

Supplemental materials for

Stomatal clustering in *Begonia* improves water use efficiency by modulating stomatal movement and leaf structure

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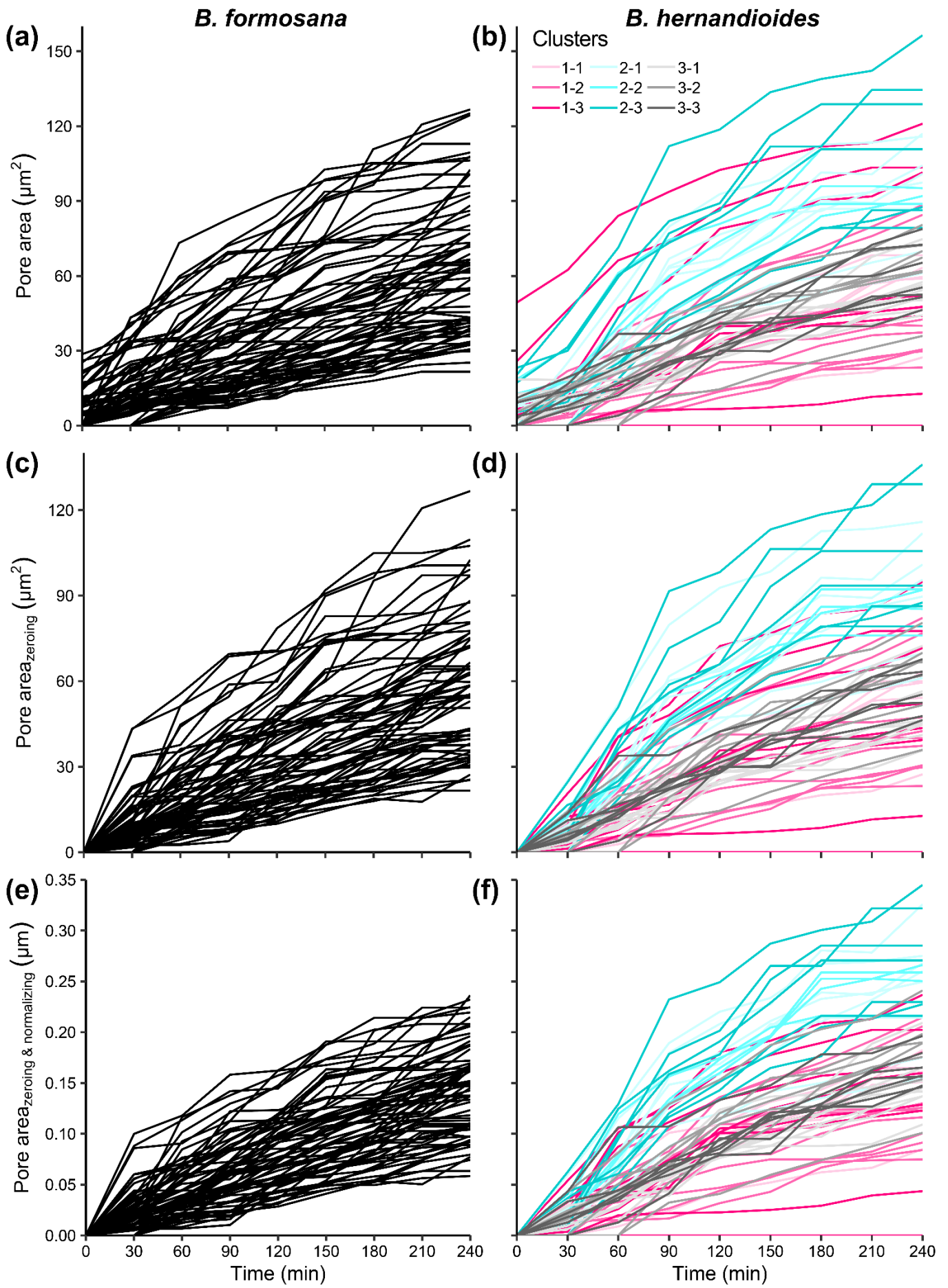
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This PDF file includes

Supplemental Figure 1 to 3

Supplemental Table 1

Supplemental References



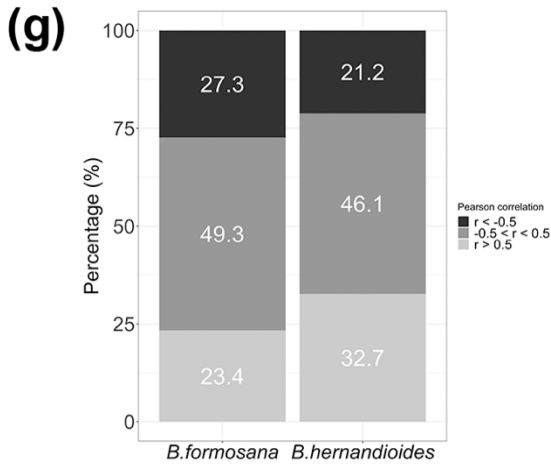


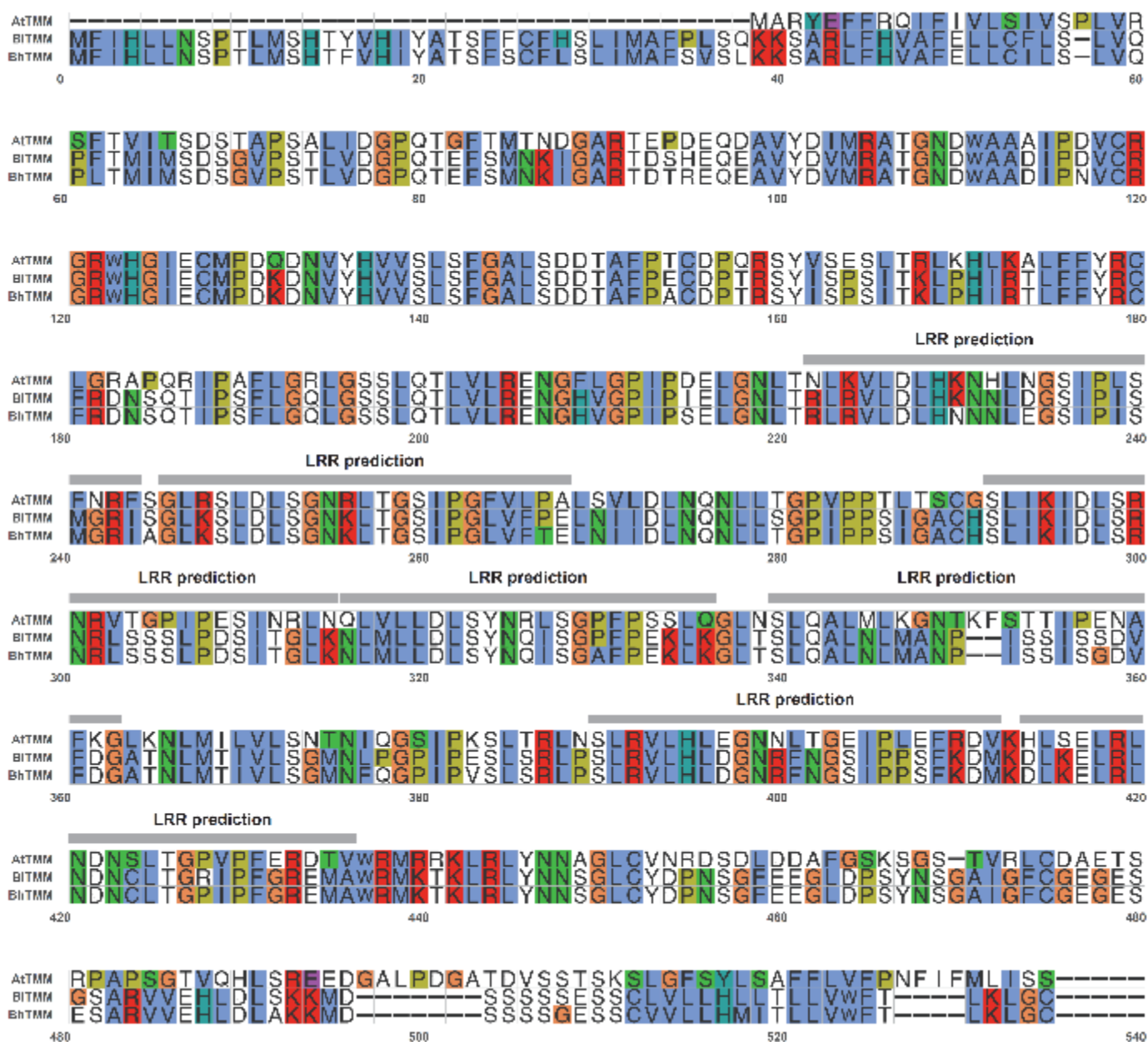
Figure S1. Response of pore area from 0 to 240 min under an irradiance of $100\text{--}200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in opening buffer.

(a and b) Raw data from changes in pore area from 0 to 240 min under an irradiance of $100\text{--}200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *B. formosana* (a) and *B. hernandioides* (b).

(c and d) Pore area subtracted by the pore area at the starting point (0 min) (zeroing) in *B. formosana* (c) and *B. hernandioides* (d).

(e and f) Normalized data after zeroing and normalization by the maximum length of each stomate in *B. formosana* (e) and *B. hernandioides* (f).

(g) Pearson's correlation coefficients (r) for pairwise comparisons between individual opening dynamics from 30 to 150 minutes using three individuals from *B. formosana* and individuals #1 and #3 from *B. hernandioides*. There were more positive correlations in *B. hernandioides* than in *B. formosana*.



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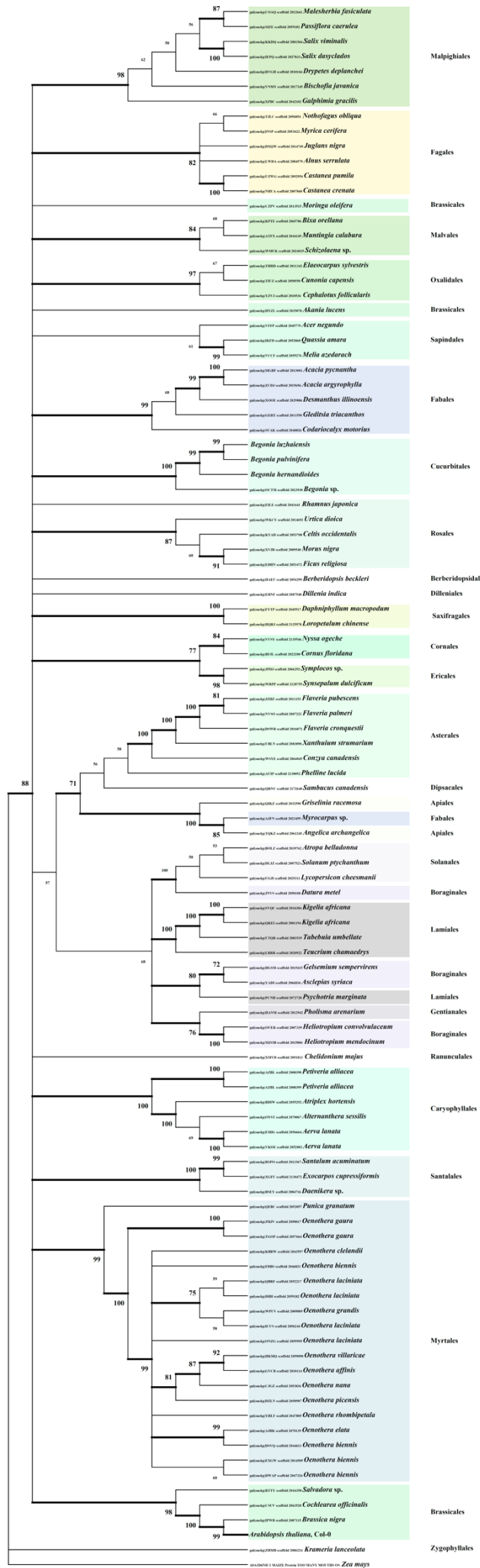
2 **Figure S2.** Amino acid alignment of AtTMM, BITMM, and BhTMM.

3 *Begonia* TMM (521 amino acids) is longer than *Arabidopsis* TMM (496 amino acids). The alignments were

4 performed with the CRAN Package *ggmsa* (<https://CRAN.R-project.org/package=ggmsa>) in R version 4.0.3.

5 Color patches were based on the ClustalX color scheme. Leucine-rich repeat (LRR) domains were predicted

6 using the PROSITE database (50).



8 **Figure S3.** The optimal tree of TMMs was shown using the Neighbor-Joining method (Saitou & Nei, 1987).
9 This analysis involved 105 amino acid sequences. The percentage of replicate trees in which the associated
0 taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein,
1 1985). For Condensed tree, Cut-off value is set to BS = 50%. Bootstrap Support (BS) \geq 70% were shown in
2 bold types and lines. Most TMMs with strong support (BS > 70 %) in order-level represented similar
3 phylogenetic relationships within orders; however, phylogenetic relationships among orders could not be
4 confidently resolved with weak support (BS < 70%). The evolutionary distances were computed using the
5 JTT matrix-based method (Jones *et al.*, 1992) and are in the units of the number of amino acid substitutions
6 per site. All positions with less than 50% site coverage were eliminated (partial deletion option). There were
7 a total of 455 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et*
8 *al.*, 2018).

Table S1. Parameters of gas exchange.

Net CO₂ assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), and transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) under three light intensities in *B. formosana* (solitary stomata) and *B. hernandioides* (clustered stomata) ($n = 3$ biological replicates). No significant differences were detected between the two *Begonia* species, as determined by Student's *t*-test.

Parameters	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Species	
		<i>B. formosana</i>	<i>B. hernandioides</i>
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	25	1.41 ± 0.25	1.35 ± 0.17
	100	2.93 ± 0.54	3.02 ± 0.35
	200	3.19 ± 0.61	3.96 ± 0.81
g_s ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$)	25	0.05 ± 0.02	0.03 ± 0.02
	100	0.04 ± 0.01	0.03 ± 0.00
	200	0.04 ± 0.01	0.04 ± 0.01
E ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	25	0.52 ± 0.16	0.30 ± 0.18
	100	0.42 ± 0.08	0.29 ± 0.05
	200	0.45 ± 0.11	0.41 ± 0.13

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *evolution* **39**(4): 783-791.

Jones DT, Taylor WR, Thornton JM. 1992. The rapid generation of mutation data matrices from protein sequences. *Bioinformatics* **8**(3): 275-282.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution* **35**(6): 1547.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution* **4**(4): 406-425.