

Supplementary Information

**The genomic timeline of cichlid fish diversification  
across continents**

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## Supplementary Notes

### Supplementary Note 1: Consequences of fossil-assignment uncertainty.

The fossil record of a clade informs about the age of origin of that clade. In particular, the oldest known fossil of a clade indicates its minimum age. Unfortunately, however, the assignment of fossils to clades is often ambiguous when fossils are fragmentary or when morphological characters evolved convergently in multiple clades. For many clades, it is therefore uncertain if one or another fossil represents the earliest record of that clade.

A prominent example of this issue is the teleost fish order Tetraodontiformes, the earliest record of which has long been assumed to be †*Plectocretacicus clarae* from the limestone of Haqil, Israel [1, 2] with an age of 100.3-98.0 Ma (Supplementary Note 2). The tetraodontiform affinity of the fossil has recently been reevaluated (see discussion in [3]), and many authors [4–9] have since opted not to accept †*Plectocretacicus clarae* as the earliest tetraodontiform record. Exhibiting more tetraodontiform synapomorphies than †*Plectocretacicus clarae*, the earliest undisputed tetraodontiform record is †*Cretatriacanthus guidottii* from the Calcari di Melissano, Italy, with an age of 89.8-83.0 Ma (Supplementary Note 2). However, ambiguity remains regarding the status of †*Plectocretacicus clarae*, and a recent combined analysis of morphological and molecular data supported its original placement in Tetraodontiformes [10]. To address this ambiguity, several recent studies of teleost divergence times conducted separate analyses with and without the age constraint based on †*Plectocretacicus clarae* [7–9]. Unfortunately, the results of these studies highlight the strong impact of the fossil on a large part of the timeline of teleost evolution. For example, the age estimate for the origin of Acanthomorpha, a group comprising roughly a third of vertebrate diversity, shifted by over 10 Myr in the analyses of Musilova et al. [9], depending on whether or not †*Plectocretacicus clarae* was used as an age constraint for Tetraodontiformes. Frustratingly, this means that conclusions drawn from these timelines may only be valid under a certain assumption for the position of †*Plectocretacicus clarae*. Furthermore, the approach of repeating phylogenetic divergence-time estimation separately with and without ambiguous constraints is only feasible when the investigated group has few such ambiguities, as the number of possible combinations of constraints grows exponentially with each clade that has an ambiguous first record.

Instead of conducting phylogenetic divergence-time estimation separately with and without all ambiguous constraints, accounting for all uncertainties in a single Bayesian analysis would have the advantages that only a single set of results would be obtained, which would facilitate the interpretation, and that the results could be obtained at lower computational cost. When morphological characters have been scored for both fossils and extant species, this type of analysis is possible with so-called “tip-dating” approaches that consider fossils as terminal taxa and can infer their position as part of the analysis [11–13]. In the absence of a morphological character matrix, a recently implemented feature of the program PhyTime [14, 15] allows specifying probabilities for the assignment of a fossil to different clades; however, PhyTime allows only uniform calibration densities with hard upper boundaries that are usually selected arbitrarily and approximate the *a priori* expectations of clade ages only poorly [16–19]. In addition, PhyTime does not allow the joint estimation of tree topology and divergence times. To address these limitations in divergence-time estimation without morphological character matrices, we here develop and apply a new approach that allows the

specification of two alternative first occurrences, with equal or unequal probability weights, for each clade, and we implement this approach in the CladeAge package v.2.0.0 for BEAST 2 [18].

**Supplementary Note 2:** Fossil constraints.

In the following, we discuss, for each clade that fulfills the conditions for use as CladeAge calibration point, the first record of the clade and the age of this record. The nomenclature follows that of Betancur-R. et al.[20], and ages of geological stages are according to the ICS international chronostratigraphic chart v.2018/8 [21] unless noted otherwise. All clades listed below are considered to include stem lineages.

**Holostei:** †*Watsonulus eugnathoides* (Piveteau, 1935) (251.9-251.2 Ma) or †*Acetrophorus varians* Kirkby, 1862 (262.1-259.1 Ma). Holostei combine Amiiformes (with the only extant species *Amia calva*), Lepisosteiformes (with the extant family Lepisosteidae) and the extinct order Semionotiformes [22–24].

†*Watsonulus eugnathoides* from the Middle Sakamena Formation at Ambilombe Bay has been placed within the crown of Holostei or as a stem member of Holostei in number of studies [3, 24]. The Middle Sakamana Formation is dated as Induan (see discussion in[3]) with an age between 251.9-251.2 Ma. †*Watsonulus eugnathoides* is predated by †*Acetrophorus varians* [25] from the Raisby Formation at Fulwell Hill (Sunderland, UK). This species has been accepted by some authors as the earliest record of Holostei [26] but its phylogenetic placement within the group has been questioned [3]. The Raisby Formation was deposited during cycle 1 of the English Zechstein sequence [27], which represents the upper part of the Capitanian [28]. This age can be interpreted as 262.1-259.1 Ma. We here consider both †*Watsonulus eugnathoides* and †*Acetrophorus varians* as equally probable first occurrences of Holostei (weights: 0.5/0.5). Our phylogenomic data set includes a single representative of Holostei, *Lepisosteus oculatus*.

**Teleostei:** †*Prohalecites porroi* (Tintori, 1990) (239.5-237.0 Ma).

The earliest records of total-group Teleostei include Pholidophoriformes as well as †*Prohalecites* [29, 30]. Of these, †*Prohalecites porroi* from the middle Kalkschieferzone (Meride Kalk) near Ca’ del Frate (Viggiú, Varese, Italy) [31] represents the first occurrence of teleosts [3, 29]. Based on palynomorphology, a latest Ladinian age has been suggested for †*Prohalecites porroi* [32]. Here, this age is interpreted as 239.5-237.0 Ma. Our phylogenomic data set includes 91 members of Teleostei.

**Osteoglossomorpha:** †*Lycoptera* (Sauvage, 1880) (131.0-124.4 Ma).

The earliest records of Osteoglossomorpha are provided by members of the *Lycoptera* assemblage of the Chinese Jehol Biota [33]. *Lycoptera* first appear in the Jingangshan Bed of the Yixian Formation [34]. The fish fossils underlie a tuff layer for which a minimum age of 124.4 was directly determined [34]. The maximum age of the *Lycoptera* fossils comes from the maximum age of the Yixian Formation, 131 Ma [35]. Our phylogenomic data set includes the osteoglossid *Scleropages formosus* as a representative of Osteoglossomorpha.

**Elopomorpha:** †*Anaethalion zapporum* Arratia, 2000 (152.6-152.1 Ma).

The oldest representative of Elopomorpha is †*Anaethalion zapporum* from the Nusplingen lithographic limestone (Swabia, Germany) [3, 36]. The ammonite fauna present at Nusplingen indicates an age within the Ulmense Subzone of the latest Kimmeridgian [37]. This subzone is estimated to

be maximally 0.5 Ma older than the Kimmeridgian/Tithonian boundary [38]. Our phylogenomic data set includes a single representative of Elopomorpha, the anguillid *Anguilla anguilla*.

**Clupeocephala:** †*Leptolepides haerteisi* Arratia, 1997, (152.1-150.9 Ma) or †*Leptolepides* sp. [39] (153.9-152.2 Ma).

In the absence of known stem-group fossils, the earliest record of Clupeocephala is provided by crown-group fossils of the euteleostomorph genus †*Leptolepides* that have been reported from Tithonian and Kimmeridgian lithographic limestones of Germany and France. The oldest of these are fossils assigned to †*Leptolepides haerteisi* from the Zandt Member of the Solnhofen Formation (Eichstätt, Germany). Based on its ammonite fauna the Zandt Member can be constrained to the Tithonian *Hybonotoceras hybonotum* Ammonite Zone [3] with an age of 152.1-150.9 Ma [40]. Older fossils assigned to †*Leptolepides* occur in Kimmeridgian deposits of Cerin (France) and Nusplingen and Wattendorf (Germany), but since these have never been analyzed in as much detail as †*Leptolepides haerteisi*, Benton et al.[3] did not consider these sufficiently reliable to be used as age constraints. The earliest Kimmeridgian records of †*Leptolepides* are from the Wattendorf Limestone [39], which can be constrained to the *Aulacostephanus eudoxus* Ammonite Zone [3] with an age of 153.9-152.2 Ma [40]. We here consider both †*Leptolepides haerteisi* and †*Leptolepides* sp. as equally probable first occurrences of Clupeocephala (weights: 0.5/0.5). Our phylogenomic data set includes 88 representatives of Clupeocephala.

**Otomorpha:** †*Tischlingerichthys vlohli* Arratia, 1997 (152.1-150.9 Ma).

No stem-group fossils are known for Otomorpha, thus the earliest crown-group member, represented by the ostariophysan †*Tischlingerichthys vlohli* from the Mörsheim Formation (Mühlheim, Germany), marks the appearance of Otomorpha in the fossil record [3, 41]. The Mörsheim Formation lies within the *Hybonotoceras hybonotum* Ammonite Zone [3] with an age of 152.1-150.9 Ma [40]. Our phylogenomic data set includes four representatives of Otomorpha.

**Clupeiformes:** †*Ezcutuberezi carmeni* Poyato-Ariza, López-Horgue, and García-Garmilla, 2000 (139.8-125.0 Ma).

A number of clupeiform genera appear in the fossil record of the Early Cretaceous. Among the oldest specimens are †*Diplomystus kokuraensis* and †*Diplomystus primotinus* from the Kanmon Group at Kitakyushu City (Kyushu Island, Japan). The two specimens of †*Diplomystus* have been discovered in the upper layer of the Wakino subgroup [42], which was claimed to be Neocomian (Berriasian-Hauterivian) by the original authors, but is more likely Barremian [43] or Hauterivian-Barremian [44]. Approximately the same age are †*Ellimmichthys longicostatus* and †*Scutatuspinosus itapagipensis* from the Marfim Formation at Itapagipe (Recôncavo Basin, Brazil), that is also assumed to be Hauterivian-Barremian in age [45, 46]. However, the four species might be predated by †*Ezcutuberezi carmeni* from the Villaro Formation in the Arratia Valley close to Bilbao (Basque Country, Spain) [47]. Through correlation with the Bárcena Mayor Formation, a Valangian-Barremanian age is supported for the Villaró Formation [48]; thus, the age of †*Ezcutuberezi carmeni* lies between 139.8-125.0 Ma. Our phylogenomic data set includes *Clupea harengus* as a single representative of Clupeiformes.

**Cypriniformes:** †*Amyzon* spp. (Wilson, 1980) (60.7-59.9 Ma).

The oldest known cypriniforms are catostomids of the genus †*Amyzon* from the Paskapoo Formation (Alberta, Canada) [49, 50]. These fossils of the Paskapoo Formation are from “Site 5 at or near Erickson’s Landing” [51], which corresponds to the Ti3 stage of the Tiffanian North American Land Mammal Age (NALMA), with an age of 60.7-59.9 Ma [52]. Cypriniformes are represented in our phylogenomic data set by *Danio rerio*.

**Characiformes:** †*Santanichthys diasii* (Silva Santos, 1958) (126.3-113.0 Ma).

The earliest record of Characiformes is provided by the stem-characiform †*Santanichthys diasii* [53–55]. The species is reported from the Brazilian Santana, Riachuelo, and Codó Formations, with the best-investigated specimens coming from the Santana Formation [53]. Of the three formations, the ages of the Santana and Riachuelo Formations are given as Aptian-Turonian [56] and Upper Aptian-Albian [57], respectively, but the age of the Codó Formation can be limited to the Aptian based on palynology [58]. Thus, †*Santanichthys diasii* dates to 126.3-113.0 Ma. Our phylogenomic data set includes a single representative of Characiformes, *Astyanax mexicanus*.

**Euteleosteomorpha:** †*Leptolepides haerteisi* Arratia, 1997, (152.1-150.9 Ma) or †*Leptolepides* sp. [39] (153.9-152.2 Ma).

The earliest records of Clupeocephala are simultaneously the oldest euteleosteomorphs. See above for details. Our phylogenomic data set includes 84 representatives of Euteleosteomorpha.

**Esociformes:** †*Estesesox foxi* Wilson et al., 1992 (84.5-83.5 Ma).

The earliest fossil record of Esociformes is provided by specimens of †*Estesesox foxi* from the Milk River Formation (Alberta, Canada) [59]. According to U–Pb geochronology, the Milk River Formation was deposited between 84.5-83.5 Ma [60]. Esociformes are represented in our phylogenomic data set by *Esox lucius*.

**Salmoniformes:** †*Eosalmo driftwoodensis* Wilson, 1977 (52.1-51.4 Ma).

The oldest salmoniform record is provided by †*Eosalmo driftwoodensis* from the Driftwood Creek beds near Smithers (British Columbia, Canada) [61, 62]. Based on palynology and U–Pb zircon analysis, the age of the Driftwood Creek beds can be constrained as 52.1-51.4 Ma [63, 64]. Our phylogenomic data set includes *Salmo salar* as a representative of Salmoniformes.

**Stomiatiformes:** †*Paravinciguerrria praecursor* Arambourg, 1954 (94.3-93.7 Ma).

†*Paravinciguerrria praecursor* is known from the deposits of Jbel Tselfat (Morocco) and the Argille Varicolori succession near Floresta and Malvagna (Sicily, Italy) and is generally regarded as the earliest member of Stomiatiformes [65]. Both the deposits of Jbel Tselfat and the bituminous shales of the Argille Varicolori succession are associated with the Oceanic Anoxic Event 2 (OAE2) close to the Cenomanian-Turonian boundary [65, 66], and can be constrained to 94.3-93.7 Ma based on nannofossils [67, 68].

**Osmeriformes:** †*Speirsaenigma lindoei* Wilson and Williams, 1991 (60.7-59.9 Ma).

Of the few osmeriform fossils predating the Oligocene, the earliest record is †*Speirsaenigma lindoei* from the Paskapoo Formation (Alberta, Canada) [69, 70]. The specimens were collected from the “fish layer” at the Joffre Bridge Roadcut locality [69], the age of which falls within the Ti3 stage

of the Tiffanian North American Land Mammal Age (NALMA) [71] that can be constrained to 60.7-59.9 Ma [52]. Our phylogenomic dataset included the two osmeriforms *Osmerus eperlanus* and *Protosalanx hyalocranius*.

**Osmeridae:** †*Enoplophthalmus schlumbergeri* Sauvage, 1880 (33.9-32.6 Ma Ma).

The earliest records of the family Osmeridae are Oligocene in age [70], with the first appearance marked by †*Enoplophthalmus schlumbergeri* in the Campagne-Calavon Formation at Céreste (Alpes-de-Haute-Provence, France) [72, 73]. Through correlation with the Ronzon (France) and Hoogbutsel (Belgium) beds, the fossil age can be constrained to MP 21 [72] and thus to 33.9-32.6 Ma [52]. Of the three families within the suborder Osmeroidei, Plecoglossidae and Salangidae are sister groups [20]; therefore, the age of Osmeridae is identical to the time of divergence between Salangidae and Osmeridae, represented in our phylogenomic data set by *Protosalanx hyalocranius* and *Osmerus eperlanus*.

**Aulopiformes:** Alepisauroidei *indet.* Kriwet, 2003 (130.8-126.3 Ma).

The appearance of Aulopiformes in the fossil record is marked by isolated teeth of indeterminate Alepisauroidei from the Blesa Formation near Alcaine (Spain) [74, 75]. Owing to the presence of charophytes in the fossil-bearing layers, these can be associated with the *Atopochara trivolvris triquetra* biozone [74], indicating a Barremian age [76, 77]. The presence of Aulopiformes in the Barremian is further supported by †*Atolvorator longipectoralis* from the Morro do Chaves Formation at São Miguel dos Campos (Sergipe-Alagoas Basin, Brazil) [75, 78, 79], for which a late Barremian age is suggested by palynomorphs and pollen occurrences [80]. Given that the age of Alepisauroidei *indet.* could be early or late Barremian, the fossils date to 130.8-126.3 Ma. In our phylogenomic data set, Aulopiformes are represented by *Parasudis fraserbrunneri*.

**Myctophiformes:** †*Sardinioides* spp. van der Marck, 1858 (81.3–80.7 Ma).

A large number of Cretaceous fossil from Germany and Lebanon have been assigned to the order Myctophiformes, but the taxonomic position of many of these remains ambiguous [81]. Among the oldest of these are fossils from the Coesfeld Member at Sendenhorst (Münster Basin, Germany) that were originally described under the name †*Osmeroides monasteri* and later assigned to the genus †*Sardinioides*. This genus is robustly placed among Myctophiformes in cladistic analyses [82]. Based on its ammonite fauna, the Coesfeld Member can be constrained to an age between the top of the †*Scaphites hippocrepis* III Zone and the base of the †*Baculites maclearni* Zone [83], and thus to 81.3–80.7 Ma [68]. Myctophiformes are represented in our taxon set by *Benthosema glaciale*.

**Acanthomorphata:** †*Pseudomonocentris microspinosus* González-Rodríguez, Schultze, and Arratia, 2013, †*Handuichthys interopercularis* González-Rodríguez, Schultze, and Arratia, 2013, †*Dalgoichthys tropicalis* González-Rodríguez, Schultze, and Arratia, 2013, and †*Muhichthys cordobai* González-Rodríguez and Fielitz, 2008, (106.8-98.0 Ma).

Acanthomorph fishes are abundant in the fossil record of the Cenomanian [3, 84], with an age of at least 98.0 Ma for fossils from the Sannine Limestone at Hajula (Lebanon) [3, 85]. These acanthomorphs, however, might be predated by recently described specimens of †*Pseudomonocentris microspinosus*, †*Handuichthys interopercularis*, †*Dalgoichthys tropicalis* [86], and †*Muhichthys cor-*

*dobai* [87] from the El Doctor Formation at the Muhi Quarry near Zimapán (Hidalgo, Mexico). The age of these deposits can be constrained roughly to Albanian-Cenomanian [88] and were interpreted as “very late Albian or very early Cenomanian” [89]. We here assume that the Muhi Quarry falls into the second half of the Albian or the first half of the Cenomanian, and that the acanthomorphs from this location thus date to 106.8-97.2 Ma. We adjust the lower boundary of this range to 98.0 Ma to account for the minimum age of the Hajula acanthomorphs [3]. In our phylogeny, Acanthomorpha are represented by a total of 76 taxa.

**Polymixiiformes:** †*Homonotichthys dorsalis* (Dixon, 1850), †*Homonotichthys rotundus* (Woodward, 1902), and †*Homonotichthys pulchellus* (Dixon, 1850) (97.1-94.0 Ma).

Following Malmstrøm et al.[90] and Rabosky et al.[91], we here consider fossils of the genus †*Homonotichthys* (rather than †*Berycopsis* or †*Omosoma* with uncertain taxonomic assignments) as the earliest records of Polymixiiformes. †*Homonotichthys dorsalis*, †*H. rotundus*, and †*H. pulchellus* are all recorded from the Cenomanian English Chalk of Kent and Sussex (United Kingdom) where they occur in the *Holoaster subglobosus* ammonite zone [92]. This traditional zone spans the *Acanthoceras rhotomagense* and *Neocardioceras juddii* modern zones [93, 94]. The top of the *Neocardioceras juddii* zone has a date of 94.0 Ma, the *Cunningtoniceras inerme* zone began about 3.0 myr earlier, and *Acanthoceras rhotomagense* is about 100 kyr younger than *Cunningtoniceras inerme* [68]; thus, the first occurrence of †*Homonotichthys* dates to 97.1-94.0 Ma. Our phylogenomic data set includes *Polymixia japonica* as the single representative of Polymixiiformes.

**Lampriformes:** †*Aipichthys minor* (Pictet, 1850) (100.5-98.0 Ma).

Lampriforms first appear in the Cenomanian, with several species being recorded from the limestones of Haqil and Hajula (Lebanon), the English Chalk of Sussex (United Kingdom), and the Sierra Madre Formation in Chiapas (Mexico) [85, 95]. The oldest of these localities is the Hajula Limestone for which a minimum age of 98.0 Ma can be given based on the presence of *Mantelliceras mantelli* in overlying layers [3]. The maximum age of the Hajula Limestone is the base of the Cenomanian at 100.5 Ma. †*Aipichthys minor* is recorded from Hajula and is consistently placed on the lampriform stem in phylogenetic analyses [94, 96, 97]. Lampriformes are represented in our phylogenomic data set by *Regalecus glesne* and *Lampris guttatus*.

**Lampridae:** †*Megalampris keyesi* Gottfried, Fordyce, and Rust, 2006, (27.8-25.2 Ma).

The earliest record of the family Lampridae is †*Megalampris keyesi* from the Otekaike Limestone near Tokarahi (North Otago, New Zealand) [98]. The lower glauconitic portion of the Otekaike Limestone, from which the specimen was recorded, has a New Zealand Duntroonian age [98]. The Duntroonian is roughly equivalent to the Chattian [99] with a minimum age of 25.2 Ma determined by isotope stratigraphy [100], thus constraining the age of †*Megalampris keyesi* to 27.8-25.2 Ma. Our phylogenomic data set includes *Lampris guttatus* as a single representative of Lampridae.

**Paracanthopterygii:** †*Xenyllion stewarti* Newbrey, Murray, Wilson, Brinkmann, and Neumann, 2013 (98.2-97.8 Ma).

Members of the extinct family Sphenocephalidae have long been regarded as the earliest representatives of Paracanthopterygii [8, 101, 102], and despite repeated changes to the composition of Paracanthopterygii, this placement of Sphenocephalidae has recently been corroborated by phyloge-

netic analyses [103]. The earliest record of Sphenocephalidae is †*Xenyllion stewarti* from the Mowry Formation near Vernal (Utah, USA) [104], occurring in the *Neogastropilites americanus* ammonite zone [105] with an age of 98.2-97.8 Ma [68]. Our phylogenomic data set includes six representatives of Paracanthopterygii.

**Percopsiformes:** †*Mcconichthys longipinnis* Grande, 1988 (66.0-63.6 Ma).

The earliest record of Percopsiformes is provided by †*Mcconichthys longipinnis* from the Tullock Formation in McCone County (Montana, USA) [8, 89, 106, 107]. The Tullock Formation is associated with the Puercan North American Land Mammal Age and its base approximates the Cretaceous-Paleogene boundary [108]. The top of the Tullock Formation is younger than the C28r/C28n magnetochron boundary at 63.6 Ma [109]; thus, †*Mcconichthys longipinnis* is dated to 66.0-63.6 Ma [110]. Our phylogenomic data set includes two members of Percopsiformes, *Percopsis transmontana* and *Typhlichthys subterraneus*.

**Percopsidae:** †*Massamorichthys wilsoni* Murray, 1996 and †*Lateopisciculus turrifumosus* Murray and Wilson, 1996 (62.2-59.9 Ma).

The earliest known occurrences of Percopsidae are fossils assigned to †*Massamorichthys wilsoni* and †*Lateopisciculus turrifumosus* from the Paskapoo Formation (Alberta, Canada) [106, 111]. Of these, †*Massamorichthys wilsoni* is known from a vast number of fossils at the same locality as the osmeriform †*Speirsaenigma lindoei* (see above); thus, they share the age of 60.7-59.9 Ma. The age estimate of †*Lateopisciculus turrifumosus* is less precise. The species is known from the Smoky Tower locality that can only be constrained to the Tiffanian (NALMA) [111] and might thus be as old as 62.2 Ma [52]. The first occurrence of Percopsidae thus dates to 62.2-59.9 Ma. The family is represented in our phylogenomic data set by *Percopsis transmontana*.

**Zeiformes:** †*Cretazeus rinaldii* Tyler et al., 2000 (89.8-83.0 Ma).

The earliest zeiform record is †*Cretazeus rinaldii* from the Calcari di Melissano at Canale near Nardò (Italy) [112]. While the Calcari di Melissano were long considered to be Upper Campanian in age [113], newer studies support an older age of the formation between the Coniacian and the basal Campanian, with a minimum age around 83 Ma [114, 115]. Thus, we here interpret the first occurrence age of Zeariae as 89.8-83.0 Ma. Zeiformes are represented in our data set by *Zeus faber* and *Cyttopsis rosea*.

**Gadiformes:** †“*Protocodus*” sp. (63.3-59.7 Ma).

†“*Protocodus*” sp., known from a nearly complete specimen, is commonly considered the earliest skeletal fossil record of Gadiformes [101, 116–120] even though it has never been formally published. According to Rosenkrantz [116], the specimen was found in the Thyasira member of the Kangilia Formation (West Greenland), which is now considered part of the Eqalulik Formation [121]. Based on dinocyst and nannoplankton occurrence, the formation has been related to the NP4-NP5 zones with an age of 63.3-59.5 Ma [52]. In addition, <sup>40</sup>Ar/<sup>39</sup>Ar age determination of volcanic rocks overlying the formation provides a minimum age of 60.7 ± 1.0 Ma [121]; thus, the age of first occurrence of Gadiformes can be constrained to 63.3-59.7 Ma. In our phylogenomic data set, Gadiformes are represented by *Gadus morhua*.



**Acanthopterygii:** †*Cryptoberyx minimus* Gaudant, 1978, †*Stichocentrus liratus* Patterson, 1967, †*Stichocentrus elegans* Gaudant, 1969, †*Stichocentrus spinulosus* Gayet, 1980, †*Stichopteryx lewisi* (Davis, 1887), †*Lissoberyx dayi* (Woodward, 1942), †*Lissoberyx arambourgi* Gaudant, 1969, †*Lissoberyx denticulatus* Gayet, 1980, †*Microcapros libanicus* Gayet, 1980, †*Libanoberyx spinosus* Gayet, 1980, and †*Hgulichthys spinus* Otero et al., 1995 (100.5-98.0 Ma).

The appearance of Acanthopterygii in the fossil record is marked by various beryciforms, trachichthyiforms, holocentriforms, and a single possible tetraodontiform from the Cenomanian deposits of Lebanon, Israel, Morocco, and the United Kingdom (see discussion in [90]). The oldest of these deposits is the Sannine Limestone at Hajula (Lebanon) [3] with an age of 100.5-98.0 Ma (see above). The species †*Cryptoberyx minimus*, †*Stichocentrus liratus*, †*Stichopteryx lewisi*, †*Lissoberyx dayi*, †*Lissoberyx arambourgi*, †*Lissoberyx denticulatus*, †*Microcapros libanicus*, †*Libanoberyx spinosus*, and †*Hgulichthys spinus* are all known from Hajula [85] and thus jointly represent the earliest record of Acanthopterygii. Our phylogenomic data set includes 67 representatives of Acanthopterygii.

**Trachichthyiformes:** †*Lissoberyx dayi* (Woodward, 1942), †*Lissoberyx arambourgi* Gaudant, 1969, †*Lissoberyx denticulatus* Gayet, 1980, †*Microcapros libanicus* Gayet, 1980, †*Libanoberyx spinosus* Gayet, 1980, and †*Hgulichthys spinus* Otero et al., 1995 (100.5-98.0 Ma).

Of the oldest members of Acanthopterygii from Hajula, several species have been placed in Trachichthyidae (now elevated to the order Trachichthyiformes): †*Lissoberyx dayi*, †*Lissoberyx arambourgi*, †*Lissoberyx denticulatus*, †*Microcapros libanicus*, †*Libanoberyx spinosus*, and †*Hgulichthys spinus* [85, 122–124]. These fossils all date to 100.5-98.0 Ma (see above). Our phylogenomic data set includes two representatives of Trachichthyiformes; *Monocentris japonica* and *Gephyroberyx darwini*.

**Berycoidei:** †*Berycomorus firdoussii* (Arambourg, 1967) (47.8-33.9 Ma).

The only known skeletal fossil record of Berycoidei is †*Berycomorus firdoussii* from the Pabdeh Formation at Ilam (Zagros Basin, Iran) [124]. The formation was erroneously assumed to be Rupelian in age by Arambourg [125] and has more recently been assigned a Middle to Late Eocene age [126]; thus, it dates to 47.8-33.9 Ma. Our phylogenomic data set includes *Beryx splendens* as a single representative of Berycoidei.

**Stephanoberycoidei:** †*Miobarbourisia aomori* Fujii, Uyeno, and Shimaguchi, 2007 (13.8-11.6 Ma), or Stephanoberycoidei indet. (56.0-47.8 Ma)

Even though several fossil species have been assigned to Stephanoberycoidei, most of them remain poorly known and their placement may not be reliable. Following Rabosky et al. [91], †*Miobarbourisia aomori* from Wadagawa Formation near Aomori City (Japan) [127] is here accepted as a member of Stephanoberycoidei. The Wadagawa Formation correlates to the middle part of the Californian Monterey Formation [128] and can be considered late Middle Miocene in age; thus 13.8-11.6 Ma. Further fossils assigned to Stephanoberycoidei have been reported from Upper Miocene deposits of California (USA) [129] and Italy [130]. Substantially older than †*Miobarbourisia aomori* is a ‘*Rondeletia*-like cetomimiform’ from the Fur Formation of Jutland (Denmark) [131]. This ‘cetomimiform’ fossil has never been formally described but a preliminary examination by Friedman [124] showed

similarities with some stephanoberycoids. The Danish Fur Formation is considered Ypresian in age [131] and thus dates to 56.0-47.8 Ma. We here consider both †*Miobarbourisia aomori* and the fossil from the Danish Fur Formation as equally probable first occurrences of Stephanoberycoidae (weights: 0.5/0.5). The suborder is represented in our phylogenomic data set by *Acanthochaenus luetkenii*.

**Holocentriformes:** †*Stichocentrus liratus* Patterson, 1967, †*Stichocentrus elegans* Gaudant, 1969, †*Stichocentrus spinulosus* Gayet, 1980 (100.5-98.0 Ma).

Of the earliest members of Acanthopterygii from Hajula, those assigned to genus †*Stichocentrus* are generally considered holocentroids (and therefore holocentriforms) [8, 85, 124, 132] and have repeatedly been accepted as the earliest records of the group [8, 91]. These fossils date to 100.5-98.0 Ma (see above). Our phylogenomic data set includes the two holocentriforms *Myripristis jacobus* and *Holocentrus rufus*.

**Percomorphaceae:** †*Cretatriacanthus guidottii* Tyler and Sorbini, 1996 (89.8-83.0 Ma), or †*Plectocretacicus clarae* Sorbini, 1979 (100.5-98.0 Ma).

Cretaceous armoured acanthomorphs of the extinct superfamily Plectocretacicoidea have frequently been considered the earliest records of tetraodontiform fishes [2, 16, 17, 133, 134], a placement that, if correct, would also make them the earliest percomorphs. Four species of plectocretacicoidea are currently known. The oldest of these is †*Plectocretacicus clarae* from the limestone of Haqil for which an age of 100.3-98.0 Ma can be given due to the cooccurrence of *Mantelliceras mantelli* [28]. While the monophyly of Plectocretacicoidea and their position as a stem group of Tetraodontiformes has been supported by phylogenetic analyses [2, 17], both have become increasingly questioned in recent years, following a preliminary reexamination of the fossil material [4] and the discovery of similar armoured acanthomorphs that were placed outside crown-group of percomorphs [86]. The phylogenetic position of the older plectocretacicoidea is therefore currently unclear, with some authors accepting the tetraodontiform affiliation [10, 17] and others arguing that at least †*Plectocretacicus clarae* and †*Protriacanthus gortanii* should not be considered part of Tetraodontiformes [3–6, 8, 115]. In the latter case, the earliest record of Tetraodontiformes would be †*Cretatriacanthus guidottii* from the Calcarei di Melissano at Canale near Nardò (Italy), exhibiting more tetraodontiform synapomorphies than the other plectocretacicoidea [3, 5, 6, 115, 135]. †*Cretatriacanthus guidottii* thus has the same age as the zeiform †*Cretazeus rinaldii*; 89.8-83.0 Ma (see above). Following the group of authors questioning the tetraodontiform placement of †*Plectocretacicus clarae*, we here consider †*Cretatriacanthus guidottii* as a more probable first occurrence of Tetraodontiformes. However, after accounting for a possibility that †*Plectocretacicus clarae* is not a tetraodontiform but still a percomorph, we consider both taxa as equally probable first occurrences of Percomorphaceae (weights: 0.5/0.5). Our phylogenomic data set includes 61 representatives of Percomorphaceae.

**Ophidiaria:** †*Pastorius methenyi* Carnevale and Johnson, 2015 (76.4-69.8 Ma).

†*Pastorius methenyi* from the Liburnica Formation near Trebiciano (Italy) has recently been described as the oldest member of Ophidiiformes [136, 137], taking that title from the Eocene †*Eolamprologus senectus*. The fossil-bearing layers at Trebiciano have a Late Campanian or Early Maastrichtian age [136, 138] and thus date to 76.4-69.8 Ma. With *Brotula barbata* and *Carapus acus*, two members of

Ophidiaria are included in our phylogenomic data set.

**Batrachoidaria:** †*Louckaichthys novosadi* Přikryl and Carnevale, 2017 (32.0-29.6 Ma).

The fossil record of Batrachoidiformes has recently been extended to the Oligocene, with the description of †*Louckaichthys novosadi* from the Menelitic Formation at the Loučka locality near Valašské Meziříčí (Czech Republic) [139] that now represents the first occurrence of the order. The Dynów Member of the Menelitic Formation, from which the fossil was collected, is classified into the Rupelian nannoplankton zone NP23 [140], and thus dates to 32.0-29.6 Ma. Our phylogenomic data set includes a single representative of Batrachoidaria, *Chatrabus melanurus*.

**Gobiaria:** †*Carlomonnius quasigobius* Bannikov and Carnevale, 2016 (49.2-48.9 Ma).

The first gobiiform specimen was recently identified from the Calcarei nummulitici at Monte Bolca near Verona (Italy) and described as †*Carlomonnius quasigobius* [141]. The age of the fossil-bearing layers at Monte Bolca can be classified into nannoplankton zone NP14 based on the presence of *Discoaster subloboensis* [3, 142]. Larger foraminiferal assemblages of *Alveolina cremae*, *A. rugosa*, *A. distefanoi*, and *A. rutimeyeri* further allow the constraint to the Shallow Benthic Zone SBZ11 [143]. The top of SBZ11 (48.9 Ma) is just above the base of NP14 (49.2 Ma); thus, the age of the Monte Bolca deposits dates to 49.2-48.9 Ma. Gobiaria are represented in our phylogenomic data set by *Lesueurigobius* cf. *sanzoii* and *Periophthalmodon schlosseri*.

**Hippocampus:** *Hippocampus* †*sarmaticus* and *Hippocampus* †*slovenicus* Žalohar, Hitij, and Križnar, 2009 (12.2-11.3 Ma).

The genus *Hippocampus* first appears in the Miocene with the two species *Hippocampus* †*sarmaticus* and *H.* †*slovenicus* from the Coprolitic Horizon at the Tunjice Hills (Slovenia) [144, 145]. Based on cooccurring diatomaceous microflora, the Coprolitic Horizon can be assigned to the Lower Sarmatian Central Paratethys age which can be constrained to 12.2-11.3 Ma [146, 147]. The genus *Hippocampus* is represented in our phylogenomic data set by *Hippocampus comes*. The genus *Syngnathus* is more closely related to other genera than to *Hippocampus* [145]; therefore, we don't constrain the age of *Syngnathus* directly.

**Tetraodontiformes:** †*Cretatriacanthus guidottii* Tyler and Sorbini, 1996 (89.8-83.0 Ma), or †*Plectocretacicus clarae* Sorbini, 1979 (100.5-98.0 Ma).

We here consider †*Cretatriacanthus guidottii*, with an age of 89.8-83.0 Ma, as a more probable first record of Tetraodontiformes than the older †*Plectocretacicus clarae* (weights: 0.67/0.33) (see above). Tetraodontiformes are represented in our phylogenomic data set by three species.

**Lophiiformes:** †*Eophryne barbutii* Carnevale and Pietsch, 2009, †*Sharfia mirabilis* Pietsch and Carnevale, 2011, †*Tarkus squirei* Carnevale and Pietsch, 2011, and †*Caruso brachysomus* Carnevale and Pietsch, 2012 (49.2-48.9 Ma).

A number of lophiiform families appear simultaneously in the fossil record of the Eocene, with †*Eophryne barbutii* [148], †*Sharfia mirabilis* [149], †*Tarkus squirei* [150], and †*Caruso brachysomus* [151] all occurring in the Calcarei nummulitici at Monte Bolca (Italy). The first occurrence of Lophiiformes therefore dates to 49.2-48.9 Ma (see above). Our phylogenomic data set includes a single representative of Lophiiformes, *Antennarius striatus*.

**Morone:** †*Morone aequalis* (Weiler, 1942) (20.4-16.0 Ma).

The genus *Morone* is represented in the fossil record by †*Morone aequalis* from the Frankfurt Formation (former Upper Hydrobia Beds) at Frankfurt (Germany) [152]. The layer producing the fossil is part of the lowermost Frankfurt Formation [153]. The Frankfurt Formation is considered Burdigalian in age [154] and thus dates to 20.4-16.0 Ma. The genus *Morone* is represented in our phylogenomic data set by *Morone saxatilis*.

**Channoidei:** †*Eochanna chorlakkensis* Roe, 1991, and †*Anchichanna kuldansensis* Murray and Thewissen, 2008 (48.9-37.8 Ma).

The earliest records of Channoidei are provided by two species of channids from the Kuldana Formation in the Kohat District of Pakistan: †*Eochanna chorlakkensis* [155] was collected near Chorlakk and †*Anchichanna kuldansensis* [156] was found in the Ganda Kas area of the Kala Chitta Hills; the two sites are about 30 km apart. The Kuldana Formation is reported as early to middle Eocene in age [155, 156] but no older than SBZ12 [157]. Thus, the earliest record of Channoidei dates to 48.9-37.8 Ma. In our phylogenomic data set, Channoidei are represented by *Channa argus*.

**Anabantoidei:** *Osphronemus goramy* Lacepède, 1801, and †*Ombilinichthys yamini* Murray, Zaim, Rizal, Aswan, Gunnell, and Ciochon, 2015 (37.8-23.0 Ma)

The only two fossils known of Anabantoidei have both been reported from the Sangkarewang Formation in the Ombilin Basin (West Sumatra, Indonesia) and have been assigned to the extant species *Osphronemus goramy* and the extinct †*Ombilinichthys yamini* [158, 159]. The age of the Sangkarewang Formation is very poorly known and various authors have considered it Cretaceous, Paleogene, or Miocene. Reviewing the available evidence, Murray et al.[159] concluded that “a late Eocene or Oligocene age is not unreasonable”. Following this assessment, we here assume an age of 37.8-23.0 Ma for fossils from the Sangkarewang Formation. Anabantoidei are represented in our phylogenomic data set by *Anabas testudineus* and *Helostoma temminckii*.

**Aplocheiloidei:** †*Kenyaichthys kipkechi* Altner and Reichenbacher, 2015 (6.0-5.7 Ma).

The only known fossil record of Aplocheiloidei is the recently described †*Kenyaichthys kipkechi* from the Lukeino Formation in the Tugen Hills (Central Rift Valley, Kenya) [160]. The Lukeino Formation is late Miocene in age and has been constrained to 6.0-5.7 Ma [161, 162]. Our phylogenomic data set includes two representatives of Aplocheiloidei; *Kryptolebias marmoratus* and *Nothobranchius furzeri*.

**Cyprinodontoidei:** †*Prolebias stenoura* Sauvage, 1874, †*Francolebias aymardi* (Sauvage, 1869), and †*Francolebias delphinensis* (Gaudant, 1989) (33.9-28.1 Ma), or Poeciliidae *indet.* (56.0-47.8 Ma). Undescribed poeciliid fossils have been reported from the Maíz Gordo Formation near Salta (Argentina) [163]. The Maíz Gordo Formation can be assigned to the Riochican or the early Casamayoran South American Land Mammal Ages (SALMA), suggesting an Ypresian age [164, 165]. However, the taxonomic assignment of these undescribed fossils is questionable. No poeciliid fossils were included in Cione and Báez’[166] list of paleocene fishes from Argentina, and according to López-Fernández and Albert[167], the only confirmed fossils of poeciliids in Argentina are of middle-late Miocene age. No other Eocene fossils are known of Cyprinodontoidei. The earliest records of

Cyprinodontoidei outside of South America are †*Prolebias stenoura* from the deposits at Coirent (Puy-de-Dôme, France), †*Francolebias aymardi* from the Calcaires marneux of Ronzon (Haute-Loire, France) and †*Francolebias delphinensis* from sediments near Montbrun-les Bains (Drôme, France) [160, 168–170], all of which have a lower Oligocene age. Thus, the first occurrence of Cyprinodontoidei dates to 56.0–47.8 Ma if the taxonomic assignment of Poecilidae *indet.* is correct, or to 33.9–28.1 Ma if it is not. We assume equal probabilities for both ages (weights: 0.5/0.5). Our phylogenomic data set includes four representatives of Cyprinodontoidei.

**Cichlinae:** *Gymnogeophagus* †*eocenicus* Malabarba, Malabarba, and del Papa, 2010, †*Plesioheros chauliodus* Alano Perez, Malabarba, and del Papa, 2010, and †*Proterocara argentina* Malabarba, Zuleta, and del Papa, 2006 (45.0–40.0 Ma).

*Gymnogeophagus* †*eocenicus*, †*Plesioheros chauliodus*, and †*Proterocara argentina* from the Lumbrera Formation of the Salta Province (Argentina) have been placed within the crown of Cichlinae [171–173]. The Lumbrera Formation has traditionally been assigned to the Casamayoran (SALMA) [174] and direct dating of a tuff layer near the top of the formation provides a minimum age of 39.9 Ma [174]. The age of fossils can therefore be assumed to be 45.0–40.0 Ma [175]. Our phylogenomic data set includes seven species representative of Cichlinae.

**Heroini:** †*Plesioheros chauliodus* Alano Perez, Malabarba, and del Papa, 2010 (45.0–40.0 Ma). Of the fossils known from the Lumbrera Formation, †*Plesioheros chauliodus* can be assigned to the tribe Heroini [172]; thus, this earliest occurrence of this tribe dates to 45.0–40.0 Ma. Our phylogenomic data set includes three representatives of Heroini. Note that we do not constrain the age of Geophagini because their probable sister lineage, the tribe Chaetobranchini (possible together with Astronotini) [18, 171, 176, 177], is not included in the data set.

**Cichlasomatini:** †*Tremembichthys garciae* Malabarba and Malabarba, 2008 (35.0–30.0 Ma) Of three fossils that have been assigned to Cichlasomatini, the oldest is †*Tremembichthys garciae* from the Entre-Córregos Formation near Aiuruoca (Minas Gerais, Brazil) [178, 179]. Palynomorphs at the base of the Entre-Córregos Formation indicate an age near the Eocene-Oligocene boundary, around 35.0–30.0 Ma [180, 181]. Cichlasomatini are represented in our phylogenomic data set by three species.

**Pseudocrenilabrinae:** †*Mahengechromis* spp. Murray, 2000 (46.0–45.0 Ma). The earliest record of Pseudocrenilabrinae is provided by specimens from the Mahenge paleolake (Tanzania) assigned to genus †*Mahengechromis* [182]. U/Pb isotopic analyses of Mahenge zircon constrain the age of the paleolake sediments to 46.0–45.0 Ma [175, 183]. Our phylogenomic data set includes eight representatives of Pseudocrenilabrinae.

**Heterochromini:** ?*Heterochromis* sp. (33.9–16.0 Ma). Three fossil specimens from the Ad Darb Formation (Tihamat Asir, Saudi Arabia) have been considered closely related to genus *Heterochromis* on the basis of predorsal bone count and scale structure [184, 185]. The Ad Darb Formation is assumed to be either Oligocene [184] or early Miocene [186] in age and therefore dates to 33.9–16.0 Ma. Our phylogenomic data set includes the single extant species of Heterochromini, *Heterochromis multidentis*.

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**Unnamed clade:** †*Mahengechromis* spp. Murray, 2000 (46.0-45.0 Ma).

Even though the clade comprising all tribes of African cichlids except Heterochromini and Tylochromini has not been named, it is strongly supported by phylogenetic analyses [18, 187, 188] and morphologically recognizable by having only a single predorsal bone [189]; therefore, the clade can (and should be [18]) used for time calibration with the CladeAge approach. A position of †*Mahengechromis* within this clade is supported by its single predorsal bone [185, 190, 191]. The unnamed clade is represented in our phylogenomic data set by six species.

**Haplotilapiini sensu Schlieven and Stiassny, 2003:** *Oreochromis* †*kabchorensis* Penk et al., 2019 (12.5-12.0 Ma).

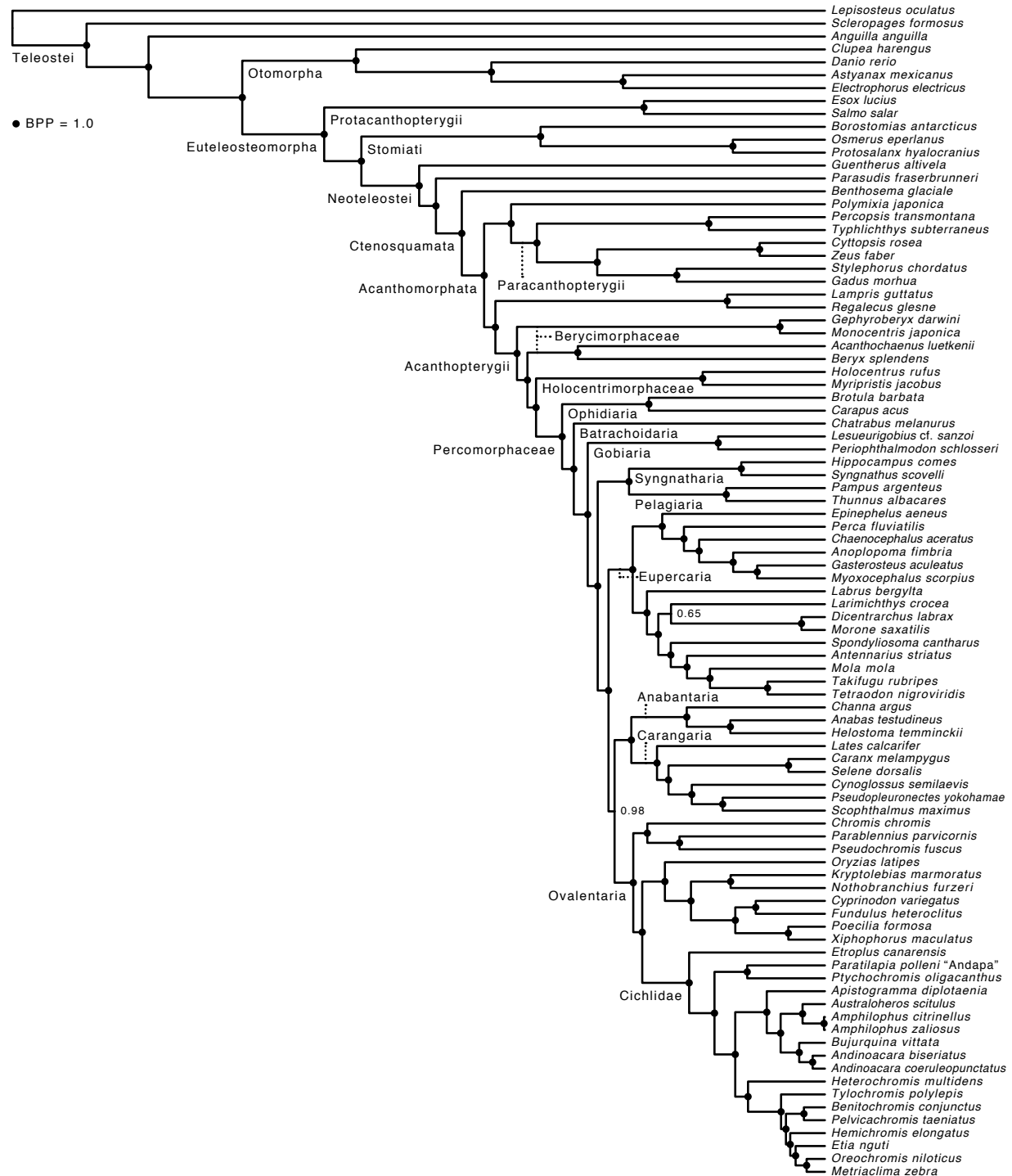
Phylogenetic analyses strongly support the monophyly of Haplotilapiini as well as a sister-group relationship with either Hemichromini or a clade combining Hemichromini and Pelmatochromini [18, 187, 188, 192]. Thus, the sister group of Haplotilapiini is included in our data set. Haplotilapiini are morphologically recognizable by having tricuspid teeth in the inner row of their dentition [193]. The oldest known record of Haplotilapiini is provided by specimens from Ngorora fish Lagerstätte at Kabchore (Tugen Hills, Central Kenya) [194]. The Kabchore locality has been assigned to Member C of the Ngorora Formation with an age of 12.5-12.0 Ma [195]. Our phylogenomic data set includes three representatives of Haplotilapiini.

**Oreochromini:** *Oreochromis* †*kabchorensis* Penk et al., 2019 (12.5-12.0 Ma).

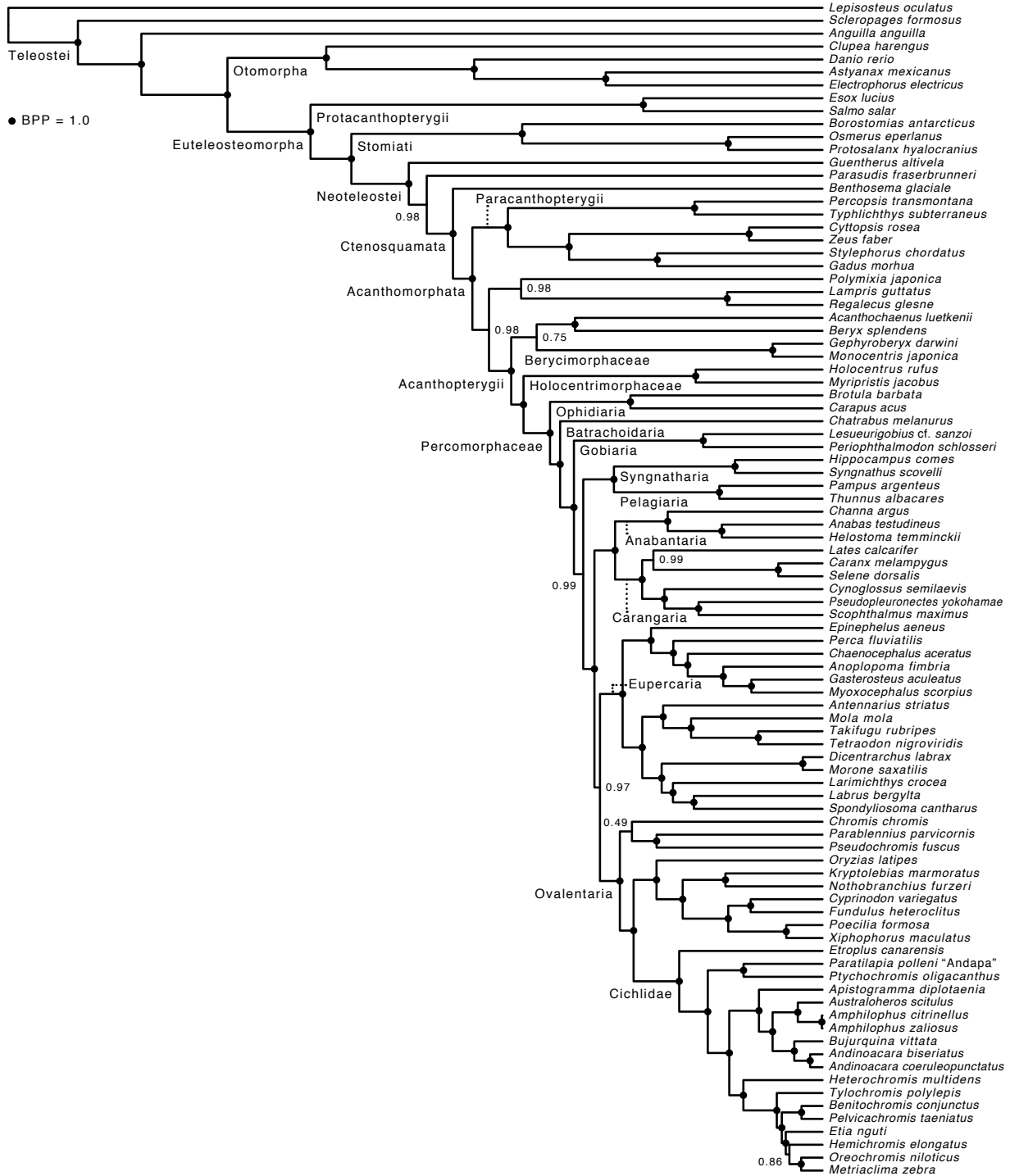
Given that the earliest records of Haplotilapiini have been placed in genus *Oreochromis* (see above), they also provide a minimum age for Oreochromini of 12.5-12.0 Ma. Oreochromini are represented in our phylogenomic data set by *Oreochromis niloticus*.

## Supplementary Figures

**Supplementary Figure 1:** Species tree inferred with the concatenated “permissive” set of gene alignments. Node values indicate Bayesian posterior probabilities (BPP) estimated with BEAST 2.

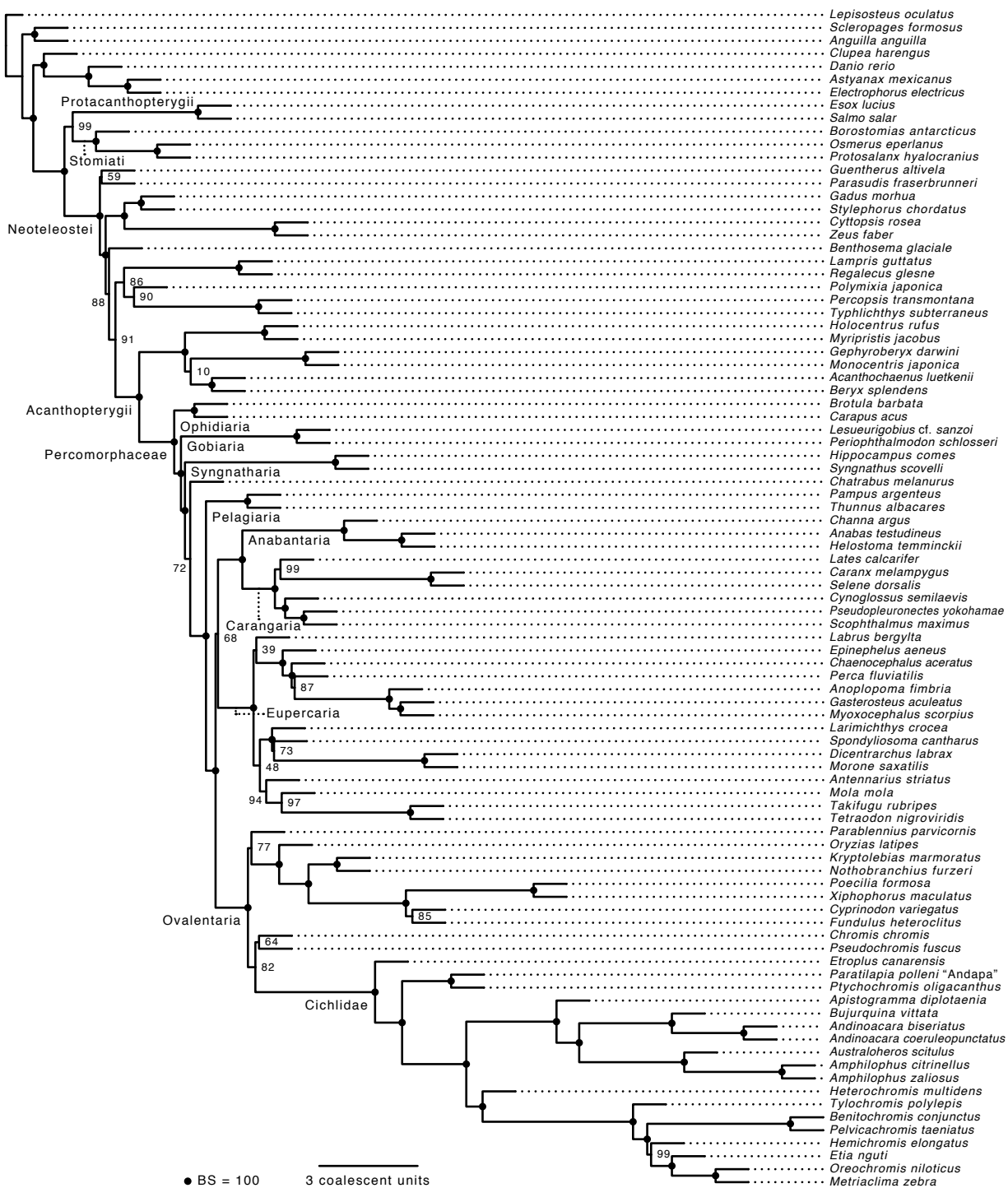


**Supplementary Figure 2:** Species tree inferred with the concatenated “strict” set of gene alignments. Node values indicate Bayesian posterior probabilities (BPP) estimated with BEAST 2.

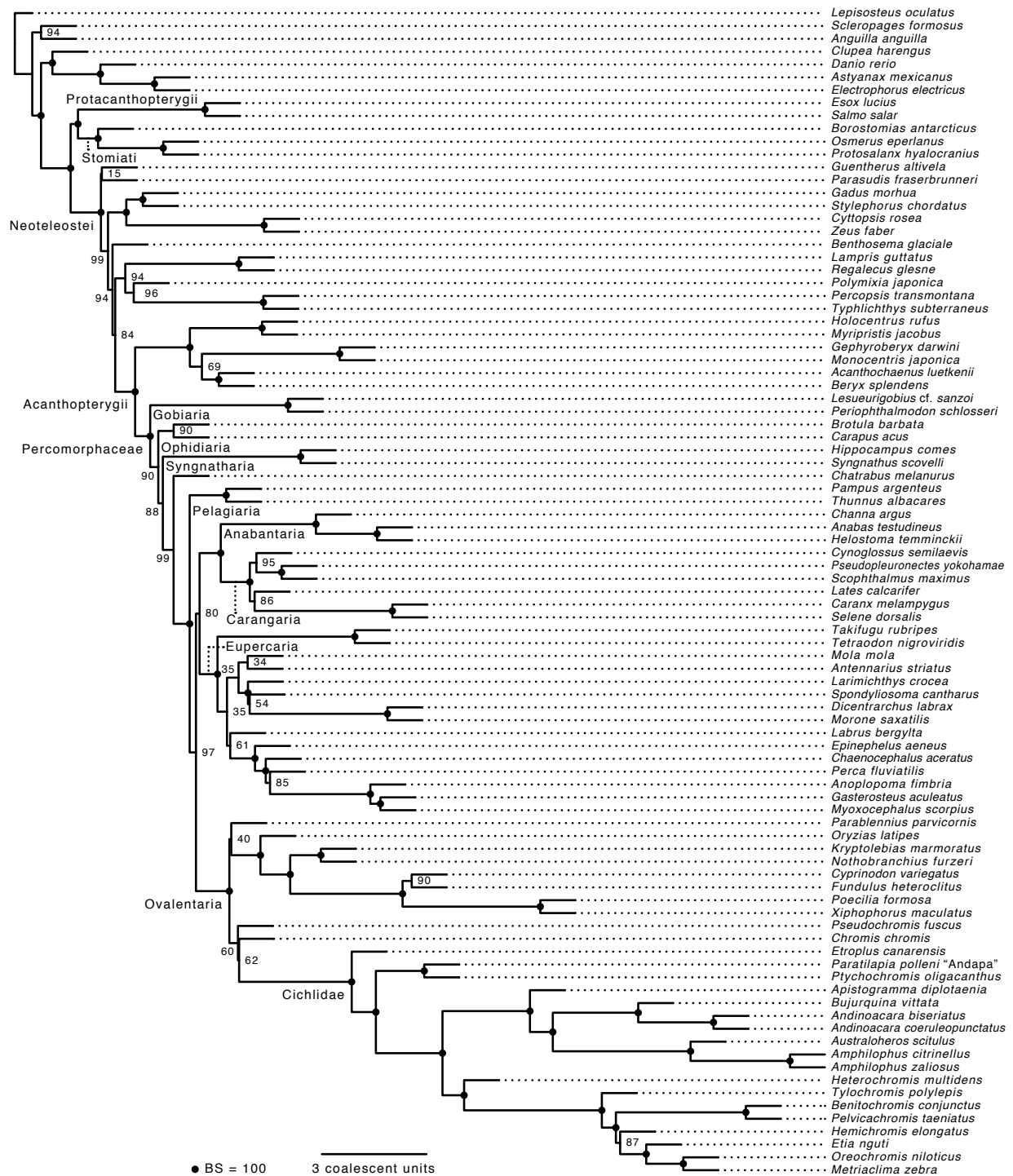




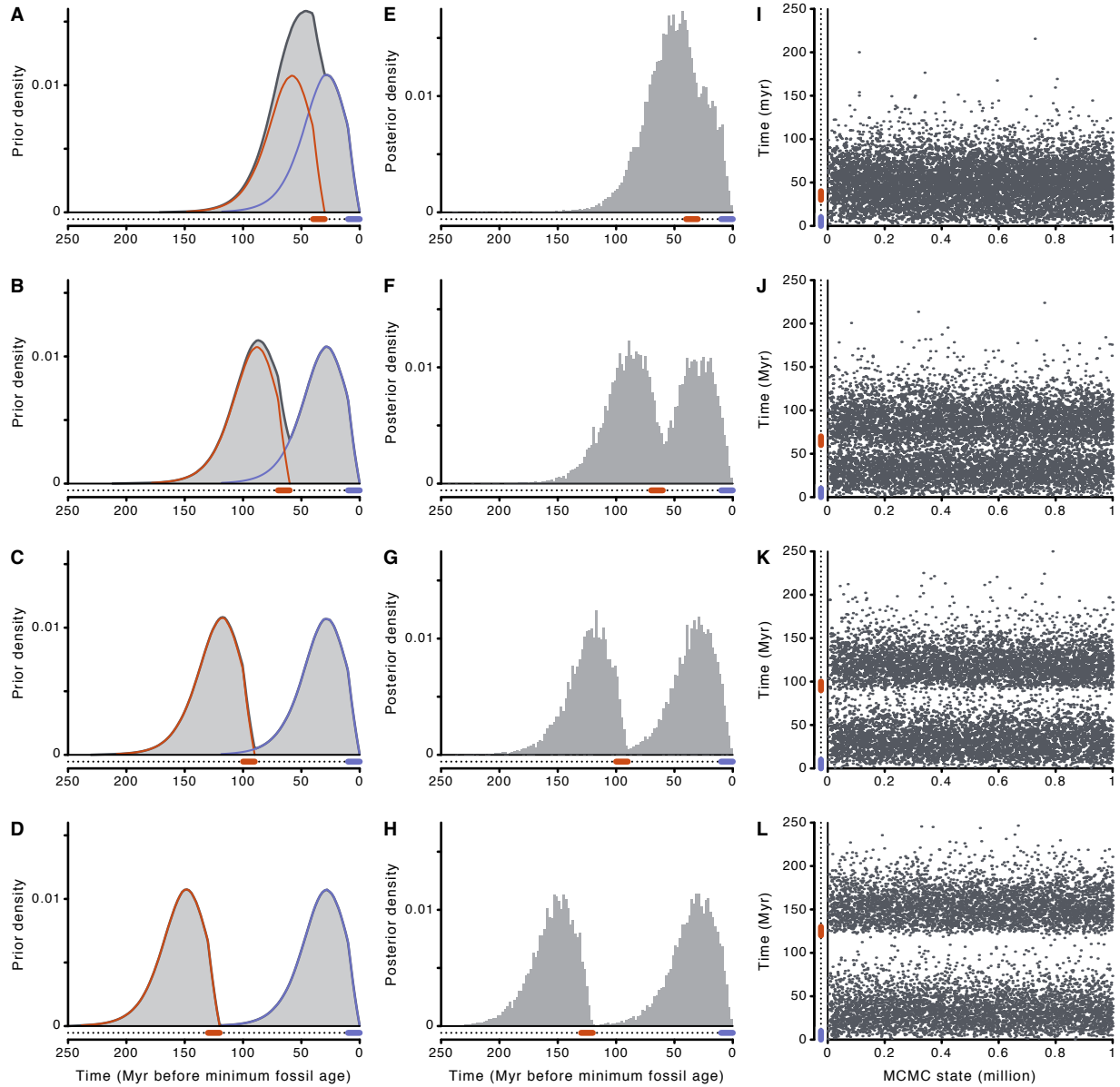
**Supplementary Figure 3:** Species tree inferred with the multi-species coalescent model and the “permissive” set of genes. Node values indicate Bootstrap support (BS) estimated with ASTRAL.



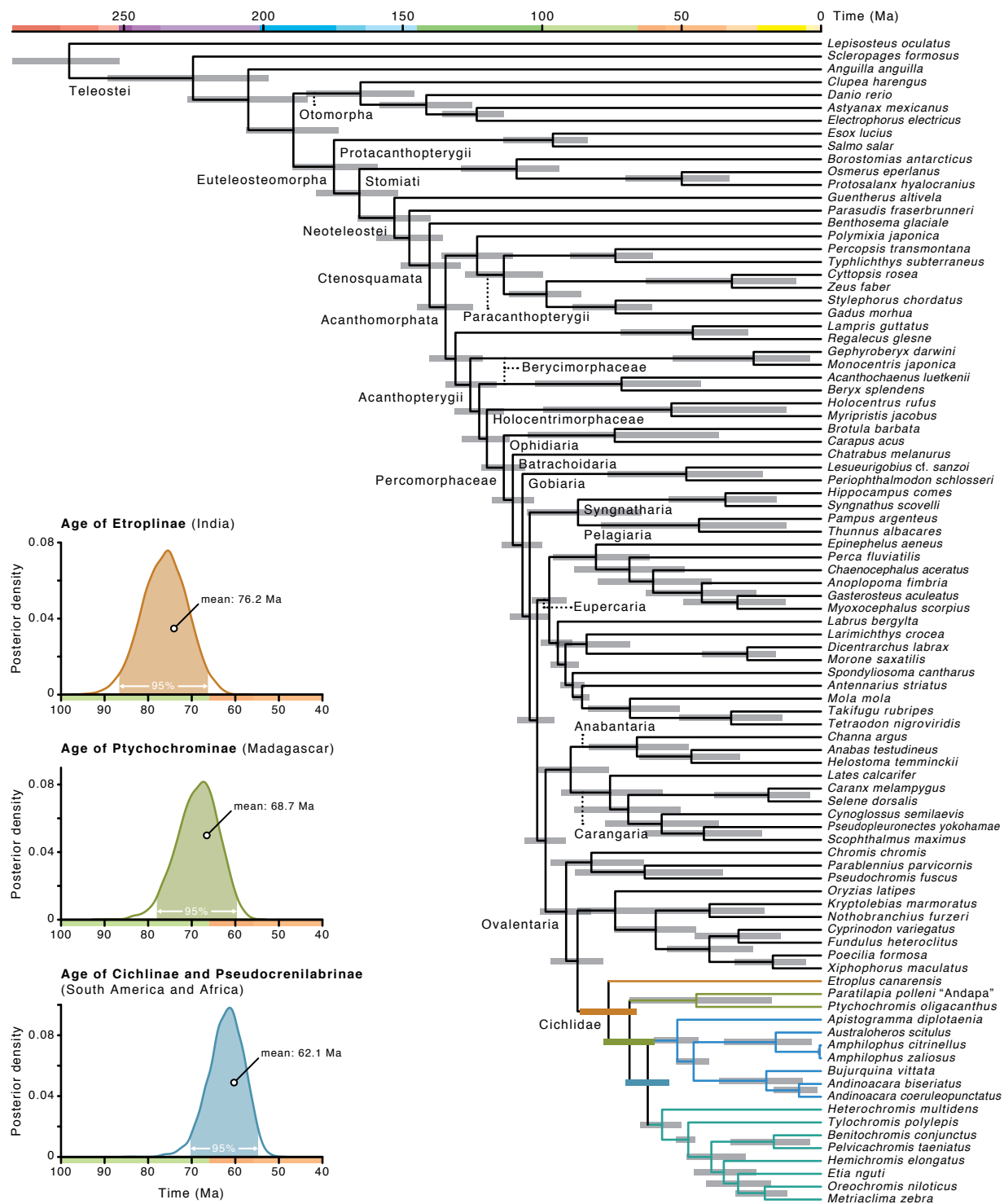
**Supplementary Figure 4:** Species tree inferred with the multi-species coalescent model and the “strict” set of genes. Node values indicate Bootstrap support (BS) estimated with ASTRAL.



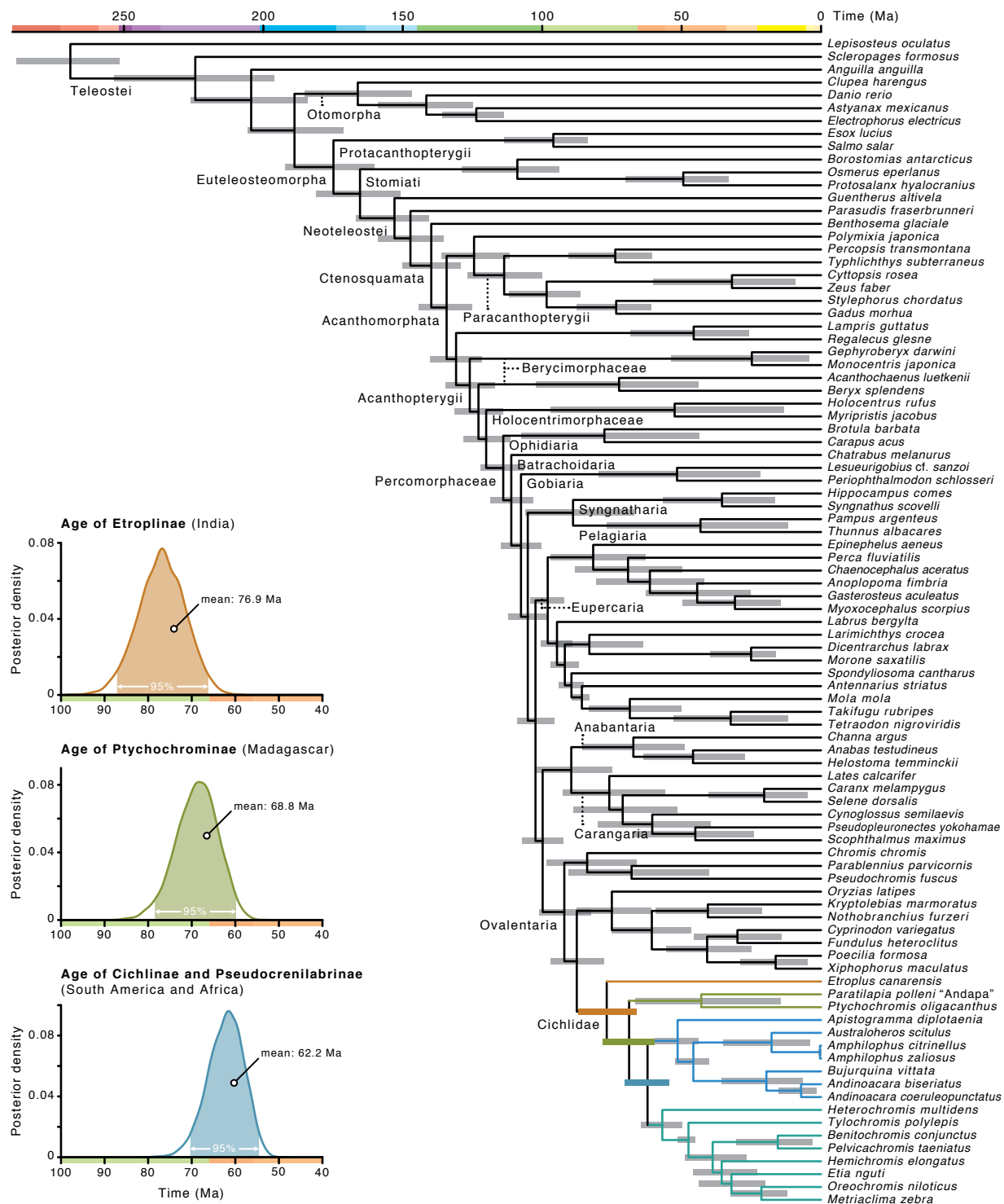
**Supplementary Figure 5:** Convergence tests with two possible first occurrences of a clade. The ages of two simulated fossils (each with an uncertainty of 10 Myr) are shown in purple (younger fossil) and orange (older fossil), with temporal distances between the fossils of 30 (A, E, I), 60 (B, F, J), 90 (C, G, K), and 120 Myr (D, H, L). The calibration densities  $f_{\text{younger}}(t)$  (purple),  $f_{\text{older}}(t)$  (orange), and  $f(t)$  (grey) are shown in A-D, corresponding posterior densities are shown in E-H, and the MCMC traces for the age of the calibrated node, sampled in every 100th iteration, are shown in I-L.



**Supplementary Figure 6:** Divergence times inferred with the “permissive” set of genes. Grey node bars indicate 95% highest-posterior density (HPD) intervals for node ages.



**Supplementary Figure 7:** Divergence times inferred with the “strict” set of genes. Grey node bars indicate 95% HPD intervals for node ages.



## Supplementary Tables

### Supplementary Table 1: Cichlid species sampled for whole-genome sequencing.

Samples with source information “Own collection” were obtained by members of the Salzburger lab during field trips to Cameroon and Zambia.

Species	Voucher	Source	Country of origin	Coverage
<i>Etroplus canarensis</i>	JWD4	Aquarium trade	India	19.01
<i>Paratilapia polleni</i> “Andapa”	JWD5	Zoo Zurich	Madagascar	23.12
<i>Ptychochromis oligacanthus</i>	FPO1	Zoo Brussels	Madagascar	7.25
<i>Apistogramma diploaenia</i>	JWD9	Z. Musilová	Venezuela	8.23
<i>Australoheros scitulus</i>	JWE2	Aquarium trade	Uruguay	10.48
<i>Amphilophus zaliosus</i>	JWE1	M. Barluenga	Nicaragua	8.34
<i>Bujurquina vittata</i>	JWD8	Z. Musilová	Paraguay	9.17
<i>Andinoacara biseriatus</i>	JWD7	Z. Musilová	Ecuador	8.98
<i>Heterochromis multidentis</i>	JWF1	Own collection	Cameroon	22.25
<i>Tylochromis polylepis</i>	JAH5	Own collection	Zambia	17.53
<i>Benitochromis conjunctus</i>	JWE4	Own collection	Cameroon	9.20
<i>Pelvicachromis taeniatus</i>	JWI4	Own collection	Cameroon	9.51
<i>Hemichromis elongatus</i>	JWE9	Own collection	Cameroon	23.07
<i>Etia nguti</i>	JWD3	Own collection	Cameroon	17.70

### Supplementary Table 2: Whole-genome assemblies generated for this study.

The results of the BUSCO analysis are given in the order complete (c), complete and single copy (c+s), complete and duplicated (c+d), fragmented (f), missing (m). The BUSCO test library contained a total of 4,584 conserved actinopterygian genes.

Species	Assembly size	N50	— BUSCOs (c/c+s/c+d/f/m) —						Accession
<i>Etroplus canarensis</i>	975,424,675	18,345	3,338	3,258	80	714	532	SAMN12123891	
<i>Paratilapia polleni</i> “Andapa”	814,091,157	21,259	3,549	3,477	72	636	399	SAMN12123892	
<i>Ptychochromis oligacanthus</i>	707,176,575	8,314	2,499	2,464	35	1,185	900	SAMN12123633	
<i>Apistogramma diploaenia</i>	627,325,156	25,146	3,875	3,803	72	421	288	SAMN12123895	
<i>Australoheros scitulus</i>	709,595,499	10,904	2,987	2,927	60	911	686	SAMN12123897	
<i>Amphilophus zaliosus</i>	692,081,349	9,617	2,611	2,563	48	1,149	824	SAMN12123896	
<i>Bujurquina vittata</i>	686,045,042	28,150	3,790	3,703	87	525	269	SAMN12123894	
<i>Andinoacara biseriatus</i>	682,812,987	25,636	3,752	3,677	75	478	354	SAMN12123893	
<i>Heterochromis multidentis</i>	833,453,564	17,755	3,285	3,212	73	794	505	SAMN12123905	
<i>Tylochromis polylepis</i>	744,326,816	18,818	3,528	3,455	73	634	422	SAMN12123821	
<i>Benitochromis conjunctus</i>	665,812,357	27,758	3,722	3,636	86	520	342	SAMN12123899	
<i>Pelvicachromis taeniatus</i>	693,375,608	26,667	3,812	3,731	81	464	308	SAMN12123930	
<i>Hemichromis elongatus</i>	694,371,948	37,079	4,056	3,947	109	302	226	SAMN12123904	
<i>Etia nguti</i>	737,212,856	33,462	3,968	3,866	102	372	244	SAMN12123890	

**Supplementary Table 3:** Teleost species used for marker selection.

The 15 species listed here were selected out of 42 teleost species included in release 94 of the Ensembl database to represent diverse teleost lineages with a focus on cichlids and their closer relatives. Exon-sequence similarity was quantified between zebrafish (*Danio rerio*) and all other species.

Species	Family	Order	Group
<i>Danio rerio</i>	Danionidae	Cypriniformes	Section Otophysa
<i>Esox lucius</i>	Esocidae	Esociformes	Subcohort Protacanthopterygii
<i>Gadus morhua</i>	Gadidae	Gadiformes	Subseries Gadariae
<i>Periophthalmus magnuspinnatus</i>	Gobiidae	Gobiiformes	Series Gobiaria
<i>Hippocampus comes</i>	Syngnathidae	Syngnathiformes	Series Syngnatharia
<i>Takifugu rubripes</i>	Tetraodontidae	Tetraodontiformes	Series Eupercaria
<i>Labrus bergylta</i>	Labridae	Labriformes	Series Eupercaria
<i>Gasterosteus aculeatus</i>	Gasterosteidae	Perciformes	Series Eupercaria
<i>Scophthalmus maximus</i>	Scophthalmidae	Pleuronectiformes	Series Carangaria
<i>Amphiprion percula</i>	Pomacentridae	incertae sedis	Series Ovalentaria
<i>Oryzias latipes</i>	Adrianichthyidae	Beloniformes	Series Ovalentaria
<i>Xiphophorus maculatus</i>	Poeciliidae	Cyprinodontiformes	Series Ovalentaria
<i>Amphilophus citrinellus</i>	Cichlidae	Cichliformes	Series Ovalentaria
<i>Oreochromis niloticus</i>	Cichlidae	Cichliformes	Series Ovalentaria
<i>Metriaclima zebra</i>	Cichlidae	Cichliformes	Series Ovalentaria

**Supplementary Table 4:** Assembly files used for marker selection.

Assembly files for the 15 species (see Supplementary Table 3) listed here are part of Ensembl databank release 94.

Species	Assembly file
<i>Danio rerio</i>	Danio_rerio.GRCz11.dna.toplevel.fa.gz
<i>Esox lucius</i>	Esox_lucius.Eluc_V3.cdna.all.fa.gz
<i>Gadus morhua</i>	Gadus_morhua.gadMor1.cdna.all.fa.gz
<i>Periophthalmus magnuspinnatus</i>	Periophthalmus_magnuspinnatus.PM.fa.cdna.all.fa.gz
<i>Hippocampus comes</i>	Hippocampus_comes.H.comes_QL1_v1.cdna.all.fa.gz
<i>Takifugu rubripes</i>	Takifugu_rubripes.FUGU5.cdna.all.fa.gz
<i>Labrus bergylta</i>	Labrus_bergylta.BallGen_V1.cdna.all.fa.gz
<i>Gasterosteus aculeatus</i>	Gasterosteus_aculeatus.BROADS1.cdna.all.fa.gz
<i>Scophthalmus maximus</i>	Scophthalmus_maximus.ASM318616v1.cdna.all.fa.gz
<i>Amphiprion percula</i>	Amphiprion_percula.Nemo_v1.cdna.all.fa.gz
<i>Oryzias latipes</i>	Oryzias_latipes_hsok.ASM223469v1.cdna.all.fa.gz
<i>Xiphophorus maculatus</i>	Xiphophorus_maculatus.X_maculatus-5.0-male.cdna.all.fa.gz
<i>Amphilophus citrinellus</i>	Amphilophus_citrinellus.Midas_v5.cdna.all.fa.gz
<i>Oreochromis niloticus</i>	Oreochromis_niloticus.Orenil1.0.cdna.all.fa.gz
<i>Metriaclima zebra</i>	Maylandia_zebra.M_zebra_UMD2a.cdna.all.fa.gz

**Supplementary Table 5:** Targeted assemblies generated for this study.

Listed below are the percentages of exon sequences that could be assembled, relative to the most successful assembly. Targeted assembly with aTRAM was not performed for the three species that had the highest assembly success rates with Kollector.

Species	Kollector	aTRAM
<i>Etroplus canarensis</i>	2.7%	82.7%
<i>Paratilapia polleni</i> “Andapa”	11.4%	70.2%
<i>Ptychochromis oligacanthus</i>	4.3%	97.4%
<i>Apistogramma diplotaenia</i>	4.4%	100.0%
<i>Australoheros scitulus</i>	9.3%	84.9%
<i>Amphilophus zaliosus</i>	6.8%	89.8%
<i>Bujurquina vittata</i>	6.7%	92.6%
<i>Andinoacara biseriatus</i>	6.8%	90.4%
<i>Heterochromis multidentis</i>	21.1%	78.3%
<i>Tylochromis polylepis</i>	71.1%	52.6%
<i>Benitochromis conjunctus</i>	64.0%	87.8%
<i>Pelvicachromis taeniatus</i>	77.4%	-
<i>Hemichromis elongatus</i>	100.0%	-
<i>Etia nguti</i>	76.2%	-



**Supplementary Table 6:** Teleost species used in phylogenetic analyses.

Species	Family	Order	Group
<i>Lepisosteus oculatus</i>	Lepisosteidae	Lepisosteiformes	Infraclass Holostei
<i>Scleropages formosus</i>	Osteoglossidae	Osteoglossiformes	Supercohort Osteoglossomorpha
<i>Anguilla anguilla</i>	Anguillidae	Anguilliformes	Cohort Elopomorpha
<i>Clupea harengus</i>	Clupeidae	Clupeiformes	Cohort Otomorpha
<i>Danio rerio</i>	Danionidae	Cypriniformes	Cohort Otomorpha
<i>Astyanax mexicanus</i>	Characidae	Characiformes	Cohort Otomorpha
<i>Electrophorus electricus</i>	Gymnotidae	Gymnotiformes	Cohort Otomorpha
<i>Esox lucius</i>	Esocidae	Esociformes	Subcohort Protacanthopterygii
<i>Salmo salar</i>	Salmonidae	Salmoniformes	Subcohort Protacanthopterygii
<i>Borostomias antarcticus</i>	Stomiidae	Stomiiformes	Subcohort Stomiati
<i>Osmerus eperlanus</i>	Osmeridae	Osmeriformes	Subcohort Stomiati
<i>Protosalanax hyalocranium</i>	Salangidae	Osmeriformes	Subcohort Stomiati
<i>Guentherus altivela</i>	Atelepodidae	Atelepodiformes	Infracohort Ateleopodia
<i>Parasudis fraserbrunneri</i>	Chlorophthalmidae	Aulopiformes	Section Cycloquamata
<i>Benthoosema glaciale</i>	Myctophidae	Myctophiformes	Subsection Myctophata
<i>Polymixia japonica</i>	Polymixiidae	Polymixiiformes	Division Polymixiipterygii
<i>Percopsis transmontana</i>	Percopsidae	Percopsiformes	Division Paracanthopterygii
<i>Typhlichthys subterraneus</i>	Amblyopsidae	Percopsiformes	Division Paracanthopterygii
<i>Cyttopsis rosea</i>	Parazenidae	Zeiformes	Division Paracanthopterygii
<i>Zeus faber</i>	Zeidae	Zeiformes	Division Paracanthopterygii
<i>Stylephorus chordatus</i>	Stylephoridae	Stylephoriformes	Division Paracanthopterygii
<i>Gadus morhua</i>	Gadidae	Gadiformes	Division Paracanthopterygii
<i>Lampris guttatus</i>	Lampridae	Lampriformes	Division Lampripterygii
<i>Regalecus glesne</i>	Regalecidae	Lampriformes	Division Lampripterygii
<i>Gephyroberyx darwini</i>	Trachichthyidae	Trachichthyiformes	Subdivision Berycimorphaceae
<i>Monocentris japonica</i>	Monocentridae	Trachichthyiformes	Subdivision Berycimorphaceae
<i>Acanthochaenus luetkenii</i>	Stephanoberycidae	Beryciformes	Subdivision Berycimorphaceae
<i>Beryx splendens</i>	Berycidae	Beryciformes	Subdivision Berycimorphaceae
<i>Holocentrus rufus</i>	Holocentridae	Holocentriformes	Subdivision Holocentrimorphaceae
<i>Myripristis jacobus</i>	Holocentridae	Holocentriformes	Subdivision Holocentrimorphaceae
<i>Brotula barbata</i>	Ophidiidae	Ophidiiformes	Series Ophidiaria
<i>Carapus acus</i>	Ophidiidae	Ophidiiformes	Series Ophidiaria
<i>Chatrabus melanurus</i>	Batrachoididae	Batrachoidiformes	Series Batrachoidaria
<i>Lesueurigobius cf. sanzoi</i>	Gobiidae	Gobiiformes	Series Gobiaria
<i>Periophthalmodon schlosseri</i>	Oxudercidae	Gobiiformes	Series Gobiaria
<i>Hippocampus comes</i>	Syngnathidae	Syngnathiformes	Series Syngnatharia
<i>Syngnathus scovelli</i>	Syngnathidae	Syngnathiformes	Series Syngnatharia
<i>Pampus argenteus</i>	Stromateidae	Scombriformes	Series Pelagiaria
<i>Thunnus albacares</i>	Scombridae	Scombriformes	Series Pelagiaria
<i>Epinephelus aeneus</i>	Serranidae	Perciformes	Series Eupercaria
<i>Perca fluviatilis</i>	Percidae	Perciformes	Series Eupercaria
<i>Chaenocephalus aceratus</i>	Channichthyidae	Perciformes	Series Eupercaria
<i>Anoplopoma fimbria</i>	Anoplopomatidae	Perciformes	Series Eupercaria
<i>Gasterosteus aculeatus</i>	Gasterosteidae	Perciformes	Series Eupercaria
<i>Myoxocephalus scorpius</i>	Psychrolutidae	Perciformes	Series Eupercaria

**Supplementary Table 6 (continued):** Teleost species used in phylogenetic analyses.

Species	Family (Subfamily)	Order	Group
<i>Labrus bergylta</i>	Labridae	Labriformes	Series Eupercaria
<i>Larimichthys crocea</i>	Sciaenidae	incertae sedis	Series Eupercaria
<i>Dicentrarchus labrax</i>	Moronidae	incertae sedis	Series Eupercaria
<i>Morone saxatilis</i>	Moronidae	incertae sedis	Series Eupercaria
<i>Spondyliosoma cantharus</i>	Sparidae	Spariformes	Series Eupercaria
<i>Antennarius striatus</i>	Antennariidae	Lophiiformes	Series Eupercaria
<i>Mola mola</i>	Molidae	Tetraodontiformes	Series Eupercaria
<i>Takifugu rubripes</i>	Tetraodontidae	Tetraodontiformes	Series Eupercaria
<i>Tetraodon nigroviridis</i>	Tetraodontidae	Tetraodontiformes	Series Eupercaria
<i>Channa argus</i>	Channidae	Anabantiformes	Series Anabantaria
<i>Anabas testudineus</i>	Anabantidae	Anabantiformes	Series Anabantaria
<i>Helostoma temminckii</i>	Helostomatidae	Anabantiformes	Series Anabantaria
<i>Lates calcarifer</i>	Centropomidae	<i>incertae sedis</i>	Series Carangaria
<i>Caranx melampygus</i>	Carangidae	Carangiformes	Series Carangaria
<i>Selene dorsalis</i>	Carangidae	Carangiformes	Series Carangaria
<i>Cynoglossus semilaevis</i>	Cynoglossidae	Pleuronectiformes	Series Carangaria
<i>Pseudopleuronectes yokohamae</i>	Pleuronectidae	Pleuronectiformes	Series Carangaria
<i>Scophthalmus maximus</i>	Scophthalmidae	Pleuronectiformes	Series Carangaria
<i>Chromis chromis</i>	Pomacentridae	<i>incertae sedis</i>	Series Ovalentaria
<i>Parablennius parvicornis</i>	Blenniidae	Blenniiformes	Series Ovalentaria
<i>Pseudochromis fuscus</i>	Pseudochromidae	incertae sedis	Series Ovalentaria
<i>Oryzias latipes</i>	Adrianichthyidae	Beloniformes	Series Ovalentaria
<i>Kryptolebias marmoratus</i>	Rivulidae	Cyprinodontiformes	Series Ovalentaria
<i>Nothobranchius furzeri</i>	Nothobranchiidae	Cyprinodontiformes	Series Ovalentaria
<i>Cyprinodon variegatus</i>	Cyprinodontidae	Cyprinodontiformes	Series Ovalentaria
<i>Fundulus heteroclitus</i>	Fundulidae	Cyprinodontiformes	Series Ovalentaria
<i>Poecilia formosa</i>	Poeciliidae	Cyprinodontiformes	Series Ovalentaria
<i>Xiphophorus maculatus</i>	Poeciliidae	Cyprinodontiformes	Series Ovalentaria
<i>Etroplus canarensis</i>	Cichlidae (Etroplinae)	Cichliformes	Series Ovalentaria
<i>Paratilapia polleni</i> “Andapa”	Cichlidae (Ptychochrominae)	Cichliformes	Series Ovalentaria
<i>Ptychochromis oligacanthus</i>	Cichlidae (Ptychochrominae)	Cichliformes	Series Ovalentaria
<i>Apistogramma diplotaenia</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Australoheros scitulus</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Amphilophus citrinellus</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Amphilophus zaliosus</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Bujurquina vittata</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Andinoacara biseriatus</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Andinoacara coeruleopunctatus</i>	Cichlidae (Cichlinae)	Cichliformes	Series Ovalentaria
<i>Heterochromis multidentis</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Tylochromis polylepis</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Benitochromis conjunctus</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Pelvicachromis taeniatus</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Hemichromis elongatus</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Etia nguti</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Oreochromis niloticus</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria
<i>Metriaclima zebra</i>	Cichlidae (Pseudocrenilabrinae)	Cichliformes	Series Ovalentaria

**Supplementary Table 7:** Assembly files used in phylogenetic analyses.

Assembly files are either hosted at the Ensembl (Ensembl.org), NCBI (ncbi.nlm.nih.gov), or EBI (ebi.ac.uk) databases or deposited on datadryad.org, figshare.com, parrot.genomics.cn, surfdrive.surf.nl, cichlid.gurdon.cam.ac.uk, efishgenomics.integrativebiology.msu.edu, or creskolab.uoregon.edu. Details about new assemblies are given in Supplementary Table 2.

Species	Assembly file(s)
<i>Lepisosteus oculatus</i>	Lepisosteus.oculatus.LepOcu1.dna.toplevel.fa.gz
<i>Scleropages formosus</i>	Scleropages_formosus.ASM162426v1.dna.toplevel.fa.gz
<i>Anguilla anguilla</i>	Anguilla.anguilla_assembly_racon_and_double_pilon_corrected.fasta
<i>Clupea harengus</i>	GCA_900323705.1_C.harengus_genomic.fna.gz
<i>Danio rerio</i>	Danio_rerio.GRCz11.dna.toplevel.fa.gz
<i>Astyanax mexicanus</i>	Astyanax_mexicanus.Astyanax_mexicanus-2.0.dna.toplevel.fa.gz
<i>Electrophorus electricus</i>	SSPACEgenomeLLT_454_10212014.fasta.gz
<i>Esox lucius</i>	Esox_lucius.Eluc_V3.dna.toplevel.fa.gz
<i>Salmo salar</i>	GCF_000233375.1_ICASASG_v2_genomic.fna.gz
<i>Borostomias antarcticus</i>	fish_65.scf.fasta.gz
<i>Osmerus eperlanus</i>	fish_54.scf.fasta.gz
<i>Protosalanx hyalocranium</i>	clearhead_icefish.fa.tar.gz
<i>Guentherus altivela</i>	fish_50.scf.fasta.gz
<i>Parasudis fraserbrunneri</i>	fish_42.scf.fasta.gz
<i>Benthoosema glaciale</i>	fish_66.scf.fasta.gz
<i>Polymixia japonica</i>	fish_24.scf.fasta.gz
<i>Percopsis transmontana</i>	fish_26.scf.fasta.gz
<i>Typhlichthys subterraneus</i>	fish_27.scf.fasta.gz
<i>Cyttopsis rosea</i>	fish_30.scf.fasta.gz
<i>Zeus faber</i>	fish_28.scf.fasta.gz
<i>Stylephorus chordatus</i>	fish_80.scf.fasta.gz
<i>Gadus morhua</i>	LN845748.fasta, LN845749.fasta, LN845750.fasta, LN845751.fasta, LN845752.fasta, LN845753.fasta, LN845754.fasta, LN845755.fasta, LN845756.fasta, LN845757.fasta, LN845758.fasta, LN845759.fasta, LN845760.fasta, LN845761.fasta, LN845762.fasta, LN845763.fasta, LN845764.fasta, LN845765.fasta, LN845766.fasta, LN845767.fasta, LN845768.fasta, LN845769.fasta, LN845770.fasta
<i>Lampris guttatus</i>	fish_48.scf.fasta.gz
<i>Regalecus glesne</i>	fish_47.scf.fasta.gz
<i>Gephyroberyx darwini</i>	CABFOQ01.fasta.gz
<i>Monocentris japonica</i>	fish_74.scf.fasta.gz
<i>Acanthochaenus luetkenii</i>	fish_79.scf.fasta.gz
<i>Beryx splendens</i>	fish_69.scf.fasta.gz
<i>Holocentrus rufus</i>	fish_35.scf.fasta.gz
<i>Myripristis jacobus</i>	fish_34.scf.fasta.gz
<i>Brotula barbata</i>	fish_32.scf.fasta.gz
<i>Carapus acus</i>	fish_33.scf.fasta.gz
<i>Chatrabus melanurus</i>	fish_40.scf.fasta.gz
<i>Lesueurigobius cf. sanzoi</i>	fish_96.scf.fasta.gz
<i>Periophthalmodon schlosseri</i>	GCA_000787095.1_PS.fa_genomic.fna.gz
<i>Hippocampus comes</i>	GCF_001891065.1_H.comes_QL1_v1_genomic.fna.gz
<i>Syngnathus scovelli</i>	ssc_2016_12_20_chromlevel.fa.gz
<i>Pampus argenteus</i>	GCA_000697985.1_PamArg1.0_genomic.fna.gz
<i>Thunnus albacares</i>	fish_83.scf.fasta.gz

**Supplementary Table 7 (continued):** Assembly files used in phylogenetic analyses.

Species	Assembly file(s)
<i>Epinephelus aeneus</i>	Epae.scf.fasta.gz
<i>Perca fluviatilis</i>	fish_55.scf.fasta.gz
<i>Chaenocephalus aceratus</i>	fish_61.scf.fasta.gz
<i>Anoplopoma fimbria</i>	GCA_000499045.1_AnoFim1.0_genomic.fna.gz
<i>Gasterosteus aculeatus</i>	Gasterosteus_aculeatus.BROADS1.dna.toplevel.fa.gz
<i>Myoxocephalus scorpius</i>	fish_91.scf.fasta.gz
<i>Labrus bergylta</i>	Labrus_bergylta.BallGen_V1.dna.toplevel.fa.gz
<i>Larimichthys crocea</i>	GCA_900246015.1_Larimichthys_crocea_chromosome_1.0_genomic.fna.gz
<i>Dicentrarchus labrax</i>	GCA_000689215.1_seabass_V1.0_genomic.fna.gz
<i>Morone saxatilis</i>	GCA_001663605.1_SBDraft1_genomic.fna.gz
<i>Spondylisoma cantharus</i>	fish_81.scf.fasta.gz
<i>Antennarius striatus</i>	fish_52.scf.fasta.gz
<i>Mola mola</i>	Mola_mola.ASM169857v1.dna.toplevel.fa.gz
<i>Takifugu rubripes</i>	Takifugu_rubripes.FUGU5.dna.toplevel.fa.gz
<i>Tetraodon nigroviridis</i>	Tetraodon_nigroviridis.TETRAODON8.dna.toplevel.fa.gz
<i>Channa argus</i>	Argus_liugm_genome.fa.gz
<i>Anabas testudineus</i>	fish_85.scf.fasta.gz
<i>Helostoma temminckii</i>	fish_84.scf.fasta.gz
<i>Lates calcarifer</i>	GCA_001640805.1_ASM164080v1_genomic.fna.gz
<i>Caranx melampygus</i>	Came.scf.fasta.gz
<i>Selene dorsalis</i>	fish_86.scf.fasta.gz
<i>Cynoglossus semilaevis</i>	GCF_000523025.1_Cse_v1.0_genomic.fna.gz
<i>Pseudopleuronectes yokohamae</i>	GCA_000787555.1_Pyoko_1.0_genomic.fna.gz
<i>Scophthalmus maximus</i>	Scophthalmus_maximus.ASM318616v1.dna.toplevel.fa.gz
<i>Chromis chromis</i>	fish_87.scf.fasta.gz
<i>Parablennius parvicornis</i>	fish_88.scf.fasta.gz
<i>Pseudochromis fuscus</i>	fish_90.scf.fasta.gz
<i>Oryzias latipes</i>	Oryzias_latipes_hsok.ASM223469v1.dna.toplevel.fa.gz
<i>Kryptolebias marmoratus</i>	Kryptolebias_marmoratus.ASM164957v1.dna.toplevel.fa.gz
<i>Nothobranchius furzeri</i>	LN609377.fasta, LN609378.fasta, LN609379.fasta, LN609380.fasta, LN609381.fasta LN609382.fasta, LN609383.fasta, LN609384.fasta, LN609385.fasta, LN609386.fasta LN609387.fasta, LN609388.fasta, LN609389.fasta, LN609390.fasta, LN609391.fasta LN609392.fasta, LN609393.fasta, LN609394.fasta, LN609395.fasta
<i>Cyprinodon variegatus</i>	Cyprinodon_variegatus.C_variegatus-1.0.dna.toplevel.fa.gz
<i>Fundulus heteroclitus</i>	Fundulus_heteroclitus.Fundulus_heteroclitus-3.0.2.dna.toplevel.fa.gz
<i>Poecilia formosa</i>	Poecilia_formosa.PoeFor_5.1.2.dna.toplevel.fa.gz
<i>Xiphophorus maculatus</i>	Xiphophorus_maculatus.X_maculatus-5.0-male.dna.toplevel.fa.gz
<i>Etroplus canarensis</i>	New
<i>Paratilapia polleni</i> “Andapa”	New
<i>Ptychochromis oligacanthus</i>	New
<i>Apistogramma diplotaenia</i>	New
<i>Australoheros scitulus</i>	New
<i>Amphilophus citrinellus</i>	Amphilophus_citrinellus.Midas_v5.dna.toplevel.fa.gz
<i>Amphilophus zaliosus</i>	New
<i>Bujurquina vittata</i>	New
<i>Andinoacara biseriatus</i>	New
<i>Andinoacara coeruleopunctatus</i>	Andinoacara_coeruleopunctatus_final_min1000bp_scaffolds.fa.gz

**Supplementary Table 7 (continued):** Assembly files used in phylogenetic analyses.

Species	Assembly file(s)
<i>Heterochromis multidens</i>	New
<i>Tylochromis polylepis</i>	New
<i>Benitochromis conjunctus</i>	New
<i>Pelvicachromis taeniatus</i>	New
<i>Hemichromis elongatus</i>	New
<i>Etia nguti</i>	New
<i>Oreochromis niloticus</i>	MKQE02.1.fsa_nt.gz, MKQE02.2.fsa_nt.gz
<i>Metriaclima zebra</i>	AGTA05.1.fsa_nt.gz, AGTA05.2.fsa_nt.gz

**Supplementary Table 8:** Markers selected for phylogenetic analyses.

“Gene ID” indicates the Ensembl ID of the zebrafish gene; “mean BPP” specifies the mean node support measured as Bayesian posterior probability in single-gene analyses with BEAST 2. “Rate” and “Coefficient” specify the mean and the coefficient of variation of the substitution-rate estimate in these single-gene analyses. The last column indicates whether the marker is included in the permissive (p) and strict (s) marker sets. var., variable.

Gene ID	# exons	Length (bp)	# var. sites	Mean BPP	Rate	Coefficient	Set
ENSDARG0000000853	4	702	356	0.673	0.00148	0.652	p
ENSDARG0000001129	4	702	338	0.655	0.00130	0.693	p
ENSDARG0000002332	3	513	237	0.570	0.00132	0.474	p+s
ENSDARG0000002402	6	1,287	572	0.735	0.00112	0.594	p+s
ENSDARG0000002600	5	930	470	0.756	0.00133	0.561	p+s
ENSDARG0000002952	4	777	356	0.716	0.00127	0.516	p+s
ENSDARG0000003449	4	645	301	0.547	0.00086	0.624	p
ENSDARG0000003495	7	1,332	652	0.797	0.00136	0.598	p+s
ENSDARG0000003963	5	1,110	494	0.777	0.00080	0.598	p+s
ENSDARG0000004173	6	1,143	522	0.769	0.00116	0.572	p+s
ENSDARG0000004302	4	807	360	0.696	0.00094	0.621	p
ENSDARG0000004581	3	483	243	0.531	0.00124	0.515	p+s
ENSDARG0000005218	4	756	443	0.819	0.00144	0.570	p
ENSDARG0000005236	4	720	312	0.669	0.00110	0.562	p+s
ENSDARG0000007708	4	708	321	0.691	0.00104	0.571	p+s
ENSDARG0000007901	6	1,095	566	0.746	0.00122	0.518	p+s
ENSDARG0000008224	3	564	268	0.553	0.00126	0.532	p+s
ENSDARG0000008575	9	1,737	937	0.841	0.00118	0.570	p
ENSDARG0000008637	4	765	454	0.720	0.00138	0.492	p+s
ENSDARG0000009830	6	1,287	647	0.879	0.00110	0.599	p+s
ENSDARG0000009953	6	1,179	608	0.814	0.00113	0.662	p
ENSDARG0000009982	4	714	342	0.755	0.00141	0.530	p
ENSDARG0000010238	3	531	259	0.620	0.00117	0.635	p
ENSDARG0000010862	3	507	307	0.659	0.00121	0.469	p+s
ENSDARG0000012138	4	699	346	0.591	0.00140	0.468	p+s
ENSDARG0000012378	3	522	241	0.614	0.00108	0.593	p+s
ENSDARG0000013079	5	942	485	0.757	0.00142	0.602	p
ENSDARG0000013150	6	1,038	506	0.768	0.00127	0.625	p
ENSDARG0000014004	3	537	269	0.611	0.00124	0.543	p+s
ENSDARG0000014634	3	483	227	0.528	0.00104	0.363	p+s
ENSDARG0000016415	6	1,131	624	0.832	0.00143	0.569	p
ENSDARG0000016753	6	1,176	550	0.812	0.00119	0.555	p+s
ENSDARG0000016775	6	1,095	553	0.836	0.00146	0.552	p
ENSDARG0000016782	5	948	432	0.739	0.00110	0.624	p
ENSDARG0000017006	4	807	396	0.678	0.00115	0.586	p+s
ENSDARG0000017986	9	1,599	822	0.807	0.00122	0.590	p+s
ENSDARG0000018192	13	2,550	1,169	0.907	0.00117	0.580	p+s
ENSDARG0000018325	6	1,254	681	0.749	0.00134	0.564	p+s
ENSDARG0000018904	3	507	227	0.445	0.00110	0.633	p
ENSDARG0000018973	3	588	258	0.746	0.00124	0.545	p+s
ENSDARG0000019000	9	1,560	644	0.800	0.00096	0.580	p+s

**Supplementary Table 8 (continued):** Markers selected for phylogenetic analyses.

Gene ID	# exons	Length (bp)	# var. sites	Mean BPP	Rate	Coefficient	Set
ENSDARG00000019451	3	873	397	0.711	0.00124	0.520	p+s
ENSDARG00000020242	3	624	257	0.688	0.00100	0.558	p+s
ENSDARG00000020693	3	618	341	0.550	0.00137	0.551	p+s
ENSDARG00000021945	6	1,182	485	0.843	0.00104	0.617	p
ENSDARG00000022974	4	897	409	0.732	0.00103	0.610	p
ENSDARG00000024317	3	498	277	0.495	0.00109	0.407	p
ENSDARG00000025011	4	771	375	0.662	0.00120	0.571	p+s
ENSDARG00000025094	3	513	257	0.619	0.00094	0.506	p+s
ENSDARG00000025212	3	576	222	0.610	0.00083	0.525	p+s
ENSDARG00000026180	19	3,696	1,366	0.925	0.00090	0.634	p
ENSDARG00000027016	3	570	327	0.603	0.00115	0.660	p
ENSDARG00000027353	6	999	550	0.822	0.00156	0.589	p
ENSDARG00000028748	3	519	223	0.720	0.00119	0.551	p
ENSDARG00000028971	4	858	416	0.746	0.00111	0.504	p+s
ENSDARG00000029157	6	1,146	533	0.765	0.00111	0.559	p+s
ENSDARG00000029472	3	549	253	0.600	0.00108	0.566	p+s
ENSDARG00000029931	3	486	213	0.675	0.00078	0.581	p+s
ENSDARG00000030022	3	561	321	0.712	0.00145	0.658	p
ENSDARG00000030665	3	534	259	0.627	0.00156	0.579	p
ENSDARG00000034396	4	774	419	0.683	0.00137	0.554	p+s
ENSDARG00000034823	3	585	232	0.599	0.00112	0.392	p+s
ENSDARG00000035535	4	630	299	0.585	0.00123	0.557	p+s
ENSDARG00000035751	5	894	463	0.737	0.00121	0.659	p
ENSDARG00000035978	6	1,218	630	0.849	0.00122	0.613	p
ENSDARG00000039931	3	630	360	0.600	0.00131	0.557	p+s
ENSDARG00000040571	5	993	555	0.782	0.00110	0.570	p+s
ENSDARG00000041133	3	693	357	0.661	0.00122	0.685	p
ENSDARG00000041217	3	504	292	0.708	0.00128	0.631	p
ENSDARG00000042087	3	525	256	0.641	0.00125	0.618	p
ENSDARG00000042147	3	486	192	0.568	0.00109	0.493	p+s
ENSDARG00000043019	6	1,107	508	0.780	0.00093	0.660	p
ENSDARG00000043359	3	480	209	0.517	0.00096	0.525	p+s
ENSDARG00000044820	3	843	377	0.607	0.00099	0.566	p+s
ENSDARG00000045275	3	501	218	0.550	0.00096	0.572	p+s
ENSDARG00000045399	3	504	222	0.704	0.00092	0.454	p+s
ENSDARG00000045560	3	528	266	0.651	0.00126	0.531	p+s
ENSDARG00000051921	3	582	284	0.625	0.00144	0.520	p
ENSDARG00000052377	3	531	255	0.606	0.00091	0.515	p+s
ENSDARG00000054224	3	540	289	0.613	0.00155	0.641	p
ENSDARG00000055554	3	1,002	528	0.801	0.00122	0.635	p
ENSDARG00000056557	6	1,119	503	0.794	0.00088	0.621	p
ENSDARG00000056587	3	1,113	679	0.813	0.00147	0.570	p
ENSDARG00000056648	4	771	385	0.653	0.00130	0.652	p
ENSDARG00000057738	3	537	272	0.582	0.00108	0.507	p+s
ENSDARG00000058419	11	2,142	1,061	0.865	0.00106	0.584	p+s
ENSDARG00000059116	10	1,926	876	0.808	0.00102	0.604	p
ENSDARG00000059323	5	1,128	639	0.844	0.00119	0.486	p+s

**Supplementary Table 8 (continued):** Markers selected for phylogenetic analyses.

Gene ID	# exons	Length (bp)	# var. sites	Mean BPP	Rate	Coefficient	Set
ENSDARG00000059553	5	975	535	0.727	0.00116	0.545	p
ENSDARG00000059701	3	504	267	0.642	0.00136	0.472	p+s
ENSDARG00000059911	3	525	306	0.654	0.00137	0.557	p+s
ENSDARG00000060323	3	597	279	0.607	0.00085	0.631	p
ENSDARG00000060785	3	789	469	0.680	0.00157	0.699	p
ENSDARG00000061039	3	585	302	0.606	0.00152	0.621	p
ENSDARG00000061203	5	1,017	461	0.578	0.00103	0.569	p+s
ENSDARG00000061590	4	693	358	0.619	0.00141	0.539	p
ENSDARG00000061723	3	552	287	0.679	0.00123	0.607	p
ENSDARG00000061960	3	543	232	0.644	0.00086	0.616	p
ENSDARG00000062198	3	591	342	0.532	0.00155	0.596	p
ENSDARG00000062472	6	1,131	549	0.789	0.00139	0.620	p
ENSDARG00000062795	3	591	324	0.661	0.00116	0.387	p+s
ENSDARG00000062846	3	612	333	0.675	0.00156	0.663	p
ENSDARG00000062973	3	537	256	0.516	0.00100	0.633	p
ENSDARG00000067913	3	513	243	0.695	0.00107	0.496	p+s
ENSDARG00000068187	4	642	325	0.659	0.00113	0.594	p+s
ENSDARG00000068910	5	930	484	0.717	0.00107	0.575	p+s
ENSDARG00000069289	3	555	253	0.668	0.00110	0.606	p
ENSDARG00000069420	3	657	291	0.494	0.00126	0.526	p+s
ENSDARG00000074524	3	555	299	0.629	0.00131	0.649	p
ENSDARG00000074686	3	612	283	0.637	0.00103	0.588	p+s
ENSDARG00000074759	3	570	217	0.624	0.00102	0.664	p
ENSDARG00000075612	3	552	284	0.599	0.00108	0.488	p+s
ENSDARG00000075884	7	1,593	882	0.784	0.00142	0.521	p
ENSDARG00000076815	7	1,434	627	0.851	0.00095	0.599	p+s
ENSDARG00000077162	3	4,638	2,316	0.796	0.00153	0.662	p
ENSDARG00000077469	3	519	298	0.719	0.00130	0.538	p+s
ENSDARG00000077536	14	2,499	1,111	0.819	0.00112	0.645	p
ENSDARG00000077570	5	1,032	522	0.675	0.00120	0.592	p+s
ENSDARG00000078127	5	849	395	0.661	0.00106	0.542	p+s
ENSDARG00000078853	4	624	320	0.690	0.00103	0.553	p+s
ENSDARG00000079163	3	456	231	0.682	0.00116	0.569	p
ENSDARG00000079308	3	678	374	0.653	0.00138	0.678	p
ENSDARG00000079848	4	702	350	0.654	0.00129	0.544	p+s
ENSDARG00000079949	6	1,158	502	0.659	0.00087	0.566	p+s
ENSDARG00000086790	3	630	269	0.671	0.00155	0.541	p
ENSDARG00000090183	4	1,191	587	0.777	0.00109	0.638	p
ENSDARG00000091367	3	537	226	0.637	0.00098	0.605	p
ENSDARG00000093413	3	519	215	0.484	0.00118	0.646	p
ENSDARG00000094380	5	882	505	0.812	0.00133	0.668	p
ENSDARG00000095890	5	1,029	531	0.871	0.00117	0.623	p
ENSDARG00000098247	3	561	248	0.521	0.00122	0.437	p+s
ENSDARG00000098916	4	684	329	0.718	0.00125	0.534	p+s
ENSDARG00000099034	4	738	395	0.516	0.00150	0.672	p
ENSDARG00000099139	3	546	277	0.615	0.00091	0.427	p+s
ENSDARG00000099458	3	576	263	0.551	0.00144	0.429	p



**Supplementary Table 8 (continued):** Markers selected for phylogenetic analyses.

Gene ID	# exons	Length (bp)	# var. sites	Mean BPP	Rate	Coefficient	Set
ENSDARG00000099798	3	561	262	0.595	0.00099	0.649	p
ENSDARG00000100013	5	858	351	0.712	0.00096	0.622	p
ENSDARG00000100419	8	1,401	726	0.902	0.00112	0.581	p+s
ENSDARG00000100524	6	1,119	574	0.832	0.00122	0.561	p+s
ENSDARG00000101062	3	549	314	0.668	0.00122	0.487	p+s
ENSDARG00000101507	4	729	376	0.819	0.00132	0.611	p
ENSDARG00000101676	4	1,029	483	0.690	0.00132	0.648	p
ENSDARG00000102184	5	888	346	0.754	0.00111	0.641	p
ENSDARG00000102307	4	726	329	0.660	0.00115	0.564	p+s
ENSDARG00000102587	3	519	246	0.603	0.00107	0.501	p+s
ENSDARG00000103799	3	510	274	0.649	0.00093	0.449	p+s
ENSDARG00000104953	4	660	255	0.551	0.00084	0.683	p
Total (permissive set):							
147 genes	646	127,638	62,190	0.688	0.00118	0.573	
Total (strict set):							
77 genes	332	62,776	30,463	0.678	0.00114	0.535	

**Supplementary Table 9:** Bayesian age estimates for the divergence of American and African cichlid fishes.

Studies reporting Bayesian molecular divergence-time estimates of African and Neotropical cichlid fishes. Taxon numbers in parentheses indicate sampled species of Pseudocrenilabrinae and Cichlinae. Calibration numbers in parentheses specify how many divergences were constrained within Cichlidae.

Study	Ref.	# taxa	# sites	% complete	# cal.	Age estimate (Ma)
Genner et al. (2007)	[196]	34 (18, 6)	905	96.0	4 (0)	98.4 (121.9-75.4)
Azuma et al. (2008)	[197]	54 (4, 2)	10 034	99.9	18 (0)	89.0 (108.0-72.0)
Santini et al. (2009)	[113]	227 (6, 1)	1 445	93.1	45 (2)	49.0 (66.0-37.0)
Matschiner et al. (2011)	[198]	67 (4, 2)	4 599	93.5	6 (0)	59.7 (80.7-40.2)
Matschiner (unpubl.)		9 (3, 1)	28 239	92.6	3 (1)	55.2 (67.4-49.7)
McMahan et al. (2013)	[199]	133 (19, 57)	2 069	88.2	4 (3)	70.8 (83.7-60.2)
Near et al. (2013)	[200]	579 (2, 3)	8 577	84.5	37 (0)	25.9 (29.6-22.0)
Friedman et al. (2013)	[5]	158 (54, 29)	7 887	93.7	10 (0)	46.4 (54.9-40.9)
Betancur-R. et al. (2013)	[201]	202 (3, 4)	17 812	51.5	59 (2)	62.1 (70.2-54.7)
Matschiner et al. (2017)	[18]	366 (64, 79)	27 650	40.7	147 (23)	81.6 (89.4-74.0)
Hughes et al. (2018)	[7]	302 (9, 2)	10 203	96.4	31 (1)	52.2 (65.9-45.7)
Musilova et al. (2019)	[9]	101 (5, 1)	71 902	93.6	28 (2)	55.1 (66.9-45.3)
Roth et al. (2010)	[202]	71 (1, 1)	108 372	93.2	34 (2)	50.3 (57.6-45.1)
This study		91 (8, 7)	127 638	93.5	51 (8)	62.1 (70.1-54.6)
This study (excl. cichlid fossils)					43 (0)	38.7 (50.8-27.1)

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