data. To avoid spurious relationships arising from the large proportion of fossil Ictiobinae taxa (57%) and Myxocyprininae taxa (50%) lacking data, samples of *Carpoides*, *Ictiobus*, Ictiobinae, and Myxocyprininae were each constrained to be monophyletic, consistent with our other phylogenetic results.

According to *Ho & Lanfear (2010)*, implementing multiple relaxed-clock models for different data subsets in a data-partitioning scheme can provide a more biologically

realistic way to model among-lineage rate variation and improves the fit of relaxed-clock models to the data. Using a relaxed-clock partitioning scheme may also yield more precise date estimates with narrower credible intervals (*Ho & Lanfear, 2010*). We statistically tested whether allocating separate uncorrelated lognormal relaxed clocks to different DNA data subsets, through “relaxed-clock partitioning,” yielded divergence time estimation models that provided a better fit to the data than assuming a single model of branch-specific rates across all data subsets. First, we estimated divergence dates and marginal likelihood scores for each of 12 different relaxed-clock partitioning models (M_1 – M_{12}) with one to eight clocks (see Results and Discussion). Second, we estimated Bayes factors and conducted Bayesian model selection to identify the best-supported model.

We ran five replicate searches of each model in BEAST (MCMC = 2×10^7 generations, sampling every 4,000) using the “BEASTRunner.sh” script in PIRANHA (*Bagley, 2017*). We then estimated log-marginal likelihoods for each model by conducting path sampling (PS) (*Baele et al., 2012*) for 100 steps (10^6 generations each), while specifying a $\sim B(0.3, 1)$ distribution for spacing the path steps (*Xie et al., 2011*). We calculated $2\log_e(B_{10})$ Bayes factors from the log-marginal likelihoods and evaluated “weight of evidence” of the models according to criteria in *Kass & Raftery (1995)*. We took posterior distributions from the best-supported model as our best estimates of the time tree and divergence dates for Catostomidae lineages. We summarized parameters from the best-supported model, and ensured convergence and adequate effective sample sizes (ESS >100–200), using Tracer v1.5 (*Rambaut & Drummond, 2014*). We calculated a maximum clade credibility tree annotated with mean node ages from 5,000 post-burn-in trees in TreeAnnotator v2.4.5.

Using phylogenetic informativeness profiles to exclude problematic characters

Among other factors influencing phylogenetic inference, such as sampling effects on branch lengths or nodal support values (*Heath, Hedtke & Hillis, 2008; Pyron, 2011*), the varying informativeness of different character sets can substantially and adversely affect phylogenetic divergence dating results (*Dornburg et al., 2014*). Fortunately, recent methods for quantifying and visualizing “phylogenetic informativeness” (PI) profiles of character sets through time (*Townsend, 2007; Townsend, Su & Tekle, 2012*) provide a framework for identifying and excluding problematic character sets (*Dornburg et al., 2014*). One common pitfall is the use of characters whose profiles exhibit a decline in informativeness towards the root. As noted by *Townsend & Leuenberger (2011)*, this decline marks a “rain shadow of noise,” with the corresponding dataset losing phylogenetic informativeness due to an increase in predicted homoplasy. In turn, homoplasious loci or character sets, but especially mtDNA datasets exhibiting high saturation or rootward declines in PI, have been shown to mislead global branch length values during divergence time estimation (*Brandley et al., 2011; Dornburg et al., 2014*).

To identify and exclude potentially problematic character sets in our database, and to evaluate whether support vs instability of subfamily relationships correlated to statistical power to resolve branching order, we evaluated the *Townsend (2007)* PI and resolution probabilities of each character set through time using PhyDesign (*López-Giráldez & Townsend, 2011*). Estimating PI requires prior information on evolutionary-genetic rates and phylogeny. Thus, we estimated rates for DNA characters and morphological characters using HyPhy (*Pond, Frost & Muse, 2005*) and BayesTraits (*Pagel & Meade, 2014*), respectively, and ran analyses along the BEAST time tree from the best-supported clock-partitioning model (see Results and Discussion). We estimated net PI for all eight data subsets in [Table 3](#) during the “subfamily divergence epoch” spanning branches leading to Myxocyprinae, Cycleptinae, Ictiobinae, and Catostominae. These analyses permitted broad comparisons of signal in the mitochondrial vs nuclear data, and molecular vs morphological character sets. We excluded character sets that exhibited steep declines in PI towards the root of our phylogeny from the final divergence time results presented below. We compared three resolution probabilities, including probability correct, probability polytomy, and probability incorrect or “phylogenetic noise” (Eqs. 11–13 in *Townsend, Su & Tekle, 2012*) for the excluded subsets vs other molecular subsets over the subfamily divergence epoch. We also evaluated sensitivity of resolution probability approximations to varying the internode time length (t_0) parameter, by recalculating over 10 t_0 values representing declining fractions of the epoch.

RESULTS

Dataset characteristics and DNA substitution models

Our final data matrices ranged in size from 123 characters in the morphological dataset to 6,048 molecular and morphological characters in the total-evidence dataset ([Table 3](#)). Proportions of missing data and parsimony informative characters ranged from 0.3% to 36.4% and from 21.9% to 97.6%, respectively, across datasets ([Table 3](#)). PartitionFinder identified seven unique DNA sequence subsets (scheme BIC = 141854.71523), and the best-fit DNA substitution model for each subset is listed in [Table S1](#). Morphological character subsets (in subset M) were assigned Mkv+ Γ models, as described above. None of the mtDNA genes sequenced in this study showed signs of NUMTs, and we found no indels in the IRBP sequences; however, GHI and *RPS7* genes aligned with ~32 and ~16 ingroup indels/gaps, respectively. We archived our sequence alignments and phylogenetic tree results in Mendeley Data ([DOI: 10.17632/trw6sb4v7w.2](https://doi.org/10.17632/trw6sb4v7w.2)).

Phylogenetic relationships among sucker subfamilies

We placed subfamilies Myxocyprinae and Cycleptinae as sister lineages in most trees, but with higher posterior support (Bayesian posterior probability (BPP) = 0.74–0.99) in runs based largely on mtDNA-encoded genes ([Figs. 2A](#) and [2D](#); [Figs. S1](#) and [S2](#)) and weak BPP support (≤ 0.69) in other runs ([Figs. 2C](#), [2E](#), [3](#); [Fig. S3](#)). Frequently, when this pattern was obtained, Myxocyprinae + Cycleptinae was resolved as sister to a monophyletic

Ictiobinae with variable posterior support (BPP = 0.69–0.98; Figs. 2, 3; Figs. S1 and S2). This “subfamily pattern 1,” with ((Myxocyprinae, Cycleptinae), Ictiobinae), departs markedly from the placement of Ictiobinae sister to Cycleptinae (sometimes including *Myxocyprinus*) + Catostominae in previous analyses of morphology (Smith, 1992) and molecular data (Dosey et al., 2010; Harris & Mayden, 2001; Mayden et al., 2008; Saitoh et al., 2006). By contrast, several trees agreed in placing Myxocyprinae as sister to all other sucker lineages, which agrees with previous mtDNA results presented in Harris & Mayden (2001). This “subfamily pattern 2” relationship was strongly supported with BPP = 1 in our total-evidence consensus topology from BEAST (Fig. 4) and resolved with low support in the four-locus topology (Fig. 2B; Fig. S4). Distinguishing between these two conflicting sets of subfamily relationships is difficult, because each is supported by molecular and total-evidence topologies herein and agrees with at least one previous molecular study. To objectively determine the arrangement of these subfamilies with the greatest weight of evidence conditional on our total-evidence dataset, we compared subfamily patterns 1 and 2 using Bayes factors. We ran MrBayes as described above, except employing topological constraints set to subfamily pattern 2, and then used stepping-stone sampling (Xie et al., 2011; Baele et al., 2012) to estimate the log-marginal likelihoods of the models, from which Bayes factor tests were conducted through comparisons to the unconstrained model matching subfamily pattern 1. Conducting 50,000 generations of stepping-stone sampling (sampling every 2,500 generations) during each of 50 steps produced a total of 250,000 MCMC generations for marginal likelihood estimation. The subfamily pattern 2 model constraining Myxocyprinae as sister to all other suckers had a higher log-marginal likelihood score (−68390.83) than the unconstrained subfamily pattern 1 model (−68910.88), and a $2\log_e(B_{10})$ Bayes factor of −1040.10 provided definitive weight of evidence against the unconstrained model.

Relationships of early-diverging sucker genera

We found that the early-diverging sucker genera *Myxocyprinus*, *Cycleptus*, *Carpiodes*, and *Ictiobus* formed well-supported clades in most analyses. However, *Ictiobus* relationships were resolved in a polytomy in our analysis of the concatenated mtDNA dataset (Fig. S1). In several other trees, including those based on our total-evidence and mtDNA + morphology datasets (Fig. 3; Fig. S2), the *Ictiobus* clade received weak BPP support and eventually collapses into a paraphyletic grade. In the analysis of the four-locus dataset with higher numerical sampling in this clade, *Ictiobus bubalus* and *I. niger* relationships had high BPP support (BPP = 0.94–1) but were para-/polyphyletic, leaving their relationship to *I. cyprinellus* uncertain (Fig. S4).

Phylogenetic relationships among the Catostominae

Within the largest sucker subfamily, Catostominae, we consistently resolved clades with the tribes Thoburniini + Moxostomatini and Erimyzonini + Catostomini across analyses. Our more robust, multilocus and total-evidence trees resolved these relationships with definitive support values of BPP = 0.99–1. Within the

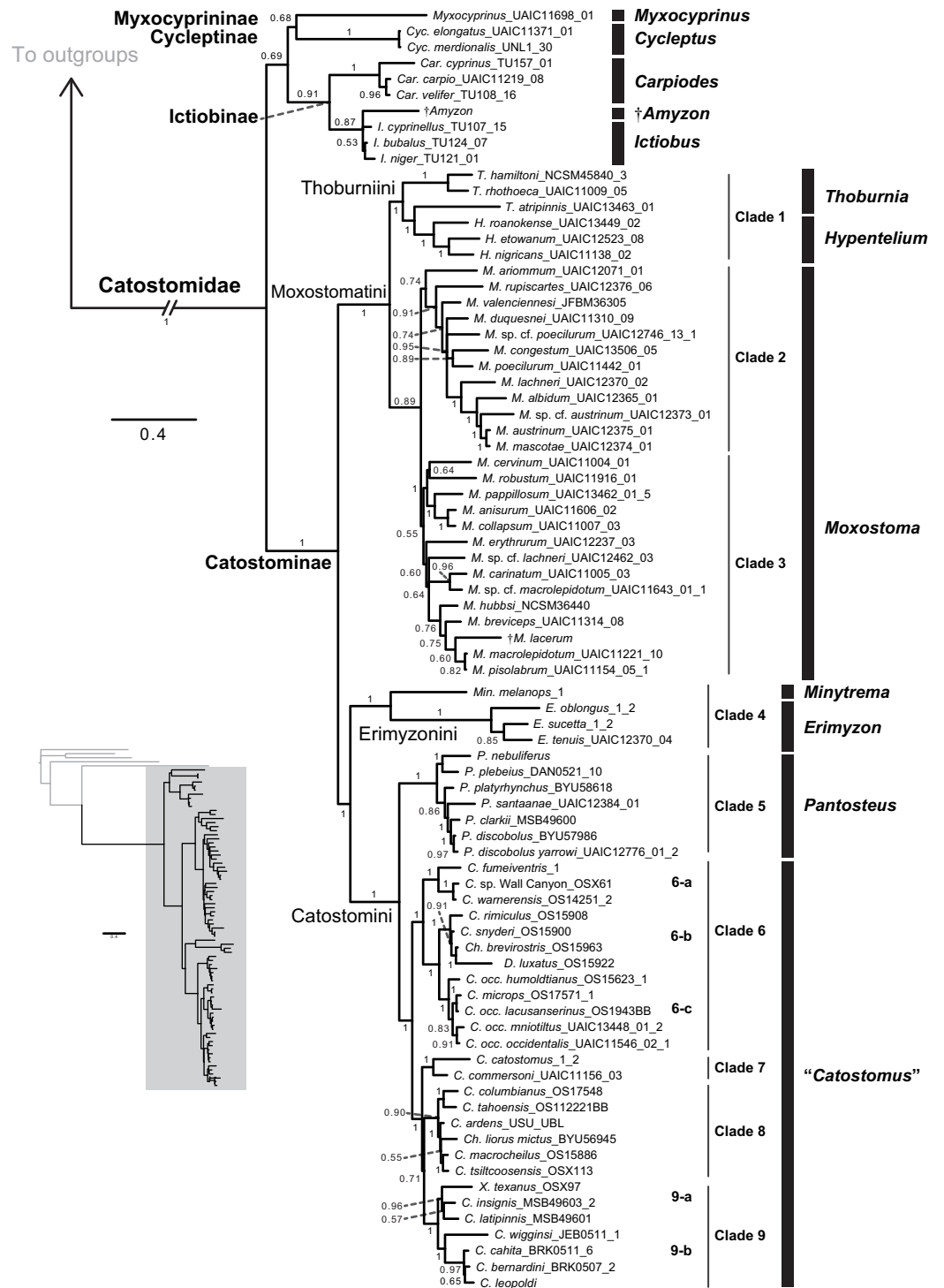


Figure 3 Phylogeny of suckers inferred from Bayesian analysis of the combined mtDNA (*cytb*, *cox1*, ND2), nDNA (IRBP, GHI, and *RPS7*), and morphological data (123 characters) in the total-evidence dataset. Within tip labels, dagger symbols indicate extinct taxa, and museum (voucher) or field numbers are followed by the number of the individual sequenced (Table 1). Nodes are labeled with Bayesian posterior probability support values above 0.50. Scale bars are in units of substitutions/site.

Full-size DOI: 10.7717/peerj.5168/fig-3

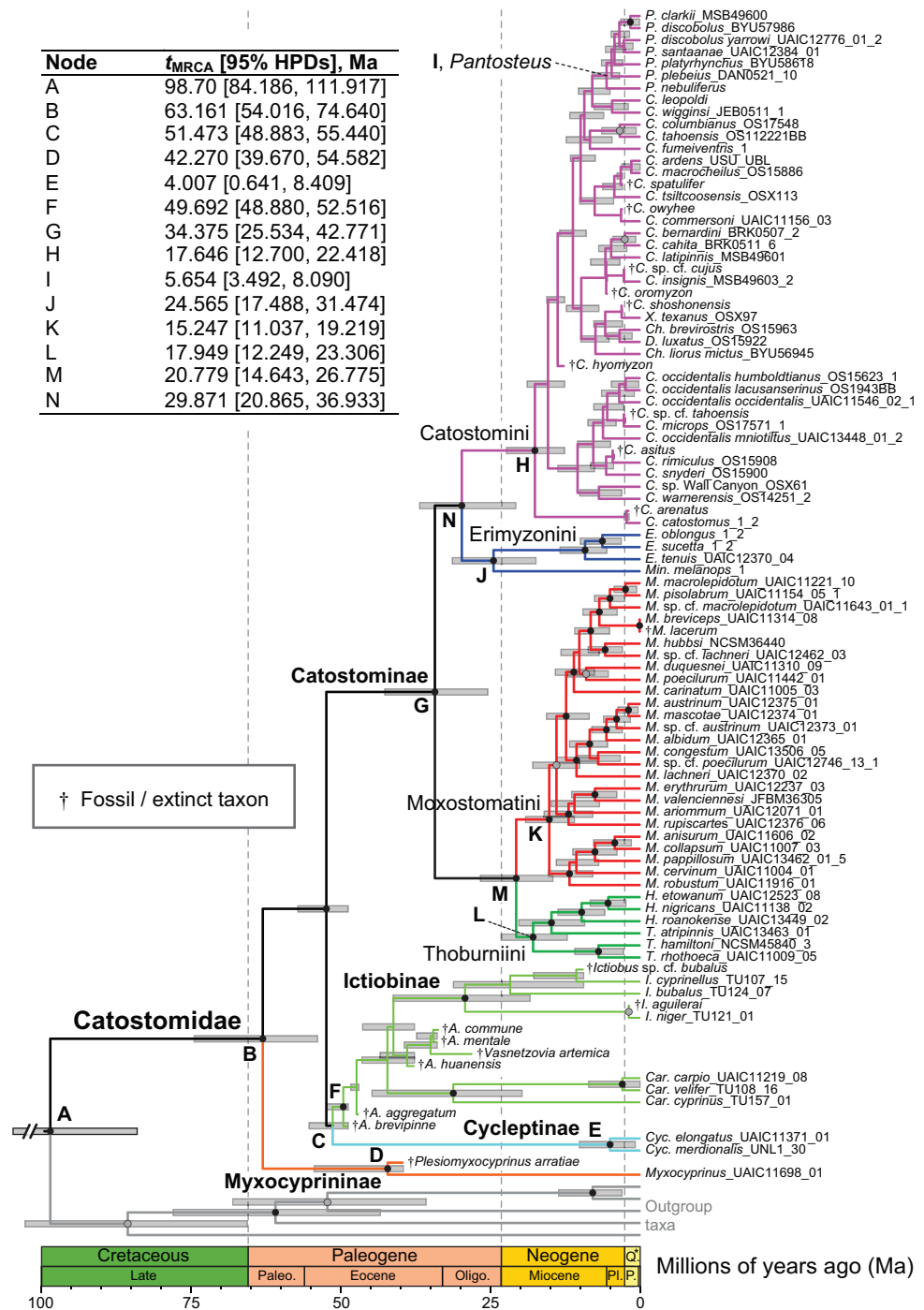


Figure 4 Time-calibrated phylogeny of Catostomidae derived from the best-supported Bayesian total-evidence dating model identified in Table 4. In parentheses beside subfamily and tribe names, numbers of extinct or fossil tips are presented out of total sample size for the corresponding clade. Along nodes, filled black circles indicate significant posterior support (BPP ≥ 0.95), gray circles indicate near-significant support (BPP = 0.80–0.94), and horizontal node bars show 95% highest posterior densities of age estimates. The inset table shows Bayesian posterior age estimates given as mean t_{MRCAs} , with their 95% highest posterior densities (HPDs; credible intervals), in units of millions of years ago (Ma). Geological epoch abbreviations: Paleo., Paleocene; Oligo., Oligocene; Q., Quaternary; Pl., Pliocene; P., Pleistocene.

Full-size DOI: 10.7717/peerj.5168/fig-4

Thoburniini + Moxostomatini clade, we consistently inferred the genus *Thoburnia* to be paraphyletic, with *Thoburnia atripinnis* sister to a clade containing the three *Hypentelium* species (mostly BPP = 1). The sole exception to this was that our morphology tree resolved *Thoburnia* as monophyletic with *T. atripinnis* sister to all other *Thoburnia* with strong support (BPP = 1). Within the *Hypentelium* clade, we inferred an identical and strongly supported set of relationships of the form (*Hypentelium roanokense*, (*H. etowanum*, *H. nigricans*)) in the mtDNA, four-locus, nDNA, and total-evidence gene trees (Fig. 3; Figs. S1, S2, S4, and S5). We obtained the same set of relationships in our morphology analysis, but with weak (BPP = 0.69) support for the *H. etowanum*–*H. nigricans* node (Fig. S3). We resolved *Moxostoma* as monophyletic with BPP = 0.89–1, except for a paraphyletic pattern in the morphology consensus tree. Within the Erimyzonini, *Erimyzon* was monophyletic (BPP = 1) and sister to *Minytrema* (e.g. Fig. 3). None of the molecular or total-evidence topologies we inferred resolved *Catostomus* as monophyletic relative to *Chasmistes*, *Deltistes*, or *Xyrauchen*. Here, yet again, results from the morphology tree departed from our other results, failing to resolve relationships among these or virtually any other catostomine lineages with strong support (Fig. 1C; Fig. S3). As a result, we do not discuss the morphology consensus topology further in this section.

Relationships within the Erimyzonini and Catostomini were similar to those in previous molecular studies (Harris et al., 2002; Doosey et al., 2010). Within *Erimyzon*, our results placed *E. sucetta* rather than *Erimyzon oblongus* as sister to *E. tenuis*. Within Catostomini, we consistently resolved nine well-supported major clades within Catostominae (e.g. Fig. 3; Figs. S1 and S4). Although relationships among these clades received varying posterior support, the species groups we identified were highly supported in multiple analyses and provide more tenable phylogenetic hypotheses than previously proposed for this tribe (Smith, 1992; Smith et al., 2002). “Clade 4” corresponded to a monophyletic Erimyzonini, while clades 5 through 9 included various Catostomini subclades composed largely of *Catostomus* samples. For conciseness, we provide an in-depth assessment of relationships only within “Clade 5,” whose results have the most important phylogenetic and taxonomic implications. More granular presentation and discussion of relationships within and among Catostomini clades 1–3 and 5–9 is provided at the end of Appendix S1. “Clade 5” corresponded mostly to the genus *Pantosteus* (Unmack et al., 2014) and was sister to the remaining Catostomini. Within Clade 5, we consistently resolved *Pantosteus nebuliferus* + *P. plebeius* as sister to a clade containing all remaining *Pantosteus*, with (*P. platyrhynchus*, (*P. santaanae*, (*P. clarkii*, (*P. d. discobolus*, *P. d. yarrowi*)))) (e.g. Fig. 3). Alternative topologies inferred for this clade involved rearrangements placing *P. santaanae* sister to *P. clarkii*, but with non-significant posterior support (e.g. Figs. S1 and S4).

Bayesian total-evidence dating and relaxed-clock partitioning

Bayes factor comparisons of 12 clock-partitioning models showed that removing mtDNA 1st and 3rd codon sites deemed to be problematic during PI profiling (see below) progressively improved model log-marginal likelihoods and posterior evidence (Table 4).

Table 4 Bayes factor tests comparing twelve Bayesian relaxed-clock partitioning models applied to the total-evidence dataset.

Model	Mitochondrial DNA	Problematic sites (#)	Relaxed clocks (#)	Clock-partitioning	Log-marginal likelihood	$2\log_e(B_{10})$ Bayes factor
M_1	1st, 2nd, and 3rd codon sites	2	1	[1, 2, 3, 4, 5, 6, 7, M]	-69,461.62	-89,832.36
M_2	1st, 2nd, and 3rd codon sites	2	2	[1, 2, 3, 4, 5, 6, 7] [M]	-69,078.47	-89,066.05
M_3	1st, 2nd, and 3rd codon sites	2	8	[1] [2] [3] [4] [5] [6] [7] [M]	-67,222.91	-85,354.94
M_4	1st + 2nd codon sites	1	1	[1, 2, 4, 5, 6, 7, M]	-38,876.51	-28,662.13
M_5	1st + 2nd codon sites	1	2	[1, 2, 4, 5, 6, 7] [M]	-38,385.92	-27,680.96
M_6	1st + 2nd codon sites	1	7	[1] [2] [4] [5] [6] [7] [M]	-37,616.86	-26,142.84
M_7	1st codon sites	1	1	[1, 4, 5, 6, 7, M]	-34,866.49	-20,625.92
M_8	1st codon sites	1	2	[1, 4, 5, 6, 7] [M]	-34,382.43	-19,673.97
M_9	1st codon sites	1	6	[1] [4] [5] [6] [7] [M]	-33,682.60	-18,237.36
M_{10}	2nd codon sites	0	1	[2, 4, 5, 6, 7, M]	-25,442.32	-1,793.75
M_{11}	2nd codon sites	0	2	[2, 4, 5, 6, 7] [M]	-25,138.71	-1,186.53
M_{12}	2nd codon sites	0	6	[2] [4] [5] [6] [7] [M]	-24,545.44	0

Notes:

Models including only 3rd codon sites of the mtDNA matrix were ruled out, because those sites had the highest amounts of predicted homoplasy during PI profiling (Results and Discussion, Fig. 5), and excluding them yielded the single greatest improvement in log-marginal likelihoods (e.g. M_1 vs M_4). Numbers and letters in brackets correspond to data subsets defined in the text and Table 3. Log-marginal likelihood estimates were derived from path sampling, and Bayes factors were estimated for each model when compared against the best-supported model.

For example, codon-partitioned relaxed-clock models were overwhelmingly supported over simpler models allocating a single relaxed clock to all data subsets, or all DNA subsets. The most complex model allocating relaxed clocks to each data subset but including only mtDNA 2nd positions, M_{12} , was decisively supported as the best model. Compared with this model, other subset and clock schemes produced negative improvements to the model, indicated by negative log Bayes factors; however, the second best model separated DNA from morphological data subsets, consistent with a decoupling of molecular rates from morphological rates (Table 4). Independent runs of the best-supported BEAST model achieved ESS scores of >100–200 for all parameters and converged on similar phylogeny and parameter estimates including mean and 95% highest posterior densities (HPDs; i.e., credible intervals) for sucker t_{MRCA} s. For example, the posterior ESS of the best model was 463 and that for the FBD model was 134. The only exception to this was the t_{MRCA} for Moxostomatini, which received ESS scores between 72 and 89. Clocks on different data subsets exhibited substantial among-lineage rate heterogeneity, with posterior means and 95% HPD intervals of the “uclStdev” (uncorrelated lognormal relaxed clock standard deviation) and “coefficientOfVariation” (coefficient of variation of branch-specific rates) statistics excluding zero (Fig. S7); thus, relaxed-clock models were warranted by the data.

In the final total-evidence dating analysis, the mean posterior age estimate for the t_{MRCA} of all suckers was 63.16 Ma in the Late Cretaceous, with credible intervals ranging from Late Cretaceous to the Paleocene–Eocene boundary (95% HPD [54.02, 74.6]; Fig. 4). The four sucker subfamilies had variable posterior age estimates ranging approximately an order of magnitude. The Cycleptinae had the youngest posterior age estimate of 5.07 Ma in the Pliocene (95% HPD [0.87, 10.23]). Following their Late

Cretaceous origin based on a stem age corresponding to the MRCA of all suckers, the posterior t_{MRCA} estimate for Myxocyprinae dated their diversification to 42.27 Ma in the early to mid-Eocene (95% HPD [39.67, 54.58]). Subsequently, Catostominae species diversified since an intermediate posterior age of 34.37 Ma near the Eocene–Oligocene boundary (95% HPD [25.54, 42.77]), and Ictiobinae species had the oldest posterior age estimate, dating to 49.69 Ma in the early Eocene (95% HPD [48.88, 52.52]). The catostomine tribes diverged approximately 29.87 Ma in the Oligocene (Catostomini + Erimyzonini) and 20.78 Ma in the early Miocene (Moxostomatini + Thoburniini). The genera *Catostomus* and *Moxostoma*, which correspond to tribes Catostomini and Moxostomatini, diversified since 17.65 and 15.25 Ma ago in the early-mid Miocene, respectively (Fig. 4).

Phylogenetic informativeness profiles

We evaluated potential impacts of phylogenetic signal on incongruent subfamily relationships by estimating resolution probabilities, over the subfamily divergence epoch (~63.2–34.4 Ma) spanning the divergence of sucker subfamilies. Overall, mtDNA 1st and 3rd codon position data subsets exhibited among the highest PI values, but with distinct Miocene peaks followed by declining PI towards the root (Fig. 5). This suggested a prominent loss of evolutionary information due to homoplasy; therefore, we excluded these sites from final divergence dating analyses, in order to avoid potentially negative effects on the topology and time-calibrated branch lengths (Dornburg *et al.*, 2014). All other molecular data subsets had substantial and relatively constant predicted PI decaying over Paleocene or Eocene to present (recent spikes are anomalies; Townsend, López-Giráldez & Friedman, 2008). Morphological characters had slightly higher signal than IRBP sites and exhibited stability before decaying 20 Ma to present (Fig. 5). In addition to nearly constant net PI (Fig. 5), the retained character subsets also had low probabilities of phylogenetic noise or polytomies, with notable increases in the probability of an incorrect topology only for internode distances less than $\sim 0.35(t_0)$, or <10.0 million years (Myr) (Fig. S9).

DISCUSSION

Sucker phylogeny and incongruence of subfamily lineages

Our phylogenetic reconstructions of Catostomidae relationships are similar to several previous morphological and molecular studies. For example, others have hypothesized that Catostomidae is monophyletic in studies focusing on suckers (Doosey *et al.*, 2010; Ferris & Whitt, 1978; Harris & Mayden, 2001; Smith, 1992; Fig. 1) and taxonomically broader analyses (Mayden *et al.*, 2008; Saitoh *et al.*, 2006). Our finding that the four currently recognized sucker subfamilies are monophyletic with definitive support also agrees with earlier phylogenetic studies based on morphology (Smith, 1992) and molecules (Chen & Mayden, 2012; Clements, Bart & Hurley, 2012; Doosey *et al.*, 2010; Harris & Mayden, 2001; Harris *et al.*, 2002; Sun *et al.*, 2007). This is perhaps unsurprising, as we reanalyzed previous morphological and molecular datasets alongside new sequence data. Yet ours are the first results definitively supporting patterns of monophyly

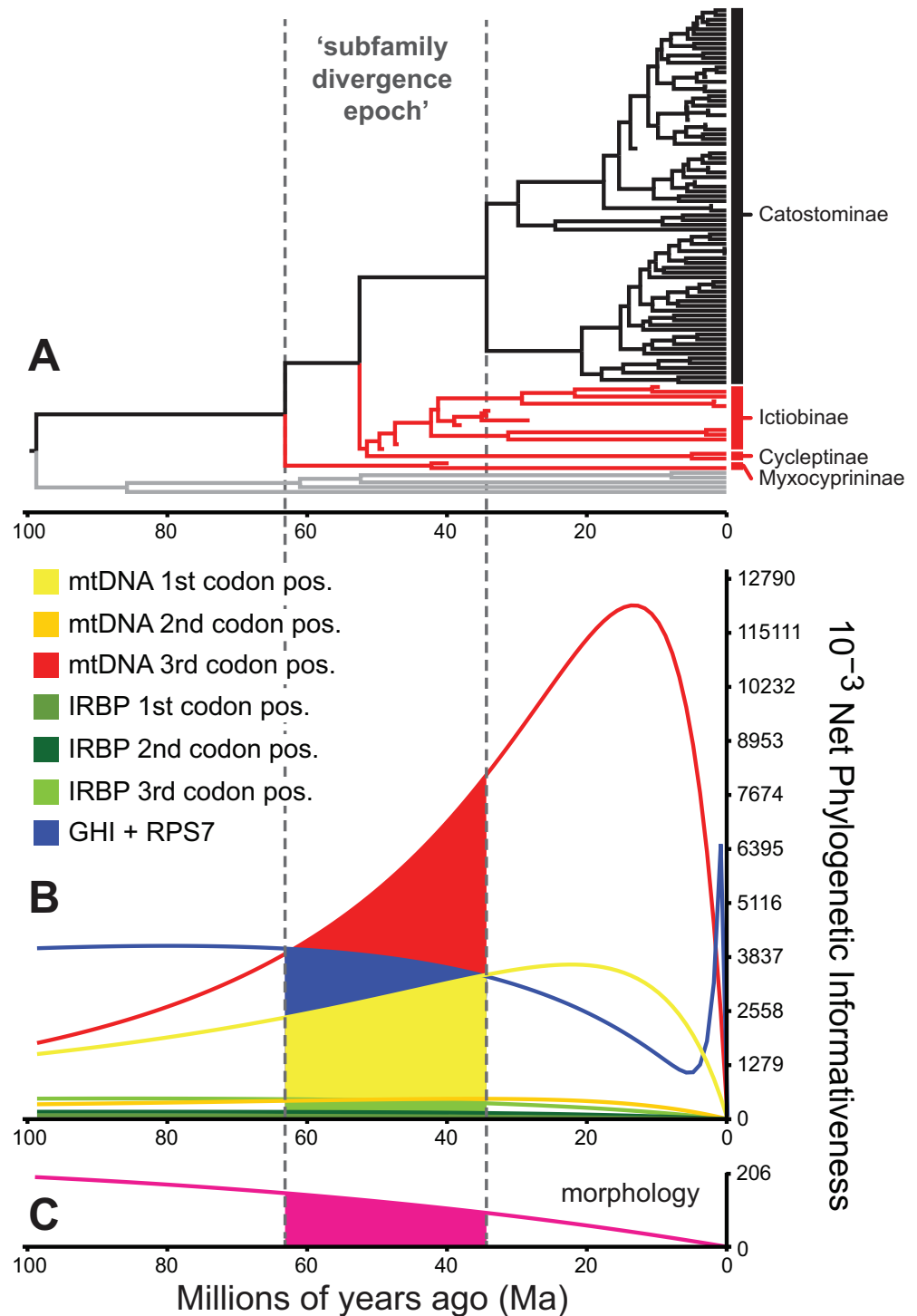


Figure 5 Phylogenetic informativeness of the sucker datasets. (A) BEAST chronogram from Fig. 4 used for site rate calculations, showing the three main subfamilies with incongruent relationships highlighted in red. Phylogenetic informativeness profiles matched to the chronogram time scale are shown for seven subsets of the four-locus dataset (B), and for the morphology dataset (C). Colored shading in (B) and (C) indicate areas integrated below each profile over the “subfamily divergence epoch” (~63.2–34.4 Ma) containing branching relationships among the four subfamilies.

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at the family and subfamily levels based on dense taxonomic sampling of mtDNA and nuclear gene sequences for all sucker genera and most species, plus total-evidence analyses, with BPP at or near 1 across datasets (Figs. 2, 3; Figs. S1–S6).

Previous molecular phylogenetic studies of higher-level sucker relationships have often encountered difficulty in resolving relationships among sucker subfamilies (Chen & Mayden, 2012; Doosey et al., 2010; Harris et al., 2002; Sun et al., 2007). Likewise, relationships among the Myxocyprinae, Cycleptinae, and Ictiobinae lineages were incongruent across analyses of different datasets (Figs. 2, 3; Figs. S1–S4), but with two main patterns that we deemed subfamily pattern 1, with the form ((Myxocyprinae, Cycleptinae), Ictiobinae), and subfamily pattern 2, with Myxocyprinae sister to all other sucker subfamilies. We distinguished between these two alternative hypotheses using a topological constraint test based on Bayes factors. The result yielded log-marginal likelihood estimates and Bayes factors giving definitive weight of evidence against the unconstrained subfamily pattern 1 model. Given this result, a placement of Myxocyprinae as sister to all other suckers seems most probable at this point, thus we favor the patterns of subfamily relationships in our four-locus and Bayesian total-evidence dating topologies that are consistent with this result. Nevertheless, the question still remains: What factors have likely influenced the difficulty of our study and previous studies to resolve phylogenetic relationships among sucker subfamily lineages? Overall, our phylogenetic informativeness analyses highlight two potential explanations for the observed incongruence in subfamily relationships across analyses. First, PI profiling identified the mtDNA 1st and 3rd codon position data subsets as problematic character sets likely compromised by homoplasy due to nucleotide saturation (Fig. 5); hence, we felt justified in excluding these sites from our final divergence dating analyses. Second, our results suggest that predicted phylogenetic noise of the combined datasets over the subfamily divergence epoch (Fig. S9) was most likely a limiting factor for resolving Cycleptinae as sister to Ictiobinae. Whereas internode distances for Catostominae and Myxocyprinae crown clades were generally longer, being ~11 to 18 Myr in length, and associated with significant posterior support, that for Cycleptinae + Ictiobinae had a short internode distance of only 1 Myr (95% HPDs [0.01,9.1]) and non-significant posterior support in our time tree (Fig. 4). Together with the more frequent incongruence and lower support for Cycleptinae compared to Ictiobinae across our MrBayes topologies, this suggests that Cycleptinae acted as a “rogue taxon” switching positions on the tree (Aberer, Krompass & Stamatakis, 2012). We hypothesize that our BEAST total-evidence tree inferred subfamily relationships that were more consistent with Bayes factor tests, and had greater nodal support for early-diverging nodes, by limiting the rogue movements of Cycleptinae.

Monophyly of early-diverging sucker genera, and relationships within Ictiobus

The genera *Myxocyprinus*, *Cycleptus*, *Carpiodes*, and *Ictiobus* formed well-supported clades in our results. However, relationships among *Ictiobus* species were resolved in a polytomy or paraphyletic grade in several cases, limiting our resolution of this clade.

These *Ictiobus* results disagree with previous mtDNA- or nDNA-based studies resolving relationships among *Ictiobus* species with strong maximum-likelihood bootstrap support (Doosey *et al.*, 2010), and Smith's (1992) hypothesis of relationships among four *Ictiobus* species based mainly on morphology. Given these findings, and that our current results fail to unquestionably place the Cycleptinae as sister to the Ictiobinae, drawing phylogenetic or taxonomic conclusions about *Ictiobus* species relationships would seem premature, and we recommend more in-depth analyses of these taxa.

Phylogenetic relationships among the Catostominae

Within the Catostominae, our multilocus and total-evidence results strongly supported sister relationships between Thoburniini + Moxostomatini, and between Erimyzonini + Catostomini. These findings agree well with previous molecular results (Harris *et al.*, 2002; Clements, Bart & Hurley, 2012), except for mitochondrial trees in Doosey *et al.* (2010) showing the Erimyzonini as sister to all other clades within Catostominae. Interestingly, however, our catostomine relationships conflict with the analysis of Smith (1992), whose morphological data we re-analyzed. In Smith's (1992) sucker phylogeny, the Erimyzonini is resolved as sister to a clade containing what are currently regarded as the Moxostomatini and Thoburniini (Harris & Mayden, 2001; Harris *et al.*, 2002). Smith pointed out that this set of relationships was supported by >20 apomorphies that changed at the node representing the MRCA of these lineages in his parsimony tree. But this conclusion is only as sound as the phylogeny upon which character state transitions were mapped by Smith (1992), which, at this node and several other key nodes, is rejected by our mtDNA, nDNA, and multilocus trees, as well as total-evidence results from analyzing Smith's data together with molecular datasets.

Regarding our Thoburniini + Moxostomatini clade, genus *Thoburnia* was inferred to be paraphyletic based on the placement of *T. atripinnis* sister to the *Hypentelium* clade, mostly with strong BPP support. Doosey *et al.* (2010) and Clements, Bart & Hurley (2012) obtained the same relationship for *T. atripinnis*. However, our morphology tree resolved *Thoburnia* as monophyletic, which is consistent with Smith's (1992) original analysis of the morphological data we used, suggesting further data or analyses are needed to clarify these relationships. We consistently inferred *H. roanokense* as sister to a clade of *H. etowanum* + *H. nigricans*, across molecular, morphological, and total-evidence analyses, though with varying BPP (Fig. 3; Figs. S1, S2, S4, and S5). These results match relationships inferred by Buth (1980) using isozyme data reflecting variation at 40 putative loci. By contrast, our results conflict with Smith's (1992) hypothesis, which resolved *H. roanokense* as sister to *H. nigricans*; however, this relationship was based on a single morphological character, dermethmoid spine shape. Taking this into consideration, the broad congruence between multiple data types, as well as our re-analysis of Smith's (1992) data, suggests high confidence in the inference that *H. roanokense* is the earliest diverging lineage in the genus. Within our Moxostomatini clade, *Moxostoma* was monophyletic consistent with previous analyses (Clements, Bart & Hurley, 2012).

Relationships within the Erimyzonini and Catostomini were very similar to those in Harris *et al.* (2002) and consistent with Doosey *et al.* (2010), but they contradicted

[Smith \(1992\)](#), especially by resolving relationships within *Erimyzon* while placing *E. sucetta* sister to *E. tenuis*. As in previous molecular results for Erimyzonini, *Erimyzon* was monophyletic and sister to *Minytrema* in our results. However, *Catostomini* genera were not generally obtained as monophyletic, and in no case was *Catostomus* monophyletic relative to *Chasmistes*, *Deltistes*, or *Xyrauchen*. Our molecular and total-evidence analyses consistently resolved nine well-supported major clades within *Catostominae* (e.g. [Fig. 3](#); [Figs. S1](#) and [S2](#)). Here, we focus on relationships within Clade 5, which corresponded to the former subgenus *Pantosteus*, which [Unmack et al. \(2014\)](#) recently elevated to genus. [Smith \(1966\)](#) recognized six species within *Pantosteus*: *P. clarkii*, *Catostomus columbianus*, *P. discobolus*, *P. plebeius*, *P. platyrhynchus*, and *P. santaanae*. We sampled all of these, including both subspecies of *P. discobolus*, but consistently inferred a polyphyletic *Pantosteus*, with *P. nebuliferus* (recognized as distinct from *P. plebeius* by [Miller, Minckley & Norris, 2005](#); [Nelson et al., 2004](#)) falling within Clade 5 but *Catostomus columbianus* placed in Clade 8 (discussed below). A clade with *P. nebuliferus* + *P. plebeius* was frequently sister to all remaining *Pantosteus* (e.g. [Fig. 3](#)). Notwithstanding incongruent results among analyses in the two papers, the consensus of results from our study and those of [Unmack et al. \(2014\)](#) seems to lend strongest support to the former relationship, with *P. santaanae* sister to a clade containing *P. clarkii* and *P. discobolus* lineages. The polyphyly of *Pantosteus* and *nebuliferus*–*plebeius* sister relationship are concordant with the results of [Doosey et al.'s \(2010\)](#) analyses using RY-coding for third position mtDNA substitutions, although they inferred *P. santaanae* as sister to a clade containing other members of *Pantosteus*. These results also agree with mitochondrial and morphological analyses of [Unmack et al. \(2014\)](#). However, our results depart from [Doosey et al. \(2010\)](#) and agree better with [Unmack et al. \(2014\)](#) in strongly supporting a sister relationship between *C. columbianus* and *C. tahoensis*. That the morphological and molecular data analyzed herein support the monophyly and diagnosability of *Pantosteus* relative to *Catostomus*, without rendering *Catostomus* paraphyletic, strongly supports [Unmack et al.'s \(2014\)](#) decision to redefine *Pantosteus* to exclude *C. columbianus*. We note that this taxonomic arrangement is also consistent with studies on morphological and biochemical variation in western suckers ([Koehn, 1969](#); [Smith, 1992](#); [Smith & Koehn, 1971](#)). Also, *C. columbianus* has an open frontoparietal fontanelle, a key diagnostic character of this clade, whereas other *Pantosteus* species have the frontoparietal fontanelle closed or reduced to a narrow slit ([Smith, 1966](#)).

“*Catostomus*” polyphyly and introgressive hybridization

As noted above, *Catostomus* was never resolved in our study as monophyletic relative to *Chasmistes*, *Deltistes*, or *Xyrauchen*, and this result is concordant with phylogenetic results of [Doosey et al. \(2010\)](#) based on mtDNA *ND4/ND5* sequences. Hybridization of *Catostomus* with *Chasmistes*, *Deltistes*, and *Xyrauchen* is well documented ([Buth, Murphy & Ulmer, 1987](#); [Markle, Cavalluzzi & Simon, 2005](#); [Mock et al., 2006](#); [Tranah & May, 2006](#)), and may be related to the non-monophyly of *Catostomus* relative to *Chasmistes* and *Deltistes*. However, while *Xyrauchen texanus* has been documented to

hybridize with *Catostomus latipinnis* and *Catostomus insignis* (Buth, Murphy & Ulmer, 1987; Hubbs & Miller, 1953), the majority of these reports evaluate hybridization between *X. texanus* and *Catostomus latipinnis* (Buth, Murphy & Ulmer, 1987). Samples of *Xyrauchen* used in this study originated from the Dexter National Fish Hatchery, which obtained the original hatchery stock of Razorback sucker from Lake Mohave, Arizona, where hybridization with *Catostomus latipinnis* has been documented but allozyme evidence indicates only low levels of introgression of *Catostomus latipinnis* with *X. texanus* (Buth, Murphy & Ulmer, 1987). Therefore, placement of *Catostomus insignis* sister to *X. texanus* here and by Doosey et al. (2010) suggests that introgression is not a factor in either study. As such, “*Xyrauchen*” embedded within *Catostomus* renders the latter polyphyletic. Even if hybridization-mediated introgression were considered as an ad hoc explanation of this pattern, this is difficult to distinguish from the more parsimonious hypothesis of common ancestry, and the available data do not demonstrate that any hybridization events among these taxa have corresponded to the Neogene–present timeframe of their divergences inferred by our time tree. Thus, we advocate the tentative placement of “*Xyrauchen*” into synonymy with *Catostomus* until additional fossil or molecular evidence rejects an inference of common ancestry in favor of Neogene hybridization of these taxa.

Bayesian total-evidence dating and relaxed-clock partitioning

In showing that the best clock-partitioned BEAST models excluded sites identified as problematic in our PI profiling analysis, the results of our Bayes factor clock-partitioning model comparisons bolster Ho & Lanfear’s (2010) recommendation that accounting for differences in substitution rates among data partitions through clock-partitioning is not only feasible but also improves phylogenetic divergence dating models. We believe that by employing a clock-partitioning scheme objectively chosen in this way allowed our final BEAST FBD analysis to more correctly estimate topology and rate variance among branches, and better handle rate heterogeneity of the retained characters. However, while the inclusion of fossil taxa in an FBD model in the final BEAST analysis certainly improved our divergence time estimates over what might be obtained using node calibration or tip-dating methods (Arcila et al., 2015; Gavryushkina et al., 2017), one limitation of this analysis was that nodal support was reduced within the Catostomini and Ictiobinae. This pattern was caused by rogue placements of fossil taxa lacking character data, which were constrained within these crown groups but made up $\geq 50\%$ of tip sampling (Fig. 4). Still, this mainly caused misleading relationships and lowered nodal support within the Catostomini; after removing fossil taxa, relationships within Ictiobinae would be essentially identical to our preferred MrBayes topologies. After pruning extinct taxa, our time tree will provide a suitable basis for interrogating the comparative biogeography and evolution of all groups of suckers, except for patterns within Catostomini. One alternate way forward for researchers interested in using our results for comparative phylogenetics would be to convert one of our preferred topologies (Fig. 3; Fig. S2) to an ultrametric tree while constraining subfamily and tribal node ages to mean t_{MRCA} estimates shown in Fig. 4.

A major goal of our study was to use our final total-evidence dating results to test hypotheses on the temporal diversification of suckers. Our divergence dating results (Fig. 4) generally agree with the fossil record but reject or confirm different molecular hypotheses about the temporal diversification of sucker subfamilies. Unsurprisingly, given our incorporation of all fossil sucker lineages in the paleontological literature under an FBD model (accounting for extant and fossil sampling levels), our BEAST results strongly support hypothesis H_4 that Catostomidae lineages have diversified since ~50 Ma in the Early Eocene, which is widely accepted as the minimum age of the origin of suckers based on stratigraphic information for the oldest sucker fossils (review and refs. in Appendix S1). Our results also support *Sun et al.'s (2007)* proposal, or our hypothesis H_3 , that catostomine lineages in the most speciose sucker subfamily went on to diversify since ~20 Ma. Indeed, initial divergences and subsequent diversification of all four catostomine tribes has proceeded since around ~34–17.6 Ma in the Eocene–Miocene, with 95% credible intervals ranging from 43 to 11.04 Ma (Fig. 4), and the t_{MRCA} s for ~81% (64/79) of extant species/lineages in our time tree (all catostomines) coincide with the last 20 Myr.

In contrast to hypotheses H_3 and H_4 discussed above, we reject two previous molecular hypotheses about the tempo of sucker evolution advanced by *Sun et al. (2007)*. First, we reject hypothesis H_1 because we infer that the Asian Myxocyprinae diverged from North American suckers during the Late Cretaceous, and the 95% credible intervals for this divergence do not overlap with their proposed ~14 Ma Miocene date for the MRCA of *Myxocyprinus* and *Cycleptus*. Second, we reject H_2 given that we infer an early Eocene origin for Ictiobinae, including the extinct †*Amyzon* and †*Vasnetzovia* ictiobine lineages, and this vastly predates *Sun et al.'s (2007)* proposed origin of the clade. Given *Sun et al. (2007)* produced divergence time estimates using only *cytb* divergences and a global molecular clock assuming a 2.0% Myr⁻¹ pairwise rate for vertebrate mtDNA, there are too many methodological distinctions between our approach and theirs to pinpoint a single factor causing our results to contrast theirs so strongly. However, our more comprehensive and nuanced approach using Bayesian total-evidence dating not only allowed us to use a realistic FBD tree prior incorporating the speciation-extinction-fossilization sampling process (*Gavryushkina et al., 2017*), but also permitted estimation of evolutionary rates for each character subset analyzed. We inferred a slower rate of evolution for mtDNA 2nd position sites, 6.53×10^{-4} substitutions site⁻¹ Myr⁻¹, and in fact all DNA subsets (mean: 0.0055 substitutions site⁻¹ Myr⁻¹), than the global rate applied by *Sun et al. (2007)*. This is partly due to our best model excluding the most variable mtDNA codon sites, but it appears that differences between our t_{MRCA} estimates and theirs are not fully accounted for based on substitution rates alone. Nevertheless, our more appropriate modeling of the evolutionary processes producing variation in sucker DNA sequences and morphological characters, and extant and fossil taxon sampling, has allowed us to estimate older and undoubtedly more accurate divergence dates, especially for deeper nodes in the sucker phylogeny.

We infer divergence times for major sucker lineages that are conspicuously older than those recently estimated from multilocus analyses of other North American freshwater

fish clades, including sunfishes and black basses (Centrarchidae; [Near, Bolnick & Wainwright, 2005](#); [Near et al., 2011](#)), bullhead and madtom catfishes (Ictaluridae; [Hardman & Hardman, 2008](#)), and darters (Etheostomatinae; [Near et al., 2011](#)). Whereas the diversification of these major lineages has occurred since around the Eocene–Oligocene transition ~34 Ma, a time of global cooling ([Zachos et al., 2001](#)), we infer an earlier Late Cretaceous–Eocene age for the onset of sucker subfamily divergences. While tip-dating approaches can lead to older divergence dates ([O’Reilly, Dos Reis & Donoghue, 2015](#)), we believe that this result accurately reflects the relatively longer timeframe of sucker evolution captured by the fossil record. This general timeframe for sucker evolution also correlates well to the Late Paleocene Thermal Maximum, a period of greater ambient and sea-surface temperatures, higher sea levels, and higher precipitation and humidity ([Zachos et al., 2001](#)). Sucker diversification thus appears to have initiated during a period of climate change and sea level rise, which may have facilitated the isolation of ancestral sucker populations. Our results also suggest that approximately 4–7 sucker genera may have been present in North America by the Oligocene, a period coinciding with the arrival of minnows in the family Cyprinidae on the continent based on the broader fossil record of North American teleost fishes ([Cavender, 1986](#)). Together with the molecular results from other studies above, this implies that the subsequent diversification of these genera, including at least *Cycleptus*, †*Amyzon*, *Ictiobus*, and *Carpionodes* as well as the speciose Catostominae, would have coincided with the diversification of most other major lineages of North American freshwater fishes.

CONCLUSIONS

We have presented the results of a phylogenetic analysis of Holarctic sucker fishes (family Catostomidae) drawing on the most comprehensive dataset to date and inferring, separately and jointly, the phylogeny and divergence times of suckers while including fossil taxa as tips. Our molecular and total-evidence results corroborated relationships hypothesized in previous molecular studies and yielded evidence in favor of some new hypotheses of relationships within and among subfamilies, for example, with Bayes factor support for Myxocyprininae sister to all other sucker lineages. Our study also highlights how using PI profiling to identify problematic character sets can subsequently improve or provide additional evidence for clock-partitioning scheme choice during Bayesian relaxed-clock divergence dating. Our divergence-dating results strongly supported the hypotheses that Catostomidae lineages have diversified since ~50 Ma in the Early Eocene ([Uyeno & Smith, 1972](#)), and that tribes within the most speciose subfamily, Catostominae, have diversified since ~20 Ma in the Eocene–Miocene ([Smith, 1992](#); [Sun et al., 2007](#)). Moreover, we hypothesized that incongruent subfamily relationships were driven in part by problematic mtDNA 1st and 3rd codon sites, and by “rogue taxon” movements of Cycleptinae and fossil taxa, for example in our FBD process time tree. Our analysis could be extended to test this latter hypothesis using additional statistical analyses of rogue taxa ([Aberer, Krompass & Stamatakis, 2012](#)) and internode uncertainty ([Zhou et al., 2017](#)), and by additional resolution analyses employing Monte Carlo simulations and tests of their assumptions ([Townsend, Su & Tekle, 2012](#)), which were

beyond the scope of the present study. Nevertheless, our results suggest that future studies of suckers will benefit from using PI profiles as a predictive tool to select loci for subsequent phylogenetic analyses (*Dornburg et al., 2014*).

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Justin C. Bagley conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Richard L. Mayden conceived and designed the experiments, contributed reagents/materials/analysis tools, approved the final draft.

- Phillip M. Harris conceived and designed the experiments, contributed reagents/materials/analysis tools, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All molecular laboratory work for this project conducted in the R. L. Mayden laboratory was approved under Saint Louis University Institutional Animal Care and Use Committee (IACUC) protocol #2467.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

GenBank accession numbers for sequences used in this study are listed in [Table 2](#), and the raw sequence files are provided in the Mendeley Data accession ([DOI: 10.17632/trw6sb4v7w.2](https://doi.org/10.17632/trw6sb4v7w.2)).

Data Availability

The following information was supplied regarding data availability:

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Supplemental Information

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REFERENCES

- Aberer AJ, Krompass D, Stamatakis A. 2012.** Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Systematic Biology* **62**(1):162–166
[DOI 10.1093/sysbio/sys078](https://doi.org/10.1093/sysbio/sys078).
- Agassiz L. 1854.** Notice of a collection of fishes from the southern bend of the Tennessee River, in the State of Alabama. *American Journal of Science and Arts 28 Second Series* **17**(297–308):353–365.
- Arcila D, Alexander Pyron R, Tyler JC, Ortí G, Betancur RR, Pyron RA. 2015.** An evaluation of fossil tip-dating versus node-age calibrations in tetraodontiform fishes (Teleostei: Percomorphaceae). *Molecular Phylogenetics and Evolution* **82**:131–145
[DOI 10.1016/j.ympev.2014.10.011](https://doi.org/10.1016/j.ympev.2014.10.011).
- Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV. 2012.** Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution* **29**(9):2157–2167
[DOI 10.1093/molbev/mss084](https://doi.org/10.1093/molbev/mss084).
- Bagley JC. 2017.** justincbagley/PIrANHA: PIrANHA Version 0.1.4 [Data Set]. *Zenodo*. Available at <http://doi.org/10.5281/zenodo.571185>.

- Bouckaert R, Heled J, Kühnert D, Vaughan TG, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* **10**(4):e1003537 DOI [10.1371/journal.pcbi.1003537](https://doi.org/10.1371/journal.pcbi.1003537).
- Brandley MC, Wang Y, Guo X, Montes de Oca AN, Fería-Ortiz M, Hikida T, Ota H. 2011.** Accommodating heterogeneous rates of evolution in molecular divergence dating methods: an example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. *Systematic Biology* **60**(1):3–15 DOI [10.1093/sysbio/syq045](https://doi.org/10.1093/sysbio/syq045).
- Bruner JC. 1991.** Comments on the genus *Amyzon* (family Catostomidae). *Journal of Paleontology* **65**(4):678–686 DOI [10.1017/s0022336000030766](https://doi.org/10.1017/s0022336000030766).
- Buth DG. 1980.** Genetics and systematic relationships in the catostomid genus *Hypentelium*. *Copeia* **1980**:280–290.
- Buth DG, Murphy RW, Ulmer L. 1987.** Population differentiation and introgressive hybridization of the flannelmouth sucker and of hatchery and native stocks of the razorback sucker. *Transactions of the American Fisheries Society* **116**(1):103–110 DOI [10.1577/1548-8659\(1987\)116.0.co;2](https://doi.org/10.1577/1548-8659(1987)116.0.co;2).
- Cavender T. 1986.** Review of the fossil history of North American freshwater fishes. In: Hocutt CH, Wiley EO, eds. *The Zoogeography of North American Freshwater Fishes*. New York: John Wiley and Sons, 699–724.
- Chang MM, Miao D, Chen Y, Zhou J, Chen P. 2001.** Suckers (Fish, Catostomidae) from the Eocene of China account for the family's current disjunct distributions. *Science in China Series D* **44**(7):577–586 DOI [10.1007/bf02875332](https://doi.org/10.1007/bf02875332).
- Chen W-J, Mayden RL. 2012.** Phylogeny of suckers (Teleostei: Cypriniformes: Catostomidae): further evidence of relationships provided by the single-copy nuclear gene IRBP2. *Zootaxa* **3586**:195–210.
- Chen WJ, Miya M, Saitoh K, Mayden RL. 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**(2):125–134 DOI [10.1016/j.gene.2008.07.016](https://doi.org/10.1016/j.gene.2008.07.016).
- Chow S, Takeyama H. 1998.** Intron length variation observed in the creatine kinase and ribosomal protein genes of the swordfish *Xiphias gladius*. *Fisheries Science* **64**(3):397–402 DOI [10.2331/fishsci.64.397](https://doi.org/10.2331/fishsci.64.397).
- Clarke JA, Middleton KM. 2008.** Mosaicism, modules, and the evolution of birds: results from a Bayesian approach to the study of morphological evolution using discrete character data. *Systematic Biology* **57**(2):185–201 DOI [10.1080/10635150802022231](https://doi.org/10.1080/10635150802022231).
- Clements MD, Bart HL Jr, Hurley DL. 2012.** A different perspective on the phylogenetic relationships of the Moxostomatini (Cypriniformes: Catostomidae) based on cytochrome-b and Growth Hormone intron sequences. *Molecular Phylogenetics and Evolution* **63**(1):159–167 DOI [10.1016/j.ympev.2012.01.001](https://doi.org/10.1016/j.ympev.2012.01.001).
- Doosey MH, Bart HL, Saitoh K, Miya M. 2010.** Phylogenetic relationships of catostomid fishes (Actinopterygii: Cypriniformes) based on mitochondrial ND4/ND5 gene sequences. *Molecular Phylogenetics and Evolution* **54**(3):1028–1034 DOI [10.1016/j.ympev.2009.06.006](https://doi.org/10.1016/j.ympev.2009.06.006).
- Dornburg A, Townsend JP, Friedman M, Near TJ. 2014.** Phylogenetic informativeness reconciles ray-finned fish molecular divergence times. *BMC Evolutionary Biology* **14**(1):169 DOI [10.1186/s12862-014-0169-0](https://doi.org/10.1186/s12862-014-0169-0).
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012.** Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**(8):1969–1973 DOI [10.1093/molbev/mss075](https://doi.org/10.1093/molbev/mss075).
- Ferris SD, Whitt GS. 1978.** Phylogeny of tetraploid catostomid fishes based on the loss of duplicate gene expression. *Systematic Zoology* **27**(2):189–206 DOI [10.2307/2412971](https://doi.org/10.2307/2412971).

- Gavryushkina A, Heath TA, Ksepka DT, Stadler T, Welch D, Drummond AJ. 2017.** Bayesian total-evidence dating reveals the recent crown radiation of penguins. *Systematic Biology* **66**(1):57–73 DOI [10.1093/sysbio/syw060](https://doi.org/10.1093/sysbio/syw060).
- Gavryushkina A, Welch D, Stadler T, Drummond AJ. 2014.** Bayesian inference of sampled ancestor trees for epidemiology and fossil calibration. *PLOS Computational Biology* **10**(12):e1003919 DOI [10.1371/journal.pcbi.1003919](https://doi.org/10.1371/journal.pcbi.1003919).
- Hardman M, Hardman LM. 2008.** The relative importance of body size and paleoclimatic change as explanatory variables influencing lineage diversification rate: an evolutionary analysis of bullhead catfishes (Siluriformes: Ictaluridae). *Systematic Biology* **57**(1):116–130 DOI [10.1080/10635150801902193](https://doi.org/10.1080/10635150801902193).
- Harris PM, Hubbard G, Sandel M. 2014.** Catostomidae: Suckers. In: Warren ML, Burr BM, eds. *Freshwater Fishes of North America: Volume 1: Petromyzontidae to Catostomidae*. Baltimore: Johns Hopkins University Press, 451–502.
- Harris PM, Mayden RL. 2001.** Phylogenetic relationships of major clades of Catostomidae (Teleostei: Cypriniformes) as inferred from mitochondrial SSU and LSU rDNA sequences. *Molecular Phylogenetics and Evolution* **20**(2):225–237 DOI [10.1006/mpev.2001.0980](https://doi.org/10.1006/mpev.2001.0980).
- Harris PM, Mayden RL, Perez HE, Garcia de, Leon F. 2002.** Phylogenetic relationships of Redhorse (*Moxostoma*) and Jumprock (*Scartomyzon*) suckers (Cypriniformes: Catostomidae) based on mitochondrial cytochrome *b* sequence data. *Journal of Fish Biology* **61**:1433–1452.
- Heath TA, Hedtke SM, Hillis DM. 2008.** Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution* **46**:239–257.
- Heath TA, Huelsenbeck JP, Stadler T. 2014.** The fossilized birth–death process for coherent calibration of divergence-time estimates. *Proceedings of the National Academy of Sciences of the United States of America* **111**(29):E2957–E2966 DOI [10.1073/pnas.1319091111](https://doi.org/10.1073/pnas.1319091111).
- Ho SYW, Lanfear R. 2010.** Improved characterisation of among-lineage rate variation in cetacean mitogenomes using codon-partitioned relaxed clocks. *Mitochondrial DNA* **21**(3–4):138–146 DOI [10.3109/19401736.2010.494727](https://doi.org/10.3109/19401736.2010.494727).
- Hubbs CL. 1930.** Materials for a revision of the catostomid fishes of eastern North America. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* **20**:1–47.
- Hubbs CL, Miller RR. 1953.** Hybridization in nature between the fish genera *Catostomus* and *Xyrauchen*. *Papers of the Michigan Academy of Science, Arts and Letters* **38**:207–234.
- Jenkins RE. 1970.** *Systematic Studies of the Catostomid Fish Tribe Moxostomatini*. Ithaca: Cornell University.
- Kass RE, Raftery AE. 1995.** Bayes factors. *Journal of the American Statistical Association* **90**(430):773–795 DOI [10.2307/2291091](https://doi.org/10.2307/2291091).
- Katoh K, Toh H. 2008.** Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9**(4):286–298 DOI [10.1093/bib/bbn013](https://doi.org/10.1093/bib/bbn013).
- 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, Meintjes 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 DOI [10.1093/bioinformatics/bts199](https://doi.org/10.1093/bioinformatics/bts199).
- Koehn RK. 1969.** Hemoglobins of fishes of the genus *Catostomus* in western North America. *Copeia* **1969**(1):21–30 DOI [10.2307/1441692](https://doi.org/10.2307/1441692).
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**(6):1695–1701 DOI [10.1093/molbev/mss020](https://doi.org/10.1093/molbev/mss020).

- Lewis PO. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50(6):913–925 DOI 10.1080/106351501753462876.
- López-Giráldez F, Townsend JP. 2011. PhyDesign: an online application for profiling phylogenetic informativeness. *BMC Evolutionary Biology* 11(1):152 DOI 10.1186/1471-2148-11-152.
- Markle DF, Cavalluzzi MR, Simon DC. 2005. Morphology and taxonomy of Klamath Basin suckers (Catostomidae). *Western North American Naturalist* 65:473–489.
- Mayden RL, Chen WJ, Bart HL Jr, Doosey MH, Simons AM, Tang KL, Wood RM, Agnew MK, Yang L, Hirt MV, Clements MD, Saitoh K, Sado T, Miya M, Nishida M. 2009. Reconstructing the phylogenetic relationships of the earth's most diverse clade of freshwater fishes—order Cypriniformes (Actinopterygii: Ostariophysi): a case study using multiple nuclear loci and the mitochondrial genome. *Molecular Phylogenetics and Evolution* 51(3):500–514 DOI 10.1016/j.ympev.2008.12.015.
- Mayden RL, Tang KL, Wood RM, Chen WJ, Agnew MK, Conway KW, Yang L, Simons AM, Bart HL, Harris PM, Li J, Wang X, Saitoh K, He S, Liu H, Chen Y, Mutsumi N, Miya M. 2008. Inferring the Tree of Life of the order Cypriniformes, the earth's most diverse clade of freshwater fishes. *Journal of Systematics and Evolution* 46:424–438.
- Miller RR. 1959. Origin and affinities of the freshwater fish fauna of western North America. In: Hubbs CL, ed. *Zoogeography*. Vol. 51. Washington, D.C.: American Association for the Advancement of Science Publication, 187–222.
- Miller RR, Minckley WL, Norris SM. 2005. *Freshwater Fishes of Mexico*. Chicago: University of Chicago Press.
- Mock KE, Evans RP, Crawford M, Cardall BL, Janecke SU, Miller MP. 2006. Rangewide molecular structuring in the Utah sucker (*Catostomus ardens*). *Molecular Ecology* 15(8):2223–2238 DOI 10.1111/j.1365-294x.2006.02932.x.
- NatureServe. 2013. *Moxostoma lacerum*. The IUCN Red List of Threatened Species. Version 2015.2. Available at www.iucnredlist.org (accessed 1 August 2015).
- NatureServe. 2014. *Chasmistes cujus*. Red List of Threatened Species. Version 2015.2. Available at www.iucnredlist.org (accessed 1 August 2015).
- Near TJ, Bolnick DI, Wainwright PC. 2005. Fossil calibrations and molecular divergence time estimates in centrarchid fishes (Teleostei: Centrarchidae). *Evolution* 59(8):1768–1782 DOI 10.1111/j.0014-3820.2005.tb01825.x.
- Near TJ, Bossu CM, Bradburd GS, Carlson RL, Harrington RC, Hollingsworth PR, Keck BP, Etnier DA. 2011. Phylogeny and temporal diversification of darters (Percidae: Etheostomatinae). *Systematic Biology* 60(5):565–595 DOI 10.1093/sysbio/syr052.
- Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences of the United States of America* 109(34):13698–13703 DOI 10.1073/pnas.1206625109.
- Nelson JS, Crossman EJ, Espinosa-Perez H, Findley LT, Gilbert CR, Lea RN, Williams JD. 2004. *Common and Scientific Names of Fishes from the United States, Canada, and Mexico*. Bethesda: American Fisheries Society Special Publication, 29.
- O'Reilly JE, Dos Reis M, Donoghue PCJ. 2015. Dating tips for divergence-time estimation. *Trends in Genetics* 31(11):637–650 DOI 10.1016/j.tig.2015.08.001.
- Pagel M, Meade A. 2014. BayesTraits (First Edition). Reading, UK. Available at <http://www.evolution.rdg.ac.uk/BayesTraits.html>.
- Pond SLK, Frost SDW, Muse SV. 2005. HyPhy: hypothesis testing using phylogenies. *Bioinformatics* 21(5):676–679 DOI 10.1093/bioinformatics/bti079.

- Pyron RA. 2011.** Divergence time estimation using fossils as terminal taxa and the origins of Lissamphibia. *Systematic Biology* **60**(4):466–481 DOI [10.1093/sysbio/syr047](https://doi.org/10.1093/sysbio/syr047).
- Rambaut A, Drummond AJ. 2014.** Tracer v1.5. Available at <http://tree.bio.ed.ac.uk/software/tracer/>.
- Robins CR, Raney EC. 1956.** Studies of the catostomid fishes of the genus *Moxostoma*, with descriptions of two new species. *Cornell University Agricultural Experiment Station Memoir* **343**:1–56.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**(3):539–542 DOI [10.1093/sysbio/sys029](https://doi.org/10.1093/sysbio/sys029).
- Saghai-Marroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984.** Ribosomal DNA spacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences of the United States of America* **81**(24):8014–8019 DOI [10.1073/pnas.81.24.8014](https://doi.org/10.1073/pnas.81.24.8014).
- Saitoh K, Sado T, Mayden RL, Hanzawa N, Nakamura K, Nishida M, Miya M. 2006.** Mitogenomic evolution and interrelationships of the Cypriniformes (Actinopterygii: Ostariophysi): the first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences. *Journal of Molecular Evolution* **63**(6):826–841 DOI [10.1007/s00239-005-0293-y](https://doi.org/10.1007/s00239-005-0293-y).
- Smith GR. 1966.** Distribution and evolution of the North American catostomid fishes of the subgenus *Pantosteus*, genus *Catostomus*. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* **129**:1–132.
- Smith GR. 1992.** Phylogeny and biogeography of the Catostomidae, freshwater fishes of North America and Asia. In: Mayden RL, ed. *Systematics, Historical Ecology, and North American Freshwater Fishes*. Stanford: Stanford University Press, 778–813.
- Smith GR, Dowling TE, Gobalet KW, Lugaski T, Shiozawa DK, Evans RP. 2002.** Biogeography and timing of evolutionary events among Great Basin fishes. In: Hershler R, Madsen DB, Currey DR, eds. *Great Basin Aquatic Systems History*. Washington: Smithsonian Institution Press, 175–234.
- Smith GR, Koehn RK. 1971.** Phenetic and cladistic studies of biochemical and morphological characteristics of *Catostomus*. *Systematic Zoology* **20**(3):282–297 DOI [10.2307/2412341](https://doi.org/10.2307/2412341).
- Sun YH, Xie CX, Wang WM, Liu SY, Treer T, Chang MM. 2007.** The genetic variation and biogeography of catostomid fishes based on mitochondrial and nucleic DNA sequences. *Journal of Fish Biology* **70**(sc):291–309 DOI [10.1111/j.1095-8649.2007.01453.x](https://doi.org/10.1111/j.1095-8649.2007.01453.x).
- Stadler T. 2010.** Sampling-through-time in birth–death trees. *Journal of Theoretical Biology* **267**(3):396–404.
- Townsend JP. 2007.** Profiling phylogenetic informativeness. *Systematic Biology* **56**(2):222–231 DOI [10.1080/10635150701311362](https://doi.org/10.1080/10635150701311362).
- Townsend JP, Leuenberger C. 2011.** Point of View: taxon sampling and the optimal rates of evolution for phylogenetic inference. *Systematic Biology* **60**(3):358–365 DOI [10.1093/sysbio/syq097](https://doi.org/10.1093/sysbio/syq097).
- Townsend JP, López-Giráldez F, Friedman R. 2008.** The phylogenetic informativeness of nucleotide and amino acid sequences for reconstructing the vertebrate tree. *Journal of Molecular Evolution* **67**(5):437–447 DOI [10.1007/s00239-008-9142-0](https://doi.org/10.1007/s00239-008-9142-0).
- Townsend JP, Su Z, Tekle YI. 2012.** Phylogenetic signal and noise: predicting the power of a data set to resolve phylogeny. *Systematic Biology* **61**(5):835–849 DOI [10.1093/sysbio/sys036](https://doi.org/10.1093/sysbio/sys036).

- Tranah GJ, May B. 2006.** Patterns of intra-and interspecies genetic diversity in Klamath River Basin suckers. *Transactions of the American Fisheries Society* **135**(2):306–316
[DOI 10.1577/t05-026.1](https://doi.org/10.1577/t05-026.1).
- Unmack PJ, Dowling TE, Laitinen NJ, Secor CL, Mayden RL, Shiozawa DK, Smith GR. 2014.** Influence of introgression and geological processes on phylogenetic relationships of western North American mountain suckers (*Pantosteus*, Catostomidae). *PLOS ONE* **9**(3):e90061
[DOI 10.1371/journal.pone.0090061](https://doi.org/10.1371/journal.pone.0090061).
- Uyeno T, Smith GR. 1972.** Tetraploid origin of the karyotype of catostomid fishes. *Science* **175**(4022):644–646 [DOI 10.1126/science.175.4022.644](https://doi.org/10.1126/science.175.4022.644).
- Warren ML Jr, Burr BM, Walsh SJ, Bart HL, Cashner RC, Etnier DA, Freeman BJ, Kuhajda BR, Mayden RL, Robison HW, Ross ST, Starnes WC. 2000.** Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. *Fisheries* **25**(10):7–31 [DOI 10.1577/1548-8446\(2000\)0252.0.co;2](https://doi.org/10.1577/1548-8446(2000)0252.0.co;2).
- Xie W, Lewis PO, Fan Y, Kuo L, Chen MH. 2011.** Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology* **60**(2):150–160
[DOI 10.1093/sysbio/syq085](https://doi.org/10.1093/sysbio/syq085).
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001.** Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**(5517):686–693.
- Zhou X, Lutteropp S, Czech L, Stamatakis A, von Looz M, Rokas A. 2017.** Quartet-based computations of internode certainty provide accurate and robust measures of phylogenetic incongruence. *bioRxiv* 168526 [DOI 10.1101/168526](https://doi.org/10.1101/168526).