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Cd_glob PVLEGLVTNMRMNRVVAALEREYEQHYSRGATELDEREFLSTDFTKFASPRFSPGHMHSMAKLRSEMPFLRSGGLSPGLHTT 386
Gn_pect_Plus P I L S G L V T D I D R M N L A L A A L E R V Y E Q H L A C S G S E L D E R E F L T D F T K F A S P R Y S P G Q M H Y V L S T L R G G S T P I R P G I L L G P G A H R T 374
Gn_pect_Minus P I L S G L V T D I D R M N L A L A A L E R V Y E Q H L A C S G S E L D E R E F L T D F T K F A S P R Y S P G Q M H Y V L S T L R G G S T P I R P G I L L G P G A H R T 374
Ym_unic_Plus/Minus P I L E G L V T D S D R M N R A V A A L E V E Y E Q H Y V R G T S E L D E R D F L S T D F T K F A S P R F S P G Q M H S V M T K L R G G P M P L R P G D L L G P G A H T T 393
Eu_sp_Male S S L E G L V T D A D R M Q R A L A A L E T E Y E Q H F N R G D S E L D E R E F L Q T D F T K F A S P R L S P A H L Q T A M A K V R A G P M P L R Q G A L L G P G A H T T 392
Eu_sp_Female S S L E G L V T D A D R M Q R A L A A L E T E Y E Q H F N R G D S E L D E R E F L Q T D F T K F A S P R L S P A H L Q T A M A K V R A G P M P L R Q G A L L G P G A H T T 392
Pl_star_Male/Female PVLEGLVTDADRMRQALAALEAEYEQHYTLGGTELDEREFLHTDFTKFAFSPRFSGHHLAAMTKLRAGPMP L R Q G A L L G P G A H T T 244
Vx_afri_Male PVLEGLVTDADRMRQALAALEAEYEQHYTKHGSELDEREFLTDFTKFAFSPRFSRHMMSA I T K L Q S G P T P L R Q G A L L G P G A H I T 391
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Vx_obve_Male PVLEGLVTDG I R M E Q A L A A L E A E Y E H Y V H G S E L D E R E F L Y T D F A K F A S P R L S P G H I H S A M V K L R S G F L P L R Q G A L L G P G A H T T 95
Vx_car_naga_Male PVLEGLVTDVARMQRALDALESEYKHYEHGSELDEREFLTDFTKFAFSPRFSGHMQSA I T K M R L G P M P L R Q G A L L G P G A H T T 387
Vx_car_naga_Female R L E G L V T D G N R M Q R A L S A L E T E Y A Q H Y T R G S E L D E R E F L T D F T K F A S P R F S P R H M Q S A V M K L R A G P L P I R Q G S L L G P G A H T T 392
Vx_car_kawa_Male PVLEGLVTDAAARMQRALDALESEYKHYALGSELDEREFLTDFTKFAFSPRFSGHMQSAVTKLRLGPMPLRQ G A L L G P G A H T T 407
Vx_car_kawa_Female R V L E G L V T D A N R M Q R A L S A M E Y E Q H Y T R G S E L D E R E F L Y T D F S K F A S P R F S P G H M Q S A V M K L R A G P L P I R Q G E F A V P G A H T T 401
Vx_car_weis_Male PVLEGLVTDVARMQRALDALESEYKHYVHGGSELDEREFLTDFTKFAFSPRFSGHMQSA I T K L R L S P M P L R Q G A L L G P G A H T T 117
Vx_car_weis_Female R V L E G L V T D A N R M Q R A L T A L E T E Y E Q H Y T R G S E L D E R E F L Y T D F T K F A S P R F S P G H M Q S A V M K L G A G P L P I R Q G A L L G P G A H I T 104

440          460          480          500
Cd_rein_Plus TPAHGPQQ --- LHL --- VPLGLHSLPMMHLNAGPGVGP T P I SEAMGASAWLRGVT SNMVAEPSPTLQRFLAALP I - - - - - 459
Cd_rein_Minus TPAHGPQQ --- LHL --- VPLGLHSLPMMHLNAGPGVGP T P I SEAMGASAWLRGVT SNMVAEPSPTLQRFLAALP I - - - - - 459
Cd_glob I PAHGPQQ --- MQL - MGPAPWLHSLPMMHLNAGPGLQG T P I SEAMGASAWLRGVT S S M A A E P S P A L Q R L L A A L P I - - - - - 459
Gn_pect_Plus APQQ - - PMR - - - SFPVSAGTPGLHSLPGVHCG - DP G P P I T P V S E L M S A S T W L R G L A T T L T A E P S C T L Q R F L A A V P V - - - - - S V 471
Gn_pect_Minus APQQ - - PMR - - - SFPVSAGTPGLHSLPGVHCG - DP G P P I T P V S E L M S A S T W L R G L A T T L T A E P S C T L Q R F L A A V P V - - - - - S V 471
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Eu_sp_Male A P S P S P Q Y - - - - L Q F K M A P S P G F H S P L P M L H L G V D L G Q P N T P V S E V M G A S A W L R G V T A S L Q T E P S A S L M R Y L E A V S V P G G V G G S 473
Eu_sp_Female A P S P S P Q Y - - - - L Q F K M A P S P G F H S P L P M L H L G V D L G Q P N T P V S E V M G A S A W L R G V T A S L Q T E P S A S L M R Y L E A V S V P G G V G G S 473
Pl_star_Male/Female A S N T S S P H - - - - P H I T M S L T G M Y S P L P M L H L G M D S G Q P N T P V S E V M G A S A W L R S V T A N L A P E P P S L L Q Y F E A V A V P G A G P S 324
Vx_afri_Male A S S V S - P H L - - - - I N F T M P I P A G T H S P L P M L N L A M D V G Q P T P V S E V M S A S A W L R A L T A N L A T E P S P L M R Y L A A I D M P L S G T R S 471
Vx_afri_Female A S S V S - P H L - - - - I N F T M P I P A G T H S P L P M L N L A M D V G Q P T P V S E V M S A S A W L R A L T A N L A T E P S P L M R Y L A A I D M P L S G T R S 471
Vx_obve_Male A P S A V S P H - - - - L N L R I P M P A M Q S P L P L N L G I D M G Q P N T P V S E V M S A S A W L R A L T A N L A E P T P G L V R H L S A A - - - - T R S 172
Vx_car_naga_Male A P S S V S P R L - - - - N F R V P S A G M H S P L P V L N L S I D T G Q P S T P V S E V M N I S A W L R G R T A N L A A E P S P G L T R Y L A A V G G - - - - T R S 463
Vx_car_naga_Female A S Y A I S P S S - - - - M N P K T S L L A R M H S P L P K L N L G V E T G P P V T P V S E I M S T S A W L R G I T A S L A A E P S P S V M R Y L A E V A V P P D G G S 473
Vx_car_kawa_Male A P S S V S P R L - - - - L H F R V P M P A G M H S P L P V L H L S I D T G Q P S T P V S E V M N I S A W L R G R T A N L A A E P S P G L A R Y L A A V G G - - - - T R S 483
Vx_car_kawa_Female A P C V I S P S L - - - - M N L K T P L W A G V H S P L P K L D L G V E T G P P V T P V S E I M S A S A W L R G V T A N L A A E P S P M R Y L A T V A V P P Y G E G S 482
Vx_car_weis_Male A P S S V S P R - - - - L N F R M P M P A G M H S P L P V L N L G I D T G Q P S T P V S E V M N I S A W L R G R T A N L A A E P S P G L T R Y L A A V G G - - - - T R S 193
Vx_car_weis_Female A P Y S I S P S S - - - - T P L K T P F W A G L H S P - - K L D L G V E A G P P V T P V S E I M S A S A W L R G V T A N L A A E P S P L M R Y L A A V A V P H D G G G S 183

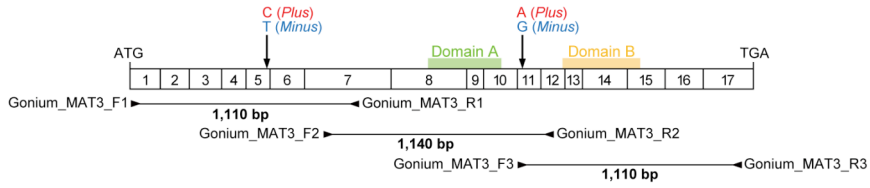
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Cd_rein_Minus N S N S S G P T P V Q L M K R V R D L V A S V I P D E A A P S L L G P F P L L N T S L G A E R R I E A T K L Y C H S L D T I L Q N E Q R T N G L P A A L A L L G S A K F 544
Cd_glob N S T G L G P T P A Q L M K R V R D L V A S V I P D E A A P S L L G P F P L L N T S F G A E R R T Q A A R L Y C H S L E T I M L N E E R T N G L P A A L A L L G S A K F 544
Gn_pect_Plus A Q A G T S L S A A E Q L S R R I R E L I S L V I P D E S S T L L G S F P - L Q P R L A A E R R M E A Y K L Y L H S L E T L L V A E E K V N G L A G V A L L S S S K F 531
Gn_pect_Minus A Q A G T S L S A A E Q L S R R I R E L I S L V I P D E S S T L L G S F P - L Q P R L A A E R R M E A Y K L Y L H S L E T L L V A E E K V N G L A G V A L L S S S K F 531
Ym_unic_Plus/Minus S G P P G S Q S L A V Q L S R R V R D M V A S V I P D E N G P S V L G F P P L L Q P N T P V S E A E R R M E A T K L Y Y H S L E T I L M S E E K A S G L T V A L L V A T K F 562
Eu_sp_Male S G S A A S A A A A Q L S R R V R D L V L S I M P D E R I P A L F G P P F L L Q T S L A A E R R T E V T K L Y Y H S L E N I L R A E E K A S G I A A A I A L L S A G K F 558
Eu_sp_Female S G S A A S A A A A Q L S R R V R D L V L S I M P D E R I P A L F G P P F L L Q T S L A A E R R T E V T K L Y Y H S L E N I L R A E E K A S G I A A A I A L L S A G K F 558
Pl_star_Male/Female T C P T G P S A A A Q L S R R V R E L V S S V M P E E K I P S L L G P F P L L Q S S L A V E R R T E I T K L Y Y H S L E I L Q N E E K A S G L A G V A P L L A S G K F 409
Vx_afri_Male T Y T S G Y A S A A E Q L G Q R V R D L V S S V M P E E E I P S L L G P F P L L Q S S L A V E R R T E I T K L Y Y H S L E I L Q N E E K A S G L A G V A P L L A S G K F 556
Vx_afri_Female T Y K S G Y A S A A E Q L G Q R V R D L V S S V M P E E E I P S L L G P F P L L Q S S L A V E R R T E I T K L Y Y H S L E I L Q N E E K A S G L A G V A P L L A S G K F 556
Vx_obve_Male - - A N G S A T A V H Q L G Q R V R D L V S S I M P E E K A P S L V G P F P L F Q S S L A A E R R S E V T K L Y Y H S L D S I L Q T E E K V S G M P G V T S L L L A G K F 255
Vx_car_naga_Male E Y V T G S A S T A H Q L G Q R V R D L I S S I I P E E K I P S L V G A F P L L Q P S L T A E R R L E V T K L Y Y H S L D N I L H T E E K V G G M A G V T S L L S A G K F 548
Vx_car_naga_Female A C V T V T L S A A E Q L N Q R V Q E L V S C I M P E E K I P S L L G S F P L L Q P S L V S E R R I E V T K V Y Y L S L E R I L Q A E E K N N G V A G V T S L L S A G K F 558
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Vx_car_kawa_Female A C V T V - - S A A E Q L N Q R I R E L V T C I M P E E K I P S L L G P F P L F Q P S L V A E R R I E V M K L Y L L G L E C I L Q A E E K S S G V P G V T S L L S S G K F 565
Vx_car_weis_Male E C A T G S A T A A H Q L G Q R V R D L I S S V I P E E K I P S L V G A F P L L Q P S L A A E R R T E V T K L Y Y H S L D S I L H T E E K V S G M A G V T S L L S A G K F 278
Vx_car_weis_Female A C M T V T V S A A K Q L T Q R V R D L V S C I M P E E K I P S L L G P F P L L Q P S L V T E R R I E V T K L Y Y L S L E R I L Q A E E K N N G V T G V I S L L S A G K F 268

600          620          640          660          680
Cd_rein_Plus Q R G L I A C C I E V V A A C Y R M V S C A F P K V D L A L R I K A F D M A N I I G T F V K S I A T L P R E L K R H L F I E E K I L E C L A W E P G S S L Y H L I V N V 629
Cd_rein_Minus Q R G L I A C C I E V V A A C Y R M V S C A F P K V D L A L R I K A F D M A N I I G T F V K S I A T L P R E L K R H L F I E E K I L E C L A W E P G S S L Y H L I V N V 629
Cd_glob Q R G L I A C C I E V V A A C Y R M S C A F P K V D L A L R I K A F D M A S I I G T F V K S I A T L P R E L K R H L F I E E K I L E C L A W Q P G S S L Y H L I V N V 629
Gn_pect_Plus H R G L V A C T V E I V A A C Y R M V S C A F P K V M D V L H I K A F D L A K M V Q G F V R S V S L P R E L K R H M F L I E E K I L E S L A W E P G S S L Y F H I V N H 616
Gn_pect_Minus H R G L V A C T V E I V A A C Y R M V S C A F P K V M D V L H I K A F D L A K M V Q G F V R S V S L P R E L K R H M F L I E E K I L E S L A W E P G S S L Y F H I V N H 616
Ym_unic_Plus/Minus H R G L V A C C V E V V A A C Y R M V S C A F P K V L D S L R I K A F D L A K M I Q G F V K S I A T L P R E L K R H L F I E E K I L E S L A W E P G S S L Y H L I I N V 647
Eu_sp_Male H R A L V A C C V E V V A A C Y R M A G C A F P K V L D A V G I K A F D L G K M I Q G F V K S V A S L P R E L K R H M F L I E E K I L E S L A W E P G S S L Y N H I A F 643
Eu_sp_Female H R A L V A C C V E V V A A C Y R M A G C A F P K V L D A V G I K A F D L G K M I Q G F V K S V A S L P R E L K R H M F L I E E K I L E S L A W E P G S S L Y N H I A F 643
Pl_star_Male/Female H R T L V A C C V E V V A A C Y R M V S C A F P K V L D A L H I K A F D L A K M I Q G F V K S I P A L P R E L K R H L F I E E K I L E S L A W E P G S S L Y H H I I N H 494
Vx_afri_Male H R A L V T C C I E V V A A C Y R M V S C A F P K V L D A L H I K A I D L P N M I Q S F V K S I E T L P R E L K R H L F I E E K I L E S L A W E P G S S L Y S H I T S I 641
Vx_afri_Female H R A L V T C C I E V V A A C Y R M V S C A F P K V L D A L H I K A F D L A K M I Q S F V K S I E T L P R E L K R H L F I E E K I L E S L A W E P G S S L Y S H I T S I 641
Vx_obve_Male H R A L I A C C V E V V A A C Y R M V S C S F P K V L D A L H I K A F D L A K M I Q S F V K S V E T L P R E L K R H L F I E E K I L E S L A W E P G S S L Y S H I I T I 340
Vx_car_naga_Male H R A L V A C C I E V V A A C Y R M V S C A F P K V L D A L H I K A F D L A K M I P S F V K S I E T L P R E L K R H L F I E E K I I E S L A W E P G S S L Y T H I V N I 633
Vx_car_naga_Female H R A L V A C S A E V V T A C Y R M V S C T F P K V L D A L H I K A F D L A K M I Q C F V R S I T T L P R D L K R H L F L V E E K I I E S L A W E A D S P L Y S L I C S T 643
Vx_car_kawa_Male H R A L V A C C I E V V A A C Y R M V S C A F P K V L D A L H I K A F D L A K M I P S F V K S V E T L P R E L K R H L F I E E K I I E S L A W E P G S S L Y T H I L D V 653
Vx_car_kawa_Female H R A L V A C S V E V V T A C Y R M V S C A F P K V L D A L H I K A F D L A K M I P S F V K S V E T L P R E L K R H L F I E E K I I E S L A W D P G S S L Y T H I A N V 363
Vx_car_weis_Male H R A L V A C C I E V V A A C Y R M V S C A F P K V L D A L H I K A F D L A K M I P S F V K S V E T L P R E L K R H L F I E E K I I E S L A W D P G S S L Y T H I A N V 363
Vx_car_weis_Female H R A L V A C S L E V V T A C Y R M V S C A F P K V L D A L H I K A F D M A K M I E C F V K S I T T L P R E L K R H L F L V E E K I L E S L A W E A G S P I Y S L I S S T 353

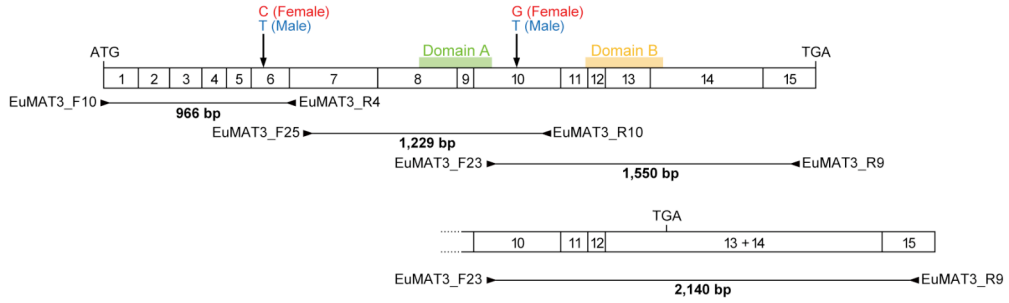
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are shown for *V. carteri* f. *nagariensis*. Positions 341-427, 447-503, 518-678, 874-969, 972-1016 and 1210-1227 (464 amino acids) were used for phylogenetic analysis (fig. 3).

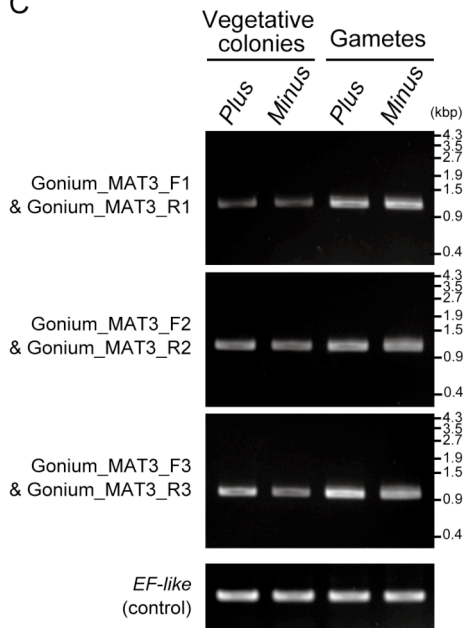
A *Gonium pectorale* MAT3 (coding sequence: 3,087 bp)



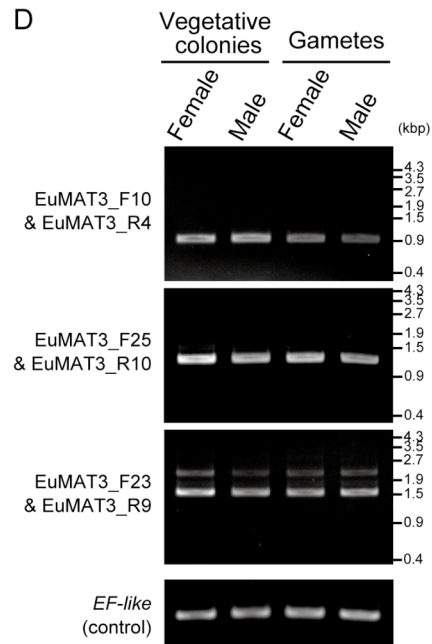
B *Eudorina* sp. MAT3 (coding sequence: 3,525 bp)



C



D



E

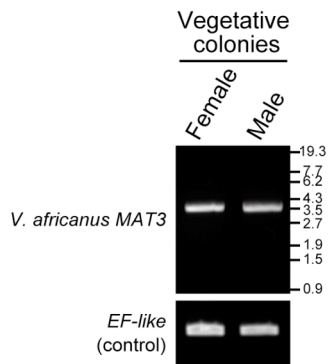


Figure S2

Semi-quantitative RT-PCR analyses of *MAT3* from different colonial Volvocales.

(A, B) Schematic representations of *Gonium pectorale* (A) and *Eudorina* sp. (B) *MAT3* coding sequences. Numbers in the open boxes indicate the exon numbers. The regions corresponding to the two conserved domains A and B are shown. The positions of the bases different between each sex are also indicated. The primer sets (supplementary information, Supplementary Material online) used in the RT-PCR experiments and their resulting product sizes are given. A partial structure of the *Eudorina* sp. *MAT3* splicing variant identified by sequencing of the PCR product (D, upper band amplified with the primer set EuMAT3_F23 and EuMAT3_R9 [supplementary information, Supplementary Material online]) is also shown (B). (C-E) Results of semi-quantitative RT-PCR analyses of *MAT3* genes from *G. pectorale* (C), *Eudorina* sp. (D), and *Volvox africanus* (E). Poly(A)⁺ mRNA from each sample was isolated and subjected to RT-PCR analysis using primer sets indicated. Loading volume for each lane was normalized to the amount of the *EF-like* gene (internal control) product. Note that no significant differences in expression level and splicing pattern were detected between the two sexes. For *V. africanus* (E), a primer set which amplifies a 3,462-bp fragment including the full-length coding sequence of female and male *MAT3* (Vol_Africa_male_F8 and Vol_Africa_CDS_R1 [supplementary information, Supplementary Material online]) was used for the analysis.

Supplementary Reference

- S1. Nakada T, Shinkawa H, Ito T, Tomita M. 2010. Recharacterization of *Chlamydomonas reinhardtii* and its relatives with new isolates from Japan. J Plant Res. 123: 67–78.