Comparative sequence analysis of the complete set of 40S ribosomal proteins in the Senegalese sole (Solea senegalensis Kaup) and Atlantic halibut (Hippoglossus hippoglossus L.) (Teleostei: Pleuronectiformes): phylogeny and tissue- and development-specific expression

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Abstract

Background: Ribosomal proteins (RPs) are key components of ribosomes, the cellular organelle responsible for protein biosynthesis in cells. Their levels can vary as a function of organism growth and development; however, some RPs have been associated with other cellular processes or extraribosomal functions. Their high representation in cDNA libraries has resulted in the increase of RP sequences available from different organisms and their proposal as appropriate molecular markers for phylogenetic analysis.

Results: The development of large-scale genomics of Senegalese sole (Solea senegalensis) and Atlantic halibut (Hippoglossus hippoglossus), two commercially important flatfish species, has made possible the identification and systematic analysis of the complete set of RP sequences for the small (40S) ribosome subunit. Amino acid sequence comparisons showed a high similarity both between these two flatfish species and with respect to other fish and human. EST analysis revealed the existence of two and four RPS27 genes in Senegalese sole and Atlantic halibut, respectively. Phylogenetic analysis clustered RPS27 in two separate clades with their fish and mammalian counterparts. Steady-state transcript levels for eight RPs (RPS2, RPS3a, RPS15, RPS27-1, RPS27-2, RPS27a, RPS28, and RPS29) in sole were quantitated during larval development and in tissues, using a real-time PCR approach. All eight RPs exhibited different expression patterns in tissues with the lowest levels in brain. On the contrary, RP transcripts increased co-ordinately after first larval feeding reducing progressively during the metamorphic process.

Conclusion: The genomic resources and knowledge developed in this survey will provide new insights into the evolution of Pleuronectiformes. Expression data will contribute to a better understanding of RP functions in fish, especially the mechanisms that govern growth and development in larvae, with implications in aquaculture.
Background

The eukaryotic ribosome is a complex macromolecular structure composed of a large (60S) and a small (40S) subunit. The large ribosomal subunit catalyses peptide bond formation and is responsible for channelling the nascent proteins through their exit tunnel. The small ribosomal subunit binds mRNA and is responsible for the fidelity of translation by ensuring the correct base pairing between aminocyt-tRNAs and codons of the mRNA in the decoding centre [1]. Biochemically, the eukaryotic ribosome is composed of four ribosomal RNA molecules and over 70 ribosomal proteins (RPs) [2]. In mammals, the 60S and 40S subunits are composed of 47 and 32 RPs, respectively [3]. Each mammalian RP is typically encoded by a single gene except RPS4 in human [4], and RPS27 in rat [5] and human [6], which are encoded by two separate genes. In contrast, in the yeast Saccharomyces cerevisiae, the 78 RPs are encoded by 137 genes, 59 of which are duplicated [7]. In fish, the complete set of RPs in Fugu rubripes [8] and Ictalurus punctatus [9] has been described. Of the 32 RPs from the 40S subunit, a duplication of RPS27 in both species and of RPS26 in I. punctatus was observed. In the 47 RPs from the 60S subunit, all of them but one (RPL5 in I. punctatus) appeared to have only one type of mRNA [10].

RPs play a critical role in protein biosynthesis. Cellular levels change as a function of growth rate in bacteria and fungi [11-14]. In fish, mRNA levels increase co-ordinately during embryogenesis and larval development [15-18]. In mammals, certain tumors have substantially increased levels of some RP transcripts [19,20]. However, different RPs have also been associated with various other cellular processes; the so-called extraribosomal functions. For example, in Drosophila, mutations in the RPS2 gene appear to cause arrest of oogenesis [21] and RPS6 functions as a tumor suppressor in the hematopoietic system [22]. Mammalian RPS3 appears to possess apurinic/apyrimidinic endonuclease activity involved in DNA repair functions [23]. Haploinsufficiency of the RPS4 genes has been suggested to contribute to anatomic abnormalities associated with the Turner syndrome in humans [4]. The gene encoding RPS19 seems to participate in embryogenesis due to its capacity to interact with FGF-2, a factor involved in the differentiation process of different cell types [24]. Finally, apoptosis can be induced by inhibiting or activating expression of RPS3a and RPS27L, respectively [6,25].

Senegalese sole, Solea senegalensis (Pleuronectiformes: Soleidae), and Atlantic halibut, Hippoglossus hippoglossus (Pleuronectiformes: Pleuronectidae), are two commercially important flatfish species. During larval development, both species change from a symmetrical morphology to an asymmetric, benthic juvenile. This metamorphic process involves dramatic morphological and physiological changes. In Senegalese sole, metamorphosis occurs very early during larval development, between 12 and 19 days after hatching (DAH) [26]. In Atlantic halibut, metamorphosis begins with the migration of the left eye about 80 DAH [27]. Apoptotic processes induced by thyroxine hormone have been associated with this tissue remodelling in flatfish [28]. In addition, Senegalese sole larvae exhibit two different growth rates during development [26,29]. Because of the key role RPs play in cellular growth and proliferation and in some cases apoptosis, it is important to elucidate the expression pattern of RPs during flatfish development.

RPs are highly represented in cDNA libraries [9,10]. The development of large-scale genomics on Senegalese sole and Atlantic halibut has made possible an efficient and systematic analysis of RP sequences in both species. In this work, we report the complete set of 32 40S subunit RP cDNAs for both Senegalese sole and Atlantic halibut and describe their main characteristics. Comparative sequence analysis revealed the existence of two and four RPS27 genes in Senegalese sole and Atlantic halibut, respectively. Real-time PCR analysis revealed different RP expression patterns during larval development and in tissues in sole.

Results

Characteristics of the 40S RPs

Sequence analysis of normalized libraries for Senegalese sole allowed the identification of 31 out of 32 40S subunit RPs (only RPS28 was absent). RPS28 was obtained from a premetamorphic stage larval library using specific primers (Table 1). Overall, 40S RP genes were not highly represented in the normalized libraries accounting for 252 (2.5%) out of the 10,099 good sequences. The number of clones for each RP ranged between 24 for RPS2 and only 1 for RPS27-2 and RPS29 (Table 2A).

Gene sizes for the complete set of 40S RPs ranged between 279 and 1,043 bp for RPS29 and RPSa, respectively. Only RPS2, RPS4 and RPS8 had partial sequences missing the 5’-ends. All cDNA sequences have been deposited in the GenBank/EMBL/DDBJ with accession numbers from AB291554 to AB291586 (Table 2A). Most RPs (63.6%) used TAA as termination codon. Only RPS8, RPS11, RPS12, RPS15, RPS17, RPS27-1, RPS28, and RPS29 used TAG, and RPS6 and RPS24, TGA. The 3’-UTRs were highly AT-rich. All RPs had a canonical AATAAA polyadenylation signal between 7–37 nucleotides from the poly(A) tail.

In halibut, sequences for all except RPS29 and RPS27-2 were identified from the Pleurogene database (Table 2B). In most cases, the complete coding sequences were obtained, but 3’-end sequencing was performed for all RP sequences to confirm the 3’ends, particularly of the long
Table 1: Primers used for real-time PCR gene expression analysis. F and R refer to forward and reverse primers, respectively.

| Target | Primer pair name | Sequence 5' - position | 3' - position |
|--------|------------------|-------------------------|--------------|
| RPS2   | SserpS2•1        | 5'-CCAAGCTTCGGTTGTCCCGGTCA-3' (F) | 434          |
|        | SserpS2•2        | 5'-CGGGGGCCAGGAGATGAGACG-3' (R) | 127          |
| RPS3a  | SserpS3a•1       | 5'-TCGAGAAAGACCTCCTACGCCAGCA-3' (F) | 560          |
|        | SserpS3a•2       | 5'-AGATCATTGCTGCAACCTACGGGTCG-3' (R) | 94           |
| RPS15  | SserpS15•1       | 5'-CATGCTTGCTGCTGCTGCTGCTGCT-3' (F) | 543          |
|        | SserpS15•2       | 5'-GGGCGACGGTGGACGGGTGATG-3' (R) | 116          |
| RPS27-1| SserpS27-1•1     | 5'-CCCGAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA...
Table 2: Structural characteristics of the cDNAs encoding RPs of (A) Senegalese sole, *Solea senegalensis* and (B) Atlantic halibut, *Hippoglossus hippoglossus*. Lengths of coding regions, available 5'-UTR, 3'-UTR and poly (A) tail distances from poly(A) signals are indicated. Asterisk (*) denotes RPS coding sequences that were derived partially or completely from sequences present in GenBank. ND, not detected.

(A)

| Gene | # clones | Accession #       | Coding region | 5'-UTR | 3'-UTR | Poly(A) from poly(A)Signal |
|------|----------|-------------------|---------------|--------|--------|---------------------------|
| RPSa | 15       | AB291586          | 942           | 60     | 41     | 17                        |
| RPS2 | 24       | AB291554          | 843           | ND     | 39     | 12                        |
| RPS3 | 16       | AB291555          | 738           | 9      | 84     | 17                        |
| RPS3a| 13       | AB291556          | 801           | 1      | 41     | 17                        |
| RPS4 | 7        | AB291557          | 792           | ND     | 57     | 17                        |
| RPS5 | 5        | AB291558          | 612           | 29     | 62     | 37                        |
| RPS6 | 14       | AB291559          | 750           | 23     | 39     | 22                        |
| RPS7 | 19       | AB291560          | 585           | 51     | 36     | 13                        |
| RPS8 | 6        | AB291561          | 627           | ND     | 52     | 24                        |
| RPS9 | 6        | AB291562          | 585           | 46     | 67     | 14                        |
| RPS10| 9        | AB291563          | 501           | 5      | 44     | 15                        |
| RPS11| 7        | AB291564          | 486           | 11     | 77     | 26                        |
| RPS12| 16       | AB291565          | 399           | 54     | 23     | 11                        |
| RPS13| 4        | AB291566          | 456           | 3      | 37     | 13                        |
| RPS14| 2        | AB291567          | 456           | 37     | 43     | 13                        |
| RPS15| 4        | AB291568          | 438           | 24     | 43     | 16                        |
| RPS15a| 4       | AB291569          | 393           | 34     | 38     | 16                        |
| RPS16| 3        | AB291570          | 441           | 18     | 39     | 14                        |
| RPS17| 2        | AB291571          | 405           | 21     | 39     | 11                        |
| RPS18| 10       | AB291572          | 459           | 5      | 39     | 15                        |
| RPS19| 7        | AB291573          | 444           | 5      | 22     | 22                        |
| RPS20| 6        | AB291574          | 360           | 98     | 54     | 24, 29                    |
| RPS21| 4        | AB291575          | 252           | 92     | 44     | 7                         |
| RPS23| 5        | AB291576          | 451           | 19     | 41     | 14                        |
| RPS24| 9        | AB291577          | 396           | 17     | 84     | 22                        |
| RPS25| 5        | AB291578          | 372           | 6      | 66     | 24                        |
| RPS26| 6        | AB291579          | 348           | 23     | 47     | 15                        |
| RPS27-1| 7      | AB291580          | 255           | 30     | 200    | 20                        |
| RPS27-2| 1      | AB291581          | 255           | 62     | 190    | 13                        |
| RPS27a| 7       | AB291582          | 471           | 77     | 36     | 19                        |
| RPS28| 1        | AB291583          | 210           | 1      | 126    | 21                        |
| RPS29| 1        | AB291584          | 171           | 5      | 103    | 14                        |
| RPS30| 8        | AB291585          | 402           | 35     | 96     | 15                        |

(B)

| Gene | # clones | Accession #       | Coding region | 5'-UTR | 3'-UTR | Poly(A) from poly(A)Signal |
|------|----------|-------------------|---------------|--------|--------|---------------------------|
| RPSa | 3        | EB034722, EB039353| 930           | 84     | 41     | 17                        |
| RPS2 | 4        | EB029719, EB030051| 858           | 3      | 41     | 12                        |
| RPS3 | 2        | EB034826, EB036030| 741           | 25     | 126    | 12                        |
| RPS3a| 6        | EB035413, EB031090| 801           | 23     | 41     | 14                        |
| RPS4 | 8        | EB032598          | 792           | 41     | 57     | 20                        |
| RPS5 | 2        | EB032359, EB036495| 612           | 44     | 44     | 14                        |
| RPS6 | 2        | EB031692, EB032095| 750           | 40     | 33     | 14                        |
| RPS7 | 7        | EB032431          | 585           | 37     | 33     | 14                        |
| RPS8 | 5        | EB035663          | 627           | 24     | 39     | 11                        |
| RPS9 | 2        | EB040140, EB032308| 585           | 46     | 60     | 19                        |
| RPS10| 2        | EB031080          | 501           | 39     | 41     | 15                        |
| RPS11| 5        | EB031513          | 486           | 23     | 67     | 11                        |
| RPS12| 1        | EB029693          | 399           | 65     | 23     | 11                        |
| RPS13| 1        | EB034224          | 456           | 21     | 41     | 14                        |
| RPS14| 3        | EB040078          | 456           | 43     | 38     | 12                        |
| RPS15| 3        | EB036829, EB029855| 438           | 25     | 42     | 18                        |
tic halibut, total lengths ranged between 499 and 963 nt for RPS27-2 and RPS27-3, respectively. The Atlantic hali-
but RPS27-4 had a slightly shorter coding region (249 nt),
whereas no putative polyadenylation signal could be
identified in RPS27-3. RPS27-1 and RPS27-3 were repre-
sented by 4 and 5 clones in the halibut libraries, respec-
tively, and RPS27-4 by only 1 EST.

Both Senegalese sole paralogs showed a high divergence at
the nucleotide level when complete cDNAs (48.4% iden-
tity) or coding regions (73.7%) were aligned. At the
amino acid level, they differed in 9 residues with a
sequence identity of 89.3% (Figure 1B; Table 4). Similarly,
low sequence similarity (36.5–49.4%) was detected
among Atlantic halibut full-length sequences. These val-
ues ranged between 66.7 (RPS27-1 and RPS27-4) and
82.0% (RPS27-1 and RPS27-3) in the coding regions. At
the protein level, RPS27-4 was 2 amino acids shorter that
the other paralogs. Amino acid similarities ranged
between 74.4 (RPS27-3 and RPS27-4 with 21 amino acid
changes) and 95.2% (RPS27-1 and RPS27-3 with 4 resi-
due differences). Among species, S. senegalensis RPS27-1
and H. hippoglossus RPS27-1 were the closest evolutionary
homologues with 82.5% similarity using full-length
sequence, 92.5% in coding sequence and 100% in amino
acid sequence (Table 4).

A phylogenetic analysis based on RPS27 coding sequences
using the NJ, MP and ML methods showed that fish RPS27
genes grouped mainly in two distinct clades (Figure 2).
Both Senegalese sole and Atlantic halibut RPS27-2 genes
clustered with their fish counterparts. The RPS27-4
appeared more closely related to RPS27-2 than the other
two Atlantic halibut RPS27 genes. Moreover, RPS27-2 and
RPS27L genes in rat and human, respectively, formed a
sister clade sharing a common ancestor with fish RPS27-2
genes (bootstrap values higher than 50%). The other fish
RPS27 gene copies appeared linked in a clade that was not
well resolved. Both Senegalese sole and Atlantic halibut
RPS27-1 grouped with P. flesus and T. nigroviridis RPS27-1
(bootstrap values higher than 70%). Curiously, this clade
contained both I. punctatus RPS27 genes.

### Gene expression analysis

We used a quantitative approach based on reverse tran-
scription followed by real-time PCR amplification to
investigate the steady-state levels of eight sole RP tran-
scripts (RPS2, RPS3a, RPS15, RPS27-1, RPS27-2, RPS27a,
RPS28, and RPS29) in liver, spleen, intestine, stomach,
head kidney, gills, muscle, brain, heart, and skin. Relative
gene expression levels were normalized by measuring
ubiquitin levels.

All eight RP genes were expressed in detectable amounts
in all tissues (Figure 3). RPS2 transcripts were the most
abundant except in brain, where RPS27-1 showed the
highest values (1.82-fold higher than RPS2). On the other
hand, RPS27-2 was expressed at the lowest level in all tis-
sues analyzed.

RP genes were expressed differentially among tissues. RPS2,
RPS15, and RPS28 exhibited lower expression levels
in brain and RPS3a transcripts were reduced in brain,
heart, and skin. In contrast, RPS27-2 was expressed more
highly in intestine and stomach, and RPS29 in heart. If we
calculate the mean ribosomal expression ratio as a global
RP expression index, all tissues showed similar values (0.74–0.99) except brain with only 0.48.

We also investigated the expression pattern of RP genes during sole larval development. mRNA levels were determined in samples extracted from whole larvae pools collected from 2 to 22 DAH (Figure 4). Expression levels of each RP gene were normalized to that of GAPDH. RPS2, RPS27-1, RPS27a, RPS28, and RPS29 showed higher transcript levels than RPS3a, RPS15 and RPS27-2 during early (2 to 3 DAH) larval development in Senegalese sole.

All RP mRNAs increased from 2 to 3 DAH, 24 hours after first external feeding (2.1-fold as global mean). Fold induction values ranged between 1.7 for both RPS27-1 and RPS28 and 3.0 for RPS27-2. These levels were reduced at 9 DAH, two days before the onset of eye migration, with the lowest values at the first metamorphic stages (13 DAH). The number of mRNA molecules for the 8 RPs analyzed declined approximately 3.5-fold as global mean from 3 (pre-metamorphosis) to 13 DAH (metamorphosis). The fold reduction values ranged between 1.7 and 10.3 for RPS28 and RPS27-2, respectively. RPS27-1 was expressed at a higher level than RPS27-2 during all pre-

Table 3: Amino acid comparisons of the RPs from *S. senegalensis* (Sse) and *H. hippoglossus* (Hhi) with those of *I. punctatus* (Ipu), *F. rubripes* (Fru) and human (Hsa). Similarity values for Senegalese sole and Atlantic halibut are separated by "/".

| Gene | Sse | Hhi | Ipu | Fru | Hsa | Sse/Hhi | Ipu Fru Hsa |
|------|-----|-----|-----|-----|-----|---------|-------------|
| S5a  | 313 | 309 | 317 | 306 | 295 | 91.3    | 88.5/88.0   |
| S2   | 280 | 285 | 278 | 279 | 293 | 94.0    | 96.3/91.0   |
| S3   | 245 | 246 | 245 | 245 | 243 | 97.6    | 98.8/96.3   |
| S3a  | 266 | 266 | 266 | 266 | 264 | 96.6    | 94.7/94.7   |
| S4   | 263 | 263 | 263 | 263 | 263 | 96.6    | 95.8/99.9   |
| S5   | 203 | 203 | 203 | 203 | 204 | 99.5    | 99.0/98.5   |
| S6   | 249 | 249 | 249 | 249 | 249 | 98.4    | 95.6/94.0   |
| S7   | 194 | 194 | 194 | 194 | 194 | 98.5    | 96.4/95.9   |
| S8   | 208 | 208 | 208 | 208 | 208 | 98.1    | 89.4/88.9   |
| S9   | 194 | 194 | 194 | 194 | 194 | 97.9    | 95.9/96.9   |
| S10  | 166 | 166 | 166 | 166 | 165 | 97.6    | 96.4/94.6   |
| S11  | 161 | 161 | 161 | 161 | 158 | 98.1    | 90.6/91.8   |
| S12  | 132 | 132 | 132 | 132 | 132 | 97.7    | 97.7/95.5   |
| S13  | 151 | 151 | 151 | 151 | 151 | 98.0    | 98.7/98.0   |
| S14  | 151 | 151 | 151 | 151 | 151 | 96.0    | 95.4/99.3   |
| S15  | 145 | 145 | 145 | 145 | 145 | 98.6    | 96.6/96.6   |
| S15a | 130 | 130 | 130 | 130 | 130 | 100     | 96.9/96.9   |
| S16  | 146 | 146 | 146 | 146 | 146 | 97.9    | 96.6/97.3   |
| S17  | 134 | 134 | 134 | 134 | 135 | 92.5    | 92.5/96.3   |
| S18  | 152 | 152 | 152 | 152 | 152 | 98.7    | 99.3/98.0   |
| S19  | 147 | 147 | 147 | 147 | 145 | 92.5    | 89.1/87.1   |
| S20  | 119 | 119 | 119 | 119 | 119 | 98.3    | 98.3/97.5   |
| S21  | 83  | 83  | 83  | 83  | 83  | 92.8    | 92.8/90.4   |
| S22  | 143 | 143 | 143 | 143 | 143 | 100     | 99.3/99.3   |
| S24  | 131 | 132 | 131 | 131 | 133 | 98.5    | 96.9/96.2   |
| S25  | 123 | 131 | 124 | 123 | 125 | 95.1    | 83.1/82.3   |
| S26  | 115 | 115 | 115 | 115 | 115 | 98.3    | 93.0/94.8   |
| S27-1| 84  | 84  | 84  | 84  | 84  | 84      | See Table 4  |
| S27-2| 84  | 84  | 84  | 84  | 84  | 84      |              |
| S27-3| 84  | 84  | 84  | 84  | 84  | 84      |              |
| S27-4| 82  |     |     |     |     |         |              |
| S27a | 156 | 156 | 156 | 156 | 156 | 100     | 100/100     |
| S28  | 69  | 69  | 69  | 69  | 69  | 98.6    | 100/98.6    |
| S29  | 56  | 56  | 56  | 56  | 56  | 98.2    | 100/98.2    |
| S30  | 133 | 133 | 133 | 133 | 133 | 94.7    | 85.8/85.0   |

Overall: 92.1

92.4/94.0

95.4/92.5

92.8/89.8

$a$ and $b$ refer to similarities to *H. sapiens* RPS4X and RPS4Y isoforms, respectively.
$c$ and $d$ refer to similarities to *I. punctatus* RPS26-1 and RPS26-2 isoforms, respectively.
metamorphic, metamorphic and post-metamorphic stages (28-fold higher on average).

Discussion
In this work, we describe the complete set of 40S RPs in the Senegalese sole and Atlantic halibut. Sequences were generated from normalized cDNA libraries constructed for expressed sequence tag (EST) analysis. The rapid development of genomics in all biological research areas, including aquaculture, and the high representation of RPs in the cDNA libraries have favoured the availability of an increasing number of RP sequences from different organisms [30]. This fact has motivated their proposed use as appropriate molecular markers for phylogenetic analysis. In fact, concatenation of orthologous RP amino acid sequences to form a single one of more than 10,000 characters has allowed the reconstruction of phylogenetic relationships between animal, fungal, and plant kingdoms

Table 4: Amino acid similarities of RPS27 from S. senegalensis (Sse) and H. hippoglossus (Hhi) with those of I. punctatus (Ipu), F. rubripes (Fru) and H. sapiens (Hsa).

|            | Sse RPS27-2 | Hsi RPS27-1 | Hsi RPS27-2 | Hsi RPS27-3 | Hsi RPS27-4 | Ipu RPS27-1 | Ipu RPS27-2 | Fru RPS27-1 | Hsa RPS27-1 | Hsa RPS27-L |
|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Sse RPS27-1| 89.3        | 100.0       | 91.7        | 95.2        | 79.3        | 96.4        | 97.6        | 100.0       | 98.8        | 95.2        |
| Sse RPS27-2| 89.3        | 96.4        | 85.7        | 74.4        | 90.5        | 90.5        | 89.3        | 89.3        | 86.9        | 95.2        |
| Hsi RPS27-1| 89.3        | 85.7        | 74.4        | 90.5        | 90.5        | 89.3        | 89.3        | 86.9        | 95.2        | 95.2        |
| Hsi RPS27-2| 88.1        | 76.8        | 92.9        | 92.9        | 91.7        | 91.7        | 91.7        | 91.7        | 89.3        | 89.3        |
| Hsi RPS27-3| 74.4        | 91.7        | 92.9        | 95.2        | 90.5        | 90.5        | 89.3        | 89.3        | 86.9        | 95.2        |
| Hsi RPS27-4| 79.3        | 79.3        | 79.3        | 78.0        | 79.3        | 79.3        | 78.0        | 75.6        | 75.6        | 75.6        |
| Ipu RPS27-1| 98.8        | 96.4        | 95.2        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        |
| Ipu RPS27-2| 97.6        | 96.4        | 95.2        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        |
| Fru RPS27-1| 98.8        | 96.4        | 95.2        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        |
| Hsa RPS27-1| 98.8        | 96.4        | 95.2        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        | 92.9        |

Figure 1
RPS27 genes from S. senegalensis (Sse) and H. hippoglossus (Hhi). (A) Coding sequence alignment. (B) Amino acid alignment.

Dots indicate identity and hyphens represent indels.
31]. With regard to this, Pleuronectiformes comprises a broad taxonomic group with 11 families and about 500 species worldwide, some of them of high commercially interest both in fisheries and aquaculture [32-34]. All flatfish species share in common an asymmetrical body development and a bottom-dwelling mode of life. However, their high phenotypic similarity has invoked great differences in the number and nomenclature of taxa depending on the relevance assigned to morphologic features [35-38]. Most phylogenetic studies focused on relationships among Pleuronectiformes have been based on partial mitochondrial DNA sequences [39-41]. The description of the complete set of RPs in one Pleuronectidae and Soleidae species provides new molecular markers to investigate the taxonomy and phylogeny among Pleuronectiformes. Also, the existence of paralogous genes exhibiting differential expression patterns in tissues, and even more important during larval development, particularly in metamorphosis, suggests RPs as interesting molecular markers to investigate flatfish genome evolution in terms of gain and loss of paralogous genes and the availability to acquire new functions (neofunctionalization) or divide the ancestral function between the paralogs (sub-functionalization) [42,43].

Three rounds of large-scale gene duplications (referred to as 1R, 2R, and 3R or fish-specific genome duplication) have been identified in fish [44,45]. These duplications are responsible, at least in part, for their speciation, adaptive radiation and high morphological complexity [45]. Although the majority of these gene duplicates have been lost or silenced during evolution, several gene copies have been described for some group of genes including glycolytic enzymes [44], Hox genes [46,47] and hormones and their receptors [48,49]. Similarly, different gene copies have been described for some RPs. In human, two differ-

**Figure 2**
Phylogenetic relationships of RPS27 genes from *S. senegalensis* (Sse), *H. hippoglossus* (Hhi) *H. sapiens* (Hsa), *R. norvegicus* (Rno), *I. punctatus* (Ipu), *P. flesus* (Pfi), *D. rerio* (Dre) and *T. nigroviridis* (Tni). *Xenopus laevis* RPS27 was used as outgroup to root tree. Bootstrap values using NJ/MP/ML are indicated on each branch.
ent RPS4 genes exist, one encoded on the X and one on the Y chromosome. Rat possesses two distinct RPS27 transcripts that are expressed differentially in the hypothalamus [5]. In fish, *I. punctatus* has two paralogous genes of RPS26 and RPS27 [9]. In *S. senegalensis* and *H. hippoglossus*, two and four different RPS27 genes have been detected, respectively. Phylogenetic analysis revealed that RPS27-1 and RPS27-2 sequences grouped in two separate clades supported by significant bootstrap values. Although Thomas et al. [5] proposed the RPS27-2 as a mammalian-specific isoform, the identification of orthologous sequences for both RPS27 genes in different fish species supports the hypothesis of at least two RPS27 paralogs as a common feature in fish as well. Moreover, two additional RPS27 genes (referred to as RPS27-3 and RPS27-4) were identified in *H. hippoglossus*. These two paralogous genes might have appeared in the 3R or fish-specific genome duplication. As a result of this, we should highlight that both RPS27 genes in *I. punctatus* grouped together in the same clade with *S. senegalensis* RPS27-1 and *H. hippoglossus* RPS27-1 and RPS27-3. This clustering suggests the existence of, at least, a third RPS27 gene in *I. punctatus* orthologous to fish RPS27-2. Such a hypothesis is also supported by the fact that both *I. punctatus* RPS27 paralogs were expressed at a similar level (represented by 7 and 10 clones for RPS27-1 and RPS27-2, respectively) [9], whereas in *S. senegalensis* RPS27-2 was expressed at a much lower level than RPS27-1 in all tissues and during larval development as determined by real-time PCR.

During embryogenesis, after mid-blastula transition in zebrafish, RP genes co-ordinately increase their expression [16-18]. In addition, in Atlantic halibut up to 40 and 41 RPs increase mRNA levels from embryos to 1 day-old yolk sac larvae and fast skeletal muscle in juveniles, respectively [15]. In this study, we provide evidence that one day after first feeding, the eight RPs analyzed by real-time PCR increase their expression levels in Senegalese sole also. During this period, larvae undergo important physiological and morphological changes such as the opening of the mouth and anus. When live prey are provided for feeding, different organs such as the liver, pancreas, and the digestive tract are activated, promoting larval metabolism [50]. Larval rearing is a critical period during which different aspects concerning important anatomical and physiological traits in the juvenile stage are modulated. There are reports on biomarkers for fish larvae fed different diets that focused specifically on oxidative stress [51] and digestive enzymes [52]. The co-ordinate changes in RP expression under important physiological events such as the first feeding suggest RPs might be considered as biomarkers that could provide broader information about the general physiological condition in fish. In addition, the high abundance of these RPs (about 50% of RNA polymerase II transcription in rapidly growing yeast cells [53]), most of which are considered as house-keeping genes, indicates that even small induction values, as observed in this survey (1.7–3.0 fold), can play an important physiological role.

Although RP transcript levels increased after first feeding, they dropped at the first metamorphic stages (13 DAH). In this respect, we should take into account that Senegalese sole exhibits two different growth rates during larval development. At pre-metamorphosis, larvae grow at almost twice the rate as at metamorphosis and accumulate energy reserves in tissues to be used during this important period [26,29]. The lower growth rate at metamorphosis has been correlated with reduced IGF-II expression levels [54], an activator of the 70-kDa ribosomal S6 kinase (S6K1), a serine/threonine protein kinase that plays a central role in cell growth and proliferation. This kinase mediates the phosphorylation of RPS6, thereby enabling efficient translation of 5′-terminal oligopyrimidine tract (5′-TOP) mRNAs. Since RPs and translation elongation factors are encoded by 5′-TOP mRNAs, signalling along the S6K1 pathway may regulate ribosome biogenesis and therefore the response to growth conditions [55,56]. Moreover, apoptosis has been shown to play an important role in the organ-rebuilding process during flatfish metamorphosis [57] and some RPs have been associated with apoptotic processes [25,58]. The reduction in RP gene expression, especially at the beginning of sole metamorphosis, suggests they could also be involved in the control of apoptosis during metamorphosis.

We evaluated gene expression of eight RPs in ten different sole tissues. Overall, all tissues except brain expressed RPs at a similar level. These data agree with those obtained in *I. punctatus* using a transcriptomic approach. Representation of RPs was reduced in brain compared with skin and head kidney [9]. Ribosome formation can vary in response to cellular demands and their protein synthetic requirements [59] and these differences in the steady-state number of RP transcripts might reflect the distinct metabolic activity of tissues.

RPs exhibited different expression levels in different sole tissues. RPS27-2 mRNA levels were up to 41.9 and 54.7-fold lower than RPS27-1 and RPS2, respectively. These differences in relative mRNA abundance among RPs were also observed in *I. punctatus* and *H. hippoglossus* larvae and juveniles [9,15]. Such difference suggests a translational regulation to facilitate the correct assembly of ribosomes. Moreover, there is increasing evidence that RPs modulate a variety of cellular activities independent of their own involvement in the protein biosynthesis such as replication, transcription, RNA processing, DNA repair, and inflammation [60]. In our study, some RPs exhibited different tissue expression patterns. For instance, RPS2 was
highly expressed in all tissues except in brain where RPS27-1 transcripts were the highest. These data agree with those described for *I. punctatus* where the number of ESTs corresponding to RPS2 and RPS27 were 10-fold lower and 3-fold higher, respectively, in brain than in skin and head kidney [9]. In addition, RPS3a transcripts were reduced in brain, heart, and skin, and RPS27-2 showed the highest expression levels in intestine and stomach. All these data underscore the necessity for new studies to elucidate the regulation of these RPs in tissues and their possible extraribosomal function.

**Conclusion**

In this work we have identified and characterized the complete set of 40S RPs in two Pleuronectiformes: Senegalese sole and Atlantic halibut. These data provide new molecular markers to investigate genome evolution and phylogenetic relationships among flatfish. Also, gene expression studies in Senegalese sole have revealed a coordinate response after first feeding in larvae suggesting a possible role of RPs as general condition biomarkers to estimate larval physiological status in response to changing environmental conditions. Moreover, the differential expression patterns in tissues suggest that RPs might perform other functions distinct from protein biosynthesis.

**Methods**

**Identification of RP cDNAs in Senegalese sole and Atlantic halibut**

Ten cDNA libraries were constructed from different larval stages and adult tissues of Senegalese sole using the ZAP Express® cDNA Syntesis kit and Zap Express cDNA Giga-pack® III Gold Cloning kit (Stratagene) following the manufacturer’s protocol (Cerdà et al., in preparation). The libraries were pooled and normalized, and approximately 11,000 randomly selected clones were sequenced from the 3’-end. Expressed sequence tags (ESTs) encoding RPs were identified after EST annotation. For RPS28 isolation, we designed specific primers (Table 1) using a partial sequence from a suppression subtractive hybridization library. RPS28 was amplified from the premetamorphic larval development library using combination of specific primers and the universal primers T3 and T7.

In halibut, normalised cDNA libraries were constructed for five different larval time points (hatching, mouth-opening, mid-way to metamorphosis, premetamorphosis, and postmetamorphosis) and eight adult tissues (testis, ovary, liver, head kidney, spleen, skin, gill, and intestine) [61], incorporated into the Pleurogene database [http://www.pleurogene.ca](http://www.pleurogene.ca) and provisionally annotated using AUTOFACT [62] implemented on the database.

**Fish sampling**

Juvenile Senegalese sole individuals (n = 3) were obtained from IFAPA Centro El Toroño facilities (El Puerto Santa María, Cádiz, Spain). They were sacrificed by immersion in tricaine methanesulfonate (MS-222). Liver, spleen, intestine, stomach, head kidney, gills, muscle, brain, heart, and skin were rapidly dissected, frozen in liquid nitrogen and stored at -80°C until use.

For larval studies, fertilized eggs from a naturally spawning Senegalese sole broodstock (IFAPA Centro El Toroño) were collected. They were incubated in a 150 L tank at 19–21°C for two days. Newly hatched larvae were transferred to a 400 L tank at an initial density from 45 to 50 larvae L-1 with a 16L:8D photoperiod and a light intensity of 600–800 lux. Larvae were fed rotifers (*Brachionus plicatilis*) 3 DAH till 9 DAH. From 7 DAH enriched artemia metamauplii were fed until the end of the experiment. Pools of larvae from 2 to 22 DAH (n = 3) were collected, washed with DEPC water, frozen in liquid nitrogen and stored at -80°C until analysis.

**RNA isolation and gene expression analysis**

Homogenization of juvenile tissues and larvae was carried out using Lysing Matrix D (Q-BioGene) for 40 s at speed setting 6 in the Fastprep FG120 instrument (Bio101). Total RNA was isolated from 50 mg of *S. senegalensis* tissues or pools of larvae using the RNeasy Mini Kit (Qiagen). All RNA isolation procedures were performed in accordance with the manufacturer's protocol. In all cases, total RNA was treated twice with DNase I using the RNase-Free DNase kit (Qiagen) for 30 min in order to avoid amplification of contaminated genomic DNA. RNA sample quality was checked using Experion (Bio-Rad) and quantification was performed spectrophotometrically. Total RNA (1 µg) from each sample was reverse-transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). Reverse transcription reactions were performed in duplicate. Lack of genomic DNA contamination was confirmed by PCR amplification of RNA samples in the absence of cDNA synthesis.

Real-time analysis was carried out on an iCycler (Bio-Rad). Reactions were performed in a 25 µl volume containing cDNA generated from 10 ng of original RNA template, 300 nM each of specific forward (F) and reverse (R) primers (Table 1), and 12.5 µl of iQ™ SYBR Green Supermix (Bio-Rad). Matching oligonucleotide primers were designed using Oligo v6.89 software (MedProbe). The amplification protocol used was as follows: initial 7 min denaturation and enzyme activation at 95°C, 40 cycles of 95°C for 15 s and 70°C for 30 s. Each assay was performed in duplicate. For normalization of cDNA loading, all samples were run in parallel with a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase (GAPDH;
sequences were carried out and the sequence similarities calculated by the MegAlign program from the LASERGENE software suite. For phylogenetic analysis, sequences of RPS27 from different species including Homo sapiens ([GenBank:HSU57847, GenBank:NM_015920; [6,63]), Rattus norvegicus ([GenBank:AF184893, EMBL:X59375]; [5,64]), Xenopus laevis ([GenBank:BC053815]; [65]), Ictalurus punctatus ([GenBank:AF402836, GenBank:AF402837]; [9]), Platicthys flesus ([GenBank:DV566302 and GenBank:DV567451]; unpublished), Danio rerio ([GenBank:BOQ77524, GenBank:BC114281]; unpublished) and Tetraodon nigroviridis ([EMBL:CR722207 and EMBL:CR642405; unpublished]) were employed. Coding sequences were aligned using MegAlign software. Neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses were carried out using PAUP*4beta10 software [66]. The TrNef + G model of sequence evolution was the most appropriate as selected by MODELTEST v3.5 [67]. The parameters of ML methods were R(a) = 1.0000, R(b) = 3.2865, R(c) = 1.0000, R(d) = 1.0000, and R(e) = 6.057. The gamma distribution shape parameter was estimated to be 0.3234. The degree of confidence assigned to nodes in trees was achieved by bootstrapping with 1,000 replicates.

Authors’ contributions
MM designed the study, carried out the phylogenetic analyses, and drafted the manuscript. CI carried out the gene expression analysis and helped to draft the manuscript. EA performed the Senegalese sole cultures and samplings. JPC participated in the study design and coordination and helped to draft the manuscript. SED participated in sequence analysis and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This work has been financially supported by PLEUROGENE project funded by the Genome Canada-Genoma España joint program. Sequencing by the Atlantic Genome Centre [http://www.tngc.ca], Halifax, Nova Scotia, Canada a partnership between Genome Atlantic and the National Research Council of Canada Institute for Marine Biosciences, is gratefully acknowledged. This is NRC publication number 42588.

References
1. Zarivach R, Bashan A, Berisio R, Harms J, Auerbach T, Schluenzen F, Bartels H, Baram D, Pyetan E, Sittner A, et al.: Functional aspects of ribosomal architecture: symmetry, chirality and regulation. J Phys Org Chem 2004, 17:901-912.
2. Wool IG, Endo Y, Chan YL, Glück A: Studies of the structure, function and evolution of mammalian ribosomes. In Ribosome Structure, Function and Evolution Edited by: Hill W, Dahlberg A, Garrett R, Moore P, Schlessinger D, Warner J. Washington DC: Society for Microbiology; 1990:203-214.
3. Wool IG, Chan YL, Glück A: Structure and evolution of mammalian ribosomal proteins. Biochem Cell Biol 1995, 73:933-947.
4. Fisher EM, Beer-Komoro P, Brown LG, Ridley A, McNeil JA, Lawrence JB, Willard HF, Bieber FR, Page DC: Homogeneous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. Cell 1990, 63:1205-1218.
5. Thomas EA, Alvarex CE, Sutcliffe IG: Evolutionarily distinct classes of 527 ribosomal proteins with differential mRNA expression in rat hypothalamus. J Neurochem 2000, 74:2259-2267.
6. He H, Sun Y: Ribosomal protein S27L is a direct target that regulates apoptosis. Oncogene 2007, 17:2707-2714.
7. Planta RJ, Mager WH: The list of cytoplasmic ribosomal proteins of Saccharomyces cerevisiae. Yeast 1998, 14:471-477.
8. Ribosomal Protein Gene Database [http://ribosome.med.miya zaki-u.ac.jp] I.
9. Karsi A, Patterson A, Feng J, Liu Z: Translational machinery of channel catfish: I. A transcriptomic approach to the analysis of 32 405 ribosomal protein genes and their expression. Gene 2000, 291(1-2):177-186.
10. Thomas EA, Alvarez CE, Sutcliffe IG: Evolutionarily distinct classes of 527 ribosomal proteins with differential mRNA expression in rat hypothalamus. J Neurochem 2000, 74:2259-2267.
11. He H, Sun Y: Ribosomal protein S27L is a direct target that regulates apoptosis. Oncogene 2007, 17:2707-2714.
12. Patterson A, Karsi A, Feng J, Liu Z: Translational machinery of channel catfish: I. Complementary DNA and expression of the complete set of 47 605 ribosomal proteins. Gene 2003, 305:151-160.
13. Cujec TP, Tyler BM: Nutritional and growth control of ribosomal protein mRNA and rRNA in Neurospora crassa. Nucleic Acids Res 1996, 24:943-950.
14. Herruer MH, Mager WH, Bartels H, Willard HF, Bieber FR, Page DC: Homogeneous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. Cell 1990, 63:1205-1218.
15. Groeneveld P, Planta RJ: Transcriptional control of yeast ribosomal protein synthesis during carbon-source upshift. Nucleic Acids Res 1987, 15(24):10133-10144.
16. Milne AN, Mak WW, Wong JT: Variation of ribosomal proteins with bacterial growth rate. J Bacterial 1975, 122:89-92.
17. Waldron C, Jund R, Lacroute F: Evidence for a high proportion of inactive ribosomes in slow-growing yeast cells. Biochem J 1977, 168:409-415.
18. Bai J, Solberg C, Fernandes JM, Johnston IA: Profiling of maternal and developmental-stage specific mRNA transcripts in Atlantic halibut Hippoglossus hippoglossus. Gene 2006, 386:202-210.
19. Linney E, Dobbs-McAuliffe B, Sajadi H, Malek RL: Microarray gene expression profiling during the segmentation phase of zebrafish development. Comp Biochem Physiol C Toxicol Pharmacol 2002, 138:351-362.
20. Lo J, Lee S, Xu M, Liu F, Ruan H, Eun A, He Y, Ma W, Wang W, Wen Z, et al.: 15,000 unique zebrafish EST clusters and their future use in microarray for profiling gene expression patterns during embryogenesis. Genome Res 2003, 13:455-466.
21. Mathavan S, Lee SG, Mak A, Miller LD, Murthy KR, Govindarajan KR, Tong Y, Wu YL, Lam SH, Yang H, et al.: Transcriptome analysis of zebrafish embryogenesis using microarrays. PLoS Genet 2005, 1:260-276.
19. Kowalczyk P, Woszczyński M, Ostrzycki J. Increased expression of ribosomal protein S2 in liver tumors, posthepaticomized livers, and proliferating hepatocytes in vitro. Acta Biochim Pol 2002, 49:615-624.

20. Pogue-Geile K, Geiser JR, Shu M, Miller C, Wool IG, Meisler AI, Pipas JM. Ribosomal protein genes are overexpressed in colorectal cancer: isolation of a CDNA clone encoding the human S2 ribosomal protein. Mol Cell Biol 1991, 11:3842-3849.

21. Cromartie SE, Laski FA. String of pears encodes Drosophila ribosomal protein S2, has Minute-like characteristics, and is required during oogenesis. Genetics 1994, 137:1039-1048.

22. Watson KL, Konrad KD, Woods DF, Bryant PJ. Drosophila homolog of the human S6 ribosomal protein is required for tumor suppression in the hematopoietic system. Proc Natl Acad Sci USA 1992, 89:1302-1306.

23. Kim J, Chubatsu LS, Admon A, Stahl J, Fellous R, Linn S. Implication of mammalian ribosomal protein S3 in the processing of DNA damage. J Biol Chem 1995, 270:13620-13629.

24. Drapchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, Ball S, Tchernia G, Klar J, Matsson H, Nelson JS. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Hum Mol Genet 1998, 7:2707-2713.

25. Naora H, Takai I, Adachi M, Naora H. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3 gene expression induces apoptosis. J Cell Biol 1998, 141:1741-753.

26. Fernández-Díaz C, Yúfera M, Cañavate JP, Moyano FJ, Alarcón FJ, Díaz M. Growth and physiological changes during metamorphosis of Solea senegalensis larvae: in vitro experiment. J Fish Biol 2001, 58:1-13.

27. Haug T. Biology of the Atlantic halibut, Hippoglossus hippoglossus (L. 1758). Adv Mar Biol 1990, 26:2-70.

28. Power DM, Llewellyn L, Faustino M, Nowell MA, Bjornsson BT, Einhorn RL, Pogue-Geile K, Geiser JR, Shu M, Miller C, Wool IG, Meisler AI, Pipas JM. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3 gene expression induces apoptosis. J Cell Biol 1998, 141:1741-753.

29. Pardos BN, Small CJ, Possani LD. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3 gene expression induces apoptosis. J Cell Biol 1998, 141:1741-753.

30. Nelson JS. Fishes of the world. 3rd edition. New York: John Wiley & Sons; 1994.

31. Helfman G, Collette B, Facey D. The diversity of fishes. 3rd edition. New York: John Wiley & Sons; 1994.

32. Pardos BN, Small CJ, Possani LD. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3 gene expression induces apoptosis. J Cell Biol 1998, 141:1741-753.

33. Neff BE, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Ramapycin suppresses 5'TOP mRNA translation through inhibition of eIF4E. EMBO J 1997, 16:3639-3704.

34. Terada N, Patel HR, Takase K, Kohna K, Nairn AC, Gelfand EW. Rapamycin selectively inhibits translation of mRNAs encoding elongation factors of ribosomal proteins. Proc Natl Acad Sci USA 1994, 91:1477-1481.

35. Baalbaki L, Gui-Mei Y, Da-Ming R. Apoptosis in the metamorphosis of Japanese flounder Paralichthys olivaceus. Acta Zool Sin 2003, 52:355-361.

36. Bushell M, Stoneley M, Sarnow P, Willis AE. Translation inhibition during the induction of apoptosis: RNA or protein degradation? Biochem Soc Trans 2004, 32:606-610.

37. Woolford JL Jr, Warner JR. The ribosome and its synthesis. In The Molecular Biology and Cellular Biology of the Yeast Saccharomyces cerevisiae. Edited by: Broach JR, Pringle JR, Jones EW. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1991:587-626.

38. Woolford JL Jr, Warner JR. The ribosome and its synthesis. In The Molecular Biology and Cellular Biology of the Yeast Saccharomyces cerevisiae. Edited by: Broach JR, Pringle JR, Jones EW. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1991:587-626.

39. Wook Y, Park S, Lee JH, Jung S, Kim K, Park H. ZnF motifs in rat ribosomal protein/sL7/S12: Characterization of the cDNA clones encoding this type of zinc finger-like motif. DNA Res 2004, 11:151-158.

40. Helfman G, Collette B, Facey D. The diversity of fishes. 3rd edition. New York: John Wiley & Sons; 1994.

41. Neff BE, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Ramapycin suppresses 5'TOP mRNA translation through inhibition of eIF4E. EMBO J 1997, 16:3639-3704.