

Table S2. Primer used in the study.

| Used primer | Sequence 5'-3'                 | Target marker region | References                             |
|-------------|--------------------------------|----------------------|----------------------------------------|
| Cob 1f      | ATG GYT TTT YTC WAC WAA TCA    | COI                  | PETERSON et al. (2006)                 |
| Gaz 2R      | ACT TCT GGA TGT CCA AAA AAY CA | COI                  | SAUNDER (2005)                         |
| ITS 1420f   | CAG GTC TGT GAT GCC C          | ITS1-5.8S-ITS2       | ROGERS et al. (2006)                   |
| ITS 4r      | TCC TCC GCT TAT TGA TAT GC     | ITS1-5.8S-ITS2       | WHITE et al. (1990)                    |
| Sogin2f     | AGG GTTCGA TTC CGG             | 18S                  | Modified after GUNDERSON et al. (1986) |
| Proto5r     | GACCGGGCGGTGTGTAC              | 18S                  | MEDINGER et al. (2010)                 |
| 82F         | GAA ACT GCG AAT GGC TC         | 18S                  | LÓPEZ-GARCÍA et al. (2003)             |
| LEUK2       | ACCCGCTGAACCTAAGCATATCACT      | LSU                  | LIU et al. (2010)                      |
| LSU-740DR   | ACCCTATTCAGGCATAGTT            | LSU                  | VESELÁ et al. (2012)                   |

Table S3. Estimates of average evolutionary divergence over sequence pairs within groups for selected gene markers. All positions containing gaps and missing data were eliminated. Analyses were conducted using the Kimura 2-parameter model. Evolutionary analyses were conducted in MEGA5 (TAMURA et al. 2011). The number of base substitutions per site from averaging over all sequence pairs within each group are shown. (n=number of analyzed sequences for the respective marker; n.a.=not analyzed)

|                                                                      | 18S     | V4      | 5.8S    | LSU     | COI     |
|----------------------------------------------------------------------|---------|---------|---------|---------|---------|
| Ochromonadales                                                       | 0.01693 | 0.04083 | 0.05654 | 0.07495 | 0.22006 |
| Synurales<br>(18S n=24, V4 n=24, 5.8S n=13, LSU n=27, COI n=10)      | 0.02162 | 0.06189 | 0.02183 | 0.09134 | 0.20718 |
| Paraphysomonas<br>(18S n=15, V4 n=16, 5.8S n= 7, LSU n=12, COI n=14) | 0.03375 | 0.10162 | 0.03267 | 0.06999 | 0.22295 |
| Apoikiida<br>(18S n=3, V4 n=3, 5.8S n= 2, LSU n= 4, COI n=3)         | 0.03087 | 0.07250 | 0.0100  | 0.02243 | 0.21266 |
| Chromuliniales<br>(18S n=3, V4 n=4, 5.8S n=1, LSU n=4, COI n=2)      | 0.03733 | 0.10502 | n.a.    | 0.10820 | 0.28381 |

Table S4. Estimates of average evolutionary divergence between groups for selected gene markers. All ambiguous positions were removed for each sequence pair. Analyses were conducted using the Kimura 2-parameter model. Evolutionary analyses were conducted in MEGA5 (TAMURA et al. 2011).

| Analyzed groups and<br>marker regions<br>(18S, V4, 5.8S, LSU,<br>COI) | Ochromonadales | Synurales | Paraophysomonadida | Apoikiida |
|-----------------------------------------------------------------------|----------------|-----------|--------------------|-----------|
|                                                                       | 0.04638        |           |                    |           |
| Synurales                                                             | 0.12165        |           |                    |           |
| (18S n=24, V4 n=24, 5.8S<br>n=13, LSU n=27, COI<br>n=10)              | 0.09461        |           |                    |           |
|                                                                       | 0.18123        |           |                    |           |
|                                                                       | 0.24974        |           |                    |           |
|                                                                       | 0.06084        | 0.06148   |                    |           |
| Paraphysomonas                                                        | 0.15426        | 0.15583   |                    |           |
| (18S n=15, V4 n=16, 5.8S<br>n= 7, LSU n=12, COI<br>n=14)              | 0.08930        | 0.09508   |                    |           |
|                                                                       | 0.15874        | 0.17760   |                    |           |
|                                                                       | 0.29907        | 0.28881   |                    |           |
|                                                                       | 0.04556        | 0.04852   | 0.06191            |           |
| Apoikiida                                                             | 0.13074        | 0.12750   | 0.15382            |           |
| (18S n=3, V4 n=3, 5.8S<br>n= 2, LSU n= 4, COI n=3)                    | 0.06918        | 0.07309   | 0.07315            |           |
|                                                                       | 0.14417        | 0.18873   | 0.16436            |           |
|                                                                       | 0.25302        | 0.22532   | 0.27931            |           |
|                                                                       | 0.04914        | 0.05546   | 0.06847            | 0.05767   |
| Chromulinales                                                         | 0.11600        | 0.13200   | 0.16487            | 0.14638   |
| (18S n=3, V4 n=4, 5.8S<br>n=1, LSU n=4, COI n=2)                      | 0.08893        | 0.08560   | 0.10924            | 0.07460   |
|                                                                       | 0.17081        | 0.20327   | 0.18059            | 0.17229   |
|                                                                       | 0.27071        | 0.27262   | 0.29395            | 0.26483   |

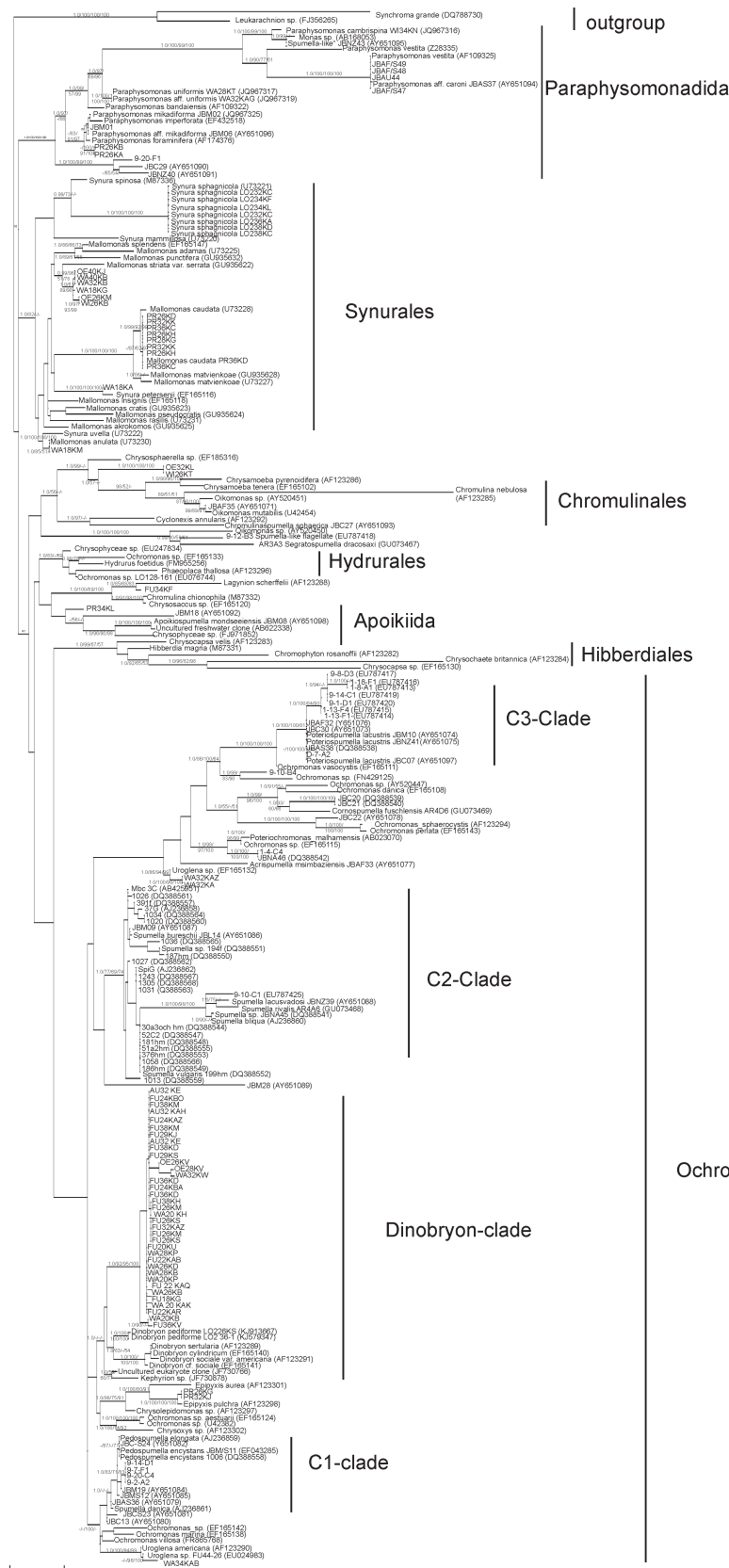
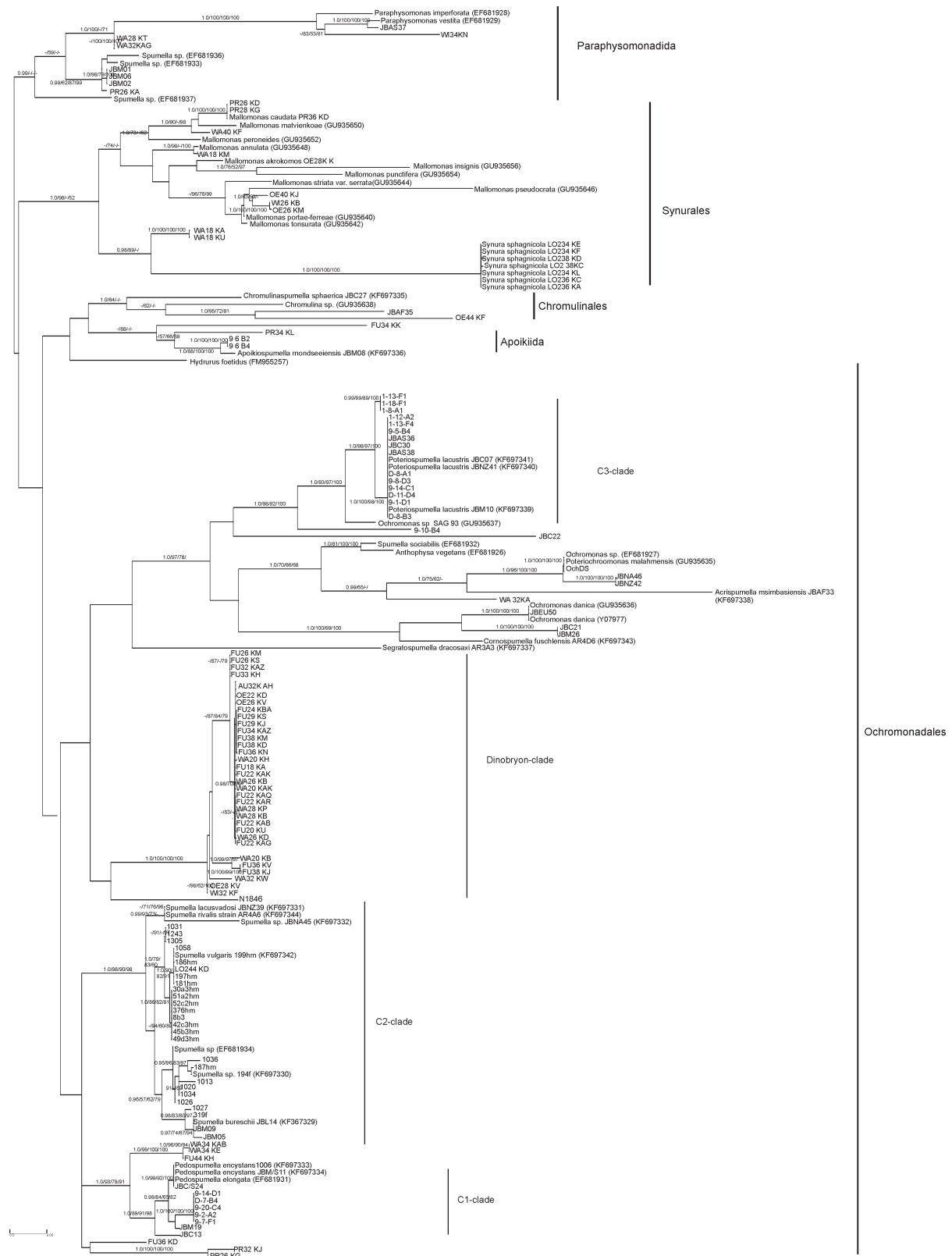


Figure S1. Complete phylogeny of the chrysophytes obtained by maximum likelihood inference of the SSU rDNA dataset. Values at the nodes indicate statistical support estimated by four methods—Bayesian inference (MB), maximum likelihood (ML), maximum parsimony (MP), distance (neighbor joining; NJ). Hyphens indicate support below 50% for ML, MP, NJ and below 0.95 for MB. Scale bar represents the expected number of substitutions per site.



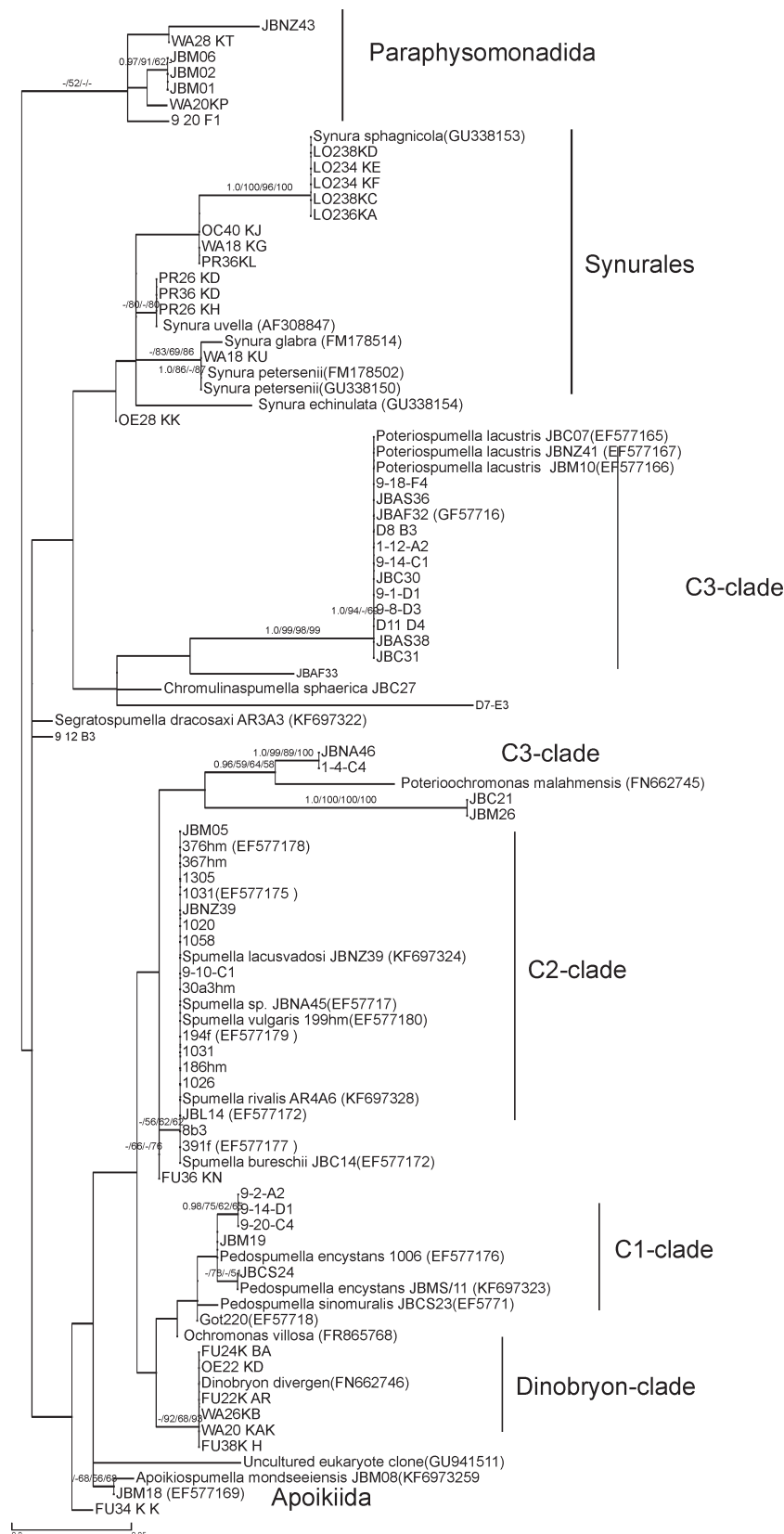


Figure S3. Phylogeny of the chrysophytes obtained by maximum likelihood inference of the 5.8S rDNA dataset. Values at the nodes indicate statistical support estimated by four methods—Bayesian inference (MB), maximum likelihood (ML), maximum parsimony (MP), distance (neighbor joining; NJ). Hyphens indicate support below 50% for ML,MP, NJ and below 0.95 for MB. Scale bar represents the expected number of substitutions per site.

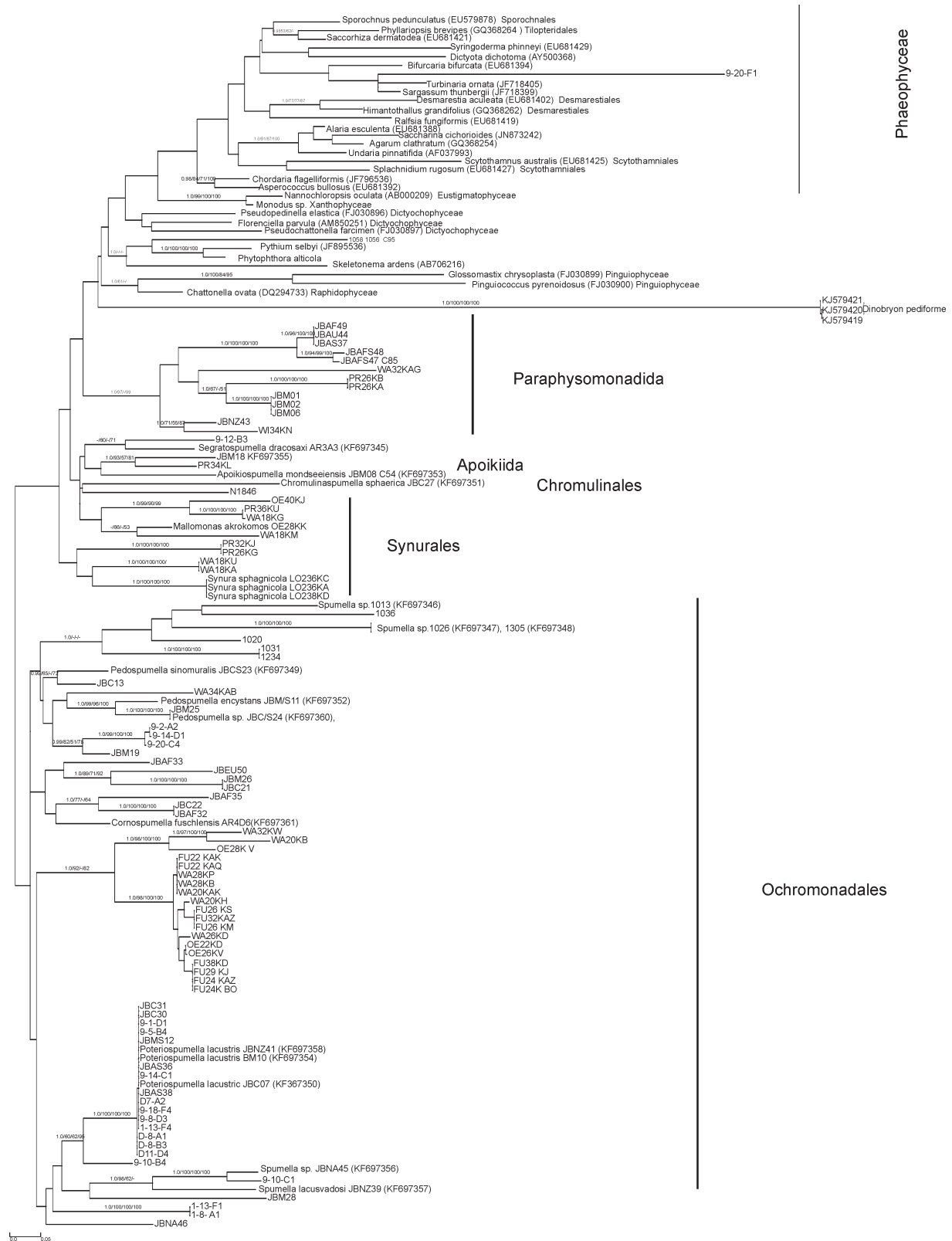


Figure S4. Complete phylogeny of the chrysophytes obtained by maximum likelihood inference of the COI dataset. Values at the nodes indicate statistical support estimated by four methods—Bayesian inference (MB), maximum likelihood (ML), maximum parsimony (MP), distance (neighbor joining; NJ). Hyphens indicate support below 50% for ML, MP, NJ and below 0.95 for MB. . Scale bar represents the expected number of substitutions per site.