Think again

Why coelacanths are not 'living fossils'

A review of molecular and morphological data

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A series of recent studies on extant coelacanths has emphasised the slow rate of molecular and morphological evolution in these species. These studies were based on the assumption that a coelacanth is a 'living fossil' that has shown little morphological change since the Devonian, and they proposed a causal link between low molecular evolutionary rate and morphological stasis. Here, we have examined the available molecular and morphological data and show that: (i) low intra-specific molecular diversity does not imply low mutation rate, (ii) studies not showing low substitution rates in coelacanth are often neglected, (iii) the morphological stability of coelacanths is not supported by paleontological evidence. We recall that intra-species levels of molecular diversity, inter-species genome divergence rates and morphological divergence rates are under different constraints and they are not necessarily correlated. Finally, we emphasise that concepts such as 'living fossil', 'basal lineage', or 'primitive extant species' do not make sense from a tree-thinking perspective.

Keywords:
coelacanth; evolutionary rate; *Latimeria*; living fossil; slow evolution; substitution rate; tree-thinking

Introduction

The term 'living fossil', was coined by Charles Darwin in the first edition of *On the origin of species*... [1], as follows:

> All fresh-water basins, taken together, make a small area compared with that of the sea or of the land; and, consequently, the competition between fresh-water productions will have been less severe than elsewhere; new forms will have been more slowly formed, and old forms more slowly exterminated. And it is in fresh water that we find seven genera of Ganoid fishes, remnants of a once preponderant order: and in fresh water we find some of the most anomalous forms now known in the world, as the Ornithorhynchus and Lepidosiren, which, like fossils, connect to a certain extent orders now widely separated in the natural scale. These anomalous forms may almost be called living fossils; they have endured to the present day, from having inhabited a confined area, and from having thus been exposed to less severe competition.

Since then, the term 'living fossil' has a double meaning: it is a species which has no close living relatives, and which underwent very few changes during the course of evolution. However, in his founding book, Darwin used 'living fossil' in two passing comments only. The term was actually popularised eighty years later after the discovery of *Latimeria chalumnae* [2], an extant species belonging to the infraclass Actinistia that was, at that time, exclusively known from the fossil record (see [3] for review). This unexpected discovery made the extant coelacanths (*L. chalumnae*, and the more recently discovered *L. menadoensis* [4]) the paradigmatic example of a 'living fossil', thus defining them as an evolutionarily conserved group of species that has evolved little over a geological time scale.

Transposing the concept of 'living fossil' to the genomic level has led to the hypothesis of genetic stasis (or at least to the idea of a reduced molecular evolutionary rate) that is in sharp contrast with the principles of evolutionary genetics [5]. Genomes change continuously under the combined effects of various mutational processes, that produce new variants, and genetic drift and selection, that eliminates or fixes them in populations [6]. In other terms, the only possibility for genomes to rep-

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licate without change implies at least one of the two following conditions: (i) new variants do not appear (i.e. no mutations), and (ii) new variants are systematically eliminated by selection (i.e. no genetic drift and very powerful selection against new variants). Of course we can consider a less extreme case, i.e. a reduced evolutionary rate of the genome, but this still implies a lower mutation rate and/or stronger selection against new variants than observed in other species.

However, the long held idea that coelacanths are 'living fossils' has been recently revitalised following several molecular studies that concluded that there is low molecular diversity within populations of *L. chalumnae* [7] and low substitution rates [8–10] in the lineage leading to the two extant coelacanths. A causal link between low molecular evolutionary rate and morphological stasis was systematically hypothesised. In order to re-evaluate the pace of genomic and anatomical evolution in coelacanths, here we review the available molecular and morphological data for this group. We question whether the data actually support the hypotheses of a slowly evolving genome and morphological stability in Actinistia.

Low molecular diversity and low geographic differentiation in coelacanths

Two population genetics studies in *Latimeria chalumnae* are available and both show low allelic diversity and low levels of geographic differentiation [7, 11]. For neutral alleles, diversity depends on two opposite forces, the rate of mutation that is the source of new alleles and the genetic drift that eliminates alleles. The effect of genetic drift depends on the population size, i.e. random changes in allele frequencies are more important in small populations and rare alleles are lost more rapidly [6] (see also [12] for recent review). Thus, for a given mutation rate, the genetic diversity is positively correlated with the population size: smaller populations are expected to show a lower level of genetic diversity. On Comoro Island, where the largest known coelacanth population is situated, the population size is about 300–400 adult individuals [13]. Even if larger populations of coelacanths existed in the past, the small sizes of extant populations are sufficient to explain low genetic diversity in this species. The geographic differentiation of populations depends on abiotic factors such as geographic barriers (e.g. land masses and ocean currents for marine fish) and biotic factors such as migration (e.g. the existence of planktonic larvae and adult migration). *L. chalumnae* and *L. menadoensis* are viviparous species that live in the deep ocean and give birth to juveniles about 30 cm in length [3]. The low genetic differentiation found in *Latimera* could thus be the result of the absence of geographic barriers, and the absence of small pelagic larvae could explain the low impact of ocean currents on population isolation.

Interestingly, although the two available analyses of *L. chalumnae* natural population genetics both detected low allelic diversity and low levels of geographic differentiation, they came to different conclusions. The first study [11] concluded that the low allelic diversity is the result of a small population size and the absence of strong geographic isolation, as has been concluded for similar findings in other species [14]. In contrast, the more recent study, Lampert et al. [7], rejected these straightforward hypotheses. On the assumption that coelacanths are 'living fossils', the authors concluded that their data 'confirm the assumed slow rate of molecular evolution in coelacanths'. As we found this conclusion surprising, we questioned to what extent low mutation rates have been detected in *Latimeria*.

Is the genome of the coelacanth slowly evolving?

The rate of molecular evolution in a lineage, the substitution rate, depends on the mutation rate and the rate of fixation of new variants, which itself depends on the selective values of these new variants and genetic drift [6]. The mutation rate depends on environmental factors and on replication and repair mechanisms that are under selective pressure. The mutation rate is the result of a trade-off between the need for new variants to adapt to environmental changes and selection for a reduced mutation rate because mutations are more often deleterious than advantageous [15]. The substitution rate depends chiefly on fixation of neutral and slightly deleterious mutations [16] and it is thus positively correlated with mutation rate and negatively correlated with population size because selection is more efficient in large populations [17]. An unexpectedly low substitution rate could thus be the result of a peculiarly low mutation rate and/or an especially strong selection against deleterious mutations [16, 17].

Molecular support for the hypothesis that *L. chalumnae* and *L. menadoensis* have slowly evolving genomes comes chiefly from two studies on HOX gene clusters [8, 9]. This is supported by an analysis of the *L. menadoensis sonic hedgehog* (*shh*) locus that has low substitution rates [10]. Assuming that the Hox gene clusters and *shh* are evolving particularly slowly, does this indicate a general trend for the whole nuclear genome? Analyses of three nuclear genes (*protocadherin* clusters [18], *vitellogenin* genes [19], and *nuclear-encoded recombination activating genes* [19, 20]) and two of the whole mitochondrial genome [21, 22] are often quoted in support of the hypothesis of slow evolution. However, a closer look at the data challenges this interpretation: depending on the analysed sequence, the coelacanth branch is not systematically shorter than the branches leading to other species. In addition, most phylogenetic analyses – including analysis of Hox sequences [23–26] – do not support the hypothesis that the *Latimeria* genome is slow evolving, i.e. they do not place coelacanth sequences on short branches nor do they detect low substitution rates [24, 27–31]. The clearest example, which involves the largest number of genes, is a phylogeny based study of forty-four nuclear genes that does not show a dramatic decrease, if any, in the rate of molecular evolution in the coelacanth lineage [32]. What we know about the biology of coelacanths does not suggest any obvious reason why the coelacanth genome should be evolving particularly slowly. They do

not live in an environment that suggests unusual pressure to improve molecular mechanisms, such as DNA replication and repair, resulting in a very low mutation rate [33]. They do not have an extremely long generation time, and we have previously discussed that they definitely do not form large populations [13]. In addition, it is well known that substitution rates vary across a genome depending on several factors, such as the local recombination rate and distance to the origin of replication; it is also known that substitution rates are not constant across lineages [34–36]. In addition, it is likely that amino acid interactions are very important in variation in the rate of protein evolution in different lineages [37]. Therefore, some genes may appear to be evolving faster and other genes more slowly when comparing genes from one species to those from another species. The genome of *L. chalumnae* has been recently sequenced (Broad institute: Coelacanth Genome Project) and the issue of substitution rates could therefore be analysed more completely. We anticipate that, as in any species, an analysis would show lineage specific gene deletions and gene duplications, some genes evolving faster and others more slowly than in other lineages.

In addition, we would like to emphasise that even if a lower substitution rate was shown at the scale of the whole genome, it is unlikely that it could explain, or be explained by, the rate of morphological evolution as the two evolutionary rates are not linked in such a simple way. For example, we and other colleagues have recently found that serially homologous structures, that is, branchial arches, somites and rhombomeres, have undergone a high rate of morphological differentiation during gnathostome evolution, but show very few differences in Hox gene expression [38]. A key factor controlling the mutation rate is the effective genome size, i.e. the fraction of the genome that is under selection. For prokaryotes, it is approximately the whole genome, whereas for eukaryotes, it can be only a tiny fraction of the genome. For comparison purposes, the number, or the sum of the length, of the protein-coding genes is a good proxy of the effective genome size. As only one highly deleterious mutation in a whole genome is sufficient to result in low fitness in this genome, for a given mutation rate, the probability of the occurrence of a highly deleterious mutation increases with the effective size of a genome. We expect that the mutation rate is negatively correlated with the effective genome size since a genome with a smaller effective size can afford a higher mutation rate than a genome with a higher effective size. Another key factor is the effective population size that is also negatively correlated with the mutation rate because it imposes a drift barrier, high in small populations and lower in larger populations, to the evolution of higher replication fidelity [15, 39]. It is known that the size of the coelacanth genome and the number of genes it contains is comparable to other vertebrate species (http://www.ensembl.org/Latimeria_chalumnae/Info/Annotation/#assembly) and the effective population size is not particularly large (see above), thus we would not expect that a lower mutation rate driven by these two factors would have evolved in this lineage. If there is no obvious biological reason to favour the hypothesis of a low substitution rate at the genome scale in *Latimeria*, one could question why conclusions of alternate studies are ignored, even when they are quoted. Symptomatically, a recent paleontological study [40] quoted the work on Hox genes in support of the slow evolving coelacanth hypothesis and omited other molecular studies. It is striking that molecular studies supporting low substitution rates are interpreted in light of the a priori hypothesis that extant coelacanths are 'living fossils' because their morphology has changed very little from that in the known fossil record (Table 1). We therefore question if the morphological conservatism of coelacanth is actually supported by the available data on fossil Actinistia.

Table 1. 'Living fossil' hypothesis in recent studies of nuclear genes of *Latimeria*

Articles (numbered as in reference list)	'Living fossil' hypothesis	Conclusion of slow evolution of coelacath genome
[7–10]	Assumed	Yes
[19]	Assumed	No
[11, 23, 24, 27, 31]	Not evoked	No
[28, 29]	Criticised	No

Publications since 2010 were scanned for keywords 'coelacanth' or 'Latimeria', 27 articles were retrieved. Articles that analysed only morphological or histological data ($n = 5$), or only mitochondrial sequences ($n = 2$), or made very indirect mention of *Latimeria* ($n = 1$), were discarded. Nineteen molecular studies were further examined and seven were discarded because they were not informative on substitution rates. The 12 remaining articles were examined for a priori hypotheses and conclusions about the rate of evolution in *Latimeria*. Note that the four recent molecular studies that explicitly concluded that the genome of *Latimeria* is slowly evolving also explicitly assumed that coelacanths are 'living fossils'. An additional study that assumed the 'living fossil' hypothesis only suggested that it could be the case. In contrast, all other studies which did not conclude that the genome is slowly evolving, criticised or did not evoke the 'living fossil' hypothesis.

Do coelacanths actually show morphological stability?

No fossil is available either for extant coelacanth species or for the genus *Latimeria* itself [3]. This suggests that palaeontologists – even those that are convinced that coelacanths are 'living fossils' – have considered morphological differences between extant and fossil coelacanths to be so extensive that they should be grouped in separate genera [41]. In fact, coelacanth body shapes are much more diverse than is generally thought (Fig. 1). The vertebral column of coelacanth genera shows considerable variation in the number of neural

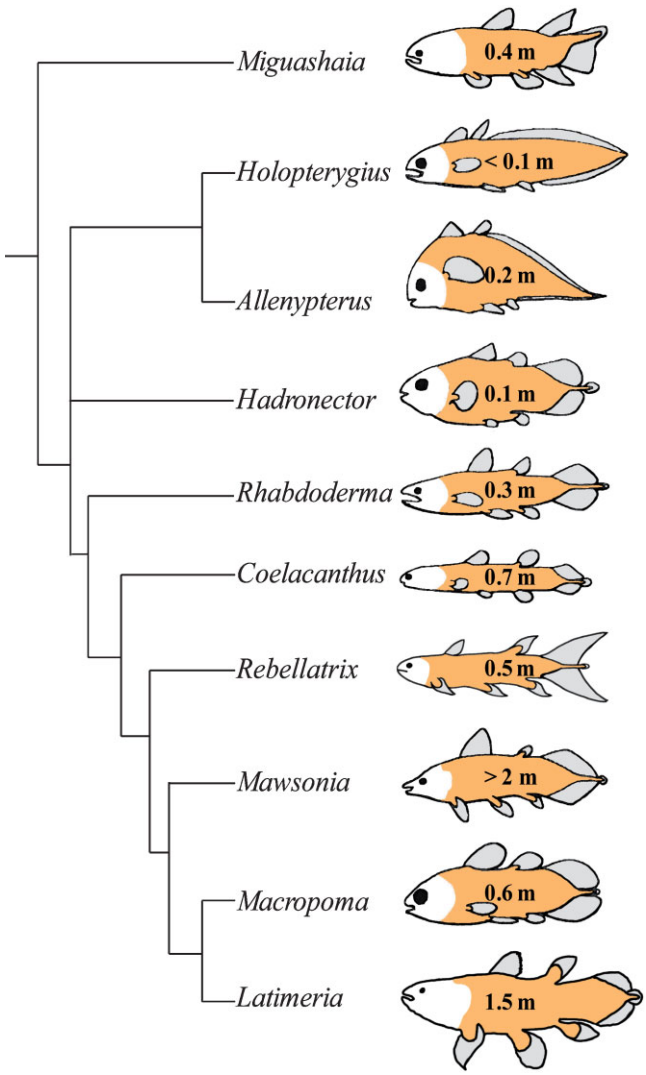

Figure 1. Comparison of extant and selected extinct actinistians, commonly known as coelacanths. A phylogeny of Actinistia; schematic sketches of body outlines and approximate body length (given in metre) illustrate the morphological diversity of extinct coelacanths: some had a short, round body (*Hadronector*), some had a long, slender body (*Rebellatrix*), some were eel-like (*Holopterygius*) whereas others resembled trout (*Rhabdoderma*), or even piranha (*Allenypterus*). Note that the body shape of *Latimeria chalumnae* differs significantly from that of its closest relative, *Macropoma lewesiensis*. Adapted from [3, 42, 43].

and haemal arches, as well as in the spacing throughout the abdominal and caudal regions, suggesting that they probably had diverse modes of locomotion [3, 42].

In addition, an examination of the skeleton of the fossil genus *Macropoma* (approximately 70 Ma), the sister group of *Latimeria* and the only known fossil actinistian record from the Cretaceous to the present [43], shows some interesting differences. Not only are the extant coelacanths three times larger than their closest extinct relatives (about one and a half metres vs. half a metre), but there are also numerous structural differences. The swim bladder is ossified in *Macropoma* but filled with oil in *Latimeria* (Fig. 2A and B), indicating they were probably found in different types of environments [3]. There are also noticeable differences in the vertebral column (the post anal region is shorter and ventral spines extend less ventrally in *M. Lewesiensis* compared with *L. chalumnae*), and in the attachment bones of the fins (Fig. 2A–D). In addition, *Macropoma* and *Latimeria* have distinctly dissimilar skull anatomies, resulting in noticeable differences in head morphology (Fig. 2C and D).

Finally, it should not be forgotten that external morphological resemblances can be based on a very different internal anatomical organisation. The most often emphasised resemblance between coelacanths is that they all have four fleshy-lobed-fins. Until recently, the anatomy of the lobed fins of coelacanths was only known in *Latimeria*, in which the pectoral fin endoskeleton is short and symmetrical (Fig. 2E). In 2007, Friedman et al. [44] described the endoskeleton of the pectoral fin of *Shoshonia arctopteryx*, a coelacanth species from the mid Devonian, and therefore contemporary with *Miguashaia* (Fig. 1). They showed that this earliest known coelacanth fin endoskeleton is highly asymmetrical (Fig. 2E), a characteristic that is probably ancestral since it resembles the condition found in early sarcopterygians such as *Eusthenopteron*, *Rhizodopsis* or *Gogonasus*. This result is additional support, if needed, that extant coelacanths have not remained morphologically static since the Devonian.

'Living fossils' from a tree-thinking perspective

During the last century, the concept of 'living fossil' as a result of evolutionary stasis has been increasingly regarded as misleading as milestones have been added to Darwin's theory of evolution. Hennig [45], clarified the speciation process (or cladogenesis) by showing that the ancestral species ceased to exist as two new sister species formed (see [46] for review). In addition, Gould [47] and Kimura [6], have reinforced the idea that evolution is a bushy process that has not stopped in any lineage (see [48] for review). However, the phylogenetic paradigm, also called 'tree-thinking' point of view [49], has encountered difficulties in spreading outside the community of evolutionary researchers [50, 51]. The most common misunderstanding of tree-thinking and evolution is the remnant misinterpretation of biodiversity as a 'ladder of progress' [52], a concept that originated in the pre-evolutionist idea of *scala naturae* that came from antiquity as it appeared in

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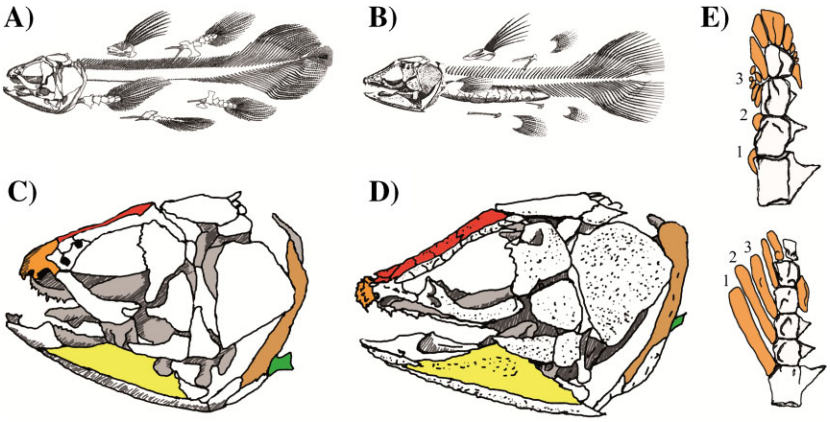

Figure 2. Comparison of the skeleton of extant and selected extinct coelacanths. **A–D:** *Latimeria* and its sister group *Macropoma* show numerous skeletal differences. **A, B:** Overall view of the skeletal organisation of the extant coelacanth and of its closest relative. **A:** *Latimeria chalumnae*. **B:** *Macropoma lewesiensis*. Relative to the body length, in *L. chalumnae* the vertebrae are smaller, the truncal region of the vertebral column is longer and the post anal region is shorter than in *M. lewesiensis*. In the latter region, the hemal arches (ventral spines) extend more ventrally in *M. lewesiensis* than in *L. chalumnae*. In addition, the swim bladder is ossified in *Macropoma* but not in *Latimeria*, and the basal bone of the first dorsal fin is characteristic of each genus. **C, D:** Comparison of the skulls of *L. chalumnae* and *M. lewesiensis*. **C:** In *L. chalumnae*, the mouth opens upward, the articular bone (yellow) is long and narrow, the parietonasal shield (red) is short, the premaxillary bone (orange) is devoid of denticle ornamentation, the dorsal part of the cleithum (light brown) is spiny, and the scapulocoracoid (green) is located on the ventral side. **D:** In contrast, in *M. Lewesiensis*, the mouth opens forward, the angular bone (yellow) is triangular, the parietonasal shield (red) is long, the premaxillary (orange) protrudes and forms a hemispherical snout which is ornamented with prominent denticles, the dorsal part of the cleithrum (light brown) is thick, and the scapulocoracoid, (green) is located more medially. Modified from [3]. **E:** Pectoral fin skeleton of *L. chalumnae* (above) and *Shoshonia arctopteryx* (below). The three first preaxial radials are numbered from proximal to distal. In *L. chalumnae* the fin appears nearly symmetrical because radial bones (orange) are arranged nearly symmetrically about the fin axis. The proximal preaxial radials 1-2 are extremely short and bear no fin ray, and the preaxial radial 3 is short and fractionated. In contrast, in *S. arctopteryx* the fin is strongly asymmetrical chiefly because proximal preaxial radials are long and all bear fin rays. Modified from [44].

Aristotle's *Historia Animalium* [53]. Although Darwin coined the term 'living fossil', it is unlikely that he actually thought that an extant species would be identical to an ancestral species. Indeed, Darwin warns his readers against identifying an extant species with an ancestor by writing:

> In the first place it should always be borne in mind what sort of intermediate forms must, on my theory, have formerly existed. I have found it difficult, when looking at any two species, to avoid picturing to myself, forms directly intermediate between them. But this is a wholly false view; we should always look for forms intermediate between each species and a common but unknown progenitor; and the progenitor will generally have differed in some respects from all its modified descendants [1].

The concept of *scala naturae* was brought formally into an evolutionary framework by Haeckel [54] who misinterpreted Darwin's metaphor of a tree of life and incorporated some extant species into the trunk of the tree of life instead of placing them as leaves (terminal nodes). In this view, 'living fossils' were considered as 'ancient' or 'primitive' suggesting that they had stopped evolving long ago. As this archetypal, or essentialist, species concept persists (consciously or not) in a lot

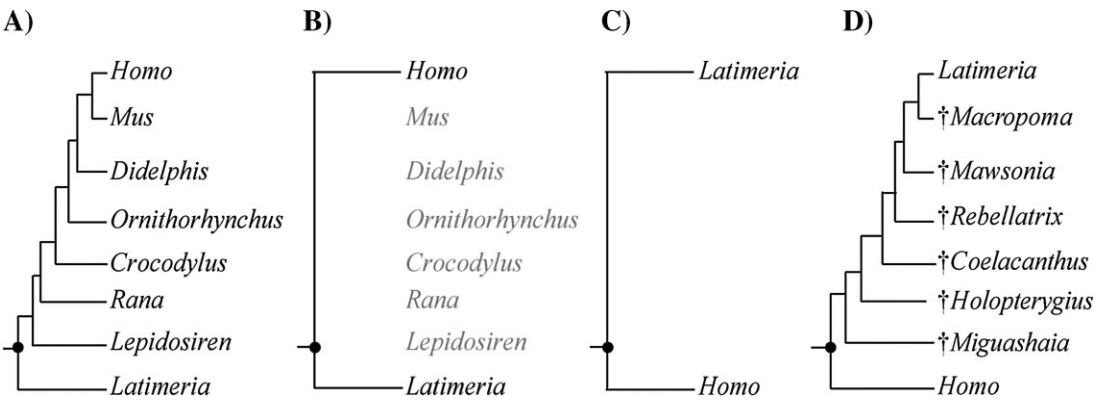

Figure 3. No species is 'early branching' or 'basal' per se. **A:** Traditional presentation of Sarcopterygii phylogeny places human beings on top and shows a detailed phylogeny of Amniota whereas few Dipnoi or Actinistia are sampled. In this tree, lungfish or coelacanths *appear* to have diverged early and to belong to poorly diversified clades. This is artefactual and depends on species sampling and tree presentation. **B:** Cutting the intermediate branches between human and coelacanth species shows that these species diverge at the level of the same node, that corresponds to the last common ancestor of Sarcopterygii (black circle). **C:** As nodes are free rotating, the tree shown in B could be presented with *Latimeria* on top and human beings at the bottom. **D:** A tree compatible with the one presented in A could be obtained by re-sampling Actinistia and taking humans as sole representative of Rhipidistia. In the latter tree, humans now *appear* early diverging and belonging to a poorly diversified clade, whereas *Latimeria appears* late diverging and belonging to a bushy clade.

of modern studies [55, 56], we would like to recall here that the coelacanths, like any extant species, should not be considered as 'ancient', or 'primitive', or 'basal', or 'early branching' in the context of a phylogenetic tree [46, 57]. Indeed, if *Latimeria* is often interpreted as an isolated species that diverged before the diversification of tetrapods, it is due to the combined misleading effect of the chosen panel of species and a misinterpretation of the divergence between Actinistia and Tetrapoda. Traditionally, the human species is placed on top and the Amniota clade is more fully developed than some others like Dipnoi or Actinistia which therefore *appear* to have diverged early and be poorly diversified, whereas human beings *appear* to be nested in a bushy clade (Fig. 3A). A closer look at tree topology shows that humans and actinistians diverged at the same node, i.e. the last common ancestor of Sarcopterygii, and therefore *Latimeria* does not diverge earlier or branch deeper than *Homo* (Fig. 3B). Of course, a node could be located deeper, or closer to the base, than another node, but no species should be flagged as 'basal' or 'early branching' per se. In addition, re-sampling a traditional phylogeny by choosing *Homo* as the sole member of Rhipidistia, and including fossil actinistians shows that *Latimeria*, like any other species, belongs to a bushy clade (Fig. 3D).

Conclusion and outlook

Latimeria was first labelled as a 'living fossil' because the fossil genera were known before the extant species was discovered, and erroneous biological interpretations have grown and reports still show little morphological and molecular evolution. A closer look at the available molecular and morphological data has allowed us to show that most of the available studies do not show low substitution rates in the *Latimeria* genome, and furthermore, as pointed out by Forey [3] long before us, the supposed morphological stability of coelacanths from the Devonian until the present is not based on real data. As a consequence, the idea that the coelacanth is a biological 'living fossil' is a long held but false belief which should not bias the interpretation of molecular data in extant *Latimeria* populations. The same reasoning could be generalised to other extant species (such as hagfish, lamprey, shark, lungfish and tatuara, to cite few examples of vertebrates) that for various reasons are often presented as 'ancient', 'primitive', or 'ancestral' even if a lot of recent data has shown that they have many derived traits [58–64]. We hope that this review will contribute to dispelling the myth of the coelacanth as a 'living fossil' and help biologists keep in mind that actual fossils are dead.

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