

S1 Fig. Putative nonfunctionalization nature of *JcERF043* and *JcERF071* genes in the physic nut genome.

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JcERF043 ATGTCATTGCCACAACCTACCCAAGATCAAGAAGCTCTCCATCATGGTCTCTGCTCTGAAA 60
JiERF043 ATGTCATTGCCACAACCTGCCCAAGATCAAGAGCTCTCCATCATGGTCTCTGCTCTGAAA 60
*****

JcERF043 AATGTTGTCTCCGGCGACGCCACCAGTAGCACAACGGTGGCTTCAATTTCTCACAGGTT 120
JiERF043 AATGTTGTCTCCGGCGACGCCACCAGTAGCACAACGGTGGCTTCAAATTTCTCACAGGTT 120
*****

JcERF043 CGTCAATCCCTCCAATGGTTATGGTTATAGTTCTAGTTACTCCTCTGCCGCTGTTACT 180
JiERF043 CGTCAATCCCTCCATTGGTTATGGTTATAGCTCTAGCTCCTCCGCTGCCGTTGTTACT 180
*****

JcERF043 GGTGCAGTTGCTACCACCACCAGTATGTTTATAACGCATCAGATCTTGACACGTGTAGC 240
JiERF043 GGTGCAGTTGCTACCACCACCGTAGTATGTTTATAACGCATCAGATCTTGACACGTGTAGC 240
*****

JcERF043 ATATGCAAAATCAACGGATGTTTAGGATGCAACTTCTCCCGCAAATCAAGAGGAAAAA 300
JiERF043 ATATGCAAAATCAACGGATGTTTAGGATGCAACTTCTCCCTCAAATCAAGAGGAAAAA 300
*****

JcERF043 AAGGGGAGAAGAAAAGAATAAAGAAAAATTACAGAGGAGTAAGGCAGAGCCGTGGGGA 360
JiERF043 AAGGGGAGAAGAAAAGAATAAAGAAAAATTACAGAGGAGTAAGGCAGAGCCGTGGGGA 360
*****

JcERF043 AAATGGGCGCGGAGATAAGAGACCCACGGCGAGCGACAAGGGTGTGGCTAGGGACTTTT 420
JiERF043 AAATGGGTGGCGGAGATAAGAGACCCACGGCGAGCGACAAGGGTGTGGCTAGGGACTTTT 420
*****

JcERF043 AACACGGCGGTG TAGGCTGCTAGAGCCTATGATAAGGCCGCGATAGATTTTCGAGGGCCG 480
JiERF043 AACACGGCGGAG GACGCTGCTAGAGCCTATGATAAGGCCGCGATAGATTTTCGAGGGCCG 480
*****

JcERF043 AGAGCGAAGCTGAATTTCCATTTCCAGATAATACAGACAGTAATGGTTCAAATTAGGA 540
JiERF043 AGAGCCAAGCTGAATTTCCATTTCCAGATAATACAGACAGTAATGGTTCAATTAGGA 540
*****

JcERF043 GAGCAAATTTATTCCTCAAGAACAAGAACAAGAAAATAGGAAAGGAAAGGAATTTGAA 600
JiERF043 GAACAAATTTATGCTCAAGAACAAGAACAAGAAAATAGGAAAGGAAAGGAATTTGAA 600
** *****

JcERF043 GTGAAATGAAAAATCGAAACGATTTGAATCTGGGAAGGAGTTGGTAAAGATGAAAT 660
JiERF043 GTGAAATGAAAAATCGAAAGGATTTGAATCTGGGAAGGAAATGGTAAAGATGAAAT 660
*****

JcERF043 GAACAATGGA-----TGATGAATTTAATAGGGATTCATCAGATTCTTCTGCTACT 711
JiERF043 GAACAATGGACGATGATGATGATGAATTTAATGGGATTCATCAGATTCTTCTGCTACT 720
*****

JcERF043 ATTATTTGTAA 723
JiERF043 ATTAATTTGTAA 732
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(A), DNA sequence alignment of ERF043 coding domain sequences from *J. curcas* and *J. integerrima*, showing a nonsense mutation site in *JcERF043*.

S1 Fig. (Continued.)

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JcERF042      MHKSSKRLKHTGPPPTTEYLPPAMLLPRLTQDQELFIMVSALKNVVSG-TTSSTSVATDL- 58
JiERF042      MHKSSKRLKHTGPPPTTEYLPPVMPLPRLTQDQELSIMVSALKNVVSG-TTSSTSVATDL- 58
JcERF043      -----MSLPQLTQDQELSIMVSALKNVVSGDATSSTTVASDFS 38
JiERF043      -----MSLPQLAQDQELSIMVSALKNVVSGDATSSTTVASNFS 38
                * *:***** ***** :****:***:::

JcERF042      -FHQFAAAAS--SSSPATAVASTAVTTTTDSLYNPIPPPSDLDTCNICKIKGCLGCNFFP 115
JiERF042      -FHQFTSAAS--SSSSATAVASTAVSTTTASLYNPIPPPSDLDTCNICKIKGCLGCNFFP 115
JcERF043      QVRQFPSNGYGYSSSYSSAAVTGAVATTTTSFYN----ASDLDTCSICKINGCLGCNFFP 94
JiERF043      QVRQFPSIGYGYSSSSAAVVTGAVATTTASFYN----ASDLDTCSICKINGCLGCNFFP 94
                .:*.:. .   *** :*:..:  **:.**  *:*   .*****. :****:*****

                AP2/ERF domain

JcERF042      PNQEEKKGTRKRVKKNYRGVQRQRPWGKWA AEIRDPRRATRVLGTFNTAE EAARAYDKAA 175
JiERF042      PNQEEKKGTRKRVKKNYRGVQRQRPWGKWA AEIRDPRRATRVLGTFNTAE EAARAYDKAA 175
JcERF043      PNQEEKGRRKR IKKNYRGVQRQRPWGKWA AEIRDPRRATRVLGTFNTAV •AARAYDKAA 153
JiERF043      PNQEEKGRRKR IKKNYRGVQRQRPWGKWA AEIRDPRRATRVLGTFNTAE DAARAYDKAA 154
                ***** *:***** ***** *****

JcERF042      IDFRGPRAKLNFPFPDNTAS----NLEGQSTPQEQQKENGKRKELEVEMQVGKEYEFW 230
JiERF042      IDFRGPRAKLNFPFPDNTASASNITNLEGQSTPQEQQKENGKRK----DMQLGNEHEFW 231
JcERF043      IDFRGPRAKLNFPFPDNTDS--NGSKLGEQIIPQEQEQKENRKGKEFEVEMENRNDFEFW 211
JiERF043      IDFRGPRAKLNFPFPDNTDS--NGSNLGEQIIAQEQEQKENRKGKEFEVEMENRKFDFEW 212
                ***** ***** *      :* * .***:**** * *      :*: :*.***

JcERF042      EKIGEDEIQWMN-MMDFNGDSSDSTGTAATTTIAN 265
JiERF042      EKIGEDEIQWMN-MMDFNGDSSDSTGTA--TTIAN 264
JcERF043      EGVGKDEIEQW---MMNFNRDSSDSSATIIL----- 239
JiERF043      EGIGKDEIEQWTMMMMNFNGDSSDSSATINL----- 243
                * :*:****:*  **:* *****:.*

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(B), Amino acid sequence alignment of ERF042 and ERF043 proteins from *J. curcas* and *J. integerrima*: the nonsense mutation in the *JcERF043* gene results in an incomplete AP2/ERF domain in the protein that it encodes.

S1 Fig. (Continued.)

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JcERF071 -----MHSLASPIMQPL----- 12
JcERF070 MEAETHVASALEFIKHHLLGDLSPVGASCYSYTLNDVNMELSSSENESENCSRTSSIDST 60
          * * . :.:*

JcERF071 -----ALPASLTQSSRSFLQP-- 28
JcERF070 ISFSDYFTSNNIDDDFFQFEAKPQIVDLTAPRSCDSPTSFEFDKAPQLISQSSNEFFEFE 120
          * * ::*:.*:

JcERF071 -----
JcERF070 SKPQIQHIQNFREFESKPQISLSNNVFNDFESKPEIPSVGSSSQGNRKPSLKI SLPKKTE 180

                                     AP2/ERF domain
JcERF071 -----VQADSGAFAVEKKRHYGGVQRQPWSKYAAE IRDPNRKGRRLWLGTFD-- 75
JcERF070 WIQFAEPNQSVQADSGASAVEEKRHYRGVQRQPWGKYAAE IRDPNRKGRRLWLGTFETA 240
          ***** *:*:*:* *:*:*:* *:*:*:*:* *:*:*:*:* *:*:*:*:*

JcERF071 -EAAKAYDQAAFKFCGSKAILNFTLKARKWNTRSSEASEQKGTGESEIVEEEAKKAVRER 134
JcERF070 IEAAKAYDQAAFKFRGSKAILNFFLEAGKWNTRASEGNERKRTRESEIVEEEAKKVVK-- 298
          ***** *:*:*:* *:*:*:* *:*:*:* *:*:*:* *:*:*:* *:*:*:*

JcERF071 SLREMWRARRRAGRLSWTGRVALG--LGILGEKKLG--AQNGEYGRFDLGLG 183
JcERF070 --REEPQGDEALTPSSWTAIMDGGDLKGFNVPPLSPFSPHPPLG--YSQLIVS 348
          ** : . *** : * **: * . : : * : * :

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(C), Amino acid sequence alignment of annotated JcERF071 and JcERF070 proteins. JcERF071 has lost 3 amino acid residues from within the AP2/ERF domain.

S1 Fig. (Continued.)

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JcERF070      ATGAGGCTGAAA--CTCATGTAGCTTCTGCTTTAGAGTTCAATCAAGCATATTACTTG 58
JcERF071      -TCCAAATTGAAAATTTTATATATTATCGCCCTAAA----TTCAAAT----TTGCTAA 51
* * * * *
JcERF070      GTGATCTTCTCTACC--TGTGGTGTCTCATGTCTTATTCCACCCCTTAATGATGTAAA 116
JcERF071      G--ATTTTAAATAAAAAATTAATAAGTG----GATCCCACAGCACCCCTT-TGGGGTTAA 104
* * * * *
JcERF070      TATGGAGCTTCGAGCTCTGAAAATGAGTC-TAATTGCTCCCGGACTTCAAGTATTGATT 175
JcERF071      CTTAA-----CCAAGGTGTCAAAGGCGTCGTTTTTGACACCATACTC--ACCATT--TT 155
* * * * *
JcERF070      CAACAATCTCATTCTCTGATTACTTTACAAGCAACAATATTGATGACGATTCTTCCAAT 235
JcERF071      CAACAATCTCAT-CTTCAATCACTGTCT--TCTTGAAATGCCAAGA--ACCCCTAC---- 206
*****
JcERF070      TTGAAGCTAAACCTCAAATCGTGTACTCTACTGCTCCAAGATCTTGGCATTGCGCAACTA 295
JcERF071      ----GC--AACCTCAAATCGTGTACTCTACTGCTCCAAGATCTTGTGATTGCGCAACCA 259
* * * * *
JcERF070      GTTTGAATTGTGATGCAAAACCTCAACTAATCTCTCAATCAAGTAACGAATTCTCGAAT 355
JcERF071      GTTTGAATTGTGATGCAAAACCTCAACTAATCTCTCAATCAAGTAACGAATCTCGAAT 319
*****
JcERF070      TCGAATCAAAACCTCAAATACAACACATTGAGATTTCAGATTGGAATCAAAA--CCTCAA 414
JcERF071      TCGAATCAAAACCTCAAATACAACACATTGAGATTTCAGATTTCAGATTGGAATCAAAA 379
*****
JcERF070      AGTATTCTCTATCAATAACGTTTTCACGATTTGCAATCAAAACCTGAAATACCATCT 474
JcERF071      AGTATTCTCAATCAATAACGTTTTCACGATTTGCAATCAAAACCTGAAATACCATCT 439
*****
JcERF070      GTTGGTTCAAGTTCTCAGGGAACCGAAGCCGCTTTGAAAATATCGTTGCCCAAAAA 534
JcERF071      GTTGGTTCAACTTCTACGACGAATTAAGTCGACTTCAAGATA-----CTCA----- 488
*****
                                Stop
JcERF070      ACCGAATGGATCCAATTGCTGAACCGAACCAGCAATCGGTCCAAGCTGACTCGGGTGCT 594
JcERF071      -----TCCAAATTGCTGAACCGAACCAGCAATCGGTCCAAGCTGACTCGGGTGCT 538
*****
JcERF070      TCTGCTGTAGAAGAAAAGGCATTACAGAGGAGTCCGACAGAGACCGTGGGGCAAATAC 654
JcERF071      TTTGCTGTAGAAAAGAAAAGGCATTACGAGGAGTCCGACAGAGACCGTGGAGCAAATAC 598
* * * * *
JcERF070      GCAGCGGAGATCCGAGACCCGAACCGAAAGGCACTCGGTGTGGCTCGGAACCTCGAA 714
JcERF071      GCAGCGGAGATCCGAGACCCGAATCGGAAAGGCGTCTGATTTGGCTCGGACATTCGA 657
*****
JcERF070      ACAGCTATCGAAGCCGCCAAGCCTACGATCAAGCAGCGTTCAAGTTTGGCGCTCAAAA 774
JcERF071      -----CGAAGCCGCCAAGCCTACGATCAAGCAGCGTTCAAGTTTGGCGCTCAAAA 709
*****
JcERF070      GCCATTCTGAACCTCCCTCTAGAAGCTGGGAAATGGAACACACGTTCCAGTGAAGGAAC 834
JcERF071      GCCATTCTGAACCTCACTTAAAAGCTAGGAAATGGAACACACGTTCCAGTGAAGCAAGC 769
*****
JcERF070      GAACGAAACGAACAGAGAAAGTGAGATTGTAGAAGGGAAGCGAAGAAGGTGGTGAAG 894
JcERF071      GAACAGAAAGGAACAGGAGAAAGTGAGATTGTAGAAGGGAAGCGAAGAAGCGGTGAAG 828
* * * * *
JcERF070      AGAGAAGAGCCTCAGGAGATGAGGCGTTGACGCCGTCGAGCTGGACGGCAATTATGGAC 954
JcERF071      AGAGAGGAGCCTACGAGAGATGTGGCGTTGTCGCCGTCGAGCTGGACGGCTATCATGGAC 888
* * * * *
JcERF070      GGAGG---CGATTGAAA--GGAATCTC-----AAT----- 981
JcERF071      GGGGAGAGTTGCTTTAGGATTAGGATTTTGGGGAAAAAAAATTTGGGGGCGCAAAACGG 948
* * * * *
JcERF070      ---GTACCGCCGTTGT----CTCCGTTT-----CTCCTC----- 1009
JcERF071      TGAGTATGGTCGTTTTGACACCTTGGGCTTGGGTTAAGCTGATCAACAGTGGTGTATG 1008
* * * * *
JcERF070      --ATCCCGGTAGGATATTCTCAGCTTATAGTTTCA-TAA----- 1047
JcERF071      GGATCCACT-TTAATATTTTTTTA-TTTAAAATTTAGCAAAAATTTGAAT 1056
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(D), DNA sequence alignment of *JcERF071* and *JcERF070* genes, showing DNA sequence divergence.