The salmon, the lungfish (or the coelacanth) and the cow: a revival?

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In the late 1970s, intense and sometimes acrimonious discussions between the recently established phylogeneticists/cladists and the proponents of the long-standing ‘gradistic’ school of systematics transcended specialized periodicals to reach a significantly wider audience through the journal Nature (Halstead, 1978, 1981; Gardiner et al., 1979; Halstead et al., 1979). As is well known, cladists ‘won’ the debate by showing convincingly that mere similarity or ‘adaptive levels’ were not decisive measures to establish kinship. The essay ‘The salmon, the lungfish and the cow: a reply’ by Gardiner et al. (1979) epitomized that debate, deliberating to a wider audience the foundations of the cladistic paradigm, advocating that shared derived characters (homologies) support a sister-group relationship between the lungfish and cow exclusive of the salmon (see also Rosen et al., 1981; Forey et al., 1991).

It is therefore noteworthy that more than 30 years later the pages of the same journal recently featured a contribution in which the foremost conclusion is that the lungfish is ‘the closest living fish relative of tetrapods’ (Amemiya et al., 2013). News that coelacanths and lungfishes are closer to humans and other mammals than they are to any ray-finned fishes, such as the tuna and salmon, and that the closest living ‘fish’ relative of tetrapods is the lungfish, echoed with great fanfare in the international media (e.g. Morelle, 2013; Morin, 2013; Wade, 2013; Woolston & Nature Magazine, 2013) obscuring other significant contributions of Amemiya et al.’s study. For example, in describing the entire genome of the African coelacanth, they filled an important gap in understanding evolutionary changes (including gene losses) underscoring adaptations to live on land by detecting which gene changes were likely involved in immunity, nitrogen excretion and development of the fins, tail, ear, brain, and olfaction. Amemiya et al. (2013) were further able to identify that protein-coding genes in coelacanths are evolving more slowly than those of tetrapods and dipnoans (while having, in contrast, a wide variety of transposable elements that resembles many other vertebrate genomes), and that changes in regulatory elements are closely associated with the transition to a terrestrial environment. Another contribution was to compare whole genomes of related species to uncover thousands of conserved, non-coding regulatory elements. Their findings will certainly provide new directions for research on genomic mechanisms involved in vertebrate evolution.

Nevertheless, the phylogenetic approach of Amemiya et al. (2013) has caused us some concern by not recognizing the irreplaceable morphological component (especially paleontological, in the present case) necessary to properly elucidate the phylogeny and evolution of ancient lineages. We concede that some of the blame may be attributed to the media, which frequently distorts or paints with vivid colors topics of scientific research that are more aligned with their business interests, but some tendencies in Amemiya et al. (2013) appear to be common in large molecular studies, requiring a critical appraisal.

The first issue is the omission of the overwhelming morphological evidence favoring the lungfish-tetrapod sister-group relationship, even though hundreds of published pages exist just in the ‘cladistic era’ expounding this evidence (e.g. Løvtrup, 1977; Miles, 1977; Wiley, 1979; Forey, 1980, 1986; Rosen et al., 1981; Maisey, 1986; Panchen & Smithson, 1987; Benton, 1990; Ahlberg, 1991; Cloutier & Ahlberg, 1996; Janvier, 1996; Zhu et al., 2001, 2009, 2010; Zhu & Yu, 2002; Clack, 2012). This well-known anatomical evidence is barely mentioned in their paper or in its 135 pages of supplementary files. Only two specialized accounts dealing with morphology are

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mentioned at all, but these are cited just to support particular assumptions: one for the inferred morphological stasis of coelacanths and one concerning the phylogenetic position of coelacanths and dipnoans (Zhu et al., 2012 and Schultze & Trueb, 1991, respectively). Morphology is clearly not the focus of their study, but ideas about basal sarcopterygian relationships can hardly avoid even a cursory glance at the evolutionary information it provides.

A second point concerns Amemiya et al.’s (2013) portrayal of the affinities between coelacanths, lungfishes, and tetrapods as a dilemma still awaiting a better-supported outcome. This may be the case in terms of molecular phylogenetic studies but the morphological evolutionary scenario is more consistent—lungfishes are regularly portrayed as the sister-group of tetrapods. Within the cladistic paradigm, the contrasting hypothesis that Latimeria, or Actinistia, is closer to tetrapods than are lungfishes was left implicit by Miles (1975) and Fritzsch (1987), and presented in a more assertive manner by Gorr et al. (1991) and Schultze (1986), while Northcutt (1986) suggested that actinists are the sister group of lungfishes. However, these proposals were abandoned as soon as all putative homologies supporting them were reexamined and interpreted and new characters and taxa were incorporated into the analyses, as prescribed by the primer of good cladistic practice (Meyer & Wilson, 1991; Stock & Swofford, 1991; Platt, 1994; Cloutier & Ahlberg, 1996; Platt et al., 2004, and many other references cited here).

Unsolved conflicts on sarcopterygian relationships may exist, no doubt, such as when analyses are built from different sources of characters and employ a distinct taxonomic sampling and methodological perspective. But in phylogenetic studies based on morphology this situation is rare because researchers are constantly reviewing the entire body of available evidence and, when appropriate, reinterpreting homologies and adding new characters and taxa. As a result the most commonly accepted hypothesis is, in general, the latest one to appear, which is also usually the most complete in terms of characters and taxa. On the other hand, conflicts emerge when morphological phylogenies are compared to molecular phylogenies, due to difficulties in reconciling different sources of characters, or even among molecular studies when they are based on different genes. Symptomatically, some molecular analyses suggest that dipnoans are the closest living relatives of land vertebrates (Meyer & Wilson, 1990, 1991; Meyer & Dolven, 1992; Hedges et al., 1993; Brinkmann et al., 2004), others contend that coelacanths are closer to tetrapods (e.g. Gorr et al., 1991), whereas others indicate a sister-group relationship between lungfishes and coelacanths (e.g. Yokobori et al., 1994; Blair & Hedges, 2005).

More importantly, morphology provides numerous insights unobtainable in the mega-phylogenomic analysis of Amemiya et al. (2013). The mapping of genomic changes associated with the water-to-land transition in vertebrates exemplifies this. Amemiya et al. (2013) stated that because Latimeria chalumnae is the species with a completely sequenced genome closest to our most recent aquatic ancestor, it provides a unique opportunity to identify genomic changes that were associated with the successful adaptation of vertebrates to the terrestrial environment. Even though lungfishes are considered more closely related to tetrapods than the coelacanth, living lungfish species have huge genomes that are presently computationally cumbersome (Gregory, 2005 and references therein). But Amemiya et al. (2013) analyzed few living sarcopterygians—the marine actinistian Latimeria, which inhabits relatively deep volcanic slopes (Fricke et al., 2000; Hissmann et al., 2006), the obligatory air-breather Protopterus, a dipnoan present in shallow, stagnant continental waters of Africa (Budget, 1901), and two species of frogs (as representatives of basal tetrapods). Clearly, only living species can provide high quality and complete genetic material, but interspersed between them are many extinct lineages displaying distinct morphologies, ecologies, behaviors, and habitats. Although not attainable by direct observation, information on the ecology, habitat, locomotion, respiration, and feeding habits of extinct sarcopterygian fishes may be inferred from an array of anatomical data including body form, axial and appendicular skeletons (form and position of fins), mouth position, size, dentition, jaw suspension, position of eyes, opercular arrangement, gill arches, and hypobranchial apparatus (e.g. Thomson, 1969; Clement & Long, 2010). In other words, our understanding of this specific evolutionary transition benefits from knowledge of living sarcopterygians but mostly relies on the detailed morphological study of numerous ‘intermediate’ fossils. These studies, capably summarized by Clack (2012), reveal the gradual sequence of acquisition of morphological features that aided the transition from water to land, such as the dorsal elongation of the pubis, the development of a ‘neck’ and a stouter, more imbricated rib cage, specific morphology of zeugo- and autopodia, flattening of the head, among numerous other featured. One could also cite the analogous situation concerning the evolution of the mammalian middle ear, which involved stepwise transformations of the maxillary and hyomandibular arches from stem gnathostomes to basal mammaliaforms, and all superbly revealed not only in ontogeny but also through the discovery and detailed study of many ‘intermediate’ fossils (e.g. Rougier & White, 2006; Meng et al., 2011).
FIGURE 1. (continued on next page)
Figure 1. Cladogram depicting phylogenetic relationships among basal sarcopterygians. Phylogenetic information was mainly compiled from: Cloutier & Ahlberg (1996) and Zhu et al. (2009, 2012) for Sarcopterygi; Zhu et al. (2009) for †Achoania, †Ligulalepis, †Meeinmania, †Gaiyu, and †Psarolepis; Lu & Zhu (2010) for Onychodontiformes; Friedman (2007b), Dutel et al. (2012), and Zhu et al. (2012) for Actinistia; Ahlberg (1991), Ahlberg et al. (2006), Cione et al. (2007), Cavin et al. (2007), Friedman (2007a), Agnolin (2010), and Clement & Long (2010) for †Diabolepis, †Ponwichthys, †Youngolepis, and Dipnoi; Cloutier & Ahlberg (1996) and Clement (2001) for Porolepisiformes; Ahlberg et al. (2003) and Swartz et al. (2012) for Tetrapodomorpha [despite Holland (2013) having proposed important changes in tetrapodomorph phylogeny, his work was not here considered because many dipnoan terminals were lacking]; Holland et al. (2007) for Rhizodontidae; Ahlberg et al. (2008) for Elpistostegalia; and Carroll (2007) for Tetrapoda. The cladogram was built with the software Mesquite, version 2.75 (Maddison & Maddison, 2011). Names of terminals that have extant members are marked in black.
To demonstrate this point we present in Figure 1 a rather complete summary to genus level of the current state of knowledge of basal sarcopterygian relationships. Represented here are only those fossils that have their positions assigned with some precision; taxa represented only by heavily fragmented specimens were excluded. For the sake of simplification, genera admittedly polyphyletic or whose monophyly is unsubstantiated (e.g. the porolepiforms †Holoptichius and †Porolepis, and the dipnoans †Chirodipterus and †Griphognathus; see Clement, 2001; Friedman, 2007a; Downs et al., 2013) were represented as single terminals (the inclusion of other species of these genera would add several new branches, substantially increasing the complexity of the tree). Living amphibians were represented by its three orders (Anura, Gymnophiona, and Urodela), despite that only two species of frogs were included by Amemiya et al. (2013). Of the 178 terminal taxa of this cladogram (Figure 1), living sarcopterygians (the coelacanth Latimeria, the dipnoans Lepidosiren, Neoceratodus, and Protopterus, the lissamphibians Anura, Urodela, and Gymnophiona, and Amniota) represent only a tiny fraction of the mostly extinct cladal diversity of the Sarcopterygii. In a very simplified perspective, the actinistian Latimeria is separated from the dipnoan Protopterus by more than 46 cladogenetic events, while the oldest common ancestor of Dipnomorpha (Dipnoi + Porolepiformes) is distant from the nearest common ancestor of living amphibians by at least 29 cladal diversification events.

The ability to breathe air is a milestone in the occupation of the terrestrial environment. But while the ability of dipnoans to breath air has been in existence in some capacity since the Middle Devonian (Clement & Long, 2010), mandatory air ventilation, accompanied by a number of physiological and anatomical modifications, as seen in the extant dipnoans Lepidosiren and Protopterus, is likely to have appeared much later. Based primarily on broader comparisons including fossils, the similarities in air-breathing between Dipnoi and Tetrapoda were, consequently, rejected as synapomorphies and deemed non-homologous (cf. Cloutier & Ahlberg, 1996; Zhu et al., 2009). In this sense, despite agreeing with the hypothesis based on morphology, strong support for the sister-group relationship between Protopterus and tetrapods obtained by Amemiya et al.’s (2013) study may have been artificially inflated by molecular homoplasies associated with anatomical and physiological adaptations to their mandatory air-breathing behaviors, a problem that could be minimized with the inclusion of the Australian lungfish Neoceratodus, which does not share those modifications to the same degree.

Moreover, some of the most basal members of Actinopterygii—the counterpart of Sarcopterygii, namely Polypterus, Amia, and Lepisosteus—have a bimodal respiration pattern, undergoing facultative air breathing by using their gas bladders as sites for gas exchange in the same way as living sarcopterygian fishes (Magid, 1966, 1967; Johansen et al., 1970; Rahn et al., 1971; Deyst & Liem, 1985; Graham, 1997). Many representatives of basal teleostean lineages, such as juveniles of the elopomorph Megalops (Megalopidae), and the osteoglossomorphs Arapaima (Arapaimidae), Heterotis (Arapaimidae), Notopterus (Notopteridae), and Pantodon (Pantodontidae), are also obligatory air breathers using highly vascularized and subdivided gas bladders (Babcock, 1951; Dehadrai, 1962; Poll & Nysten, 1962; Schwartz, 1969; Greenwood & Liem, 1984; Merrick & Schmida, 1984; Graham, 1997). Therefore, taking into account that Latimeria is understood to comprise highly divergent, water-breathing fishes, these basal, air-breathing actinopterygians would be more suitable candidates for a comparative genomic research program aiming to trace evolutionary changes relevant to the water-to-land transition. Furthermore, it is not unlikely that air breathing and terrestriality may have taken place on more occasions during evolution, as indicated by the large spiracular openings on top of the skull and advanced internal spiracular architecture of †Gogonasus, a fish-like tetrapodomorph from the Late Devonian of Australia (Long et al., 2006; Clement & Long, 2010), and by the digit-like radials of †Sauripterus, a rhipidistian from the Late Devonian of Pennsylvania (Daeschler & Shubin, 1997).

The lack of a comprehensive phylogenetic framework suitable to support interpretations of genomic structure and its possible implications for the evolution of a given group can lead to erroneous interpretations. In the present case, living coelacanths are repeatedly considered to be morphologically conserved in relation to older actinistsians, and hence commonly dubbed ‘living fossils’, a condition that has been endorsed by their low rates of protein-coding gene evolution (Noonan et al., 2004; Amemiya et al., 2010, 2013). Apart from the fact that low intra-specific molecular diversity does not necessarily imply in a low mutation rate (cf. Casane & Laurenti, 2013), the issue is that coelacanths along with lungfishes are the few fish-like survivors of the Sarcopterygii, a Devonian lineage (over 410 million years old) that diversified into more than 32,700 species of living tetrapods (cf. Schipper et al., 2008; AmphibiaWeb, 2013; Clements et al., 2013; Uetz, 2013) but that possessed, also, a stunning diversity of extinct fish-like forms (Figure 1). Differing from the most typical habitus of coelacanths, which is shared by the
well-known *Latimeria*, are actinistians as aberrant as the small sized, tear-drop shaped *Allenpyerus* and the stout and small-sized (about 10 cm long) *Hadromector*, both from the Early Carboniferous of Montana (Melton, 1969; Lund & Lund, 1984, 1985). The mid-Late Devonian *Holopterygius* is an eel-like actinistian, with a diphycercal caudal fin (Friedman & Coates, 2006) reminiscent of the extant cusk-eels and brotulas of the order Ophidiiformes, fishes that typically live within confined spaces such as caves and even inside body cavities of echinoderms (cf. Nielsen et al., 1999; Nelson, 2006). The recent discovery of the tuna-like *Rebellatrix divaricera* from the Lower Triassic of British Columbia, which bears a streamlined body and a deeply forked caudal fin, suggestive of a fast-swimming and active lifestyle (Wendruff & Wilson, 2012), also undermines the ingrained notion that coelacanths are morphologically static over their roughly 410 million years of existence (cf. Friedman & Coates, 2006; Wendruff & Wilson, 2012; Casane & Laurenti, 2013). Even within the crown clade Latimerioidei, the differences between head shape, dentition, structure of swim bladder (lung), and several other anatomical structures, as well as in paleohabitats, are striking, such as those exhibited by the Lower Cretaceous marine *Axelrodichthys* (e.g. Maisey, 1986, 1991) and the Triassic freshwater *Chinlea* (cf. Elliott, 1987; Dutel et al., 2012; Casane & Laurenti, 2013).

The essay by Amemiya et al. (2013) may also highlight a broader issue at the very core of systematics. Recent technological developments have prompted the advancement of phylogenetic hypotheses based on whole genomes, most of which have been featured in leading scientific journals (e.g. Merchant et al., 2007; Hackett et al., 2008; Putnam et al., 2008; Faircloth et al., 2013). It is of course true that these mega-genomic approaches are highly promising and exciting for evolutionary biology (see Eisen & Fraser, 2003). However, as currently conducted, these studies may unintentionally reinforce an undesired conflict centered on the nature of the data employed (morphology vs. molecules). This antagonism is apparent in the repeated exclusion of morphological data and literature—molecular phylogenies are commonly identical to known morphological hypotheses but are repackaged as ‘new’ and portrayed as innovative simply because they are built from matrices of thousands of characters that decipher entire genomes. Phylogenomics is thereby perceived as a Rosetta stone for solving outstanding evolutionary questions, supposedly untangling conflicts between different phylogenetic hypotheses based on molecules or on so-called ‘traditional’ morphology (e.g. Delsuc et al., 2005, 2006; Hackett et al., 2008; Amemiya et al., 2013; Faircloth et al., 2013).

While it is true that these macro-genomic initiatives potentially clarify controversies between phylogenies derived from different sections of the genome, this reasoning does not apply to all phylogenetic hypotheses generated from morphological characters. That is because the phenotype is only a partial expression of the genome, shaped by the evolution of developmental and ecological interactions. Despite this fact, complex morphological phylogenies are frequently presented as only one hypothesis among many. This is exemplified by the treatment given to the monumental work of Livezey & Zusi (2006, 2007) on the higher-level phylogeny of Aves in the phylogenomic study by Hackett et al. (2008), which gave it the same importance as the molecular phylogeny of Sibley & Ahlquist (1990), an influential but outdated work, and Peters et al.’s (1931–1979) ‘Check-list of Birds of the World’, whose classification was entirely phenetic. Another example of this lack of due credit is the molecular phylogenetic study of archosaurs by Xia et al. (2003), who fail to acknowledge the overwhelming morphological evidence in support of the close relationship between birds and crocodiles (e.g. Gauthier et al., 1988a, b; Kemp, 1988; Benton, 1990; Patterson et al., 1993), maintaining an unrealistic uncertainty about the issue.

Combining mega-genomic data and morphology, or simply understanding the relative contribution of both kinds of data within their specific contexts, can generate a clearer picture of major events in evolution. Evolutionary scenarios, even those founded on massive genetic evidence, are incomplete without the interpretation of specific morphological transformations. The effort to establish a functional correspondence between complex gene networks and phenotypes is still in its infancy, so to choose a molecular phylogeny over a morphological one based simply on the number of characters (base-pairs) and the appeal of technology is inappropriate. Furthermore, the increasingly more common trend of selectively sorting those anatomical features that corroborate a ‘true’ molecular phylogeny (e.g. Jiménez-Guri et al., 2007; Amemiya et al., 2013) is epistemologically questionable (see critique in Assis & Carvalho, 2010).

One issue at the heart of the original ‘salmon-lungfish-cow’ debate (Gardiner et al., 1979) concerned the importance then given to the fossil record in explicating deep phylogenetic divergences. At the time, the fossil record was departing center stage to become just one more source of information for phylogenetic reconstruction, with a greater significance being given to comparative morphology of extant organisms (see Rosen et al., 1981).
Cladistics rose to prominence, to some extent, as a reform of paleontology (Nelson, 2004). Reflecting further on that debate in *Nature*, and with Amemiya *et al.* (2013) in hand, it may appear that the privileged data set that was previously ‘set in stone’ has now switched to the genome—in other words, *genomics as the new fossil record* (D. Williams, pers. comm.). Even though we praise the significant contributions advanced by Amemiya *et al.* (2013), we hope to see more studies that are truly integrative, such as in the sense envisioned by Eisen & Fraser (2003), or that place the very important contributions of morphology into proper context. Exemplary integrative studies involving developmental biology, anatomy, paleontology, molecular biology and biogeography have surfaced in recent years with remarkable overall achievements (e.g. Kuratani *et al*., 2012; Hirasawa *et al*., 2013). Whereas the progress to genomics put forth by Amemiya *et al.* (2013) is not in dispute, the impact their study may have on the evolution of basal tetrapods and the terrestrialization of vertebrates is still premature if the numerous ‘intermediate’ fossils and relevant morphological transformations are not taken into account.

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**Literature cited**


COMMENTS ON THE SALMON-LUNGFISH-COW DEBATE

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http://dx.doi.org/10.1007/bf02106050

http://dx.doi.org/10.1038/353219a0

http://dx.doi.org/10.1016/0031-0182(75)90162-7


http://dx.doi.org/10.1011/gr.2972804

http://dx.doi.org/10.1002/jmor.1051900418

http://dx.doi.org/10.1111/j.1469-185x.1987.tb01635.x

http://dx.doi.org/10.1146/annurev.ecolsys.24.1.153


http://dx.doi.org/10.1016/0034-5687(71)90003-x

http://dx.doi.org/10.1002/jmor.1051900418


http://dx.doi.org/10.1126/science.1165115


http://dx.doi.org/10.1002/jmor.1051900407


http://dx.doi.org/10.1007/bf00298136


http://dx.doi.org/10.1038/353217a0


http://dx.doi.org/10.1371/journal.pone.0033683


http://dx.doi.org/10.1111/j.1469-185X.1969.tb00823.x


http://dx.doi.org/10.1080/02724634.2012.657317


http://dx.doi.org/10.1111/j.1096-3642.1979.tb01110.x


http://dx.doi.org/10.1038/nature00871


http://dx.doi.org/10.1038/35065078


http://dx.doi.org/10.1038/ncomms1764


http://dx.doi.org/10.1038/nature07855