

Supplementary material

Mate perception and gene networks regulating the early phase of sex in *Pseudo-nitzschia multistriata*

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Table S1. List of the strains used. MT, strain name, isolation date, isolation site and experiments (figure in the paper) in which they were employed are indicated.

Mating Type	Strain name	Isolation date	Isolation site	Experiment
MT-	1334-1	21/06/2019	LTER-MC	Supp. Fig. 2, Fig. 2a-e, Fig. 2f, Fig. 3a-c
	1334-3	21/06/2019	LTER-MC	Fig. 3b,c
	OGS - PN 62	9/12/2019	Adriatic Sea	Fig.2f
	MF1	04/06/2020	Laboratory cross between 1334-1 and 1334-5	Supp. Fig. 2, Fig. 3d, Fig. 4
	1365-17	14/7/2020	LTER-MC	Supp. Fig. 2
	MF9	25/11/2020	Laboratory cross between 1364-3 x 1365-16	Fig. 3d, Fig. 4
MT+	1334-5	21/06/2019	LTER-MC	Supp. Fig. 2, Fig. 2a-e, Fig. 2f, Fig. 3a-c
	1343-43	10/9/2019	LTER-MC	Fig. 3d
	1364-9	7/7/2020	LTER-MC	Supp. Fig. 2, Fig. 3d
	1365-16	14/7/2020	LTER-MC	Supp. Fig. 2

Table S2. List of the primers used.

Gene	Primer Name	Primer sequence (5'-3')	Application
MRP1	13283EcoF 13283EcoR	ggcggccgaattcatgatgacctcaacttctt ggcggccgaattcgttgatcatgttctttaccg	PmH4pMRP1-YFPAt construction
MRP1 GFP	MRP1fw1 GFP_down	gtatggcgctcaccacttc aactccagcaggaccatgtg	Transformants screening
7488	7488 Fw 7488 Rv	agcaaagccgacgatgcc aattcgtgcgattctccgttg	qPCR
Cathepsin D	Cathepsin Fw Cathepsin Rv	aagagcaagtacgacgcctc gagaccctgccaagtcctg	qPCR
MRP1	MRP1 Fw MRP1 Rv	gtatggcgctcaccacttc cgtcttcgactgcgtcttc	qPCR
Rad51A1	Rad51-A1 Fw Rad51-A1 Rv	catcggcggaacatcattg atcggtagcatcgcaaactc	qPCR
MRM2	46228 Fw 46228 Rv	ccaccgaactaggcaactgtc ggcacagaaccctcaac	qPCR
9830	9830 Fw 9830 Rv	tggactcaactcctgcatcg tcaaatgctacgtcggatgg	qPCR
77510	77510 Fw 77510 Rv	ccaagaccgctcattacgc actccgaccgtctttccg	qPCR
102250	102250 Fw 102250 Rv	atcgaccggaattggtgagg cgttctccgттаатgatttc	qPCR
36800	36800 Fw 36800 Rv	actcattatccaaccgcca cccgggtggcaaacagaacag	qPCR
14720	14720 Fw 14720 Rv	tattatcgggtcgctctggt cggaatcctcgttactcct	qPCR

31150	31150 Fw 31150 Rv	catcggcgacaaggtaaagg acttttccttgggcagcaag	qPCR
59280	59280 Fw 59280 Rv	tgaaggctttgacaaacggg gtcttcggtgaccaatccag	qPCR
29310	29310 Fw 29310 Rv	acgaggaagtgatcaaggca ggattcgttcaagggttcc	qPCR
67000	67000 Fw 67000 Rv	attgcgagctttcacaggg atccaaagctcaactgcac	qPCR

Table S3. Complete table of all the target genes with an FC >1,5 selected from the transcriptomic databases [27,28] to study mate perception in *P. multistriata*. For each of them the level of induction in sexualized and cross samples are indicated.

Gene name	Gene ID	Log2 Fold Change (Basu et al. 2017[27])				Log2 Fold Change (Annunziata et al. 2022[28])					
		MT+		MT-		Cross vs MT+			Cross vs MT-		
		2h	6h	2h	6h	1h	24h	5d	1h	24h	5d
Calmodulin	PSNMU-V1.4_AUG-EV-PASAV3_0102250.1	2,8	1,5	3,2	1,3	5,3	0,6	0,0	3,4	-1,3	-2,3
FAS1	PSNMU-V1.4_AUG-EV-PASAV3_0009830.1	4,6	1,3	1,6	0,9	2,9	0,7	0,0	1,2	-1,0	-2,0
Peptidase_M1 1	PSNMU-V1.4_AUG-EV-PASAV3_0036800.1	2,4	1,3	2,5	0,5	4,0	-1,6	-1,8	2,4	-3,2	-3,4
59280	PSNMU-V1.4_AUG-EV-PASAV3_0059280.1	2,1	2,4	1,8	1,5	3,6	1,0	1,2	2,3	0,4	0,6
77510	PSNMU-V1.4_AUG-EV-PASAV3_0077510.1	3,2	2,3	3,2	2,2	2,8	0,6	0,0	2,3	0,0	0,0
29310	PSNMU-V1.4_AUG-EV-PASAV3_0029310.1	1,7	1,2	1,6	0,4	2,3	-1,7	-2,0	2,4	-2,3	-2,7
14720	PSNMU-V1.4_AUG-EV-PASAV3_0014720.1	2,2	1,0	-0,3	-0,3	1,7	0,0	0,6	0,7	-0,8	0,0
31150	PSNMU-V1.4_AUG-EV-PASAV3_0031150.1	2,1	1,6	0,2	0,8	1,7	1,2	1,2	0,7	0,0	0,0
67000	PSNMU-V1.4_AUG-EV-PASAV3_0067000.1	1,5	0,7	1,4	0,5	1,7	-3,6	-3,2	0,9	-4,4	-4,0

-10 0 10

* The color pattern indicates Log2 Fold Change, ranging from -10 (red) to +10 (green).

Figure S1. Outputs of prediction software for transit peptides for the 7488 protein. **(a)** Output of the SignalP 3.0 software, which can predict the presence of a secretory signal peptide, a ubiquitous protein sorting signal that targets proteins for translocation across the endoplasmic reticulum (ER) membrane. **(b)** Output of the AsaFind software, a prediction tool that identifies proteins with a signal peptide for transport to diatom plastids. The 7488 protein contains a signal peptide, but it is not predicted to go to the plastid.

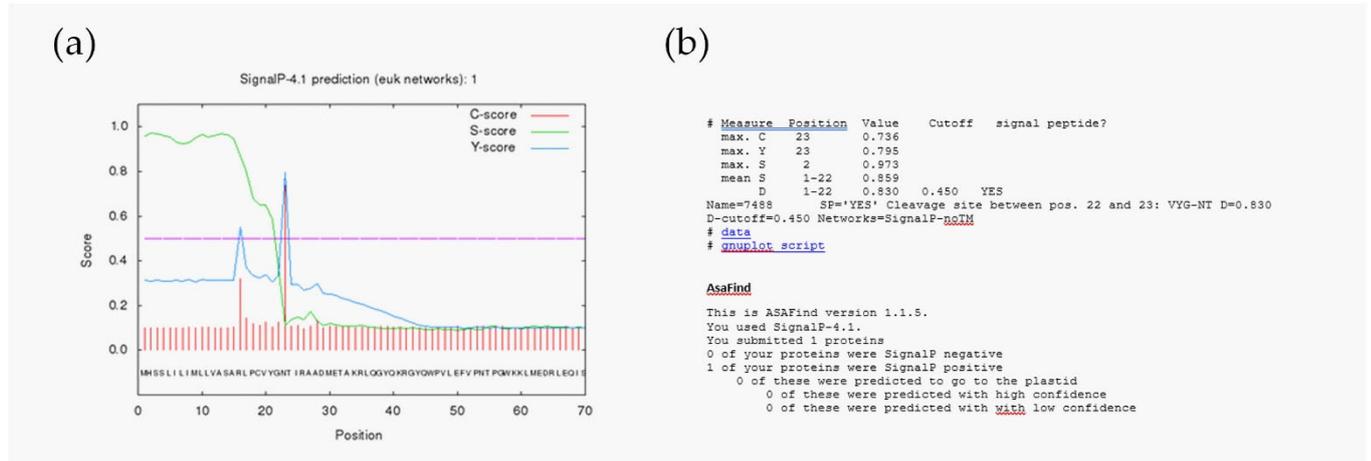


Figure S2. Identification of MT-specific sexualization markers. (a) Real-time qPCR performed on MT- and MT+ strains to evaluate the expression level of the selected genes. The values are presented as fold changes (FC) of MT+ expression with respect to MT-. (b-c) Real-time qPCRs to evaluate the MT- and MT+ genes expression during the onset of sexual reproduction; qPCRs were performed on co-cultures of opposite MTs, and the values are expressed as FC with respect to the correspondent monoculture. 7488 and *MRP1* have been used as sexualization controls. (b) means \pm SD (n=4, with different strains); (c) means \pm SD (n 77510=3, n Calmodulin=2, n 29310=2, n 67000=1, n *MRP1*=4, with different strains).

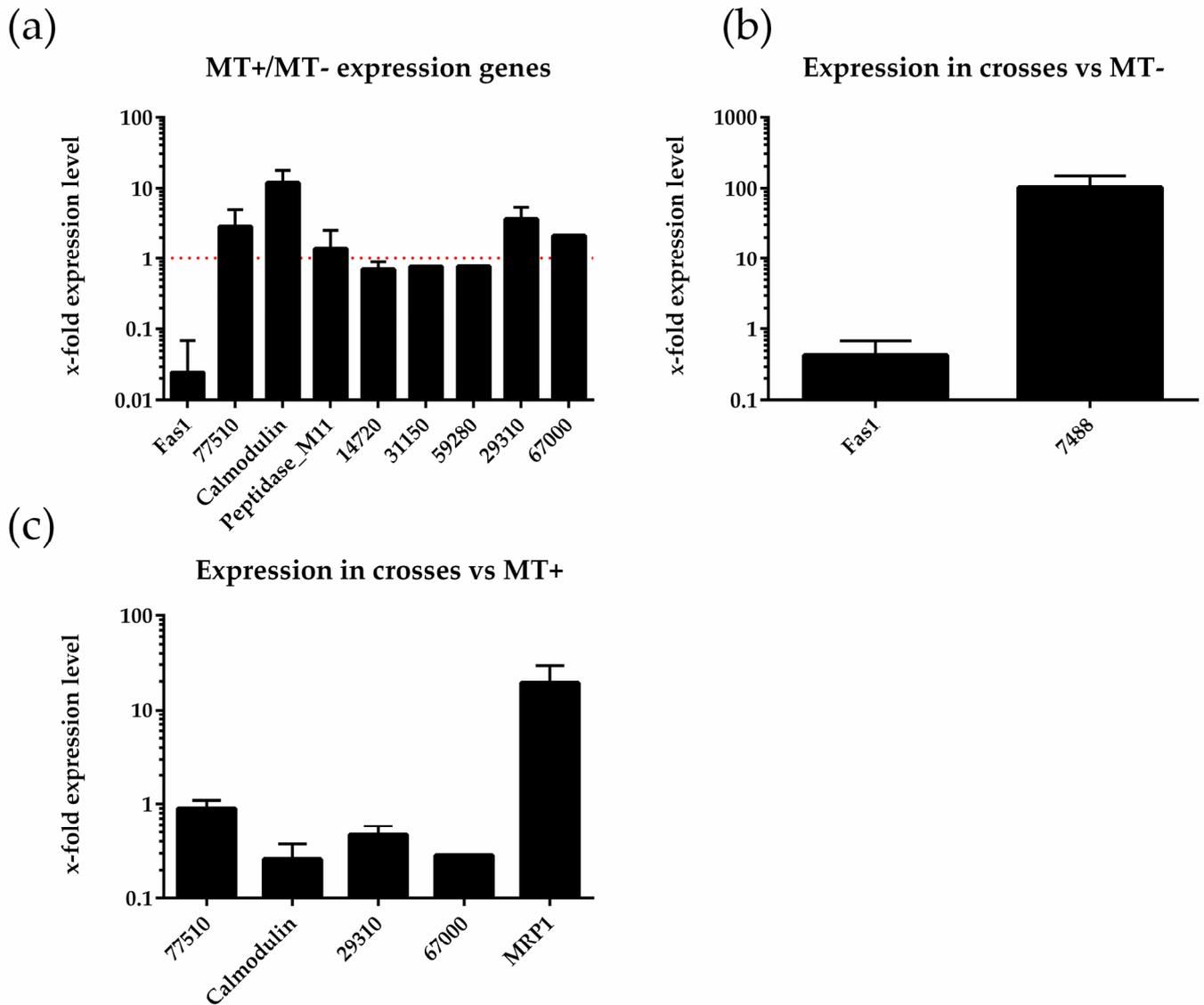


Figure S3. Chemical-physical characterization of *P. multistriata* pheromones. (a) 7488 and Cathepsin D qPCR performed on MT- cells conditioned with the raw medium and the fractions between 30 and 10 kDa and below 10 kDa of an MT+ vegetative culture medium; dots represent the expression levels obtained in distinct experiments with different couples of opposite MTs. (b) 7488 and Cathepsin D qPCR performed on MT- cells conditioned with the native and the heat-inactivated medium of an MT+ vegetative culture medium; dots represent the expression levels obtained in distinct experiments with different couples of opposite MTs. (c) *MRP1* qPCR performed on MT+ cells conditioned with the native and the heat-inactivated raw medium of an MT- vegetative culture medium. Lines represent the median of the different experiments. Each expression value is normalized for the correspondent MT- or MT+ untreated cells.

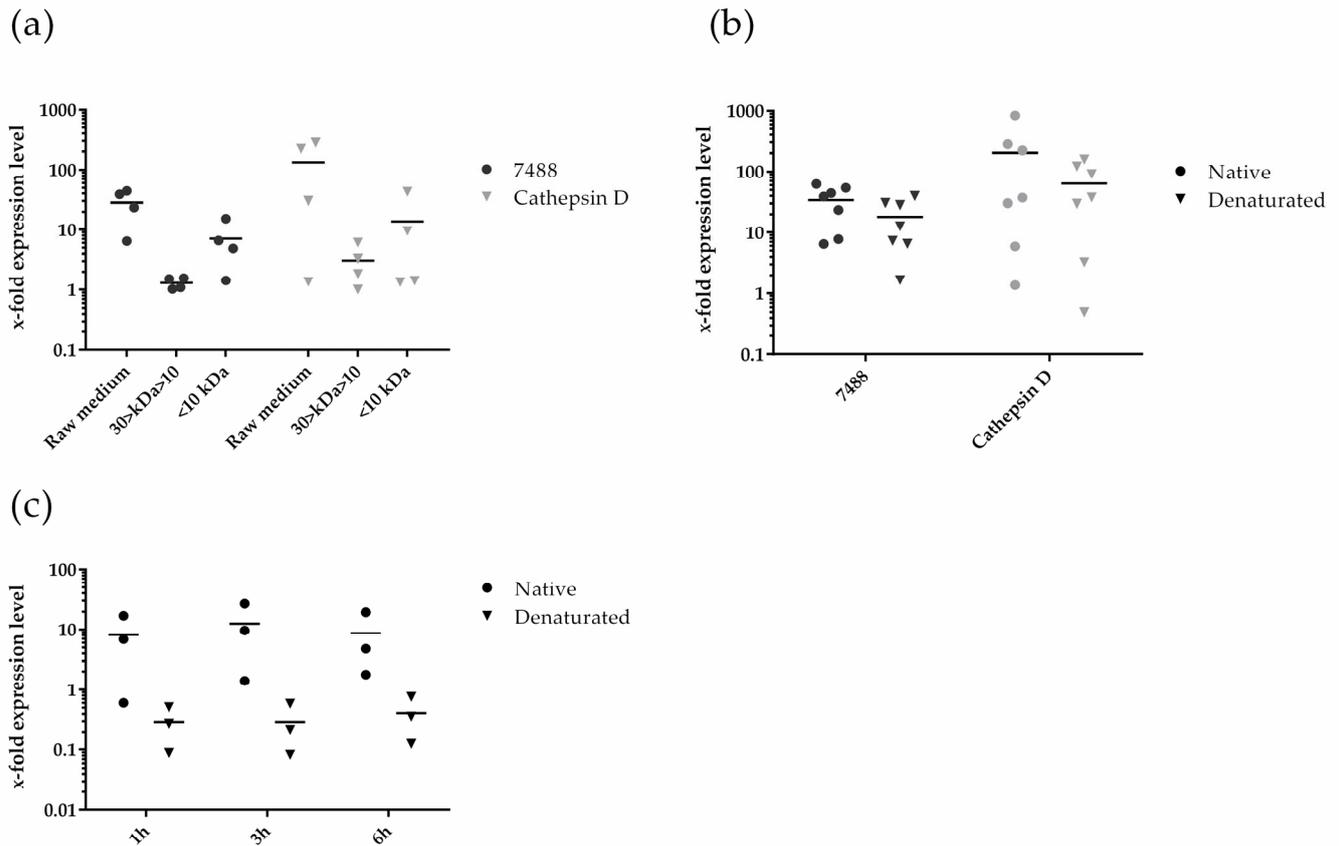


Figure S4. Scheme of the MRP1 construct and screening of the resistant clones. (a) Diagram of the *MRP1* overexpression plasmid; the coding sequence of *MRP1* was cloned in frame with the CDS of the *YFP* and put under the transcriptional control of the histone *H4* promoter. Red arrows indicate the position of primers used for the screening of the resistant clones; the size of the PCR band is also reported. (b) Representative PCR analysis performed on the cDNA of the 3 positive clones obtained from the biolistic transformation of the MF1 strain. WT: wild type; 1kb: molecular ladder.

