

Zhangfan Chen*, Xihong Li, Peng Cheng, Rui Shi, Na Wang, Songlin Chen

State Key Laboratory of Mariculture Biobreeding and Sustainable Goods, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China

*E-mail: chenzf@ysfri.ac.cn

Sexual size dimorphism (SSD) with faster-growing females challenges sustainable aquaculture of Chinese tongue sole (*Cynoglossus semilaevis*), an economically important flatfish in northeastern Asia. Females grow faster than males, and eventually reached over twice in weight and length. We explored the steroid pathway's role in SSD using comparative transcriptomics. Notably, genes involved in this pathway, such as emopamil-binding protein (*ebp*) and its downstream genes, lathosterol 5-desaturase (*sc5d*), 7-dehydrocholesterol- Δ 7 reductase (*dhcr7*) and 24-dehydrocholesterol Reductase (*dhcr24*), were highly expressed in female individuals of *C. semilaevis*. EBP is the key catalyzing enzymes in cholesterol biosynthesis. Its mutation caused abnormal bone development and growth retardation in humans. In the study, we characterized the *C.semilaevis* EBP gene (*Cs-ebp*) and revealed its possible involvement in the regulation of growth performance in teleost.

1. EBP domain was conserved in mammals and teleost. *C.semilaevis* EBP was grouped together with those of two flatfish species.

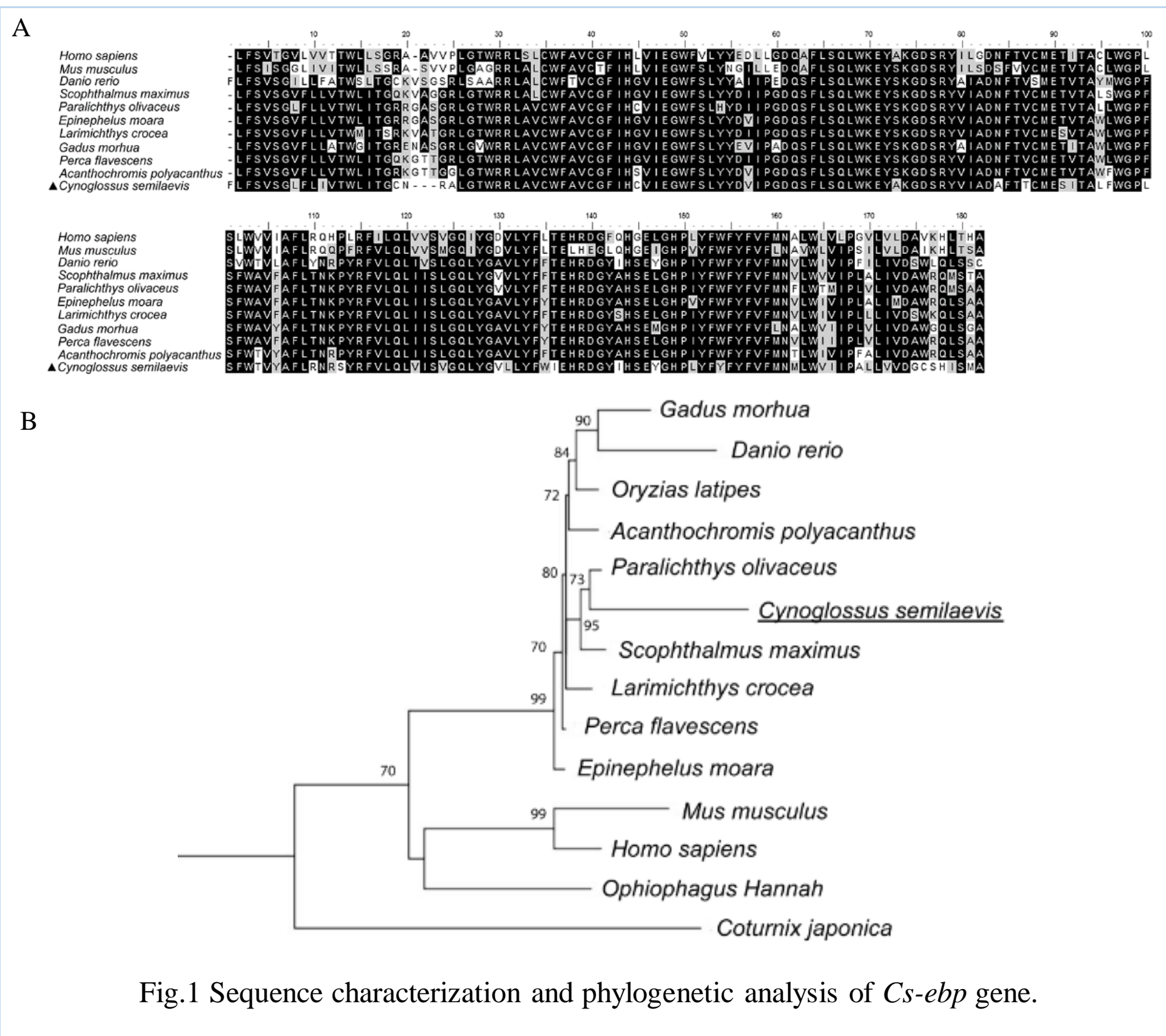


Fig.1 Sequence characterization and phylogenetic analysis of *Cs-ebp* gene.

2. *Cs-ebp* exhibited female-dominant expression in gonads and livers. Its abundance in ovaries were in accordance with the ovarian development and oocyte growth.

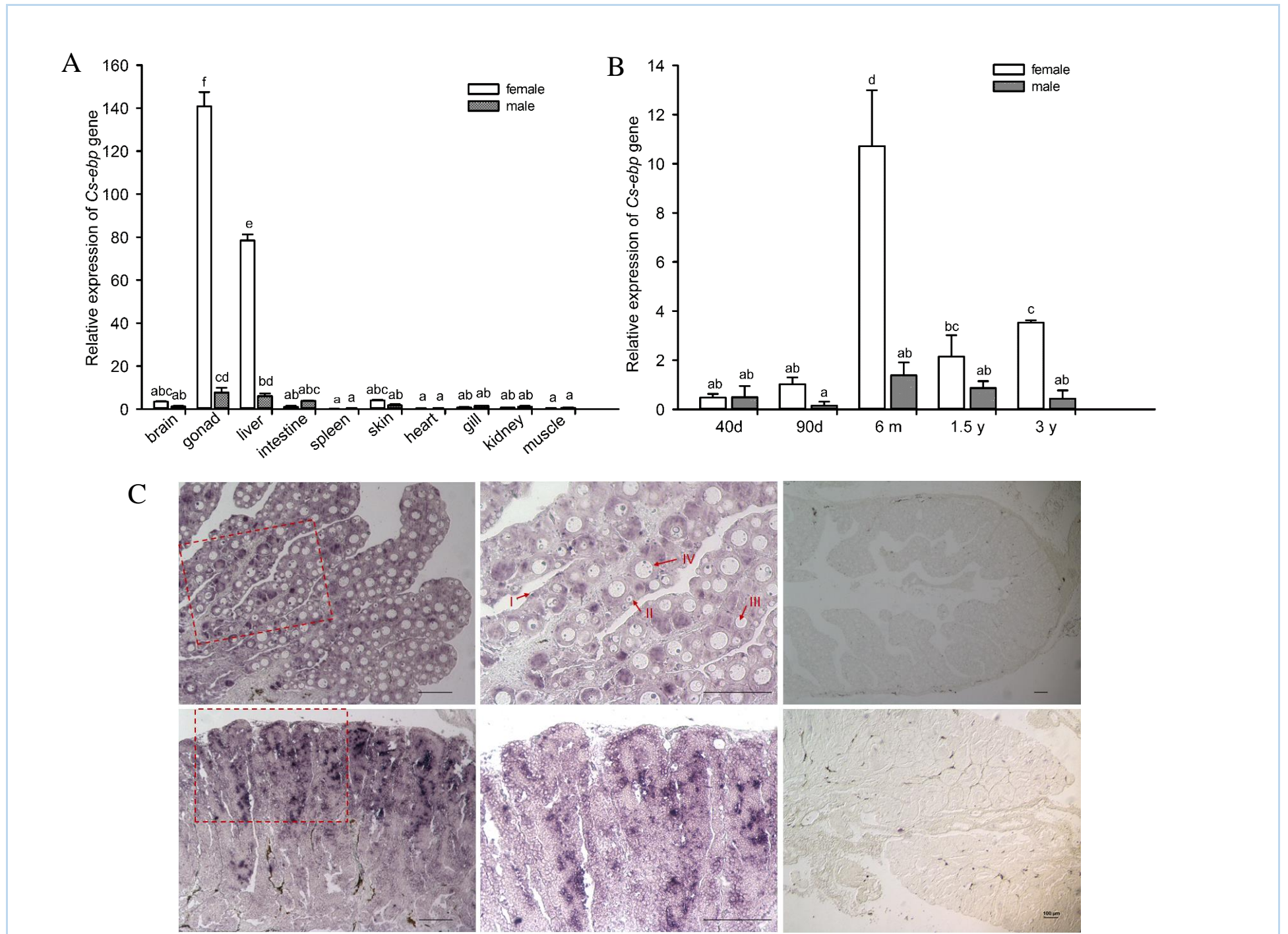


Fig.2 Tissue distribution of *Cs-ebp* gene and its temporal and spatial expression in gonad.

3. *Cs-ebp* transcription was regulated by transcriptional factors JUNB and POU1F1. *Cs-ebp* gene knockdown triggered the down-regulation of its downstream genes in cholesterol biosynthesis pathway (*sc5d*, *dhcr7*, and *dhcr24*), and genes related to estrogen biosynthesis (*cyp19a1a*) and body growth (*akt1* and *bmp2b*).

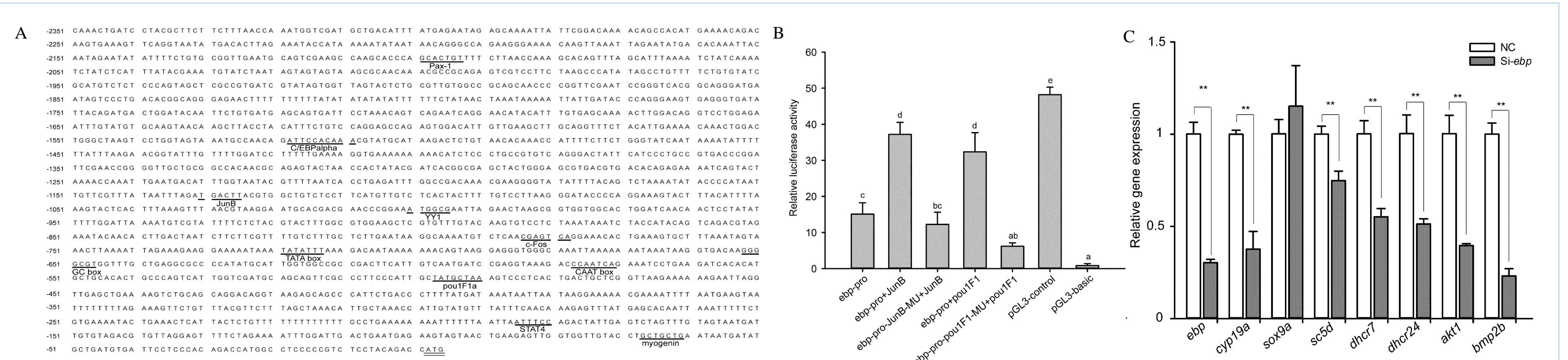


Fig.3 The effect of transcription factor on *Cs-ebp* promoter and the inhibitory effects of *Cs-ebp* siRNA in *C. semilaevis* ovarian cells.

Conclusions:

1. Emopamil binding protein (*Cs-ebp*) showed high expression in gonads in 6 months post hatching, and the expression was female biased.
2. *Cs-ebp* RNAi led to decreased transcription of genes involved in steroid and estrogen biosynthesis, and body growth.
3. By screening the transcription factors on *Cs-ebp* promoter, we found it is positively regulated by transcription factors JUNB and POU1F1.
4. Based on these findings, *Cs-ebp* is a potential regulator in sexual size dimorphism of Chinese tongue sole, possibly through PI3K/Akt and TGF- β /Bmp signaling pathway.
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