New Phytologist Supporting Information

The distinctive flagellar proteome of *Euglena gracilis* illuminates the complexities of protistan flagella adaptation

Michael Hammond, Martin Zoltner, Jack Garrigan, Erin Butterfield, Vladimir Varga, Julius Lukeš and Mark C. Field

Accepted 15 July 2021

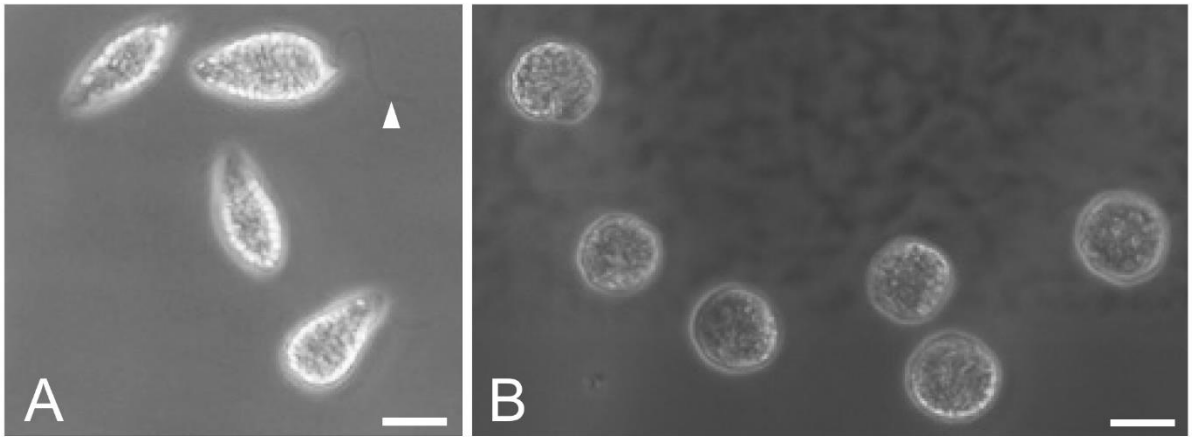

Figure S1: Light microscopy of aflagellated *Euglena gracilis* cells after cold shock ethanol treatment. Micrographs showing *E. gracilis* cells before (A) and after one round of cold-shock treatment (B). Arrow indicates visible emergent flagellum (bar=20 μm).

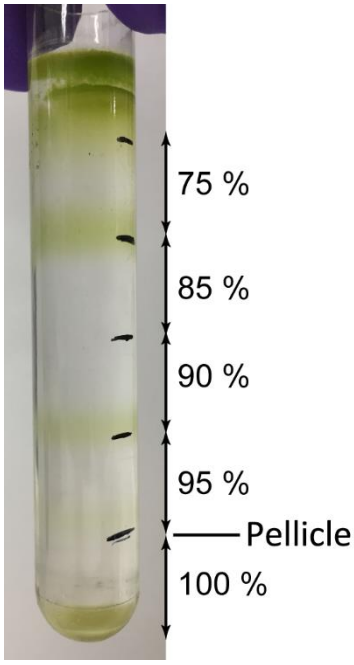

Figure S2: Density gradient centrifugation for isolation of the pellicle fraction. The pellicle fraction was recovered from the discontinuous gradient (sucrose percentage (w/V) is indicated for each layer) from the 95-100% sucrose interface.

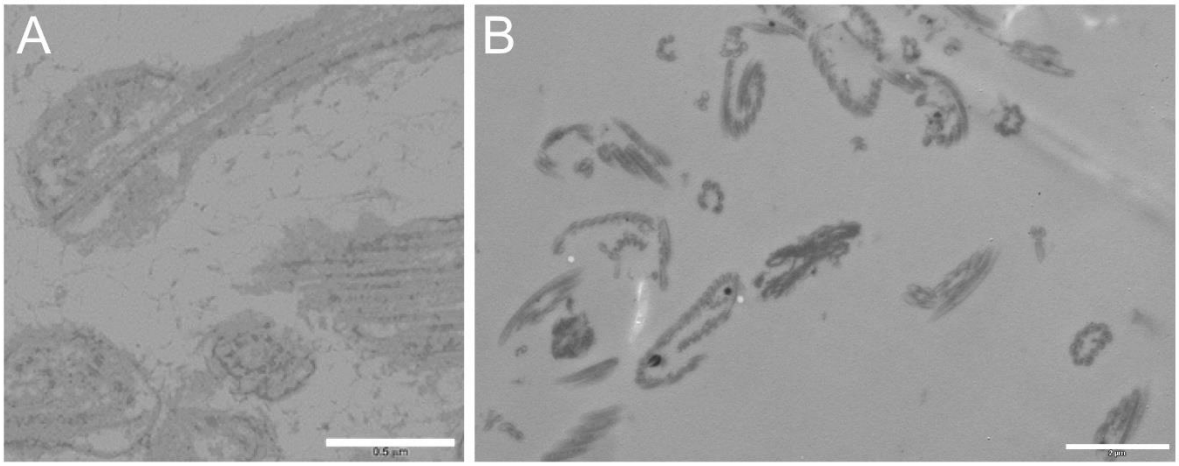

Figure S3: Transmission electron microscopy isolated samples showing. Isolated flagellum (A) and pellicle (B) fractions (Scale bar A = 0.5μm, B = 2μm).

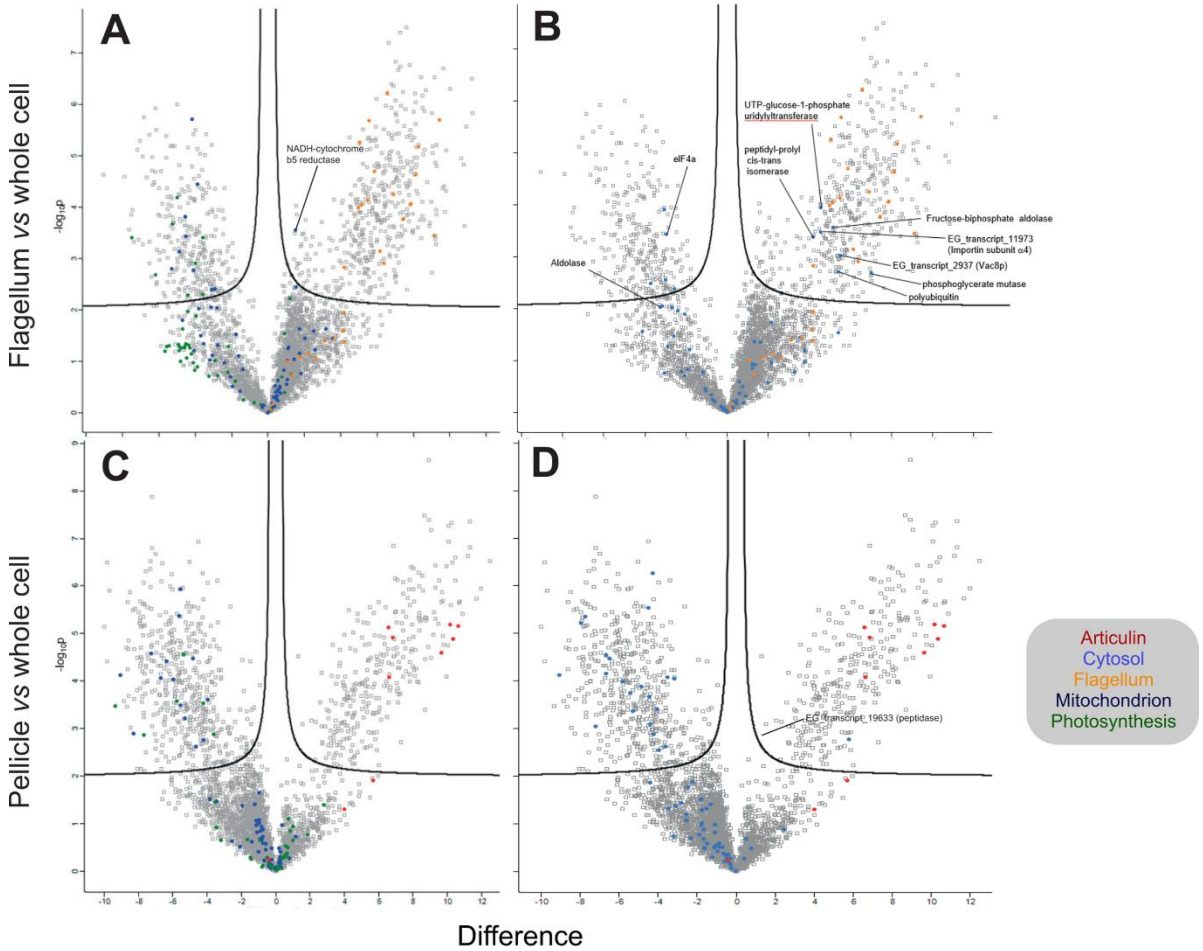

Figure S4: Volcano plots of flagella and pellicle proteomes. Plots from *p*-values versus the corresponding *t*-test difference of 3,787 protein groups quantified in the whole cell lysate fraction and the flagellum (A,B) or pellicle fraction (C,D), respectively. Green, dark blue and light blue dots represent proteins assigned to "photosynthetic", "mitochondrial" and "cilium" GO categories, respectively. Articulins are labelled in red. Stringent cutoff curves for statistically significant enrichment (black curves) were calculated from a false discovery rate (FDR) of 1%. Potential contaminant proteins in the respective fractions and selected marker proteins are labeled.

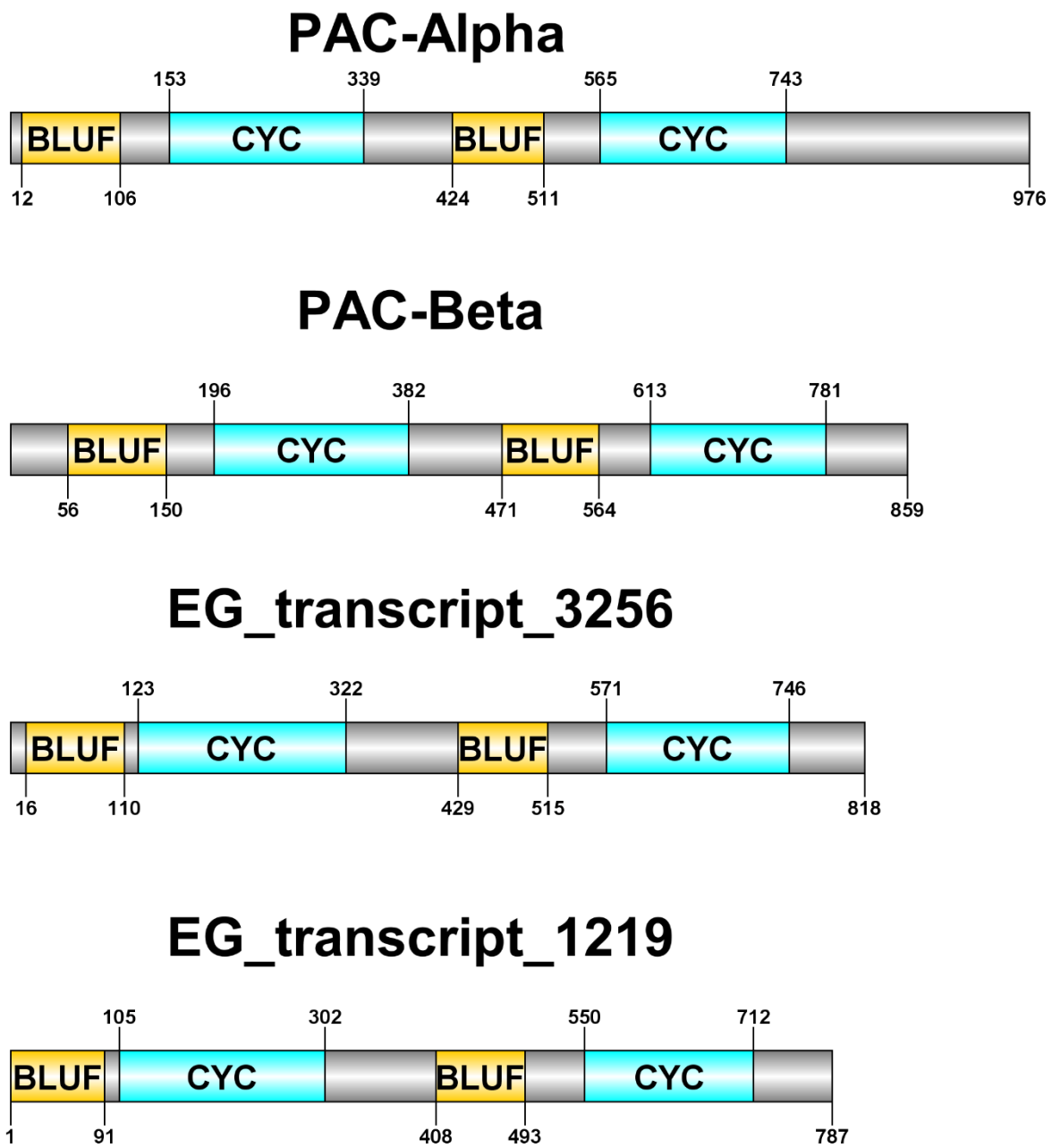

Figure S5: Domain architecture of photoactivated adenyl cyclase subunits, as well as hypothetical candidate subunits for mediating light based 'step down' response. Blue Light Using FAD (BLUF) and Adenylate Cyclase (CYC) domains indicated.

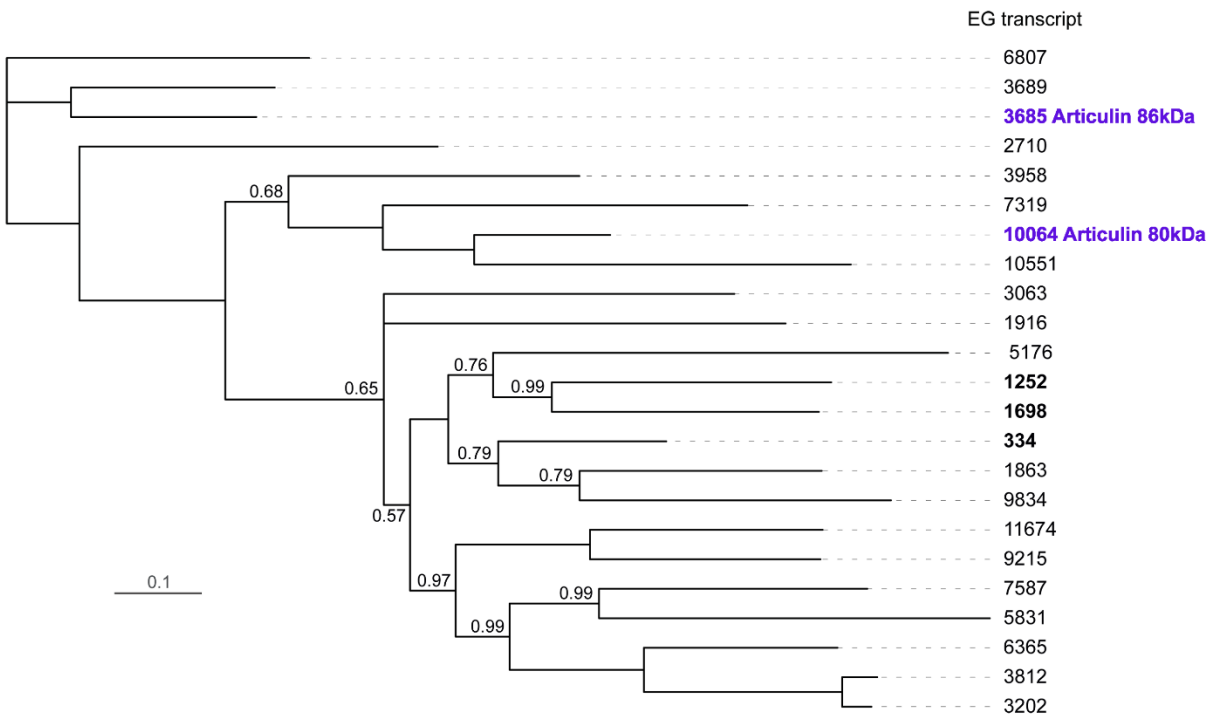

Figure S6: Phylogenetic distribution of articulins and articulin-related proteins of *E. gracilis* pellicle. Original articulins highlighted in purple. Bold sequences indicate those analysed for repeats in Suppl. Figure 8.

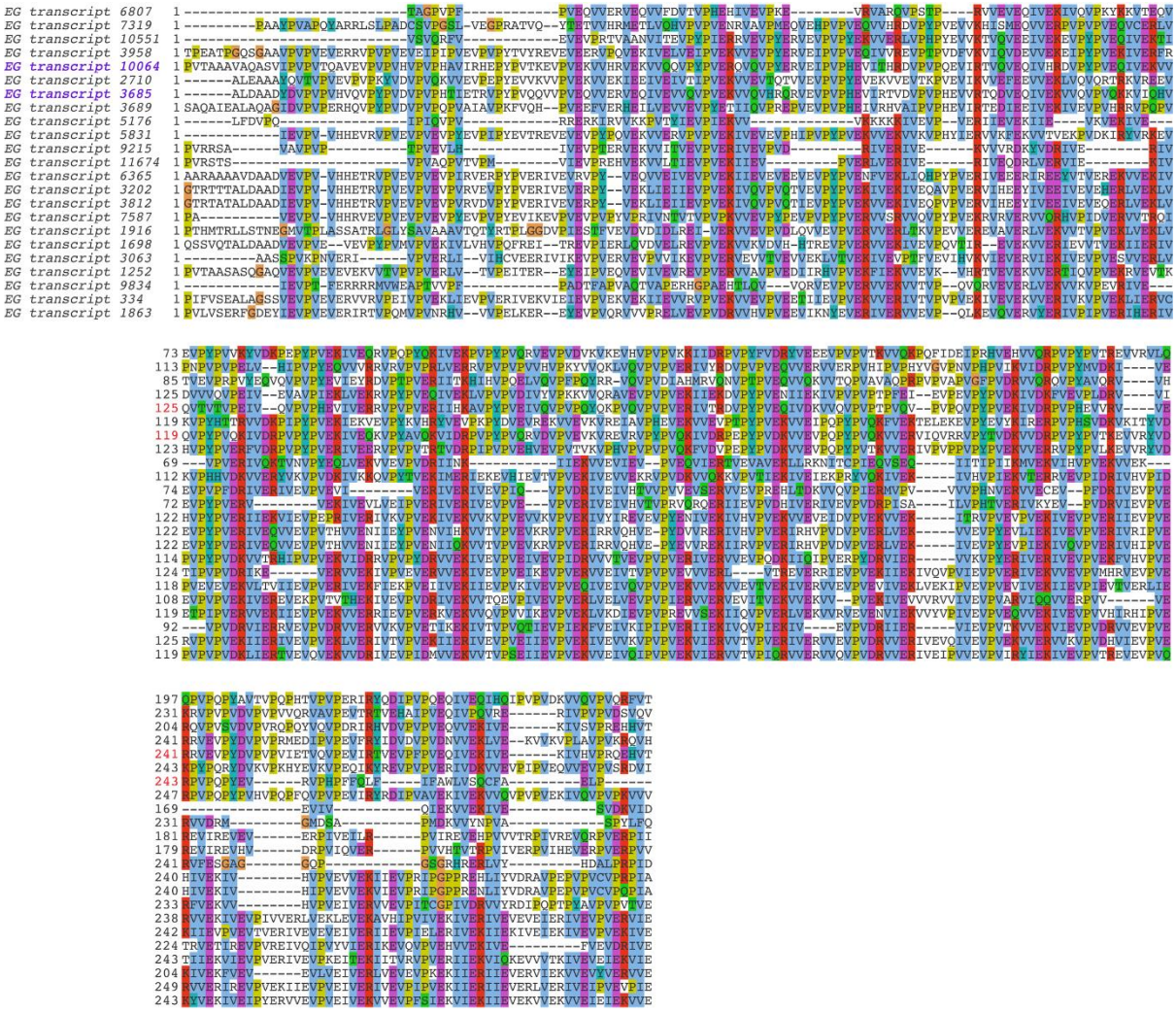

Figure S7: Alignment of articulins and articulin-related proteins of *E. gracilis*. Clustal coloration used, with original articulins highlighted in purple.

334

```
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
          31|     887.56|      15|      38|     410|     424|        1

  230-  244 (26.39/ 6.67)        RIVQVPVDRVVEVPV
  250-  264 (25.82/ 6.34)        RIVRVPEVRTVEVPV
  290-  304 (27.69/ 7.41)        RVVRVPEIHEIEVPV
  410-  424 (26.78/ 6.89)        RVVQTHTRQVIEVPV
  430-  444 (29.31/ 8.34)        RIVRVPQETLIEVPV
  450-  464 (29.17/ 8.25)        RVVRVPQHTTVEVPV
  470-  484 (28.83/ 7.60)        RVVQTHTRQIIEVPV
  490-  504 (32.09/ 9.91)        RVVRVPQETIIEVPV
  510-  524 (28.64/ 7.95)        RIVTVPQHTTVEVPV
  530-  544 (26.78/ 6.89)        RVVQTHTRQVIEVPV
  550-  564 (31.51/ 9.59)        RIVKVPQETIIEVPV
  570-  584 (31.58/ 9.62)        RVCKVPQETIIEVPV
  590-  604 (25.36/ 6.09)        RVSQTHVRQIVEVPV
  610-  624 (28.10/ 7.65)        RIVTVPRETVIEVPV
  630-  644 (31.50/ 9.58)        RIVKVPQETIVEVPV
  650-  664 (27.54/ 7.32)        RVCQTHTRQIIEVPV
  670-  684 (31.49/ 9.58)        RVVKVPQETIIEVPV
  690-  704 (29.45/ 8.41)        RVVTVPQETIEEVPV
  710-  724 (28.03/ 7.60)        RVVQTHTRQIIEVPV
  730-  744 (31.48/ 9.57)        RVVKVPQETIVEVPV
  770-  784 (31.51/ 9.59)        RIVKVPQETIIEVPV
  810-  824 (32.11/ 9.93)        RIVRVPQETIIEVPV
  862-  876 (28.85/ 8.07)        RVVKVPVETIKEVPV
  918-  932 (27.28/ 7.17)        RLVEVPVTHTVEVPV
  978-  992 (27.92/ 7.54)        RVVTVPVEKRIEVPV
  998- 1012 (25.68/ 6.26)        RIKEVPVEKIEVPV
 1018- 1032 (29.50/ 8.44)        RIKVPVEKIVEVPV
 1038- 1052 (27.03/ 7.03)        RIVQVPVTQTVEVPV
 1078- 1092 (28.49/ 7.87)        RVVKVPVDHGIEVPV
 1098- 1112 (26.38/ 6.66)        RIREVPVEKIIQVPV
 1210- 1224 (26.09/ 6.50)        RIVEVPITNVVEVPV

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           9|     479.63|      37|      37|     313|     349|        2

  198-  223 (37.87/ 7.61)        KVP..VEI.DVDID................T..YREiV......EVPIEKIVE
  313-  349 (76.71/22.57)        RVPEIHEI..EVPVERIVE....KVIRVP..E..IRQ.V......EVPVERIIE
  353-  389 (73.13/21.19)        RVPEIHEI..EVPVEKRIE....TVVRVP..E..VMQ.I......EVPVEKIIE
  830-  861 (40.41/12.06)        RV.....G..EVQVEKIVEvqvqQIVRVP.........V......EVPVEHIVE
  885-  917 (58.80/15.67)        PVEVIHHI..EVPVERIVE....RVV.....E..VLR........EVPVDRVVE
  941-  977 (55.79/14.51)        EVPV..EIirEVPVERIVE....RVVTVRVdN..Y...I......EVPVEKIIE
 1172- 1209 (45.52/10.56)        .VPKV......VERIVE....RIVEVP..deyIRE.VTvTSiKEVPVEKIVE
 1242- 1265 (39.69/ 8.32)        .....RIV..EVPVERIIE....RII..................EVPVERVVE
 1266- 1289 (42.71/ 9.48)        .....HII..EVPVERLVE....RIV..................EIPVEKIIE

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           3|      76.58|      14|      14|    1308|    1321|        3

 1308- 1321 (25.02/ 6.62)        VPIEIEKVVEIEKI
 1324- 1337 (27.09/ 7.85)        VPVVVEKVVEVEKI
 1359- 1372 (24.47/ 6.28)        VPVVVEKVIETEKI

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           5|     213.20|      33|      37|    1428|    1460|        4

 1390- 1409 (28.91/11.17)        ....YSTSYST......GSKGTSS......SISASG
 1428- 1460 (72.10/37.37)        GGG.YAVSGGTGGA.GYGSIGTSG.GTGYSSITTGG
 1462- 1482 (29.50/11.53)        G............T.GYSSIGGTGaGVGYSSATT..
 1499- 1528 (32.38/13.28)        .........SGAPGGAgGYSS.......TSYSSGTYGA
 1521- 1548 (50.32/24.16)        GGGtYG.AGAPGAG.GYGS....YS.STSYSG..TGG
```

1698

```
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
          13|     364.85|      19|      19|     625|     643|        1

  285-  301 (30.74/ 9.82)        EVPVEKIVE....K..DKLVHVP
  307-  325 (29.70/ 9.24)        EVPIEVPVS....HVIEHLVQVD
  331-  345 (25.42/ 6.84)        EVP....IE...RVVERILQVD
  351-  365 (26.17/ 7.26)        EVP....VE...RLVERVVVVD
  371-  385 (33.89/11.58)        EVP....VE....RVVERIVEVP
  469-  483 (31.54/10.26)        EVPVE........QIVEKIVQVP
  529-  539 (24.14/ 6.13)        EV...........VVEKIVEVP
  595-  611 (30.35/ 9.60)        ..PVEVPVE....VIVEKIIEVP
  625-  639 (27.60/ 8.06)        EVPVEVTVE....RIVERI....
  641-  655 (27.02/ 7.74)        EVPVEVEVE....RIVERI....
  717-  739 (24.68/ 6.38)        EVPVPVHDDgsqIRVVEKIVEIE
  743-  757 (28.38/ 8.50)        EVPVEVE.......VVRIVEVEE
  761-  776 (24.51/ 6.34)        EVPVEVV......KIIEKYIEV.

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|     138.20|      38|      39|      75|     112|        2

   75-  112 (68.61/50.99)        RVNSVGAVEAVAQALDAADGTLDGRFFGKPIVAQSSSA
  115-  152 (69.59/51.82)        HASSASPNAVAEALDAADGVLDGAFFGKRIVTSGAGA

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           7|     241.75|      29|      41|     512|     552|        3

  391-  411 (36.50/11.28)        EVPVE........KVVE..R.............IEV.VT..VEKIIE.......
  414-  433 (26.28/ 6.17)        .VPVEH....IVE...VE...RV.............LTVT..LQRVI........
  435-  468 (35.68/10.86)        EVPVEH....IVEKFIEkqV...............TVEII..VEKIIEVpvkhiV
  489-  512 (32.03/ 9.04)        EVPVEH....VEKVX.....................VEYTVEkiVE........vV
  513-  551 (37.12/12.40)        EVPVEV....IVEKLVE..VVkevvvvkivevpVEVTVQ..VDRIVE.......
  657-  684 (36.62/11.33)        EVPIEV......EKIVE..............VERIVT..VEKI..IevekiV
  685-  712 (37.51/11.78)        EVPIEVelerIVEKII................EVPPE..VEKIVEI......

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      46.45|      13|      29|     588|     592|        5

  580-  592 (23.56/13.61)        IPVEVERLVEKII
  612-  624 (22.88/12.97)        IEVTVERLIEKII

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           4|     120.76|      21|      37|     855|     875|        6

  811-  831 (32.57/18.34)        ADSPDFGSPQMSAPSLLPSGQ
  855-  875 (40.12/24.84)        QQSWGGGGPSYSSAASNNTGA
  880-  897 (24.72/12.40)        SGRWGGAAQGASQGGNG...
  900-  912 (23.35/11.36)        QQTFSTGG.......WGSFA

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      35.95|      10|      15|     245|     254|        7

  245-  254 (18.77/ 8.62)        TEYIEHVPIE
  262-  271 (17.18/ 7.19)        THEIVEVPVE

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      47.90|      12|      19|     204|     215|        8

  204-  215 (24.68/13.45)        PPCQSLPAAVSP
  226-  237 (23.21/12.27)        PLCSSAIRRVSP
```

1252

```
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
          18|     488.00|      17|      18|     484|     500|        1

  268-  288 (23.59/ 6.65)        EVEKVwtvpQLVEVPVERLVE
  292-  308 (26.04/ 8.06)        TVPEI....TERDVPVEELVE
  312-  328 (27.82/ 9.08)        QVPEV....FEYEIPVEQLVE
  384-  400 (27.21/ 8.73)        EVPVD....IIRHVPVERFIE
  424-  440 (27.92/ 9.14)        QVPQL....TLVEVPVEKRVE
  444-  460 (28.44/ 9.43)        ETPQI....TVKEIPVERVVE
  464-  480 (26.69/ 8.43)        QVDQQ....MIIEVPVEKVVE
  484-  500 (29.42/10.00)        EVPKV....TMQAVPVERVKE
  504-  520 (28.80/ 9.18)        QVPPV....RIKEVPVEKLVK
  524-  540 (27.40/ 8.84)        EVPKV....TIREVPKEVVSE
  544-  560 (31.00/10.90)        QVPVT....HITEVPVERLVE
  576-  592 (30.71/10.73)        QVPKV....VTQEVPVERLIE
  628-  644 (24.72/ 7.30)        EVTKE....YITEVPVVKVVE
  648-  664 (27.66/ 8.99)        YVPVE....KIVEVPVEQVVE
  668-  684 (28.76/ 9.62)        EVPVV....HIRHIPVETIIE
  715-  730 (24.82/ 7.36)        .VPQE....NIVEVPVERIIE
  740-  756 (22.46/ 6.00)        EVPIE....VLKEVVVTKIVE
  794-  810 (25.33/ 7.65)        EVEKI....VEVPVPIEHIIE

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           6|     187.36|      21|      26|     871|     891|        2

  826-  852 (23.20/ 6.70)        TAGtvqsGSS.....rrV.SSITGSSFQT.G..GTK
  865-  885 (41.59/17.74)        TST....MTG.......G.GSISGSYGAS.G..GGY
  886-  919 (34.38/13.41)        TAG....GSGaggyraG.GSISSGYGGSaG..GGY
  915-  939 (33.55/12.92)        AAG....GSG.....vtgGySSYGSYGGS.T..GGY
  940-  956 (25.53/ 8.10)        AAG....GSG.....aaG.G......YTA..G..GPV
 1003- 1025 (29.11/10.25)        LGS....GSA.......G.YYQESYSYGSS.GgaGGP

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           3|     242.28|      66|      71|      92|     161|        3

   40-   95 (83.29/41.17)        R..RPVTAASYQ...ESGLRTYSSSGAH.IT..PS..LVSGRSLYDAGSQ..SV.....LGGQQFVSVTEGDK...............
   96-  161 (101.49/58.76)       ....YVSVSASQ...GAQARLAGSQYAT.VG..SQGvLGSQPRQVAGGSQIVSV.....SEGDQYVSVTEGGQlLS........GGAVQ
  162-  235 (57.50/30.92)        GaqS..AVAASQysvGSQSALARSQFVSgvGysSRG.......QYAGAGQYYTVgsqaasARSQYVS.T.....LSaqrtlgrlGEAVQ

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           5|     195.50|      25|      83|     601|     627|        4

  339-  367 (36.59/11.79)        REVPVERLiqVERVVEraVPVEHLVDQVV
  403-  423 (34.70/10.66)        VEVKVHRT..V....E..IPVEKVVERTI
  603-  627 (49.81/19.66)        VEVPVERL..VEKIIE..VPVEHLVEKVV
  687-  711 (43.81/16.09)        IEVPVERL..VQNIVE..VPKEFITEKII
  757-  773 (30.60/ 8.22)        VEVPVP....IEKVIE..V......EKIV

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      43.48|      11|      31|     957|     967|        7

  957-  967 (22.78/11.30)        TGGLSGYTQGG
  991- 1001 (20.70/ 9.52)        TTGVSGATQEG
```

10064 (80kDa)

```
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           9|     638.10|      44|      73|     214|     257|        1

   61-  105 (70.66/13.50)        PVQHNHEVELIEREFIVPVEKIVQRHVPVPV..ERIVQREVPVPR..QV
  112-  155 (81.87/16.74)        EIPVPVE.RIQHRQVPYPVEQIVEKRIPVPV..TQIVEQAVEVPV..PV
  164-  207 (69.54/13.10)        PVPHAVV.HEVEVHEPVPVTVEVIRQVPVEV..PREVVRQVTVDV..PV
  214-  257 (90.81/19.32)        QVPYPVE.KVVHRQVPYPVEKVVQRQVPYPV..QKIVERQVQVPY..EV
  288-  319 (63.68/11.49)        QVPVPVE.QIVHRDVPVPVEQ.............IVEKYVQVTR..QV
  326-  369 (80.13/16.24)        QVPVPHE.KIVERHVPVPVEHIRKAVPYPV..EQIVEKIVQVPV..PQ
  376-  413 (66.96/12.44)        QVPVPVE.RIVTRDVPYPVEQI.......V..DKVVERQVPVPTpvQV
  414-  450 (67.52/12.60)        PVPTPV......QVPYPVEHIVDRPVPHEV..VRVVERRVEVPT..D.
  470-  503 (46.93/ 6.65)        EVPFPVE.QIVEK....IVEKIV..HVPVPVtrQEHVTRQV.......

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      34.77|       9|     171|     277|     287|        2

  277-  287 (15.37/11.11)        VPVPqeVIRTV
  451-  459 (19.40/ 7.26)        VPVP..VIETV
```

3685 (86kDa)

```
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           8|     414.19|      23|      23|     292|     314|        1

  114-  138 (33.97/ 7.67)        PVPHEVVRTVDVPE..HYdvP.VPHAVH
  140-  162 (42.95/11.64)        QVPYPVDRFVDVPV..PH..T.IQKIVE
  164-  187 (39.50/10.12)        RVPYPVQQVVQRRVerPY..D.VP.VVE
  190-  212 (45.26/12.67)        NVPPVEQVVERRV..PV..P.VEQIVE
  242-  262 (35.33/ 8.27)        PVPHEVIRTVDVPV..PH..E.V..VRT
  292-  314 (51.44/15.40)        QVPYPVDKIVDRPV..PY..P.VEKIVE
  316-  338 (45.77/12.89)        KVPYAVQKIDRPV..PY..P.VQKIVE
  354-  376 (49.16/14.39)        KVPYPVDKIVDRPE..PY..P.VDKVVE
  378-  401 (36.69/ 8.87)        PQPYPVDKVVERRV..EV..PhVIQVRE
  404-  421 (34.11/ 7.73)        RVPYTVDKVVDRPV..PY..P.V.....

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      72.63|      22|      47|     215|     238|        2

  215-  236 (41.55/16.62)        VQVPVERLVE......KVVQVHRQVPVP
  439-  466 (31.08/ 6.68)        VRVPHPDFFQlfifawLVSQCFAELPIP

No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|    Level
           2|      57.27|      16|      16|      36|      51|        3

   36-   51 (32.68/21.13)        PGAPVTYGA.AAPVTSY
   54-   70 (24.59/14.02)        PAAVTSFAATAAPVTSF
```

Figure S8: Repeated motifs and sequences displayed in select articulins and articulin-related proteins.

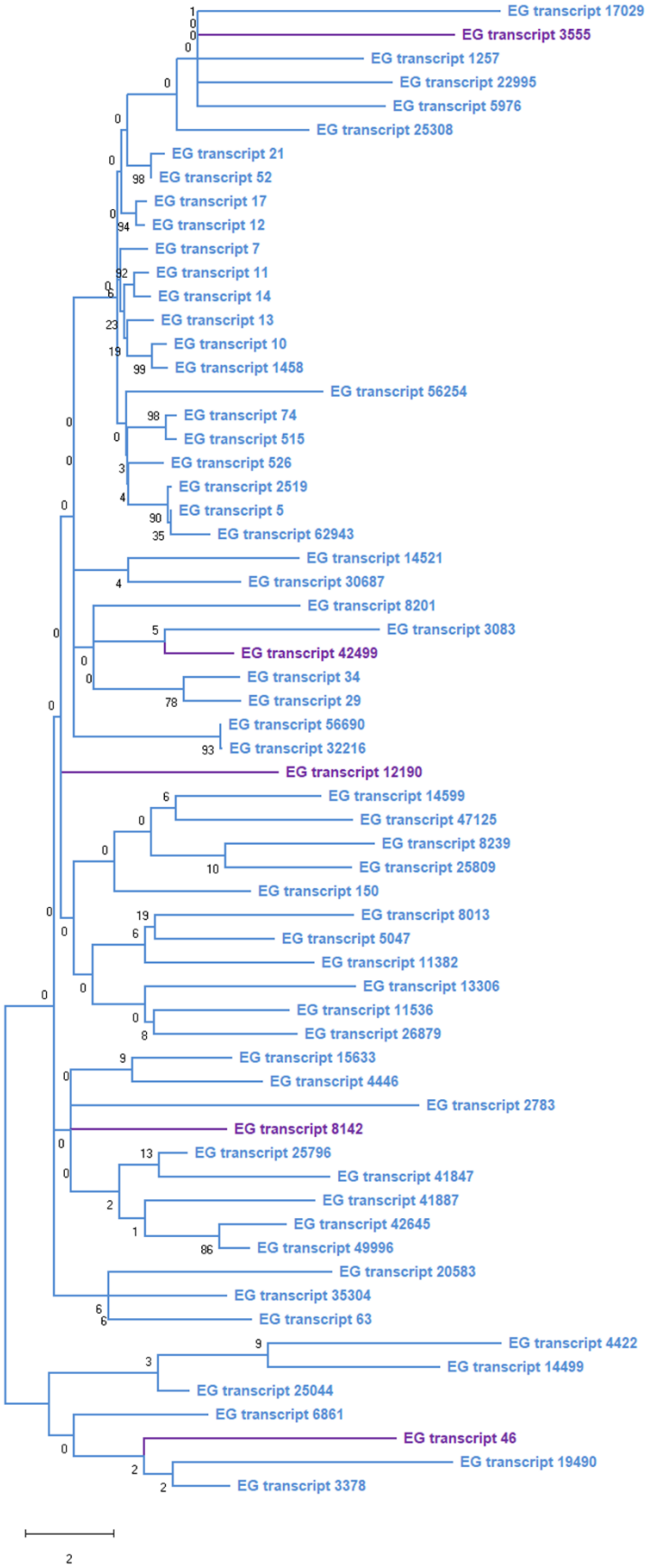
EG transcript 17029
EG transcript 3555
EG transcript 1257
EG transcript 22995
EG transcript 5976
EG transcript 25308
EG transcript 21
EG transcript 52
EG transcript 17
EG transcript 12
EG transcript 7
EG transcript 11
EG transcript 14
EG transcript 13
EG transcript 10
EG transcript 1458
EG transcript 56254
EG transcript 74
EG transcript 515
EG transcript 526
EG transcript 2519
EG transcript 5
EG transcript 62943
EG transcript 14521
EG transcript 30687
EG transcript 8201
EG transcript 3083
EG transcript 42499
EG transcript 34
EG transcript 29
EG transcript 56690
EG transcript 32216
EG transcript 12190
EG transcript 14599
EG transcript 47125
EG transcript 8239
EG transcript 25809
EG transcript 150
EG transcript 8013
EG transcript 5047
EG transcript 11382
EG transcript 13306
EG transcript 11536
EG transcript 26879
EG transcript 15633
EG transcript 4446
EG transcript 2783
EG transcript 8142
EG transcript 25796
EG transcript 41847
EG transcript 41887
EG transcript 42645
EG transcript 49996
EG transcript 20583
EG transcript 35304
EG transcript 63
EG transcript 4422
EG transcript 14499
EG transcript 25044
EG transcript 6861
EG transcript 46
EG transcript 19490
EG transcript 3378
2

Figure S9: Phylogenetic distribution of dynein motor proteins from *E. gracilis* flagella and pellicle fractions. Maximum likelihood tree showing highest log likelihood distribution (-16343.88). Dyneins enriched in flagella fraction highlighted in blue and those enriched in pellicle fraction in pink. Numbers next to branch show percent of other trees in which taxa cluster together similarly. Scale bar represents number of substitutions per site.

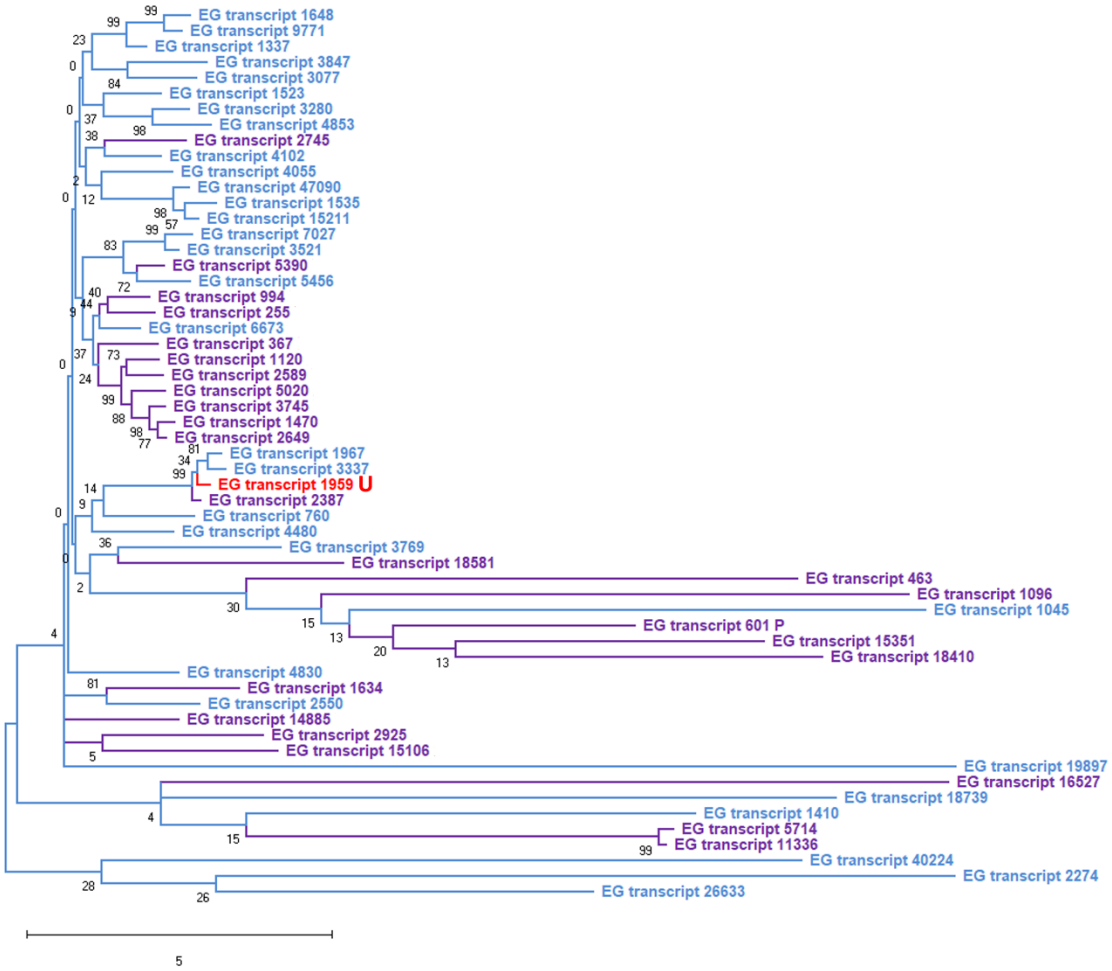

Figure S10: Phylogenetic distribution of kinesin motor proteins from *E. gracilis* flagella and pellicle fractions.

Maximum likelihood tree showing highest log likelihood distribution (-21995.81). Kinesins enriched in flagella fraction highlighted in blue, those enriched in pellicle fraction in pink, kinesin recovered in both fractions in red, U indicates presence in putative ancestral Urglenid pellicle, as shown in Figure 6. Numbers next to branch show percent of other trees in which taxa cluster together similarly. Scale bar represents number of substitutions per site.