



# Comparative genomic and transcriptional profile analysis of bone morphogenetic protein (BMP) gene family in Chinese soft-shell turtle (*Pelodiscus sinensis*)

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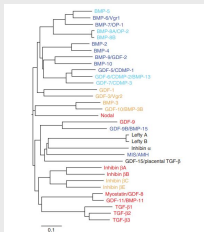
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## Abstract

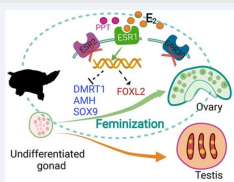
Bone morphogenetic proteins (BMPs) play a crucial role in bone formation and differentiation. Recent RNA-Seq results suggest that BMPs may be involved in the sex differentiation of *P. sinensis*, yet more relevant studies about BMPs in *P. sinensis* are lacking. Herein, we identified BMP gene family members, analyzed the phylogeny, collinear relationship, scaffold localization, gene structures, protein structures, transcription factors and dimorphic expression by using bioinformatic methods based on genomic and transcriptomic data of *P. sinensis*. A total of 11 BMP genes were identified, 10 of which were localized to their respective genomic scaffolds. Phylogenetic analysis revealed that BMP genes were divided into eight subfamilies and shared similar motifs ("WII", "FPL", "TNHA", "CCVP", and "CGC") and domain (TGF- $\beta$  superfamily). The results of the sexually dimorphic expression profile and qRT-PCR showed that *Bmp2*, *Bmp3*, *Bmp15l*, *Bmp5* and *Bmp8a* were significantly upregulated in ovaries, while *Bmp2lb*, *Bmp7*, *Bmp2bl* and *Bmp10* were remarkable upregulated in testes, suggesting that these genes may play a role in sex differentiation of *P. sinensis*. Collectively, our comprehensive results enrich the basic data for studying the evolution and functions of BMP genes in *P. sinensis*.

## Introduction

The transforming growth factor-beta (TGF- $\beta$ ) superfamily is a large group of extracellular growth factors controlling many aspects of development. As a dominant subfamily of the TGF- $\beta$  superfamily, the activity of BMPs was initially investigated in the 1960s. BMPs play a major role in osteoblast differentiation and bone formation.



Recently, studies have shown that BMP family members have a role in sex differentiation. Meanwhile, *Amh*, *Gsdf*, and *Gdf9* share the same domain with BMPs, belonging to the TGF- $\beta$  superfamily, and also are identified as the key members of sex differentiation. Taken together, BMPs are connected to the process of sex differentiation and seem to be conserved in multiple species.



Likewise, sex differentiation in the Chinese soft-shell turtle (*Pelodiscus sinensis*) is the consequence of multi-gene regulation. Recently, a novel study indicates that *Bmp2* was significantly more expressed in the gonads of female *P. sinensis* than in males by RNA-Seq, suggesting that *Bmp2* may be a potential gene for sex differentiation in *P. sinensis*. Nevertheless, further information about BMP family members in *P. sinensis* is not yet clarified.

## Materials and Methods

- Genome-wide identification of BMP genes
- Phylogenetic analysis and collinear analysis of BMP gene family
- Analysis of scaffold location, gene structure, conserved motif, and domain
- Multiple sequence alignment and prediction of protein structure
- Analysis of transcription factors
- Analysis of sexually dimorphic expression profiles of BMP genes based on transcriptomic data

## Results

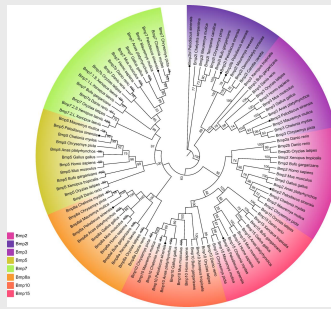


Figure 1. Phylogenetic tree of the BMP gene family among different vertebrates. Different colors represent different BMP gene subfamilies. The black circles indicate the BMP genes in *P. sinensis*.

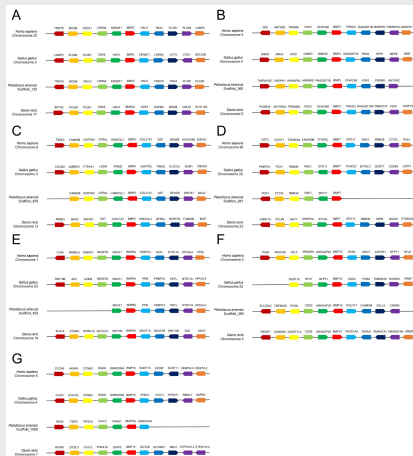


Figure 2. Collinear analysis of *Bmp2*(A), *Bmp3*(B), *Bmp5*(C), *Bmp7*(D), *Bmp8a*(E), *Bmp10*(F), *Bmp15*(G) and their adjacent genes in *Homo sapiens*, *Gallus gallus*, *P. sinensis* and *Danio rerio*. Direction of the arrows indicates gene orientation.

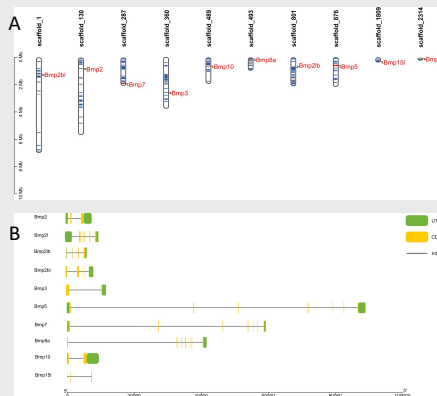


Figure 3. Scaffold distribution and gene structure of BMP genes in *P. sinensis*. (A) The gene density is defined as the number of genes within a 50 kb genome. (B) The green boxes show untranslated regions, the yellow boxes show coding sequences, and the black lines show introns.



Figure 4. Conserved motifs and domains of BMP genes in *P. sinensis*. (A) Different colored boxes indicate different motifs. (B) The green boxes and yellow boxes indicate the protein domains of the TGF- $\beta$  superfamily and the TGF- $\beta$  propeptide superfamily, respectively.

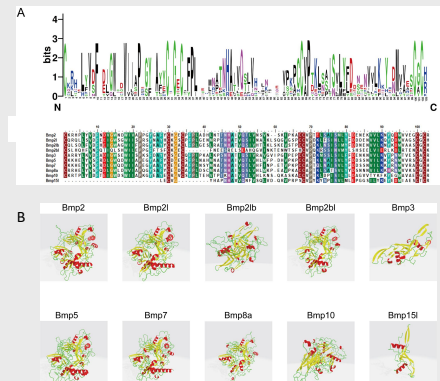


Figure 5. Multiple sequence alignment of TGF- $\beta$  superfamily domains and three-dimensional modeling of BMP genes in *P. sinensis*. (B) Red indicates alpha helix, yellow represents beta turn, green shows random coil.

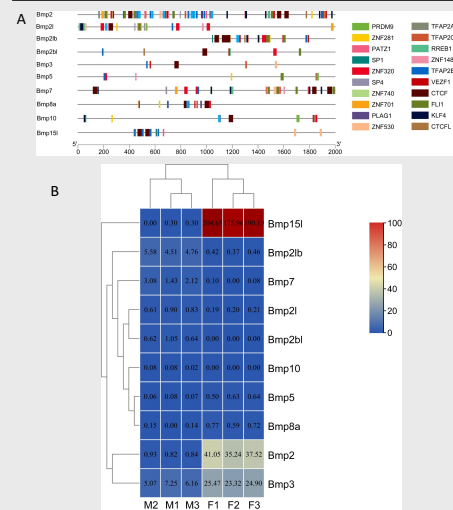


Figure 6. Promoter analysis and expression analysis of BMP genes in *P. sinensis*. (A) Different colored rectangles indicate different transcription factors. (B) The expression levels of BMP genes are presented with FPKM values.

**Conclusion:** We performed an initial characterization of the BMP genes to explore its evolution and functions in *P. sinensis*, especially in the sex differentiation. Our study not only provides a completed data of BMP genes in *P. sinensis* genome, but also offers a novel insight to study the regulatory mechanism of sex differentiation in *P. sinensis*, even in other turtles.