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# Functional PTB phosphate transporters are present in streptophyte algae and early diverging land plants



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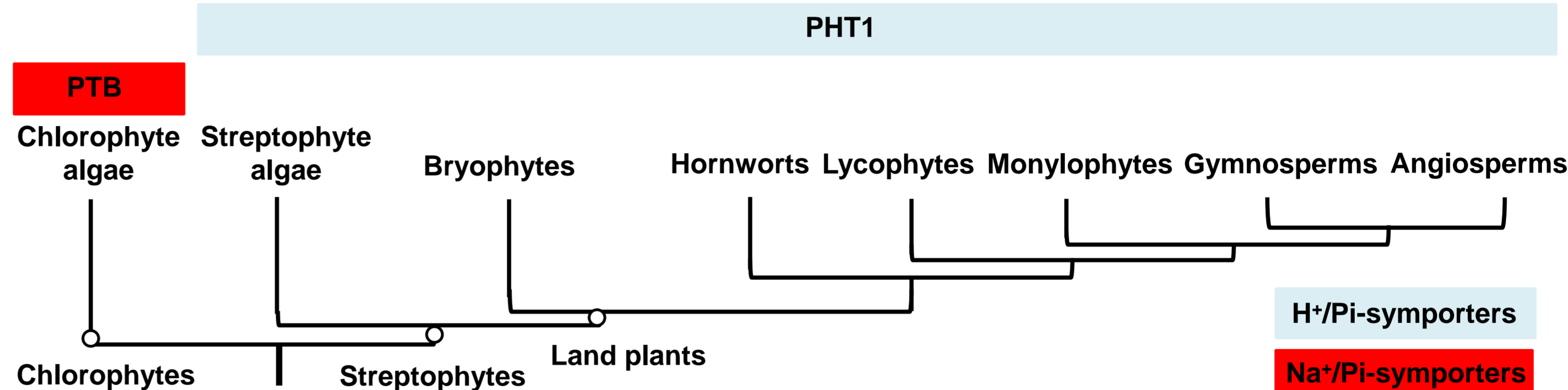
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## Introduction

- Angiosperms, the most diverged group of land plants, use plasma membrane H<sup>+</sup>/Pi-symporters called PHT1 to absorb Pi from the environment.
- In chlorophytes, the PTB proteins are hypothesised to be plasma membrane Na<sup>+</sup>/Pi-symporters responsible for Pi-uptake.
- PHT1 sequences are present in early diverging land plant and streptophyte algae but Na<sup>+</sup>-uptake is required for Pi-uptake in streptophyte algae implying that both H<sup>+</sup>/Pi- and Na<sup>+</sup>/Pi-symporters are active in streptophytes.

To understand the evolution of Pi-uptake during land adaptation we set out to determine if PTB proteins were present and functional in streptophyte lineages.

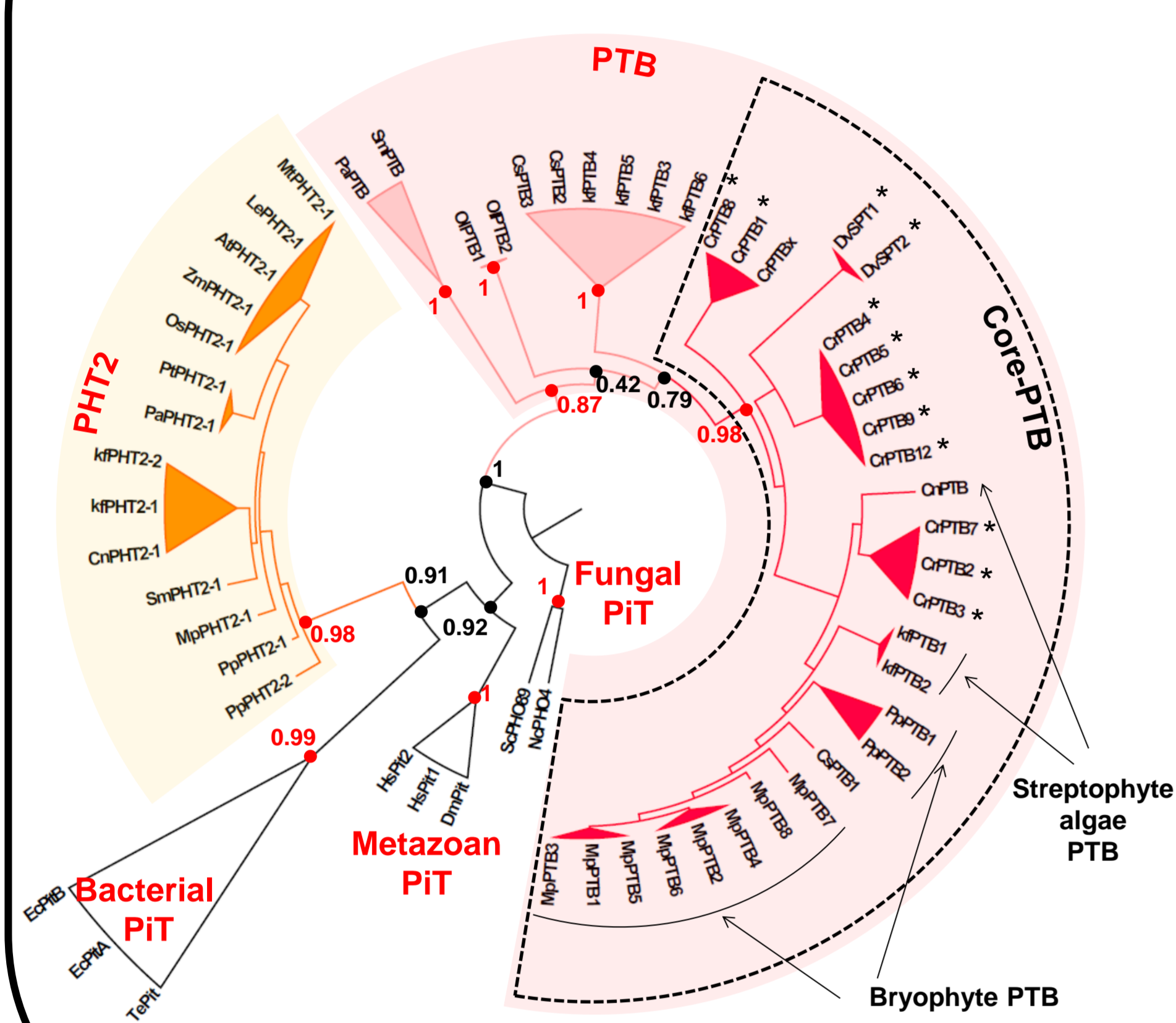
### Pi-uptake mechanisms and Pi-symporters in the streptophyte phylogeny



After Wickett *et al.*, 2014, *PNAS*; Moseley *et al.*, 2006, *Euka cell*; Li *et al.*, 2006, *BBRC*; Nussaume *et al.*, 2011, *Front plant Sci*; Delaux *et al.*, 2015, *PNAS*

## I. PTB proteins are present in streptophytes

### Maximum-likelihood phylogeny of the PTB proteins across the green plants



- Core-PTB clade = PTB proteins from streptophyte algae (*Kelbsormidium flaccidum* (Kf) and *Coleochaete nitellarum* (Cn)) and bryophytes (*Marchantia polymorpha* (Mp) and *Physcomitrella patens* (Pp)) and published chlorophytes PTB proteins (\*) (*Dunaliella viridis* (Dv) and *Chlamydomonas reinhardtii* (Cr)).

### PTB and PTB-related protein amino acid conserved motifs

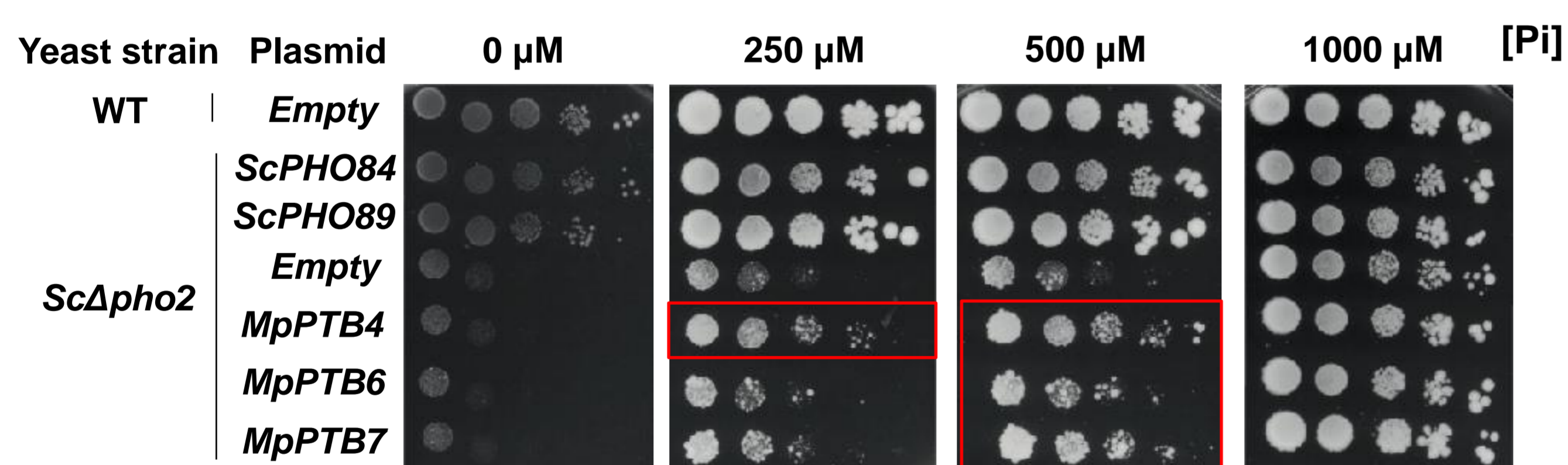
Motifs	AA	Sequence	PHT2	Bacterial PIT	Metazoan PIT	Fungal PIT	Chlorophyte Core-PTB	Streptophyte Core-PTB
Motif 1	14	GaNDVANafgTsvG	ok	ok	ok	ok	ok	ok
Motif 2	15	lpsStTHxixGaxxG	ok	ok	ok	ok	ok	ok
Motif 3	6	SWfxSP	ok	ok	ok	ok	ok	ok
Motif 4	14	FaHGaNVDVxnaXGP	ok	ok	ok	ok	ok	ok
Motif 5	34	TPxRgFxaElaxaxxxxxASxxGLPxStTxxxxG	ok	ok	ok	ok	ok	ok
Motif 6	12	HaxAEVfDpXtE	-	-	-	ok	ok	ok
Motif 7	8	FpyxxGxV	-	-	-	-	ok	ok
Motif 8	12	kaAWVxaxxaaG	-	-	-	-	ok	ok
Motif 9*	8	KxaaxxGx	-	-	-	-	ok	ok
Motif 10	12	AAXfAXGayaPS	-	-	-	-	ok	ok
Motif 11	7	kdxkYe	-	-	-	-	ok	ok
Motif 12	5	lNxxl	-	-	-	-	ok	ok
Motif 13*	8	nxSxxTxG	-	-	-	-	ok	ok

- Core-PTB are extremely conserved between streptophytes and chlorophytes.

There is little sequence diversification of the core-PTB proteins since the divergence of chlorophytes and streptophytes

## II. *M. polymorpha* PTB genes expression rescues a yeast mutant defective in Pi-uptake

### Growth phenotype of the yeast mutant Scpho2 expressing *M. polymorpha* (Mp)PTB

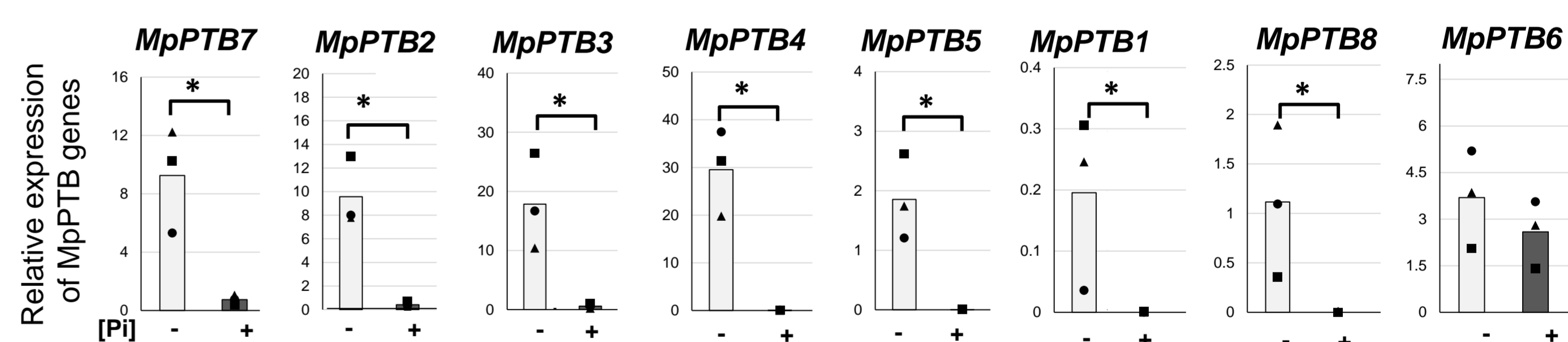


- The transcription factor *ScPHO2* is required for the expression of the *S. cerevisiae* high-affinity Pi-transporters (*ScPHO84* and *ScPHO89*) (Pinson *et al.*, 2004, *J Bio Chem*).
- MpPTB4*, *MpPTB6* and *MpPTB7* expression rescue *Scpho2* mutant growth in low-Pi.

This suggests that core-PTB proteins are Pi-transporters

## III. *M. polymorpha* PTB genes expression is induced in low-Pi environments

### Effect of the Pi-concentration of the environment on MpPTB genes expression



The star indicates that the transcript levels measured are significantly different (P<0.05, Kruskal-Wallis test).

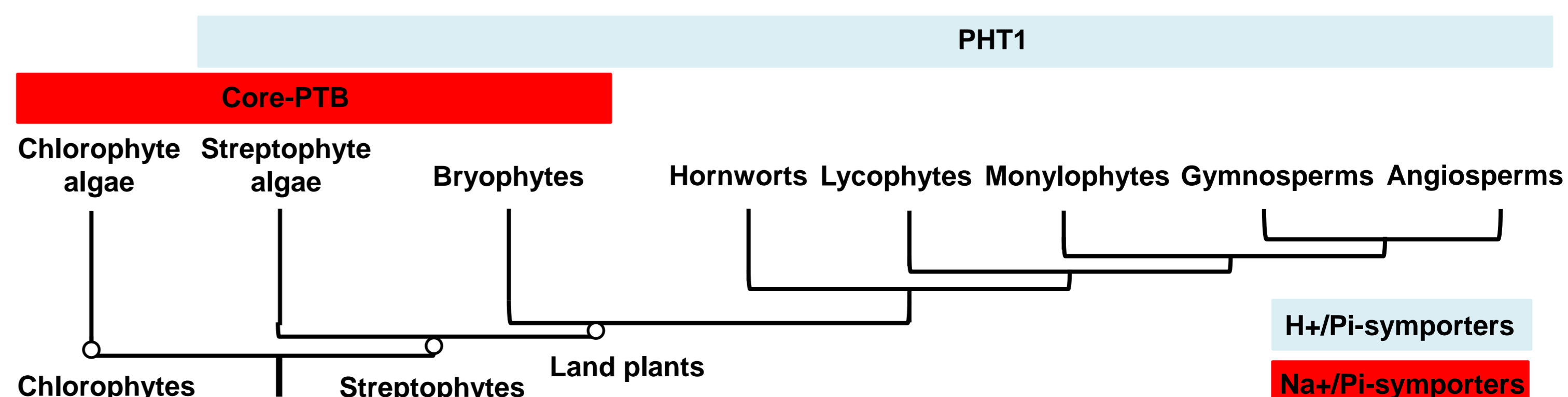
- The expression of *CnPTB* and of 7 *MpPTB* genes is higher in low-Pi than replete-Pi.

This suggests that core-PTB proteins are involved in Pi-uptake

## Conclusion

- PTB Pi-transporters are present in streptophytes.
- Two Pi-uptake systems co-exist in streptophyte algae and bryophytes

### Pi-uptake mechanisms and Pi-symporters in the streptophyte phylogeny



After Wickett *et al.*, 2014, *PNAS*; Moseley *et al.*, 2006, *Euka cell*; Li *et al.*, 2006, *BBRC*; Nussaume *et al.*, 2011, *Front plant Sci*; Delaux *et al.*, 2015, *PNAS*