

## Supplementary Note

### RT-PCR detection of Preproghrelin

#### RT-PCR

Total RNA was extracted from different tissues of an adult individual of *P. sinensis* with RNeasy Lipid Midi kit (Life Technologies) and cDNA was synthesized using an anchored oligo-dT<sub>(20)</sub> primer and Superscript III reverse-transcriptase (Life Technologies) following manufacturer's protocols. *P. sinensis* GAPDH gene was used as control. PCR was performed with the following conditions: 95° C, 2 min.; 35 cycles of 95° C, 30 sec., 60° C 30 sec., 72° C 1 min., and a final hold of 72° C 5 min. using GoTaq DNA polymerase (Promega). Primer sequences are as follows: *Preproghrelin* forward: 5'-atgtttctcaggagcacta-3', based on the sequence reported by [2]; *Preproghrelin* reverse: 5'-tgcaaaccttttcagctttatgc-3', based in the sequence of the scaffold JH211518 of *P. sinensis* genome v1.0, as in Ensemble release e74; *GAPDH* forward: 5'-ctgagctgaacgggaaactc-3' and *GAPDH* reverse: 5'-ctgagctgaacgggaaactc-3', based in the gene ENSPSIG00000016258 from Ensembl release e74.

### Supplementary Figure 1. Cloned Preproghrelin mRNA sequence of *P. sinensis*

>Pelodiscus\_sinensis\_Preproghrelin

**ATGTTTCTCAGGAGCACTA**AGATGGGAATTCTCCTACTCTGCATTCTCTGGACAGAGACTGCCATGACTGGATCT  
AGTTTTTTAAGTCCTGAATATCAAAACACACAGCAACGAAAGGAACCAAAAAACATACAAAGTTAAATCGCCGG  
GCTACACAAGGATTTTTGGATGCAGATGCAAGGCAGGCAGAAGGAGACAATGAAATAGAAATTAAGCTTAATGTT  
CCCTTTGAAATTGGTGTCAAGATAACAGAAGATCAGTACCAGGAGTACGGGCAAGTGCCTGGAGAAAATTCCTAGAG  
GATGTTCTTGCAAGGACACAAAAGAAATCCAAACATTGACAAGAGCTGAAACATGAagacagtacaacatcatt  
ccagttcaatgcagttccacttactggcttgatttcttttaaagtgtgacttgtgtaattgatgacaatagcatgc  
tttaaaagtcaatgcataaagctgaaaagtttgca

Bold: sequence reported by Larhammar and Lagman.

Regular: new sequence added from RT-PCR cloning.

Uppercase: coding region.

Lowercase: partial 3'-UTR.

Underlined: STOP codon

Red: Primers used in the RT-PCR

### Supplementary Figure 2. Predicted amino acid sequence of Preproghrelin of *P. sinensis*.

MFLRSTKMGILLLCILWTETAMTGSSFLSPEYQNTQQRKEPKKHTKLNRRATQGFLDADARQAEKDNEIEIKLNV  
PFEIGVKITEDQYQYGVLEKILEDVLAEDTKEIQTLTRAET(Stop)

Predicted amino acid sequence of Preproghrelin of *P. sinensis*, based on the cloned and sequenced ghrelin cDNA of *P. sinensis*.

### References

1. Wang, Z., et al. Nature Genet. 45, 701-706 (2013).
2. Larhammar, D. & Lagman, D. Nat. Genet. 46, aaa-bbb (2014).
3. Seim, I., Jeffery, P.L. & Chopin, L.K. Nat. Genet. 46, aaa-bbb (2014).