

The background features a network of white dots connected by thin lines, overlaid on a blue-tinted photograph of laboratory glassware. Two Erlenmeyer flasks are visible, each containing a green plant stem submerged in a clear liquid. The overall aesthetic is scientific and modern.

CINÉTICA E REGULAÇÃO ENZIMÁTICA | ENZYMOLOGY IN EVOLUTION STUDIES

A circular inset on the right side of the slide shows a close-up of a hand in a blue nitrile glove holding a small, clear petri dish. The dish contains a small green seedling growing in dark soil. The background of the inset is a blurred laboratory setting with a microscope and other equipment.

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Global seagrass distribution and diversity: A bioregional model

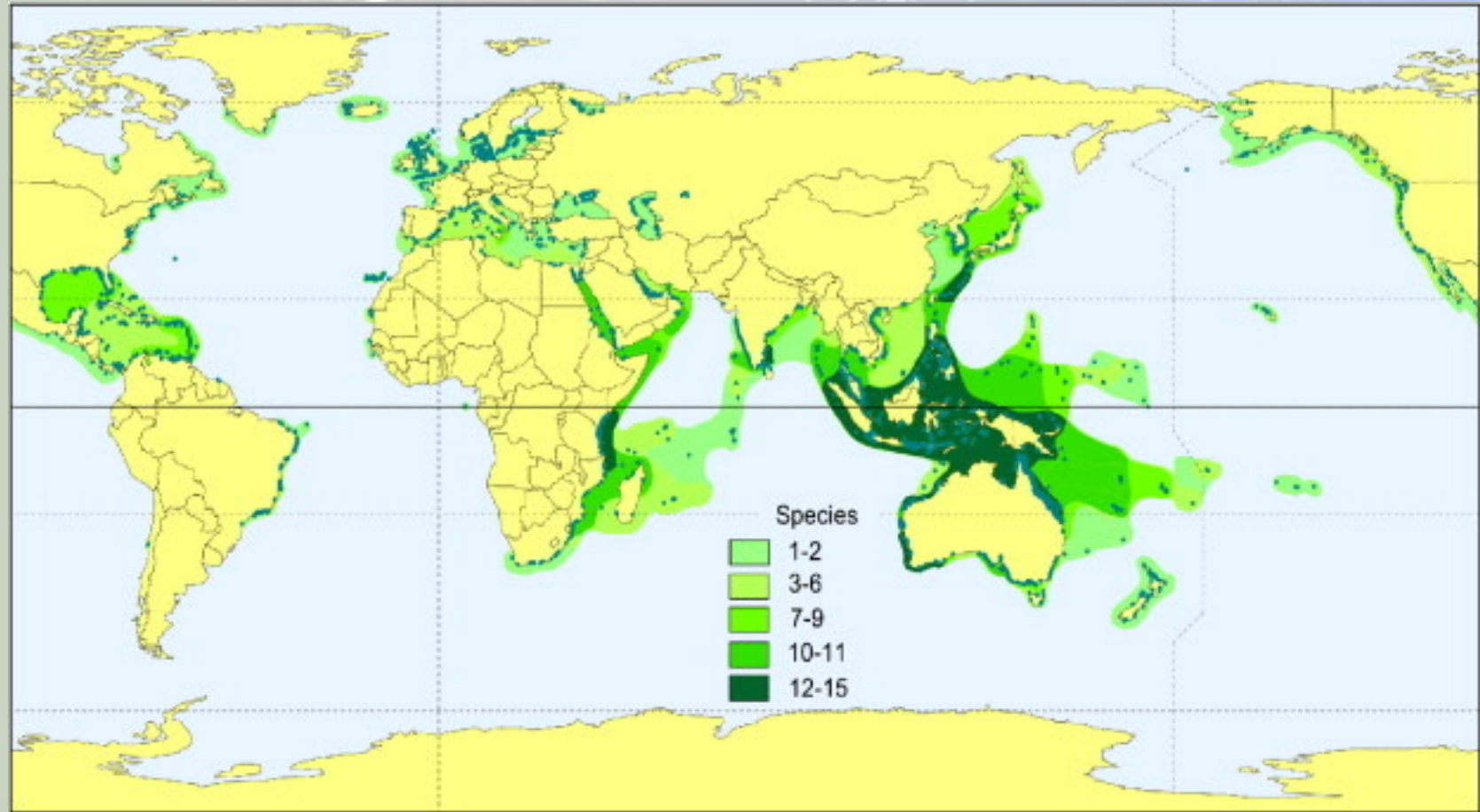
F. Short^{a,*}, T. Carruthers^b, W. Dennison^b, M. Waycott^c

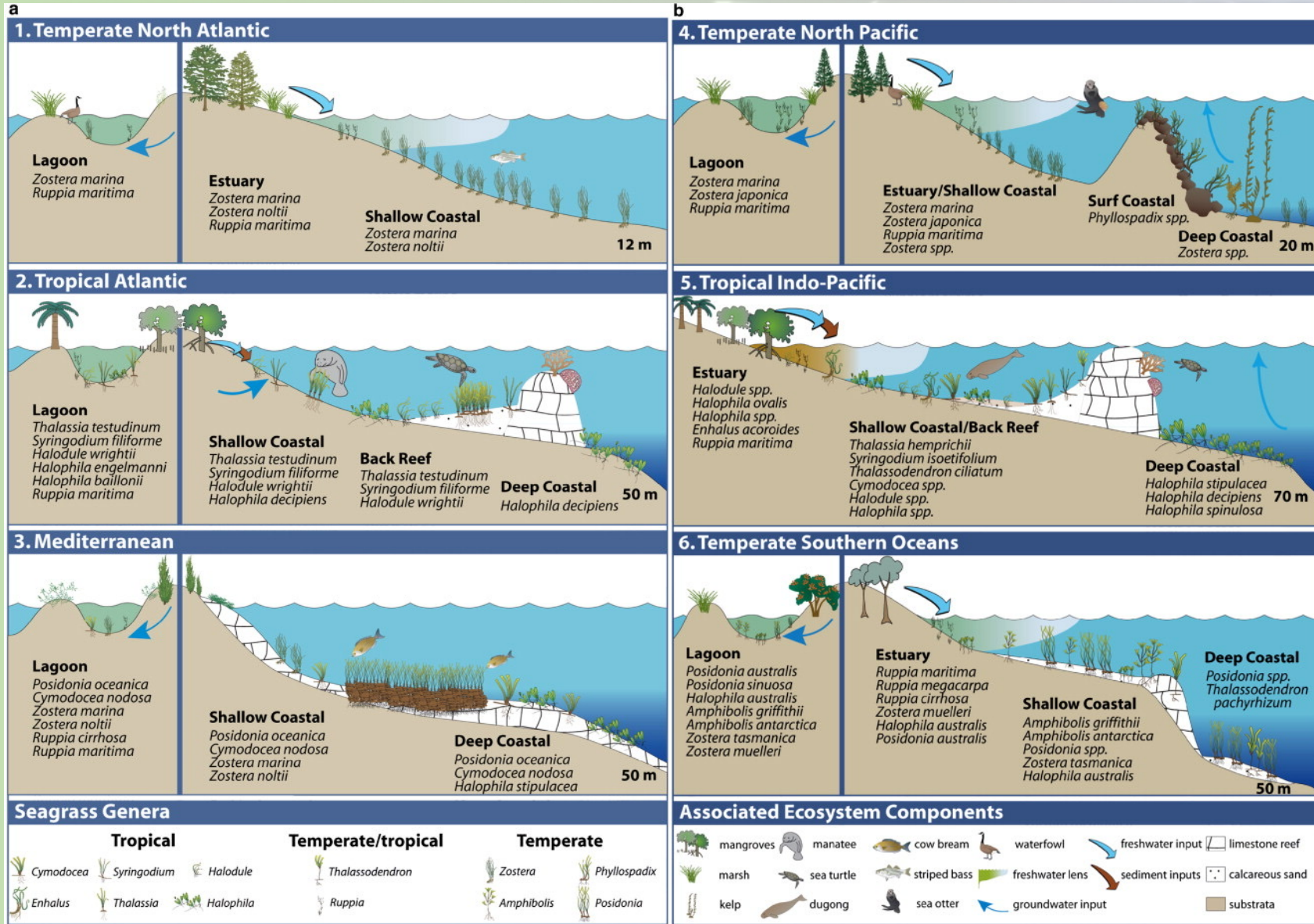
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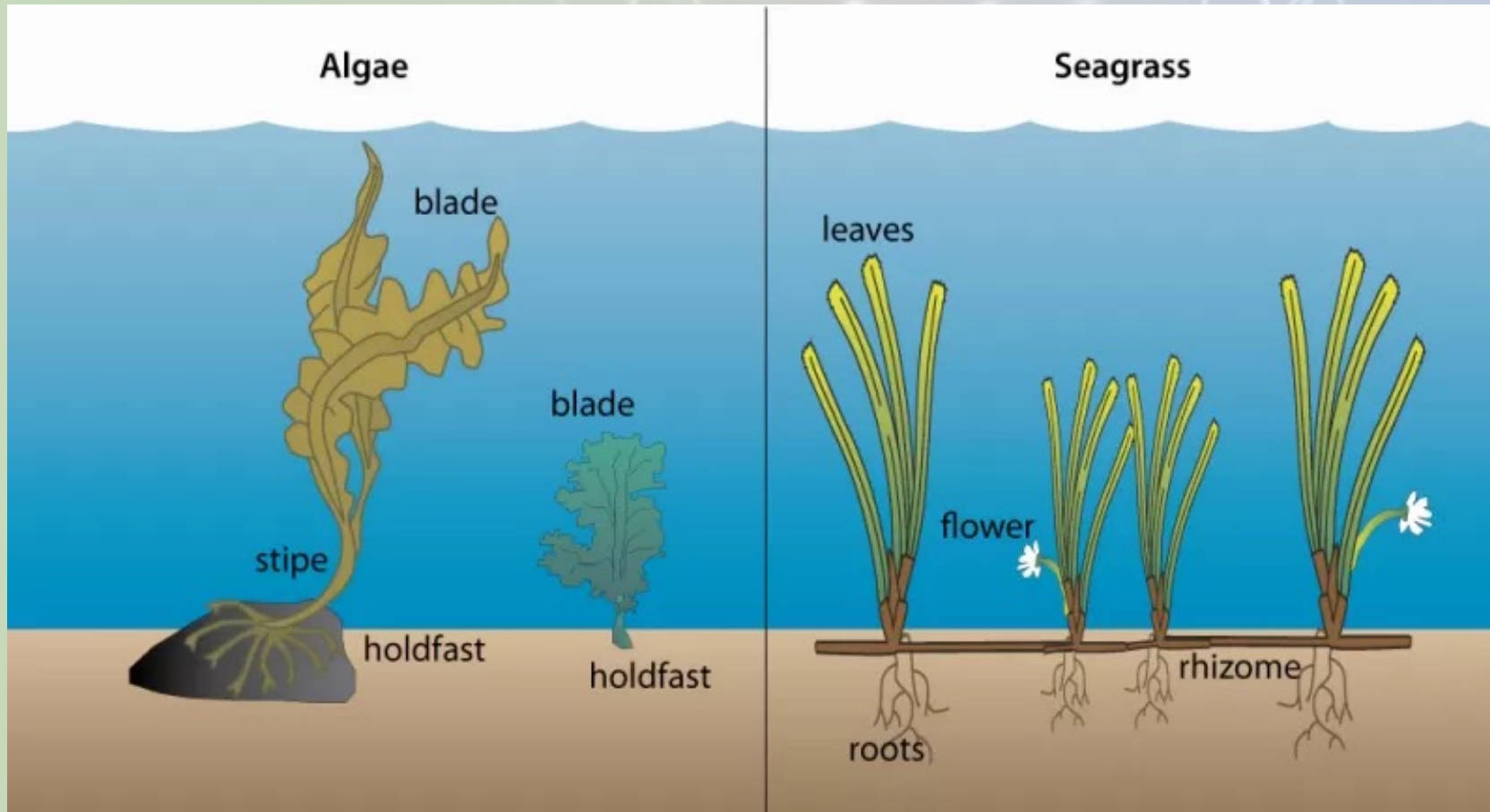
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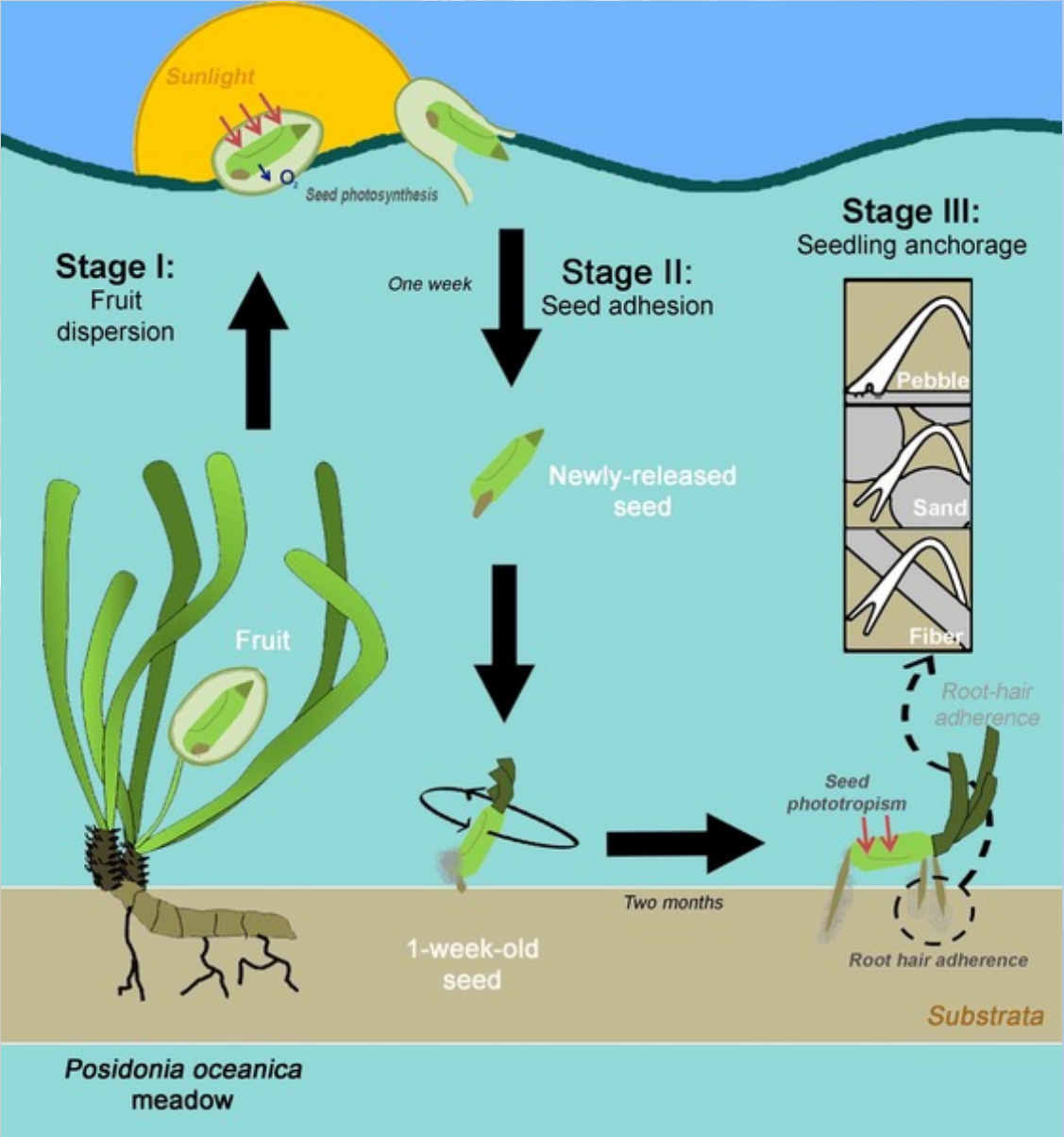
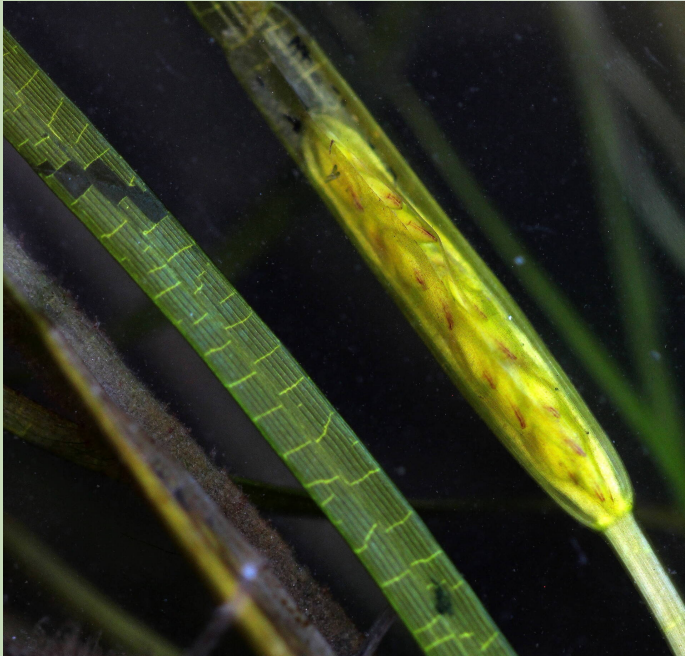
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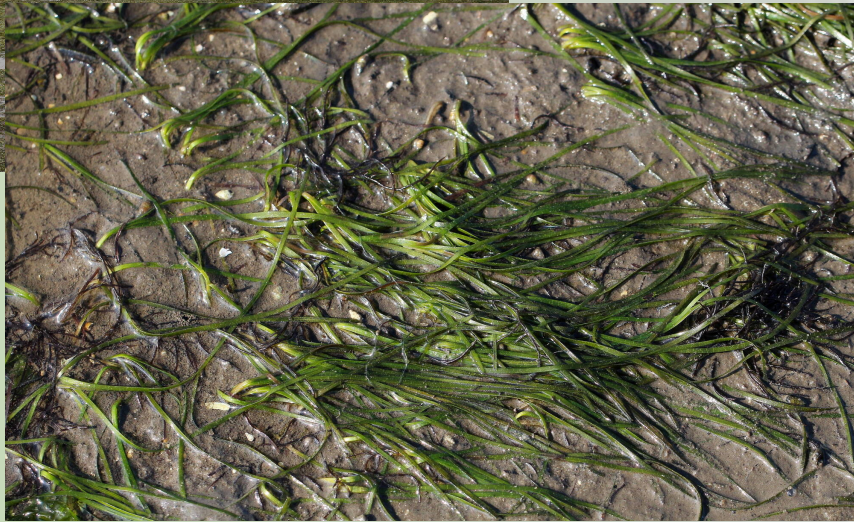
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SEAGRASSES ARE ANGIOSPERMS
VASCULAR PLANTS
PRODUCE FLOWERS, FRUITS AND SEEDS





Zostera noltii



Zostera marina



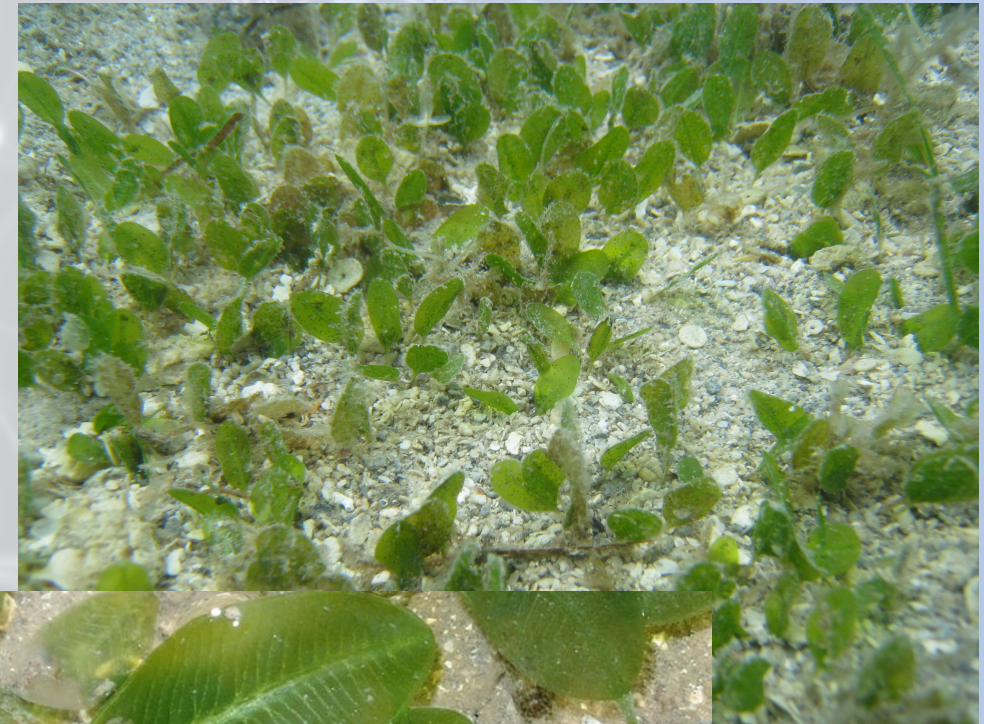
Cymodocea nodosa



Posidonia oceanica



Rupia maritima



Halophila ovalis

THREATS TO SEAGRASS ECOSYSTEMS

THREATS AFFECTING:

- HABITAT SUITABILITY
- WATER QUALITY
- GRAZING/RECRUITMENT



TEMPERATURE INCREASE

HABITAT LOSS THROUGH HEAT STRESS, INCREASED DISEASE RISK AND POTENTIAL DECREASED GRAZING ANIMAL COMMUNITIES



ALTERED RAINFALL

SEAGRASSES AFFECTED BY CHANGING SALINITY, AND IN CATCHMENTS WHERE RAINFALL INCREASES, BY INCREASES IN SEDIMENT AND NUTRIENTS



INCREASED FREQUENCY OF DESTRUCTION OF COASTAL SEAGRASS, DECREASING WATER CLARITY FOLLOWING MAJOR RAINFALL

AGRICULTURAL RUN-OFF

EXCESSIVE NUTRIENT AND SEDIMENT INPUTS REDUCE LIGHT FOR PHOTOSYNTHESIS

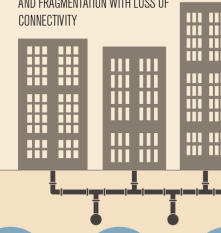


BOATING

SCARRING OF MEADOWS BY BOAT PROPELLERS AND MOORINGS, REDUCED WATER CLARITY THROUGH BOAT WAKE RESUSPENDING SEDIMENT

URBAN INFRASTRUCTURE

DIRECT DESTRUCTION OF SEAGRASS MEADOWS AND FRAGMENTATION WITH LOSS OF CONNECTIVITY



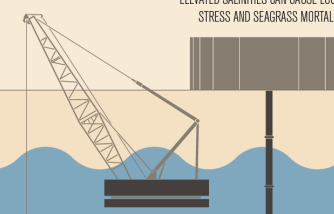
URBAN & INDUSTRIAL RUN-OFF

EXCESSIVE NUTRIENT INPUTS REDUCE LIGHT FOR PHOTOSYNTHESIS



DESALINATION PLANTS

ELEVATED SALINITIES CAN CAUSE LOCAL STRESS AND SEAGRASS MORTALITY

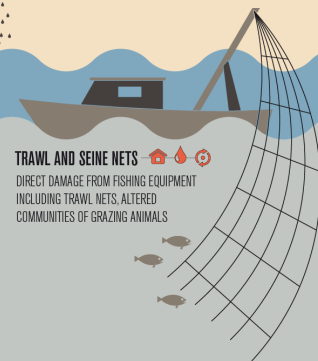


SEA LEVEL RISE

LOSS OF HABITAT AREA WHERE SEAGRASS MIGRATION UP THE SHORELINE IS INHIBITED

TRAWL AND SEINE NETS

DIRECT DAMAGE FROM FISHING EQUIPMENT INCLUDING TRAWL NETS, ALTERED COMMUNITIES OF GRAZING ANIMALS



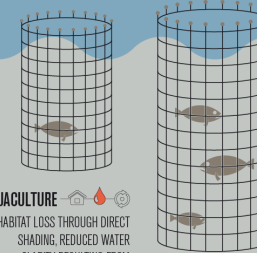
SHIPPING ACCIDENTS

POLLUTION BY OIL AND OTHER CONTAMINANTS AFTER MAJOR AND MINOR SPILLS



AQUACULTURE

HABITAT LOSS THROUGH DIRECT SHADING, REDUCED WATER CLARITY RESULTING FROM EXCESSIVE NUTRIENTS



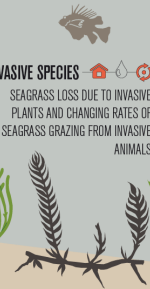
HARVESTING

LOCAL LOSSES DUE TO HARVESTING OF PLANTS



INVASIVE SPECIES

SEAGRASS LOSS DUE TO INVASIVE PLANTS AND CHANGING RATES OF SEAGRASS GRAZING FROM INVASIVE ANIMALS



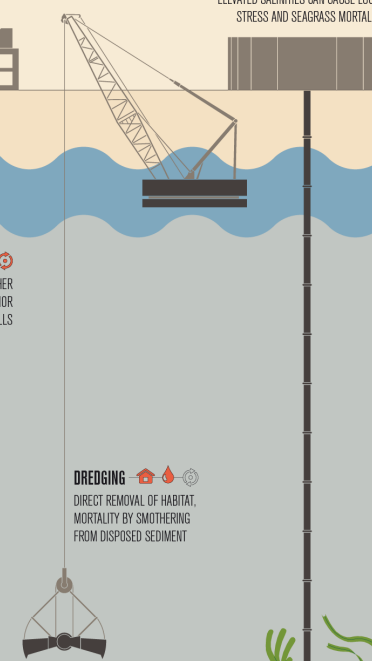
OCEAN ACIDIFICATION

BALANCE BETWEEN POTENTIAL POSITIVE EFFECTS ON PLANT GROWTH AND NEGATIVE EFFECTS ON GRAZING FAUNA



DREDGING

DIRECT REMOVAL OF HABITAT, MORTALITY BY SMOTHERING FROM DISPOSED SEDIMENT



MOST SEAGRASSES GROW IN DEPTHS LESS THAN 15 METERS

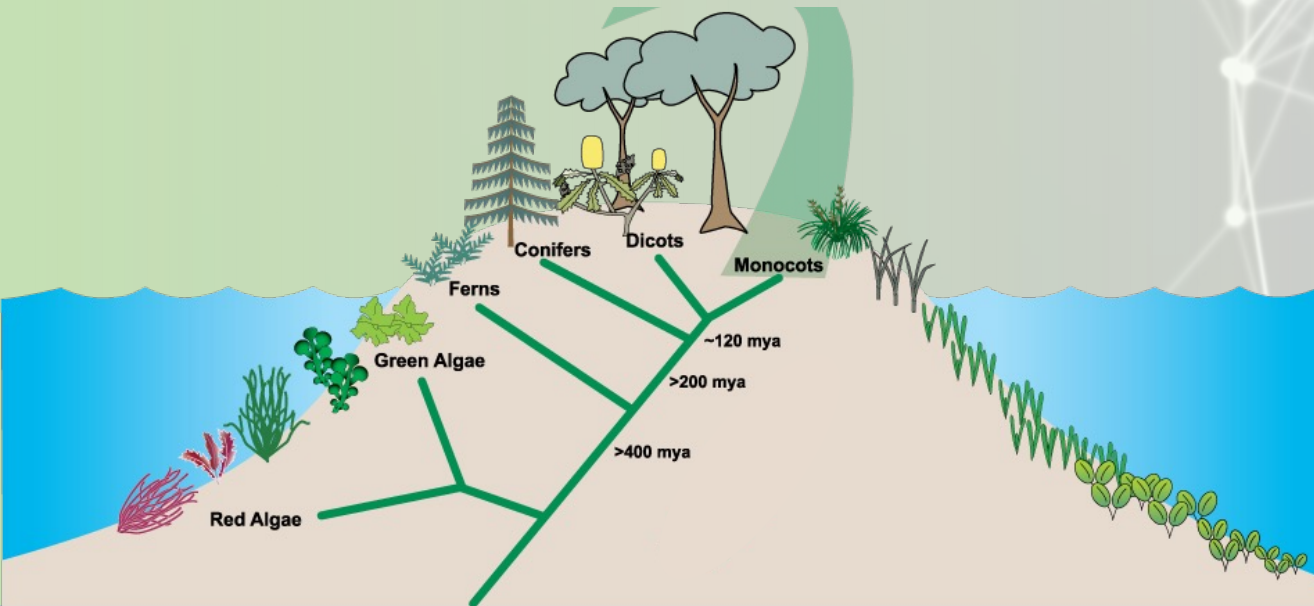
OBJECTS IN THIS VISUAL ARE NOT DRAWN TO SCALE SOME THREATS ARE EVEN CLOSER THAN THEY APPEAR

Source: GRID-Arendal (2020).

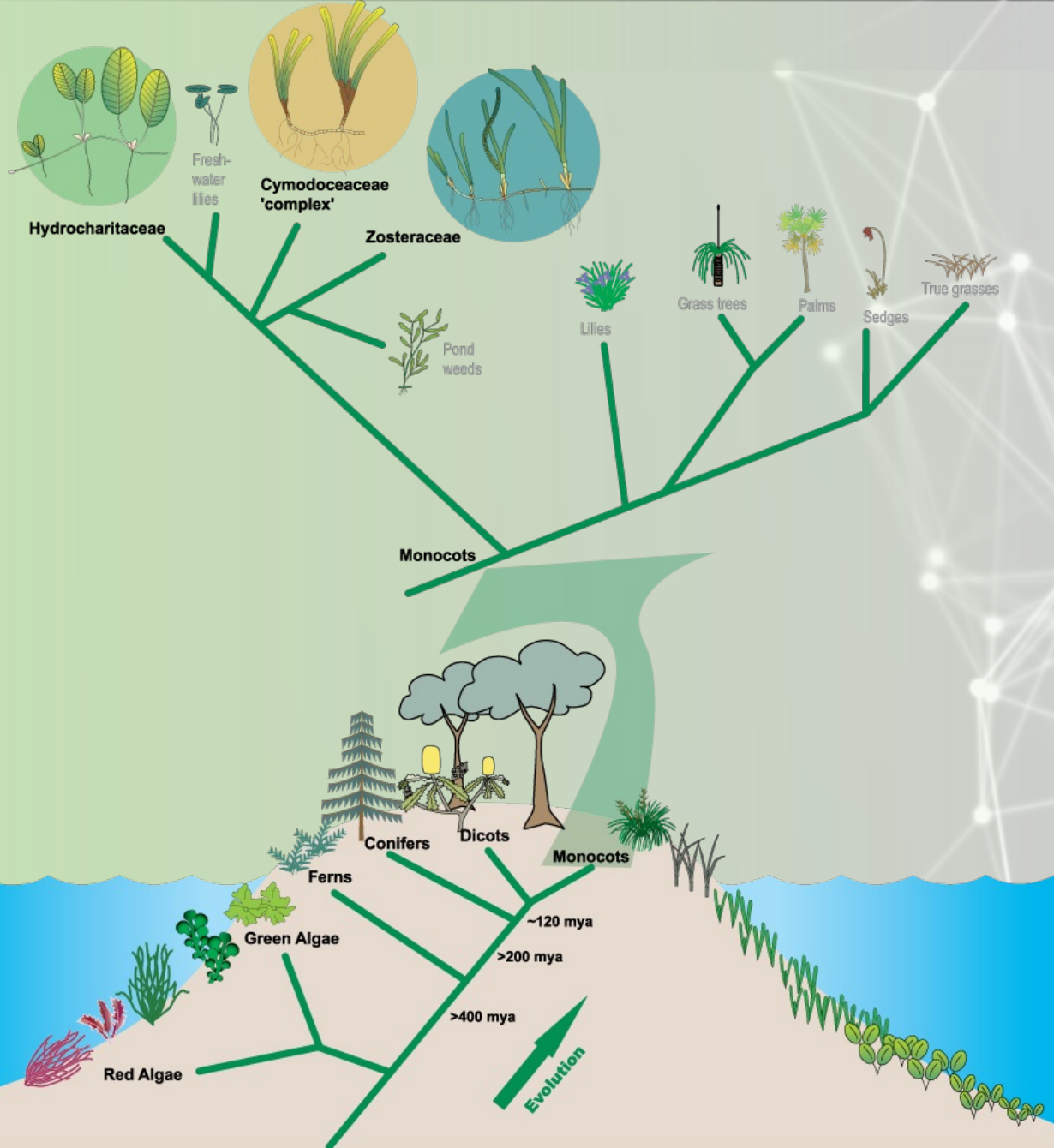
HOW DID SEAGRASSES EVOLVE?

HYPOTHESIS 1 – FROM ALGAE AT AN INTERMEDIATE STEP BEFORE THE EVOLUTION TO TERRESTRIAL VASCULAR PLANTS?

HYPOTHESIS 2 – FROM TERRESTRIAL VASCULAR PLANTS?



Conceptual diagram illustrating the evolution of seagrass species.
Diagram courtesy of James Cook University, Australia.



HOW DID SEAGRASSES EVOLVE?

HYPOTHESIS 1 – FROM ALGAE AT AN INTERMEDIATE STEP BEFORE THE EVOLUTION TO TERRESTRIAL VASCULAR PLANTS?

HYPOTHESIS 2 – FROM TERRESTRIAL VASCULAR PLANTS?

LETTER

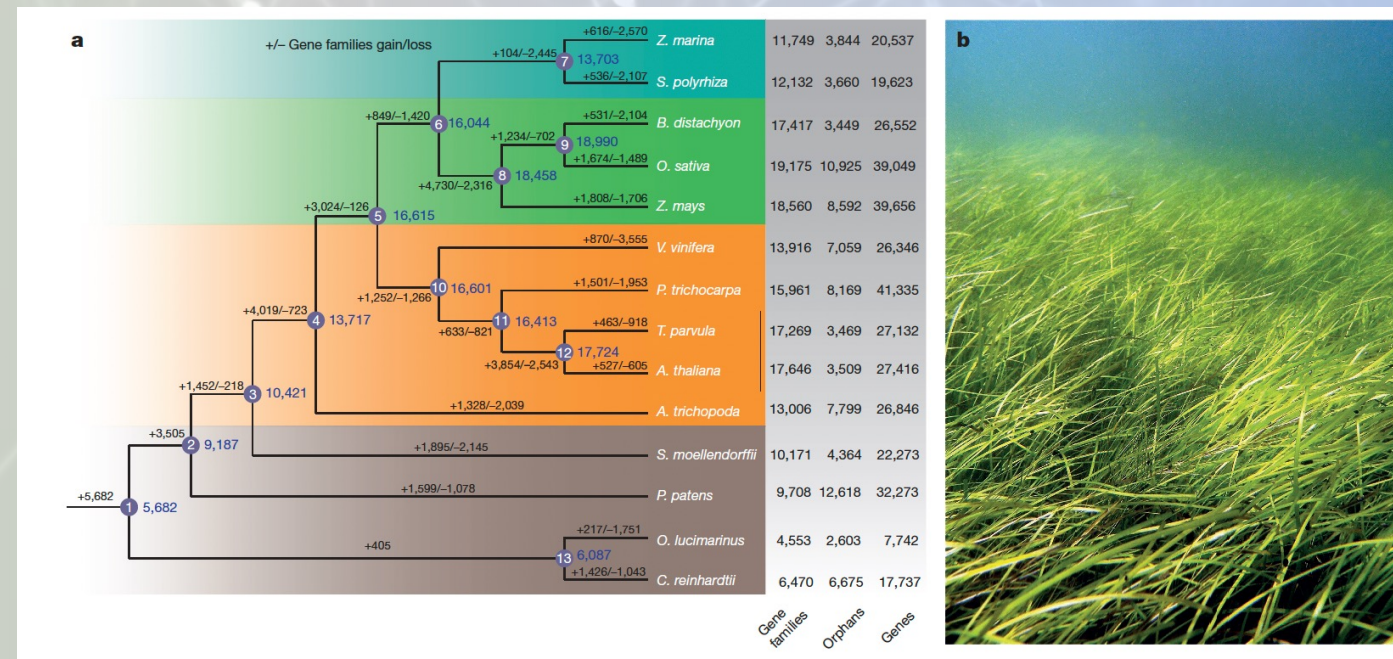
OPEN

doi:10.1038/nature16548

The seagrass *Zostera marina*, or eelgrass, is widely distributed throughout the Northern Hemisphere. It is therefore of considerable ecological importance but — as with other seagrasses — its coastal habitats are among the world's most threatened ecosystems. Jeanine Olsen and colleagues report the whole-genome sequence of *Zostera*. Their analyses provide insights into the evolutionary changes associated with the **'back to the sea' reverse evolutionary trajectory** that has occurred in this angiosperm lineage.

The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea

Jeanine L. Olsen^{1*}, Pierre Rouzé², Bram Verhelst², Yao-Cheng Lin², Till Bayer³, Jonas Collen⁴, Emanuela Dattolo⁵, Emanuele De Paoli⁶, Simon Dittami⁴, Florian Maumus⁷, Gurvan Michel⁴, Anna Kersting^{8,9}, Chiara Lauritano⁵, Rolf Lohaus², Mats Töpel¹⁰, Thierry Tonon⁴, Kevin Vanneste², Mojgan Amirebrahimi¹¹, Janina Brakel³, Christoffer Boström¹², Mansi Chovatia¹¹, Jane Grimwood^{11,13}, Jerry W. Jenkins^{11,13}, Alexander Jueterbock¹⁴, Amy Mraz¹⁵, Wytze T. Stam¹, Hope Tice¹¹, Erich Bornberg-Bauer⁸, Pamela J. Green¹⁶, Gareth A. Pearson¹⁷, Gabriele Procaccini^{2*}, Carlos M. Duarte¹⁸, Jeremy Schmutz^{11,13}, Thorsten B. H. Reusch^{3,19*} & Yves Van de Peer^{2,20,21*}



Key angiosperm innovations that were **lost** include the **entire repertoire of stomatal genes, genes involved in the synthesis of terpenoids and ethylene signaling, and genes for ultraviolet protection and phytochromes for far-red sensing**. Seagrasses have also regained functions enabling them to adjust to full salinity. Their cell walls contain all of the polysaccharides typical of land plants, but also contain polyanionic, low-methylated pectins and sulfated galactans, a feature shared with the cell walls of all macroalgae and that is important for ion homeostasis, nutrient uptake and O₂/CO₂ exchange through leaf epidermal cells.

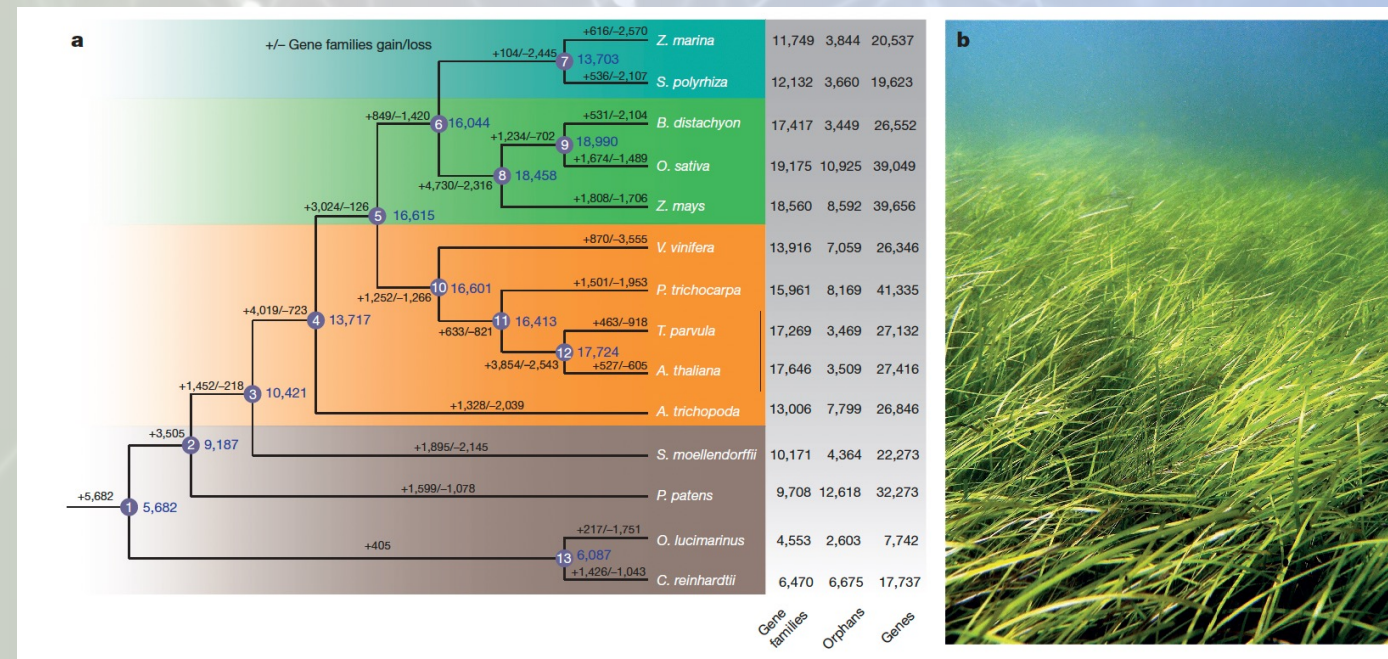
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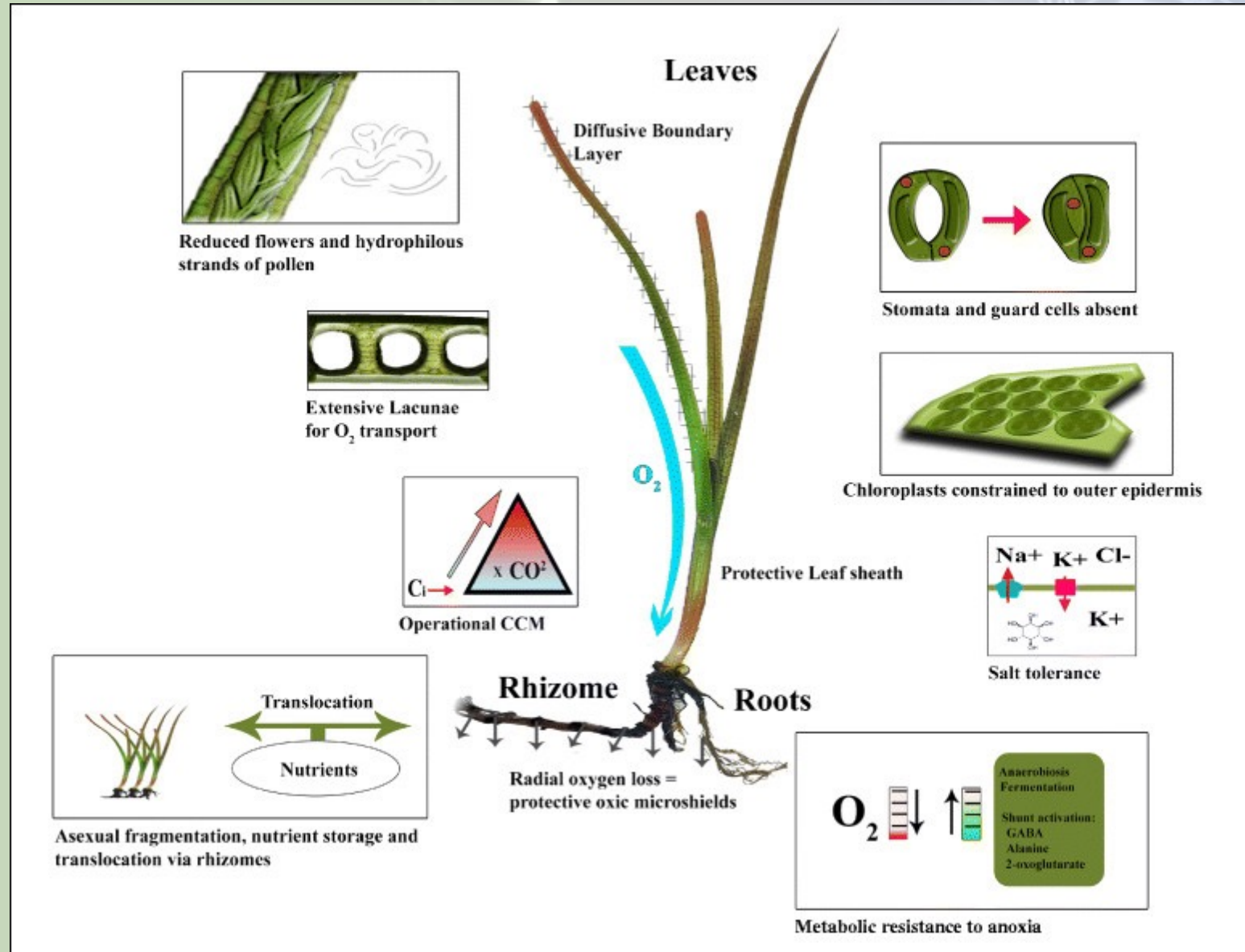
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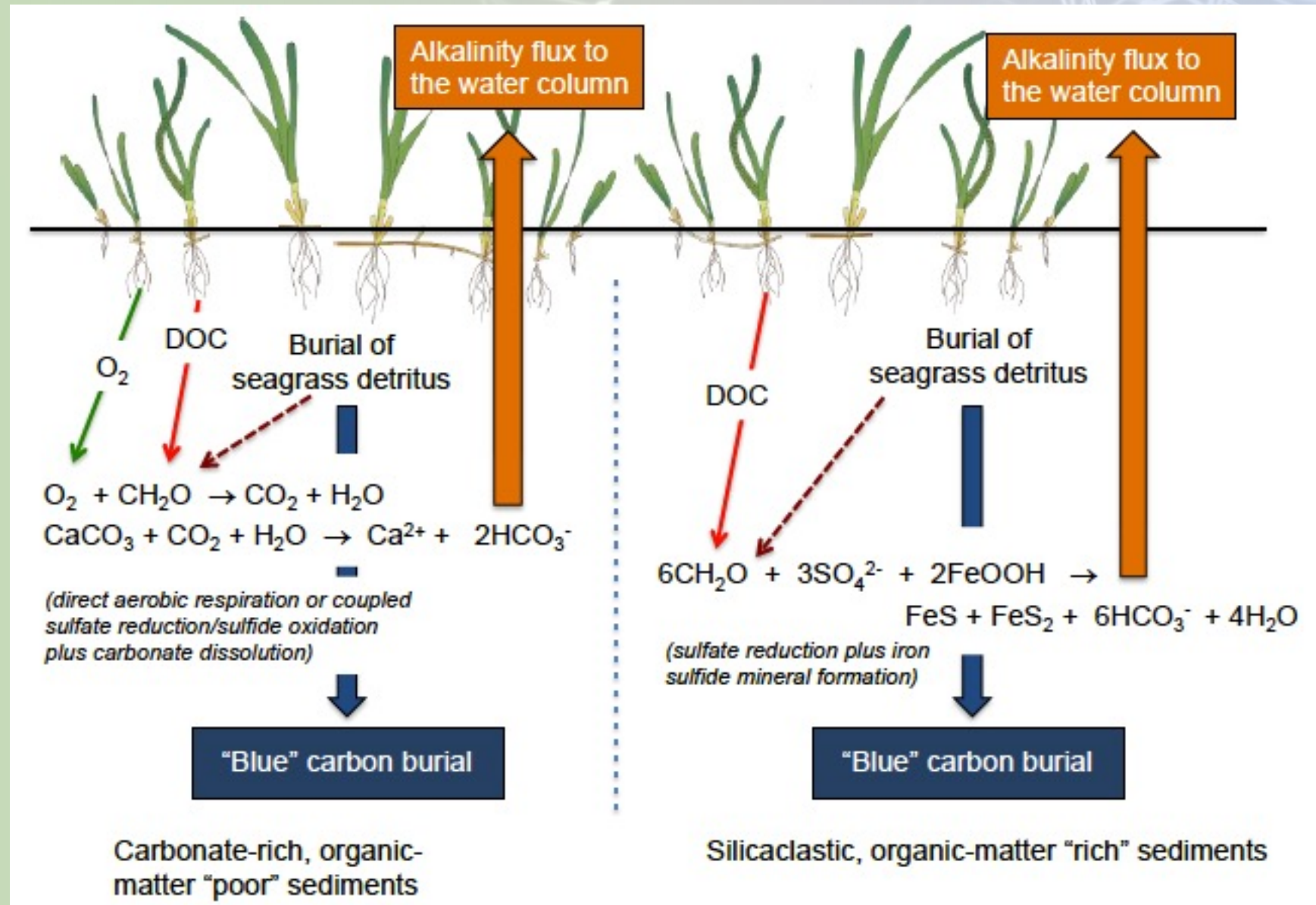
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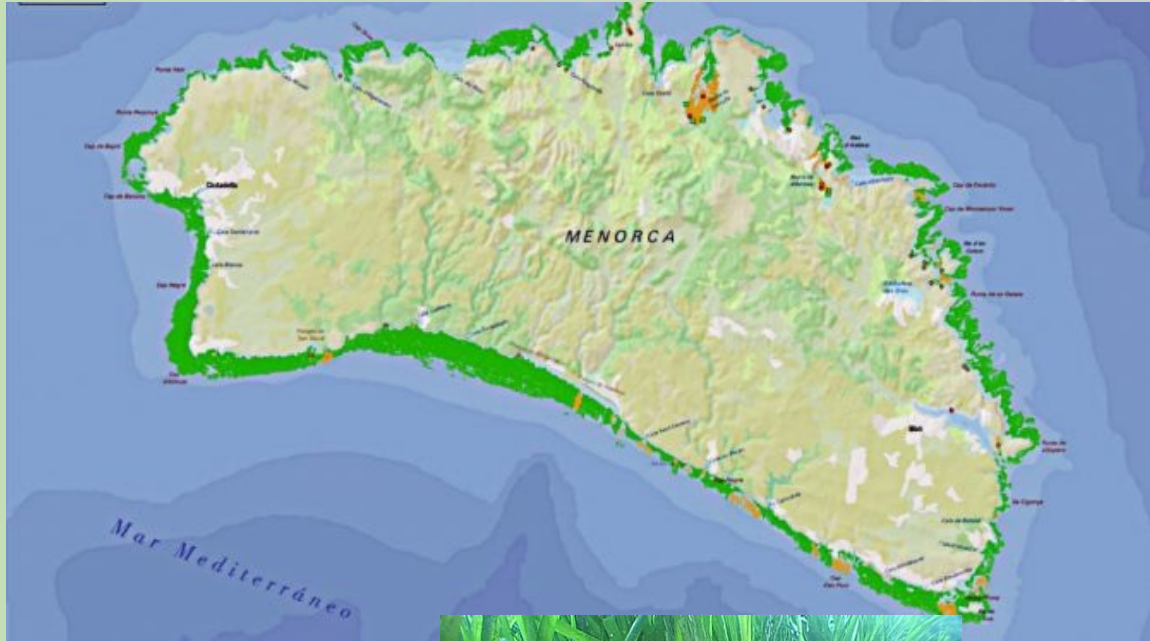
The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea

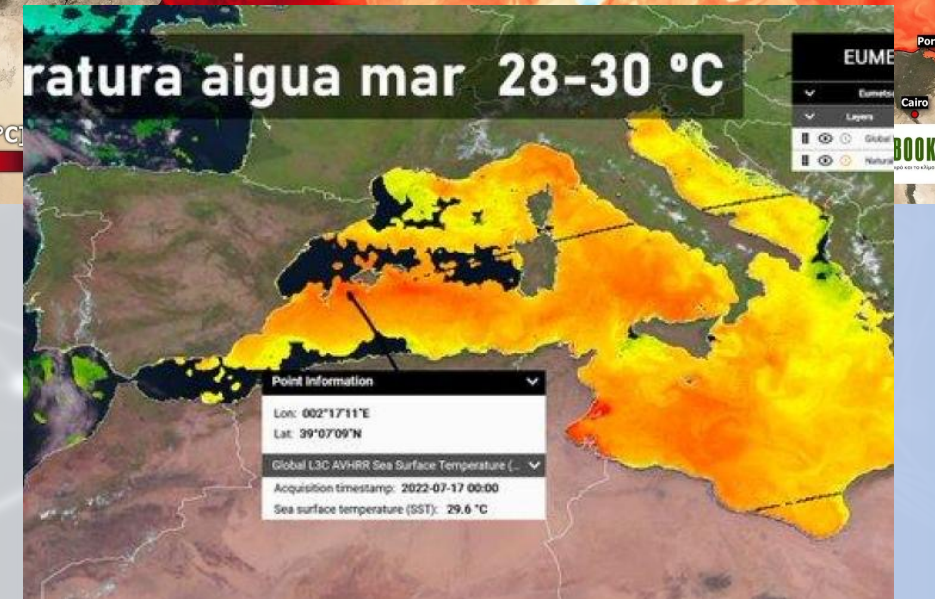
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WHY?



Posidonia oceanica massive death 2022







The lack of stomata makes these plants' leaf temperature prone to water temperature.

Increase temperature reduces CO₂ dissolution in seawater, reducing its availability for photosynthesis.





The trajectory in catalytic evolution of Rubisco in *Posidonia* seagrass species differs from terrestrial plants

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C.I. and J.G. conceived the study. S.C.-B. and S.W. measured the Rubisco biochemistry and S.C.-B. carried out the rest of experiments, analyzed the data, and produced the figures with help from all authors. T.R. performed the rbcL sequencing and O.S. collected the Australian *Posidonia* species. S.C.-B. drafted the manuscript with editing contributions from all authors.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (<https://academic.oup.com/plphys/pages/general-instructions>) is: Sebastià Capó-Bauçà (sebastia.capo@uib.cat).

Abstract

The CO₂-fixing enzyme Ribulose biphosphate carboxylase-oxygenase (Rubisco) links the inorganic and organic phases of the global carbon cycle. In aquatic systems, the catalytic adaptation of algae Rubiscos has been more expansive and followed an evolutionary pathway that appears distinct to terrestrial plant Rubisco. Here, we extend this survey to differing seagrass species of the genus *Posidonia* to reveal how their disjunctive geographical distribution and diverged phylogeny, along with their CO₂ concentrating mechanisms (CCMs) effectiveness, have impacted their Rubisco kinetic properties. The Rubisco from *Posidonia* species showed lower carboxylation efficiencies and lower sensitivity to O₂ inhibition than those measured for terrestrial C₃ and C₄-plant Rubiscos. Compared with the Australian *Posidonia* species, Rubisco from the Mediterranean *Posidonia oceanica* had 1.5–2-fold lower carboxylation and oxygenation efficiencies, coinciding with effective CCMs and five Rubisco large subunit amino acid substitutions. Among the Australian *Posidonia* species, CCM effectiveness was higher in *Posidonia sinuosa* and lower in the deep-living *Posidonia angustifolia*, likely related to the 20%–35% lower Rubisco carboxylation efficiency in *P. sinuosa* and the two-fold higher Rubisco content in *P. angustifolia*. Our results suggest that the catalytic evolution of *Posidonia* Rubisco has been impacted by the low CO₂ availability and gas exchange properties of marine environments, but with contrasting Rubisco kinetics according to the time of diversification among the species. As a result, the relationships between maximum carboxylation rate and CO₂- and O₂-affinities of *Posidonia* Rubiscos follow an alternative path to that characteristic of terrestrial angiosperm Rubiscos.

The genus *Posidonia* is one of the most relevant genera among seagrasses and contains relevant species in terms of biomass and carbon sequestration capacity (Duarte and Chiscano, 1999), being distributed in **seven species along the Australian coastlines and a single *Posidonia oceanica* species endemic to the Mediterranean Sea** (Campey et al., 2000).

This **disjunct species distribution** suggests there may have been a **loss of *Posidonia* species from those that originally connected the Mediterranean and Australian populations during Pangea** (Larkum et al., 2006).

Within these **phylogenetically separate clades of *Posidonia* species** it is unclear the extent to which **growth habitat differences** within and between both geographical locations have **impacted the adaptive evolution of their CCM and Rubisco**.

Here, we **examine the ecophysiology and Rubisco biochemistry at 25 °C among the Mediterranean *P. oceanica* and four distinct lineages of *Posidonia* species from the seven morphologically described species found in Australia** (Aires et al., 2011).

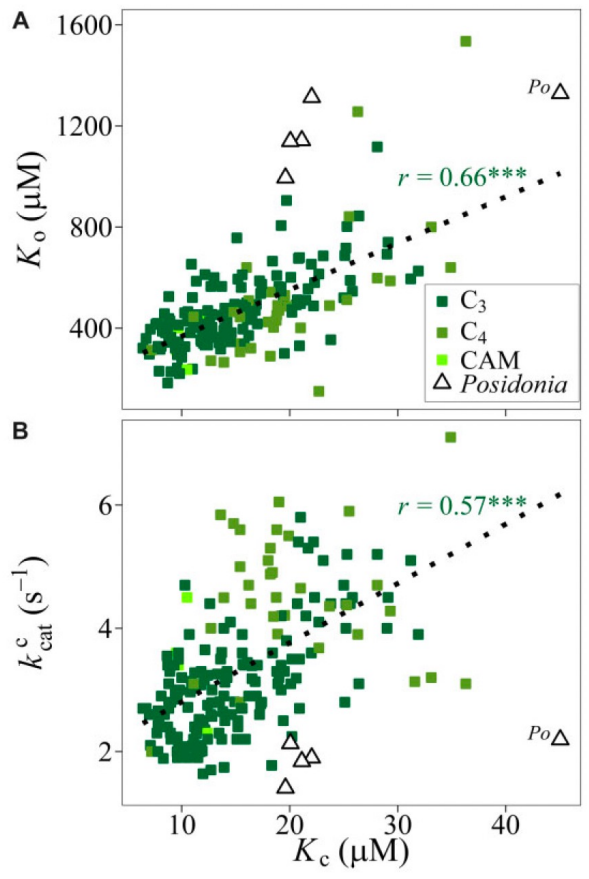


Figure 1 Diversity in the catalytic properties of *Posidonia* Rubisco relative to C₃, C₄, and CAM plants. A, Correlation between the Michaelis–Menten constant for CO₂ (K_c) and the Michaelis–Menten constant for O₂ (K_o); (B) and between K_c and the maximum carboxylation rate (k_{cat}^c). Empty triangles are the species of *Posidonia* measured in this study ($n = 5$) and *Po* indicates the Mediterranean endemic *P. oceanica*. Light and dark green squares belongs to terrestrial plants compiled by [Iñiguez et al. \(2020\)](#) ($n = 178$ in A and 203 in B). r is the reported Pearson’s regression coefficient of terrestrial plants and asterisks show the significance of the correlation test (*** $P < 0.001$).

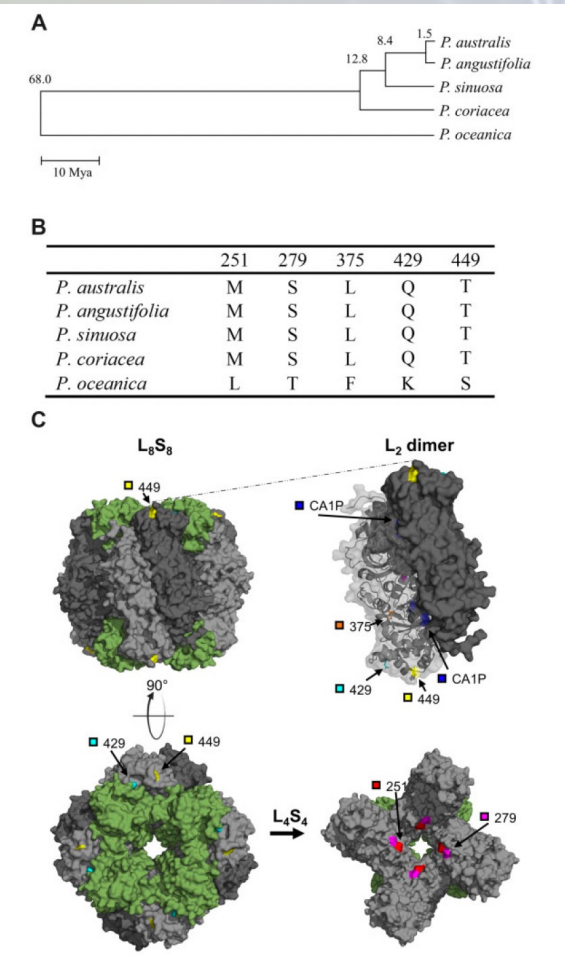
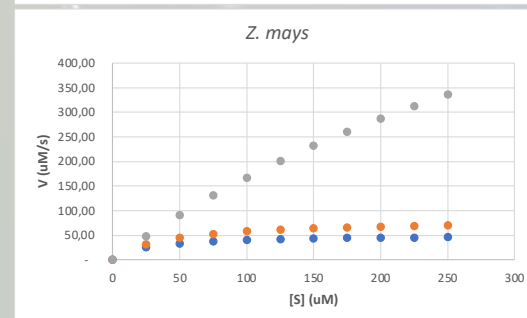
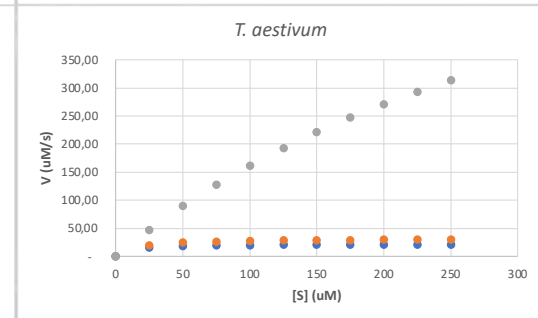
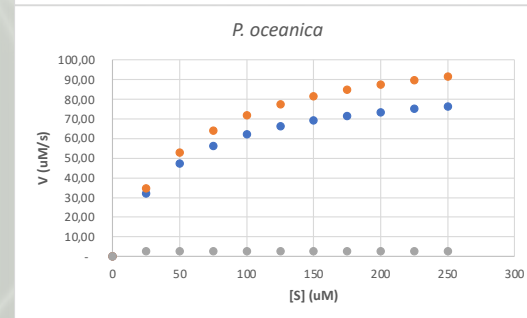
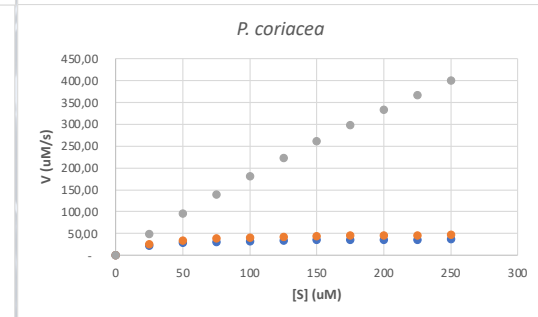
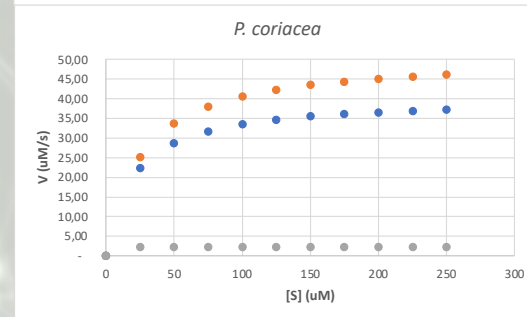
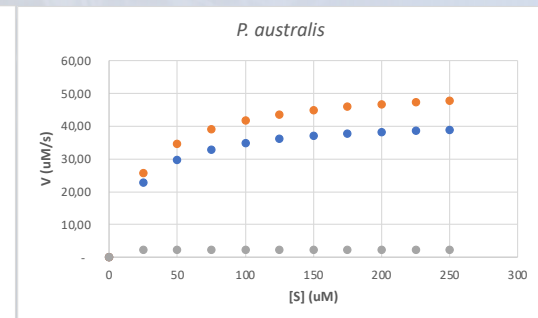
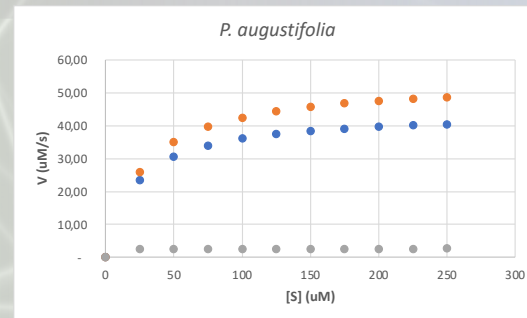


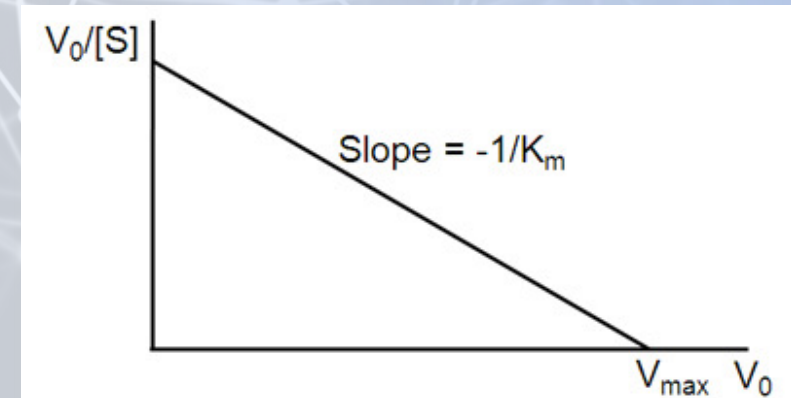
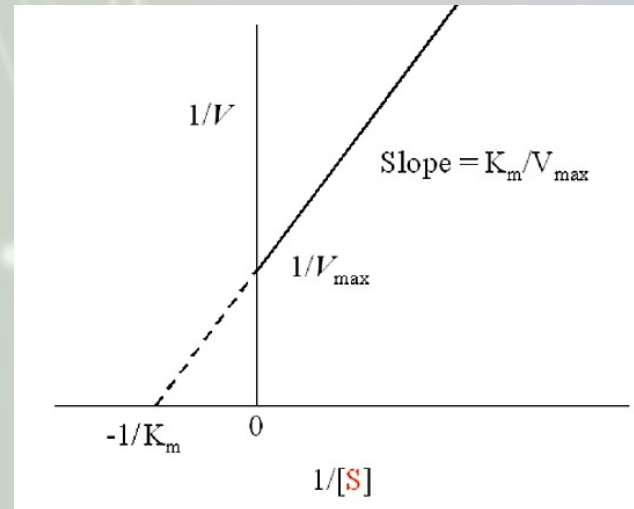
Figure 2 Phylogenetic and Rubisco molecular diversity across *Posidonia* species. A, Phylogenetic tree of *Posidonia* species analyzed in the study abbreviated from that derived by [Aires et al. \(2011\)](#) from mapping the rRNA-internal transcribed spacer (ITS) region; numbers above branches are the divergence dates (in Mya) estimated on the maximum-likelihood topology derived from the ITS data set; B, amino acid changes among *rbcl* of *Posidonia* species; C, location of the variable amino acid positions of *P. oceanica* Rbcl mapped onto the crystal structure of spinach Rubisco (8RUC) using Pymol V1.8.x. Residues 251, 279, 375, 429, and 449, and the inhibitor CA1P are highlighted.

Rubisco Kinetic Parameters

[S] μM	<i>P. augustifolia</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
	v (Carboxylase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	23,40	22,89	22,28	21,97	32,14	15,56	25,15
50	30,56	29,68	28,67	28,16	47,37	18,43	33,60
75	34,02	32,93	31,70	31,08	56,25	19,64	37,84
100	36,07	34,85	33,47	32,78	62,07	20,31	40,38
125	37,41	36,11	34,63	33,89	66,18	20,73	42,08
150	38,37	37,00	35,45	34,67	69,23	21,02	43,30
175	39,09	37,66	36,06	35,25	71,59	21,23	44,21
200	39,64	38,17	36,53	35,70	73,47	21,39	44,92
225	40,08	38,58	36,90	36,06	75,00	21,52	45,49
250	40,44	38,92	37,21	36,35	76,27	21,62	45,95
	v (Carboxylase+Oxygenase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	25,96	25,68	25,20	25,35	34,57	19,59	31,03
50	35,06	34,55	33,69	33,95	52,83	24,36	44,99
75	39,71	39,05	37,95	38,28	64,12	26,51	52,93
100	42,52	41,77	40,51	40,89	71,79	27,73	58,06
125	44,41	43,59	42,22	42,63	77,35	28,53	61,63
150	45,76	44,90	43,44	43,88	81,55	29,08	64,27
175	46,78	45,88	44,36	44,82	84,85	29,49	66,30
200	47,58	46,64	45,08	45,55	87,50	29,80	67,91
225	48,21	47,26	45,65	46,13	89,68	30,05	69,22
250	48,74	47,76	46,11	46,61	91,50	30,25	70,30
	v (Oxygenase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	2,49	2,18	2,18	48,77	2,52	47,20	47,67
50	2,56	2,23	2,23	95,22	2,59	89,41	91,10
75	2,58	2,25	2,24	139,49	2,61	127,36	130,83
100	2,59	2,26	2,25	181,74	2,62	161,69	167,32
125	2,60	2,26	2,26	222,10	2,63	192,87	200,94
150	2,60	2,27	2,26	260,70	2,63	221,33	232,02
175	2,61	2,27	2,26	297,65	2,64	247,40	260,84
200	2,61	2,27	2,27	333,05	2,64	271,38	287,64
225	2,61	2,27	2,27	367,01	2,64	293,51	312,62
250	2,61	2,27	2,27	399,60	2,64	313,99	335,96

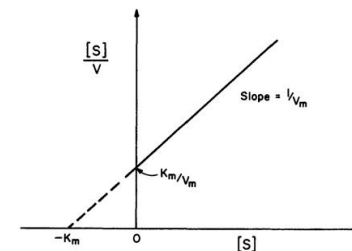


[S] μM	<i>P. augustifolia</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
	v (Carboxylase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	23,40	22,89	22,28	21,97	32,14	15,56	25,15
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75	34,02	32,93	31,70	31,08	56,25	19,64	37,84
100	36,07	34,85	33,47	32,78	62,07	20,31	40,38
125	37,41	36,11	34,63	33,89	66,18	20,73	42,08
150	38,37	37,00	35,45	34,67	69,23	21,02	43,30
175	39,09	37,66	36,06	35,25	71,59	21,23	44,21
200	39,64	38,17	36,53	35,70	73,47	21,39	44,92
225	40,08	38,58	36,90	36,06	75,00	21,52	45,49
250	40,44	38,92	37,21	36,35	76,27	21,62	45,95
	v (Carboxylase+Oxygenase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	25,96	25,68	25,20	25,35	34,57	19,59	31,03
50	35,06	34,55	33,69	33,95	52,83	24,36	44,99
75	39,71	39,05	37,95	38,28	64,12	26,51	52,93
100	42,52	41,77	40,51	40,89	71,79	27,73	58,06
125	44,41	43,59	42,22	42,63	77,35	28,53	61,63
150	45,76	44,90	43,44	43,88	81,55	29,08	64,27
175	46,78	45,88	44,36	44,82	84,85	29,49	66,30
200	47,58	46,64	45,08	45,55	87,50	29,80	67,91
225	48,21	47,26	45,65	46,13	89,68	30,05	69,22
250	48,74	47,76	46,11	46,61	91,50	30,25	70,30
	v (Oxygenase) $\mu\text{M/s}$						
0	-	-	-	-	-	-	-
25	2,49	2,18	2,18	48,77	2,52	47,20	47,67
50	2,56	2,23	2,23	95,22	2,59	89,41	91,10
75	2,58	2,25	2,24	139,49	2,61	127,36	130,83
100	2,59	2,26	2,25	181,74	2,62	161,69	167,32
125	2,60	2,26	2,26	222,10	2,63	192,87	200,94
150	2,60	2,27	2,26	260,70	2,63	221,33	232,02
175	2,61	2,27	2,26	297,65	2,64	247,40	260,84
200	2,61	2,27	2,27	333,05	2,64	271,38	287,64
225	2,61	2,27	2,27	367,01	2,64	293,51	312,62
250	2,61	2,27	2,27	399,60	2,64	313,99	335,96

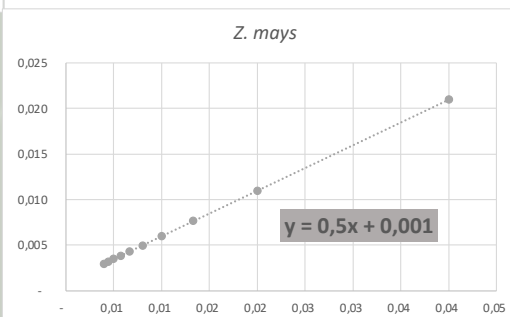
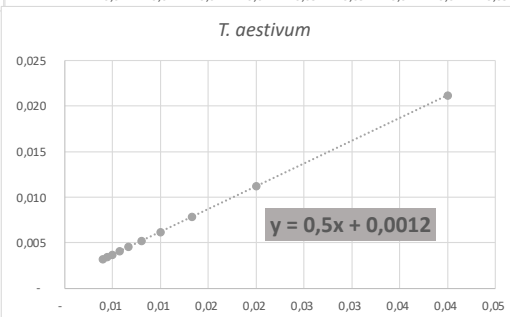
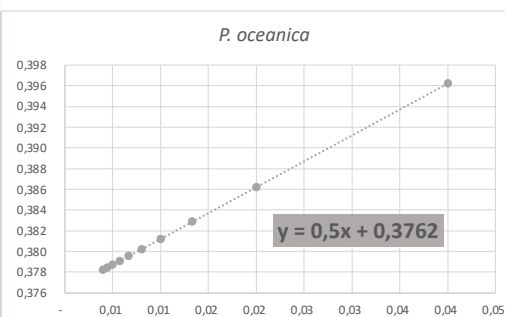
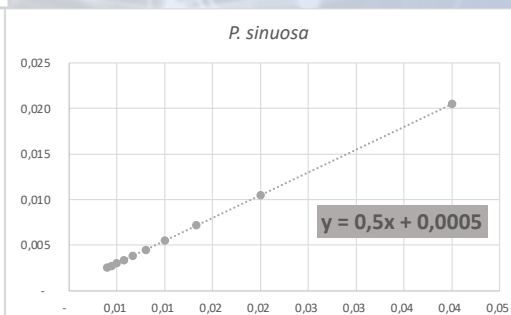
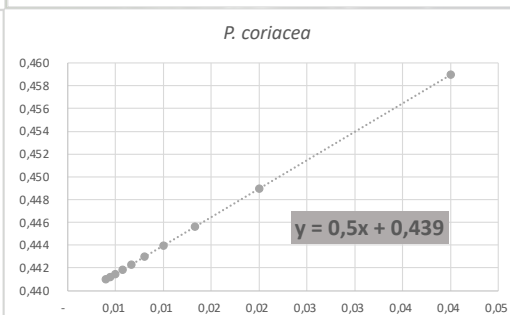
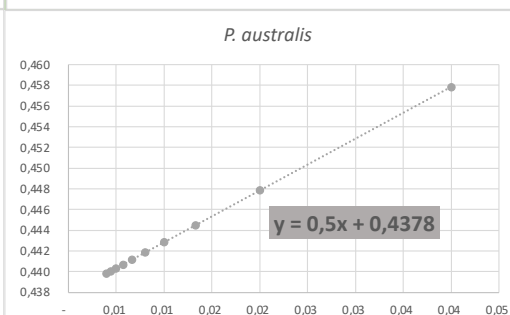
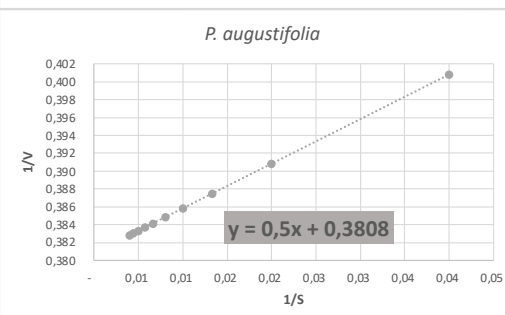
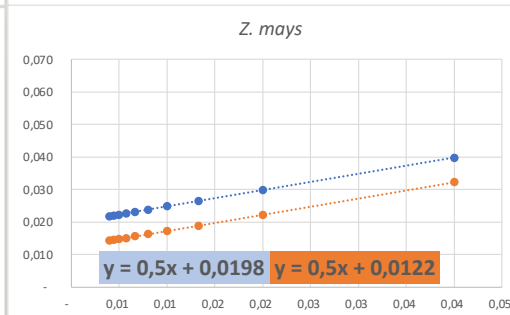
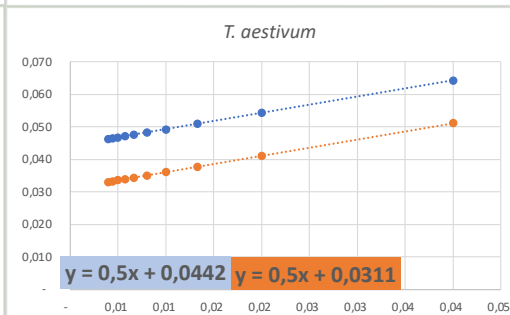
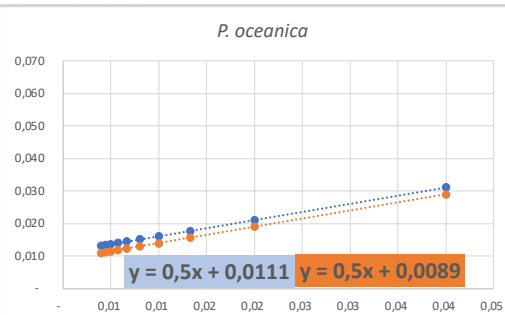
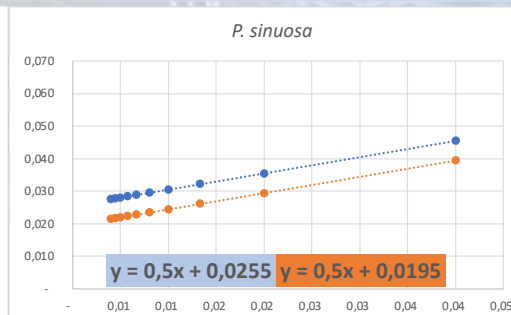
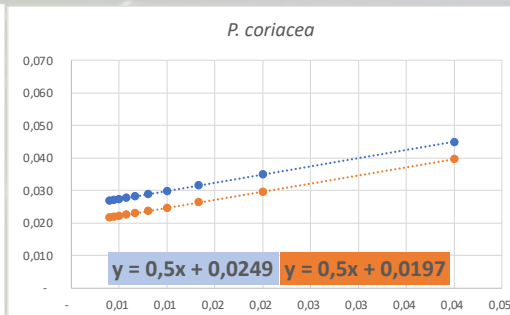
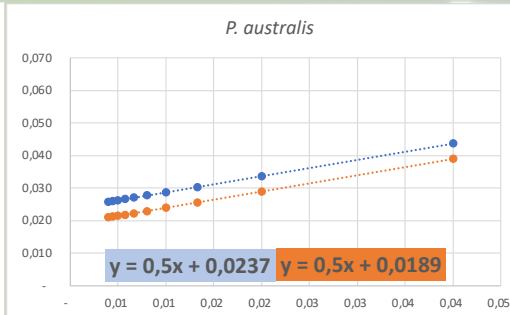
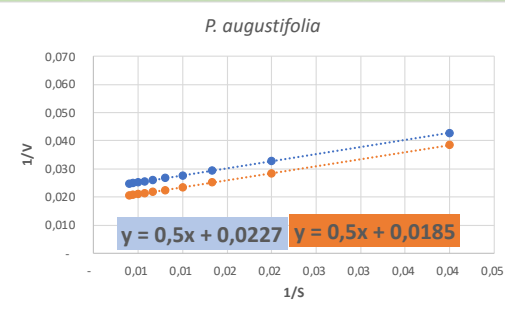


Hanes-Woolf (Langmuir) Plot

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m}[S]$$

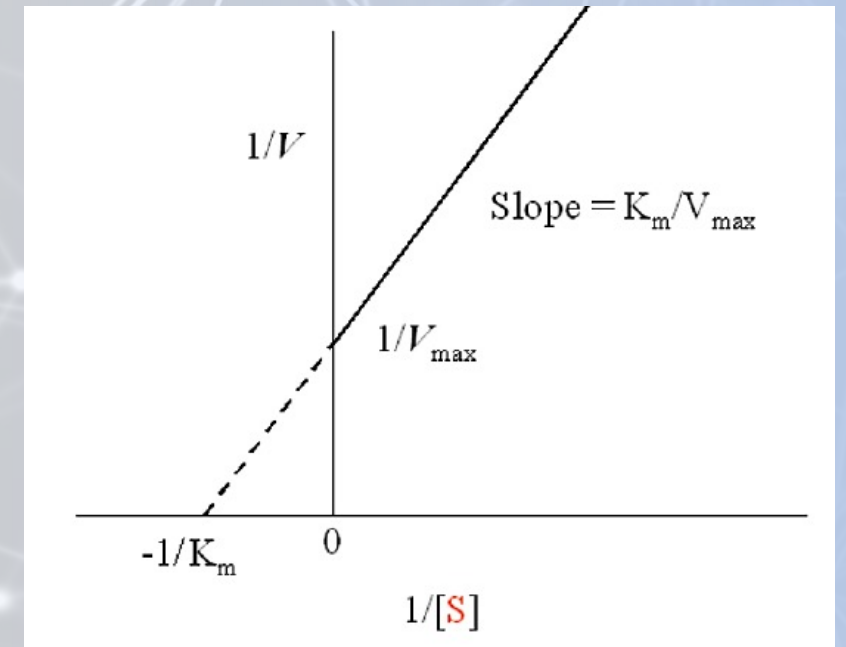


Rubisco Kinetic Parameters - Lineweaver-Burke

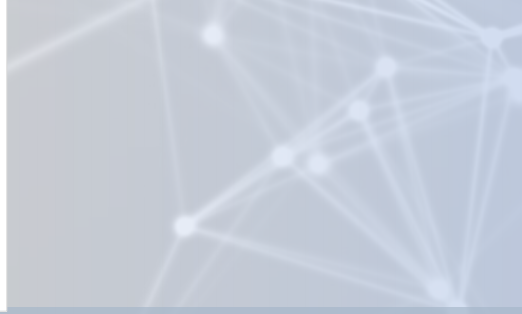
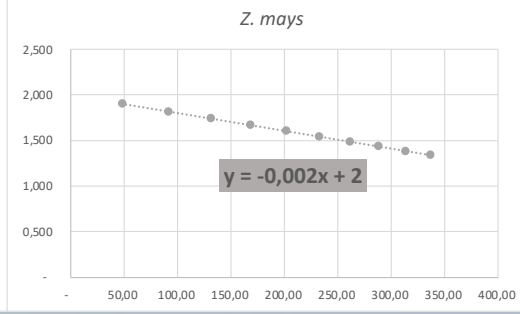
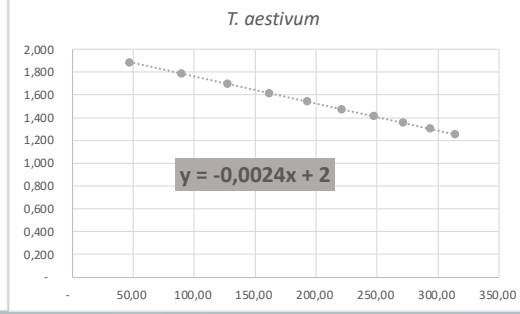
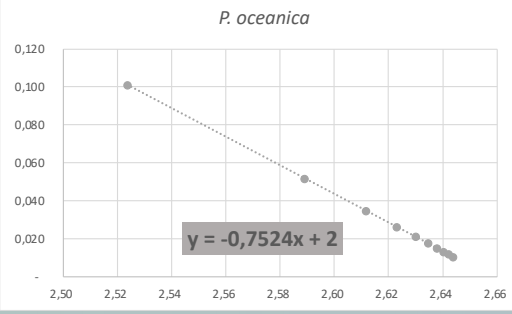
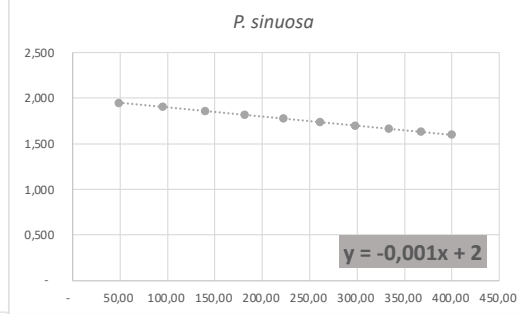
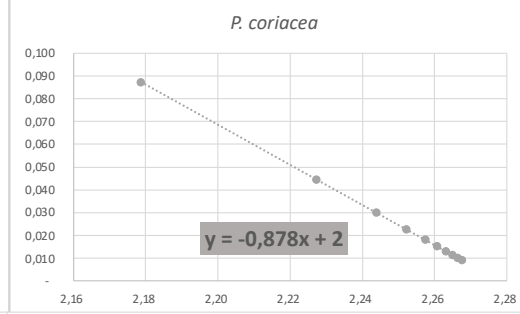
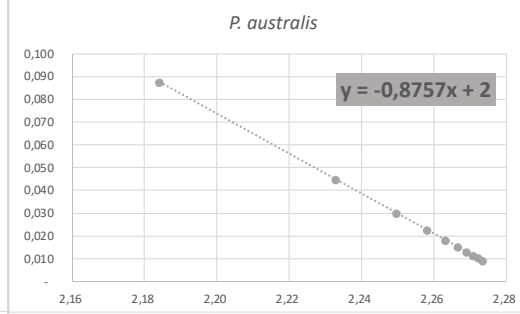
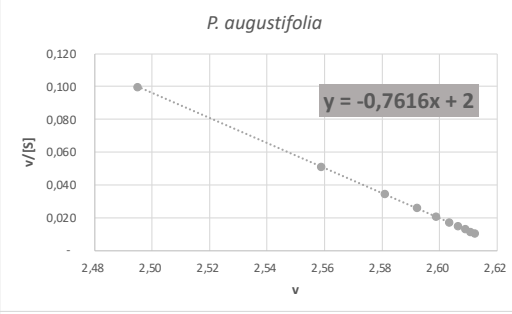
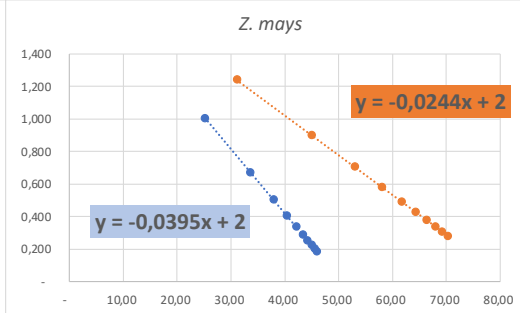
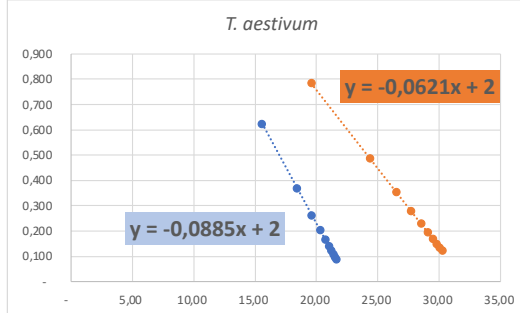
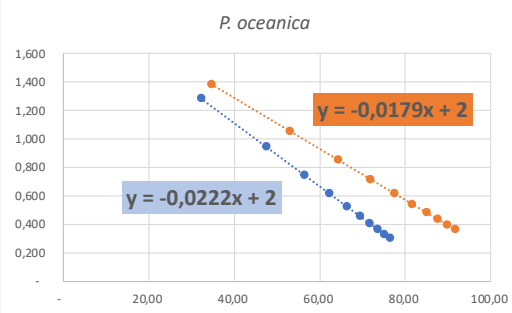
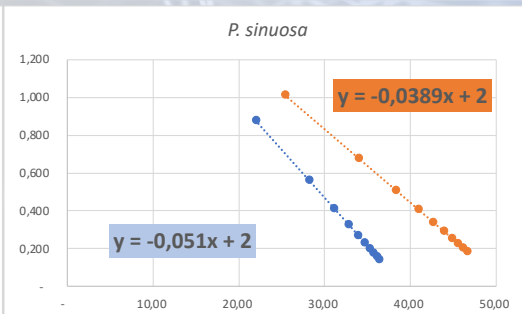
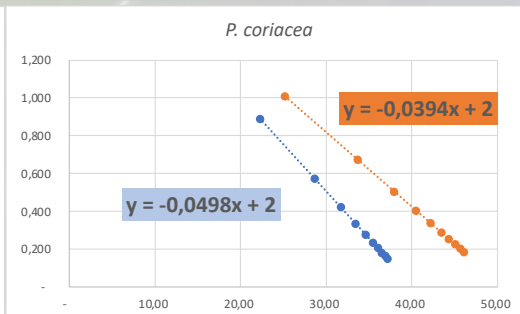
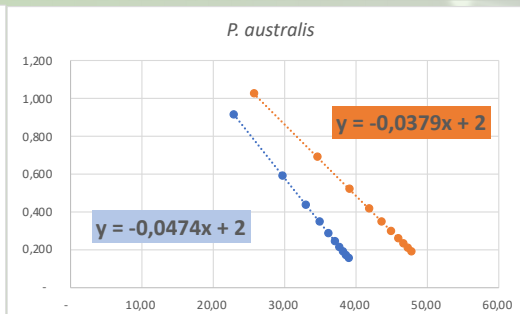
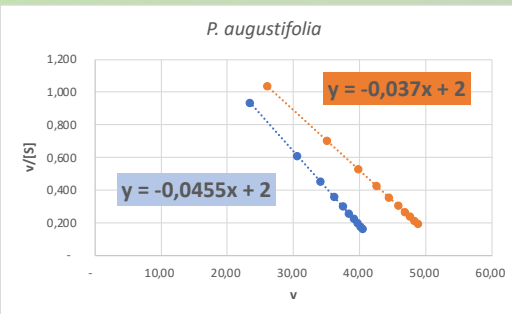


Rubisco Kinetic Parameters - Lineweaver-Burke

	<i>P. augustifol.</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
Km (c)	22,03	21,10	20,08	19,61	45,05	11,31	25,25
Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
Vmax (c)	44,05	42,19	40,16	39,22	90,09	22,62	50,51
Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00

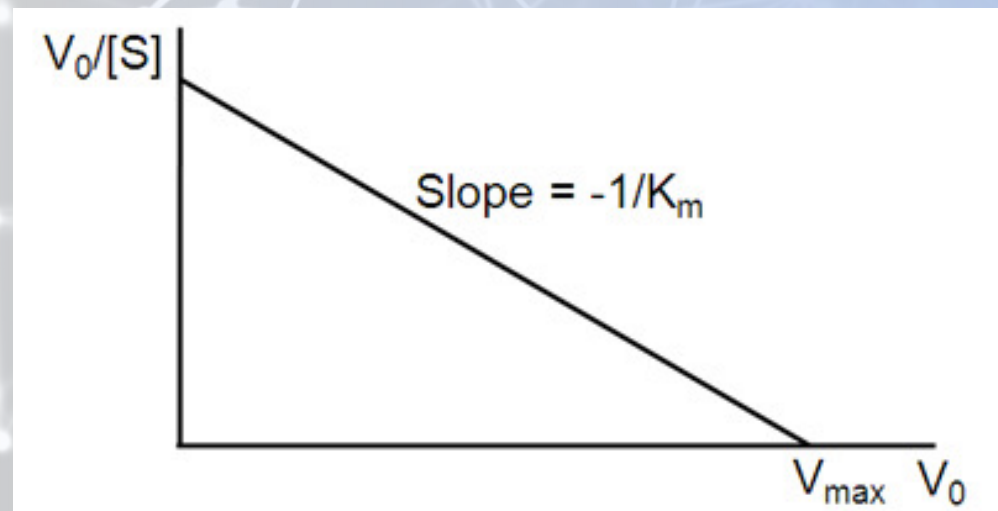


Rubisco Kinetic Parameters - Eadie-Hofstee

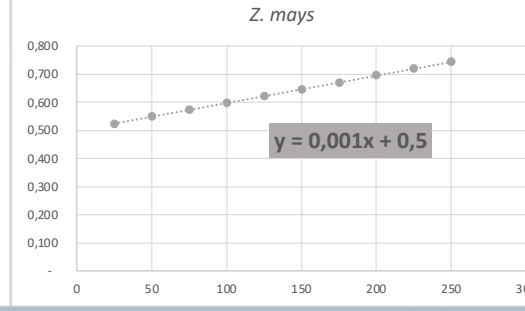
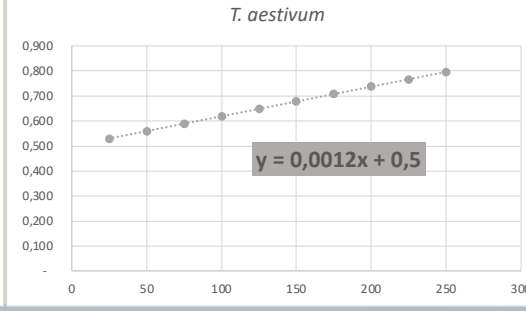
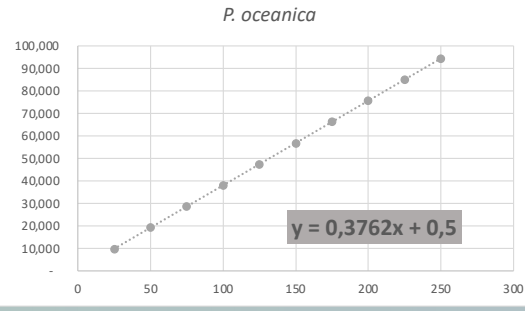
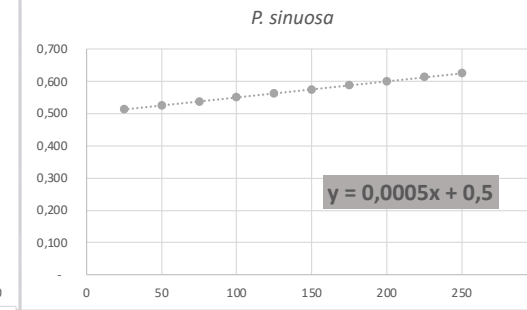
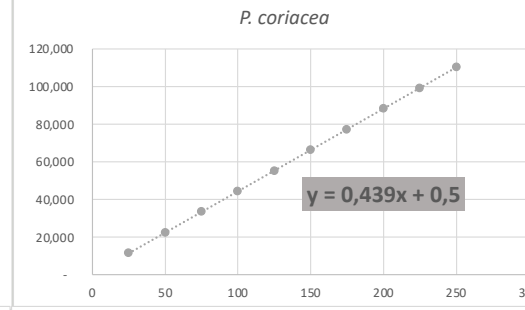
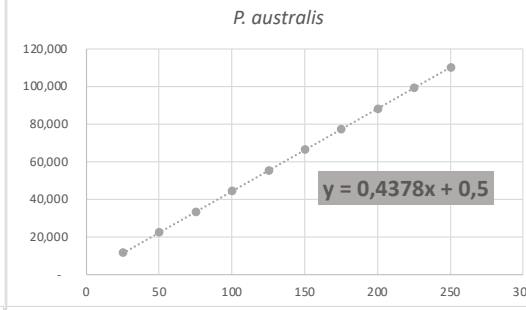
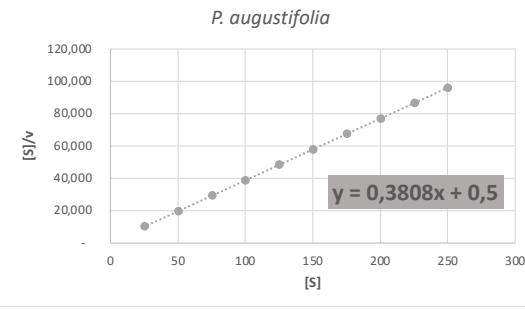
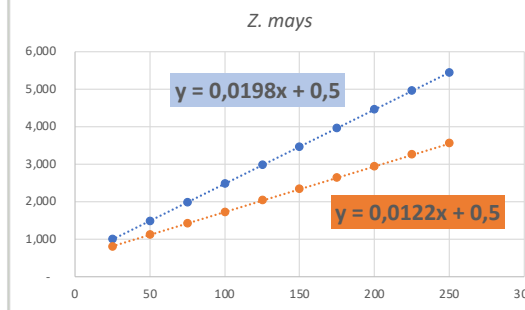
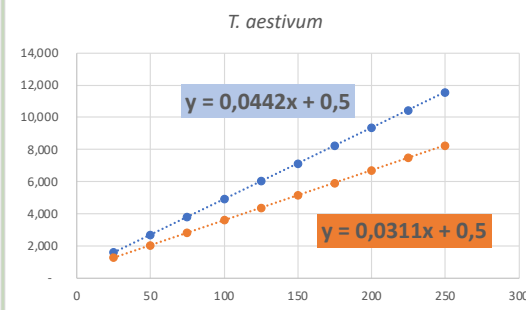
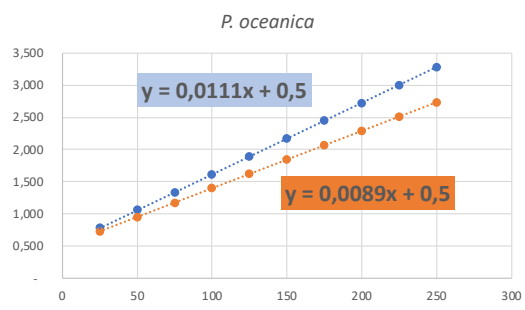
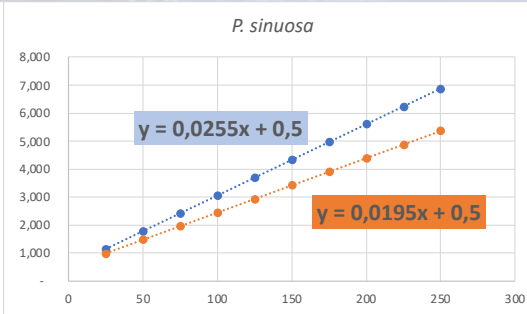
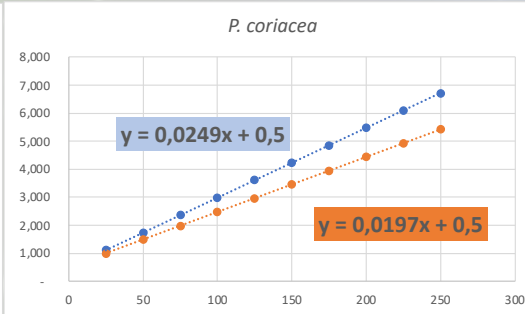
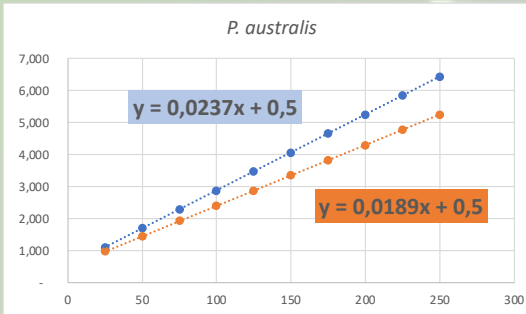
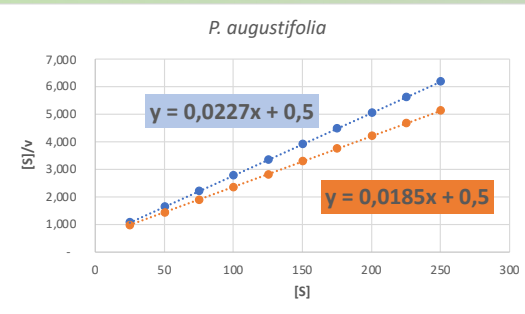


Rubisco Kinetic Parameters - Eadie-Hofstee

	<i>P. augustifol.</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
Km (c)	21,98	21,10	20,08	19,61	45,05	11,30	25,32
Km (c+o)	27,03	26,39	25,38	25,71	55,87	16,10	40,98
Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
Vmax (c)	43,96	42,19	40,16	39,22	90,09	22,60	50,63
Vmax (c+o)	54,05	52,77	50,76	51,41	111,73	32,21	81,97
Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00



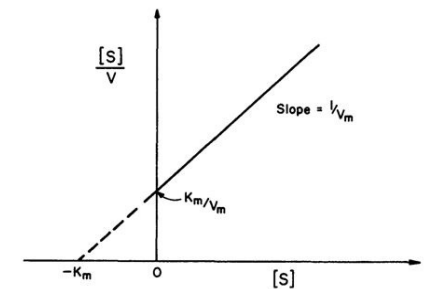
Rubisco Kinetic Parameters - Hanes-Woolf



	<i>P. augustifol.</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
Km (c)	22,03	21,10	20,08	22,22	45,05	11,31	25,25
Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
Vmax (c)	44,05	42,19	40,16	44,44	90,09	22,62	50,51
Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00

Hanes-Woolf (Langmuir) Plot

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m} [S]$$



Rubisco Kinetic Parameters - Hanes-Woolf

		<i>P. augustifol.</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
MM	Km (c)	22,00	21,10	20,10	19,60	45,00	11,30	25,30
	Km (c+o)	27,00	26,40	25,40	25,70	56,00	16,10	40,90
	Km (o)	1.31	1.14	1.14	995.00	1.33	422.00	512.00
	Vmax (c)	44,00	42,20	40,20	39,20	90,00	22,60	50,60
	Vmax (c+o)	54,00	52,80	50,80	51,40	112,00	32,20	81,80
	Vmax (o)	2,63	2,28	2,28	1 990,00	2,66	844,00	1 024,00
LB	Km (c)	22,03	21,10	20,08	19,61	45,05	11,31	25,25
	Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
	Km (o)	1.31	1.14	1.14	1 000.00	1.33	416.67	500.00
	Vmax (c)	44,05	42,19	40,16	39,22	90,09	22,62	50,51
	Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00
EH	Km (c)	21,98	21,10	20,08	19,61	45,05	11,30	25,32
	Km (c+o)	27,03	26,39	25,38	25,71	55,87	16,10	40,98
	Km (o)	1.31	1.14	1.14	1 000.00	1.33	416.67	500.00
	Vmax (c)	43,96	42,19	40,16	39,22	90,09	22,60	50,63
	Vmax (c+o)	54,05	52,77	50,76	51,41	111,73	32,21	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00
HW	Km (c)	22,03	21,10	20,08	22,22	45,05	11,31	25,25
	Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
	Km (o)	1.31	1.14	1.14	1 000.00	1.33	416.67	500.00
	Vmax (c)	44,05	42,19	40,16	44,44	90,09	22,62	50,51
	Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00

Lower Km(carboxylase) than Z. mays (C4) -> Higher affinity to CO2.

Higher Vmax than C3 and lower than C4.

Rubisco Kinetic Parameters - Hanes-Woolf

		<i>P. augustifol</i>	<i>P. australis</i>	<i>P. coriacea</i>	<i>P. sinuosa</i>	<i>P. oceanica</i>	<i>T. aestivum</i>	<i>Z. mays</i>
MM	Km (c)	22,00	21,10	20,10	19,60	45,00	11,30	25,30
	Km (c+o)	27,00	26,40	25,40	25,70	56,00	16,10	40,90
	Km (o)	1,31	1,14	1,14	995,00	1,33	422,00	512,00
	Vmax (c)	44,00	42,20	40,20	39,20	90,00	22,60	50,60
	Vmax (c+o)	54,00	52,80	50,80	51,40	112,00	32,20	81,80
	Vmax (o)	2,63	2,28	2,28	1 990,00	2,66	844,00	1 024,00
LB	Km (c)	22,03	21,10	20,08	19,61	45,05	11,31	25,25
	Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
	Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
	Vmax (c)	44,05	42,19	40,16	39,22	90,09	22,62	50,51
	Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00
EH	Km (c)	21,98	21,10	20,08	19,61	45,05	11,30	25,32
	Km (c+o)	27,03	26,39	25,38	25,71	55,87	16,10	40,98
	Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
	Vmax (c)	43,96	42,19	40,16	39,22	90,09	22,60	50,63
	Vmax (c+o)	54,05	52,77	50,76	51,41	111,73	32,21	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00
HW	Km (c)	22,03	21,10	20,08	22,22	45,05	11,31	25,25
	Km (c+o)	27,03	26,46	25,38	25,64	56,18	16,08	40,98
	Km (o)	1,31	1,14	1,14	1 000,00	1,33	416,67	500,00
	Vmax (c)	44,05	42,19	40,16	44,44	90,09	22,62	50,51
	Vmax (c+o)	54,05	52,91	50,76	51,28	112,36	32,15	81,97
	Vmax (o)	2,63	2,28	2,28	2 000,00	2,66	833,33	1 000,00

Lower Km(oxygenase) than *Z. mays* (C4) -> Higher affinity to O₂.

Very low Vmax compared to C3 or C4.

This study revealed differences in the kinetic evolution of Rubisco among *Posidonia* species consistent with their disjunct geographic distribution and phylogenetic divergence, leading to five unique amino acid substitutions in the RbcL of *P. oceanica* that possibly account for its two-fold poorer Rubisco CO₂-affinity.

Among Australian *Posidonia* species, we can attribute some of the Rubisco kinetics and quantity variation with differences in their CCM efficiency, as the 40% slower k_{cat} and 20%–35% lower carboxylation efficiency in *P. sinuosa* or the two-fold higher Rubisco content in the deep-living *P. angustifolia*.

Nevertheless, the distinctly slower k_{cat} , lower CO₂ and O₂ affinities, and lower carboxylation efficiency shared by *Posidonia* Rubiscos compared with those of their distant terrestrial angiosperm relatives show how the enzyme in these seagrasses have clearly followed an alternative pathway in their kinetic evolution.

However, further refined experiments are needed to accurately map evidence of correlations in the adaptive evolution of *Posidonia* Rubisco kinetics and their photosynthetic adaptation. Such analyses necessitate an assessment of the contrasting temperature, nutrient, CO₂ concentration, and irradiance characteristics of the habitats of each species, as well as an appreciation of their adaptive Rubisco temperature kinetic response and intracellular O₂ concentration.

