

Gene body methylation plays a homeostatic role in stress adaptation of extremophiles

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ABSTRACT

Whether and how epigenetics may contribute to long-term adaptation remain open questions. Gene body methylation (gbM), a common epigenetic modification in both plants and animals, has been believed to modulate local adaptation, but the evidence for the role of gbM in long-term adaptation thus far has been lacking. Here we show that mangroves thriving in intertidal zones have acquired much more gbM in comparison with their terrestrial relatives, resulted from accelerated gain rate relative to loss rate. We identified thousands of genes with convergent gbM gains specific in mangroves (convBMM), presumably driven by positive selection. Despite considerable methylation changes induced by salt stress, mangrove maintained robust transcriptomes accompanied with a very good correlation between the salt-induced gains of gbM and the reduction in salt-responsive expression changes. convBMM genes showed a strong preference for stress-induced gbM gain, exhibited reduced expression changes under stress, and had little overlap with genes that are known to have undergone convergent sequence evolution. Our findings suggest that transgenerational inheritance of acquired gbM states play a homeostatic role that facilitates the long-term stress adaptation for extremophiles such as mangroves.

1. Accelerated accumulation of gbM in mangroves compared with their non-mangrove relatives

Using whole-genome bisulfite sequencing (BS-seq), we compared genome-wide methylation distribution between mangroves and their non-mangrove relatives. While mCG levels in gene body are comparable between pairs of mangroves and non-mangroves (Fig 1A), mangroves have acquired much more body-methylated (BM) genes in comparison with their terrestrial relatives (Fig.1B), which is resulted from accelerated gbM gain rate relative to loss rate (Fig.1A).

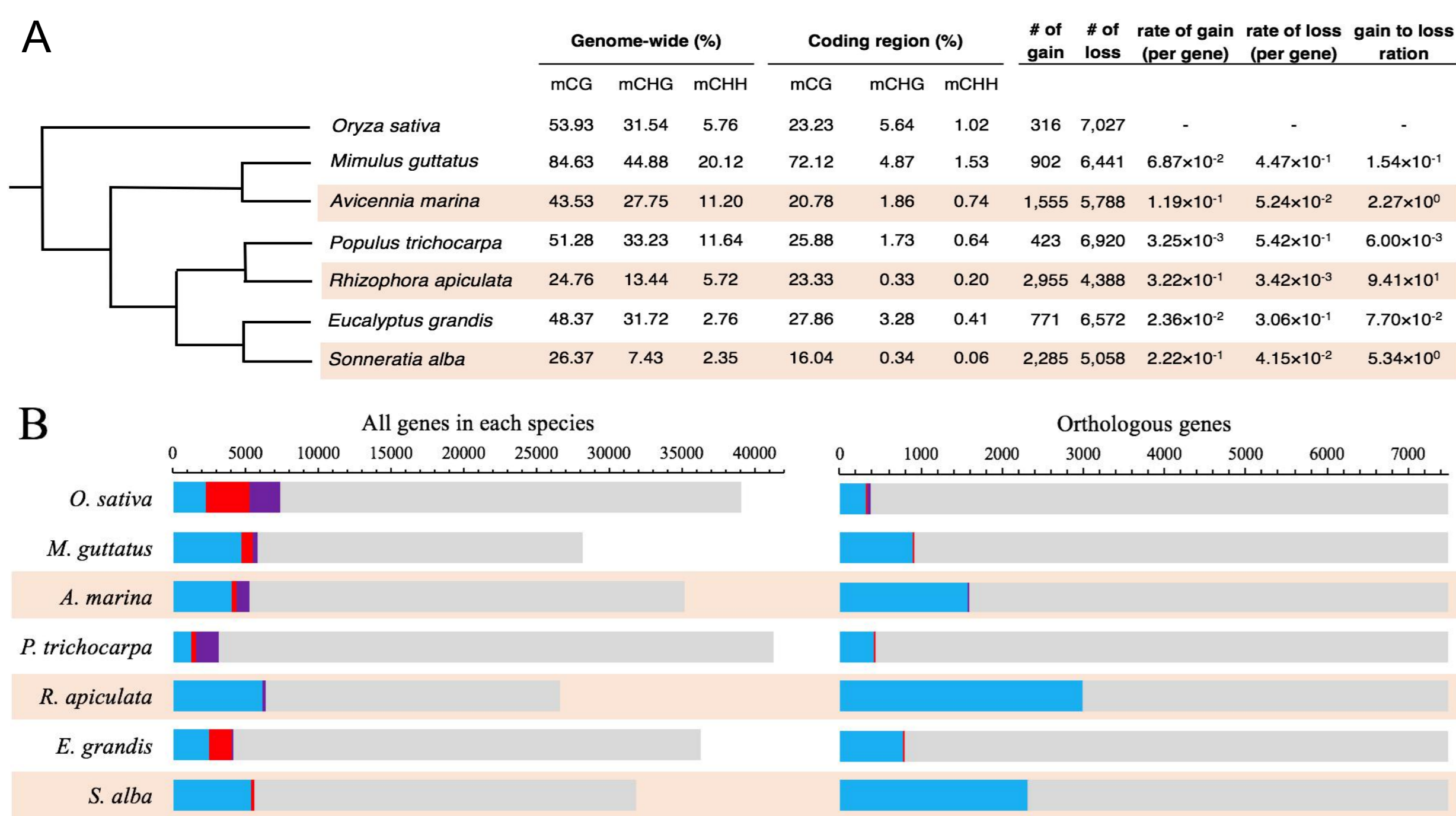


Fig. 1 Genome-wide methylation distribution between mangrove and non-mangrove lineages. (A) Genome-wide methylation levels, and gain or loss rates of gbM in leaves of mangroves and non-mangrove species. (B) Number of different kinds of methylated genes in each species.

2. Convergent gbM gains specific to mangrove lineages

Using a revised version of the convergence at conservative sites method (Xu et al. 2017), we identified 764 ortholog clusters that convergently gained gbM status in two (M2M in Fig. 2) and 360 in all three (M3M in Fig. 2) mangrove species. In contrast, there are fewer genes that convergently gained gbM status in the non-mangroves, only 12 in two (N2M in Fig. 2) and one in all three non-mangrove relatives (N3M in Fig. 2).

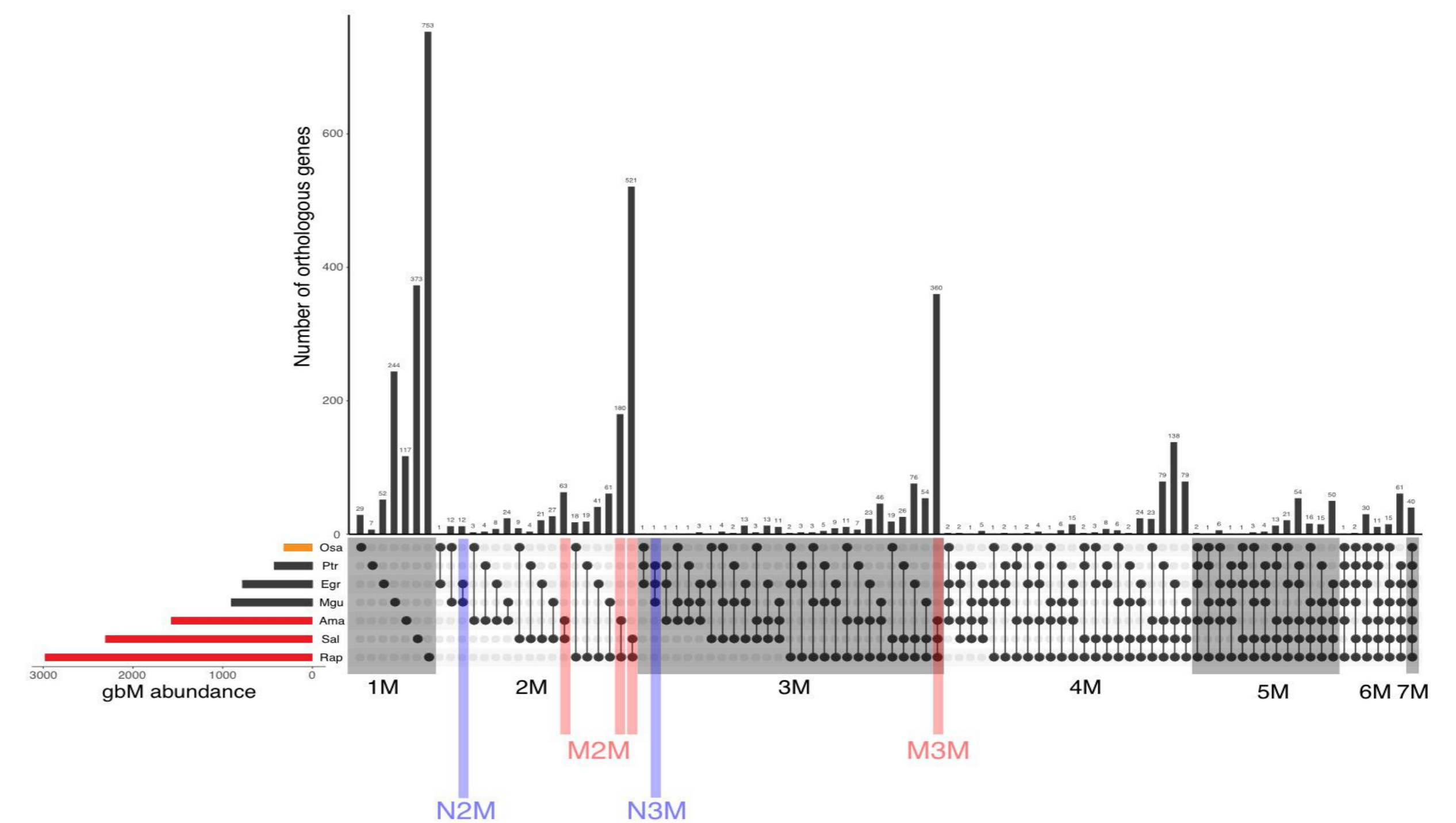


Fig. 2 Conservation of gbM status across orthologs of the three mangroves and their non-mangrove relatives. An UpSetR plot shows all kinds intersecting sets of the gbM status between species on survey. gbM and non-gbM genes are indicated with filled and open circles, respectively.

3. Mangroves exhibited homeostatic transcriptomes in salt stress despite considerable methylation changes

We treated seedlings of *Avicennia marina* (Ama) and *Rhizophora apiculata* (Rap) with 500 mM NaCl for seven days, and conducted RNA-seq and BS-seq using leaves of treated and untreated plants (Fig. 3A). Despite considerable differentially methylated positions (DMPs) in CG context (Fig. 3B), which arose more frequently in genic than non-genic regions (Fig. 3C), few genes were differentially expressed (DEGs) between salt-treated and untreated samples (Fig. 3D).

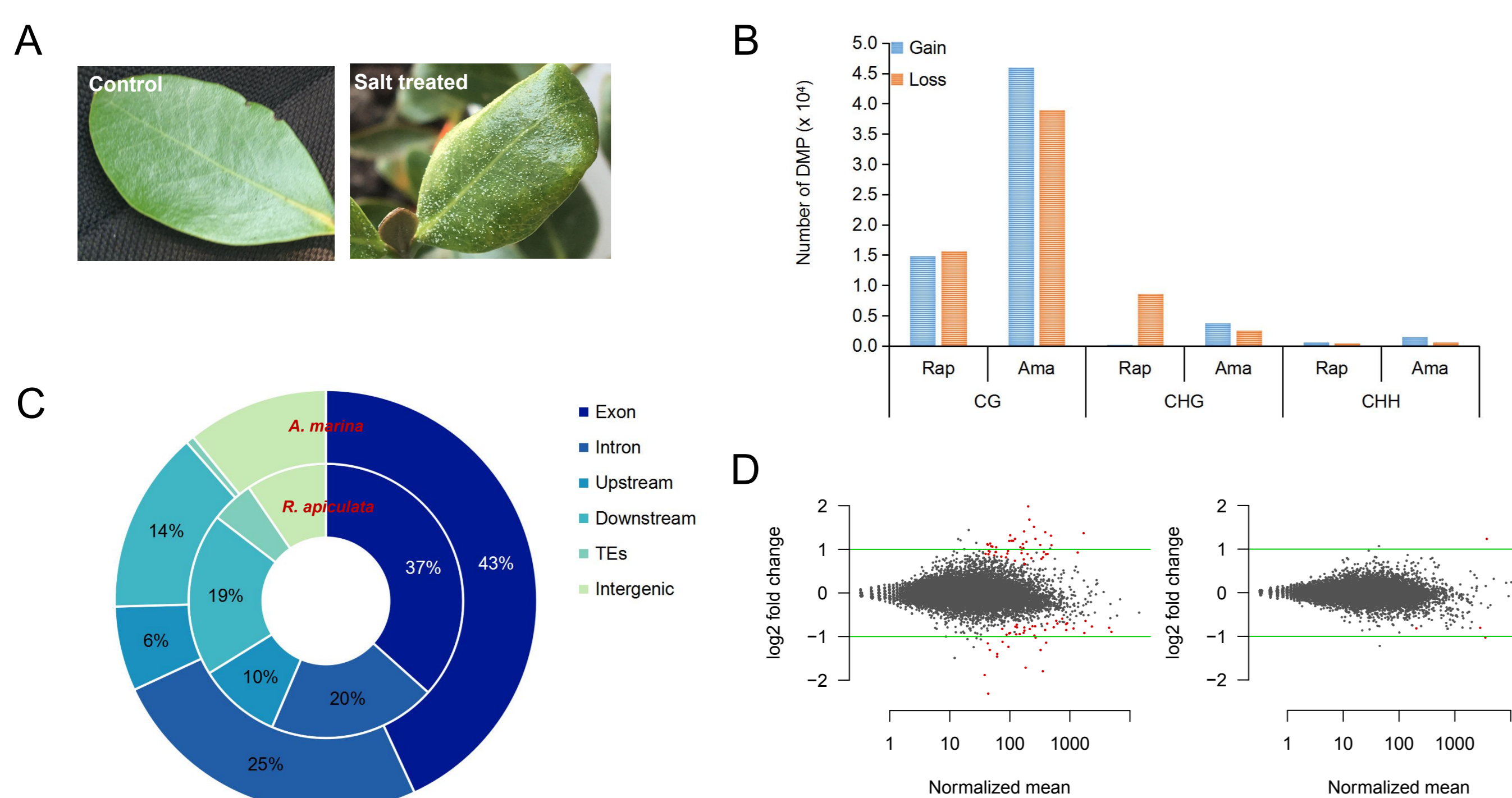


Fig. 3 Salt-induced changes of methylation and gene expression in *A. marina* and *R. apiculata*. (A) Salt-treated and untreated leaves of *A. marina*. (B) The number of DMPs in different sequence contexts. (C) The genomic distribution of CG-DMPs. (D) Fold changes of gene expression between salt-treated and untreated samples plotted against expression levels.

5. Gains of gbM correlated with reduction in stress-responsive expression variation

Genes that gained gbM under high salinity exhibited lower expression difference than UM genes, while genes that lost gbM showed the opposite trend in comparison with the BM genes (Fig. 5A). convBMM genes exhibited the smallest expression differences in response to salt compared with control body-methylated genes in *A. marina* (Fig. 5B). convBMM genes were also expressed at a higher level than non-convBM and convBMN genes (Fig. 5C).

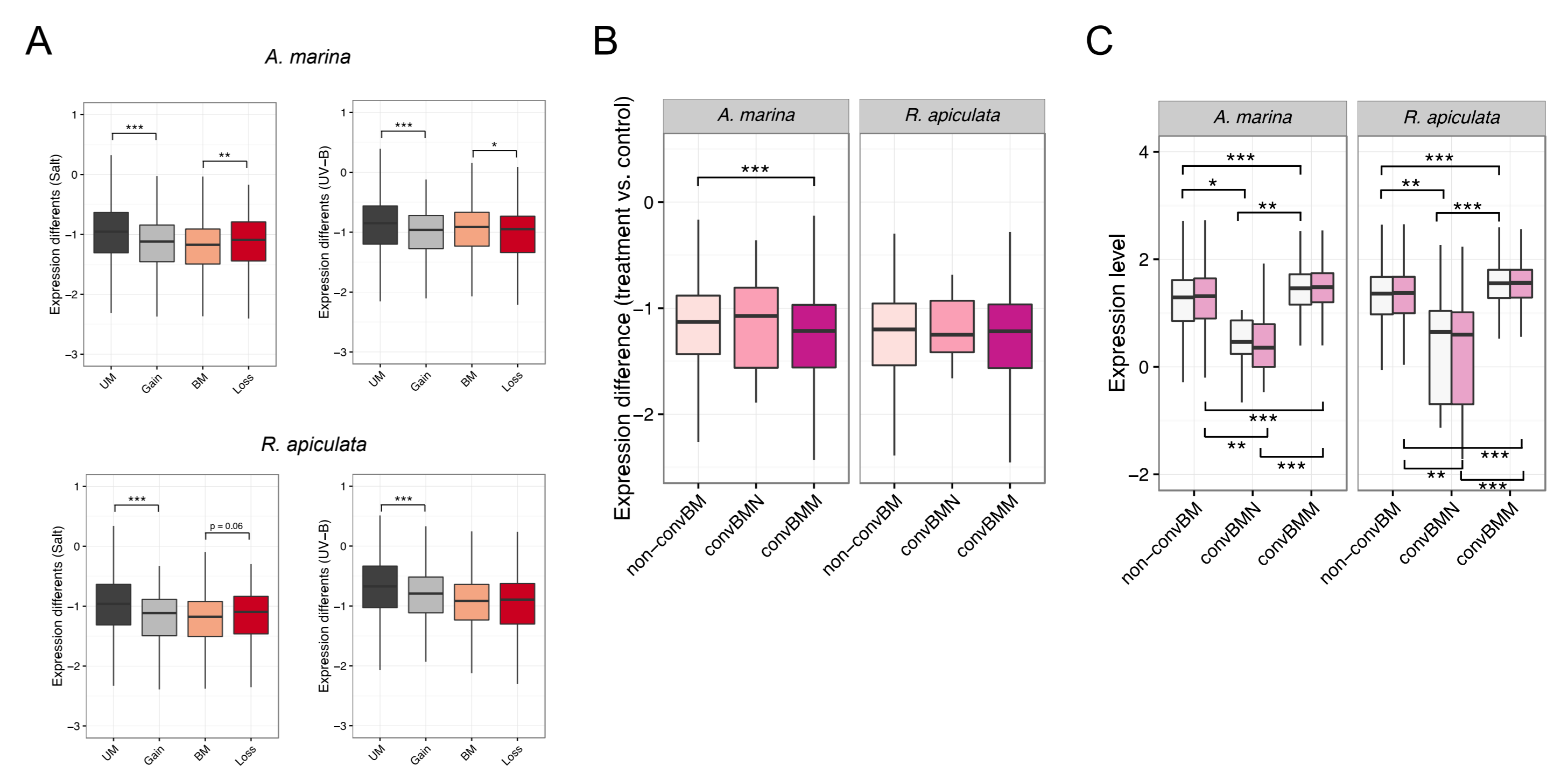


Fig. 5 Expression patterns in relation to gbM status in *A. marina* and *R. apiculata* under salt stress. (A) Expression differences of genes that gained or lost gbM in response to salt stress. (B) Salt-responsive expression differences in relation to gbM status. (C) Expression levels in relation to gbM status in salt treated (pink) and control samples (white).

4. Mangrove-specific convergent BM genes (convBMM) preferentially gained gbM in response to salt stress

Table 1 Salt-responsive methylation changes for convBMM genes in comparison with the others. BM, body-methylated, UM, unmethylated.

Species	Methylation Type	Category	unchanged	changed	% of DMG	X ² test (P value)
			n	n		
Ama	BM	convBMM	541	36	6.7	n. s
		others	3337	269	8.1	
	UM	convBMM	479	56	11.7	
		others	28875	311	1.1	
Rap	BM	convBMM	984	13	1.3	< .01
		others	4817	155	3.2	
	UM	convBMM	96	19	19.8	
		others	20243	180	0.9	

6. Little overlap between genes with convergent gbM gains and genes with signatures of convergent sequence evolution

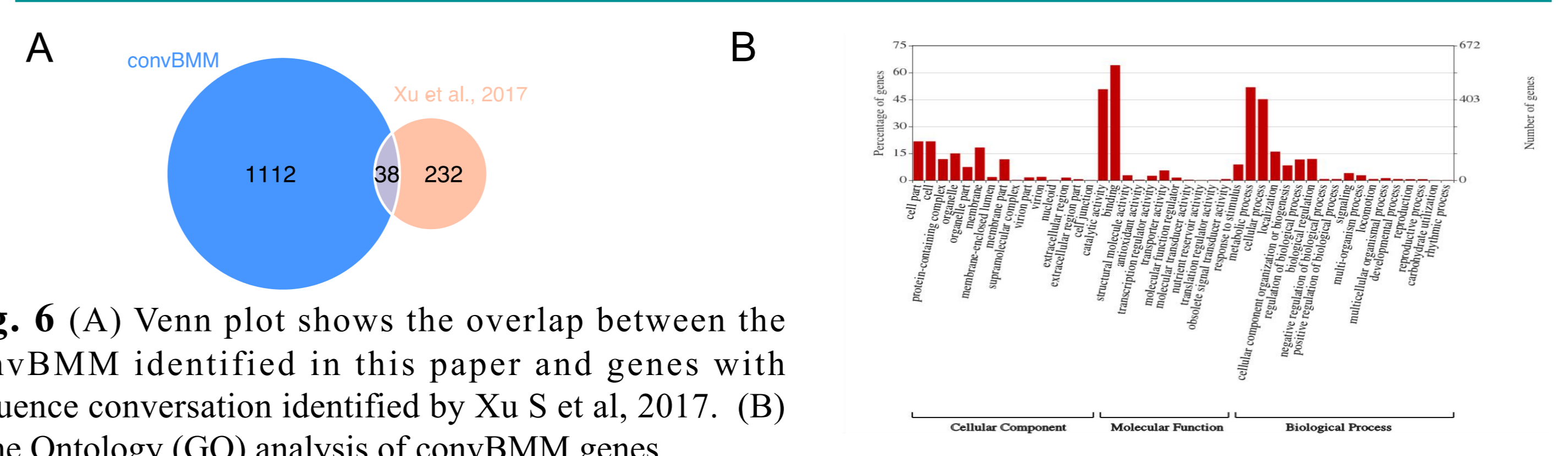


Fig. 6 (A) Venn plot shows the overlap between the convBMM identified in this paper and genes with sequence conversation identified by Xu S et al, 2017. (B) Gene Ontology (GO) analysis of convBMM genes.

Conclusion

Our results indicate that gbM plays an independent role in expression robustness to cope with environmental stress. This may allow accelerated accumulation of genetic variation and eventually facilitate long-term stress adaptation.

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