J Mol Evol (1993) 37:644–649

Journal of Molecular Evolution

Molecular Phylogeny of Bony Fishes, Based on the Amino Acid Sequence of the Growth Hormone

Giacomo Bernardi,* Giuseppe D'Onofrio, Simone Caccio', Giorgio Bernardi**

Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2, Place Jussieu, 75005 Paris (France)

Received: 15 November 1992 / Revised: 8 December 1992 / Accepted: 30 March 1993

Abstract. Bony fishes (Osteichthyes) comprise over 22,000 species, about half of all vertebrate species. In order to investigate the phylogenetic relationships within this vertebrate class, we have studied the only protein whose primary structure is known in a rather large (27) number of fish species belonging to seven orders—the growth hormone. The phylogeny obtained using the maximum parsimony method based on amino acid sequences represents the first molecular phylogeny of teleostean fishes based on an extensive set of data. This phylogeny agrees remarkably well with the generally accepted phylogeny based on morphological characters and paleontological data.

Introduction

The bony fishes (Osteichthyes) are the largest class of vertebrates (see Fig. 1 for the systematics of the fishes under consideration here), comprising as many species (>22,000) as all other classes together (MacAllister 1987). Within Osteichthyes, the subdivision Teleostei has been shown by morphological systematics to be monophyletic (Patterson 1973, 1977; Patterson and Rosen 1977; Rosen 1982; Lauder and Liem 1983), whereas several phylogenetic relationships among Teleostei and other groups of fishes are still unclear.

In the case of fishes, molecular approaches have not been widely used in phylogenetic studies. So far, only mitochondrial DNA sequences (Kocher et al. 1989; Meyer et al. 1990; Meyer and Wilson 1990; Normark et al. 1991; Martin and Palumbi 1992) or nuclear ribosomal sequences (Stock et al. 1991; Bernardi et al. 1992) have been used for reconstructing some fish phylogenies (in fact, either for several closely related taxa, or for a small number of taxa separated by large evolutionary distances). Indeed, very few genes coding for proteins have been sequenced in fishes (less than 200 for all fishes vs over 3,000 for man). Among them, the coding sequences of the growth hormone gene are the most widely known in primary structure and are potentially useful for phylogenetic studies covering broad phylogenetic spectra both in cold- and warm-blooded vertebrates.

In the present work we have used all the available amino acid sequences (27) for the growth hormone gene of fishes for phylogenetic reconstructions. The results obtained agree remarkably well with the generally accepted phylogenies based on morphology and paleontology (Fig. 1).

* *Present address:* Hopkins Marine Station, Stanford University, Department of Biological Sciences, Pacific Grove, CA 93950-3094, USA

** *Present address:* Stazione Zoologica, Naples, Italy

Correspondence to: G. Bernardi

Materials and Methods

Sequences. We studied the amino acid sequences from 24 euteleostean fishes (see Table 1 for a list and Fig. 1 for the system-

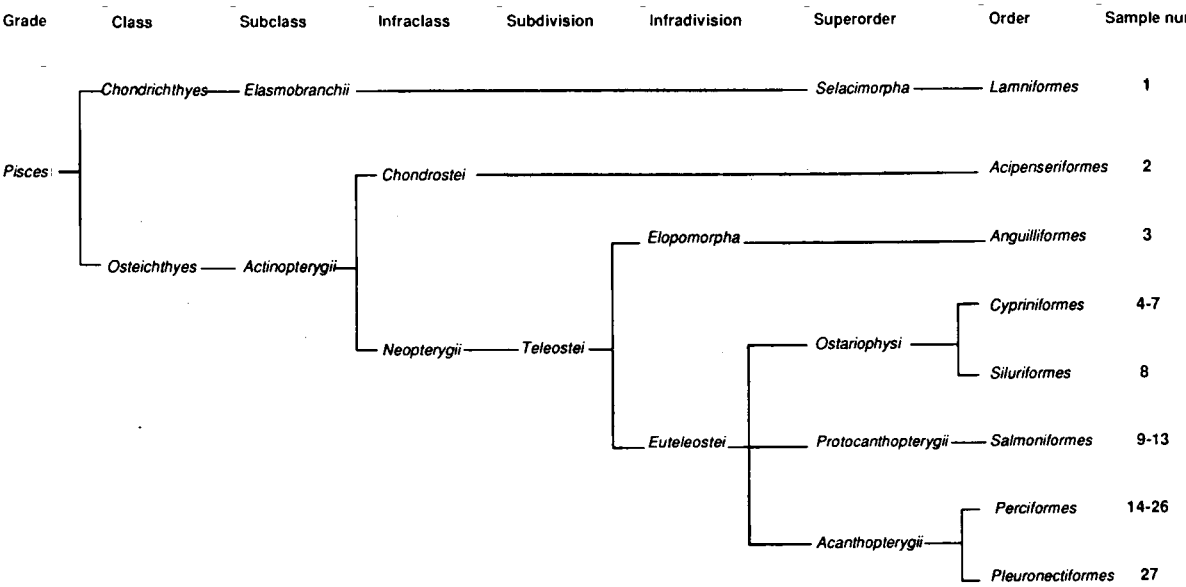

Fig. 1. Hierarchy of higher categories of fishes (Nelson 1984), based on morphological characters.

atics of the species) ranging from the older superorders Ostariophysi and Protacanthopterygii to the most recent order Pleuronectiformes, one elopomorph, the japanese eel *Anguilla japonica* (Teleostei, Anguilliformes), and one chondrostean fish, the sturgeon (Osteichthyes, Chondrostei). A cartilaginous fish, the blue shark *Prionace glauca* (Chondrichthyes, Lamniformes), was used as the outgroup. The source of most sequences was the NBRF (National Biomedical Research Foundation), release 33 (June 1992); sequences 6 and 7 were from GenBank release 71 (June 1992); sequences 1, 2, 8, 9, and 16 were from recently published data not yet available in databanks; sequence 22 was from a personal communication; the sequences for *Tilapia buttikofferi* and *Oreochromis grahami* (sequences 14 and 15, respectively, in Fig. 1) were determined in our laboratory (S. Caccio', G. D'Onofrio, G. Bernardi, and G. Bernardi, manuscript in preparation). Table 1 lists the mnemonics and references of the analyzed sequences.

Alignments of Sequences. Amino acid sequences were aligned (Fig. 2) using the CLUSTAL program (Higgins and Sharp 1988). Out of 193 residues for the consensus sequence, only 150 were used for the phylogenetic analysis; insertions, deletions, and regions of ambiguous alignments were removed from the analysis. Of the 150 positions, 108 were variable, and 96 were phylogenetically informative.

Phylogenetic Analyses. Phylogenetic analyses were done using the maximum parsimony (MP) method. The most parsimonious trees were determined using the heuristic or branch and bound options of the phylogenetic analyses using parsimony (PAUP) program (Swofford 1990). The degree of confidence that could be assigned to the various groupings in the most parsimonious trees was determined by bootstrapping with 100 replicates, using the corresponding options in the program. The recommended 2,000 replicates (Hedges 1992) were not performed because of the excessive computer time involved. Grouping is usually considered to be significantly supported if it appears in 95% or more of the most parsimonious trees (Felsenstein 1985).

Results and Discussion

Sequences

The longest among the growth hormone sequences analyzed here, those of cyprinids and salmonids, contained 186 amino acids, while the shortest one, that of the flounder, was only 168 residues long (Fig. 2). Apart from a few deletions and insertions, the growth hormone is a remarkably conserved protein. The molecule is composed of four conserved regions, which are likely to be functionally important, Ag, Bg, Cg, Dg, and four variable regions, V1, V2, V3, V4 (Kawauchi and Yasuda 1989; Watanabe et al. 1992). These variable regions may create ambiguities responsible for the slight differences found between the alignment presented here (Fig. 2) and those previously published (Watanabe et al. 1992). To avoid ambiguities for the phylogenetic reconstructions, insertions and deletions were excluded from our analysis, as already mentioned. However, it should be stressed that these differences are informative. Some deletions were characteristic of particular groups. A deletion of 14 amino acids (at position 144) was only found in Pleuronectiformes; a deletion of five residues (at position 102) in Acanthopterygii; another one of two amino acids (at position 89) in Salmonidae; and two deletions of one amino acid each (positions 139 and 150) in Ostariophysi.

Sequence Homologies

Several closely related fishes from the same genus did not show any difference at the protein level.

Table 1. List of fish species analyzed in this work[a]

Number	Species	Mnemonics	Source
1	*Prionace glauca*		Yamaguchi et al. (1989)
2	*Acipenser guldenstadt*		Yasuda et al. (1992)
3	*Anguilla japonica*	A27268	NBRF
4	*Cyprinus carpio*	S02764	NBRF
5	*Ctenopharyngodon idella*	A32424	NBRF
6	*Hypophthalmychtis multitrix*	Hyppgh	GenBank
7	*Hypophthalmychtis nobilis*	Hyppgi	GenBank
8	*Ictalurus punctatus*		Watanabe et al. (1992)
9	*Esox lucius*		Schneider et al. (1992)
10	*Oncorhyncus keta*	A23154	NBRF
11	*Oncorhyncus kisutch*	STONC	NBRF
12	*Salmo gairdneri*	A25791	NBRF
13	*Salmo salar*	S03709	NBRF
14	*Tilapia buttikofferi*		Our data[b]
15	*Oreochromis grahami*		Our data[b]
16	*Oreochromis mossambicus*		Yamaguchi et al. (1991)
17	*Oreochromis niloticus*	A32478	NBRF
18	*Euthynnis pelamis*	JK0021	NBRF
19	*Thunnus albacares*	JU0030	NBRF
20	*Thunnus thynnus*	S01746	NBRF
21	*Acanthopagrus butcheri*	X59377	NBRF
22	*Acanthopagrus latus*		H.J. Tsai, personal comm.
23	*Pagrus major*	S00747	NBRF
24	*Lates calcarifer*	X59378	NBRF
25	*Sparus auratus*	S54890	NBRF
26	*Seriola quinqueradiata*	STFI	NBRF
27	*Paralichthys olivaceus*	S04355	NBRF

[a] Fish species have been listed in the order used in Figs. 1–4. Mnemonics from NBRF (release 33, June 1992), Genbank (Release 71, June 1992), literature data, or personal communication were used to obtain the sequences

[b] S. Caccio', G. D'Onofrio, G. Bernardi, and G. Bernardi (paper in preparation)

Within the genera *Oreochromis, Oncorhynchus,* and *Hypophthalmichtys,* amino acid identity was 100%. Within families, a high identity was also found: 95.8% for salmonids, 99.5% for cichlids (tilapiine cichlids), and 97.7% for cyprinids. This situation clearly rules out any problems that might potentially arise from the fact that salmonids and cyprinids, being ancient tetraploids, contain several copies of the gene. Since the growth hormone gene is a highly conserved protein, it provided a better resolution for more distantly related taxa. Nucleic acid sequences may, however, be more informative for studying the relationships between closely related taxa (paper in preparation).

Phylogenetic Trees

The maximum parsimony (MP) analysis of our data using the program PAUP resulted in 12 most parsimonious trees (length = 340 steps; the Consistency Index [CI] was 0.862; the CI excluding uninformative characters was 0.853). One of these trees is shown in Fig. 3. The branching orders were very highly statistically supported, with bootstrap results often higher than 95%. The results of the bootstrap analysis are shown in Fig. 4 superimposed with the consensus tree (majority rule, 50%) for the 6 most parsimonious trees.

The 6 most parsimonious trees differed only for relationships below the order level, such as the relationship within salmonids, which corresponded to taxa with few differences in sequence, as mentioned before. On the other hand, the relationships between the major lineages, Teleostei, Elopomorpha, Euteleostei, Ostaryophysi, Protacanthopterygii, and Acanthopterygii, were always the same for all the 6 most parsimonious trees. Their positions were statistically well supported (Fig. 4) with bootstrap values ranging from 94 to 100% (with the exception of the Protacanthopterygii, which showed a bootstrap value of 69%).

At the major-lineages level, the Ostariophysi seem to be the sister group of the Protacanthopterigyi + Acanthopterygii (a group present in 99% of the generated trees). The Ostariophysi would be, in this case, at the start of the Euteleostei, in agreement with Rosen (1973) and Greenwood and Lauder (1981). The Acanthopterygii seem to be derived from the Paracanthopterygii, confirming previous hypotheses (Nelson 1984). Interestingly, the posi-

```
                      -22                     1         .         .         .         .         .         .         .         .
                                                   ---------------------------------------------------------------------------------
 1 P.glauca           YPLLPLS*******************DLFAKAVHRAQHLHLVAAETTKDFERKYIPEEQRHSHKSSPSAFCQSETIPAPTGKEDAQQRSDRELLLYSLLLIQSWLNPINQL

 2 A. guldenstadt     *******************YPMIPLSSLFTNAVLRAQYLHQLAADIYKDFERTYMPNEQRHSSKNSPSAFCYSETIPAPTGKDEAQQRSDVELLQFSLALIQSWISPLQSL
 3 A.japonica         VEPISLY*******************NLFTSAVNRAQHLHTLAAEIYKEFERSIPPEAHRQLSKTSPLAGCYSDSIPTPTGKDETQEKSDGYLLRISSALIQSWVYPLKTL
 4 C.carpio           MARALVLLSVVLVSLLVNQGRASDNQRLFNNAVIRVQHLHQLAAKMINDFEDSLLPEERRQLSKIFPLSFCNSDYIEAPTGKDETQKSSMLKLLRISFRLIESWEFPSQTL
 5 C.idella           MARALVLLSVVLVSLLVNQGTASENQRLFNNAVIRVQHLHQLAAKMINDFEDNLLPEERRQLSKIFPLSFCNCDSIEAPTGKDETQKSSMLKLLRISFRLIESWEFPSQTL
 6 H.mulitrix         MARALVLLSVVLVSLLVNQGTASENQRLFNNAVIRVQHLHQLAAKMINDFEDNLLPEERRQLSKIFPLSFCNSDSIEAPTGKDETQKSSMLKLLRISFRLIESWEFPSQTL
 7 H.nobilis          MARALVLLSVVLVSLLVNQGTASENQRLFNNAVIRVQHLHQLAAKMINDFEDNLLPEERRQLSKIFPLSFCNSDSIEAPTGKDETQKSSMLKLLRISFRLIESWEFPSQTL
 8 I.punctatus        **********************FESQRLFNNAVIRVQHLHQLAAKMMDDFEEALLPEERKQLSKIFPLSFCNSDSIEAPAGKDEAQKSSVLKLLHTSYRLIESWEFPSRNL
 9 E.lucius           MGQVFLLMPVLLVAGYLSLGAAMENQRLFNIADNRDQHLHLLAQKMFNDFEGTLLPDERRQLNKIFLLDFCNSDSIVSPIDKHETEKSSDLKLLHISFRLIESWEYPSQTL
10 O.keta             MGQVFLLMPVLLVSCFLSQGAAIENQRLFNIAVSRVQHLHLLAQKMFNDFDGTLLPDERRQLNKIFLLDFCNSDSIVSPVDKHETQKSSVLKLLHISFRLIESWEYPSQTL
11 O.kisutch          MGQVFLLMPVLLVSCFLSQGAAIENQRLFNIAVSRVQHLHLLAQKMFNDFDGTLLPDERRQLNKIFLLDFCNSDSIVSPVDKHETQKSSVLKLLHISFRLIESWEYPSQTL
12 S.gairdneri        MT********MITN***SRGSAIENQRLFNIAVSRVQHLHLLAQKMFNDFDGTLLPDERRQLNKIFLLDFCNSDSIVSPVDKHETQKSSVLKLLHISFRLIESWEYPSQTL
13 S.salar            MGQVFLLMPVLLVSCFLSQGAAMENQRLFNIAVNRVQHLHLMAQKMFNDFEGTLLPDERRQLNKIFLLDFCNSDSIVSPIDKLETQKSSVLKLLHISFRLIESWEYPSQTL
14 T.buttikofferi     MNSVVLLLSVVCLG**VSSQQITDSQRLFSIAVNRVTHLHLHAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRTSVLKLLSISYRLVESWEFPSRSL
15 O.grahami          MNSVVLLLSVVCLG**VSSQQITDSQRLFSIAVNRVTHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYGLVESWEFPSRSL
16 O.mossambicus      ******************QQITDSQRLFSIAVNRVTHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYGLVESWEFPSRSL
17 O.niloticus        MNSVVLLLSVVCLG**VSSQQITDSQRLFSIAVNRVTHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYGLVESWEFPSRSL
18 E.pelamis          ******************QPITESQRLFSIAVSRVQNLHLLAQRLFSDFESSLQTQEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL
19 T.albacares        ******************QPITDSQRLFSIAVSRIQHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVQSWEFPSRSL
20 T.thynnus          MDRVFLLLSVLSLG**VSSQPITDSQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL
21 A.butcheri         MDRVVLMLSVLSLG**VSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL
22 A.latus            MDRVVLMLSVLSLG**VSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQRQLDKIFLQTFCNSDYIISPIDKHQTQRSSVLKLLSISYRLVESWEFPSRSL
23 P.major            MDRVVLMLSVLSLG**VSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQLKLNKIF*PDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL
24 L.calcarifer       MDRVVLLLSVLSLG**VSSQPITDSQRLFSIAVSRVQHLHLLAQRRFSEFESSLQTEEQRHVNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFSSRSL
25 S.aurata           MDRVVLMLSVMSLG**VSSQPITDGQRLFSSAVSRVQHLHLLAQRLFSDFESSLQTEEERQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL
26 S.quinqueradiata   MDRVVLLLSVLSLG**VSSQPITDSQHLFSIAVSRIQNLHLLAQRLFSNFESTLQTEDQRQLNKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFSSRFL
27 P.olivaceus        MNRVILLLSVMCVG**VSSQPITENQRLFSIAVGRVQYLHLVAKKLFSDFENSLQLEDQRLLNKIASKEFCHSDNFLSPIDKHETQGSSVQKLLSVSYRLIESWEFFSRFL
                      .         .         .         .         .         .         .         .         .         .         .
                                          -------------------- ------- --  ----              ------------------------------
 1 P.glauca           R**********TSDRVYDKLRDLEEGIFALMKTLEDGG*SSQGFAWLKF*SYERFDGNLSEEA*LMKNYGLLACFKKDMHKVETYLKVMNCKRFAESNCTV

 2 A. guldenstadt     SRVFTNSLVFLTSDRVFEKLKDLEEGIVALMRDLGEGG*FGSSTLL*KL*TYDKFDVNLRNDDALFKNYGLLSCFKKDMHKVETYLKVMKCRRFVESNCTL
 3 A.japonica         SDAFSNSLMFGTSDGIFDKLEDLNKGINELMKVVGDGG*IYIEDVR*NL*RYENFDVHLRNDAGLMKNYGLLACFKKDMHKVETYLKVTKCRRFVESNCTL
 4 C.carpio           SGTVSNSLTVGNPNQITEKLADLKMGISVLIKGCLDGQPNMDDNDSLPLP*FEDFYLTMG*ENNLRESFRLLACFKKDMHKVETYLRVANCRRSLDSNCTL
 5 C.idella           SGQVSNSLTVGNPNQITEKLADLKVGISVLIKGCLDGQPNMDDNDSLPLP*FEDFYLTMG*ESSLRESFRLLACFKKDMHKVETYLRVANCRRSLDSNCTL
 6 H.mulitrix         SGAVSNSLTVGNPNQITEKLADLKVGISVLIKGCLDGQPNMDDNDSLPLP*FEDFYLTMG*ESSLRESFRLLACFKKDMHKVETYLRVANCRRSLDSNCTL
 7 H.nobilis          SGAVSNSLTVGNPNQITEKLADLKVGISVLIKGCLDGQPNMDDNDSLPLP*FEDFYLTMG*ESSLRESFRLLACFKKDMHKVETYLRVANCRRSLDSNCTL
 8 I.punctatus        **********GNPNHISEKLADLKMGIGVLIEGCVDGQTGLDEEDSLAPP*FEDFYQTLS*EGNLRKSFRLLSCFKKDMHKVETYLSVAKCRRSLDSNCTL
 9 E.lucius           THTMSNNL***NQNQMSEKLSNLKVGINLLIKGNQEDVPSLDDNDSQQLLPYGNYYQNLGDNDNDRRNYELLACFKKDMHKVEYYLTVAKCRKSLEANCTL
10 O.keta             **IISNSLMVRNANQISEKLSDLKVGINLLITGSQDGVLSLDDNDSQQLPPYGNYYQNLGGDGNVRRNYELLACFKKDMHKVETYLTVAKCRKSLEANCTL
11 O.kisutch          **IISNSLLVGNANQISEKLSDLKVGINLLIMGSQDGLLSLDDNDSQQLPRYGNYYQNPGGDGNVRRNYELLACFKKDMHKVETYLTVAKCRKSLEANCTL
12 S.gairdneri        **IISNSLMVRNANQISEKLSDLKVGINLLITGSQDGVLSLDDNDSQQLPPYGNYYQNLGGDGNVRRNYELLACFKKDMHKVETYLTVAKCRKSLEANCTL
13 S.salar            **TISNSLMVRNSNQISEKLSDLKVGINLLIKGSQDGVLSLDDNDSQQLPPYGNYYQNLGGDGNVRRNYELLACFKKDMHKVETYLTVAKCRKSLEANCTL
14 T.buttikofferi     SGGSSLRNQIS*P*****RLSELKTGILLLIRANQDEAENYPDSDALQHAPYGNYYQSLGGNESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
15 O.grahami          SGGSSLRKQIS*P*****RLSELKTGILLLIRANQDEAENYPDTDTLQHAPYGNYYQSLGGNESLRQTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
16 O.mossambicus      SGGSSLRNQIS*P*****RLSELKTGILLLIRANQDEAENYPDTDTLQHAPYGNYYQSLGGNESLRQTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
17 O.niloticus        SGGSSLRNQIS*P*****RLSELKTGILLLIRANQDEAENYPDTDTLQHAPYGNYYQSLGGNESLRQTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
18 E.pelamis          SG**AQRNQISE******KLSDLKMGIQLLIRANQDGAEMFADSSALQLAPYGNYYQSLGGDESLRRNYELLACFKKDMHKVETYLMVAKCRLSPEANCTL
19 T.albacares        SGGSALRNQIS*P*****KLSELKTGIHLLIRANQDGAEMFADSSALQLAPYGNYYQSLGADESLRRSYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
20 T.thynnus          SGGSAPRNQIS*P*****KLSELKTGIHLLIRANQDGDEMFADSSALQLAPYGNYYQSLGADESLRRSYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
21 A.butcheri         AGGSAPRNQIS*P*****KLSELKTGIHLLIRANEDGAELFPDSSALQLAPYGDYYHSPGTDESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
22 A.latus            AGGSAPRNQIS*P*****KLSELKTGIHLLIRANEDGAELFPDSSALQLAPYGNYYQSPGTDESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
23 P.major            SGGSAPRNQIS*P*****KLSELKMGIHLLIRANEDGAEIFPDSSALQLAPYGNYYQSLGADESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
24 L.calcarifer       SGGSAPRDQISSP*****KLSELKTGILLLIRANQDGAEMFSDSSALQLAPYGNYYQSLGADESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
25 S.aurata           SGGSAPRNQIS*P*****KLSELKTGIHLLIRANEDGAEIFPDSSALQLAPYGNYYQSLGTDESLRRTYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
26 S.quinqueradiata   SGGSALRNQIS*P*****RLSELKTGIQLLITANQDGAEMFSDVSALQLAPYGNFYQSLGGEELLRRNYELLACFKKDMHKVETYLTVAKCRLSPEANCTL
27 P.olivaceus        VASFAVRTQVTS******KLSELKMGLLKLIEANQDGAGGFSESSVLQLTPYGNS**************ELFACFKKDMHKVETYLTVAKCRLFPEANCTL
```

Fig. 2. Aligned amino acid sequences from the pregrowth hormone gene for 26 teleostean fishes, compared with the corresponding sequence from the blue shark, *P. glauca*. Entire sequences are shown, with gaps (*) inserted to increase sequence similarity. A line above the sequences indicates the regions used in the phylogenetic analysis. The first amino acid after the signal peptide is numbered, and *dots* mark every 10th position.

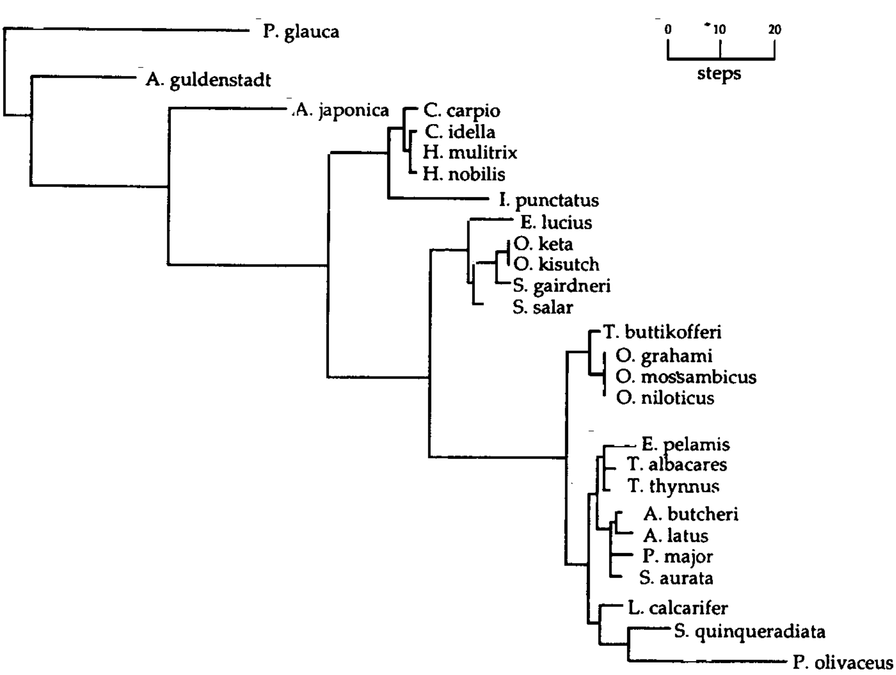

Fig. 3. One of the six most parsimonious trees obtained for the growth hormone. (See Materials and Methods.) The length of each branch (steps) is indicated above the tree, as a legend. For each of the shortest trees, the number of steps was 340, the Consistency Index (C.I.) was 0.862, and the C.I. excluding uninformative characters was 0.853.

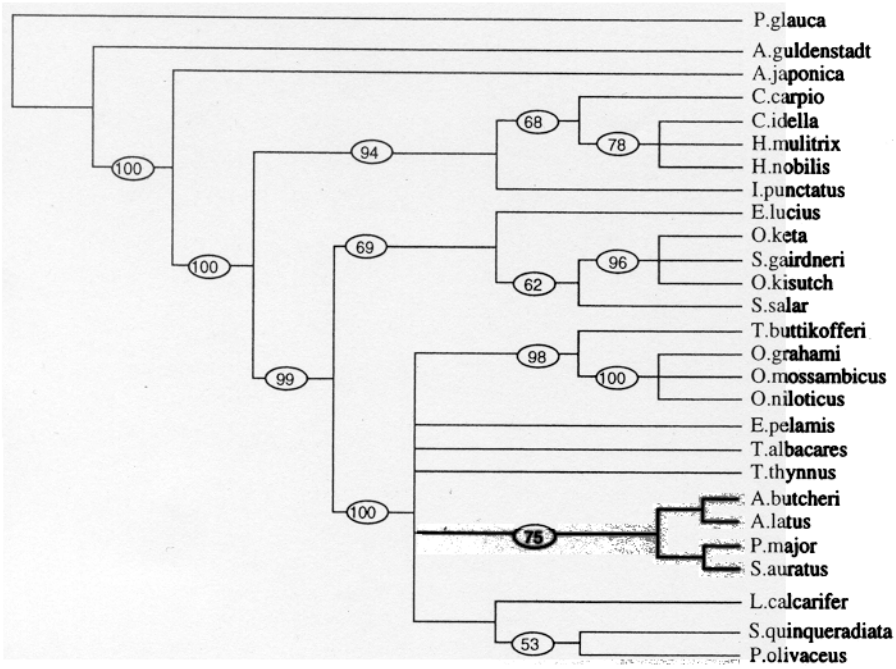

Fig. 4. Consensus tree (majority rule) obtained from the six most parsimonious trees. The *circled numbers* indicate the bootstrapping results for the node. See Results for details.

tion of the recent order Pleuronectiformes, derived from the order Perciformes, is in agreement with classical phylogenies (Lauder and Liem 1983; Nelson 1984).

The excellent agreement of the molecular data obtained in the present work (Figs. 3 and 4) with phylogenies (Fig. 1) based on morphological characters and paleontological data (Nelson 1984) is of interest in two respects: (1) It is in sharp contrast with the frequent divergencies between molecular and morphological-paleontological data found in the case of mammals (even when using the growth hormone sequence), a point which will be discussed elsewhere in detail (G. D'Onofrio, G. Bernardi, S. Caccio', and G. Bernardi, paper in preparation); and (2) it is the first case in which such a congruence has been found over extended evolutionary time in the case of vertebrates; in turn, this suggests that phylogenetic studies based on the growth hormone might be useful in order to solve issues like the controversial positions of Crossopterygii and Dipneusti.

Acknowledgments. We thank H.J. Tsai (Institute of Fisheries Science, National Taiwan University) for having kindly provided unpublished data on the growth hormone gene from *A. latus*.

References

Bernardi G, Sordino P, Powers DA (1992) Nucleotide sequence of the 18S ribosomal ribonucleic acid gene from two teleosts and two sharks and their molecular phylogeny. Mol Mar Biol Biotechnol 1:187–194

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

Greenwood PH, Lauder GV (1981) The protractor pectoralis muscle and the classification of teleost fishes. Bull Br Mus Nat His (Zool) 41:213–234

Hedges SB (1992) The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. Mol Biol Evol 9:366–369

Higgins DG, Sharp PM (1988) CLUSTAL: a package for performing multiple sequence alignments on a microcomputer. Gene 73:237–244

Kawauchi H, Yasuda A (1989) Evolutionary aspects of growth hormone from nonmammalian species. In: Muller EE, Cocchi D, Locatelli V (eds) Advances in growth hormone and growth factor research. Pythagora Press, Roma-Milano, pp 51–68

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6196–6200

Lauder GV, Liem KF (1983) The evolution and interrelationships of the actinopterygian fishes. Bull Museum Comp Zool 150:95–197

MacAllister DE (1987) A working list of the fishes of the world. Ichthyology section, National Museum of Natural Science, National Museums of Canada, Ottawa

Martin AP, Naylor GJP, Palumbi SR (1992) Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357:153–155

Meyer A, Wilson AC (1990) Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. J Mol Evol 31:359

Meyer A, Kocher TD, Basasibuaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 247:550–553

Nelson JS (1984) Fishes of the world, 2nd ed. Wiley, New York

Normark BB, McCune AR, Harrison RG (1991) Phylogenetic relationships of Neopterygian fishes, inferred from mitochondrial DNA sequences. Mol Biol Evol 8:819–834

Patterson C (1973) Interrelationships of holosteans. In: Greenwood PH, Miles RS, Patterson C (eds) Interrelationships of fishes. Academic Press, New York, pp 233–605

Patterson C (1977) The contribution of Paleontology to teleostean phylogeny. In: Hecht MK, Goody PC, Hecht BM, (eds) Major patterns in vertebrate evolution. Plenum, New York, pp 579–643

Patterson C, Rosen D (1977) Review of the ichthyodectiform and other Mesozoic teleost fishes and the theory and practice of classifying fossils. Bull Am Museum Nat His 158:81–172

Rosen DE (1973) Interrelationships of higher euteleostean fishes. In: Greenwood PH, Miles RS, Patterson C (eds). Interrelationships of fishes. J Linn Soc (Zool) 53:397–513 (Suppl 1), New York, Academic

Rosen D (1982) Teleostean interrelationships, morphological function, and evolutionary inference. Am Zool 22:261–273

Schneider JF, Myster SH, Hackett PB, Guise KS, Faras AJ (1992) Molecular cloning and sequence analysis of the cDNA for northern pike (*Esox lucius*) growth hormone. Mol Mar Biol Biotechnol 1:106–112

Stock DW, Moberg KD, Maxson LR, Whitt GS (1991) A phylogenetic analysis of the 18S ribosomal RNA sequence of the coelacanth *Latimeria chalumnae*. Environmental Biology of Fishes 32:99–117

Swofford DL (1990) PAUP: Phylogenetic Analysis Using Parsimony, version 3.0. Illinois Natural History Survey, Champaign

Watanabe K, Igarashi A, Noso T, Chen TT, Dunham RA, Kawauchi H (1992) Chemical identification of catfish growth hormone and prolactin. Mol Mar Biol Biotechnol 1:239–249

Yamaguchi K, Yasuda A, Lewis U, Yokoo Y, Kawauchi H (1989) The complete amino acid sequence of growth hormone of an elasmobranch, the blue shark (*Prionace glauca*). Gen Endocrinol 73:252–259

Yamaguchi K, King DS, Specker JL, Nishioka RS, Hirano T, Bern HA (1991) Amino acid sequence of growth hormone isolated from medium of incubated pituitary glands of tilapia (*Oreochromis mossambicus*). Gen Comp Endocrinol 81:322–331

Yasuda A, Yamaguchi A, Noso T, Papkoff H, Plenov AL, Nicoll CS, Kawauchi H (1993) The complete aminoacid sequence of growth hormone from sturgeon (*Acipenser guldenstadt*). Biochim Biophys Acta (in press)