Short communication

Evolutionary history of African lungfish: a hypothesis from molecular phylogeny

Masayoshi Tokita*, Taku Okamoto, Tsutomu Hikida

Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan

1. Introduction

Lungfish (Dipnoi) are recognized as living fossils whose evolutionary history dates back to the early Devonian (390 MYA). Their close phylogenetic relationship with tetrapods is supported by recent studies (reviewed in Brinkmann et al., 2004). Extant lungfish include six species: one in Australia, one in South America, and four in Africa (Table 1). The Australian species (*Neoceratodus*) is considered to be a primitive type of lungfish, having a single lung and a high dependence on gill respiration, and is classified into the family Ceratodontidae (Marshall, 1987a). South American (*Lepidosiren*) and African (*Protopterus*) species with paired lungs and well developed air-breathing ability are closely related to each other (Marshall, 1987b; Miles, 1977), and are grouped into the same family Lepidosirenidae (Marshall, 1987a).

Although *Protopterus* is distinguished conventionally from *Lepidosiren* by a few characters (Marshall, 1987a; Wake, 1987), the monophyly of these genera has not yet been established unambiguously. Furthermore, irrespective of their morphological and ecological differences, phylogenetic relationships among the four African lungfish species are still unknown (Greenwood, 1987). Although morphometric data of the four African species have been obtained by some authors (Poll, 1961; Trewavas, 1954), these characters seem to be not suitable for phylogenetic analyses due to such limitations as changes of body proportion according to the age of animals, and

the accidental amputation of limbs (pers. obs.). In the present study, we determined partial sequences of the mitochondrial 16S rRNA gene from four African lungfish species (Table 1) to infer a hypothesis of phylogenetic evolution in African lungfish.

2. Materials and methods

Fresh tissues were obtained from *Protopterus amphibius*, *Protopterus aethiopicus*, *Protopterus annectens*, and *Protopterus dolloi* purchased from pet dealers and stored in 99% ethanol. Two or three specimens of each species were included in the analyses. These specimens were deposited as vouchers of the Kyoto University Museum (Table 1). The corresponding DNA sequences of *Neoceratodus forsteri*, and *Lepidosiren paradoxa* were obtained from GenBank (Table 1). We also got the already published sequence data of *P. dolloi* from the database to increase the amount of information.

DNA samples for sequencing were prepared using PCR amplification after total DNA extraction from tissues, following the methods of Wada et al. (1992) and Honda et al. (1999). A part of the 16S rRNA gene of mitochondrial genome was amplified by PCR System GeneAmp 2700 (Applied Biosystems, Lincoln, USA), using an Ex *Taq* polymerase kit (Takara Shuzo, Otsu, Japan) and the primers L2206s (5′-GGCCTAAAAGCA GCCACCTGTAAAGACAG-3′) and H2741 (5′-AAG CTCCACAGGGTCTTCTCGTCTTATG-3′) (Honda et al., 1999), and L2606 (5′-CTGACCGTGCAAAGGT AGCGTAATCACT-3′) and 16SR.0 (5′-TAGATAGA AACCGACCTGGATT-3′) (Hedges et al., 1993b; Whiting et al., 2003). The thermocycling regime was 30 cycles of 94 °C for 1 min, 52 °C for 2 min, and 72 °C for 3 min.

---

* Corresponding author. Fax: +81 75 753 4114.
E-mail address: tokky@zoo.zool.kyoto-u.ac.jp (M. Tokita).

Table 1
A list of extant lungfish, with information on distribution, GenBank accession numbers and references

Species	Voucher Nos.	Family	Distribution	Accession Nos.	Reference
Neoceratodus forsteri		Ceratodontidae	Australia	AF302933	Denk et al. (unpublished)
Lepidosiren paradoxa		Lepidosirenidae	South America	AF302934	Denk et al. (unpublished)
Protopterus aethiopicus	FAKU88466	Lepidosirenidae	Africa	AY677094	This study
	FAKU88467			AY677095	This study
	FAKU88468			AY677096	This study
Protopterus amphibius	FAKU88469	Lepidosirenidae	Africa	AY677097	This study
	FAKU88470			AY677098	This study
	FAKU88471			AY677099	This study
Protopterus annectens	FAKU88472	Lepidosirenidae	Africa	AY677100	This study
	FAKU88473			AY677101	This study
	FAKU88474			AY677102	This study
Protopterus dolloi		Lepidosirenidae	Africa	L42813	Zardoya and Meyer (1996)
	FAKU88475			AY796306	This study
	FAKU88476			AY796307	This study

Before sequencing, uncorporated primers were removed from PCR products by PEG precipitation, adding 0.6 volume of PEG solution (20% PEG6000, 2.5 M NaCl). The amplified DNA templates were then sequenced using a Big Dye Terminator Cycle Sequencing Ready Reactions Kit v1.0 and an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Lincoln, USA), using the four primers described above.

In a χ^2 test of base composition homogeneity among sequences, *Neoceratodus* sequence deviated significantly from the null hypothesis ($P = 0.045$). Therefore, we inferred phylogeny using the minimum evolution criterion based on LogDet distances that are robust against base composition heterogeneity (Barry and Hartigan, 1987; Lockhart et al., 1994) after multiple alignments of sequences. The DNA sequences were firstly aligned using ClustalX 1.81 (Thompson et al., 1997), and then manually adjusted considering the secondary structure hypothesis for *Xenopus laevis* 16S rRNA, available in CRW site (Cannone et al., 2002; Supplementary material). The gaps inserted in alignment procedure were excluded before phylogenetic inference. Supports for the inferred clades were assessed by bootstrap method (Felsenstein, 1985) with 1000 pseudoreplicates.

We treated the Australian lungfish *Neoceratodus* as an outgroup in this analysis because previous phylogenetic studies based on both morphological (Marshall, 1987b; Wake, 1987) and molecular (Brinkmann et al., 2004; Hedges et al., 1993a) data have shown it diverged firstly from the ancestor of remaining extant lungfish taxa.

The divergent times among *Protopterus* species were estimated under assumption of a molecular clock, after the molecular clock hypothesis was tested between *Lepidosiren* and *Protopterus*, and among *Protopterus* species with two-cluster test (Takezaki et al., 1995). In this test, standard errors of branch lengths were estimated through bootstrapping instead of original method by Takezaki et al. (1995). The clock was calibrated based on the assumption that the divergence between *Lepidosiren* and *Protopterus* was caused by the continental split of Africa and South America. According to Lévéque (1997), the separation of two continents started in early Cretaceous (125 MYA) but was not completed until the late Cretaceous (80 MYA). Therefore, we used 102.5 MYA, the middle point between these ages, for calibration. The errors of divergent times were estimated based on ranges of bootstrap pseudoreplicates: (depths of particular branching points)/(depth of branching point of *Lepidosiren–Protopterus*). The phylogenetic inference and the optimization of branch lengths under molecular clock hypothesis were undertaken with PHYLIP 3.62 (Felsenstein, 2004).

3. Results

After excluding the positions with gaps the aligned sequences were as long as 860 bp. There were 329, 265, and 201 variable sites among all specimens, within ingroup specimens, and within *Protopterus*, respectively.

The inferred phylogenetic tree demonstrated the monophyly of *Protopterus*, which was strongly supported (BP; 100%; Fig. 1). A close relationship between *P. aethiopicus* and *P. annectens* was also apparent (BP = 98%). Contrary to our expectations, *P. dolloi* used in Zardoya and Meyer (1996) was located within *P. annectens* assemblage. We suspect the authors have misidentified lungfish species owing to DNA extraction from egg samples. In contrast to them, we identified *Protopterus* species surely based on the examination of morphological features such as the number of ribs (Table 2). Numbers of ribs in our *P. dolloi* specimens were 48 and 50. While, that in our *P. annectens* specimens were 34 or 36. Within *Protopterus*, branching order among three basal clade: (1) *P. amphibius*, (2) *P. dolloi*, and (3) *P. aethiopicus* + *P. annectens* was not resolved clearly (BP = 56%).
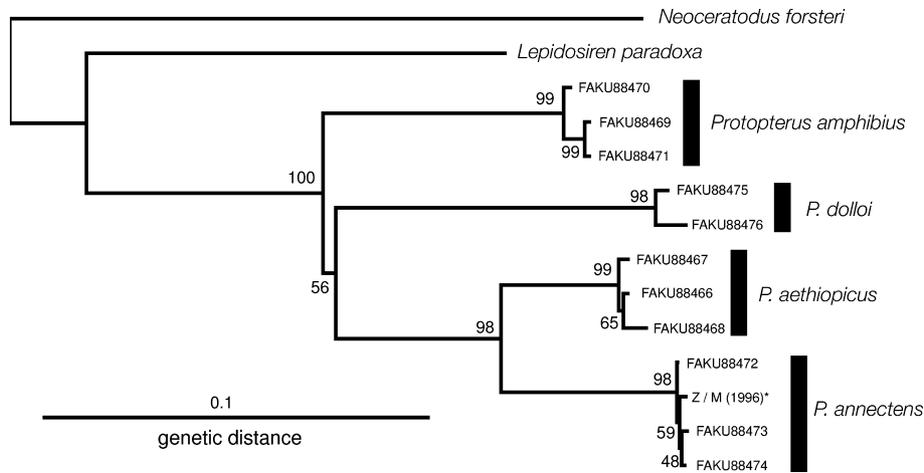
Fig. 1. The phylogeny of lungfish based on a comparison of partial nucleotide sequences of the mitochondrial 16S rRNA gene. The phylogram inferred by minimum evolution criterion using LogDet distances. The numbers nearby each branch are bootstrap probabilities. ∗A lungfish specimen used in Zardoya and Meyer (1996).

Table 2
Morphological characteristics seen in each *Protopterus* species

Species	Number of ribs	HL/AL (%)	DL/AL (%)	External gills
Protopterus aethiopicus	38–39	24–29.6	62.1–67.1	Absent in adult
Protopterus amphibius	27–30	33.2	45–56	Present in adult
Protopterus annectens	32–37	22.8–28.2	51–57.5	Present in adult
Protopterus dolloi	47–55	16.2–19.6	63.7–66	Absent in adult

Numerical data were taken from Poll (1961). Only effective characters to identify each species are shown.
Abbreviations: DL, distance from snout to most anterior point of dorsal fin; HL, head length; AL, distance from snout to most anterior point of anal fin.

It has been said that the mithochondrial rRNA genes are suited for analyses of lineage divergence back to 300 MYA, especially 150 MYA or later (Mindell and Honeycutt, 1990). The divergence of lungfish genus is usually considered to be caused by split of Gondwana (<150 MYA). This time scale is in the range possible to be inferred by the mitochondrial rRNA genes. In fact, the deeper and shallower parts of the tree seemed reasonably resolved. Hence, the relationships among the three lineages should be regarded as the result from considerably close divergent times among them, rather than the result of shortage of information given by the present data.

A likelihood ratio test based on parametric bootstrapping also supported the polytomy hypothesis of basal divergence within *Protopterus*. For that, a maximum likelihood analysis was firstly applied to the present data after excluding *Neoceratodus* sequence because of its significant difference in base composition from those of the other taxa. Then, likelihood ratio of polytomy against ML topology was computed. Stochastic model for molecular evolution assumed in the analysis was REV + G, which was selected through the method of Huelsenbeck and Rannala (1997). The null distribution of likelihood ratio was approximated with likelihood ratio on 1000 data sets generated through simulation of molecular evolution assumed the polytomy phylogeny and the evolutionary model selected above. Because the observed statistic (1.343) was in 95% confidence interval of null hypothesis (0–32.897), the null hypothesis of polytomy was tentatively accepted (Supplementary material). The maximum likelihood optimizations and data simulation were conducted with PAML 3.14 (Yang, 1997).

The two-cluster test could not reject molecular clock hypothesis ($P > 0.05$ for all nodes tested). Then, we applied a molecular clock to the inferred phylogeny. The 95% confidence intervals of divergent times were 75.7–53.1 and 42.1–25.6 MYA for basal divergence of *Protopterus* and divergence between *P. annectens* and *P. aethiopicus*, respectively.

4. Discussion

A sister-group relationship between South American *Lepidosiren* and African *Protopterus* was strongly demonstrated by recent molecular phylogenetic studies (Brinkmann et al., 2004; Hedges et al., 1993a). However, these studies failed to verify the monophyly of each taxon clearly because only one species representing the genus *Protopterus* was included in analyses.

By including of all *Protopterus* species into analyses, the present study demonstrated unequivocally the monophyly of African lungfish for the first time. *Protopterus* might have arisen after the late Cretaceous split

of the African-South American continents, and diversified morphologically and ecologically after this time (Lévéque, 1997; Lundberg, 1993; Patterson, 1975).

The basal divergence of *Protopterus* was probably occurred in relatively short span around late Cretaceous to early Tertiary periods. During those periods, rain forests, a suitable habitat for them, were distributed along the Northern coast of Africa due to a shift of the Equator to north (Lévéque, 1997). Such dramatic changes in climatic conditions might have triggered simultaneous divergence of basal *Protopterus* lineages. The evidence that many fossils assigned to different *Protopterus* species were found from layers around late Cretaceous to early Tertiary in Africa (Churcher and de Iuliis, 2001; Greenwood, 1974; Mahboudi et al., 1984; Martin, 1984) may consolidate this possibility.

Within *Protopterus* clade, *P. aethiopicus* and *P. annectens* were closely related to one another with high statistical support. However, the result is not so unexpected. It has been said that to distinguish these two species is somewhat difficult due to morphological resemblance, especially in the numbers of ribs and some morphometric features (Table 2). While, some noticeable differences between these animals are also known. *P. aethiopicus* is the largest lepidosirenid species whose maximum total length (TL) is 200 cm and maximum weight is 17 kg. On the other hand, *P. annectens* never grows to such large size: TL = 90–100 cm, weight = 4 kg. Egg size of *P. aethiopicus* is also bigger than that of *P. annectens*, the greatest egg diameter being in the ranges of 4.5–5.0 mm and 3.5–4.0 mm, respectively (Greenwood, 1987). It has been said that external gills are usually disappeared in *P. aethiopicus* greater than 15 cm TL (Table 2; Van Oijen, 1995). While, *P. annectens* retains these structures for life (Table 2; Poll, 1961; Trewavas, 1954). The types of habitat occupied by these species are also different. It is said that *P. annectens* is common in shallow swamps effectively isolated from permanent water and aestivates at dry-season with cocoons (Greenwood, 1987; Jardine, 1841). Unlike *P. annectens*, *P. aethiopicus* inhabits at large lakes (e.g., Victoria, Edward, and Tanganyika), smaller but permanent lakes (e.g., Lakes George, Nabugabo, and Kyoga), and a major river system (the upper and middle Nile and their tributaries). Because such types of habitat are not relevant to seasonal changes in water-level, cocoon formation appears to be a rare event in this species (Greenwood, 1987).

Judging from divergence time between *Protopterus* species calculated by us, speciation of these two species appears to be occurred around middle Tertiary. It has been said that the land rifting took place in extensive areas of central and eastern Africa during those periods, accompanied by a number of other disruptive activities like faulting and volcanism (Lévéque, 1997; Schlüter, 1997). Such geological events might have brought about the diversification of the environment to promote their adap-
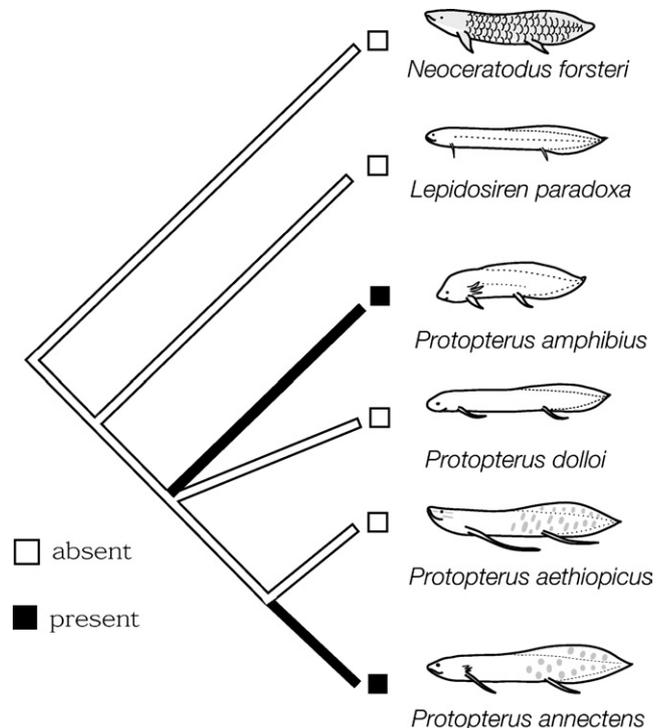

Fig. 2. Reconstruction of the evolution of paedomorphic character: retention of external gills in adult fish, using Mesquite (Maddison and Maddison, 2004). The basal divergence of *Protopterus* is regarded as polytomy. The most parsimonious character reconstruction based on this phylogeny indicates that neoteny is likely to have evolved at least twice within *Protopterus*.

tive radiation into different habitats and increase further morphological differences between them thereafter.

Larvae of lepidosirenids have conspicuous external gills (Budgett, 1901; Kerr, 1901), unlike *Neoceratodus* in which these structures are not generated throughout ontogeny (Kemp, 1987; Semon, 1901). Although the external gills of these animals are generally resorbed at younger age (Poll, 1961; Van Oijen, 1995), two *Protopterus* species (*P. amphibius* and *P. annectens*) retain this larval character for life (Table 2). Such condition is broadly recognized in amphibians, especially urodeles (Dent, 1968; Gould, 1977; Lynn, 1961) and this type of phenomenon is called "neoteny." Neoteny is regarded as one of the processes causing paedomorphosis in which a descendent undergoes less growth during ontogeny than its ancestor. (reviewed in Gould, 1977 and McNamara, 1997).

The evolution of such paedomorphic character was investigated, on the basis of our molecular phylogeny, with Mesquite (Maddison and Maddison, 2004). The resulting pattern suggests that neoteny has occurred at least twice independently in the evolution of *Protopterus* (Fig. 2).

Bearing a superficial resemblance to neotenic amphibians, *P. amphibius* retains well-developed three external gills on each side of the body even in adult stage (Trewavas, 1954). On the other hand, neoteny in *P.*

annectens is less remarkable than that in *P. amphibius*. In the former species, the length of external gills relative to snout-vent length is usually shorter and individual variations in the number of retained external gills are also reported (Trewavas, 1954; Poll, 1961; pers. obs.).

Some authors investigated the role of internal gills of lungfish and revealed its supplementary function in respiration (Fullarton, 1931; Johansen and Lenfant, 1967; Wright, 1974). While, little is known as to the biological function of external gills in neotenic lungfish. In larval stages of lepidosirenid lungfish, external gills are main respiratory organ (Fullarton, 1931). In adult one, on the other hand, well-developed lungs play an appreciable part in respiration. Although external gills of neotenic urodeles have respiratory function (Baldwin and Bentley, 1982), their function seems to be auxiliary to air-breathing by lungs. We suspect that external gills seen in two *Protopterus* species might be not so functional and rudimentary organ resulted from a slower rate of somatic development rather than the one with adaptive significance, because a relationship between life history of animals and the character state at external gills is vague.

It has been shown that obligate neotenic urodeles such as *Necturus* cannot metamorphose due to the absence of a thyroid hormone receptor (Yaoita and Brown, 1990). Therefore, it is likely that neoteny in two *Protopterus* species might have evolved using similar genetic pathway. Further studies should consider possible factors that generate paedomorphosis in two African lungfish species, as well as differences in developmental mechanisms between them. Also, it seems that to investigate the difference between paedomorphic species and non-paedomorphic one such as *Lepidosiren* and *P. aethiopicus* that resorb external gills in the early stages of development would be interesting. Of course, needless to say, further comparative ecological works on *Protopterus* would be necessary to understand evolutionary history of such peculiar animals fascinating biologists for a long time.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2004.11.025.

References

Baldwin, G.F., Bentley, P.J., 1982. Roles of the skin and gills in sodium and water exchanges in neotenic urodele amphibians. Am. J. Physiol. 242, R94–R96.

Barry, D., Hartigan, J.A., 1987. Statistical analysis of hominoid molecular evolution. Stat. Sci. 2, 191–210.

Brinkmann, H., Venkatesh, B., Brenner, S., Meyer, A., 2004. Nuclear protein-coding genes support lungfish and not the coelacanth as the closest living relatives of land vertebrates. Proc. Natl. Acad. Sci. USA 101, 4900–4905.

Budgett, J.S., 1901. On the breeding habits of some African fishes, with an account of the external features in the development of *Protopterus annectens* and a description of the larva of *Polypterus laparadei*. Trans. Zool. Soc. Lond. 16, 115–136.

Cannone, J.J., Subramanian, S., Schnare, M.N., Collett, J.R., D'Souza, L.M., Du, B.F.Y., Lin, N., Madabusi, L.V., Muller, K.M., Pande, N., Shang, Z., Yu, N., Gutell, R.R., 2002. The comparative RNA Web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinform. 3, 2 Correction: BMC Bioinformatics. 3, 15.

Churcher, C.S., de Iuliis, G., 2001. A new species of *Protopterus* and a revision of *Ceratodus humei* (Dipnoi: Ceratodontiformes) from the late Cretaceous Mut Formation of eastern Dakhleh oasis, western desert of Egypt. Paleontology 44, 305–323.

Dent, J.N., 1968. Survey of amphibian metamorphosis. In: Etkin, W., Gilbert, L.I. (Eds.), Metamorphosis. North-Holland, Amsterdam, pp. 271–311.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 2004. PHYLIP (Phylogeny Inference Package) version 3.62. Department of Genome Sciences, University of Washington, Seattle.

Fullarton, M.H., 1931. Notes on the respiration of *Lepidosiren*. Proc. Zool. Soc. Lond., 1301–1306.

Gould, S.J., 1977. Ontogeny and Phylogeny. Harvard University Press, Cambridge, MA.

Greenwood, P.H., 1974. Review of Cenozoic freshwater fish faunas in Africa. Ann. Geologic. Surv. Egypt IV, 211–232.

Greenwood, P.H., 1987. The natural history of African lungfishes. J. Morphol. 1986 (Suppl. 1), 163–179.

Hedges, S.B., Hass, C.A., Maxson, L.R., 1993a. Relations of fish and tetrapods. Nature 363, 501–502.

Hedges, S.B., Nussbaum, R.A., Maxson, L.R., 1993b. Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12S rRNA and 16S rRNA genes (Amphibia:Gymnophiona). Herpetol. Monogr. 7, 64–76.

Honda, M., Ota, H., Kobayashi, M., Nabhitabhata, J., Yong, H.-S., Hikida, T., 1999. Phylogenetic relationships of the flying lizards genus *Draco* (Reptilia, Agamidae). Zool. Sci. 16, 535–549.

Huelsenbeck, J.P., Rannala, B., 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276, 227–232.

Jardine, W., 1841. Remarks on the structure and habitats of *Lepidosiren annectens*. Ann. Mag. Nat. Hist. 7, 21–26.

Johansen, K., Lenfant, C., 1967. Respiratory function in the South American Lungfish, *Lepidosiren paradoxa* (Fitz.). J. Exp. Biol. 46, 205–218.

Kemp, A., 1987. The biology of the Australian lungfish, *Neoceratodus forsteri* (Krefft 1870). J. Morphol. 1986 (Suppl. 1), 181–198.

Kerr, J.G., 1901. Normal plates of the development of *Lepidosiren paradoxa* and *Protopterus annectens.* In: Keibel, F. (Ed.), Normentafeln zur Entwicklungsgeschichte der Wirbelthiere. Gustav Fischer, Jena.

Lévêque, C., 1997. Biodiversity Dynamics and Conservation: the Freshwater Fish of Tropical Africa. Cambridge University Press, Cambridge, UK.

Lockhart, P.J., Steel, M.A., Hendy, M.D., Penny, D., 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Mol. Biol. Evol. 11, 605–612.

Lundberg, J.G., 1993. African-South American freshwater fish clades and continental drift: problems with a paradigm. In: Goldblatt, P. (Ed.), Biological Relationships between Africa and South America. Yale University Press, New Haven, CT, pp. 156–199.

Lynn, W.G., 1961. Types of amphibian metamorphosis. Am. Zool. 1, 151–161.

Maddison, W.P., Maddison, D.R., 2004. Mesquite: a modular system for evolutionary analysis. Version 1.03. Available from: <http://mesquiteproject.org>.

Mahboudi, M., Ameur, R., Crochet, J.Y., Jaeger, J.J., 1984. Implications paléogéographiques de la découverte d'une nouvelle localité Eocène à vertébrés continentaux en Afrique nord-occidentale: El Kohol (sud-oranais, Algérie). Geobios 17, 625–629.

Marshall, C.R., 1987a. A list of fossil and extant dipnoans. J. Morphol. 1986 (Suppl. 1), 15–23.

Marshall, C.R., 1987b. Lungfish: phylogeny and parsimony. J. Morphol. 1986 (Suppl. 1), 151–162.

Martin, M., 1984. Deux lepidosirenidae (Dipnoi) crétacs du Sahara, *Protopterus humei* (Priem) et *Protopterus protopteroides* (Tabaste). Paläontologische Zeitung 58, 265–277.

McNamara, K.J., 1997. Shapes of Time: the Evolution of Growth and Development. Johns Hopkins University Press, Baltimore, MD.

Miles, R.S., 1977. Dipnoan (lungfish) skulls and the relationships of the group: a study based on new species from the Devonian of Australia. Zool. J. Linn. Soc. 61, 1–328.

Mindell, D.P., Honeycutt, R.L., 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic application. Annu. Rev. Ecol. Syst. 21, 541–566.

Patterson, C., 1975. The distribution of Mesozoic freshwater fishes. Mémoires du Muséum d'Histoire naturelle de Paris 88, 156–173.

Poll, M., 1961. Révision systmatique et raciation gographique de Protopteridae de l'Afrique centrale. Annls. Mus. R. Afr. Cent. 103, 1–50 +6pl.

Schlüter, T., 1997. Geology of East Africa. Gebrüder Borntraeger, Berlin.

Semon, R., 1901. Normentafel zur Entwicklungsgeschichte des *Ceratodus forsteri*. In: Keibel, F. (Ed.), Normentafeln zur Entwicklungsgeschichte der Wirbelthiere. Gustav Fischer, Jena.

Takezaki, N., Rzhetsky, A., Nei, M., 1995. Phylogenetic test of the molecular clock and linearized tree. Mol. Biol. Evol. 12, 823–833.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Trewavas, E., 1954. The presence in Africa east of the Rift Valleys of two species of *Protopterus*, *P. annectens* and *P. amphibius.* Ann. Mus. Congo Belge sér. Quarto Zool. 1, 83–100.

Van Oijen, M.J.P., 1995. Appendix I. Key to Lake Victoria fishes other than haplochromine cichlid. In: Witte, F., van Densen, W.L.T. (Eds.), Fish Stocks and Fisheries of Lake Victoria: A Handbook for Field Observations. Samara Publishing Limited, Dyfed, Great Britain, pp. 209–300.

Wada, H., Makabe, K.W., Nakauchi, M., Satoh, N., 1992. Phylogenetic relationships between solitary and colonial ascidians, as inferred from the sequence of the central region of their respective 18S rDNA. Biol. Bull. 183, 448–455.

Wake, M.H., 1987. Urogenital morphology of dipnoans, with comparisons to other fishes and to amphibians. J. Morphol. 1986 (Suppl. 1), 199–216.

Whiting, A.S., Bauer, A.M., Sites Jr., J.W., 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). Mol. Phylogenet. Evol. 29, 582–598.

Wright, D.E., 1974. Morphology of the gill epithelium of the lungfish, *Lepidosiren paradoxa.* Cell Tissue. Res. 153, 365–381.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS 13, 555–556.

Yaoita, Y., Brown, D.D., 1990. A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. Genes Dev. 4, 1917–1924.

Zardoya, R., Meyer, A., 1996. The complete nucleotide sequence of the mitochondrial genome of the lungfish (*Protopterus dolloi*) supports its phylogenetic position as a close relative of land vertebrates. Genetics 142, 1249–1263.