## Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes

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## **Additional files**

## Additional file 3 – Description of timetree calibration points

To obtain a molecular timetree, we assigned fossil calibrations to the root and 44 additional nodes on the Bayesian consensus tree (Fig. 1-3). For each constraint below, the age of the fossil provided a hard lower bound on the minimum age of the node. Upper limits on the ages of constrained nodes are generally difficult to determine. We used log normal priors in our BEAST analysis because this distribution allowed us to express our prior belief, based on the occurrence of related taxa in the fossil record, of the maximum age of each constraint as a 95% cumulative probability while still allowing for the possibility that a constrained node could be older. Thus, fossils in our analysis provided hard lower and soft upper bounds. Justification for these bounds for each constraint is given below.

Age of the Root between Osteichthyes and Outgroups. The sister group of the Osteichthyes (bony fishes) is currently unknown. It may be represented by the Chondrichthyes (cartilaginous fishes), or by the Acanthodii. The fossil record of the Chondrichthyes dates to at least the early-middle Caradoc of the Harding Sandstone,

Colorado (Ordovician, 456-454 Ma) [1]. The acanthodian *Onchus clintonii* dates to the Sheinwoodian and Homerian (Silurian, 436-428 Ma)2 [2]. Due to the uncertainty in the sister group relationships between Osteichthyes, Acanthodii, and Chondrichthyes, we assigned to the root a minimum age of 428 My. Placing an upper limit on the root is problematic, due the uncertain relationships of many worm-like Cambrian fossils that have been tentatively referred to as chordates. *Pikaia*, from the Burgess Shale fossil deposits of the late Cambrian of British Columbia, Canada (505 Ma) is a prevertebrate that has been identified as probably being related to cephalochordates [3]. Our prior uses the age of these deposits to set the 95% confidence interval.

**Elasmobranchii (Lamnidae vs. Scyliorhinidae)** (Fig. 1, node 1). While Elasmobranchii (sharks) have a fossil record that spans back to the Ordovician, crown sharks are much more recent, having appeared in the fossil record during the Mesozoic. The earliest fossils that can be used to date the split between the two species that we used in this analysis is an unnamed and undescribed taxon from deposits of the Bathonian (Jurassic, 167.7-164.7 Ma), which was assigned to the family Scyliorhinidae [4]. Our prior assumed a minimum age of 165 My, and placed 95% of the weight on divergence times within the time span marked by the first Elasmobranchii (415 Ma) [4].

**MRCA of Osteichthyes (Sarcopterygii vs. Actinopterygii)** (Fig. 1, node 2). The oldest known fossil from this clade is the stem actinopteygian *Andreolepis hedei* from the Lludlowian of Gotland, Sweden (Silurian, 422-418 Ma) [5]. Our prior assumed a

minimum age of 418 My, and used the age of the Burgess Shale deposits (505 My) for the 95% upper limit.

**MRCA of Sarcopterygii (Dipnotetrapodomorpha vs. Coelacanthimorpha)** (Fig. 1, node 3). The oldest sarcopterygian is *Psarolepis romeri* from the Lludlow-Pridoli of China (Silurian, 420-418 Ma), but this is likely a stem taxon. The oldest taxon that marks the split between coelacanths, lungfishes, and tetrapods is *Eoactinistia foreyi*, the oldest coelacanthimorph from the Pragian (Devonian, 409-407 Ma) [6]. The prior thus assumed 407 My as the minimum age of the split, and a 95% upper bound of 505 My (reflecting the upper age of the Burgess shale deposit).

MRCA of Actinopterygii (Actinistia vs Acipenseriformes vs Neoteleostei) (Fig. 1, node 4). The oldest fossil known to belong to this clade is the stem actinopteygian *Andreolepis hedei* from the Lludlowian of Gotland, Sweden (Silurian, 422-418 Ma) [5]. The oldest taxon that can be assigned to the crown is the neopterygian *Brachydegma caelatum*, from the Artkinsian of Texas (early Permian, 284 Ma) [7]. Our prior thus assumed 284 My as the minimum age, and 418 My for the upper bound.

**MRCA of Neopterygii (Amilformes vs Lepisosteiformes vs Teleostei)** (Fig. 1, node 5). The oldest crown taxon is the stem teleost *Pholidophoretes salvus*. The oldest stem is *Brachydegma caelatum*, from the Artkinsian of Texas (early Permian, 284 Ma) [7]. Our prior thus assumed 225 My as the minimum age, and 284 My for the upper bound.

MRCA of Teleostei (Elopomorpha vs Osteoglossomorpha vs Clupeocephala) (Fig.

1, node 6). The oldest taxon assigned to this clade is *Anaethalion*, from the late Kimmeridgian lithographic limestone of Nusblingen, Germany, and Cerin, France (Jurassic, 152 My) [8]. The oldest stem teleost is *Pholidophoretes salvus* (Pholidophoridae), from the early Carnian/Julian of Polzberg bei Lunz, Austria (Triassic, 228-225 Ma). Our prior assumed 152 My as the minimum age and 225 My for the upper bound.

**MCRA of Osteoglossomorpha** (Fig. 1, node 7). The oldest crown osteoglossomorphs are several taxa from the Lycoptera assemblage of the Barremian of China (Early Cretaceous, 130 Ma), such as the Hiodontidae *Yanbiania wangqingica* [9]. To establish an upper boundary we used the elopomorph *Anaethalion*, from the late Kimmeridgian lithographic limestone (Jurassic, 152 Ma) [8]. Our prior assumed 130 My as the minimum age, and 152 My as the upper bound.

MCRA of Elopomorpha (Fig. 1, node 8). The oldest crown elopomorph is the albulid *Albuloideorum ventralis*, from the Early Hauterivian (Jurassic/Cretaceous border, 135 Ma) [10]. The oldest stem elopomorph is *Anaethalion*, from the late Kimmeridgian lithographic limestone of Nusblingen, Germany, and Cerin, France (Jurassic, 152 Ma) [8]. Our prior assumed 135 My as the minimum age, and 152 My for the upper bound.

**MCRA of Ostarioclupeomorpha (Ostariophysi vs Clupeomorpha)** (Fig. 1, node 9). The oldest known crown ostarioclupeomorph fossil is *Tischlingerichthys viohli*, upper Tithonian of Muhlheim, Bavaria, Germany (Jurassic, 149 Ma) [11]. To establish a 95% upper boundary we used the elopomorph *Anaethalion*, from the late Kimmeridgian lithographic limestone of Nusblingen, Germany, and Cerin, France (Jurassic, 152 Ma) [8]. Our prior assumed 149 My as the minimum age, and 152 My for the upper bound.

*Pellona vs Alosa* (Fig. 1, node 10). The oldest fossil that can be assigned to this clade is *Gasteroclupea branisai*, from the Maastrichtian of the El Molino Formation, Cayara, Bolivia (Late Cretaceous, 72-67 Ma) [12]. A number of clupeiform fossils from the Lower Cretaceous (e.g., '*Clupea*' spp.; *Ellimichthys longicostatus*; *Paraclupea chetungensis*; 130-125 Ma) provide us with the upper boundary [13]. The prior assumed a minimum age of 67 My, and an upper boundary of 125 My.

**MCRA of Ostariophysi** (Fig. 1, node 11). The oldest crown ostariophysans are the Chanidae *Gordichthys* and *Rubiesichthys* from the Berriasian/Barremian (Jurassic/ Cretaceous, 145-125 Ma) [14]. The ostarioclupeomorph *Tischlingerichthys viohli*, from upper Tithonian of Muhlheim, Germany (Jurassic, 149 Ma), was used to establish an upper boundary. Our prior thus assumed 125 My as the minimum age, and 149 My as the 95% upper boundary.

**MCRA of Characiformes** (Fig. 1, node 12). *Santanichthys diasii* from the Santana formation in Brazil, Albian (Early Cretaceous, 112-100 Ma) is the oldest stem characiform [15]. The oldest crown characiforms are various Serrasalminae *indet*. and Tetraogonopterinae *indet*. from the middle Maastrichtian of the El Molino Formation,

Bolivia (Late Cretaceous, 69-68 Ma) [16]. Our prior assumed 68 My as the minimum age, and 100 My as the 95% upper boundary.

**MCRA of Cyprinidae** (Fig. 1, node 13). *Parabarbus* spp. from the Ypresian of the Obailinskaya Formation, Zaissan Basin, Kazakhstan (early-middle Eocene, 51-49 Ma) is the oldest crown cyprinid [17] and provided the minimum age for the cyprinid calibration. The stem characiform *Santanichthys diasii* from the Santana formation in Brazil, Albian (Early Cretaceous, 112-100 Ma) was used to set the 95% upper boundary [15]. Our prior assumed 49 My as the minimum age, and 100 My as the 95% upper boundary.

**MCRA of Siluriformes** (Fig. 1, node 14). The oldest fossils that have been assigned to the crown Siluriformes are incomplete remains of Ariidae from the Campanian-Maastrichtian of Argentina (Late Cretaceous, 73 Ma) [18]. The oldest siluriforms are *incertae sedis* materials from the Adamantina Formation of Brazil, Turonian-Santonian (Late Cretaceous, 93.6-83.5 Ma) [19]. Our prior thus assumed 73 My as the minimum age, and 83.5 My as the upper boundary.

**MCRA of Bagridae** (Fig. 1, node 15). The oldest bagrid fossils, *Eomacrones wilsoni*, *Nigerium gadense*, *Nigerium wurnoënse*, have all been assigned to deposits in Niger of Paleocene age (~59 Ma) [18]. Our prior assumed 59 My as the minimum age, and 73 My, age assigned to the crown siluriforms, as the upper boundary [18].

**MCRA of Callichthyidae** (Fig. 1, node 16). The oldest callichthyid, *Corydoras revelatus*, dates from the Thanetian of Argentina (Paleocene, ~55 Ma) [18]. Our prior assumed 55 My as the minimum age, and 73 My, age assigned to the crown siluriforms, as the upper boundary [18].

**MCRA of Ictaluridae** (Fig. 1, node 17). *Astephus* sp. from the Thanetian of Wyoming (Paleocene, 56 Ma) provides minimum age for this group [18]. Our prior assumed 55 My as the minimum age, and 73 My, age assigned to the crown siluriforms, as the upper boundary [18].

**MCRA of Argentiniformes** (Fig. 2, node 18). The argentiniform *Nybelinoides brevis* from the Barremian-Aptian of Bernissart, Belgium (Early Cretaceous, 127-124 Ma) marks the earliest appearance of the crown argentiniforms [20]. *Leptolepides sprattiformis* (Orthogonikleithridae) from the late Kimmeridgian lithographic limestone of Cerin, France (Jurassic, 152 Ma) is the oldest euteleost [11]. Our prior assumes 124 My as the minimum age, and 152 My as the upper boundary

**MCRA of Osmeridae** (Fig. 2, node 19). *Speirsaenigma lindoei*, from the Thanetian of the Paskapoo Formation, Alberta, Canada (Paleocene, ~58.7 Ma) is the oldest osmerid fossil [21]. The otoliths from the Late Santonian of Font de las Bagasses, Spain (Late Cretaceous, 87-84 Ma) are used to set the upper boundary [22]. Our prior assumes 58.7 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Galaxiidae** (Fig. 2, node 20). *Stompooria rogersmithi* from the Maastrichthian of South Africa (late Cretaceous, 70 Ma) [23] indicates the appearance of the crown galaxiids. The Argentinidae *Nybelinoides brevis* from the Barremian-Aptian of Belgium (Early Cretaceous, 127-124 Ma) is used to establish the upper boundary [20]. The prior thus assumes 70 My as the minimum age, and 124 My for the 95% upper bound.

**MCRA of Esociformes** (Fig. 2, node 21). The oldest Esociformes is *Estesesox foxi* from the Campanian of the Milk River Fomation, Alberta, Canada (Late Cretaceous, 85 Ma) [24]. *Leptolepides sprattiformis* (Orthogonikleithridae) from the late Kimmeridgian lithographic limestone of Cerin, France (Jurassic, 152 Ma) is the oldest euteleost [11]. Our prior assumes 85 My as the minimum age, and 152 My as the upper boundary.

**MCRA of Salmoniformes** (Fig. 2, node 22). The origin of Salmoniformes is indicated by *Helgolandichthys schmidi*, from the early Aptian of Tock, Helgolang, Germany (Early Cretaceous, 125 Ma) [25]. Due to uncertainty on the placement of this taxon we assign its age to the upper boundary. The minimum age of the crown salmoniforms in our prior is that of *Eosalmo driftwoodensis* from the Lutetian (Eocene, 48 Ma) [26]. Our prior assumes 48 My as the minimum age, and 125 My as the upper boundary.

**MCRA of Aulopiformes** (Fig. 2, node 23). An undescribed alepisauroid from the early Barremian of Alcaine, Oliete subbasin, NE Spain (Early Cretaceous, 130-128 Ma) [27] is used to mark the appearance of the earliest aulopiforms. This taxon is a stem to the crown alepisauriforms present in our analysis, so we assign its age to the upper boundary. The minimum age of the crown alepisauriforms is assigned on the basis of the fossils *Nematonotus* spp. (Aulopodidae) and *Acrognathus dodgei* (Chlorophthtalmidae) from the Cenomanian of Hakel, Lebanon (Late Cretaceous, 98-96 Ma) [13]. The prior assumed 96 My as the minimum age, and 128 My as the upper boundary.

**MCRA of Acanthomorpha** (Fig. 2, node 24). The fossil otoliths assigned to the "genus *Acanthomorphorum*" *forcallensis* from the early Aptian of the Maestrazgo, Castellon Province, Spain (Early Cretaceous, 124-122 Ma) [28]. Various Beryciformes (e.g., *Hoplopteryx, Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) represent the oldest record of crown acanthomorphs [13]. The prior assumed a minimum age of 99 My, and an upper boundary of 122 My.

**MCRA of Beryciformes** (Fig. 2, node 25). Various Beryciformes (e.g., *Hoplopteryx*, *Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) represent the appearance of the crown [13]. The fossil otoliths assigned to the "genus *Acanthomorphorum*" *forcallensis* from the early Aptian of the Maestrazgo, Castellon Province, Spain (Early Cretaceous, 124-122 Ma) [28] is used to identify the upper boundary. The prior assumed a minimum age of 99 My, and an upper boundary of 122 My.

**MCRA of Lampridiformes** (Fig. 2, node 26). *Nardovelifer altipinnis* is the oldest crown lampridiform currently known, from the Late Campanian of Nardó, Italy (Late

Cretaceous, 75-70 Ma) [29]. Undescribed taxa from the Cenomanian of Hakel, Lebanon (Late Cretaceous, 97-98 Ma) [30] represent the earliest appearance of lampridiforms in the fossil record. The prior assumed 70 My as the minimum age, and 98 My as the upper boundary.

**MCRA of Ophiididae** (Fig. 2, node 27) The otoliths of "genus *Ophidiidarum*" *cavatus* from the early Maastrichtian of Coon Creek Tongue, Blue Spring, Mississippi (Late Cretaceous, 70-68 Ma) marks the appearance of the group in the fossil record [31]. The Beryciformes (e.g., *Hoplopteryx, Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) are used to establish an upper boundary [13]. The prior assumed a minimum age of 68 My, and an upper boundary of 99 My.

**Fundulidae vs Poeciliidae** (Fig. 3, node 28). This split is dated by Poeciliidae indet. from the Thanetian of the Maiz Gordo Formation, Argentina (Paleocene, 58-55 Ma) [32]. The Beryciformes (e.g., *Hoplopteryx*, *Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) are used to establish an upper boundary [13]. The prior assumed a minimum age of 55 My, and an upper boundary of 99 My.

**MCRA of Channoidea** (Fig. 2, node 29). *Eochanna chorlakiensis* from the Ypresian and Lutetian (Middle Eocene, 49-48 Ma) marks the appearance of the clade [33]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an

upper boundary [34]. The prior assumed 48 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Cichlidae** (Fig. 3, node 30). *Mahengechromis* spp. From the early Lutetian of the Mahenge paleolake, Tanzania, Africa (Eocene, 46 Ma) [35] is the oldest taxon that can be assigned to the crown cichlids. The otoliths assigned to the "genus *Epigonidarum" weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 46 My as the minimum age, and 84 My as the upper boundary.

**MCRA of African Cichlidae** (Fig. 3, node 31). *Heterochromis* fossils from the late Chattian Ad Daib, Baid Formation, Tihamat Asir, SW Saudi Arabia (Oligocene, 23.3 Ma) are used to date this clade [36]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 23.3 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Gerreidae** (Fig. 3, node 32). *Gerres latidens* from the Ypresian of the Wittering Formation, London Clay, Southern England, UK (Eocene, 54-52 Ma) marks the appearance of the clade [37]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 52 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Gobiidae** (Fig. 2, node 33). Gobiidae indet. From the Lutetian (Eocene, 48-40 Ma) is used to determine the minimum age [38]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 40 as the minimum age, and 84 as the upper boundary.

MCRA of Labridae (Fig. 3, node 34). Several taxa (e.g., Eocoris bloti,

*Phyllopharyngodon longipinnis*) from the late Ypresian of Monte Bolca, Italy (Eocene, 50 Ma) are used to determine the minimum age of the crown labrids [39]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 50 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Moronidae** (Fig. 3, node 35). *Morone* sp. from the Late Campanian of Coffee Sand, Northeastern Mississippi (Late Cretaceous, 74-73 Ma) is the oldest fossil assigned to moronids [40]. Due to the uncertainty of the phylogenetic relationships within the "Perciformes", it is not currently known which is the sister group of the Moronidae. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma), however, indicate the earliest appearance of taxa belonging to the clade of "Perciformes" that likely includes the Moronidae, and are thus used to establish an upper boundary [34]. The prior assumed 74 My as the minimum age, and 84 My as the upper boundary.

**MCRA of Pomacentridae** (Fig. 2, node 36). Several taxa (e.g., *Palaeopomacentrus orphae, Lorenzichthys olihan*) from the late Ypresian of Monte Bolca, Italy (Eocene, 50 Ma) are used to determine the minimum age of the crown pomacentrids [41]. The otoliths assigned to the "genus *Epigonidarum*" *weinbergi* from the Coniacian of Tiefe Gosau, Ennstaler Alpen, Austria (Late Cretaceous, 89-84 Ma) are used to establish an upper boundary [34]. The prior assumed 50 My as the minimum age, and 84 My as the upper boundary

**MCRA of Pleuronectiformes** (Fig. 2, node 37). The otolith of several taxa assigned to the families Bothidae, Citharidae, and Psettodidae from the Late Ypresian of Tuilerie de Gan, Argile de Gan, Gan, Pyrénées-Atlantique, France (Eocene, 52-51 Ma) indicate the appearance of the crown [42]. The Beryciformes (e.g., *Hoplopteryx, Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) are used to establish an upper boundary [13]. The prior assumed a minimum age of 51 My, and an upper boundary of 99 My.

**MCRA of Tetraodontiformes** (Fig. 3, node 38). This oldest fossil that can be assigned to the crown tetraodontiforms is the stem balistoid *Moclaybalistes danekrus*, from the Moclay deposits of Denmark (Palaeocene, 59 Ma) [43]. The oldest tetraodontiform fossil is *Plectocretacicus clarae*, from the Cenomanian of Lebanon (Cretaceos, 98 Ma) [44].

We thus assigned a lower bound of 59 My and an upper bound of 98 My to this calibration.

*MCRA* of Balistoidea (Balistidae vs Monacanthidae) (Fig. 3, node 39). The stem balistids *Balistomorphus*, which includes the species *B. orbiculatus*, *B. ovalis* and *B. spinosus*, and *Oligobalistes robustus* are all from the early Oligocene of, respectively, Switzerland and Caucasus (35 Ma) [43]. They provide a minimum age estimate for the split between balistids and monacanthids. Our prior assigns a minimum age of 35 My to this calibration, and an upper bound of 50 My to this calibration (reflecting the appearance of several other tetraodontiform families in Monte Bolca) [43].

**MCRA of Ostracioidea (Aracanidae vs. Ostraciidae)** (Fig. 3, node 40). *Eolactoria sorbinii* is a stem ostraciid, and *Proaracana dubia* is a stem aracanid [43]. Both are from the Ypresian of Monte Bolca, Italy (middle Eocene, 50 Ma). We assigned a lower bound of 50 My and an upper bound of 70 My, likely age of crown tetraodontiforms, as derived from Alfaro et al. [45] to this calibration.

**MCRA of Tetraodontoidea (Tetraodontidae vs. Diodontidae)** (Fig. 3, node 41). Several stem diodontids, *Prodiodon erinaceus, Prodiodon tenuispiis*, *Heptadion echinus* and *Zignodon fornasieroae* [43], and the stem tetraodontid *Eotetraodon pygmaeus* [43, 46] are known from the Ypresian of Monte Bolca, Italy (middle Eocene, 50 Ma). We used this date as a lower bound and assigned an upper bound of 70 My, likely age of crown tetraodontiforms, as derived from [45] to the calibration. **MRCA of Tetraodontidae** (Fig. 3, node 42). The fossil *Archaeotetraodon winterbottomi* [47] from the Oligocene of Caucasus, Russia, has been assigned to the crown Tetraodontidae, and provides a minimum age estimate of 35 Ma for the MRCA of the family [48]. We assigned an upper bound of 50 My (age of stem tetraodontids, see above) to this calibration.

**MCRA of Caproidae** (Fig. 3, node 43). *Eoantigonia veronensis* from the late Ypresian of Monte Bolca, Italy (Eocene, 50 Ma) marks the split between *Antigonia* and *Capros* [49]. The Beryciformes (e.g., *Hoplopteryx*, *Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) are used to establish an upper boundary [13]. The prior assumed a minimum age of 50 My, and an upper boundary of 99 My.

**MCRA of Zeiformes** (Fig. 2, node 44). *Cretazeus rinaldii* from the Late Campanian of Nardó, Italy (Late Cretaceous, 72 Ma) marks the appearance of the crown zeiforms [50, 51]. The Beryciformes (e.g., *Hoplopteryx*, *Trachichthyoides*) from the Cenomanian of the Lower Chalk, SE England (Late Cretaceous, 99 Ma) are used to establish an upper boundary [13]. The prior assumed a minimum age of 72 My, and an upper boundary of 99 My.

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