Molecular evidence on the origin of tetrapods and the relationships of the coelacanth

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Coelacanths were believed to have gone extinct more than 80 million years ago – until the sensational rediscovery of one surviving member of this lineage, *Latimeria chalumnae*, in 1938. Since then, paleontologists and comparative morphologists have argued whether coelacanths or lungfish (two groups of lobe-finned fish) are the living sistergroup of the third extant lineage, the tetrapods. Recent molecular phylogenetic data on this debate tend to favor the hypothesis that lungfish are the closest relatives of land vertebrates. Somewhat surprisingly, the strongest molecular support for this hypothesis stems from mitochondrial rather than nuclear DNA sequences, despite the expectation that the more-slowly evolving nuclear genes should be more appropriate in addressing a phylogenetic issue involving taxonomic groups that diverged around 400 million years ago. This molecular estimate might serve as a framework to test paleontological phylogenies and hypotheses about morphological and physiological innovations and preadaptations that allowed Devonian lobe-finned fish to colonize land.

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The origin of land vertebrates, among the major transitions in the history of organismal diversity, is a question of importance and contention[1]. The origin of tetrapods and the identification of their living sistergroup are two separate issues, but an answer to the latter question could rule out some historical scenarios of the origin of land vertebrates[2,3]. Although extinct groups of lobe-finned fish are likely to be more closely related to tetrapods than to any living piscine relative (reviewed in Refs 1,4) the identification of the living sistergroup of tetrapods can make an important contribution to this larger issue nonetheless. Molecular phylogenetic approaches can aid the more time-honored ones like comparative morphology and paleontology in achieving this goal.

Despite much debate and generations of investigators, no consensus on the identity of the closest living relative of tetrapods has yet been reached. Some workers considered land vertebrates to be related to actinistians (i.e. coelacanths), lungfish, actinopterygians (ray-finned fish), or lungfish and coelacanth equally (reviewed in Ref. 5). Even a diphyletic origin of tetrapods has been proposed[6], with porolepiform† (Box 1) fish being the sistergroup of urodels, and osteolepiform† fish, the sistergroup to anurans and all other land vertebrates. In a controversial paper reviewing paleontological and morphological data that included the extinct groups of lobe-finned fish, Rosen *et al.*[7] considered lungfish to be the sistergroup to tetrapods. The vast literature on the ancestry of tetrapods from fish-like ancestors based on morphological and paleontological data has been reviewed elsewhere[1,5,7–9]; here, I will focus on biochemical data sets.

Lungfish were initially believed to be amphibians, and their relationship to tetrapods has been debated for more than 150 years (for review, see Refs 7,8) (Fig. 1). After the sensational discovery of the 'living fossil', *Latimeria chalumnae* (reviewed in Ref. 9), the last known surviving species of another lineage of lobe-finned fish, the coelacanth (rather than the lungfish) was generally thought to be the 'missing link' between aquatic and terrestrial vertebrates (Fig. 1). This is still the prevailing opinion in most general biology texts. The suggestion that the coelacanth and cartilaginous fish are most closely related, which was largely based on similarities of their pituitary glands and common mode of osmoregulation, has been soundly dismissed and will not be considered further (reviewed in Ref. 5).

The monophyly of tetrapods, the coelacanth, and lungfish, and the legitimacy of the outgroup position of the monophyletic Actinopterygii (Box 1) for this question is widely accepted. There is no debate that lungfish (Fig. 1, hypothesis 1a) or the coelacanth (Fig. 1, hypothesis 1b) or both equally (Fig. 1, hypothesis 1c) are more closely related to tetrapods than to actinopterygian fish – leaving three alternatives to be tested for the relationships among extant lineages of lobe-finned fish (Fig. 1). Support for each of the three hypotheses can be found, based on cladistic approaches using neontological and paleontological phenotypic characters, but there is no strong consensus opinion for any one of these hypotheses (reviewed in Ref. 5).

Only if all four groups of bony fish are included in molecular phylogenetic studies will they have the potential to address the question of the relationships among the extant lobe-finned fish lineages (Fig. 1). Ideally, in such studies, as many species as possible should be represented for each of the lineages, and thousands could theoretically be included on the tetrapod and the actinopteryian branches. However, there are only three genera of lungfish and one coelacanth species alive and available for molecular phylogenetic work. Several studies that aim to add to the understanding of the relationships among the lobe-finned fish fall short because either the coelacanth or the lungfish lineage are not included. These studies typically only reaffirm the well-established sistergroup relationship of either the lungfish or the coelacanth to the tetrapods (Fig. 2a, Fig. 3a).

During the past five years, various forms of biochemical data have been collected with the explicit goal of testing alternative hypotheses on the molecular evolutionary relationships among lobe-finned fish. This review aims to summarize the current information on this issue. Knowledge of

Box 1. Classification of extant (and extinct†) bony fish

Class: Chondrichthyes (sharks, rays and other cartilaginous fish)
Class: Osteichthyes (bony fish)
Subclass: Actinopterygii (ray-finned fish)
Cladistia (birchir, *Polypterus*, reedfish, *Erpetoichthys*)
Actinopteri
Chondrostei (sturgeons, paddlefish)
Neoterygii (gars, *Amia* and modern, ray-finned fish)
Subclass: Sarcopterygii (lobe-finned bony fish)
Actinistia (coelacanth, *Latimeria*)
Rhipidistia
Dipnoi (lungfish)
Porolepiformes†
Osteolepiformes†
Tetrapoda (land vertebrates)
Lissamphibia (modern amphibians)
Amniota (all other tetrapods except modern amphibians)

The assignment of groups into this classification varies greatly among researchers; this one is modified from Ahlberg (Ref. 47) and Patterson (Ref. 52). Some authors do not include the lungfish with rhipidistian fish (e.g. Refs 4,53). Classifications change depending largely on the phylogenetic position of the lungfish and the coelacanth. The Crossopterygia and Rhipidistia are considered natural or unnatural groups by different researchers, depending on the phylogeny on which the classification is based. The Crossopterygia, a group that includes the coelacanths and the rhipidistians (but excludes the lungfish) is an unnatural group under this classification (reviewed in Ref. 4).

the phylogenetic relationships among the living and extinct representatives of the major groups of lobe-finned fish would aid the understanding of the sequence of morphological events and innovations that facilitated the conquest of land.

Neurobiological data

The search for neurobiological similarities between the tetrapod and piscine conditions is an active area of research. Although in most cases these data were not intended to elucidate this phylogenetic issue, and are typically not analyzed in a rigorous phylogenetic fashion, they nonetheless contain relevant evolutionary information. Immunocytochemical techniques allowed mapping of the distribution of substance P, leu-enkephalin, serotonin, tyrosine hydrolase and pancreatic polypeptide-immunoreactive neurons, and seemed to suggest that the conditions in the brain of the African lungfish, *Protopterus annectens*, are similar to those in land vertebrates[10] – supporting hypothesis 3a (Fig. 3). Furthermore, the distribution of alpha-melanocyte-stimulating hormone (α-MSH) in neurons of *P. annectens* was characterized using immunohistochemical and radioimmunoassay methods and was reportedly more similar to that in amphibians than in teleost fish[11]. High Pressure Liquid Chromatography (HPLC) analyses also suggested a high degree of sequence similarity between the α-MSH of lungfish and tetrapods[11]. Other neuroanatomical and neuropeptide studies also argue that lungfish are the sistergroup to tetrapods, weakly supporting hypothesis 3a. However, other neuroanatomical investigations support hypothesis 1b (Ref. 12) or 1c (Ref. 13). There is a higher similarity of brain myelin components, assessed by graded measured antibody responses in cross-reactivity tests, between the coelacanth and tetrapods than between lungfish or cartilaginous fish and tetrapods[14,15]. Moreover, immunochemical analyses of the myelin proteolipid protein (PLP) revealed that the carboxyl terminal epitope was conserved in lungfish, coelacanth and tetrapods, but that the staining intensity was higher for the coelacanth, seemingly indicating a closer phylogenetic relationship to tetrapods. The coelacanth myelin protein assays were judged to be more akin to those of tetrapods than to those of the myelin of lungfish, and the authors concluded that these data reject the coelacanth–chondrostei link and support hypothesis 1b.

Because of the nature of the studies, these results typically do not provide information on the sequence homology within the non-immunogenic domains of the proteins studied. In general, since immunological techniques can only provide crude quantitative measures of supposed homology that cannot be analyzed cladistically, all results from these techniques need to be interpreted cautiously in terms of the value of their phylogenetic information content. In the future, these studies need to be expanded to include the cloning of the myelin (and other; see above) components to

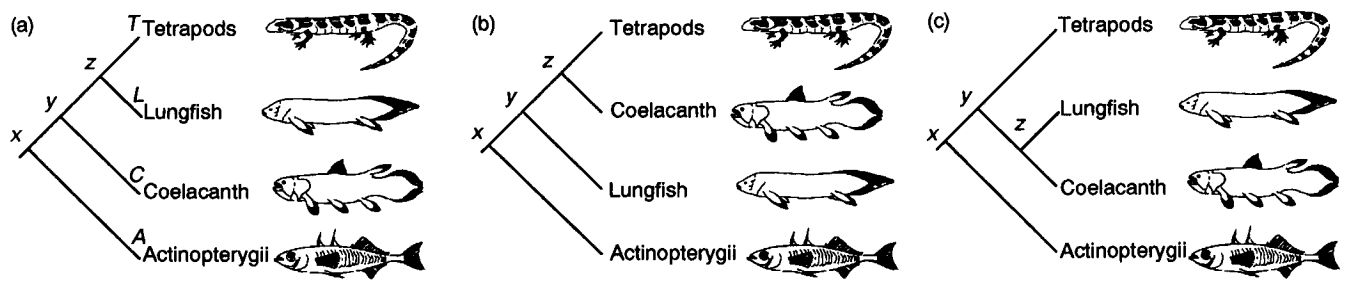

Fig. 1. Hypothesized phylogenetic relationships among the major groups of living bony fish (Osteichthyes). The species that are diagrammatically depicted are: the threespine stickleback, the Australian lungfish, the coelacanth and a salamander. (a) Tree relating the ray-finned fish (Actinopterygii) and the three groups of lobe-finned fish (Sarcopterygii): coelacanth, lungfish (Dipnoi) and tetrapods. In this tree *x* refers to the ancestor of all bony fish and *y* to the common ancestor of all lobe-finned fish. In this phylogenetic hypothesis the lungfish are the sistergroup of the tetrapods and the coelacanth is the sistergroup to the lungfish+tetrapod clade. Among paleontologists this view is, for example, supported by Refs 7,46–49. (Ref. 49 reports on the unpublished PhD thesis of X-B. Yu, Yale University, CT, USA, 1990.) *zL* is the lungfish lineage, *yC* is the coelacanth lineage, *zT* is the tetrapod lineage, and *xA* is the ray-finned fish lineage. (b) Phylogenetic hypothesis depicting tetrapods and the coelacanth as sistergroups and placing lungfish as the sistergroup to those two groups of sarcopterygians. Among paleontologists this view is supported by, for example, Refs 12,45. (c) Phylogenetic hypothesis relating the coelacanths and lungfish equally closely related as sistergroups of tetrapods. Among paleontologists this hypothesis is supported by Refs 50,51. *Illustrated by Vincaine Milinkovitch.*

allow for direct assessment of amino acid and nucleic acid similarity and rigorous phylogenetic testing.

Nuclear ribosomal data

Initially through direct RNA sequencing (a fast but comparatively inaccurate way to determine sequences), and more recently through polymerase chain reaction facilitated DNA sequencing, both the small (18S) and the large (28S) ribosomal genes have been frequently applied to a range of usually 'deep', that is, phylogenetically inclusive, questions. The 18S ribosomal RNA (rRNA) is overall a highly conserved molecule, but also has surprisingly variable regions that are, typically, difficult to align. Unfortunately, most efforts using this molecule to address the question of the origin of tetrapods have met with limited success. One study[16] based on about 500 sites and 11 species did not include *Latimeria* and was unable to distinguish among any hypotheses with confidence. More-extensive sequences of almost complete 18S sequences (about 1800 nucleotides for eight taxa with 202 variable sites of which 68 were phylogenetically informative) for a large group of species resulted in a single shortest tree of 'anomalous' relationships with weak bootstrap support[17–19].

An analysis of over 2000 nucleotides of the large ribosomal gene sequences (28S) for a small number of species has so far not included sequences from lungfish and thus cannot address the competing main hypotheses (Fig. 1a–c)[20,21]. These studies provided strong support for hypothesis 2a (Fig. 2) and called into question previous (not cladistically based) morphological studies that advocated a sistergroup relationship between the Actinopterygii and the tetrapods[22] (Fig. 2b) or the coelacanth+cartilaginous fish hypothesis. These 28S data were encouraging, and future work should include lungfish. However, a smaller data set of about 500 nucleotides obtained through direct RNA sequencing from the 5′ end of the 28S rRNA gene provided only largely unorthodox and weakly supported groupings for a large number of species that did not include the coelacanth[23].

Both nuclear ribosomal genes, and in particular the small one, may be inappropriate for addressing this phylogenetic question. There are several potential reasons for this: (1) unequal rates of evolution among portions of the molecule and among lineages hinder the recovery of true phylogenetic relationships; (2) gene duplications[17,18,23]; (3) extinctions of lineages; and (4) the potential for a rapid radiation of the lobe-finned fish lineages. The last potential difficulty is not specific to nuclear ribosomal genes and will affect all phylogenetic studies of this question. Ribosomal genes, as compared to protein coding genes, have to contend with the additional difficulty that alignment problems may compound the phylogenetic analysis.

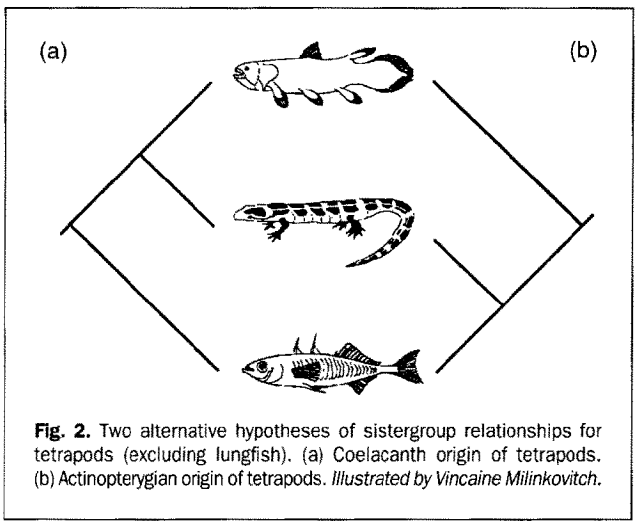

Fig. 2. Two alternative hypotheses of sistergroup relationships for tetrapods (excluding lungfish). (a) Coelacanth origin of tetrapods. (b) Actinopterygian origin of tetrapods. *Illustrated by Vincaine Milinkovitch.*

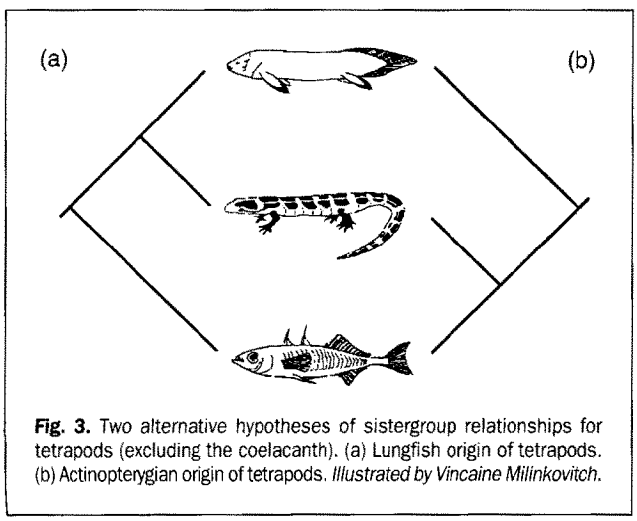

Fig. 3. Two alternative hypotheses of sistergroup relationships for tetrapods (excluding the coelacanth). (a) Lungfish origin of tetrapods. (b) Actinopterygian origin of tetrapods. *Illustrated by Vincaine Milinkovitch.*

Structural genes

The coelacanth has high urea concentrations in its blood (as do cartilaginous fishes), which serves as an osmolyte aiding in osmoregulation. The high levels of urea do not denature the coelacanth's hemoglobin or affect its O_2 binding capacity as they would in tetrapods. Studies on the evolution of urea synthesis pathways and the analysis of the carbamoyl phosphate synthetases (CPSs) allowed the mapping of a parsimonious distribution of changes in the biochemical pathway of the urea cycle[24]. These data link lungfish rather than the coelacanth more closely to tetrapods[24], supporting hypothesis 1a.

Phylogenetic analyses of parvalbumins did not provide conclusive results[25]. Several unorthodox groupings were found; the most parsimonious solution tentatively favored an actinopterygian over a coelacanth origin for tetrapods (hypothesis 2b). This study did not include lungfish and was therefore unable to address the main hypotheses. Several attributes of parvalbumins, for example, their relatively small size (110–115 amino acids), uneven and probably too fast rates of evolution, and multiple duplication events, make the α and β parvalbumin genes inappropriate for this question.

Prolactins (PRL) and growth hormone (GH) belong to the same gene family; their amino acid and DNA sequences have been determined from a relatively large number of vertebrates, including the African lungfish *Protopterus aethiopicus*[26]. The PRL sequence of the lungfish seems to be more similar to amphibians, reptiles, birds (34% divergence) and mammals (42% divergence) than to teleosts (62% divergence); however, no formal phylogenetic analysis was conducted. The lungfish PRL protein also has three disulfide bonds, which is similar to tetrapods but different from the actinopterygians, which lack the amino terminal disulfide bond – both observations potentially support hypothesis 3a (Ref. 26). Since the PRL or GH sequences for the coelacanth have not been collected, these genes cannot yet address the main hypotheses (Fig. 1).

The gene organization of the immunoglobulin heavy-chain variable region (V_H) has recently been determined for *Latimeria* (lungfish were not tested)[27] and compared to tetrapods. These results were interpreted to support the coelacanth+elasmobranch hypothesis. However, other aspects of the coelacanth V_H gene organization are more similar

to those of ray-finned fish and tetrapods. Sequence comparisons of *Latimeria* V_H DNA sequences showed 65–70% sequence similarity with mouse, rabbit, caiman, *Xenopus* and an actinopterygian (*Elops*) but less than 61% identity with a shark V_H sequence[27]. However, none of these comparisons can be considered powerful phylogenetic indicators, since they were not subjected to any phylogenetic analysis. Furthermore, the V_H gene is a member of a multigene family for which identification of homologous sequences is difficult, and as long as the orthologies for V_H genes are unclear they remain of limited phylogenetic use. The exon–intron organization of the major histocompatibility complex (MHC) of the coelacanth was found to be identical to that of mammals[27]; so far, lungfish have not been studied. Moreover, the DNA sequences of *Latimeria* class I MHC genes were found to be more similar to those of amphibians than to those of actinopterygians, supporting hypothesis 2a (Ref. 28).

Hemoglobin amino acid and DNA sequences have been used for a variety of phylogenetic questions (e.g. Ref. 29). A recent study of 20 α and β hemoglobin-chain amino acid sequences[30,31] claimed to have found support for hypothesis 1b. Gorr *et al.*[30] noted that pairwise comparisons of hemoglobins of actinopterygian fish tend to be more similar to larval than to adult amphibian hemoglobin amino acid sequences. More specifically, for the β chains the coelacanth and for the α chains actinopterygians were most similar to those of larval amphibians. The conflicting data for the two chains were interpreted as evidence for hypothesis 1b. As has been pointed out previously[32–36], results of this hemoglobin study are rendered suspect because of difficulties with (1) alignment, (2) unequal rates of evolution (which violates the assumptions of UPGMA, the method that was used for phylogenetic inference), (3) flawed phylogenetic analyses, (4) the assumption of larval-amphibian and fish hemoglobin orthology, and (5) the inferred pattern of hemoglobin gene duplication events. When these sequences were analyzed correctly, they tended weakly to support hypothesis 1b (Refs 32–36). Numerous duplications of globin genes during the evolution of vertebrates make the phylogenetic analysis of this gene family problematic, since orthology needs to be established before any phylogenetic analyses. Other potential problems with hemoglobins include the facts that they are quite rapidly evolving molecules, their rates of molecular evolution might be increased after gene duplications, and different selection constraints on oxygen-binding proteins might differ in the aquatic and the terrestrial environment[37]. Several of the potential problems mentioned for globin genes might render them unsuitable for phylogenetic inquiry at this level of divergence.

Mitochondrial data

Six hundred and sixty four nucleotides of two slowly evolving mitochondrial (mt) genes (12S rRNA and cytochrome *b*) from a ray-finned fish, the South American lungfish (*Lepidosiren*), the coelacanth and the frog *Xenopus laevis* favored hypothesis 1a (Ref. 2). Of the two genes, the 12S rRNA gene contained a higher density of phylogenetically informative sites (17 of 240 alignable nucleotides) than the cytochrome *b* gene (17 of 360) for which transitional changes at third positions were ignored since they are likely to be saturated with back mutations[2,3]. Based on this data set, the statistical confidence supporting hypothesis 1a was strong. Further inclusion of African and Australian lungfish into an extended mtDNA study of the 12S gene continued to support hypothesis 1a, but with lowered statistical confidence[3]. For several ray-finned fish and for another lungfish lineage, the African *Protopterus* sp., additional mtDNA sequences for a piece of the cytochrome *b* gene (and inferred 97 amino acids) were determined[38]; these data weakly supported the lungfish–tetrapod sistergroup relationship (hypothesis 1a).

Recently, amino-acid maximum likelihood, maximum parsimony and neighbor-joining analyses of new sequences of the complete mitochondrial cytochrome oxidase I (COI) gene (about 1550 nucleotides), and a re-analysis of previous mtDNA data were published[39]. Whereas COI seems to favor hypothesis 1c over the other two alternatives, the combination of COI[39] with the earlier 12S rRNA and cytochrome *b* data[2,3] could rule out 1b, but could not discriminate between alternatives 1a and 1c (Ref. 39).

The strongest support for one of the main hypotheses (1a) is based on the largest data set so far (almost 3000 nucleotides, of which 2073 could be aligned and 830 were variable) from the $tRNA^{Val}$ gene and the two rRNA genes, for which the three lungfish and the coelacanth were compared to eight tetrapods and one actinopterygian outgroup[40]. Also a portion of the cytochrome *b* gene was determined for the Australian lungfish *Neoceratodus*[40]. Parsimony and neighbor-joining analyses, as well as several statistical tests, provided strong, unequivocal support for the lungfish–tetrapod sistergroup relationship (hypothesis 1a).

Mitochondrial DNA is a molecule that has a rapid rate of evolution overall and has been used largely for population-level work and to address questions of relationships among closely related species. It was therefore initially surprising[2], but seems to be confirmed[40,41], that the clearest answers to the main question seem to be coming from slowly evolving genes (rRNA, tRNA and maybe some protein coding genes) of the mitochondrial genome. Most of the mitochondrial data that have been collected so far appear to support hypothesis 1a (Refs 2,3,38,40) but possibly also hypothesis 1c (Ref. 39), whereas hypothesis 1b receives no support.

Molecular phylogenies, morphological preadaptations and extinct lineages

The molecular data (particularly mtDNA data) provide support for the lungfish (hypothesis 1a) and not the coelacanth (hypothesis 1b) as the living sistergroup of tetrapods. This is a result that had not been agreed upon using morphological data (reviewed in Ref. 5). The conclusion based on molecules may lead to an increased understanding of which morphological traits might have preadapted the common ancestor of lungfish and tetrapods to life on land, and what the sequence of morphological changes was that led to the colonization of land. Once the attachment of the extinct lobe-finned fish in a tree on lineages (e.g. *zT*, *zL*, *xy* or *yz* in Fig. 1a) is achieved, morphological changes can be ordered and reinterpreted cladistically.

Since molecular data can only be collected from living representatives, molecular phylogenetic studies cannot provide direct information on the evolutionary position of extinct groups. However, a molecular phylogenetic hypothesis can provide a skeleton for a more inclusive phylogeny reconstruction that also considers extinct lineages (for discussion of this contentious issue see Refs 2,3,42–45). If a well-supported molecular phylogenetic hypothesis of living groups (e.g. hypothesis 1a) was accepted, it would have the power to support or rule out some of the proposed phylogenetic relationships that include lineages of extinct lobe-finned fish. Some paleontological phylogenies (e.g. Fig. 4b, 4d and 4f) are incongruent with hypothesis 1a and would need to be re-examined. A molecular framework might provide guidance for the interpretation of morphological characters as ancestral or derived and aid in the assignment of fossils to particular lineages. Therefore, molecular phylogenetic

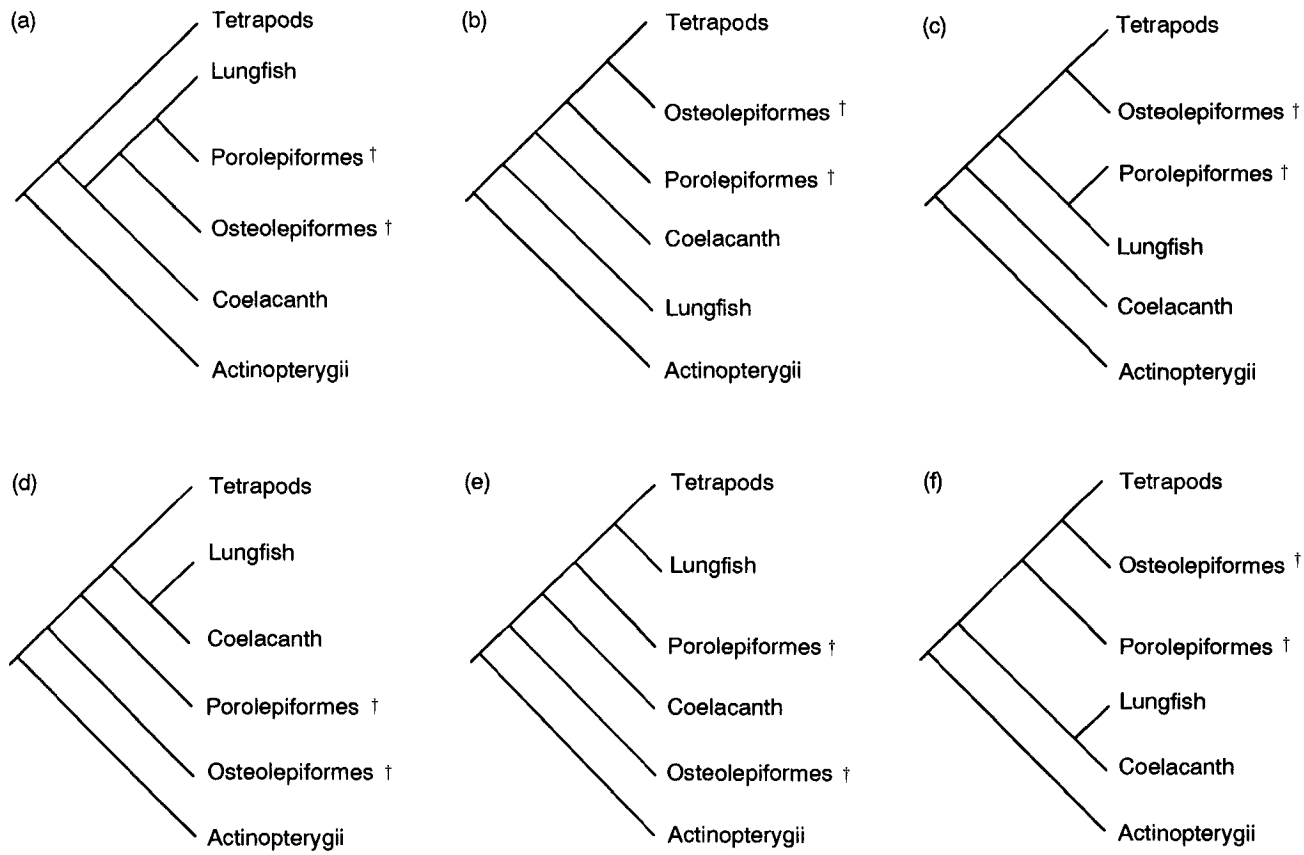

Fig. 4. Subset of cladistically derived phylogenetic hypotheses (references given after figure number) relating the major sarcopterygian groups including two of the major extinct lineages (Osteolepiformes† and Porolepiformes†). (a)[50], (b)[45], (c)[47–49], (d)[51], (e)[7]; (f) is based on Ref. 4, a result of a reanalysis of 216 (partially recoded) morphological characters from major previous investigations (but excludes other studies, for example, Ahlberg's[47]).

hypotheses also have the capacity to advance understanding beyond the identification of the living sistergroup of tetrapods[2,3].

Character modification along a lineage may happen in all clades, and paleontologists alone can determine what common ancestors might have looked like, based on the interpretation of sistergroup relationships of living and extinct lineages and the assignment of fragmentary fossils to these groups. Since characters can change and evolve repeatedly and be lost along a particular lineage, not all character states found in the terminal taxa of a tree will represent true synapomorphies (shared derived) but may be homoplasies (phylogenetic noise of various kinds). Without the firmly established relationships of fossil and living forms, it seems premature to try to trace single characters, since the interpretation of traits as synapomorphies or symplesiomorphies (shared primitive) or homoplasies depends on the branching pattern and has to be made *a posteriori* (although decisions about what character states are homologous are made *a priori* by character analysis). The decision about whether a particular character is attributable to recent common ancestry or convergent evolution can only be made based on a phylogenetic tree that uses all available information (genetic as well as phenotypic).

Difficulties and future directions

The common problem that plagues all studies of tetrapod origins, morphological as well as molecular, is the fact that these lineages originated within a small (20–30 million years) window in time, almost 400 million years ago[1]. Thus, there was little time for lineage-specific molecular changes to occur, but the chance for multiple and parallel changes to have accumulated since the origin of these lineages is great. This rapid origin of lineages might be one reason why slowly evolving nuclear ribosomal genes have been generally unsuccessful in providing an answer to this question[17].

Morphological changes may come in leaps and bounds, yet may be static for long periods as well. Unlike molecular ones, they are not likely to accumulate linearly with time, but rather, by their very nature, morphological innovations might be responsible for increased cladogenesis. Several important morphological innovations (e.g. involving lungs or limbs) might have been responsible for the evolutionary success of the first tetrapods; however, the lack of fossils at this crucial point in time[1] obstructs the reconstruction of the sequence of steps that led to life on land, and hinders the assignment of fragmentary fossils to particular lineages of lobe-finned fish.

Molecular studies have several advantages over morphological studies: (1) in principle, virtually unlimited amounts of molecular data can be collected from every relevant genome in the anticipation that phylogenetic information will prevail over noise, whereas only a limited number of morphological characters typically can be extracted from incomplete fossil material; (2) issues of homology, which have plagued morphological inquiries into this question (e.g. Refs 4,7,44,45), are, aside from alignment difficulties with some genes (e.g. ribosomal genes), not quite so problematic for molecular phylogenetic work.

Some studies that employed biochemical information fall short of providing support to any of the hypotheses since no real phylogenetic analysis was conducted and/or because of

an incomplete representation of major lineages. The simple reporting of percent values of similarity has little value for phylogenetic inference. Similarities (statements about percent sequence similarity) are phenetic statements, but provide no information (unless phylogenetic analyses are conducted) on whether the similarity represents an indication of common descent or is a result of convergence, homoplasy or other factors (e.g. similar base compositional, mutational or codon biases in different lineages). All of these factors can obscure true phylogenetic information.

So far, based on molecular data, no general consensus on the competing hypotheses of the relationships among lobe-finned fishes has been reached. It seems fair to say, however, that the overall molecular (particularly mitochondrial) data favor the lungfish+tetrapod hypothesis (Fig. 1a), and do not support the coelacanth+tetrapod (Fig. 1b) hypothesis. However, the evidence for hypothesis 1a over hypothesis 1c is not overwhelmingly strong, leaving room for the latter still to be considered seriously. Both phenotypic and genotypic traits have experienced the same evolutionary history and are expected, if analyzed correctly, to support the same phylogenetic hypothesis. The combination ('total evidence approach') of both kinds of data set might be desirable in future work, however it is a contentious issue with ample potential problems (e.g. relative weights, different tempos and modes of evolution) that still need to be worked out. Surely, the establishment of a general consensus for a phylogeny of the extant lobe-finned fish (and fossils) will require the collection of more data (both genetic and phenotypic) and their careful evolutionary analyses.

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