

Supplementary Material

Characterisation, expression and possible functions of prohibitin during spermatogenesis in the silver pomfret *Pampus argenteus*

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Fig. S1. The test of antibody specificity.

Fig. S2. Predicted tertiary structure of PHB in several animals.

Table S1. The primer and probe sequence used in this study for *phb* cDNA full-length cloning and fluorescent *in situ* hybridization.

Table S2. PHB sequence information and corresponding GenBank accession numbers used in this study.

Table S3. Nuclear diameter, cross-sectional area and volume of spermatogenic cell.

Table S4. Amino acid consensus/identity positions among PHB of animals.

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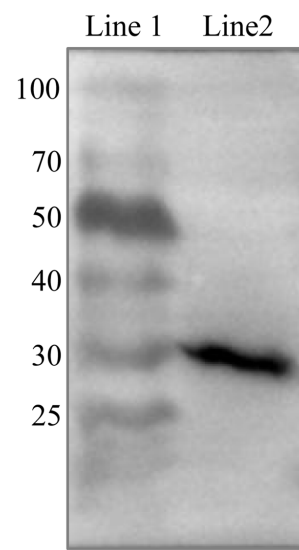


Fig. S2. Predicted tertiary structure of PHB in several animals.

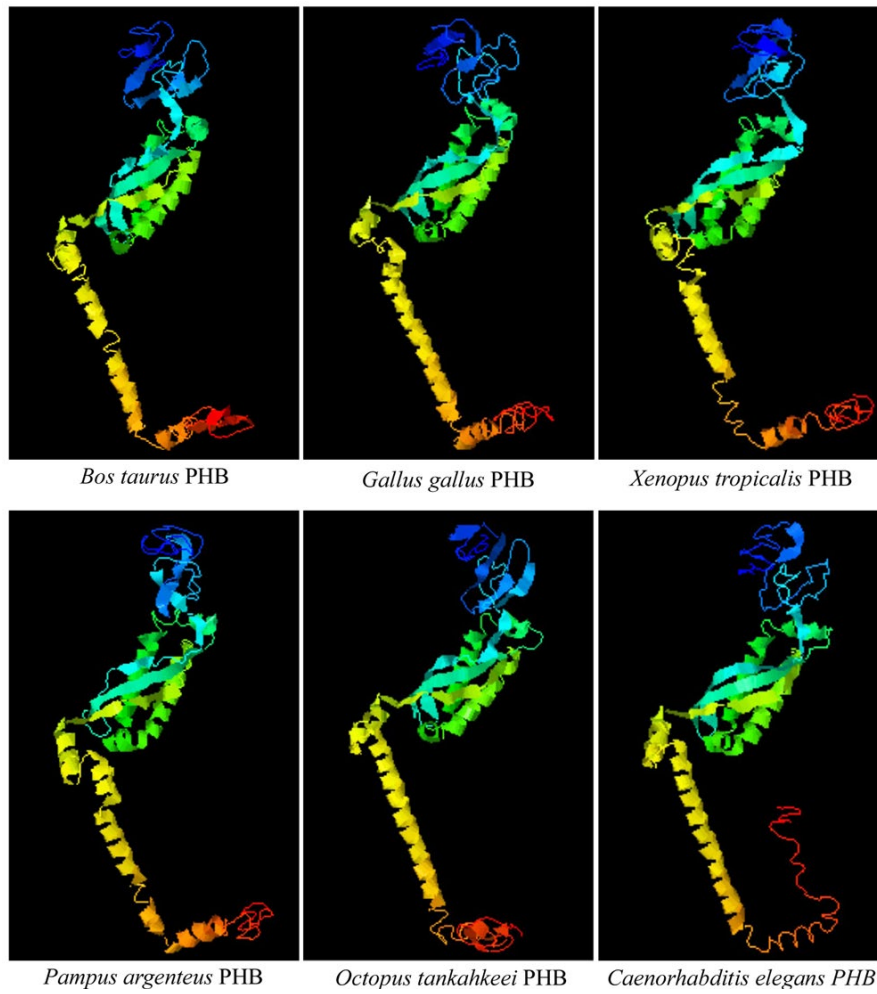


Table S1. The primer and probe sequence used in this study for *phb* cDNA full-length cloning and fluorescent *in situ* hybridization.

Primer/probe	Sequence (5'-3')	Purpose
UPM-Long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCA GAGT	5' RACE
UPM-Short	CTAATACGACTCACTATAGGGC	5' RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	5' RACE
5'RACE R1	CAATACTGGTGAAGATGCGTGGGAG	5' RACE
5'RACE R2	CTTTGCTGCCTGTGATGACGGAA	5' RACE
Outer Primer	TACCGTCGTTCCACTAGTGATTT	3' RACE
Inner Primer	CGCGGATCCTCCACTAGTGATTTCACTATAGG	3' RACE
3'RACE F1	TGATGGTCTGGTAGAGTTA	3' RACE
3'RACE F2	TTACTTGCCCTCAGGACAG	3' RACE
qPCR-F	CCCACGCATCTTCACCAGT	qPCR
qPCR-R	CTGACCTGCCGAGACACAAG	qPCR
β -actin F	TGAAATCGCCGCACTGGTTG	qPCR
β -actin R	ACCAACGTAGCTGTCCTTCTG	qPCR
anti-sense probe	CACAACACCTCCACCGACGGCAA	FISH
sense probe	TTGCCGTCGGTGGAGGTGTTGTG	FISH

Table S2. PHB sequence information and corresponding GenBank accession numbers used in this study.

Species	Order/Phylum	Accession numbers
<i>Homo sapiens</i>	Mammalia/Vertebrata	AAB21614.1
<i>Bos taurus</i>	Mammalia/Vertebrata	NP_001029744.1
<i>Mus musculus</i>	Mammalia/Vertebrata	NP_032857.1
<i>Gallus gallus</i>	Aves/Vertebrata	NP_001171206.1
<i>Manacus vitellinus</i>	Aves/Vertebrata	XP_017943376.1
<i>Pelodiscus sinensis</i>	Reptilia/Vertebrata	XP_006120504.1
<i>Cynops orientalis</i>	Amphibia/Vertebrata	AJF36071.1
<i>Xenopus tropicalis</i>	Amphibia/Vertebrata	NP_001079486.1
<i>Danio rerio</i>	Pisces/Vertebrata	NP_958454.1
<i>Salmo salar</i>	Pisces/Vertebrata	NP_001133602.1
<i>Pampus argenteus</i>	Pisces/Vertebrata	MH215678
<i>Procambarus clarkii</i>	Malacostraca/Arthropoda	AGU02225.1
<i>Eriocheir sinensis</i>	Crustacea/Arthropoda	ADM64319.1
<i>Octopus tankahkeei</i>	Cephalopoda /Mollusca	AEI91930.1
<i>Phascolosoma esculenta</i>	Phascolosomatidea/Sipuncula	KY807538
<i>Caenorhabditis elegans</i>	Secernentea/Nematoda	NP_490929.1
<i>Clonorchis sinensis</i>	Trematoda/Platyhelminthes	AAAY32923.1

Table S3. Nuclear diameter, cross-sectional area and volume of spermatogenic cell.

Spermatogenic cell types	Nuclear diameter (μm)	Nuclear cross-sectional area (μm^2)	Nuclear volume (μm^3)
Spermatogonium	4.35 ± 0.51 (minor axis length)/ 6.24 ± 0.34 (major axis length)	21.17 ± 3.27	61.69 ± 16.30
Primary spermatocyte	4.33 ± 0.41	14.83 ± 2.85	43.50 ± 12.80
Second spermatocyte	3.09 ± 0.29	7.55 ± 1.44	15.81 ± 4.56
Early spermatid	2.36 ± 0.21	4.40 ± 0.78	7.03 ± 1.86
Middle spermatid	2.01 ± 0.14	3.17 ± 0.40	4.27 ± 0.81
Late spermatid	1.27 ± 0.15	1.29 ± 0.31	1.12 ± 0.40
Sperm	1.22 ± 0.09	1.17 ± 0.17	0.96 ± 0.20

The fluorescence staining of nucleus was used to measure the nuclear diameter which was measured from 15 cells for each type of spermatogenic cell. The spermatogonium nucleus is oval. Its cross-sectional area (S) was calculated by formula $S=\pi ab$, and its volume (V) was calculated by formula $V=4\pi abc/3$; a and c are the semi-minor axis length and b is the major semi-axis length. The nucleus of primary spermatocyte, second spermatocyte, early spermatid, middle spermatid, late spermatid and sperm is approximately circular. Their cross-sectional area was calculated by formula $S=\pi r^2$, and their volume was calculated by formula $V=4\pi r^3/3$; r is the radius of the nucleus.

Table S4. Amino acid consensus/identity positions among PHB of animals.

Species	<i>Homo sapiens</i>	<i>Bos taurus</i>	<i>Mus musculus</i>	<i>Gallus gallus</i>	<i>Manacus vitellinus</i>	<i>Pelodiscus sinensis</i>	<i>Cynops orientalis</i>	<i>Xenopus tropicalis</i>	<i>Danio rerio</i>	<i>Salmo salar</i>	<i>Pampus argenteus</i>	<i>Procambarus clarkii</i>	<i>Eriocheir sinensis</i>	<i>Octopus tankahkeei</i>	<i>Phascolosoma esculenta</i>	<i>Caenorhabditis elegans</i>	<i>Clonorchis sinensis</i>
<i>Homo sapiens</i>	100/100%	100/100%	100/99.6%	98.5/96.0%	98.9/96.0%	97.1/91.9%	97.8/93.8%	96.7/91.5%	96.0/91.5%	94.9/92.6%	94.5/89.7%	85.1/70.5%	85.5/69.8%	86.8/73.3%	84.9/72.8%	78.9/64.7%	83.0/67.1%
<i>Bos taurus</i>		100/100%	100/99.6%	98.5/96%	98.9/96.0%	97.1/91.9%	97.8/93.8%	96.7/91.5%	96.0/91.5%	94.9/92.6%	94.5/89.7%	85.1/70.5%	85.5/69.8%	86.8/73.3%	84.9/72.8%	78.9/64.7%	83.0/67.1%
<i>Mus musculus</i>			100/100%	98.5/95.6%	98.9/95.6%	97.1/91.5%	97.8/93.4%	96.7/91.2%	96.0/91.2%	94.9/92.3%	94.5/89.3%	85.1/70.9%	85.5/70.2%	86.8/73.6%	84.9/73.2%	78.9/65.1%	83.0/67.5%
<i>Gallus gallus</i>				100/100%	98.9/98.9%	96.7/92.6%	96.3/93.4%	96.3/93.0%	96.0/90.8%	95.6/90.8%	94.9/89.7%	85.5/70.9%	85.5/70.5%	87.2/74.0%	84.9/72.8%	78.5/65.5%	82.3/67.1%
<i>Manacus vitellinus</i>					100/100%	96.7/92.6%	97.4/94.5%	96.3/93.0%	96.7/91.5%	96.0/91.2%	95.2/90.1%	85.5/70.5%	86.2/71.3%	87.5/74.0%	85.3/72.8%	79.3/65.5%	83.0/67.5%
<i>Pelodiscus sinensis</i>						100/100%	96.0/91.2%	94.9/90.1%	95.2/89.0%	94.1/87.5%	93.4/86.4%	84.0/70.5%	84.7/70.9%	86.1/75.1%	84.6/74.6%	78.5/65.5%	82.3/67.9%
<i>Cynops orientalis</i>							100/100%	96.7/91.5%	95.6/91.2%	94.5/90.1%	94.1/90.1%	84.7/70.9%	85.1/71.3%	86.4/74.7%	84.6/74.6%	78.9/65.5%	82.7/68.2%
<i>Xenopus tropicalis</i>								100/100%	95.2/89.3%	94.1/87.5%	94.5/88.6%	84.7/71.3%	84.7/72.0%	87.2/74.7%	84.6/72.1%	78.2/65.1%	81.6/67.1%
<i>Danio rerio</i>									100/100%	98.2/95.9%	97.8/96.3%	86.5/72.0%	86.5/70.9%	86.8/74.0%	84.9/73.2%	79.3/66.5%	83.0/66.8%
<i>Salmo salar</i>										100/100%	98.5/95.9%	86.2/71.3%	85.5/69.8%	86.1/73.6%	84.9/73.5%	78.9/65.1%	82.7/66.4%
<i>Pampus argenteus</i>											100/100%	85.5/71.6%	85.1/70.2%	86.6/74.7%	83.8/72.8%	78.5/65.8%	82.7/65.7%
<i>Procambarus clarkii</i>												100/100%	96.0/90.9%	81.2/66.3%	80.7/69.1%	80.4/66.5%	80.2/66.9%
<i>Eriocheir sinensis</i>													100/100%	81.5/66.3%	80.7/67.6%	80.4/66.5%	80.6/67.6%
<i>Octopus tankahkeei</i>														100/100%	85.0/73.6%	80.0/69.6%	83.0/67.9%
<i>Phascolosoma esculenta</i>															100/100%	81.1/70.2%	79.4/67.1%
<i>Caenorhabditis elegans</i>																100/100%	79.1/69.1%
<i>Clonorchis sinensis</i>																	100/100%

This consensus/identity positions analysis was accomplished by Vector NTI. *Pampus argenteus* was marked by red word. The animals on the left/top of *P. argenteus* were vertebrate and the animals on the right/underside of *P. argenteus* were invertebrate.